

PROCEEDINGS
OF
PAKISTAN CONGRESS OF ZOOLOGY

Volume 35, 2015

*All the papers in this Proceedings were refereed
by experts in respective disciplines*



THIRTY FOURTH PAKISTAN CONGRESS OF ZOOLOGY

held under auspices of

THE ZOOLOGICAL SOCIETY OF PAKISTAN

at

**CENTRE OF EXCELLENCE IN MARINE BIOLOGY,
UNIVERSITY OF KARACHI, KARACHI**

MARCH 1 – 4, 2015

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PROCEEDINGS OF THE CONGRESS

Editor

Dr. A.R. Shakoori

Composed & Designed by: Amjad Ali

ACKNOWLEDGMENTS

Centre of Excellence in Marine Biology, University of Karachi, Karachi hosted the 35th Pakistan Congress of Zoology (International).

The Zoological Society of Pakistan expresses its deep gratitude to the Vice Chancellor, Centre of Excellence in Marine Biology, University of Karachi, Karachi and faculty members and students of the Department of Zoology for extending warm hospitality.

Grants were received from Higher Education Commission, Islamabad, COMSTECH, Islamabad, Pakistan Atomic Energy Commission, Islamabad, Welcare Chemical Corporation Limited and Hamdard Foundation, Pakistan.

35th PAKISTAN CONGRESS OF ZOOLOGY (INTERNATIONAL)

**CENTRE OF EXCELLENCE IN MARINE BIOLOGY,
UNIVERSITY OF KARACHI, KARACHI**

March 1 – 4, 2015

PROGRAMME

SUNDAY, MARCH 1, 2015

VENUE:	BAHRIA AUDITORIUM, KARSAZ, KARACHI
04:30 PM	REGISTRATION
04:50 PM	Guests to be Seated
05:00 PM	Tilawat'e Kalam-e-Pak & National Anthem
05:10 PM	Welcome Address by Prof. Dr. Pirzada Jamal A. Siddiqui <i>Director, Centre of Excellence in Marine Biology</i>
05:25 PM	Address by Prof. Dr. Abdul Rauf Shakoori, T.I., A.K., FPAS <i>President, Zoological Society of Pakistan</i>
05:40 PM	Address by Mr. Abdul Aziz Khan, Secretary General, <i>Zoological Society of Pakistan</i>
05:55 PM	Inaugural Address - Prof. Dr. Muhammad Qaiser, Vice Chancellor, University of Karachi
06:10 PM	Awards & Medals Distribution Ceremony
06:40 PM	Address by Guest of Honor - Senator Abdul Haseeb Khan, <i>Chairman, Brookes Pharma</i>
06:55 PM	Address by the Chief Guest - Dr. Abdul Qadeer Khan, N.I., <i>H.I., FPAS Distinguished National Scientist</i>
07:00 PM	Invited Lecture by Dr. Manzoor Hussain Soomro, <i>Chairman,</i> <i>ECO Science Foundation, Pakistan</i>
	Inquiry based science education (IBSE) for S&T innovation led economic development and international Cooperation
07:40 PM	Vote of Thanks - Prof. Dr. Shahina Fayyaz, <i>Director, N.N.R.C,</i> <i>University of Karachi</i>
07:55 PM	Dinner
08:40 PM	Musha'era & Mehfil-e-mosequi

DAY TWO: MONDAY, MARCH 2, 2015

JOINT SESSION I: (Plenary Lectures)

Chairman: Prof. Dr. A.R. Shakoori

Co-chairman: Dr. Manzoor Soomro, Islamabad

- Speakers: 1. **Prof. Dr. Abdullah G. Arijo,**
*Department of Parasitology, Sindh Agriculture University,
Tandojam, Pakistan*
**Soft Ticks in Tharparker: A molecular approach towards
zoonosis.**
2. **Prof. Dr. Salah A. Soliman,**
*Professor of Pesticide Chemistry & Toxicology, Alexandria
University, Alexandria, Egypt*
**Harmonization of pesticide regulation and MRLs: An
African initiative serving global health, environment and
trade**
3. **Dr. Jan Johan Ter Poorten,** *The Netherlands.*
**Biodiversity in the bivalve family Cardiidae: Larger the
island of knowledge, longer the shoreline wonder.**
4. **Dr. Hui Zhang,**
*Institute of Evolution & Marine Biodiversity, Ocean
University of China, P.R. China*
Genetic study of marine rockfish, *Sebastes schlegelii*

11:00 AM Tea Break

JOINT SESSION II: Plenary Lectures

Chairperson: Prof. Dr. Salah A. Soliman, Alexandria, Egypt

Co-chairperson: Dr. Hui Zhang, People's Republic of China

- Speakers: 1. **Prof. Dr. M.A. Bashar,**
*Chairman, Department of Zoology, University of Dhaka,
Dhaka-1000, Bangladesh.*
**Conservation of plants and successive trophic levels by
colonizing butterflies in Bhawal National Park of
Bangladesh**

2. **Dr. Naveed Ahmed Khan,**
The Feerasta Family Chair & Professor, Department of Biological & Biomedical Sciences, Agha Khan University, Stadium Road, Karachi
Animals living in polluted environments are a potential source of antimicrobials against infectious agents.

1:30 PM Lunch Break & Zuhur Prayer

JOINT SESSION III: Plenary Lectures

Chairperson: Dr. Jan Johan Ter Poorten, The Netherlands

Co-chairperson: Prof. Dr. Pirzada Jamal Siddiqui, Karachi,

- Speakers:
1. **Mr. Muhammad Moazzam Khan,**
Technical Advisor (Marine Fisheries), WWF-Pakistan, Karachi
Bycatch of commercial fishing practices: A major threat to the marine biodiversity in Pakistan
 2. **Prof. Dr. Jun Sun,**
College of Marine Science and Engineering, Tianjin University of Science and Technology, Tianjin, P.R.China
Planktonic tintinnids community in the East Indian Ocean
 3. **Dr. Gao Chen-Rin,**
Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, P. R. China
Effect of stocking density on adult turbot is growth, physiology and water quality in recirculating aquaculture system
 4. **Dr. Yan Zhang,**
Chinese Academy of Fisheries Science, Beijing, China
A genome scanning for analysis of economic trait-related QTLs in common carp (*Cyprinus carpio* L.)

4:45 PM Tea Break

JOINT SESSION IV: Plenary Lectures

Chairperson: Dr. Yan Zhang, Beijing, China

Co-chairperson: Dr. Muhammad Moazzam Khan, Karachi, Pakistan

- Speakers: 1. **Dr. Hengjiu Tian, China.**
Beijing Wildlife Rescue & Rehabilitation Centre, China.
2. **Prof. Dr. Jianguang Xiao, Prof. Dr. Gao Tiexiang,**
Fisheries College, Ocean University of China, China
Description and DNA barcoding of the Indian sillago,
***Sillago indica* (Perciformes: Sillaginidae) from the coast of**
Pakistan

6:10 PM Maghrib Prayer

JOINT SESSION V: Plenary Lectures

Chairperson: Prof. Dr. Imtiaz Ahmad, Karachi, Pakistan

Co-chairperson: Dr. M.A. Bashar, Bangladesh

- Speakers: 1. **Dr. Hengjiu Tian, China.**
Beijing Wildlife Rescue & Rehabilitation Centre, China.

- Speakers: 1. **Dr. Abdul Aleem Chaudhry,**
84-B-III, Johar Town, Lahore, Pakistan - 54770
Wildlife and its Management in Cholistan desert with
special reference to conservation efforts

8:10 PM DINNER & EXECUTIVE COUNCIL MEETING

DAY THREE: TUESDAY, MARCH 4, 2015

HALL – 1

**SECTION I: CELL BIOLOGY, BIOCHEMISTRY, GENETICS,
MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS**

SESSION I

	Chairperson:	Dr. Shamsuddin Shaikh
	Co-chairperson:	Dr. Naveed Ahmad Khan
08:30 AM	Paper reading	
11:30 AM	Tea Break	

SESSION II

	Chairperson:	Dr. Farah Rauf Shakoori
	Co-chairperson:	Dr. Rubina Mushtaq
12:00 AM	Paper reading	
01:30 PM	Lunch and Prayer	

SESSION III

	Chairperson:	Prof. Dr. Qazi Javed Iqbal
	Co-chairperson:	Dr. Bushra Mateen
02:00 PM	Paper reading	
04:45 PM	Tea Break	

SESSION IV

	Chairperson:	Dr. Abdul Rehman
	Co-chairperson:	Dr. Soumble Zulfiqar
05:00 PM	Paper reading	
07:00 PM	General Body Meeting	
08:30 PM	Dinner	

HALL – 2**SECTION II: PEST AND PEST CONTROL****SESSION I**

	Chairperson:	Mr. Abdul Aziz Khan
	Co-chairperson:	Dr. Noor-un-Nisa
08:30 AM	Paper reading	
11:30 AM	Tea Break	

SESSION II

	Chairperson:	Prof. Dr. Nasreen Memon
	Co-chairperson:	Dr. Syeda Azra Qamar
12:00 AM	Paper reading	
1:30 PM	Lunch and Prayer (Zuhur)	

SECTION III: ENTOMOLOGY**SESSION I**

	Chairperson:	Dr. Kamal ud Din Ahmad
	Co-chairperson:	Dr. Muhammad Saeed Wagan
02:30 PM	Paper reading	
04:45 PM	Tea Break	

SESSION II

	Chairperson:	Dr. Riffat Sultana
	Co-chairperson:	Dr. Muhammad Hamid Bashir
05:00 PM	Paper reading	
07:00 PM	General Body Meeting	
08:30 PM	Dinner	

HALL – 3

**SECTION V: FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER
BIOLOGY, MARINE BIOLOGY**

SESSION I

	Chairperson:	Dr. Shahid Amjad
	Co-chairperson:	Dr. Ansar Rizvi
08:30 AM	Paper reading	
11:30 AM	Tea Break	

SESSION II

	Chairperson:	Dr. Javed Mustaquim
	Co-chairperson:	Dr. Rashida Qari
12:00 AM	Paper reading	
1:30 PM	Lunch and Prayer (Zuhur)	

SESSION III

	Chairperson:	Prof. Dr. Ghazala Siddiqui
	Co-chairperson:	Dr. Quddusi B. Kazmi
02:30 PM	Paper reading	
04:45 PM	Tea Break	

SESSION IV

	Chairperson:	Prof. Dr. Ali M. Yousafzai
	Co-chairperson:	Dr. Zafar Iqbal
05:00 PM	Paper reading	
07:00 PM	General Body Meeting	
08:30 PM	Dinner	

DAY FOUR: WEDNESDAY, MARCH 4, 2015

HALL – 1

**SECTION I: CELL BIOLOGY, BIOCHEMISTRY, GENETICS,
MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS**

SESSION V

	Chairperson:	Dr. Dil Ara Bukhari
	Co-chairperson:	Dr. Nadeem Sheikh
08:30 AM	Paper reading	
11:45 AM	Tea Break	

SESSION VI

	Chairperson:	Dr. Shahid Nadeem
	Co-chairperson:	Dr. Zulfiqar Ali Saqib
12:00 PM	Paper reading	
01:30 PM	Lunch Break & Prayer (Zuhur)	

SESSION VII

	Chairperson:	Prof. Dr. Muhammad Akhtar
	Co-chairperson:	Dr. Muhammad Akbar Khan
02:00 PM	Paper reading	
02:30 PM	Concluding Session	

HALL – 2

SECTION IV: PARASITOLOGY

SESSION I

	Chairperson:	Prof. Dr. Fatima Mujib Bilaqees
	Co-chairperson:	Prof. Dr. Abdullah G. Arijo
08:30 AM	Paper reading	
11:45 AM	Tea Break	

SESSION II

	Chairperson:	Dr. Shahina Fayyaz
	Co-chairperson:	Prof. Dr. Asmatullah Khan
12:00 PM	Paper reading	
01:30 PM	Dinner and Prayer (Zuhur)	

**SECTION V: FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER
BIOLOGY, MARINE BIOLOGY**

SESSION VIII

	Chairperson:	Prof. Dr. M. Nasim Siddiqui
	Co-chairperson:	Dr. Noor un Nisa
02:00 AM	Paper reading	
02:30 AM	Concluding Session	

HALL – 3

**SECTION V: FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER
BIOLOGY, MARINE BIOLOGY**

SESSION V

	Chairperson:	Prof. Dr. Naeem Tariq Narejo
	Co-chairperson:	Dr. Irfan Zia Qureshi
08:30 AM	Paper reading	
11:45 AM	Tea Break	

SESSION VI

	Chairperson:	Prof. Dr. Muhammad Javed
	Co-chairperson:	Dr. Abdul Minan Sheikh
12:30 AM	Paper reading	
01:30 PM	Lunch & Prayer (Zuhur)	

SESSION IX

	Chairperson:	Prof. Dr. Iftikhar Hussain
	Co-chairperson:	Dr. Zaib un Nisa Memon
02:00 PM	Paper reading	
02:30 PM	Concluding Session	
02:30 PM	Concluding Ceremony	
02:30 PM	Recitation	
02:35 PM	Congress Report by President ZSP	
02:45 PM	Award Ceremony	
03:00 PM	Concluding Remarks by the Chief Guest	
03:10 PM	Vote of Thanks	
03:15 PM	Refreshments	

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Zafar, Z.

SWABI, KPK

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Solangi, B.K.

Syed, T.S. (Dr.)

China

Tian, H.

Xiao, J.

Zhang, H.

Zhang, Y. (Dr.)

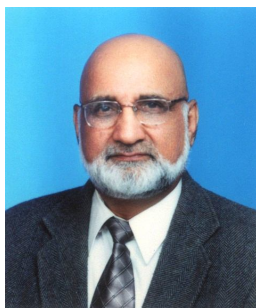
Xiao, J.

The Netherlands

Poorten, J.J.T.

CITATIONS

RECIPIENT OF LIFE TIME ACHIEVEMENT AWARD 2015



**Late Prof. Dr. Shahzad Ahmad Mufti
(1942-2013)
Ex-President, Zoological Society of Pakistan**

Prof. Dr. Shahzad A. Mufti, Adviser (Biosciences), COMSATS Institute of Information Technology, Islamabad and Ex-Chairman, Pakistan Science Foundation, obtained his M.Sc. degree in Zoology, from University of the Punjab in 1963. Soon after his M.Sc., he was selected as Lecturer in Zoology in the University of the Punjab. He proceeded to USA for his doctoral degree under the Central Overseas Training Scheme of Government of Pakistan. He obtained his Ph.D. degree from Case Western Reserve University, Cleveland, Ohio, USA in 1970 in the field of Anatomy/ Developmental Biology. He served as Associate Professor of Biomedical Sciences, Ohio University, USA during 1980-1984, and later became Professor of Zoology in the University of the Punjab in 1987. In 1990, Prof. Mufti was first appointed as Director General, Pakistan Museum of Natural History, and then later became Chairman, Pakistan Science Foundation. Because of his eminence in the field, Prof. Mufti was elected Fellow of Pakistan Academy of Sciences in 2003. He also served as Treasurer, Pakistan Academy of Sciences in 2011-2012.

Prof. Mufti was associated with Zoological Society of Pakistan, ever since its inception in 1968. He served the Zoological Society of Pakistan in various capacities: Secretary General in 1984-89, 1997-99; Vice-President in 1995-1997 and President in 2001-2003. During his tenure of different offices,

he made significant contributions towards development and promotion of Zoological Sciences in the country.

Dr. Mufti breathed his last in the early morning of Monday, December 30, 2013 after a brief illness in Islamabad. We have lost in him a very competent Developmental Biologist, a kind teacher, and a thoughtful friend, In recognition of his excellent contributions for the promotion of Zoological Sciences in Pakistan, the Executive Council of Zoological Society of Pakistan decided to give him Life Time Achievement Award 2015 Posthumous.

**RECIPIENT OF
LIFE TIME ACHIEVEMENT AWARD 2015**



Dr. Quddusi B. Kazmi

Ex-Director, Marine Reference Collection & Resource Centre, University of Karachi

Dr. Quddusi B. Kazmi began her career as Research Assistant after BSc. at the Zoology Department, Karachi University. After working for 41 years, she retired from the same institution as the Director, Marine Reference Collection & Resource Centre and Professor of Zoology.

She obtained her masters degree in Marine Zoology and wrote a thesis on the taxonomy of marine shrimps of Pakistan under the supervision of late Prof. Dr. Nasima M. Tirmizi. Her thesis was later published by the FAO. Likewise her M.Phil dissertation on anatomy was published as a handbook by Dr. R.B. Manning of the Smithsonian Institution, Washington, D.C. Later she worked for her doctoral dissertation on the marine crabs of Pakistan at the Natural History Museum, London. Her thesis was highly appreciated by Prof. L.B. Holthuis of the Netherlands, Dr. R.B. Manning of the Smithsonian Institute and Dr. R. W. Ingle of U.K.

Dr. Kazmi has received 2 gold medals from scientific organizations, a Best Paper Award, a *Star Woman of the Year Award*, two travel grant awards to work in the British Museum and two consecutive Productivity Awards of Ministry of Science and Technology.

She has guided several M.Sc.s, MAS, M.Phils and Ph.Ds. Dr. Kazmi has written 9 books, 4 monographs and 137 research papers. Presently she is preparing a document on the marine molluscs of Pakistan.

Her areas of concentration are marine biodiversity and its conservation, anthropogenic changes of macrobenthic ecology in coastal waters, and invasive species. She has delivered several Invited Lectures on Wetland conservation and Management.

On the basis of her academic achievement and excellent services for promotion of marine biology in Pakistan, ZSP has decided to give their Life Time Achievement Award 2015 to her, which comprises a Shield and a Certificate.

**RECIPIENT OF
LIFE TIME ACHIEVEMENT AWARD 2015**



Dr. Muhammad Ramzan Mirza

Ex-Chairman, Department of Zoology, Government College, Lahore

Dr. Muhammad Ramzan Mirza obtained his M.Sc. (Zoology) from the University of the Punjab, Lahore in 1962 and Ph.D. from University of Amsterdam in 1976. Dr. Mirza's book on freshwater fishes of Pakistan was published by the Urdu Science Board in 1990, which later was used as resource book on the freshwater fishes of Pakistan by the Word Bank and cited by several authors in their research works. Dr. Mirza has published articles on fishes, reptiles, zoogeography and history of science in Pakistan and abroad. He wrote monograph entitled Freshwater Fishes and Zoogeography of Pakistan which was published in the Bijdragan tot de Dierkunde (contribution to zoology) Amsterdam in 1975.

Dr. Ramzan Mirza was awarded *Aizaz-i-Fazeelat* by the President of Pakistan in 1996, Zoologist for the Year Award 1997 by Zoological Society of Pakistan and Award of Honor by PARC in 1998.

Dr. Mirza has been Life Fellow of the Biological Society of Pakistan, and Zoological Society of Pakistan, Founder Fellow of the Pakistan Fisheries Society, Member, Editorial Board, *Biologia* (1971 to date), and Member Advisory Board, *Pakistan Journal of Zoology* (1993 to 2001), Editor *Pakistan Journal of Fisheries*, Member Editorial Board *Records of Zoological Survey of Pakistan* (Karachi), President *Biological Society of Pakistan* (2014 to date). He has published More than 200 scientific papers, 15 books in Urdu and English and 25 popular articles in Urdu and Pashto (uls, Quetta).

**RECIPIENT OF
LIFE TIME ACHIEVEMENT AWARD 2015**



Abdul Aziz Khan

Secretary General, Zoological Society of Pakistan

Mr. Abdul Aziz Khan FZSP is a Vertebrate Pest Management (VPM) specialist of national as well as of international repute. He has devoted 42 years of professional career to this subject activity.

After doing his M.Sc. in Zoology in 1968, Mr. Khan started his career as Technical / Research Assistant in the Department of Plant Protection. In 1974, he was selected as Assistant Rodent Control Officer in FAO / UNDP Project, Vertebrate Pest Control Center, Karachi. In 1975, he was awarded FAO fellowship for M.S. degree in VPM from the University of Wisconsin, USA. In 1989, he was promoted as Principal Scientific Officer and in this capacity he established Vertebrate Pest Control Laboratory at National Agricultural Research Center (NARC), Islamabad. In 2000, he was appointed as Director, Integrated Pest Management Institute, NARC. In March, 2005 Mr. Khan worked as Senior Director (Research), Plant Protection, PARC, Islamabad. At present, Mr. Khan is Vice-President of Crop Protection Association of Pakistan, Senior Vice-President Pakistan Wildlife Foundation and member Executive Council, Zoological Society of Pakistan.

Mr. Khan's research interests are Rodent Control in Agriculture and Urban Environments, Wildlife Damage Control in Agricultural, Forestry, and Range Land Systems, Vermiculture and Vermicomposting, Environment impact assessment and risk analysis of wildlife (small and large mammals), Risk Analysis of pesticides

Mr. Khan to his credit has more than 175 research papers and technical articles published in journals of international repute and in peer reviewed

proceedings of symposia / congresses / conferences, etc. He is author of several chapters of FAO Handbook of Vertebrate Pest Control in Pakistan, seven chapters in Training Manual on Vertebrate Pest Management, a monographs titled “Progress of Vertebrate Pest Management in Pakistan”, and “Bio-Economic Impacts of Vertebrate Pests on Crops with Special Reference to Rodent Pests in Pakistan and Other Countries”.

In recognition of his contribution and published work, he was awarded Hamdard Award and National Farm Guide Gold Star Prize in 1994, Bhutto Agriculture Gold Medal in 1996 and several Commendation Certificates by USDA; Pakistan Institute of Medical Sciences, PARC Scientists Association, and Zoological Society of Pakistan, and was declared “**Zoologist of the Year**” in 1999.

**RECIPIENT OF
ZOOLOGIST OF THE YEAR AWARD 2015***



Prof. Dr. Abdullah G. Arijo

Associate Professor,

Department of Parasitology, Sindh Agriculture University, Tandojam

Dr. Abdullah G Arijo is among the leading Parasitologists of Pakistan. Dr. Arijo obtained his Ph.D. from University of North Wales, Bangor United Kingdom in 1997 under "World Bank Award" scheme.

His teaching career started as lecturer in Pakistan Air Force College Base Masroor, Karachi in 1986, and later he shifted to Sindh Agriculture University Tandojam, Pakistan. Under his supervision a significant number of students have completed their MSc research projects. He has published four books and is author and co-author of many publications that have appeared in international and national journals.

The research areas of Dr. Arijo are (a) Schistosomiasis and (b) Tick-borne Diseases. Presently he is Principal Investigator in a PAK-US Science & Technology Research Project and has established a State-of-Art Molecular Parasitology Laboratory at Sindh Agriculture University Tandojam. Under this project he has established collaboration with University of Rhode Island, USA.

Dr. Arijo has delivered several invited lectures in various universities including University of Southern Mississippi, USA and University of Rhode Island USA on Stem Cell Technology for replacement of affected cells in myocardial infarction. Recently he has won another collaborative research project funded by University of Cologne, Germany.

*Other nominees of this award were Prof. Dr. Nasreen Memon, Prof. Dr. Hamid Bashir and Prof. Dr. Mazhar Qayyum.

**RECIPIENT OF
PROF. A.R. SHAKOORI GOLD MEDAL 2014***



Dr. Nadeem Sheikh

Department of Zoology, University of the Punjab, Lahore

Dr. Nadeem Sheikh did his M.Sc. (Zoology) from University of the Punjab, Lahore in 1999 securing overall first class first position in the University. He was conferred Dr. Muzzafar Ahmad Gold Medal, Sir William Roberts Gold Medal and University of the Punjab Gold Medal for outstanding performance. He joined the Department of Zoology, University of the Punjab in Feb. 2001 as Lecturer in Zoology. In October, 2003 he was awarded scholarship by Deutsche Forschungsgemeinschaft (DFG) for higher studies leading to Ph.D. in Georg-August University, Goettingen, Germany. His field of specialization is Cellular, Molecular and Clinical Biology of internal organs especially iron regulation during inflammation, hepatic injury, regeneration and iron overload conditions. In 2008, he was awarded Charles Wallace trust fellowship to visit Imperial College, London for one Month. Later in year 2009, he was awarded HEC postdoctoral fellowship to work in Brain Tumor Research Center of Harvard Medical School at Harvard University (USA).

Dr. Sheikh is HEC recognized supervisor for Ph.D., M.S. /M. Phil research. He has authored more than 40 research articles. He has been awarded research productivity award by PCST. His h-index and i-10 index is 9. He has contributed 3 chapters in open access books. He has supervised 3 Ph.D. scholars, 11 M.S. /M. Phil, 16 B.S. / M.Sc. research thesis. He successfully brought EXPERTS (Exchange by Promoting Quality Education, Research and Training in South and South-East Asia) project for the students of the University of the Punjab and other universities of Pakistan for exchange as well as degree courses in EU Universities.

*Other applicants for this award were Dr. Riffat Sultana, Dr. Muhammad Saleem Akhtar, Dr. Touqeer Ahmed, Dr. Muhammad Hamid Bashir, Dr. Hafiz Muhammad Tahir, Dr. Shaukat Ali, Dr. Abdul Rehman and Mr. Salman Ahmed.

**RECIPIENT OF
PROF. DR. MIRZA AZHAR BEG GOLD MEDAL 2015***



Dr. Hafiz Muhammad Tahir

Assistant Professor,

Department of Zoology, University of Sargodha, Sargodha

Dr. Hafiz Muhammad Tahir joined Department of Biological Sciences, University of Sargodha, Sargodha in 2010, where he is currently working as Assistant Professor (Zoology). Dr. Tahir received his M.Sc and M.Phil degrees from the GC University Lahore, and Ph.D. from University of the Punjab, in 2009. He did his post doctorate from American Museum of Natural History, New York, USA in 2013-2014. His research interests include Applied Ecology, Insect Biochemistry, Molecular Systematics and Insect Pest Management. He is using scorpions and spiders as model organisms in his studies.

He has published over 55 original peer reviewed articles. He was awarded the “Research productivity award” by Pakistan council for Science and Technology in 2011. He was awarded Young scientist Award by International Society of Arachnology in 2010.

*Other applicant for this award is Dr. Muhammad Kashif Nadeem.

**RECIPIENT OF
PROF. IMTIAZ AHMAD GOLD MEDAL 2015***



Dr. Muhammad Hamid Bashir,
Associate Professor

Department of Agriculture Entomology, University of Agriculture, Faisalabad.

Dr. Muhammad Hamid Bashir obtained his Ph.D. from University of Agriculture, Faisalabad in 2006 and described 49 species belonging to eight genera of family Cunaxidae (Acarina) from Punjab, out of which 40 species were new to science. His area of interest is Acarology embodying mainly taxonomy and pest management.

Dr. Hamid has supervised 4 Ph.D and 31 M.Sc (Hons) students. He has 91 research publications out of which 38 are in journals with Impact Factor. He has successfully completed several projects as PI and Co-PI.

*Other applicant for this award were Dr. Hafiz Muhammad Tahir, Dr. Muzammil Sattar and Dr. Muhammad Razaq.

**RECIPIENT OF
PROF. DR. NASIMA M. TIRMIZI MEMORIAL GOLD MEDAL 2015***



Prof. Dr. Pirzada Jamal Ahmed Siddiqui

Director,

Centre of Excellence in Marine Biology, University of Karachi, Karachi.

Dr. Siddiqui obtained BSc (Hons) in 1982 and M.Sc. in Marine Sciences 1983 with first class first position in both these examinations. He did M. Phil. in Marine Biology from University of Karachi in 1987 and Ph. D. in Coastal Oceanography from State University of New York at Stony Brook, USA in 1992.

Before joining the Centre, Dr. Siddiqui worked as Research Assistant at Marine Science Research Centre, State University of New York, Stony Brook, USA (1989-1992); Research Officer at Biological Research Centre, University of Karachi (1987-1988); and served as a post-doctoral research associate at Rensselaer Polytechnic Institute, Troy, NY, USA (1992- 1993). He also served as Commonwealth Honorary Research Fellow in Glasgow University in 2002-2003.

Dr. Siddiqui won several scholarships including S&T Scholarship awarded by Government of Pakistan (1988-1992), “Hierta-Retzius Stipendiefond” awarded by Royal Swedish Academy of Sciences, Sweden (1991-92).

*Other applicant for this award were Dr. Zakia Khatoon, Dr. Alia Bano Munshi, Dr. Farhana Shafiq Ghory and Dr. Razia Sultana.

Dr. Siddiqui has supervised 06 M. Phil. and 11 Ph. D. degree research students. He has published 82 research papers.

His research interest includes mangrove and microbial ecology, biodiversity assessment, conservation and aquaculture.

Dr. Siddiqui has been coordinating and managing several national and international projects and Higher Education Linkages including DFID funded HE link with Scottish Association for Marine Sciences, UK during 1992-2002 and 2003-2006; HEC (Islamabad) funded link with University of London, Marine Biological Station at Millport (2004-2007), DEFRA UK funded Darwin Initiative Project (2005-2008) and DFID funded DelpHE project (2006-2009).

**RECIPIENTS OF
GOLD MEDALS AWARDED BY THE ZOOLOGICAL SOCIETY OF
PAKISTAN**

1. Muzaffar Ahmad Gold Medal 2015

Sixteenth Muzaffar Ahmad Gold Medal 2015 was received by Ms. Zunaira Siddique for obtaining first position in the M.Sc. Zoology examination of the University of the Punjab.



Ms. Zunaira Siddique

2. Ahmed Mohiuddin Memorial Gold Medal 2015

Eleventh Ahmed Mohiuddin Memorial Gold Medal 2015 was given to Ms. Shumila Zahid, who obtained first position in the M.Sc. Zoology examination of the University of Sindh, Jamshoro.

3. Muhammad Afzal Hussain Qadri Memorial Gold 2015

Fifteenth Muhammad Afzal Hussain Qadri Memorial Gold 2015 was given to Miss. Aaisha Siddique for obtaining first position in Parasitology for her M.Sc. Zoology examination of the University of Karachi.



Miss Aaisha Siddique

- 3. Afsar Mian Gold Medal 2015**
Sixth Afsar Mian Gold Medal 2015 was given to Miss. Shiza Nasreen who obtained first position in the M.Sc. Biology/Zoology examination of the Arid Agriculture University, Rawalpindi.
- 4. Mujib Memorial Gold Medal 2015**
Nineteenth Mujib Memorial Gold Medal 2015 was given to Miss Samina, who obtained first position in the M.Sc. Zoology examination of the University of Sindh, Jamshoro.
- 5. Prof. Dr. S.S. Akbar Memorial Gold Medal 2015**
Second Prof. Dr. S.S. Akbar Memorial Gold Medal 2015 was given to Ms. Shumila Zahid, who obtained first position in the M.Sc. Zoology examination with specialization in Entomology of the University of Sindh, Jamshoro.
- 6. M.A.H. Qadri Memorial Gold Medal 2015**
Ninth Dr. M.A.H. Qadri Memorial Gold Medal 2015 was given to Dr. Fozia Batool for his Ph.D. degree in Zoology specializing in the field of Parasitology from University of Karachi, Karachi.
- 7. Prof. Dr. S.N.H. Naqvi Gold Medal 2015**
Ninth Prof. Dr. S.N.H. Naqvi Gold Medal 2015 was given to Dr. Habibullah Rana for her Ph.D. degree in Zoology specializing in the field of Toxicology from University of Karachi, Karachi.



Dr. Habibullah Rana

CERTIFICATE OF APPRECIATION**Dr. Zulfiqar Ali Saqib**

In recognition of his diligence, devotion and outstanding performance in the affairs of Zoological Society of Pakistan, the Executive Council of Zoological Society appreciates the services of Dr. Zulfiqar Ali Saqib (Ex. Treasurer, ZSP).

**Syed Mubarak Ali Zaidi**

In recognition of his diligence, devotion and outstanding performance in the affairs of Zoological Society of Pakistan, the Zoological Society of has decided to appreciate the services of Syed Mubarak Ali Zaidi (Office Assistant, ZSP).

**IN SILICO ANALYSIS OF STRUCTURE-FUNCTION RELATIONSHIP
OF A NEUTRAL LIPASE FROM *TRIBOLIUM CASTANEUM***

AMTUL JAMIL SAMI,¹ BASIT JABBAR,¹ NURRAIZ AHMAD¹,
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Abstract.- Lipases are of immense importance in insects since lipids constitute a major dietary component of insects such as *T. castaneum*. We performed computational analysis for exploring structural features including catalytic properties of a neutral lipase from *T. castaneum*. The protein shared significant identity with human pancreatic lipase (HPL) and other mammalian pancreatic lipases and related proteins. Two major domains were identified: N-terminal domain and C-terminal domain, connected by a hinge region. N-terminal domain had the following features, similar to pancreatic lipases: signal peptide, α/β hydrolase fold, catalytic triad, nucleophilic elbow, oxyanion hole, lid domain, $\beta 5$ loop, $\beta 9$ loop, three N-glycosylation sites, and four disulfide bonds. Lid domain covers active site and renders it inaccessible to the solvent; displacement of lid should expose the active site for substrate binding by interfacial activation. Arrangement of the catalytic triad, lid domain, $\beta 5$ loop and $\beta 9$ loops of *T. castaneum* neutral lipase and a previously reported *T. castaneum* acidic lipase were compared to highlight the differences. More experimental data is needed to ascertain the function and mechanism of *T. castaneum* neutral lipase.

Key words: *Tribolium castaneum*, *T. castaneum* neutral lipase, structural features, catalytic properties.

INTRODUCTION

Lipids, indispensable for all life forms, perform a diversity of roles in insects and include molecules such as fats, sterols, waxes, triacylglycerol, monoglycerides, diglycerides and phospholipids (Canavoso *et al.*, 2001; Hahn and Denlinger, 2007; Nelson *et al.*, 2008). Lipids have various functions in living organisms such as energy storage, forming components of membranes, and signaling processes (Fahy *et al.*, 2009). All insect species, to varying extents, are dependent on lipids, which form essential part of their diet, since lipids are important for insect life cycle, metamorphosis, diapause, synthesis of secondary metabolites, such as pheromones, and some other processes (Hahn and Denlinger, 2007; Horne and Haritos, 2008; Horne *et al.*, 2009; Klowden, 2013).

Lipases (E.C. 3.1.1.3), generally known as triacylglycerol hydrolases, hydrolyze ester linkages in triacylglycerol (TAG) and can also cleave diacylglycerols (DAG), galactolipids and phospholipids, thus lipases play a pivotal role in absorption, transport and assimilation of lipids (Horne *et al.*, 2009). Lipases from an organism can be classified into six families, which share some common features such as relations with the α/β -hydrolase superfamily having Gly-X-Ser-X-Gly as the consensus sequence and a two-step reaction mechanism involving catalytic triad of Ser-His-Asp/Glu (Holmquist, 2000; Zimmermann *et al.*, 2004).

There are numerous reports on lipases from insects. Horne *et al.* (2009) have studied the comparative and functional genomics of lipases in hematobolous insects. Mrdaković *et al.* (2008) reported partial characterization of a lipase from gypsy moth (*Lymantria dispar* L.) larval midgut. Arreguín-Espinosa *et al.* (2000) published purification and properties of lipase from a beetle, *Cephaloleia presignis*. Zibae and Fazeli-Dinan (2012) reported purification and characterization of a digestive lipase in *Naranga aenescens*. In another study Zibae *et al.* (2011) reported TAG lipase activity in the salivary secretions of a predaceous bug, *Andrallus spinidens*. Brabcova *et al.* (2013) characterized a neutral lipase BT-1 isolated from *Bombus terrestris*. Delkash-Roudsari *et al.* (2014) determined lipase activity in larvae of *Bacterocera oleae*. Ranjbar *et al.* (2015) reported purification and characterization of a digestive lipase from *Ectomyeloides ceratoniae*. Weidlich *et al.* (2015) studied secretion of digestive tract lipases in *Gryllus bimaculatus*.

In red flour beetle, *Tribolium castaneum*, there are 54 genes for lipase enzyme activity. It may be noted that out of which 25 genes are for neutral lipases, 25 genes are for acidic lipases, no gene for lipase type 2, one gene for lipase type 3, two genes for GDSL and one gene for hormone sensitive lipase. This shows that neutral and acidic lipases predominate in red flour beetle. *T. castaneum* is a major stored grain pest and relies on lipids and triglycerides for its diet, therefore, inhibition of its lipases using site specific inhibitors can be of commercial importance on a large scale, such as inhibitors can bring about growth reduction and mortality in insect pests. Recently, we have reported the computational analysis of an acidic Lipase of *Tribolium castaneum* (Sami *et al.*, 2014).

In this study, we have discussed the characteristics of a neutral lipase from *Tribolium castaneum* (*T. castaneum* -L) revealed by its computational analysis

using the structure of human pancreatic lipase (HP-L) as template and compared some important features with the previously reported *T. castaneum* acidic lipase.

MATERIALS AND METHODS

PDB-blast (NCBI) was used to find homologous proteins with high sequence similarity to *T. castaneum* neutral lipase. *T. castaneum* neutral lipase showed significant homology with the pancreatic lipase family. The query sequence (XP_969219) together with the sequences of homologues, with high sequence identities, were retrieved in FASTA format to perform multiple sequence alignment using T-Coffee alignment server (CRG). Espresso alignment option was selected in T-Coffee to align sequences based on structural information. Jalview (EBI) was used for alignment viewing and editing to locate and highlight conserved features between the sequences. 3D structure was modelled using Phyre2 and structural alignment with human pancreatic lipase (1N8S) was done with TM-align. Many features of *T. castaneum* neutral lipase were identified from the sequence and structure alignment with pancreatic lipase family.

RESULTS AND DISCUSSION

T. castaneum neutral lipase shared 42% identity with human pancreatic lipase (HPL), with 79% query coverage, indicated by PDB-blast. Other proteins with known 3D structure, structurally conserved with *T. castaneum* neutral lipase were: human pancreatic lipase related proteins 1 and 2, dog pancreatic lipase, guinea pig chimeric pancreatic lipase related protein 2 and horse pancreatic lipase (sequence alignment has been shown in Fig. 1).

Homology modelled (*T. castaneum* neutral lipase) structure was aligned with template (HPL) using TM-align. TM-score was obtained to be 0.8 and RMSD of 1.56 indicating the proteins were in similar fold. *T. castaneum* neutral lipase is composed of two domains (1) N-terminal domain (Met1- Leu381) and (2) C-terminal domain (Arg392-Ser543). These two domains are connected by a loop of 10 residues from Thr382 to Arg391 which is a short, unstructured stretch of amino acids. This is called the hinge region which can undergo an angular change on binding of the substrate that is the two domains can slightly rotate around the hinge region due to which the lid is displaced, and the catalytic triad is positioned for the catalytic effect (Aoubala *et al.*, 1995). Modelled 3D structure has been shown in Figure 2, indicating main features of *T. castaneum* neutral lipase.

		Beta5 loop
<i>T. castaneum_neutral_lipase/1-543</i>	70 - PRNVIINTRF ILYTRFNPNEGTYLKVVE--DESTIEESFNPNKSTKF I	VHG F I D T P F S N W R D M - K D E L 134
<i>Human_Pancreatic_Lipase_(chain_A)1-449</i>	31 SPKDV - NTRFLLYTNENPNN - -FOEVAAD - SSS I S G S N F K T N R K T R F I	H G F I D K G E E N W L A N V C K N - L 94
<i>Human_PNLPRP2_(chain_B)1-466</i>	33 SPEDI - DTRFLLYTNENPNN - -FQLITGTEPDTIEASNFQDRKTRFI	H G F L D K A E D S W P S D M C K K - M 97
<i>Human_PNLPRP1_(chain_A)1-485</i>	56 SPEKI - GTRFLLYTNENPNN - -FQILLSDPSTIEASNFQDRKTRFI	H G F I D K G D E S W V T D M C K K - L 120
<i>Dog_Pancreatic_Lipase_(chain_A)1-450</i>	31 SPEKI - GTRFLLYTNENPNN - -FQTLPSDPSTIEASNFQDKKTRFI	H G F I D K G E E N W L L D M C K N - M 95
<i>Guinea_Pig_Chimeric_PNLPRP2/1-432</i>	31 DPKKI - NTRFLLYTNENQNS - -YQLITATDIATIKASNFNLNRKTRFI	H G F T D S G E N S W L S D M C K N - M 95
<i>Horae_Pancreatic_Lipase_(chain_A)1-449</i>	31 SPEKV - NTRFLLYTNENPDN - -FQEIIVAD - PSTIQSSNFNTGRKTRFI	H G F I D K G E E S W L S T M C N - M 94
		Nucleophilic elbow
<i>T. castaneum_neutral_lipase/1-543</i>	135 I KAGDMNVI VVDWAGGSLPLYTQATANTRLVGMEIAHLIKFLMEKYDIDASDFHI	I G H L G A H T A G Y A G 203
<i>Human_Pancreatic_Lipase_(chain_A)1-449</i>	95 F KVESVNCICVDWKGGSRGTGYTQASQNI RIVGAEVAYFVEFLQSAF GYS P S N V H V I	G H L G A H A A G E A G 163
<i>Human_PNLPRP2_(chain_B)1-466</i>	98 F EVEKVCNICVDWRHGSRAMYTQAVQNI RIVVGAETAFLIQALSTQLGYSLEDVHV	I G H L G A H T A A E A G 160
<i>Human_PNLPRP1_(chain_A)1-485</i>	121 F EVEEVNCICVDWKGGQATYTQAAANNRVRVGAQAQMLDILLTEYSYPPSKVHL	I G H L G A H V A G E A G 189
<i>Dog_Pancreatic_Lipase_(chain_A)1-450</i>	96 F KVEEVNCICVDWKGGQSTYSYTAANNRVRVGAQAQMLSLSANYSYSPSQVQL	I G H L G A H V A G E A G 164
<i>Guinea_Pig_Chimeric_PNLPRP2/1-432</i>	96 FQVEKVCNICVDWKGGSKAQYSQASQNI RIVVGAEVAYLVQVLSLSTLNYPENVH	I G H L G A H T A G E A G 164
<i>Horae_Pancreatic_Lipase_(chain_A)1-449</i>	95 F KVESVNCICVDWKGGSRATYSSQASQNI RIVGAEVAYLVQVLQSSFDYS P S N V H I	I G H L G S H A A G E A G 163
		Beta9 loop
<i>T. castaneum_neutral_lipase/1-543</i>	204 SLVP - KLGRITGLPAEPFFGQMPHVRLDPSDAEFVDVIHTDGGK	I L F L G Y G M S Q P G G H L D F Y P N N G 270
<i>Human_Pancreatic_Lipase_(chain_A)1-449</i>	164 RRTNGTIGRITGLPAEPCFQGTPELVRLDPSDAKFVDVIHTDGGAP	I V P N L G F G M S Q V G V G H L D F F P N G G 232
<i>Human_PNLPRP2_(chain_B)1-466</i>	187 RRLGGRVGRITGLPAGPCFQDEPEEVRLDPSDAFVVDVIHTDSSP	I V P S L G F G M S Q K V G V G H L D F F P N G G 235
<i>Human_PNLPRP1_(chain_A)1-485</i>	190 SKTP - GLSRITGLPVEASFESTPEEVRLDPSDAFVVDVIHTDAPL	I P F L F L G F T N C D M G H L D F F P N G G 267
<i>Dog_Pancreatic_Lipase_(chain_A)1-450</i>	165 SRTP - GLSRITGLPVEASFQGTPEEVRLDPTDAFVVDVIHTDAPL	I P F L G F T S Q M G H L D F F P N G G 232
<i>Guinea_Pig_Chimeric_PNLPRP2/1-432</i>	165 KRLNLVGRITGLPAEPYFQGTPEEVRLDPSDAKFVDVIHTDISP	I L P S L G F G M S Q K V G H M D F F P N G G 233
<i>Horae_Pancreatic_Lipase_(chain_A)1-449</i>	164 RRTNGAVGRITGLPAEPCFQGTPELVRLDPSDAQFVDVIHTDIAP	I P N L G F G M S Q T A G H L D F F P N G G 232
		Lid domain
<i>T. castaneum_neutral_lipase/1-543</i>	271 KEQPGG.....ITQIPLVPLITLIDGGLLEASRVLVACN	V R A I K L F I D S I N T O C P Y I G H G C S S 329
<i>Human_Pancreatic_Lipase_(chain_A)1-449</i>	233 VEMPGCKK.....LSD...IVD...DG IWEQTRDF AACN	L R S Y K Y Y T D S I V N P D G F A G F P C A S 288
<i>Human_PNLPRP2_(chain_B)1-466</i>	236 KEMPGCKKNVLSITITDIDG.....IWEIGGFVSCN	L R S F E Y Y S S V L N P D G F L G Y P C A S 291
<i>Human_PNLPRP1_(chain_A)1-485</i>	258 ESMPGCKK.....ALSD...IVDL...DG IWAQTRDF VACN	L R S Y K Y Y L E S I L N P D G F A A Y P C T S 313
<i>Dog_Pancreatic_Lipase_(chain_A)1-450</i>	233 EEMPGCKK.....ALSD...IVDL...DG IWEQTRDF VACN	L R S Y K Y Y S E S I L N P D G F A S Y P C A S 288
<i>Guinea_Pig_Chimeric_PNLPRP2/1-432</i>	234 KDMPGCK.....TGISCN	H R S I E Y Y H S S I L N P E G F L G Y P C A S 271
<i>Horae_Pancreatic_Lipase_(chain_A)1-449</i>	233 KEMPGCKK.....VLSG...IVD...DG IWEQTRDF AACN	L R S Y K Y Y T D S I L N P D G F A G F P C A S 288

Fig. 1. Sequence alignment of *T. castaneum* neutral lipase with mammalian homologue pancreatic lipases and related proteins, with known 3D structures. Many conserved regions have been highlighted. Catalytic residues indicated in brown.

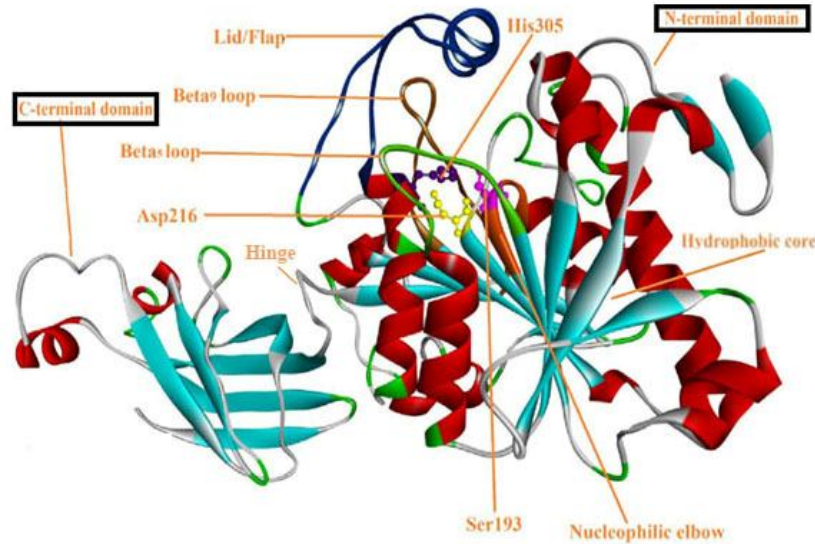


Fig. 2. Two domains of *T. castaneum* neutral lipase: N-terminal domain and C-terminal domain, connected by hinge region. Many features have been indicated including catalytic triad, lid, beta5 loop and beta9 loop and nucleophilic elbow.

Signal peptide

A signal peptide of 17 amino acids was identified in *T. castaneum* neutral lipase using the signal peptide prediction tool, Phobius (from ExPASy server) suggesting its extracellular destination. Acidic lipase from *T. castaneum* was predicted to possess signal sequence of 19 amino acids and its resemblance to human lysosomal lipase implicates its localization to lysosomes, the main function of this acidic lipase being intra-lysosomal hydrolysis of triglycerides and cholesteryl esters (Roussel *et al.*, 1999).

N-terminal domain

The N-terminal domain (381 amino acids) of *T. castaneum* neutral lipase, containing the α/β hydrolase fold, is globular and has beta sheets at the center, in a fan-like arrangement, surrounded by α -helices and loops (Remington *et al.*, 1992; Schrag and Cygler, 1997). Nine β strands are present at the core region of N-terminal globular domain and two additional β -strands close to the N-terminal next to the signal peptide (Fig. 2). It is the N-terminal that contains the active catalytic site of the enzyme and is composed of many motifs that are conserved throughout evolution in homologues as can be seen in sequence alignment in Fig. 1. The central portion composed of beta sheets which is responsible for the formation of a more compact hydrophobic core inside the N-terminal. Alpha helices surrounding beta sheets seem to protect the inner hydrophobic core of the enzymes' N-terminal and renders maximum stability to the enzyme. In lipases α/β hydrolase fold is characterized by the presence of a central β -sheet containing eight parallel β -strands, with the exception of β_2 , which is antiparallel with respect to the others.

Disulfide bonds are present in the neutral lipase, in the N-terminal domain, at the positions Cys43-Cys49, Cys276-Cys303, Cys327-Cys338 and Cys341-Cys348. All disulfide links in *T. castaneum* neutral lipase are comparable to those in human pancreatic lipase with one disulfide bond (Cys276-Cys303) stabilizing the lid domain (Lowe, 2002) and together, all disulfide bonds maximize the stability of enzyme.

The structure of acidic lipase of *T. castaneum* has already been discussed (Sami *et al.*, 2014) which, like *T. castaneum* neutral lipase, seems to have a central arrangement of β -sheets; however, the 3D structure of *T. castaneum* acidic lipase is quite different from N-terminal region of *T. castaneum* neutral lipase (Fig.3). No disulfide bonds are detected in previously reported acidic lipase of *T. castaneum*.

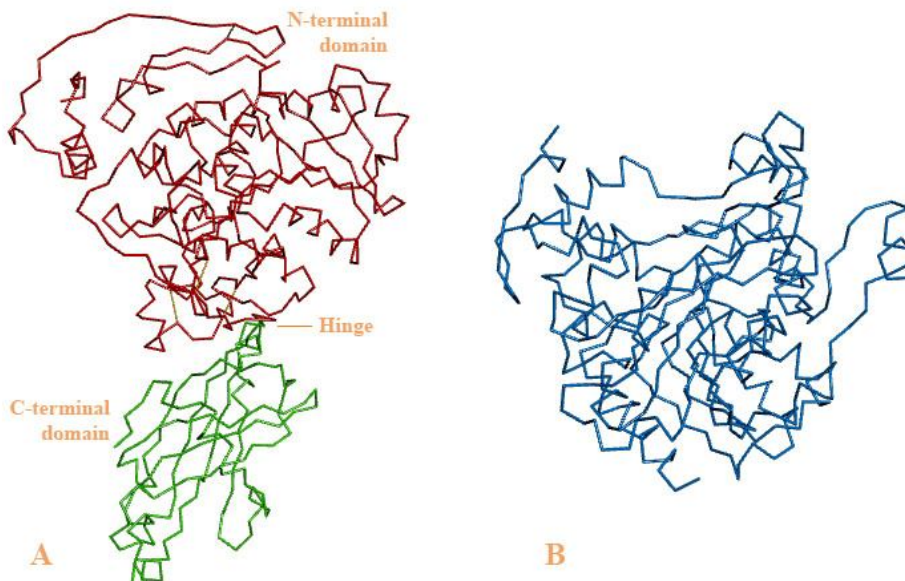


Fig. 3. Ca stick representation of 3D structure of *T. castaneum* neutral lipase (A) with acidic lipase (B). Acidic lipase is devoid of C-terminal domain.

Arrangement of catalytic triad

Three conserved residues (His, Ser, Asp), collectively known as catalytic triad in the N-terminal portion make up the active site of the enzyme. The active site of $\alpha\beta$ hydrolases consists of a highly conserved catalytic triad: one nucleophilic residue (serine, cysteine, or aspartic acid), one catalytic acidic residue (aspartic or glutamic acid), and one histidine residue. In lipases, the nucleophile has always been characterized as a serine residue (Bordes *et al.*, 2010; Bornscheuer, 2002; Jaeger *et al.*, 1999). In *T. castaneum* neutral lipase, the amide group of His305 forms two hydrogen bonds: first one with oxygen of side chain of Ser193 and second one with oxygen of side chain of Asp216 (Fig. 4). Ser193 is present at a distance of 5.580Å from Asp216 and 7.594Å from His305.

Moreover, Asp216 and His305 are located at a mutual distance of 6.511Å and taking Asp216 as centre, Ser193 makes an angle of 77.39° with His305. The amino acid residue His305 is closely spaced to the lid covering the active site. The arrangement of residues in catalytic triad of *T. castaneum* neutral lipase is different from that of acidic lipase with reference to bond angles and the

orientation of residues (Sami *et al.*, 2014). Catalytic triad of both lipases, mimicking serine protease catalytic triad, is expected to work in a similar mechanism of triglyceride hydrolysis, involving the catalytic triad with Ser acting as a nucleophile (Holmquist, 2000).

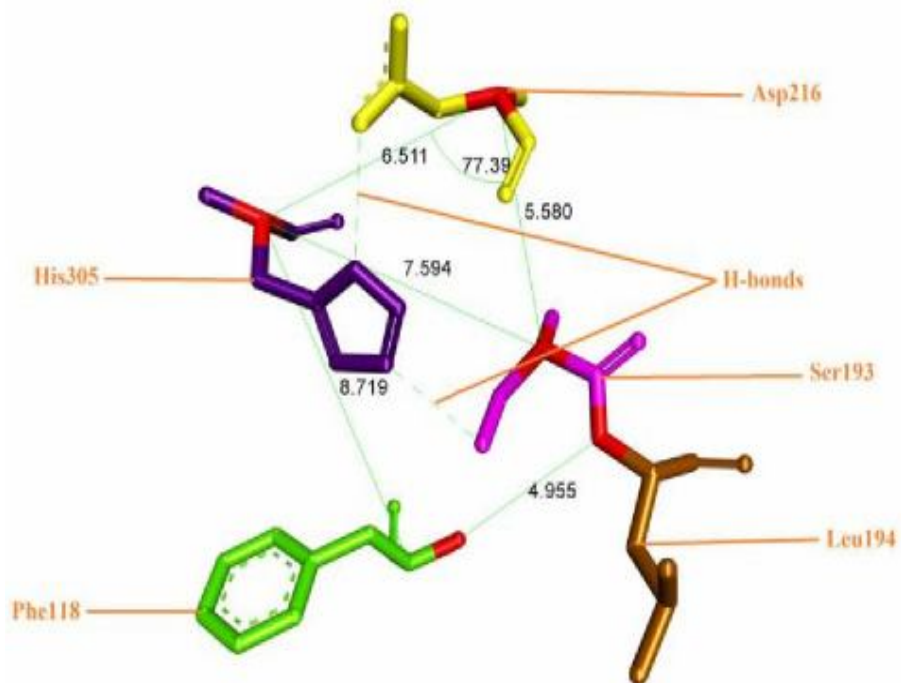


Fig. 4. Position of His305 is shown for hydrogen bonding: first one with oxygen of side chain of Ser193 and second one with oxygen of side chain of Asp216. Mutual distances between residues indicated and angle between Ser193 and His305 being 77.39, from Asp216.

Nucleophilic elbow and oxyanion hole

The nucleophilic elbow is a structure that is comprised of 5 amino acid residues having a consensus sequence of “Gly-X-Ser-Y-Gly” (Derewenda and Derewenda, 1991). In both acidic and neutral lipase of *T. castaneum*, X and Y belong to His and Leu. Active site serine residue (Ser193) of the nucleophilic elbow is present in a curved region between a β -strand and α -helix (Remington *et al.*, 1992). This position is comparable to Ser163 in acidic lipase of *T. castaneum* and the importance of Ser, at this curved position, in nucleophilic attack has been

discussed (Sami *et al.*, 2014). Similar to that in *T. castaneum* acidic lipase, glycine residues (Gly191 and Gly195) are present, to avoid steric hindrance, at the turns of nucleophilic elbow on both ends in *T. castaneum* neutral lipase. Also, for stabilization of the nucleophilic elbow, oxygen atom of carboxyl group of His192 makes a strong H-bond with amide group of Gly195 with a distance of 2.791Å (Fig. 5).

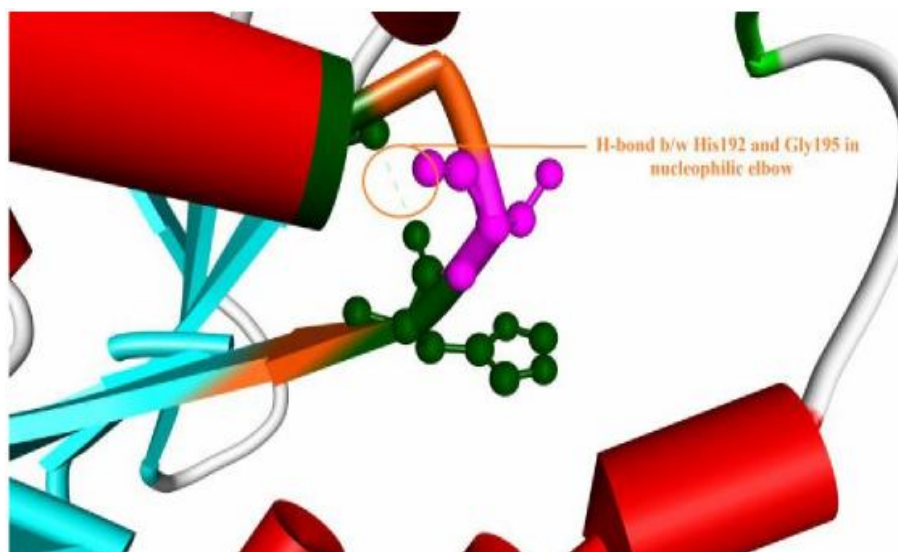


Fig. 5. The oxygen atom of carboxyl group of His192 makes a strong H-bond with amide group of Gly195 with a 2.791Å distance which stabilizes the nucleophilic elbow.

Leu194 of the nucleophilic elbow forms oxyanion hole, during the enzymatic activation on binding of the substrate, with Phe118. The amide group of Leu194 faces the amide group of Phe118 (from β 5 loop) with an interatomic distance of 4.955Å and both are involved in interaction with the substrate through hydrogen bonding (Fig. 6). On substrate binding, the oxygen of carbonyl group moves down into the active site and occupies a previously unoccupied position where it makes two H-bonds with amide groups of Phe118 and Leu194.

Active site enclosure by lid, β 5 and β 9 loops

Lid domain, in the N-terminal region of *T. castaneum* neutral lipase, consists of residues from Cys276 to Cys303 acts as a protective loop for the

active site and renders it inaccessible to solvent and also contributes to hydrophobicity of the protein core (Winkler *et al.*, 1990). A disulfide bond between Cys276 and Cys303 stabilizes the lid domain; further, numerous hydrogen bonds in the curved loop impart extra stability to it. The displacement of the lid is imperative in exposing the active site so that the catalytic attack on the substrate can be initiated (Egloff *et al.*, 1995; van Tilbeurgh *et al.*, 1992). Another loop, known as β_9 loop, extends from His243 to His262 gives additional protection to the active site from another dimension. The sequence of β_9 loop of *T. castaneum* neutral lipase is conserved in proteins homologous to human pancreatic lipase except a deletion of Pro212 (present in human PTL) (Fig. 1).

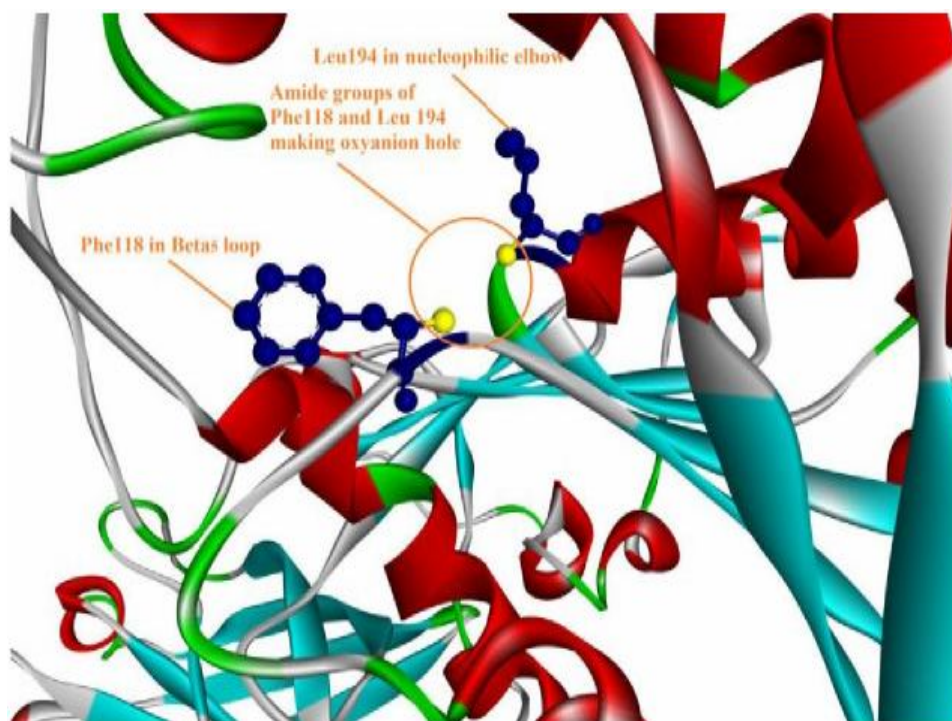


Fig. 6. Positioning of amide group of Leu194, of nucleophilic elbow, in front of amide group of Phe118, belonging to beta5 loop, with an interatomic distance of 4.955Å

β_5 loop of *T. castaneum* neutral lipase (His116 to Asn125) also confers additional protective effect to the active site. Further, as mentioned earlier, Phe118 in β_5 contributes to the formation of oxyanion hole by having amide

group of Phe118 in close proximity to Leu194. Lid domain, $\beta 5$ and $\beta 9$ loop of *T. castaneum* neutral and acidic lipase, though serving similar functions in the respective enzymes are, however, are quite different in sequence and structure as can be seen in Table I and Figure 7.

TABLE I.- SEQUENCES OF B5 LOOP, B9 LOOP AND LID DOMAIN OF *T. CASTANEUM* NEUTRAL AND ACIDIC LIPASE.

	$\beta 5$ Loop	B9 Loop	Lid Domain
<i>T. castaneum</i> acidic lipase	QATRPPVLLMH GLSSSDWVN M	IKQLLHYFQEIKSFNF SQYDYG	ETFCTNSSDYKDACY WILNII
<i>T. castaneum</i> neutral lipase	VHGFIDTPLSNW V	DGKGHIFLGYGMSQP CGHL	CDITQTPLVPLTLIRDG LEEASRVLVAC

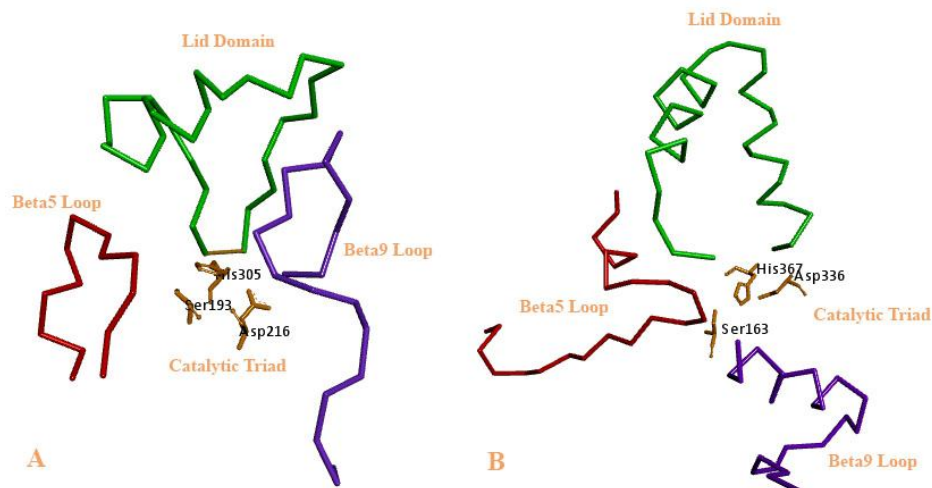


Fig. 7. Arrangement of protective regions ($\beta 5$ loop, $\beta 9$ loop and lid) around catalytic triad of *T. castaneum* neutral lipase (A) and *T. castaneum* acidic lipase (B).

N-glycosylation sites

Three *N*-glycosylation sites were detected in neutral lipase of *T. castaneum*: Asn39, Asn51 and Asn108, while acidic lipase contained four *N*-glycosylation sites. These sites may contribute additional stability to the protein

by facilitating proper folding of PTL-like lipase. N-glycosylation sites in lipases can be implicated in some functions like enzyme activity, secretion and stability as it has been revealed for some lipases through site-directed mutagenesis studies (Wicker-Planquart *et al.*, 1999; Wolle *et al.*, 1993; Zschenker *et al.*, 2005). Similar studies should be conducted for *T. castaneum* acidic and neutral lipases for ascertaining the exact function of N-glycosylation sites.

C-terminal domain

The C-terminal region is composed of 152 amino acids having a sandwich-like structure formed by seven β -strands (making two parallel β -sheets to form a sandwich), connected by loops and short helices (Fig. 2). C-terminal, in human pancreatic lipase is responsible for the binding with colipase, a protein required for the activation of the lipase to ensure the substrate binding (van Tilbeurgh *et al.*, 1993). However, C-terminal, although contributing stability and enhanced activity, is not a stringent requirement for lipase reactivation as observed for human PTL by Jennens and Lowe (1995) and Lowe (1997a).

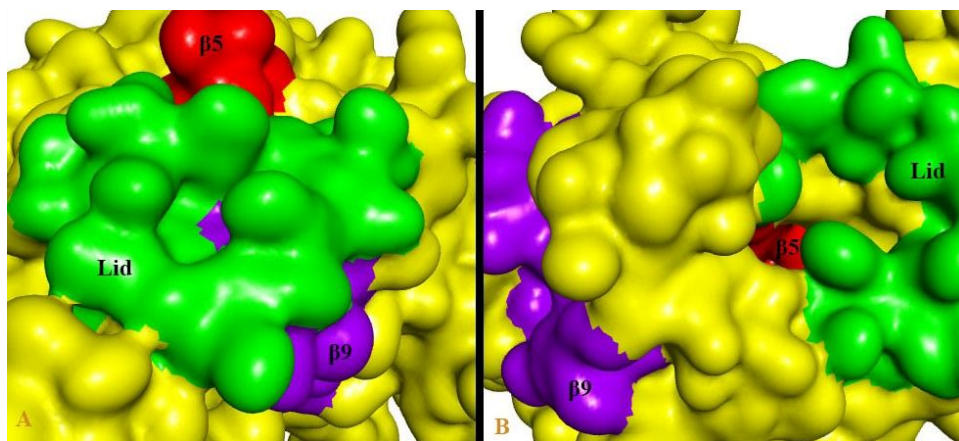


Fig. 8. Close view of molecular surface of *T. castaneum* neutral lipase (N-terminal domain) (A) and *T. castaneum* acidic lipase (B). For both enzymes lid domain at the surface encloses the active site (not visible) inside the sphere, making it inaccessible to the solvent. Displacement of the lid, initiated by substrate binding, is requisite for exposing the active site for catalytic attack.

In insects, no colipase is found (Chapman *et al.*, 2013) which suggests that there must be some alternative mechanism of activation of enzyme at the lipid-

water interface because C-terminal domain, which should be absent in insect neutral lipases, is present in neutral lipase of *T. castaneum*. Also, among neutral lipases from different sources, more similarity is found between the N-terminal sequences as compared to C-terminal sequences indicating that N-terminal is more conserved during the course of evolution than the C-terminal.

Interfacial activation of T. castaneum neutral lipase

Similar to that for acidic lipase of *T. castaneum*, the access of the substrate to the active site of *T. castaneum* neutral lipase is sterically blocked by the lid, $\beta 5$ loop and $\beta 9$ loop (Fig. 8). Due to strong homology with the pancreatic lipases and similar structural features with HPL, *T. castaneum* neutral lipase can be capable of hydrolyzing water insoluble substrate and its activity may be stimulated at the oil-water interface (Lowe, 1997b). Structural studies on human PTL-colipase complex have revealed that binding of PTL C-terminal to colipase is accompanied by movements of the C-terminal and lid domain, henceforth connecting colipase with the lid domain. This subsequently changes the conformation of $\beta 5$ loop, which moves back on the core of the protein, thereby enhancing the accessibility of the active site to the substrate (van Tilbeurgh *et al.*, 1993).

Nevertheless, there is a lack of evidence regarding the presence of colipase and bile salts in insects. Also, the presence of C-terminal domain in *T. castaneum* neutral lipase may be representative of any other mechanism of enzyme activation distinct from that of human pancreatic lipase and related proteins. Alternatively, it can be suggested that the C-terminal may not be involved in enzyme activation at all, and the only function of C-terminal domain can just be the stabilization of overall protein structure. Due to these reasons, the mechanism of interfacial activation of *T. castaneum* neutral lipase is unclear and biochemical studies are needed to explore the function of C-terminal domain in enzyme activation. On the other hand, the mechanism of interfacial activation of acidic lipase of *T. castaneum* can be predicted with a greater certainty in that the interaction of substrate with the lid domain should occur without any possible involvement of the C-terminal domain (Sami *et al.*, 2014).

Substrate selectivity

Lid domain of *T. castaneum* lipases acts as barrier for water soluble substrates. Removal of lid domain from *T. castaneum* neutral lipase will render it capable of hydrolyzing water soluble substrates according to what has been

observed with human pancreatic lipase (Jennens and Lowe, 1994). Based on the structural homology with human lipases, the acidic lipase of *T. castaneum* should be capable of hydrolysis of triglycerides as well as cholesteryl esters, yet the substrate preference for *T. castaneum* neutral lipase is strictly towards triglycerides. In addition to various other factors, the amino acid content of the lid domain and $\beta 5$ loop are crucial in determining the substrate selectivity of lipases as it has been observed for human pancreatic lipase and pancreatic lipase related protein 2, where a few differences in amino acids of lid domain and $\beta 5$ loop contributed to different substrate specificities of the two enzymes (Xiao and Lowe, 2015). Similarly, sequence of $\beta 9$ loop and the conformation of active site of lipase are also crucial in mediating substrate selectivity (Carriere *et al.*, 1998; Dridi *et al.*, 2013; Lowe, 1997a). Further, a few reports have also been found, linking substrate specificity with cysteine residues. Pagani *et al.* (1997) reported that cysteine residues are indirectly involved in determining a high activity of human lysosomal acid lipase towards cholesteryl oleate. Reduction of some of the cysteine residues (especially Cys-227) attenuated the enzyme activity to considerable degree towards cholesteryl oleate, while retaining the activity for trioleylglycerol.

CONCLUSION AND FUTURE PERSPECTIVES

In this article, we have explored catalytic features of a neutral lipase of *T. castaneum*. *T. castaneum* neutral lipase has many features similar to human pancreatic lipase and, intriguingly, it possesses C-terminal domain which could be a unique feature of some insect neutral lipases, as insects are devoid of co-lipase. N-terminal domain contains signal peptide, α/β hydrolase fold, catalytic triad, nucleophilic elbow, three N-glycosylation sites, and four disulfide bonds. Catalytic triad is protected by lid domain, $\beta 5$ loop and $\beta 9$ loops and movement of lid domain is imperative for exposing the active site residues, through interfacial activation, for initiating catalytic attack. Sequences of lid domain, $\beta 5$ loop and $\beta 9$ loops are expected to contribute towards substrate selectivity, as it has also been observed for mammalian lipases. Comparison of *T. castaneum* neutral lipase with a previously reported *T. castaneum* acidic lipase shows a different arrangement of catalytic triad, lid domain, $\beta 5$ loop and $\beta 9$ loops which can be correlated with the different substrate preferences of the two enzymes.

While we have identified many features, the involvement of C-terminal domain in enzyme activation is still questionable due to lack of empirical evidence and due to the fact that insect lipases do not have co-lipase, which binds

to the N- and C-terminal domain for lipase activation. Lipases are essential for satisfying the dietary requirement of *T. castaneum* through lipid hydrolysis; inhibition of lipase activity by targeting particular lipases using specific inhibitors can be useful in control of red-flour beetle. However, more conclusive evidence needs to be obtained for fully elucidating the function of structural components of *T. castaneum* lipases, and for ascertaining the role of sequence motifs in substrate specificity and enzyme activity. This requires its complete biochemical characterization from determination of its crystallographic 3D structure to *in-vitro* studies such as site-directed mutagenesis.

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SURVEILLANCE OF *Aedes* MOSQUITO IN SWABI AND HARIPUR DISTRICTS OF KHYBER PAKHTUNKHWA, PAKISTAN

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Abstract.- Dengue has caused much havoc in Pakistan in the recent years. The disease is now spreading from Southern part of Pakistan to the Northern areas. Vector surveillance studies including key breeding containers and ovitrapping is needed to understand vector biology. The present study was carried out to examine oviposition preference of *Aedes* mosquitoes and key breeding containers in four villages each of two districts (Swabi and Haripur) of Khyber Pakhtunkhwa province of Pakistan where the disease has been reported in the past. The results showed that five different types of containers i.e drinking water storage containers, tyres, tarcoal drums, cement tanks and small disposable containers were preferred containers for *Aedes*. High number of *Aedes* larvae (100%), were recorded from tyres in Swabi and Haripur (58.3%) as compared to the other containers in both districts. The House index (HI) and Breteau index (BI) in Swabi was 62.5, 70.8 respectively whereas in Haripur these indices were HI=54.1, BI=54.1 respectively. *Aedes* mosquito showed their preference for black ovitraps (47.3) as compared to green (19.1) or white color ovitraps (3.9). It was concluded that fresh water storage containers, black color ovitraps are attractive to *Aedes*. Thus black color ovitraps are recommended in surveillance studies for dengue vectors in Pakistan and elsewhere.

Key Words: *Aedes* sp., ovitraps, mosquito breeding containers, surveillance.

INTRODUCTION

Insect-transmitted diseases remain a major cause of illness and death worldwide. Mosquitoes alone transmit diseases to more than 700 million people annually. (Taubes *et al.*, 1997). Mosquitoes are placed in 39 genera including more than 3000 species worldwide; in which three genera, *Culex*, *Aedes* and *Anopheles* are the key vectors due to their obligate hematophagy (Aregawi *et al.*, 2008).

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Anopheles spp. are the only mosquitoes that spread malaria in humans. *Aedes* mosquitoes are aching and relentless biters that bother during day time and have a preference to bite mammals (humans). *Culex* mosquitoes are also painful but their attacks happen in dark and dusk. *Culex tarsalis* is notorious to transmit sleeping sickness (encephalitis) to horses and man. Generally, *Culex* mosquitoes are fragile fliers and do not normally go far, and they have been known to fly up to 2 miles.

Aedes is formerly found in subtropical and tropical zones and can be found in Malaysia and its adjoining countries like Thailand and Indonesia. The name of the *Aedes* mosquito originated from Greek, in which *Aedes* meant "unpleasant". These mosquitoes have typical black and white stripes on their body and legs and are the vectors of yellow fever and dengue fever (Lawson *et al.*, 2007). It usually bites in early morning or late afternoon, but females can get a blood meal at night under artificial lighting. They prefer human blood over other animals whereas; the ankle area is the favorite biting site. Adults reside in dwellings in darkly lighted cabinets, closets or cupboards. *Ae. aegypti* is recorded to fly only a few hundred meters from their breeding source. The species is active all year in the south and summer-active in the north. In colder climates, *Ae. aegypti* does not lie dormant in the egg stage but southern population stay reproductively vigorous during winter season and are occasionally inactive in colder times. Temperatures below freezing point kill the adults, and on temperatures below 5°C do not survive well (Aregawi *et al.*, 2009). Essential oils or plant extracts have been used as alternative sources of mosquito control strategies (Khan *et al.*, 2015a,b), because they contain certain bioactive constituents which are usually biodegradable into non-toxic products. Similarly insect growth regulators (IGRs) have been applied both at laboratory and field for the control of dengue and other arbovirus vectors (Khan *et al.*, 2016; Gubler *et al.*, 2008, Lee 2001).

MATERIALS AND METHODS

Identification of the key breeding containers for Aedes

Research was conducted in two districts (Swabi and Haripur) of Khyber Pakhtunkhwa. Survey for the presence of *Aedes* larvae was carried out from June 2012 to August 2012 to identify the key breeding containers of *Aedes* mosquitoes in four villages in each district. Baja bamkhel, Anbar, Gadoon and Tarbella colony was selected in district Swabi and Sobra colony, Ghazi, Khallo and Serikot in Haripur district of Khyber Pakhtunkhwa (KPK). For assessment of key breeding containers water storage containers, tyres, tarcoal drums, cement tanks

and small disposable containers (bowls, shoes) were investigated for the presence of *Aedes* larvae. The data so recorded was used to calculate House index (HI), Container index (CI) and Breteau index (BI) using formulae of WHO, 2009.

Container index

$$\frac{\text{Number of containers positive for } Aedes}{\text{Total number of containers}} \times 100$$

Breteau index

$$\frac{\text{Number of containers positive for } Aedes}{\text{Total number of inspected houses}} \times 100$$

House index

$$\frac{\text{Number of houses positive for } Aedes}{\text{Total number of houses surveyed}} \times 100$$

Evaluation of ovitraps for Aedes

Oviposition preference of *Aedes* species was assessed from various color Ovitrap that were installed in four villages each in two districts of Khyber Pakhtunkhwa. Baja bamkhel, Anbar, Gadoon and Tarbella colony was selected in district Swabi and Sobra colony, Ghazi, Khallo and Serikot in Haripur district of Khyber Pakhtunkhwa from October 2012 to November 2012. Different color ovitraps constructed from pepsu disposable plastic bottles of black, green and white color were used to see oviposition preference of *Aedes*. Each trap consisted of white paper on inner side of bottles, 1x4 inch wooden stick as oviposition substrate and a pinch of NIFA larval food (Khan *et al.*, 2013) for attraction. All traps were placed in protected areas in the houses and monitored weekly for the presence of *Aedes* larvae. The oviposition activity index (OAI) was expressed by total number of *Aedes* larvae in black and green bottles in comparison to white bottle (control traps). The oviposition activity index (OVI) was calculated by the following formula (Kramer and Mulla, 1979).

For black bottle

$$OAI = \frac{NB-NW}{NB+NW}$$

For green bottle

$$OAI = \frac{NG-NW}{NG+NW}$$

The OAI is the oviposition activity index, NB is the number of larvae in the black bottle, NG is the number of larvae in the green bottle and NW is the

number of larvae in the white bottle.

The percent effective attractancy (EA %) was also calculated for both black bottle and green bottle using the formula (Govindaraan *et al.*, 2008).

For black bottle

$$EA\% = \frac{NB-NW}{NB} \times 100$$

For green bottle

$$EA\% = \frac{NG-NW}{NG} \times 100$$

Data analysis

The data was analyzed by using statistical package Statistix 8.1 (Analytical Software, 2005) by analysis of variance in three factorial completely randomized designs. Means was compared using LSD Test at 5% level of significance (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

Key breeding containers and house index and Breteau index (BI) for Aedes in Swabi

Ae. aegypti and *Ae. albopictus* are the major vectors for the transmission of dengue virus for which there are no vaccine developed yet therefore, the vector control strategy is the top priority recommended preventive measure against dengue control (Gubler *et al.*, 2008). The present study was conducted to understand the key breeding sites and containers for *Aedes* mosquitoes in the dengue affected area of KPK and to evaluate oviposition preference of gravid females to various color containers. We surveyed 24 suspected houses out of which 15 houses were found positive (62.5%) for *Aedes* larvae. Results of house index (HI), Container Index (CI) and Breteau Index (BI) are shown in Table I. The Breteau index of district Swabi were calculated respectively in which same number of houses was inspected out of which 17 containers were found positive (70.8%) for *Aedes* mosquito. Anbar village was found with the highest house and Breteau index (HI and BI=83.3, CI=91.8) showing the high number of *Aedes* larvae followed by Gadoon (HI=50, BI=66.6, CI=66.6) and other tow villages. Lowest HI, and BI was recorded for Baja bamkhel village (HI=50, BI=50) in Swabi district. These results are similar with the research conducted by Warabhorn *et al.*, 2000 on the prevalence of *Aedes* species in three topographical

TABLE I.- VILLAGE-WISE DETAILS OF HOUSE INDEX (HI), CONTAINER INDEX (CI), AND BRETEAU INDEX (BI) IN SWABI, PAKISTAN

Villages	House Index (HI)		Index %	Inspected	Container index (CI)		Breteau Index (BI)
	Total houses	Positive			Positive	Index	
Baja bamkhel	6	3	50	45	29	64.4	50
Anbar	6	5	83.3	45	49	91.8	83.3
Gadoon	6	3	50	45	30	66.6	66.6
Tarbella colony	6	4	66.6	45	22	48.8	83.3
Total	24	15	62.5	180	130	72.2	70.8

TABLE II.- VILLAGE-WISE DETAILS OF HOUSE INDEX (HI), CONTAINER INDEX (CI), AND BRETEAU INDEX (BI) IN HARIPUR, PAKISTAN.

Villages	House index (HI)		Index %	Inspected	Container index (CI)		Breteau Index (BI)
	Total houses	Positive			Positive	Index	
Sobra	6	2	33.3	45	25	55.5	50
Khalo	6	4	66.6	45	28	62.2	50
Ghazi	6	4	66.6	45	23	51.1	66.6
Serikot	6	3	50	45	23	51.1	50
Total	24	13	54.1	180	99	55	54.1

TABLE III.- VILLAGE-WISE INFESTATION OF *Aedes* SPECIES IN VARIOUS CONTAINERS IN DISTRICT SWABI, KPK

Village Name	Total of each type	Type of water storage containers											
		Water storage containers		Tyres		Tarcoal drums		Cement tanks		Disposable containers			
		Positive	% positive	Positive	% positive	Positive	% positive	Positive	% positive	Positive	% positive		
Baja bamkhel	12	6	50	8	66.6	6	50	4	33.3	5	41.6		
Anbar	12	9	75	12	100	9	75	8	66.6	11	91.6		
Gadoon	12	5	41.6	5	41.6	7	58.3	7	58.3	6	50		
Tarbella	12	4	33.3	5	41.6	4	33.3	6	50	3	25		

TABLE IV.- VILLAGE-WISE INFESTATION OF *Aedes* SPECIES IN VARIOUS CONTAINERS IN DISTRICT HARIPUR, KPK

Village Name	Total of each type	Type of water storage containers											
		Water storage containers		Tyres		Tarcoal drums		Cement tanks		Disposable containers			
		Positive	% positive	Positive	% positive	Positive	% positive	Positive	% positive	Positive	% positive		
Sobra	12	3	25	6	50	6	50	5	41.6	5	41.6		
Khalo	12	5	41.6	7	58.3	5	41.6	6	50	4	33.3		
Ghazi	12	4	33.3	6	50	4	33.3	5	41.6	5	41.6		
Serikot	12	4	33.3	5	41.6	4	33.3	4	33.3	6	50		

areas from samples collected from 26 types of water containers in 300 households in nine districts. They reported both *Aedes* species from 17 out of 26 types of water containers and observed that the House index (HI) and Breteau index (BI) were not different in the three topographical areas.

In the current survey tyres (62.5%) were found as the highest attractive containers for *Aedes* larvae followed by tarcoal drums (54.1%) and water storage containers (50%) in the same district. Phong and Nam (1999) investigated 6,357 water containers in four districts of Hanoi for the presence of *Ae. aegypti* larvae. They found six types of water containers as breeding sites of *Aedes* in which more larvae were collected from water drums (38.8%) followed by concrete tanks (26.2%), clay jars (26.0%), discarded objects (5.0%), aquariums (3.5%) and buckets (0.5%).

Key breeding containers and houses index and Breteau index (BI) for Aedes in Haripur

Results of house index (HI), Container Index (CI) and Breteau Index (BI) are shown in Table II. Ghazi village in Haripur was found with high indices (HI=66.6, CI=51.1, BI=66.6) as compared to others villages. Sobra village was least infested with (HI=33.3, BI=50) for the *Aedes* species where as 24 houses were investigated in which 13 houses were found positive (54.1%) for *Aedes* larvae and the Breteau index (BI) in Haripur, 24 houses were inspected in which 16 containers were found positive (54.1%) for *Aedes* larvae with House Index (HI) and Breteau Index (BI) ratios in relation with the research done by Thavara, *et al.* (2001) in which he observed dengue hemorrhagic fever (DHF) outbreak in Samui Island in Thailand. They reported the three *Aedes* larval indices, Breteau index (BI), house index (HI) and container index (CI) which ranged 93-310, 43-89 and 16-50 respectively.

In the survey of larval containers in Haripur, we observed that the tyres (50%) are the more effective containers as compared to cement tanks and small disposable containers (41.6%) for the positivity of *Aedes* species. The water storage containers (33.3%) were found less attractive which was similar to earlier research in which Adeleke *et al.* (2008) reported about the different breeding sites of the mosquito fauna in Abeokuta, Nigeria. They collected samples from August 2005 to July 2006; the habitats visited by them were ground pools/ponds, gutters/open drains, tyres, domestic containers and tree holes/leaf axils. They observed 10 different species of mosquitoes in five habitats, in which the *Aedes aegypti* was recorded to breed in all the habitats while *Aedes albopictus* was

present in three habitats. Ground pools and domestic containers were recorded for the highest number of species followed by gutters/open drains. Tree holes/leaf axils was the least preferred habitat with the lowest number of species occurrence as shown in Table VI.

TABLE V.- OVIPOSITION PREFERENCE OF *Aedes* TO VARIOUS COLOR OVITRAPS IN TWO DISTRICTS OF KPK.

Ovitrap	Districts		Mean
	Swabi	Haripur	
Black bottle	50.5 a	44.1 b	47.3 a
Green bottle	20.2 c	18.1 c	19.1 c
White bottle	4.1 d	3.7 d	3.9 d

LSD value of (districts x traps) at 0.05 = 3.1

TABLE VI.- INTERACTION OF DIFFERENT COLORED OVITRAPS WITH WEEK IN TWO DISTRICTS OF KPK.

Ovitrap/Week	Black bottle	Green bottle	White bottle
Oct 1 st Week	50.6 ab	19.8 de	3.5 f
Oct 2 nd Week	40.3 c	14.8 e	3.6 f
Oct 3 rd Week	47.1 b	20.6 d	3.5 f
Oct 4 th Week	41.6 c	18.1 de	4.6 f
Nov 1 st Week	51.3 ab	20.8 d	4.5 f
Nov 2 nd Week	52.8 a	20.8 d	3.8 f
Mean	47.2 b	19.1 d	3.9 f

LSD value of (week x traps) at 0.05 = 5.48

Oviposition preference of Aedes for various color ovitraps

For the attraction of *Aedes* mosquitoes three different colored (Black, Green and White) ovitraps were used in Swabi and Haripur districts of KPK. The black ovitraps were found more attractive (47.3a) followed by green ovitraps, while the white ovitraps were the least attractive for oviposition (Table V). Significant variations were observed in ovitraps attractiveness in Swabi and Haripur districts. Maximum number of larvae (47.3a) were collected from black bottle in district Swabi, whereas, minimum number of larvae (3.9d) were collected from white bottle in district Haripur. Overall, *Aedes* larvae were abundant in district Swabi in black bottle. Research by Hoel *et al.* (2011) also indicated enhanced attractiveness of a standard black ovitrap routinely used in

surveillance of the Asian tiger mosquito, *Aedes albopictus* and now being used as lethal ovitraps in *Aedes aegypti* dengue control programs. Table IV shows the attractiveness of the three different colored ovitraps to *Aedes*. Significant variations were observed in different traps over weeks. The data showed that highest number of larvae (52.8) was collected from black bottle in the 2nd week of November. White bottle was least effective in terms of attractiveness as minimum number of larvae (3.5) were collected in 1st week of October, while green bottle was moderately effective. Similar results were obtained by Budiyanto (2010) from red, yellow, blue, black and white ovitraps. And recorded more eggs from red and black colored ovitraps.

CONCLUSION

Fresh water storage containers are attractive to *Aedes*. Their surveillance studies can be efficiently done with use of black color ovitraps.

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ISOLATION AND CHARACTERIZATION OF *BACILLUS* SPP. FOR THE PRODUCTION OF α -AMYLASE FROM DIFFERENT SOIL SAMPLES

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Abstract.- This work was conducted with the aim of isolating α -amylase producing *Bacillus subtilis* from local soil samples. Fifty bacterial strains were isolated from 28 soil samples collected from food industries dumping sites. On the basis of the morphological and biochemical characterization, five bacterial strains were screened. Through 16S rRNA sequencing it was found that the GCU-DAB-02A, GCU-DAB-12E (a) and GCU-DAB-23A showed high homology to *Bacillus subtilis*, while GCU-DAB-09A (b) showed high homology to *Bacillus amyloliquefaciens* and GCU-DAB-22A showed high percentage homology to *Bacillus licheniformis*. The maximum enzyme production was obtained on M1 medium containing molasses, dextrin, sucrose and Trypton. These strains exhibited maximum α -amylase production at following optimum conditions: 18 h inoculum age; 37°C temperature; 48 h incubation time; 2% inoculum size; GCU-DAB-09A at pH 6, GCU-DAB-12E(a), GCU-DAB-02A and GCU-DAB-22A at pH 8, and GCU-DAB-23A at pH 9; GCU-DAB-02A, GCU-DAB-09A(b) and GCU-DAB-12E(a) at 25ml, GCU-DAB-23A at 50ml and GCU-DAB-22A at 75ml GCU-DAB-22A; GCU-DAB-02A, GCU-DAB-22A and GCU-DAB- 23A at 100rpm, GCU-DAB-09A (b) at 150rpm and GCU-DAB-12E (a) at 200rpm. The enzyme produced from all strains was found to be stable at 1% starch concentration and pH 7. The enzyme produced from GCU-DAB-02A, GCU-DAB-12E (a) and GCU-DAB-23A was stable at 45°C, however the other two *i.e.*, GCU-DAB-09A (b) and GCU-DAB-22A alpha-amylase showed maximum stability at 40°C. These bacterial isolates possesses the high ability to degrade starch into its basic components, may find potential biotechnological applications in industry.

Key words: Starch; α -amylase; *Bacillus* spp., enzyme activity.

INTRODUCTION

Amylases are wide spread in nature and possible sources of amylases include animals, plants, bacteria and fungi (Mamo and Gessesse, 1999; Burhan *et al.*, 2003; Reddy *et al.*, 2003; Kandra, 2003; Octavio *et al.*, 2000).

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Microorganisms like bacteria and fungi are screened extensively for the production of α -amylase (Ivanova *et al.*, 2001). Microbial sources are used for industrial production of α -amylase because of their advantages as they are cost effective, their consistency, small time and ease of modification of process, rapid growth of microorganisms on inexpensive media and also the optimization (Mamo and Gessesse., 1999; Burhan *et al.*, 2003). Microbial enzymes are also more stable than their corresponding plant and animal enzyme and their production is more convenient and safer. However amylases of bacterial origin are generally preferred over amylases of fungal origin because of their some characteristic advantages that they offer (Goyal and Khadeparker, 1979). *Bacillus* species can be used for good production of α -amylase in shake flask (Mamo and Gessesse, 1999). For the industrial purposes, α -amylase is obtained mostly from the good producers of members of Genus *Bacillus* such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus stearothermophilus* and *Bacillus amyloliquefaciens* (Pandy *et al.*, 2000; Prakash and Jaiswal, 2009).

Microbial amylases are available in large number at commercial level (Bernfeld, 1955). Amylases that produce free sugars are known as “saccharogenic” and amylases that liquefy starch without producing free sugars are termed as “starch-liquefying” enzymes (Pandey *et al.*, 2000).

α -Amylase (EC 3.2.1.1, 1 \rightarrow 4- α -D-glucan-glucanohydrolase) has a wide range of occurrence in living organisms ranging from human saliva to several species of bacteria and fungi (Reed *et al.*, 1996). α -Amylase is considered to be one of the most important enzyme industrially that is used in biotechnological applications (Pandey *et al.*, 2000; Gupta *et al.*, 2003). The α -amylases are the class of enzymes that make about 30% of enzyme production in whole world (Vander and Maarel *et al.*, 2002). All industrial world enzyme 65% market are met by amylases and is represented as the 3rd largest group of industrial enzyme (Nimkas *et al.*, 2010). Bacteria almost completely replaced chemical hydrolysis of starch in starch processing industry (Pandey *et al.*, 2000). Among the industrial enzymes, the enzymes that are thermostable are considered more useful than the thermo labile enzymes (Fogroth *et al.*, 1974). The thermostable enzymes have more shelf life at higher temperatures (Niehaus *et al.*, 1999). Hence there is a need to find out microorganisms that are able to produce thermostable enzymes, such as thermo stable α -amylases. The thermo stable α -amylases can be used in wide range of industrial processes. For the production of thermo stable α -amylase bacteria that belong to the genus *Bacillus* have been broadly used (Leveue *et al.*, 1989).

Bacillus subtilis is also known as “hay bacillus”. *Bacillus subtilis* is a gram positive. It is found in soil and is also catalase positive (Medigan and Martinko, 2003). *B. subtilis* is a rod shaped bacterium having the property of forming a rigid, tough, resistant and protective endospore that permits the organism to tolerate harsh and extreme environmental conditions. *B. subtilis* synthesizes enzymes which are thermostable, having lesser risk of contaminants (Peixoto *et al.*, 2003). Some mutant strains of *B. subtilis* were found best for bioproduction of α -amylase. *B. subtilis* has many advantages over other microorganisms. First and greatest advantage of it is its characteristic of secretion of proteins directly into the culture medium and also the accumulation of them in elevated levels in restively pure state (Goeddle, 1990). Another advantage of *Bacillus subtilis* is its non-pathogenicity due to which it is safe biologically (Lee *et al.*, 1997; Ye *et al.*, 1999).

Enzyme production depends upon the strains, media composition, cultivation methods, growth of cells, and requirement of nutrient, metal ions, incubation time, pH, temperature and thermostability. Temperature from *B. subtilis* on relative activity of α -amylase was measured and also the temperature was optimized as between 60^o -70^oC for greatest activity (Kim *et al.*, 1995). Synthesis of α -amylase by *B. subtilis* occurs during the exponential as well as stationary phases of growth, rate was slightly rapid during exponential phase and then stationary phase. *B. subtilis* grows at pH between 4.5-10.5 (Ivanova *et al.*, 1993).

α -Amylase extensively used in manufacturing process of detergents for the removal of stains (Hewitt and Solomon, 1996; Lin *et al.*, 1998), high fructose corn syrup preparation, desizing in textile industry (Pedersen and Frantzen, 1999). Many fields such as clinical, medicinal, biotechnological, analytical chemistry have been flourishing since the beginning of usage of it (Reddy *et al.*, 2003).

The pH of the starch slurry industry is usually about 4.5 (Shivaramakrishnan *et al.*, 2006). But most of the commercially used bacterial α -amylase works optimally at 6.5. However it is relatively unstable at low pH. Thus the liquefaction step is set to operate between pH 5.8-ted from 6.2 (Sajedi *et al.*, 2005).

The present study is focused basically on the isolation and characterization of *Bacillus subtilis* strains from soil which produce α -amylase in comparably high enough yield on cheaper medium. This was accomplished through various

steps which include isolation of α -amylase producing microorganisms from different soil samples, selection as well as identification of most potent α -amylase producing isolate using liquid medium, optimization of different cultural conditions which affect the bioproduction and also the enzyme kinetics were studied.

MATERIALS AND METHODS

Collection of samples

Twenty eight samples of soil from food, industrial and agricultural waste dumping sites (*e.g.*, baggase dumps, banana's leaves and stalks) as well as from gardens and fields etc. were collected in sterile polythene bags from areas around Lahore.

Isolation and characterization

One gram of soil was dissolved in 100ml of distilled water. After 10-15 min, 10ml of supernatant was taken in a test tube and heat shocked in boiling water bath for 5 min. LB agar medium was used for the isolation purpose. The characterization of the bacterial isolates was done on morphological, biochemical (Benson, 2002) as well as on molecular basis.

Antibiotic resistance

To determine the antibiotic resistance of the isolated bacteria antibiotic assay was performed. For this purpose following antibiotics *i.e.*, tetracyclin, ampicillin, canamycin, pencillin and chlorophenicol were used.

Enzyme Assay

Preparation of inoculum

In order to prepare inoculum, LB broth was prepared, and sterilized in an autoclave for 15 min at 121°C and 15 lbs pressure. A colony from each isolate was inoculated in steam sterilized broth under sterilized conditions and then placed in shaking incubator for 18 h at 37°C.

Extraction and assay of α - amylase

After 48 h of submerged fermentation centrifugation of broth was carried

out at 8000rpm at 4°C for 5 min. The supernatant was separated used as enzyme source. The quantitative assay was performed according to Fisher and Stein (1961). One milliliter of 1% starch solution was taken in a test tube; 1ml of enzyme was added and then incubated for 3 min in shaking incubator at 200rpm at 25°C. After 3 min 2ml of DNS reagent was added and heated on a water bath for 5 min until red coloration appeared. After cooling the optical density values were taken at 550nm by using spectrophotometer. Under the assay conditions, 1.0 mg maltose hydrates (reducing sugar) was liberated by one unit of α -amylase.

Protein estimation

Estimation of protein contents of enzyme extract, using BSA as standard, was carried out by Lowry *et al.*(1951). Expression of activity of enzyme is as specific activity, *i.e.*, equivalent to U/mg of protein.

Culture medium selection for optimum production of α -amylase

For determining the optimum culture medium for the production of α -amylase, five different culture medias were used.

- Medium-1: Trypton 0.5%, Sucrose 0.1%, Dextrin 0.5%, Molasses 3%
- Medium-2: Soya bean meal 1.5%, Rice bran 3.5%, Wheat bran 2.0%, Magnesium sulphate (MgSO₄) 0.08%, Calcium chloride (CaCl₂) 0.1%, Potassium dihydrogen phosphate (KH₂PO₄) 0.1%.
- Medium-3: Rice bran 1.5%, Soya bean meal 0.5%, CaCl₂ 0.16%, KCl 0.15%, MgSO₄ 0.05%, Diammonium hydrogen phosphate 0.74%.
- Medium-4: Wheat bran 2%, Potato starch 1.5%, MgSO₄ 0.05%, KCl 0.02%, Lactose 0.5%, CaCl₂ 0.15%, Diammonium hydrogen phosphate 2.5%.
- Medium-5: Trypton 10%, Yeast extracts 5%, NaCl 5%.

They were inoculated with the fresh bacterial culture and were then placed in shaking incubator (250rpm) for 48 h at 37°C. After 48 h, the enzyme was extracted for enzyme assay. The results of enzyme assay were recorded to determine optimum medium production of α -amylase.

Determination of optimum conditions for α -amylase production

The effects of culture conditions for the production of α -amylase was determined by applying different conditions such as medium pH (6.0-10.0), temperature (25-55°C), incubation period (0-72 h), inoculum size (1-10

ml/100ml), volume of medium (25, 50, 75, 100 and 120 ml in 250 ml Erlenmeyer flasks), rpm (100-350) and age of inoculum (6-36 h.).

Enzyme kinetics

Effect of pH on α -amylase activity

For determining the effect of pH on α -amylase activity, the buffer solution was prepared having different pH *i.e.*, from 3.0-10.0. The enzyme was incubated in these buffers for 3 min at 25°C.

Effect of temperature on α -amylase activity

The effect of temperature on enzyme activity was determined by a range from 27-55°C.

Effect of starch concentration on α -amylase activity

In order to determine the effect of starch concentration on the activity of α -amylase, 1ml of different starch concentrations *i.e.*, ranging from 0.5%-3.0%, were taken in test tube and 1ml of crude enzyme was added in it and incubated at 25°C for 3 min.

RESULTS AND DISCUSSION

Possibility of depuration of food industry wastes, using them as substrates for different bioproduction of economic interest had been reported earlier (Murado *et al.*, 1993; Guerra and Pastrana, 2003). Amylases are important enzymes used in the industries of starch processing for the hydrolysis of the polysaccharides (Alva *et al.*, 2007).

An objective of present study was the isolation and selection of bacillus strains for the production of α -amylase as members of *Bacillus* genus are best known producers of the α -amylase (Pandey *et al.*, 2000; Prakash and Jaiswal, 2009). In present study twenty eight soil samples from dumping as well as gardens were collected to isolate the *Bacillus* species which are good producers of α -amylase. Heat shock was given to each soil sample, for the selection of *Bacillus* species, so that only the spore former could survive. This also provides us the advantage to isolate thermostable enzyme producing bacterial isolate as it

has been reported that for the production of thermostable α -amylase bacteria of genus *Bacillus* have been broadly used (Leveue *et al.*, 1989). Among the industrial enzymes, the enzymes that are thermostable are considered more useful than thermo labile enzymes (Fogarth *et al.*, 1974). The thermostable enzymes have more shelf life at higher temperatures (Niehaus *et al.*, 1999). Pore plate technique was used to isolate *Bacillus* species. In this way, 50 bacterial isolates were isolated were screened qualitatively and quantitatively using M-1 medium in shake flasks. *Bacillus* species GCU-DAB-02A, GCU-DAB-09A (b), GCU-DAB-12E (a), GCU-DAB-22A and GCU-DAB-23A showed the maximum enzyme activity of 877.1, 1608.2, 1636.4, 1023.4 and 701.7 U/ml.

The strains were determined according to “Benson, 2002” and it was confirmed through morphological and physiological studies that isolates GCU-DAB-02A, GCU-DAB-09A (b), GCU-DAB-12E (a), GCU-DAB-22A and GCU-DAB-23A belong to genus *Bacillus* (Tables I, II).

For molecular characterization the 16S rDNA was amplified and The PCR product was then visualized in 0.8% agarose gel containing DNA ladder (Fig.1a). On the basis of 16S rDNA gene sequencing, GCU-DAB-02A was 99.9% homologous to *Bacillus subtilis*, GCU-DAB-09A (b) was 99% homologous to *Bacillus amyloliquefacians*, GCU-DAB-12E (a) was 99.9% homologous to *Bacillus subtilis*, and GCU-DAB-22A was 98% homologous to *Bacillus licheniformes* and GCU-DAB-23A was 99% homologous to *Bacillus subtilis* (Fig. 1b)

In order to determine the antibiotic resistance of the selected bacterial isolates, the Petri plates were spreaded with fresh cultures and five different antibiotic discs were placed. It was found that bacterial isolate *i.e.*, GCU-DAB-02A was resistant against only one antibiotic and that antibiotic was chloramphenicol. However it was not resistant against ampicillin, tetracyclin, kanamycin and pencillin. GCU-DAB-09A (b) was resistant against ampicillin and chloramphenicol. It was sensitive against cephradine, kanamycin and pencillin. GCU-DAB-12E (a) showed no resistant against any of the used antibiotic discs. GCU-DAB-22A showed resistance against chloramphenicol, kanamycin and pencillin and was sensitive against ampicillin and cephradine. GCU-DAB-23A was resistant against all antibiotics used *i.e.*, ampicillin, cephradine, kanamycin, chloramphenicol and pencillin (Table III).

TABLE I.- MORPHOLOGICAL CHARACTERS OF BACTERIAL ISOLATES.

Sample no	Colony morphology				Gram staining	Endospore staining	Motility test
	Colony color	Colony size	Colony edges/appearance	Colony texture			
GCU-DAB-02A	Off-white	Medium	Sharp/ Non-elevated	Wet	+ve	+ve	+ve
GCU-DAB-09A(b)	Off-white	Medium	Smooth/ Non-Elevated	Wet	+ve	+ve	+ve
GCU-DAB-12E(a)	Pale-yellow	Large	Smooth/ Non-elevated	Wet	+ve	+ve	+ve
GCU-DAB-22A	Off-white	Medium	Sharp/ Non-elevated	Wet	+ve	+ve	+ve
GCU-DAB-23A	Pale-yellow	Small	Smooth/ Elevated	Wet	+ve	+ve	+ve

TABLE II.- BIOCHEMICAL CHARACTERIZATION OF THE ISOLATED BACTERIAL CULTURES

Sample no	Catalase test	Indole test	Nitrate reduction test	Citrate test	VP test	Casein test	Starch test	Carbohydrate tests		
								Glucose	Sucrose	Lactose
GCU-DAB-02A	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve
GCU-DAB-09A(b)	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve
GCU-DAB-12E(a)	+ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve
GCU-DAB-22A	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve
GCU-DAB-23A	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve

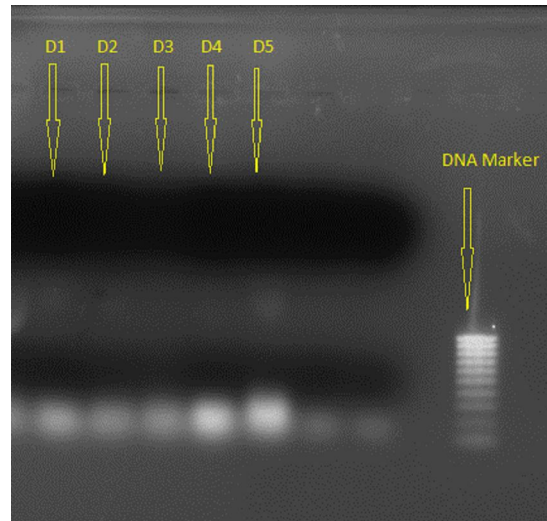


Fig. 1a: 0.8% agarose gel D1 (GCU-DAB-02A), D2 (GCU-DAB-09A (b)), D3 (GCU-DAB-12E (a)), D4 (GCU-DAB-22A) and D5 (GCU-DAB-23A) indicates the genomic DNA isolated from α -amylase producing bacterial isolates.

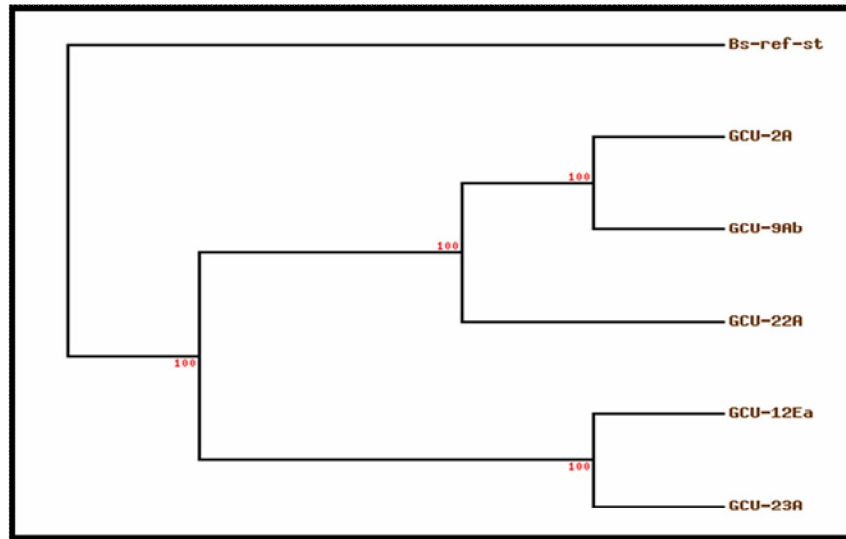


Fig. 1b: Dendrogram showing the homology (%) of α -amylase producing bacterial isolates with the standard bacterial strain (BS-ref-st).

TABLE III.- ANTIBIOTIC RESISTANCE OF SELECTED α -AMYLASE PRODUCING BACTERIAL ISOLATES.

Antibiotics	GCU-DAB-02A	GCU-DAB-09A (b)	GCU-DAB-12E (a)	GCU-DAB-22A	GCU-DAB-23A
Ampicillin	Non-resistant	Resistant	Non-resistant	Non-resistant	Resistant
Tetracyclin	Non-resistant	Non-resistant	Non-resistant	Non-resistant	Resistant
Kanamycin	Non-resistant	Non-resistant	Non-resistant	Resistant	Resistant
Pencillin	Non-resistant	Non-resistant	Non-resistant	Resistant	Resistant
Chloramphenicol	Resistant	Resistant	Non-resistant	Resistant	Resistant

The sequences of all the α -amylase producing bacterial isolates are given below:

Sequence of 16S rRNA gene (GCU-DAB-02A)

CCCCGGTTCACCCGGGGAGGGTCATTGGAAACTGGGGAACCTTGAGTGCAGAAGAGGAGAGT
GGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGC
GACTCTCTGGTCTGTAAGTACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGA
TACCCTGGTAGTCCACGCCGTAACCGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCTT
AGTGCCTGCAGTAACGCATTAAGCACTCCGCCCTGGGGAGTACGGTCGCAAGACTGAAACT
CAAAGGAATTGACGGGGGCCAGCACAAAGCGGTGGAGCATGTGGTTTAATTGAAAGCAACG
CGAAGAACCCTTACCAGGTCTTGACATCCTCTGACAATCCTAGAGATAGGACGTCCCCTTC
GGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGAGATGTTGGGTT
AAGTCCCACAACGAGCGCAAACCTTGATCTTAGTTGCCAGCATTAGTTGGGCAC

Sequence of 16S rRNA gene [GCU-DAB-09A (b)]

GGCCTCGCAGCGGTTTCTTAAGTCTGATGTGAAAGCCCCGGCTCAACCGGGGAGGGTC
ATTGAAACTGGGGAACCTTGAGTGCAGAAGAGGAGAGTGGAAATCCACGTGTAGCGGTG
AAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAAGTAC
GCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTA
AACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCTTAGTGCTGCAGCTAACGCATTAA
GCACTCCGCCCTGGGGAGAACGGTGCAGACTGAAACTCAAAGGAATTGACGGGGGGCCG
CACAAAGCGGTGGAGCATGTGGTTTAATTGAAAGCAACGCGAAGAACCCTTACCAGGTCTTG
ACATCCTCTGACAATCCTAGAGTTAGGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTG
CATGGTTGTCGTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCACAACGAGCGCAACC

Sequence of 16S rRNA gene [GCU-DAB-12E (a)]

CACGACTGAAACTCAAAGGAATTGACGGGGGCCGACAAAGCGGTGGAGCATGTGGTTTA
ATTCGAAGCAACGCGAAAAACCTTACCAGGTCTTGACATCCTCTGACAATCCTAGAGATA

GGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGGT
 GAGATGTTGGGTTAAGTCCCAGCAACGAGCGCAACCCCTTGATCTTAGTTGCCAGCATTTCAG
 TTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAAT
 CATCATCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
 GAAACCGCAGGTTAAGCCAATCACACAAATCTGTCTCAGTTCGGATCGCAGTCTGCAAC
 TCGACTGCGTGAAGCTGGAATCGCTAGTAATTGCGGATCAGCATGTGCCGATT

Sequence of 16S rRNA gene (GCU-DAB-22A)

CGTGTAGGGGTGAAATGCGTAGAGATTTGGAGGAACACCAGTGGCGAAGGCGACTCTCTG
 GTCTGTAAGTACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGT
 AGTCCACGCCGTAACGATGAGTGCTAAGTGTAGGGGGTTTCCGCCCTTAGTGCTGCA
 GTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTTCGCAAGACTGAAACTCAAAGGAAT
 TGACGGGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTAATTTGAAGCAACCGCAAGAACC
 TTACCAGTCTTGACATCCTCTGACAATCCTAGAGATAGGACGTCCCCTTCGGGGGCAGA
 GTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTTTCGTGAGATGTTGGGTTAAGTCCCGC
 AACGAGCGCAAACCTTGATCTTAGTTGCCAGCATTAGTTGGGCACCTAAGGTGACTGC
 CGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGC

Sequence of 16S rRNA gene (GCU-DAB-23A)

CGAAAAGGATTAGATATCCTGGTAGTCCACGCCGTAACGATGAGTGCTAAGTGTAGGG
 GGTTTCCGCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTC
 GCAAGACTGAAACTCAAAGGAATTGACGGGGCCCGCACAAACGGTGGAGCATGTGGTTT
 AATTTGAAGCAACCGCAAGAACCTTACCAGGTCTTGACATCCTCTGACAATCCTAGAGAT
 AGGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAACTCGTGTG
 TGAGATGTTGGTTAAGTCCCAGCAACGAGCGCAACCCCTTGATTTTAGTTGCCAGCATTCA
 GTTGGGCACCTAAGGTGACTGCCGGTGAAAAACCGGAGGAAGGTGGGGATGACGTCAA
 TCATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAG
 CGAAACCGCAGGTTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCA
 ACTCAACTGC

Successful fermentation is dependent on more appropriate strain and also the optimal cultural conditions (Gupta *et al.*, 2003; Saxena *et al.*, 2007). The choice of the suitable fermentation medium depends upon the availability, cost and quality of required enzymes and is most essential for maximum production of α -amylase in the fermenter. Usually raw materials which are used in the laboratory are replaced by the cheaper medium in the industry (Keay, 1971). Five different media were optimized and tested for the production of α -amylase. The M1 medium was selected, keeping in view the above mentioned factors, for the production of α -amylase from *B. subtilis*, *B. amyloliquifaciens*, *B. subtilis*, *B. licheniformes*, and *B. subtilis* and fermentation was carried out under submerged fermentation as α -amylase could be produced best at submerged cultural conditions (Bose and Das, 1996; Egas *et al.*, 1998; Aguilar *et al.*, 2000). The fermentation medium having suitable levels of carbon, mineral nutrients and nitrogen is usually preferred (De-Souza and Martins, 2000; Gupta *et al.*, 2003).

In the present work the fermentation medium having (g/l) trypton 5g, Sucrose 1g, Dextrin 5g and Molasses 30g in 1000 ml of distilled water was selected. Some of the researchers reported that for the production of the α -amylase organic nitrogen sources are preferred and maximum production of α -amylase was reported by peptone, yeast extract or beef extract (Krishnan and Chandra, 1982; Emanuilova and Toda, 1984; Hayashida *et al.*, 1988; Hamilton *et al.*, 1999).

For maximum enzyme production, five different media were assessed. Out of these five media the maximum production of α -amylase was observed in M-1 medium. The *Bacillus* species GCU-DAB-02A, GCU-DAB-09Aa, GCU-DAB-12Ea, GCU-DAB-22A and GCU-DAB-23 showed maximum enzyme production of 877.1, 1608.2, 1636.4, 1023.4 and 701.7 U/ml (Fig. 2). This might be due presence of readily available substance and might also be due to presence of some nutritive components that are essential for their proper growth. The other four media might lack quantity of some of these components because there was less enzyme production as compared to M-1 medium. Maximum amylase production was reported in sucrose medium by Dharani (2004).

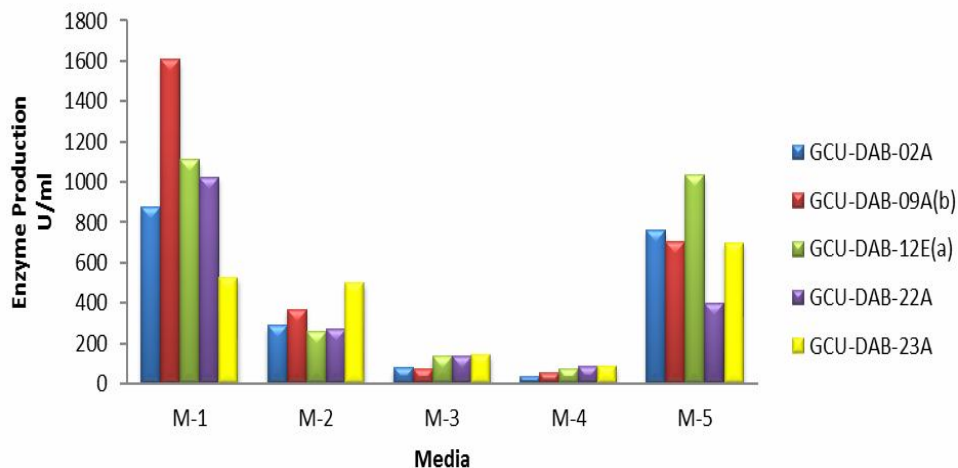


Fig. 2. Effect of culture media on production of α -amylase.

The pH of fermentation media has a pronounced effect on the synthesis of enzyme as well as on the growth of organism (Horikoshi and Akiba, 1982). Asgher *et al.* (2007) reported that with increase in pH from 5.0 to 7.0, stimulation of synthesis of enzyme was also increased. He further reported that this increased

enzyme production at pH 7.0 is due to increased growth of bacteria. The pH of the medium plays a crucial role as it induces certain morphological changes in organism and also in the enzyme secretion. The change in pH also affects the stability of the product in the medium during the growth of the organism. Mostly the *Bacillus* strains have optimum pH between 6.0 to 9.0 for growth and production of enzyme, which are available commercially and are being used for the production of α -amylase through submerged fermentation (Castro *et al.*, 1992; Jin *et al.*, 1999; Burhan *et al.*, 2003). The *Bacillus* sp. (GCU-DAB-02A) showed the maximum enzyme activity of 87.7 U/ml at pH 7 and 8. The GCU-DAB-09A (b) showed the maximum enzyme activity of 877.2 U/ml at pH 6. The GCU-DAB-12E (a) and GCU-DAB-22A showed maximum enzyme activity of 1637.4 U/ml and 877.2 U/ml, respectively at pH 8 and GCU-DAB-23A showed the maximum enzyme activity of 1754.3 U/ml at 9 pH (Fig. 3). Our results are in good agreement to findings reported earlier (Castro *et al.*, 1992; Jin *et al.*, 1999; Burhan *et al.*, 2003).

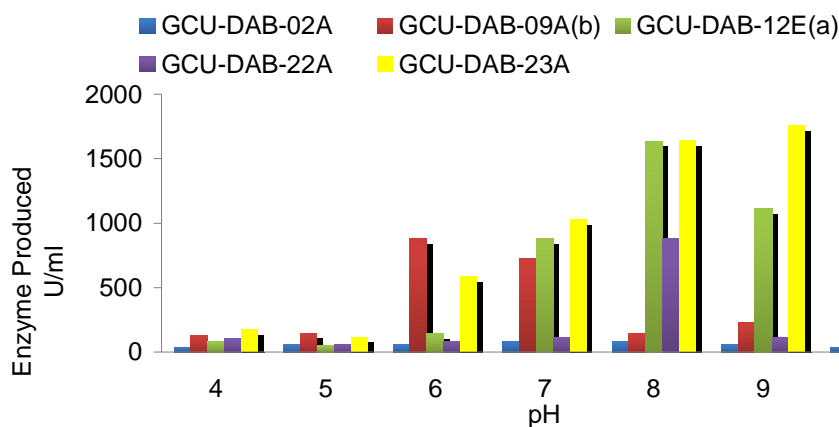


Fig. 3. Effect of pH on α -amylase production.

In the present study, rate of enzyme production was increased as the length of fermentation period increased. Rate of enzyme production was noted from 0 to 78 h and maximum production of enzyme was determined at 48 h of incubation period. The *Bacillus* species GCU-DAB-02A, GCU-DAB-09A (b), GCU-DAB-12E (a), GCU-DAB-22A and GCU-DAB-23A showed maximum enzyme production of the 1111.11, 1696, 1608, 731 and 1462 U/ml respectively (Fig. 4). Amylase production started during the exponential phase of strain and After 48 h of incubation the enzyme production started to decline. This decline was might

be due to rapid depletion of nutrients from the medium or might be due to accumulation of some byproducts in the medium. This decline might also be because of the reason that in start the substrate was more likely to be influenced by the hydrolysis that made an increased rise in enzyme amount. With intervals of time most of the susceptible part of the substrate was hydrolyzed and crystalline portion was left over there that cannot be hydrolyzed (Reese *et al.*, 1989).

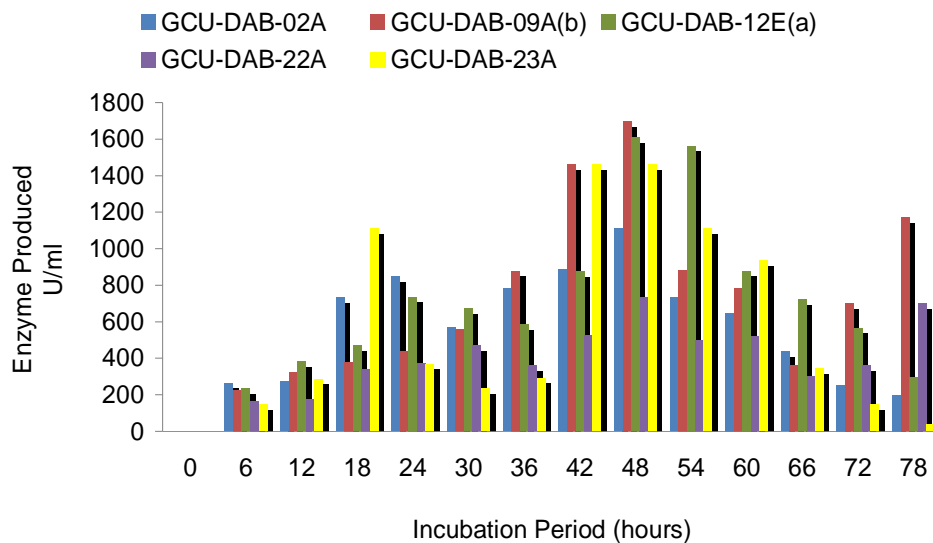


Fig. 4. Effect of incubation period on α -amylase production

Bacillus sp. is aerobic bacteria, and hence there is a strong effect of aeration on the production of enzyme. In the present study, different volumes of fermentation medium ranging from 25 ml to 120 ml were assessed in 250ml conical flasks. The *Bacillus* sp. GCU-DAB-02A, GCU-DAB-09A (b) and GCU-DAB-12E (a) showed the maximum enzyme production of 1666.67, 1462.75 and 1754.38 U/ml, respectively in 25 ml of fermentation medium. This was might be due to high supply of oxygen which was needed for their metabolic activities. The *Bacillus* sp. GCU-DAB-23A showed maximum enzyme production of 1462 U/ml in 50ml of fermentation media. The *Bacillus* sp. GCU-DAB-22A showed maximum enzyme production of 555.56 U/ml in 75 ml of fermentation medium, less production of enzyme in lower volume was might be due to low concentration of nutrients present (Fig. 5). The decline in the

production of enzyme in higher volumes was due to insufficient supply of oxygen needed for the proper growth of bacteria and this also causes the anaerobic conditions that greatly affect the metabolism and physiology of bacteria (Tonkova *et al.*, 1993).

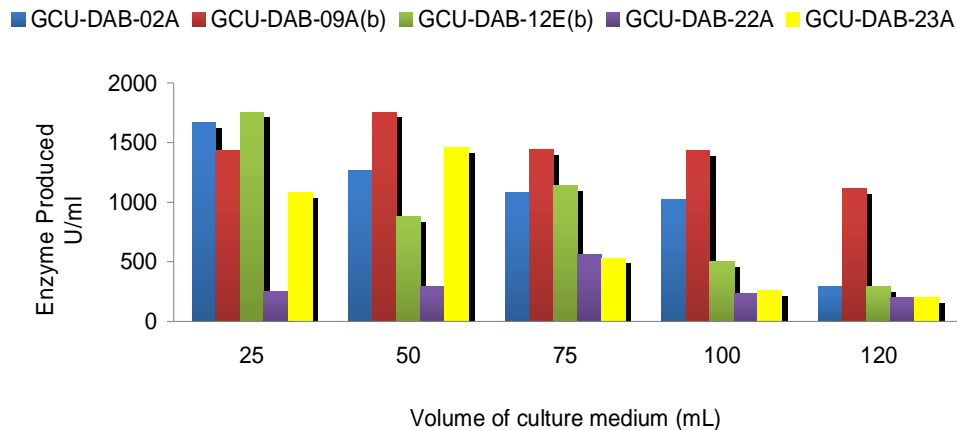


Fig. 5. Effect of aeration on α -amylase production.

Incubation temperature has serious effect on the production of enzyme by many *Bacillus* species (Morgan and Priest, 1981). Incubation at room temperature *i.e.*, 24-28°C was reported as most suitable and favorable temperature for the production of enzyme in both of solid state fermentation (SSF) as well as in submerged fermentation. Amylases of bacterial origin are produced at a very broad range of temperatures. *Bacillus subtilis* and *B. licheniformis* are the reported *Bacilli* which produce α -amylase at temperature 37-60°C (Mishra *et al.*, 2005; Mendu *et al.*, 2005). It is also reported that recovery of enzyme from SSF depends upon extraction temperature (Padmanabhan *et al.*, 1992). The *Bacillus* species GCU-DAB-02A, GCU-DAB-09A (b), GCU-DAB-12E (a), GCU-DAB-22A and GCU-DAB-23A showed maximum enzyme production at temperature 37°C as 257, 300, 309, 289.6 and 257U/ml respectively (Fig. 6). The production of enzyme becomes increased as the temperature was enhanced till 37°C and maximum enzyme production was achieved at this temperature. There was a significant decrease in the production of enzyme as temperature rises beyond 37°C. This might be due to elevated temperature, the growth of bacteria was increasingly inhibited and therefore enzyme synthesis was also prohibited. In addition, elevated temperature might reduce the moisture contents of the medium and it has a direct effect on the

growth of bacteria therefore the enzyme production is reduced.

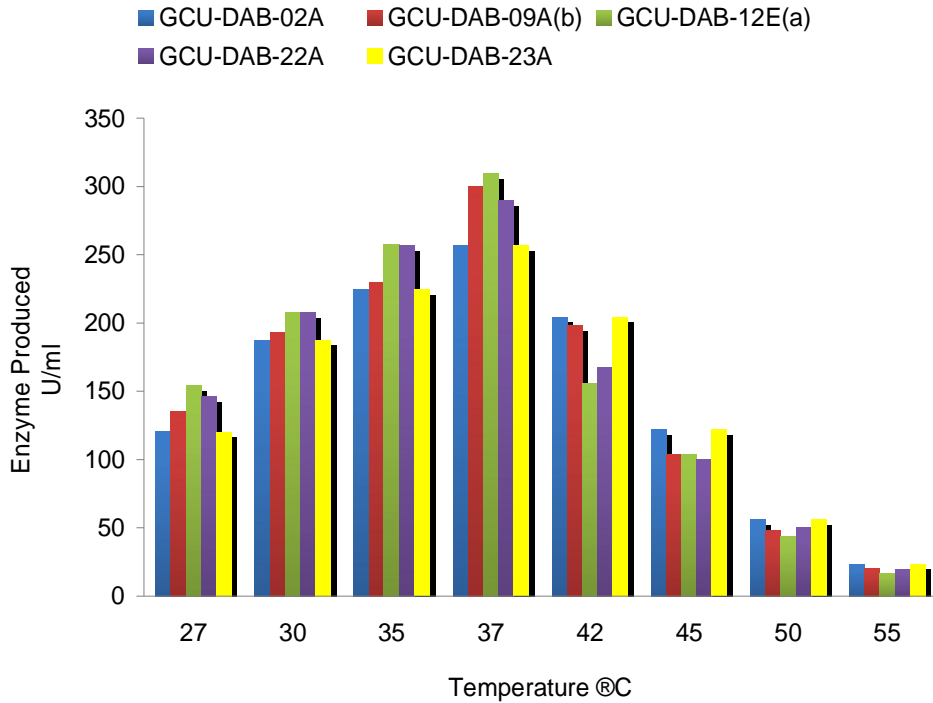


Fig. 6. Effect of incubation temperature on α -amylase production.

Allan *et al.* (1996) reported that the size of inoculum has a pronounced effect on production of α -amylase as well as on bacterial growth. Adequate quantity of the inoculum has a crucial role in fermentation of enzyme (Lin *et al.*, 1997). In the present study, evaluation of effect of inoculum size ranging from 1-10% was performed. The *Bacillus* species GCU-DAB-02A, GCU-DAB-09A (b), GCU-DAB-12E (a), GCU-DAB-22A and GCU-DAB-23A showed maximum activity of 2046.78, 2046, 1006.4, 380 and 526.3 U/ml at 2% inoculum (Fig. 7). Fazal Adnan (2008) reported that inoculum of 8% is required for optimum production of α -amylase in stirred fermenter for *Bacillus amyloliquefaciens*. Narang and Satyanaryana (2001) reported that inoculum of 5% is required for optimal production of α -amylase in stirred fermenter. The production of enzyme was increased with an increase in inoculum size but after achieving the maximum production of enzyme at optimum inoculum level there was rapid decline in α -amylase production. This was probably due to the fact that growth of bacteria

becomes rapid and all the nutrients are taken by them rapidly at initial stages of fermentation which might result into production and accumulation of some of the products in medium that prohibited the growth of bacteria and also the enzyme production. At low level of inoculum, the insignificance of the results was might be due to the fact that the organism grew slowly the time was increased to achieve the stationary phase of growth (Akher *et al.*, 1973). Therefore, the enzyme was produced in low quantity at low inoculum level.

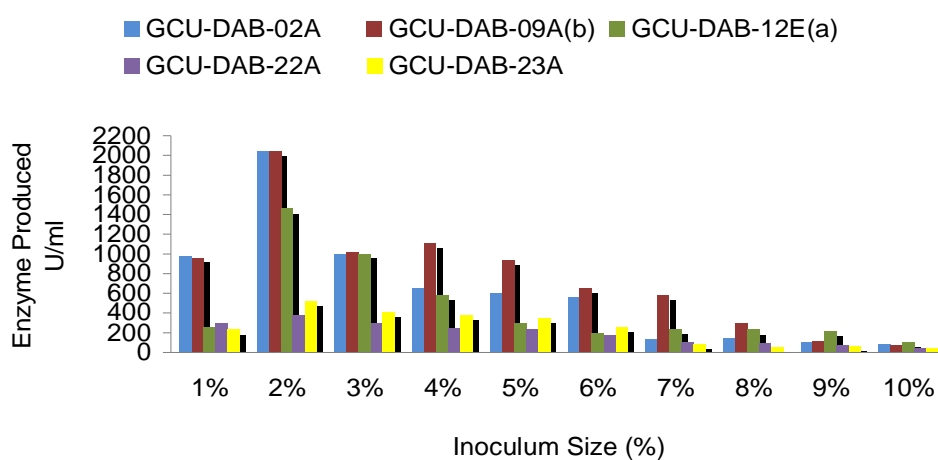


Fig. 7. Effect of inoculum size on α -amylase production.

Another important factor for the production of enzyme is the age of inoculum. Different ages of inoculum ranging from 0-30 h, were evaluated for the optimum production of enzyme. It was observed that 18 h old inoculum gave the maximum production of enzyme. The *Bacillus* species GCU-DAB-02A, GCU-DAB-09A (b), GCU-DAB-12E (a), GCU-DAB-22A and GCU-DAB-23A gave maximum units of enzyme as 257 U/ml, 1178.9 U/ml, 877.2 U/ml, 877.2 U/ml and 1637.4 U/ml, respectively on 18 h old inoculum (Fig. 8). The age of inoculum greater than 18 h did not give significant results. This was might be due to the fact that at 18 h the bacteria were at log phase therefore they produced good amounts of α -amylase, but inoculum greater than 18 h of age, the bacteria entered in stationary phase and were not present in lag and log phases. The growth of bacteria in inoculum of above 18 h of age was declining and also achieving death phase, therefore resulted in decreased amount of enzyme.

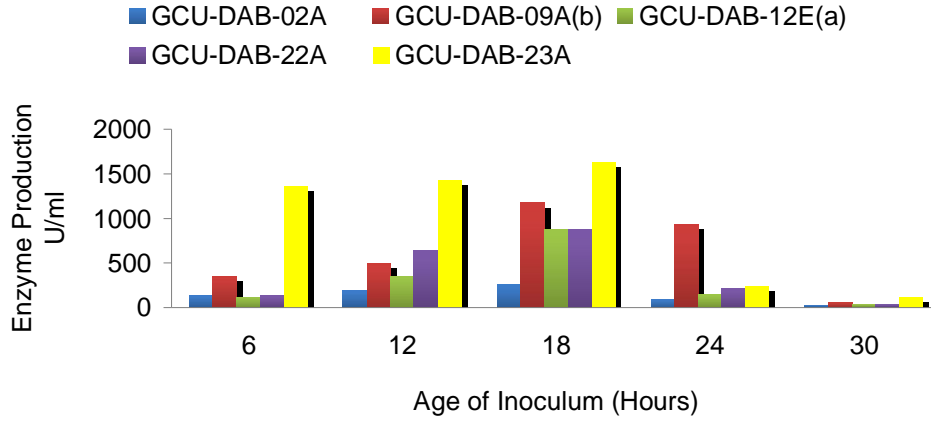


Fig. 8. Effect of age of inoculum on α -amylase production.

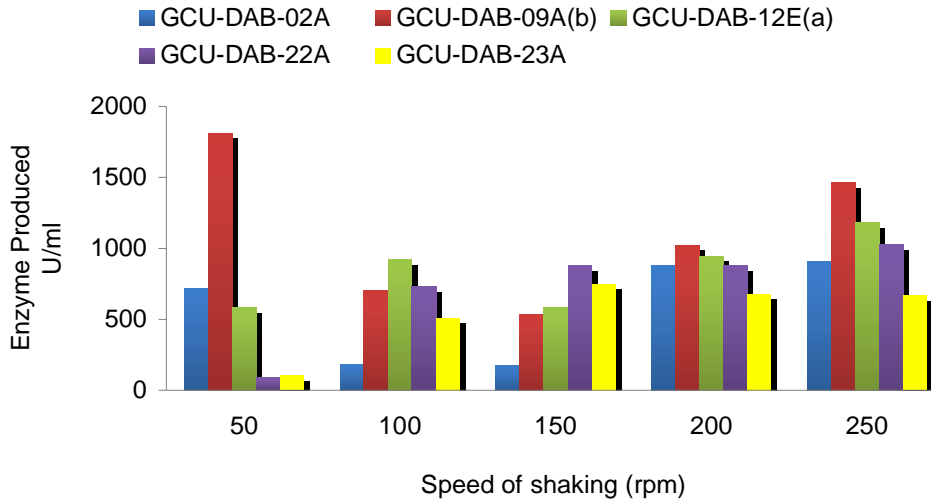


Fig. 9. Effect of Speed of shaking incubator (rpm) on α -amylase production.

Sensitivity towards the agitation was also found by the enzyme activity. Kammoun *et al.* (2008) reported that there are limited positive effects of speed of agitation on the enzyme production. On these types of limits, any increase in the agitation causes enhancement in production of enzymes, but aeration is also an important factor associated with it for the growth of aerobic bacteria. Agitation speed ranging from 50 rpm to 250 rpm was assessed for determining the

optimum activity of α -amylase. It was found that the *Bacillus* species GCU-DAB-02A, GCU-DAB-09A (b), GCU-DAB-12E (a), GCU-DAB-22A and GCU-DAB-23A gave maximum enzyme activity as 1813U/ml at 100 rpm, 924U/ml at 150rpm, 877.2 U/ml at 200rpm, 1023.34 U/ml at 100 rpm and 14622U/ml at 100 rpm, respectively (Fig. 9). It was found that greater rpm decreases the production of α -amylase, it was might be due to formation of foam, which is accordance to the work of Markkanen and Bailey (1975) who reported that the production of biomass is inhibited by the formation of foam. It might also be due to mechanical breakdown of cells which resulted into less production of enzyme. Low agitation speed has no significant effect on enzyme production as growth of cells is not favored by low agitation speed.

Enzyme activity

Temperature has a very sensitive and crucial role on the α -amylase stability (Hosrik, 1983; Young *et al.*, 1989; Suk *et al.*, 1995). The effect of temperature was carried out, in the crude enzyme, on the stability of α -amylase. It was found that the maximum stability of the *Bacillus* sp. GCU-DAB-02, GCU-DAB-12E (a) and GCU-DAB-23 was observed at 45°C, however the other two *i.e.*, GCU-DAB-09A (b) and GCU-DAB-22 showed the maximum stability at 40°C (Fig. 10). Stability of enzyme was greatly inhibited at elevated temperatures. It might be due to break down or denaturation of enzyme at elevated temperatures. The stability and activity of enzyme is too sensitive to temperature (Welker and Campbell, 1967a,b).

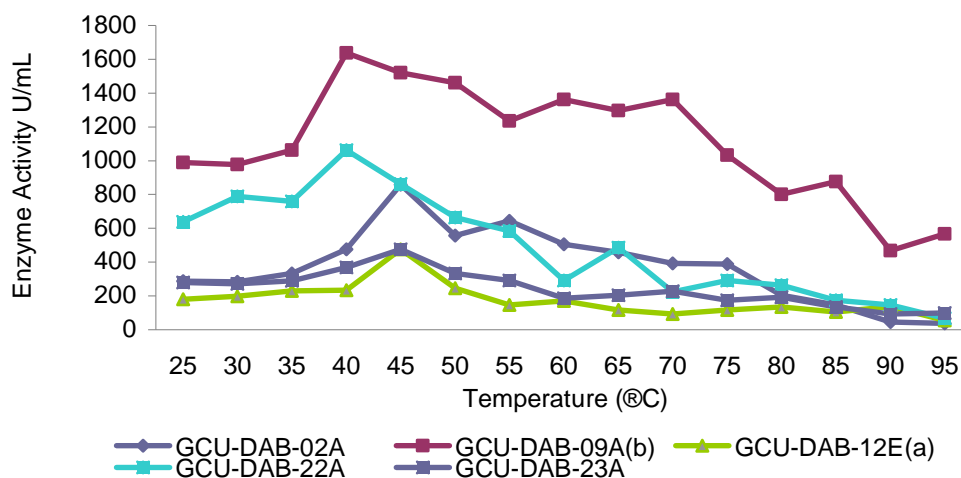


Fig. 10. Effect of temperature on α -amylase activity.

Enzyme stability was evaluated on different pH ranging from 4-12. It was found that the enzyme was stable at pH 7.0 and showed maximum activity and stability at neutral pH (Fig. 11). In highly acidic and highly basic pH enzyme was not stable. It might be due to the fact that in high acidic and basic pH the instability of enzyme is due to change in concentration of hydrogen ions. It is in accordance with the fact that inhibition of enzyme active site occurs due to change in hydrogen ion concentration due to which a decrease in activity of α -amylase was observed (Castro *et al.*, 1992).

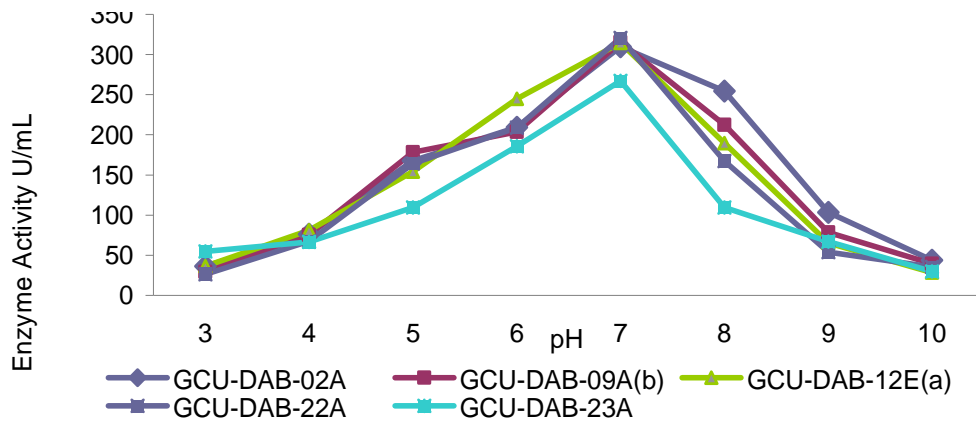


Fig. 11. Effect of pH on α -amylase activity.

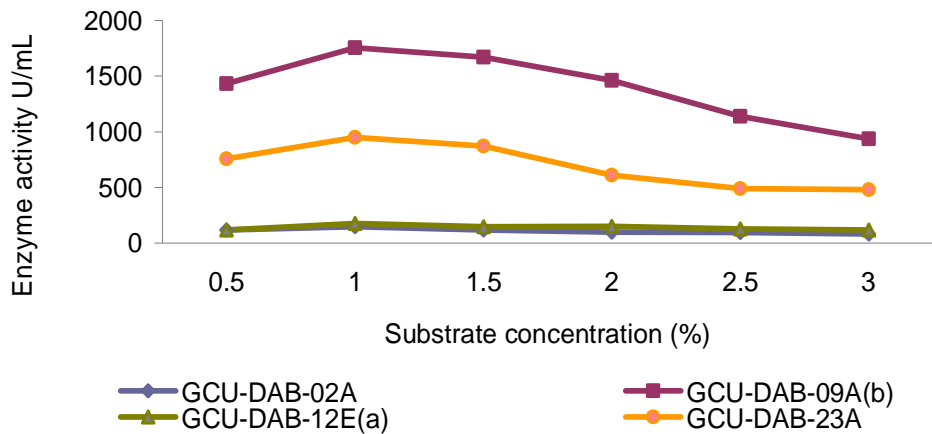


Fig. 12. Effect of temperature on α -amylase activity.

Concentration of substrate also has great effect on the activity of enzyme. All α -amylases produced from the five bacterial strains showed maximum activity with 1% substrate solution (Fig. 12). With an increase in concentration of starch there was no further increase in the activity of enzyme. This might be due to the fact that all the active sites of enzymes were occupied and there was no more active site for binding of substrate with enzyme. Therefore no further increase in activity was observed.

CONCLUSION

The newly isolated thermophilic *Bacillus* strains which produces α -amylase is novel and offers interesting hydrolytic properties. They produced α -amylase in comparably high enough yield on cheaper medium containing molasses (waste product of sugar industries). The most striking feature of the enzyme is its thermostability which makes it potential for industrial applications such as textile desizing, paper industries and liquefaction of starch for syrups and sweeteners that requires the multi steps processes to be carried out at high temperature.

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Some Abstracts

PLENARY LECTURES

INQUIRY BASED SCIENCE EDUCATION (IBSE) FOR S&T INNOVATION LED ECONOMIC DEVELOPMENT AND INTERNATIONAL COOPERATION

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Economic development has been and remains the target and priority of each entity, community and country around the globe. However, the ways and means and the way of thinking have changed. In the present day global ecosystem, name of the game really is; "Cooperation and Collaboration", may it be bilateral between two entities, two countries or multilateral. Cooperation and collaboration is pursued even to compete in the market; thus so many mergers of companies take place around the world! All this is based on each other's strengths and are targeted at mutual learning & benefit for all partners. The concept of alliances and togetherness enables communities and countries to perform better when it comes to competition in the dynamic world that we have today! There are many examples around the world of Regional and Global Economic Cooperation Organizations (G-8, G-20, ASEAN, ECO etc.), Unions, Federations etc. as well as those on political, cultural and even religious basis. Airline Alliances, Internet and other services providers are getting together to handle their affairs around the globe. Trend of franchise across the world of various companies, schools and institutions of higher learning is taking place. In all the happenings, science and technology (for good or bad) plays a major role- though S&T is never "bad", it is always the use or abuse of a technology that causes the good or bad effects. Nevertheless, we as global responsible citizens should be promoting science, technology and education based economic development or the knowledge economy for overall good of the people at large and for a better tomorrow. For economic development, S&T plays a major role and to sustain scientific and technological development leading to economic development, education plays the crucial role to maintain the feed in of thinkers and innovators. And to nurture scientific and technological innovation, critical thinking and observation, the concept of "inquiry" especially among the young children is vital. It has been this feeling that thinkers and scientists around the globe have been promoting "inquiry based science education (IBSE)" which in turn inculcates innovation among the children. Thus there are programs and institutions around the world who bring in, even the Nobel Laureates, in promoting IBSE for their future generations. IBSE in one way or the other, is being promoted by National Academies of Sciences and Engineering as well as NGOs and Government Organizations around the globe. Examples are; USA (e.g. Smithsonian Institute, Intel), France (*La main a la pate* Foundation), Australia (*Primary Connections*), China (*China Association of Science and Technology- CAST*), Latin America and many more with different local names and brands. The global forum of Inter-Academy Panel (IAP) comprising 107 Science Academies have initiated a Science Education Programme (SEP) for promoting science education and science literacy. Local examples include Pakistan Science Club, Intel-Pakistan, Pakistan Science Foundation, Pak-Turk Schools etc. It is in this context that the ECO Science Foundation (ECOSF) is supporting S&T research projects of applied nature in collaboration among its 10 member countries viz., Afghanistan, Azerbaijan, Kazakhstan, Kyrgyzstan, Iran, Pakistan, Tajikistan, Turkmenistan, Turkey and Uzbekistan. ECOSF is also promoting and linking IBSE with advance research and industrial development to ensure a sustainable supply of talent to feed the process of development in line with post 2015 UN Sustainable Development Agenda. In the opinion of ECOSF, it collectively and ultimately leads to economic development in the region. ECOSF is also encouraging international

cooperation on the basis of two common goals; advance S&T research and IBSE. It has partnered with International institutions such as UNESCO, ISTIC Malaysia, La main a la pate France. The paper revolves around the above concepts and will discuss the strategic steps for S&T based innovation and economic development in the ECO region.

A GENOME SCANNING FOR ANALYSIS OF ECONOMIC TRAIT-RELATED QTLs IN COMMON CARP (CYPRINUS CARPIO L.)

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Common carp (*Cyprinus carpio* L.) is one of the major fishery production sources, and is widely distributed. Many strains with different economic traits have been found and these strains are the best genetic resource to improve some less well known strains. So, QTL study is a better method to improve economic traits through genetic breeding. Linkage maps were constructed based on F1, F2 and BC families by microsatellite and SNP markers and then were used to scan common carp genome. Numerous important traits were studied such as standard body shape related traits, muscle fiber, body mass and swimming ability, etc. a lot of major QTLs were found in common carp genome with a phenotypic variance of >20 % and some QTLs with different traits were located in similar interval. These genomic regions will be the major sources for the discovery of important genes and pathways associated with economic trait-related traits in common carp.

THE SPECIFICS OF FORMATION OF THE LOCAL BIRD POPULATION IN THE MOUNTAIN AREA IN SOUTH EASTERN SIBERIA

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The formation the local bird population in mountains is interested by researchers last time a lot . The long-time climate changes have a great influence on this process. The summer bird registrations on the fixed routes in mountain forest, sub-alpine, and bald mountain altitudinal zones in Barguzin range (460-1700 m) were carried out. The researches were carried out in the Barguzin biosphere Reserve in 1984-2014 (Eastern Siberia, north-east coast of Lake Baikal) . The total length of registration routes are 7600 km long. Coming back from wintering areas birds look for the most favorable nesting sites in this year and make significant flights within the habitat. Many species have the volatility of the local population. This change of nesting sites is determined by the ecological conditions during the migration period and the beginning of nesting time. The annual population of birds is formed by abiotic environmental factors in the spring-timing. The phenological events, temperature, water regimes in spring and early summer affect the accessibility of food resources and nesting habitat suitability. The variability of these parameters is the cause of the annual redistribution of population density in the altitude-zones and in the neighboring river valleys. The population structure can be considered as the result of individual bird choice of suitable conditions for habitat in this year. The density of the species in the local habitat is determined by the following:

- bird numbers that reaches the nesting area and "settles" in a particular habitats is

- determined by spring migration prolongation and survival changes in wintering areas;
- main feed availability and the level of prey species in the previous and current years are important for specialized seedeaters and predatory bird species.
 - phenological phenomena (melting period of snow cover and vegetation development) creates the necessary conditions for nesting.
 - there is a correlation between the nesting abundance and the first spring nesting registration date of some migratory birds; -the increase of nesting density for both earlier and late birds arrivals is registered.
 - the changes of bird arrival in spring are registered as the result of global climate modification. It is affected for the nesting population formation and the transformation of the local breeding bird abundance.
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HARMONIZATION OF PESTICIDE REGULATION AND MRLs: AN AFRICAN INITIATIVE SERVING GLOBAL HEALTH, ENVIRONMENT AND TRADE

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Every year new pesticides are introduced to the global market. Before these new products are granted approval, distributed, and made available to users they must be rigorously assessed by national governments to ensure that they meet current health, environment and safety standards. Older pesticides already on the market are also re-evaluated to guarantee that they continue meeting these standards. The world uses about 2.5 million ton of pesticidal active ingredients each year and there are nearly about 1,000 of these are in the market worldwide. About 5-8% of which is being used in Africa. This process of pesticide regulatory review and registration is mostly carried out by each country and hence is often duplicative and resource-intensive. Since the late 1980s, many countries have agreed to work jointly through bilateral or multilateral programs to harmonize approaches to pesticide registrations and maximum residue level (MRL) standard setting. This process is critical in order to help governments work jointly to evaluate the risks of individual pesticides more quickly and thoroughly, and ensure the smooth flow of global trade and information. These “work-share” programs allow experts from collaborating countries, and sometimes from invited countries, to discuss, share and combine their knowledge, expertise, methods, findings and processes. As an outcome, these governments are able to approve the introduction of much safer, effective products to their market and replace more hazardous ones more quickly and efficiently. This also results in an increase of the number of pesticide products that are available to specialty crop growers worldwide. This has encouraged us to call for a continental initiative to harmonize regulation of pesticides in Africa. This initiative, if adopted, will also facilitate the global harmonization of pesticide MRLs by supporting Codex as the common standard. With the cooperation of the USDA, two preliminary meetings related to this initiative were held in Nairobi, Kenya and in Alexandria, Egypt. Four other meetings were organized in collaboration with the African Union. These were held in Addis Ababa, Ethiopia (two meetings), Alexandria, Egypt and Lusaka, Zambia where issues of harmonization of pesticide regulation and MRLs were addressed. Furthermore, a multinational field study to test similarity in measured residues of a pesticide on/in tomato was performed in 22 worldwide distributed locations with help and support given by the US Department of Agriculture, USDA. This study was supervised by the USDA and its results will help throwing light on possibility of global harmonization of Pesticide

MRLs. Analysis of perception and importance of this initiative will be discussed in this conference. The methodology used to clarify its benefits and the experimental method used to worldwide standardize measuring residues of a pesticide on/in a crop will be also presented.

SECTION - I

CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS,
PHYSIOLOGY, TOXICOLOGY

1. BIOCHEMISTRY, BIOTECHNOLOGY

COMPARISON OF LIPID PROFILE AND BMI AMONG OBESE AND OVERWEIGHT
ADULT POPULATION OF KARACHI, PAKISTAN

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Presently we investigated body mass index (BMI), waist hip ratio (W/R), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose (FBG) and lipid profile among obese and overweight persons. An overnight fasting venous blood samples were drawn for lipid profile *i.e.* total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c). A total of 99 subjects both males and females were included in the study. Subjects were divided into Control, Overweight and Obese according to BMI and then into males and females. The average mean BMI of control, overweight and obese subjects female was 1.03 ± 0.47 kg/m² (n=12, age 21.17 ± 0.63), 24.75 ± 0.27 (n=7, age 21.14 ± 1.0) and 32.39 ± 1.2 (n=20 with mean age 29.3 ± 2.7) respectively, while in males mean BMI was 20.95 ± 0.71 (n=6, mean age 24.67 ± 2.1), 25.41 ± 0.26 (n=15, mean age 27.20 ± 2.3) and 32.14 ± 0.71 (n=39 with mean age 32.2 ± 1.9) in control, overweight and obese subjects respectively. Comparison of control with overweight females exhibit the highly significant values *i.e.*, BMI (p < 0.0001), FBG (p < 0.05), SBP (P < 0.001), DBP (P < 0.001), HDL (P < 0.01), TG (P < 0.01). Relationship of control and overweight males showed significant results with BMI (p < 0.01), SBP (P = 0.05), DBP (P = 0.05), HDL (P < 0.01). Control versus obese females displayed significant values with age (p < 0.01), BMI (p < 0.0001), SBP (p < 0.001), DBP (P < 0.05), T-CH (P < 0.01), HDL-C (P < 0.0001), LDL-C (P < 0.0001) and TG (P < 0.001). Comparison of control versus obese females the significant values age (p < 0.05), BMI (p < 0.0001), SBP (P < 0.01), DBP (P < 0.05), HDL-C (P < 0.01).

PURIFICATION AND PARTIAL CHARACTERIZATION OF LIVER AND KIDNEY
CATALASE ENZYME FROM WILD AND FARMED *CATLA CATLA*

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During oxidative stress, reactive oxygen species (ROS) react with vital organic biomolecules such as nucleic acids, proteins, lipids and ultimately leads to death of an organism.

For protection from these ROS, each and every organism possess a defensive system called antioxidant defense system. Catalase (CAT) is an important part of this antioxidant defence system. The purpose of present study was to purify and characterize CAT enzyme from liver and kidney of wild captured and farmed cultured *Catla catla*. For this purpose, ten experimental fish were collected from each source (wild and pond). After biopsy and extraction of studied organs, purification of liver and kidney CAT enzyme was done with the help of ammonium sulphate $\text{NH}_4(\text{SO}_3)_4$ precipitation and ion exchange chromatography at 4°C. Both sources experimental fish habitat water physico chemical parameters were also measured to observe the effects of these parameters on enzyme activity. In ion exchange chromatography, specific enzyme activity measured in wild and farmed *C. catla* liver was remained 551.40 U/mg with 7.24 fold purification and 443.48 U/mg with 3.49 fold purification, respectively. Similarly, the specific activity in wild and farmed *C. catla* kidney was noted 463.02 U/mg with 6.24 fold purification and 377.94U/mg with 5.74 fold purification, respectively. Optimum pH and temperature for both wild and farmed liver and kidney purified CAT enzyme was found 7 and 25°C, respectively at which enzyme showed maximum stability and activity. Optimum substrate concentration for purified liver and kidney CAT from both experimental source fishes were measured 50mM. The results of present research work further showed that there is a significant difference in wild and farmed *C. catla* liver and kidney CAT enzyme activity when analysed statistically.

COMPARATIVE ANALYSIS OF PEROXIDASE ENZYME ACTIVITY IN HEPATIC AND RENAL TISSUES OF CULTURED AND WILD *LABEO ROHITA*

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Reactive oxygen species are produced during oxidative stress that ultimately leads to death of an organism. Antioxidant defensive system is present in every organism that protects them from reactive oxygen species. The fish body possesses oxidative reluctant guard system that defends them against oxidative pressure produced by toxic compounds. The peroxidase is a powerful antioxidant enzyme involved in catalyzing thousands of oxidative/reductive reactions in living systems. Peroxidase is an important part of antioxidant defense system. The purpose of present research work was to purify and characterize liver and kidney peroxidase enzyme from farmed culture and wild captured *Labeo rohita*. For this purpose fish was collected from pond and wild sources. The enzyme peroxidase was purified by using the techniques of ammonium sulfate precipitations and ion exchange chromatography. The specific activity for the liver of farmed culture and wild captured *L. rohita* was recorded 896.05 and 977.09 U/mg, respectively in ion exchange chromatography. Similarly the specific activity for the kidney of cultured and wild conditioned *L. rohita* was remained 659.066 and 428.76 U/mg, respectively in ion exchange chromatography. Purified liver peroxidase showed fold purification value 13.75 for farm cultured *L. rohita* and 12.02 for wild captured *L. rohita*. Similarly for kidney, Purified peroxidase showed fold purification value of 6.41 for farm cultured *L. rohita* and 10.19 for wild captured *L. rohita*. The enzyme was active in wide range of pH (4.0-12.0) and temperature (4°C-80°C). Optimum pH and temperature for purified liver and kidney peroxidase were recorded 7 and 40°C, respectively. Optimum substrate concentration for purified liver and kidney peroxidase from both cultured and wild sources was recorded 30.4mM. In wild ecosystem the enzyme activity was higher due to the

presence of toxic materials thrown by industries and sewage. In farmed environment, the water of the pond was free from toxic materials like heavy metals etc., so lesser activity of enzyme was observed than wild ecosystem.

**ALKALINE PHOSPHATASE (AKP) AND SUPEROXIDE DISMUTASES (SOD)
RELATED QUANTITATIVE TRAIT LOCUS (QTL) DETECTION IN COMMON CARP**

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Quantitative trait locus (QTL) analyses were conducted to identify chromosomal regions that contribute to variability in alkaline phosphatase (AKP) and Superoxide dismutases (SOD) enzyme activity in common carp. These are enzymes found in essentially all tissues that catalyze the hydrolysis of inorganic or organic phosphate esters with a broad specificity for ligand, including pyrophosphate. Importantly the status of these enzymes is to be said as disease indicator. Therefore, identifying chromosomal region related with these important enzymes are essential for genetic breeding of such important aquaculture species. A F1 family of common carp consisted on 92 individuals was utilized for QTL and analysis. In present investigation a total of ten QTLs were identified, five for each, on eight linkage groups (LGs) *i.e.* LG1, LG2, LG3, LG4, LG7, LG18, LG21 and LG22. LG1 and LG21 were found with the QTLs related with both enzymes, AKP and SOD. Three significant ($P < 0.05$) QTLs (AKP-I, AKP-4 and AKP-22) were found related with AKP. While only two significant ($P < 0.05$) QTLs (SOD-7 and SOD-21) were found associated with SOD. A maximum LOD 5.02 was found for SOD-3 and minimum 3.0 for SOD-7. Maximum and minimum phenotype variance was found 77.1% and 58.7% respectively. These findings would lead to produce strain of common carp with strong immunity and will contribute to highest aquaculture production.

**RELATION OF RAMADAN FASTING TO C REACTIVE PROTEIN LEVELS IN
OBESITY**

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The study was carried out to investigate Ramadan fasting on serum levels of C Reactive Protein (CRP) in overweight and obese subjects in the populations residing in Karachi. Total of 56 subjects of both the genders with 18-55 years of age participated in the study. The status of obesity etc. was assessed on World Health Organization international criteria based on Body Mass Index (BMI) values for Asiatic criteria. The sampled population included normal weight, overweight and obese subjects. An average of 15 hours fasted blood samples were collected on 1st and 28th of Ramadan in the solar year 2014. Measurements for Body Mass Index (BMI) were taken and C reactive protein was estimated by ELISA kit in each subject on first and 28th day of Ramadan. On comparison of first and the last day of the month in each category based on BMI all the groups of normal weight ($n = 9$), overweight ($n = 4$) and obese females ($n = 18$) demonstrated significant reduction in BMI ($p > 0.05$). The comparison of CRP revealed significantly increase ($p > 0.002$) in

obese females only. In males normal weight ($p > 0.029$) ($n = 4$) and overweight ($p > 0.004$) ($n = 4$) subjects displayed significant decrease in BMI. A highly significant reduction ($p > 0.006$) ($n = 18$) was however observed in CRP in obese subjects only.

LIPID CONSTITUENTS FROM *RHINCODON TYPUS* (WHALE SHARK)

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Whale shark *Rhincodon typus* is the biggest shark and the biggest extant fish in the world. It is sole member of family Rhincodontidae. These highly migratory species with a cosmopolitan distribution are found in all tropical and temperate seas. These are currently classified as vulnerable to extinction. Ecology and Biochemistry of giant marine elasmobranchs (cartilaginous fish) are studied by lipid analysis. Lipids are natural, hydrophobic, biological substances. Broadly lipids include eight categories; fatty acids, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides. Important natural constituent generally isolated from whale shark oil are vitamin A, D, and E. In addition, triglycerides of whale shark are also of importance. Certain fatty acids or the combination of fatty acids are species specific. Such fatty acids are called as signature fatty acids. Dietary preferences and level in the trophic food chain are analyzed through signature fatty acids. In wild whale shark, fatty acids are used as energy source, have structural role, involved in reproduction processes, and incorporate into various metabolic pathways. Those persistent organic pollutants, which are lipophilic in nature predominantly, get accumulated in the fatty deposits like adipose tissue because of their poor excretory capabilities. Bioaccumulation within food chain is determined through the study of adipose tissue, which is a store house of lipid constituents. The studied fish was dragged to Karachi Fish Harbor. Its liver was extracted for lipid constituents and qualitative and quantitative analysis was performed using GC-MS. The chemically derivatized portion of lipids showed the presence of various constituents, which included tetradecanoic acid, hexadecanoic acid, heptadecanoic acid, octadecanoic acid, 7Z-hexadecenoic acid, 9Z-octadecenoic acid, and 7, 10, 13, 16, 19-docosapentaenoic acid.

EFFECT OF HONEY ON LIPID PROFILE & BLOOD PRESSURE IN DIFFERENT ETHNIC HYPERTENSIVE FEMALE GROUPS OF QUETTA, PAKISTAN

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Hypertension is one of the leading causes of death worldwide. The aim of this study was to evaluate the effect of honey on blood pressure and lipid profile in hypertensive female subject in

different ethnic groups, which includes Pathan, Baloch, Hazaa and Punjabi residing in Quetta city and their surroundings. These subjects were asked to take daily 40g of honey dissolved in tap water with their normal eating habit for four weeks. The results of this study showed that honey intake caused a significant reduction in females, Pathan and Punjabi subjects demonstrated profound decline in SBP as compared to DBP. In Pathan groups significant ($P < 0.009$) and ($P < 0.002$) reduction was observed in SBP and DBP respectively, while in Punjabi subjects a noticeable and statistically significant reduction of ($P < 0.007$) in SBP and ($P < 0.001$) in DBP was observed in this ethnic group. The total cholesterol was markedly decreased [B ($P < 0.01$), P ($P < 0.05$), H ($P < 0.001$), and PU ($P < 0.01$)] in all studied female sub-population. The fraction of LDL-C was reduced in females was observed in B ($P < 0.01$), H ($P < 0.05$) and PU ($P < 0.001$) subjects. Marked and significant elevated levels of HDL-C were observed in all ethnic groups. The significant decline was observed in triglycerides of B ($P < 0.01$), P ($P < 0.001$), H ($P < 0.001$), and PU ($P < 0.001$).

BIOLOGICAL STUDIES OF VARIOUS MEDICINAL PLANTS AND INTERVENTION OF ZONOTIC DISEASES

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There is an escalating requirement for the advancement of novel drugs with secure and enhanced profile for the treatment of zoonotic diseases. Herbal plants have always been the best source of therapeutic agents. In current research work, biological properties of various extracts of different parts of medicinal plants *i.e.* roots and leaves of *Atropa acuminata* and *Atropa belladonna*, stem and leaves of *Zanthoxylum armatum* and fruit of *Morus nigra* were analyzed. Extracts of these plants were prepared through both solvent extraction and boiling extraction methods. Antibacterial activity against zoonotic pathogens such as *Serratia odorifera*, *Enterobacter amnigenus*, *Shigella flexneri*, *Salmonella typhimurium* and *Pseudomonas aureginosa* was determined using agar well diffusion method. Antibioassay was recorded by agar disc diffusion method. It was found that boiling extracts showed maximum antibacterial activity. The results of antioxidant potential that was measured by DPPH scavenging method, revealed the maximum activity in root extracts of *A. belladonna*, leaf extracts of *Z. armatum* and all extracts of *M. nigra* while extract of *A. acuminata* showed lowest scavenging activity. Phytochemical screening indicated the presence of bioactive compounds such as alkaloids, flavonoids, quinones, phenols, tannins, terpenoids, glycosides, steroids and carbohydrates. Thin layer chromatography (TLC) also showed the presence of phytochemical constituents which were further analyzed for flavonoids (by $AlCl_3$ spray) and antioxidant constituent (by DPPH spray). TLC- developed plates showed the maximum presence of both antioxidant constituents and flavonoids contents in all extracts of *Z. armatum* in case of boiling extracts. Bio-autobiography by agar overlying assay indicated the significant inhibition of tested pathogens. Ethanol extracts of *A. acuminata* roots showed the presence of oligo's, glucose and xylose sugar constituents at 6.66 (P2), 9.90 (P6), and 10.54 (P7) through High Performance Liquid Chromatography. The current study provides scientific confirmation to support the therapeutic potency of these medicinal plants for medicinal uses and identifies gaps for future research to facilitate commercial utilization.

BIOTECHNOLOGICAL POTENTIAL OF A THERMOPHILIC BACTERIUM ISOLATED FROM TATTA PANI HOT SPRING, AZAD KASHMIR (PAKISTAN)

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A thermophilic facultative anaerobic bacterium (TP-4) was isolated from Tatta Pani hot spring in Azad Kashmir and was characterized using phenotypic and genotypic characters. The strain developed off-white, round, flat and slimy colonies with smooth margins while the cells were Gram positive rods. Analysis of 16S rRNA gene sequence showed that isolate TP-4 had 96% homology with *Geobacillus vulcani*. It grew within the pH range of 5.5 to 9.0 with optimal growth observed at pH 7.0. It grew optimally at 70°C and gave positive results for sodium citrate and produced acid from sucrose, mannose and maltose. It utilized maltose, fructose, lactose, sucrose, starch, wheat bran extract, xylan and CMC as sole carbon source. It gave extracellular activities of α -amylase, CMCase, lipase, protease, phytase and intracellular activities of CMCase.

DECOLORIZATION OF TEXTILE EFFLUENT WITH LACCASE ENZYME BY USING RESPONSE SURFACE METHODOLOGY; ISOTHERMS, KINETICS AND THERMODYNAMIC STUDIES

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A local strain of white rot fungus *Pleurotus ostreatus*- P1 was cultured and showed the highest laccase activity with synthetic medium; as reacting substrate for dye decolorization. For textile dye degradation three factors temperature, crude laccase concentration & pH were optimized with Response Surface Methodology (RSM) and find 80% decolorization when the temperature, crude laccase enzyme and pH were 25°C, 13.5 U/ml and 5 respectively. In addition the predicted values were also validated with experimental values, confirms the steadiness of the model. Moreover the pseudo-first-order and pseudo-second-order were applied to kinetically define the biosorption of synthetic dye solution and found the second one have best experimental data furthermore the Freundlich isotherm equations also have the highest value of R² compared with the Langmuir isotherm. The thermodynamics parameters of biosorption process (ΔG° , ΔH° , ΔS°) were also assessed at three different temperatures (5, 15 & 25 °C) for synthetic dye solution, the variations of Gibbs free energy (ΔG°) was negative (-1.384 to -9.529 to -13.723 kJ/mole) whereas the enthalpy ΔH° (472.94 kJ/mole) was positive depicting the biosorption process was spontaneous and endothermic process. Present exercise climaxes the potential of crude laccase enzyme as an effective biosorbent for the degradation of dyes.

OPTIMIZATION OF AMYLASE PRODUCTION FROM MICROBES ASSOCIATED WITH CONTAMINATED SOIL USING SOLID STATE FERMENTATION**Amber Khatak¹, Shaukat Ali¹, Nazish Mazhar Ali² and Saiqa Andleeb^{1*}**¹*Microbial Biotechnology Laboratory, Department of Zoology, University of Azad Jammu & Kashmir, Muzaffarabad, Pakistan*²*Microbiology Laboratory, Department of Zoology, GC University, Lahore, Pakistan**Corresponding author: drsaiqa@gmail.com

Microbial enzymes are widely used in industrial processes and amylase is one of the most important industrial enzymes that catalyzes the breakdown of starch into sugars and plays a pivotal role in a variety of areas like usage as digestives, for the production of ethanol and high fructose corn syrup, detergents, desiring of textiles, modified starches, and paper recycling. Current research of amylase production was carried out with soil contaminated bacterial strains. 16 isolates were screened for amylolytic activity using starch agar medium. Among all bacterial isolates, 6 were selected for production of maximum amylase activity. These 6 isolates (H2, i2, B3, J2T, J1, d2) were identified through various biochemical tests. Optimization of the fermentation medium for maximum amylase production was carried out by three production media (AM1, AM2, AM3) with different protein and carbon sources. Different fermentation periods (24, 48, 72 and 96 h) and various pH values (4, 6, 8 and 10) were used to produce amylolytic activity. Amylase activity was recorded in both cell free supernatant and culture samples of microbes. Cell free supernatant of (H2) showed maximum activity (12154.3±0.39 U/ml) in AM1 media at pH 6.9 after 48 h of incubation period and culture sample showed activity (24915.7±0.00 U/ml) in AM2 media at pH 6.9 after 96 h of incubation period. Interestingly Culture and cell free supernatant of (B3) showed maximum activity (9629.2±0.10 and 16586.2±0.37 U/ml) in AM3 media after 24h of incubation period at pH 8. Cell free supernatant of (J2T) showed maximum amylase activity (12446.3±0.38 U/ml) in AM3 media after 24 h of incubation period at pH 6 while culture showed maximum activity (27592.2±0.0 U/ml) in AM2 media after 96 h of incubation period at pH 6.9. Culture and cell free supernatant of (J1) showed maximum activity (27592.2±0 and 16440.0±0.07 U/ml) in AM1 media at pH 6.9 after 96 and 72h of incubation period respectively. Similar results were recorded by (d2) as 27592.2±0 U/ml in AM1 media after 96 h at pH 6.9 and 14216.8±0.17 U/ml in AM1 media after 96 h at pH 6. In case of culture and cell free supernatant of (i2) showed maximum activity (26992.0±0 and 16592.1±0.23 U/ml) in AM1 media at pH 6.9 after 96 h and 24 h of incubation period respectively. It was concluded that the optimum conditions for amylase production were pH 6.9, 96 h of incubation, starch and yeast extract as carbon source and nitrogen source. The results of current research revealed the production of amylase from tested microbes that could be used to hydrolyze starch on industrial scale.

2. CELL BIOLOGY, GENETICS

STUDY OF THE EFFICACY OF PHOTOSENE MEDIATED PHOTODYNAMIC THERAPY ON HUMAN RHABDOMYO SARCOMA (RD) CELL LINE

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Cancer is an uncontrolled growth and increase of abnormal cells in the body. Cancer can be treated using different modalities like surgery, radiation therapy, Chemotherapy and Photodynamic therapy (PDT). Photodynamic therapy is inimically noninvasive cancer treatment modality. It utilizes light and photo sensitizer to produce different oxygen species which lead to the cell death. In this project photosens was used as photosensitizer (PS) while rhobdomyosarcoma(RD) was used as cancerous cell line. Different parameters like maximum uptake time of photosensitizer, concentration of photosensitizer, cytotoxicity and phototoxicity on the rhobdomyosarcoma cell line were judged. The results show that the PS accumulated rapidly within the RD cells and reached to a maximum level within 45 minutes. This time was taken as accumulation time. Different concentrations of PS were taken to get the cytotoxicity on the RD cells. It was determined that cytotoxic effect was more pronounced at 45 μM concentration of PS. Cells were irradiated with different laser doses. Phototoxic effect appeared at 35 J/cm^2 of laser dose. Now PDT was performed on RD cells with 35 μM of photosens and then irradiated with laser light of 35 J/cm^2 . It was observed that there was 84 % cell killing with this combination. It was concluded that if RD cell line is incubated for 45 minutes with 35 μM of photosens and then irradiated with laser light of 35 J/cm^2 then 84 % cell death can be achieved. Hence these results confirm the effectiveness and efficacy of the Photodynamic Therapy of RD cells with photosens as a photosensitizer irradiated at a wavelength of 630 nm.

CLONING, EXPRESSION AND PURIFICATION OF A(V2) PROTEIN OF TOMATO LEAF CURL NEW DELHI VIRUS IN pET EXPRESSION SYSTEM BL21 (DE3)

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The pET expression vectors are the basic tool for biotechnology and the production of desired proteins. The desired DNA fragment was cloned into pET32a(+) expression vector, protein was expressed in *Escherichia coli* BL21 (DE3) and purified under native conditions in *E. coli* lysates. Tomato leaf curl New Delhi virus is an Old World begomovirus belonged to family *Geminiviridae*. Virion strand of DNA A component encodes A(V2) protein that involved in the movement of monopartite viruses and may be involved in the virus fitness at least in their natural host. On the basis of A(V2) importance, the current study was designed to clone, express and purify the A(V2) protein of *Tomato leaf curl New Delhi virus* (ToLCNDV) through prokaryotic

expression system. In case of bacterial system AV2 was cloned into pET32a(+) expression vector, in-frame with the 6X His tag and also purified with Ni-NTA resin columns. The findings of current research reveal the potential use of BL21 expression system for protein expression and its purification. The purified protein could be used in the study protein-protein interactions in the host and useful for making antibodies for viral detection.

COMPARISON OF DIFFERENTIATION POTENTIAL OF BONE MARROW DERIVED AND ADIPOSE DERIVED MESENCHYMAL STEM CELLS TOWARDS NEPHROGENIC LINEAGE

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Kidneys are major homeostatic organ or highly specialized “Natural filters” of the body. Both acute and chronic losses of kidney function are becoming a major public health problem worldwide. Stem cells based cellular therapy may provide a more efficacious method for both prevention and amelioration of renal disease of many etiologies. Present study was designed to evaluate the comparative differentiation potential of rat mesenchymal stem cells (MSCs) derived from two different sources; bone marrow and adipose towards renal lineage. Both types of MSCs were treated with a cocktail of growth factors; FGF2, TGFβ2 and LIF and further analysed. Expression analysis of nephrogenic markers showed that both BMSCs and ADSCs can be successfully differentiated towards nephrogenic lineage. Moreover, the differentiation potential of both types of cells is comparable. These differentiated cells could be further transplanted and analysed for their role in protecting renal injury. In conclusion, data provides a vital step towards kidney regeneration therapy.

CHARACTERIZATION OF *TETRAHYMENA FARAHENSIS* MTT2 AND QUANTITATIVE ANALYSIS OF ITS EXPRESSION UNDER COPPER STRESS

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T. farahensis copper metallothionein (*TfCuMT*) is a new member of ciliate metallothioneins family, which showed 83% homology with already reported copper metallothioneins. This protein is encoded by 327 nucleotide chain. Like most of the metallothioneins cysteine residues contribute nearly 30%. Aromatic amino acids are missing like most of metallothioneins. The peptide sequence has copper metallothionein characteristic CXC motifs and devoid of any cadmium metallothionein specific CCC motif. Structural repeats present in peptide sequence of *TfCuMT* indicate internal duplication of gene at some stage of gene evolution. The theoretical PI is slightly basic, a characteristic of most of metallothioneins. The predicted irregular structure of *TfCuMT* shows that it is functional in the presence of some metal ions. Functional analysis using metalmine software showed that most of the cysteine residues are involved in copper binding. Real time PCR based quantitative analysis showed that *TfCuMT* was a copper inducible gene. Gene had a basic

expression level which increased by the induction of copper ions. Maximum expression was observed within 15min after copper exposure. The pattern of metal uptake appeared bimodal. Initial uptake was similar to the pattern of metallothionein expression.

IDENTIFICATION OF ALLELIC AND GENOTYPIC FREQUENCIES OF GDF₁₀ GENE IN PAKISTANI CATTLE BREEDS

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GDF₁₀, also known as BMP-3b and closely related to bone morphogenetic protein 3 (BMP3), is one of the important members of this superfamily of proteins. It can induce endochondral bone formation. Body measurement traits are known to play numerous important roles in the assessment of productivity and economic value. They are influenced by several factors, among which genetic factors are predominant. The gene GDF₁₀ is involved in skeletal morphogenesis and is associated with body measurement traits. It may be an important candidate gene for marker-assisted selection. We used the PCR-SSCP technology to examine a possible association of the single nucleotide polymorphism (SNP), (G142A) of the bovine GDF₁₀ gene with Allelic and Genotypic frequencies in 247 animals belonging to five different Pakistani cattle populations: Red Sindhi (RDS), Dhani (DH), Cholistani (CH), Thari (TH) and Dajal (DJ). The sequencing map of the novel SNP of the bovine GDF₁₀ exon 1 region revealed a G > A synonymous mutation at 142 bp and the χ^2 test showed that genotype distributions in all the population examined agreed with Hardy-Weinberg equilibrium ($P > 0.05$). We conclude that the SNP of the GDF₁₀ gene could be a very useful genetic marker for quantitative traits in cattle reproduction and breeding.

IDENTIFICATION OF ALLELIC AND GENOTYPIC FREQUENCIES OF MYOG GENE AMONG PAKISTANI CATTLE BREEDS

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Meat quality is affected by genetic and environmental factors which estimate the metabolic process in muscle tissue and in postmortem transition of muscle in to meat. The myogenic determination gene family (MyoD) determine the postnatal tissue growth and myogenesis, and comprising of four genes MyoD1 (MyF-3), Myf-4 or MyoG (Myogenin), Myf-5 and MyF-6. MyoG gene is mapped on BTA16 and diverse QTLs for carcass weight and marbling are situated at 25 to 73 cm interval. Myogenin is considered as a candidate gene for meat production and quality traits because of its probable role in muscle fiber development. Myogenin (MyoG) gene has mapped at 25 to 73 cm interval on BTA 16 where several quantitative trait loci for carcass weight and marbling are located. In this study, we determined the associations between gene-specific single nucleotide polymorphisms (SNP) in MyoG gene, to evaluate the allelic and genotypic frequencies

of five native Pakistani cattle breeds. The breeds were Red Sindhi (RDS), Dhani (DH), Cholistani (CH), Thari (TH) and Dajal (DJ). The χ^2 -test revealed the genotype distributions among all the five cattle breeds (RDS, DH, CH, TH and DJ) agreed with Hardy-Weinberg equilibrium ($P > 0.05$). Genotypic frequencies in five cattle breeds (RDS, DH, CH, TH and DJ) showed small diversity. The AA genotype was the most dominant genotype in all populations examined ranging from 0.608 to 0.808; Contrarily AB genotype frequency was lower in all the breeds studied ranging from 0.218 to 0.391. The BB homozygous genotype was not found in all the populations, possibly because: (1) the low frequency of the BB genotype in homozygous condition; (2) homozygous BB null is lethal for Myogenin in Pakistani cattle breeds. Further, gene heterozygosity (H_e), effective allele numbers (N_e) and PIC (polymorphism information content) of MyoG gene locus in six cattle breeds deviated from 0.173 to 0.315, 1.209 to 1.460 and 0.158 to 0.265, respectively. Based on the PIC classification RDS, DH, CH, TH and DJ showed low polymorphism level. Thus, our findings marked the high frequency of MyoG-A allele could be used to characterize the *Bos indicus* breeds.

IDENTIFICATION OF NOVEL SNPs AND EVALUATION OF ALLELIC FREQUENCIES OF MyoD GENE IN PAKISTANI CATTLE BREEDS

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Recent advances in genomics have made single nucleotide polymorphisms (SNPs) the marker of choice for genome-wide genetic analyses. Since SNPs occur at much higher density than other markers such as microsatellites, they are particularly useful in distinguishing closely related individuals. Studies with different populations are required to properly characterize the robustness of associations of polymorphisms in candidate genes with economically important traits across beef cattle populations before this sort of genetic information can be used efficiently in breeding and management decisions. Present research was performed to identify the Novel SNPs and evaluation of Allelic frequencies of MyoD gene in coding and non-coding regions of the 04 Pakistani cattle breeds. PCR –SSCP technique was employed for the screening of novel SNPs and Genotyping of the bovine samples. The PCR product were separated on agarose gel and photographed under UV-illumination. Hereafter, the PCR product were purified and sequenced on ABI310 genetic analyzer. The sequenced were aligned by using MEGA software. We have sequence selected samples of each breed, which include 04 samples of Red Sindhi (RDS), 05 samples of Dhani (DH), 02 samples of Cholistani (CH) and 02, samples of Thari (TH) breed. Overall four sequence variants were observed, one in the coding (exon 2) region (which not only caused the change of Nucleotide c.679G>A and codon GAC>AAC but also altered the amino acid sequence as p.Asp227Asn) and the other three variants were in non-coding (intronic) region (Intronic regionT>A, Intronic C>G and Intronic T>A that only altered the Nucleotide but did not cause any change in the codon sequence). Furthermore, the χ^2 -test revealed the genotype distributions among 03 cattle breeds (RDS, DH, CH were not in Hardy-Weinberg equilibrium ($P > 0.05$), whereas, TH breeds were in Hardy-Weinberg equilibrium ($P > 0.05$) and this could be due to the intensive selection during the long term commercial breeding.

GENETIC EPIDEMIOLOGICAL ESTIMATES OF OBESITY AMONG STUDENTS OF GC UNIVERSITY, FAISALABAD, PAKISTAN

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Genetic epidemiological study was performed to estimate the incidence, prevalence and risk factors responsible for obesity and its genetical basis among the students of GC University, Faisalabad by using a standardized questionnaire. Obesity was calculated by using Body Mass Index employing standardized procedures. Data was statistically analyzed for percentage (%) distribution, mean, chi-square test (χ^2 test) and coefficient of inbreeding (F). Out of 950 subjects, 406 (42.73%) were male subjects and 544 (57.26%) were female subjects that was highly significant ($p < 0.001$). The sex ratio/100 females of study subjects ($100\text{♀} : 74.63\text{♂}$) was significant ($p < 0.005$). According to the standard values of American Body Mass Index (BMI), the prevalence of overweight 20.21% (5.68% Male; 14.53% Female) and obesity was 10.21% (3.37% Male; 6.84% Female). Whereas according to (Ethnicity Specific) the Asian-Indian Body Mass Index (BMI) the prevalence of overweight 33.68% (14.73% Male; 18.84% Female) and obesity was 20.94% (7.47% Male; 13.47% Female). The age range was 15.0 - 40.0 years and mean age at present of male and female overweight subjects was 21.40 ± 0.137 and in obese subjects it was 22.10 ± 0.211 years. Majority overweight (68.5% Male; 62.31% Female) and obese (71.87% Male; 67.69% Female) were observed in the same age group of 21-25 year. More than 75% subjects belonged to urban area. Majority subjects were placed in first three birth orders. The socioeconomic status of subjects revealed that majority of students belonged to middle class status in each category of population. While in case of overweight and obese category, 70% subjects showed similar preferences to have snacks taking between breakfast and lunch, sweetened tea or coffee, fried/fast food and soft drinks. More than 23% overweight and obese subjects took their breakfast at university and 7.21% (male only) at market. More than 29% overweight and obese subjects took their lunch at university and 5% at market. More than 60% overweight and obese subjects were living in the vicinity of fast food restaurant. In case of physical activity, 52% subjects were not used to walk and exercise. Overall, more female subjects were used to sedentary mode of life than male subjects. Abdominal obesity in general population according to the American standard values and Indo Asian (Ethnicity Specificity) was observed in 11.1% and 36.5% subjects, respectively. Family history revealed more than 43% parental and 23% grandparental cousin's marriages.

COMPLETE MITOCHONDRIAL GENOME OF CATFISH *EUTROPICHTHYS VACHA* (HAMILTON, 1822) FROM SINDH PAKISTAN

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Complete mitochondrial genome of catfish, *Eutropichthys vacha*, was isolated by LA PCR (TakaRa LAtaq, Dalian, China); and sequenced by Sanger's method to obtain the complete

mitochondrial genome, which is listed critically endangered and red listed species. The complete mitogenome was 16,478 bp in length and contains 13 typical vertebrate protein-coding genes, 2 rRNA and 22 tRNA genes. The whole genome base composition was estimated to be 31.28% A, 27.80% C, 15.31% G, and 25.57% T. The complete mitochondrial genome of catfish, *Eutropiichthys vacha* provides the fundamental tools for genetic breeding and conservation studies.

MOLECULAR ANALYSIS OF FAMILIES SEGREGATING AUTOSOMAL RECESSIVE PRIMARY MICROCEPHALY

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MCPH is a neurodevelopmental disorder. The patients suffering from this anomaly show decreased cranial circumference ($> -3SD$) owing to structurally normal and smaller sized brain accompanied with mild to severe intellectual disability. The most affected part of brain in this disorder is the cerebral cortex which is immensely reduced due to less neuron production. The reduced number of neurons led to the simplification of gyral pattern but the thickness of cerebral cortex remains normal. Overall almost 2-2.5% of the world population is affected by MCPH, especially the populations of Arab and Asia where the frequency of consanguineous marriages is relatively high. The mode of inheritance of MCPH is mostly autosomal recessive. MCPH is a genetically heterogeneous malformation. Eight gene loci with corresponding 8 genes have been identified for this disorder to date. Two families A and B were examined for the present study. In both the families, homozygosity mapping was performed for identification of causative gene by genotyping microsatellite markers linked to currently known MCPH loci. Data for all the genotyped loci was analyzed using Merlin the multipoint LOD score calculation, which failed to yield significant LOD score, indicating the exclusion of known loci. Thus both the families were finally excluded for linkage to all known loci, which points towards the involvement of potentially novel loci. The identification of novel genes by genome wide scanning using microsatellite markers may be supportive in future for better understanding and diagnosis of MCPH.

LINKAGE ANALYSIS OF FAMILIES WITH HEREDITARY MICROCEPHALY FROM PAKISTAN

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Autosomal recessive primary microcephaly is a developmental defect of brain. Head circumference is common diagnostic tool for MCPH. Deficiency of neurons cause small size of head so can be defined as "when skull is smaller than average in respective population at respective age this is called microcephaly". Microcephalics are usually looking delightful, cheerful and cannot declare their expression completely. Autosomal recessive microcephaly is mostly in those rural areas where educational, economical; health as well as cultural, traditional customs and consanguineous marriages are very common. So, homozygosity for mutation inherited identical by descendants from MCPH patients of consanguineous families. Microcephaly caused by

environmental factors (radiations, drugs and infections etc.) and genetic factors (cytogenetic abnormalities, gene disorders and genetic contrivances etc.). Known genes are discovered which are involved in MCPH. Autopsy of brain in microcephalics having severe abnormalities in organization levels of brain. If neither normal developmental process nor regression of establishment of neuronal tissues takes then MCPH seems to arise and sometimes mild to moderate mental retardation is consequenced. In present study two families "A" and "B" of hereditary microcephaly were selected from rural areas of Pakistan. Family history of affected individuals was selected by conventional pedigree analysis. Linkage analysis for affected families was carried out by genotyping with STS marker for MCPH1-MCPH6. The results of genotyping data and LOD score calculation indicates the linkage to any of these loci is conclusively excluded in these families. It indicates the involvement of any novel loci or gene in these families.

IDENTIFICATIONS OF GENES INVOLVED IN NON-SYNDROMIC PRIMARY MICROCEPHALY

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Microcephaly is heterogeneous, autosomal recessive trait with reduced head circumference of at least 4 SD below age and sex means due to reduction in neuron production. The brain of microcephalic patient is architecturally normal but severe to mild mental retardation. It is rare disease affecting 2-2.5% of total population specifically in Asia and Arab where the incidence of cousin marriages is relatively high. From seven known currently mapped loci *ASPM* is found to be the frequent causative agent. In the current investigations exclusion mapping of a microcephalic family was done. DNA from all blood samples was extracted using standard procedure and after gene specific PCR amplifications, 8% non-denaturing PAGE was done. Linkage was observed at MCPH5 locus where *ASPM* is a candidate gene on chromosome 1q31. The results of DNA sequencing showed G to A transition and Leucine (CTG) to Leucine (CTA) was noted. There are six triplet codons which differ by single nucleotide encoding for Leucine. Hence, no overall change in the effect of protein expression was observed due to the degeneracy of codons. Therefore, the sequencing of the entire *ASPM* gene with intervening sequences was suggested in order to find the actual cause of microcephaly.

EFFECTS OF VARIOUS CONCENTRATIONS OF ETHANOL ON WEIGHT, LENGTH AND EXTRA-EMBRYONIC VASCULATURE OF CHICK EMBRYO DURING EARLY STAGES OF DEVELOPMENT

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The developing Chick has been widely used as a model organism for research studies in developmental biology. Various aspects of ethanol (EtOH) toxicity during embryonic development have been documented in experimental studies. The studies on chick embryo address the effects of EtOH on weight, length, survival rate, craniofacial abnormalities and angiogenesis. The purpose of this study was to assess the effects of a single dose of ethanol on weight, length and vasculature of

chick embryos during early stages of development. Chick eggs were incubated at 37°C in the humidified incubator, after 72 hours of incubation these eggs were removed from incubator, and treated with a single doses of 1%, 5%, 10%, 13% and 15% EtOH. The effects on survival rate and extra embryonic vasculature were recorded on ethanol treated chick embryos. Weight and length was slightly reduced with 10% and 15% EtOH. Survival rate was reduced to half at 10% and at 15% EtOH complete death was observed. Our results also indicate that treatment of EtOH with 1% and 5% did not have any obvious effects on vasculature of chick embryo, however at 10% and 15% severe inhibition of vasculature of chick embryo was observed.

THE FREQUENCY OF ABO BLOOD GROUP IN KHAIRPUR MIR'S, DISTRICT SINDH, PAKISTAN

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Research on ABO group system has been of immense interest, due to its medical importance in different diseases. The ABO blood group system is not only important in blood transfusions, cardiovascular diseases, organ transplantation, erythroblastosis in neonates, but also one of the strongest predictors of national suicide rate and a genetic marker of obesity. The present research was conducted on five densely populated and interrelated Taluka of Khairpur Mir's District including Khairpur, Kingri, Gambat, Kot Digi, Thari Mirwah allocated at 27° 32' and 68° 46' E. Primarily, Blood samples were collected from 500 individuals (100 samples per Taluka) of both sexes randomly and Open slide method of ABO blood groups testing was used as suggested by Vandana and Kumar. Our results suggested the prevalence of overall frequencies of four blood groups in the five Taluka as: B 38.40% (n=192), A (30%; n=150), O (22%; n=110) and AB (9.60%; n=48). Net result was; B>A>O>AB. Furthermore, it was found that Blood group B as the most prevalent blood group among all the individuals of five Taluka of Khairpur Mir's district in highest frequency 38.40% (n=192).

HERMAPHRODITISM: A PRIMITIVE AND UNIQUE TYPE OF REPRODUCTION WHICH IS A SOURCE OF MALE & FEMALE SEX EVOLUTION IN PLANTS, ANIMALS & HUMANS

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The human being which is taking birth today as well in this modern scientific age, having XXY chromosomes $2N+1 = 46 + 1 = 47$ are hermaphrodite (online information). This hermaphroditic sex character in human beings may be due to the heritage from genetic stem tree of past forefather, (Aadam). This may be accepted according to the re-capitulation theory, in which the parent's character(s) of a species are repeated in its individuals of coming progeny/offspring/descendants during its embryonic stage. In the recent years presence of mammary glands / organs in 100% humans in both sex (male and female) confirms the human as hermaphrodite having (XXY) chromosomes, in its forefather (Aadam). This evidence for humans

as hermaphrodite may be accepted as the 1st strongest and universal evidence, because it is found in 100% humans, weather they may be from any part / country of this universe. The 2nd evidence may be accepted as the evidence from Klinefelter's syndrome (XXY) in which one male baby out of 1000 male babies is found with XXY *i.e.*, 0.001%. When once the human being is accepted as hermaphrodite then according to recent scientific research, the clone by tissue culture method from hermaphrodite will be hermaphrodite. In natural cloning as takes place in Hydra, Metridium, Sponges (Porifera) and in many plants, the clone (2nd individual) has same chromosomes as the 1st individual from which the clone has been cloned. Keeping in mind if the 1st human (Aadam) on earth planet was hermaphrodite having (XXY) chromosomes then after natural budding / natural cloning by NMP, the 2nd human, Eve (Hawwa) was also hermaphrodite having (XXY) chromosomes, may be called as an identical female. The other example identical female is Maryam, who gave birth to a male child (Issa / Essa) due to presence of "Y" chromosome in her karyotype. The two types of hermaphroditism are described. Theory of Aadam Hypothesis "Eve" evolved from the pre-existing Human species Aadam, asexually by means of budding / cloning naturally, to maintain the pair level of Human being for sexual reproduction" and AC-Theory for Human Evolution has been described, which states. " The 1st human, Aadam (identical male) was created by nature for the 1st time as pre-existing human species, the 2nd human, Eve / Hawwa (identical female) was evolved / originated from the pre-existing 1st human (Aadam) by means of natural cloning / natural budding asexually for the 1st time, to maintain the 1st pair level of human species to start the sexual reproduction for the 1st time in human species in the world and then all men and women were spread through this pair, which is continued till today with biological evolution of height, age, colour and language ".

DISTRIBUTION OF ABO & RH (D) BLOOD GROUPS AMONG DIFFERENT COMMUNITIES OF KARACHI

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Blood groups are the most widely used genetic markers. The ABO and Rh blood grouping data of any population set is of particular interest. A total of 400 individuals were examined from May 2014- December 2014. Five groups of communities were examined including Muslims, Christians, Ismailis, Bohri and Hindu. Direct agglutination technique was adopted using glass slide and antisera A, Band D for the ABO and Rh grouping, respectively. In Muslim community ABO blood grouping in male A (11.1 %), B (36.5%), O (25.39%) and AB (26.98%). The Rh grouping show, Rh positive (85.71%) and Rh negative (14.28%). In female, A (17.27%), B (36.36%), O (20.90) and AB (25.45%) while Rh grouping show, Rh positive (83.63%) and Rh negative (16.36%). In Christian community ABO blood grouping in male A (09.75%), B (36.58%), O (39.02%) and AB (14.63%) and the Rh grouping show, Rh positive (82.92%) and Rh negative (17.07%). In female A (8.86%), B (28.30%), O (37.73%), AB (15.09%) and Rh grouping show, Rh positive (83.01%) and Rh negative (16.98%). In Ismailis (Aga Khani) community ABO blood grouping in male A (23.52%), B (35.29%), O (23.52%), AB (17.64%) and Rh grouping show, Rh positive (58.805%) and Rh negative (41.17%). In female A (24.24%), B (30.30%), O (39.30%), AB (06.06%) and Rh grouping show, Rh positive (69.69%) and Rh negative (30.30%). In Bohri community ABO blood grouping in male A (09.09%), B (54.54%), O (18.10%) and AB (12.12%) and Rh grouping show, Rh positive (60.60%) and Rh negative (39.30%). In female ABO blood grouping, A (08.33%), B (41.60%), O (08.33%) and AB (33.30%) and Rh grouping, Rh positive

(45.83%) and Rh negative (54.16%). In Hindu community ABO blood grouping in male A (36.30%), B (18.10%), O (45.40%) and AB (09.09%) and Rh grouping show, Rh positive (72.70%) and Rh negative (27.20%). In female ABO blood grouping, A (33.30%), B (06.66%), O (46.6%) and AB (13.30%) and Rh grouping, Rh positive (73.30%) and Rh negative (26.60%). Present record of blood groups, not only provides data about the availability of blood in case of regional calamities, but also serves as a future burden of disease.

ASSESSMENT OF GENETIC DEGRADATION IN THE STOCK OF *CIRRHINUS MRIGALA* IN RIVER JHELUM

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Survival of a species depends on its resilience and adaptability to environmental change. During the last several decades, anthropogenic and unsuitable stocking practices have negatively influenced the fisheries genetic resources. The current research work pay attention toward assessment of genetic degradation, genetic makeup and management of natural populations of *Cirrhinus mrigala* of river Jhelum using microsatellite markers. Assessment of genetic diversity in fish is imperative for preservation and exploitation of sustainable fisheries reserves. The samples were collected from three different sites Mangla Dam, Rasul Barrage, Trimmu Barrage of river Jhelum. Six primers of *C. mrigala*, MFW-1, MFW-2, MFW-17, Barb 54, Bgon-22, R-3R for amplification of microsatellite loci. The maximum numbers of alleles were present at locus R-3R (8) and minimum numbers of alleles were present at locus MFW-1 (1). The locus R-3R was highly polymorphic suggesting greater contribution of this locus to the existing genetic variation. Locus MFW-1 was least polymorphic. The mean values of observed heterozygosity (H_o) of three populations are 0.5058, 0.4118, and 0.5127 in populations, MD, RB, and TB respectively. The mean values of expected heterozygosity (H_e) of three populations were 0.3361, 0.3437 and 0.3530 in populations MD, RB and TB respectively. The largest value of genetic distance was 0.0516 found between RB and TB, whereas the smallest value of genetic distance was 0.0409 obtained between MD and TB populations. The major reason of genetic degradation is human exploitation with natural populations of *C. mrigala*. Knowledge on genetic structure of selected sites of river Jhelum populations would be helpful for effective management and achieving the goal of sustainable fisheries in the country.

IMPACT OF RESTOCKING ON GENETIC STATUS OF *CATLA CATLA* IN RIVER CHENAB

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The study was designed to evaluate the impact of restocking on genetic variety of *Catla catla* in wild stocks by using the microsatellite markers. Due to anthropogenic activities such as overfishing, hydrological alterations and habitat degradation substantial decline in *C. catla*

populations of river Chenab has been observed. To access the extent of genetic diversity, *C. catla* samples were taken from Chenab River. Genetic markers are important tool to investigate the genetic structure of natural population. The sampling locations included Head Trimu, Head Khanki and Head Marala. Total 30 samples of *C. catla* were randomly collected averaging 10 samples for each population. Six microsatellite markers were used to generate genotypic data of the *C. catla* populations. Genomic DNA was isolated by phenol chloroform protocol and confirmed by 0.8% agarose gel. The genotypic data was analyzed to know various parameters of genetic diversity including allelic diversity, inbreeding coefficient, heterozygosity, population differentiation test and deviation from *HWE* by various software. For SSR loci, genetic variability in *C. catla* was relatively high (H_o : 0.448-0.818) when compared with values described for many freshwater fish species (0.043-0.046). Overall the number of alleles ranged between 2 to 8. The Ccat G2 loci show maximum number of alleles is 8. The maximum value of *Ar* was found in HK population are 4.514. Various phenomena may be responsible for the decreased value of the *Ar* including founder effect, bottleneck effect, and selection during population establishment or increase in gene flow. The genetic distance was built by comparing all the population. The values of *Fst* indicate that all the populations are not homogenous. The average value of *Fst* all populations range from 0.00176 to 0.01685. The largest value of genetic distance was obtain between the HK and HM ($D = 0.1201$) whereas smallest value was observed between HT and TK ($D = 0.0693$). In UPGMA dendrogram two cluster were formed indicating high variation between geographic distance and genetic drift.

3. HUMAN AND ANIMAL DISEASES

STUDY OF ENVIRONMENTAL AND GENETIC FACTORS CAUSING CONGENITAL CATARACT IN PATIENTS VISITING LRBT HOSPITAL FROM LAHORE

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Present retrospective, analytical study was performed between October 2013 to May 2014 on all congenital cataract cases seen from Lahore during ophthalmologic consultation at the Layton Rahamatullah Benevolent Trust (LRBT) Hospital. The purpose of this research was to determine the incidence of environmental and genetic factors causing congenital cataract in Lahore. The techniques used for the diagnosis of congenital cataract were visual acuity by Snellen's chart, TORCH test for infections causing congenital cataract, ophthalmoscopy both direct and indirect and slit lamp examination. The main complaint were the infections, trauma and syndromes in congenital cataract. In this study 120 patients of congenital cataract, including four cases with family history were identified from Lahore corresponding to hospital prevalence of 3.16/1000. The CC was studied in 27 females and 93 males. The average age was 2.5 years in CC cases. About 75.83% of the cases were bilateral in our study. Rubella syndrome was very common in CC patients. People with congenital cataract are often diagnosed late and thus have difficulty accepting the outcomes of congenital cataract. Careful clinical evaluations, early diagnosis, genotyping of CC patients to identify genes and mutations, genetic counselling and effective control of CC should be done to avoid the severe results caused by congenital cataract.

PREVALENCE OF BLINDNESS, NYSTAGMUS AND LENS EXTRACTION IN CONGENITAL CATARACT PATIENTS FROM LAHORE

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Present retrospective, analytical study was performed between October 2013 to May 2014 on all congenital cataract cases seen from Lahore during ophthalmologic consultation at the Layton Rahamatullah Benevolent Trust Hospital (LRBT). The aim of this research was to determine the incidence of lens extraction and blindness in CC patients in Lahore. The diagnosis of congenital cataract was done using visual acuity test by Snellen's chart, ophthalmoscopy and biomicroscopy (slitlamp examination). The main issue was blindness and nystagmus in congenital cataract patients as a result of CC, lens extraction was also done in patients suffering from CC. In this study 120 patients were identified from Lahore corresponding to hospital prevalence of 3.33/1000. The CC was studied in 27 girls and 93 boys. The average age was $\leq 1-5$ years in CC patients. About 68.33% of the cases were bilateral in our study. People with CC are diagnosed late in life and therefore have difficulty in accepting the results caused by CC. Careful clinical evaluations, government approval, genetic counselling, nature of inheritance and implications of genetic disorder that help them make informed medical and personal decisions, early rehabilitation and treatment and effective control of congenital cataract should be done to avoid severe outcomes of congenital cataract.

EPIDEMIOLOGY OF HCV INFECTION IN BANNU DISTRICT, KPK, PAKISTAN**Farhat Ullah Khan and Zia Ur Rahman Awan****Department of Zoology, Govt. Post Graduate College Banuu, Pakistan***email: Ziabiotech78@yahoo.com*

Hepatitis C virus (HCV) is endemic throughout the world, affected an estimated 180 million people globally and it causes cirrhoses and other complications that often lead to death. However our knowledge about the disease and its mechanisms is limited. Approximately 10 million people have been infected with HCV in Pakistan. The study was conducted in general population of District Bannu, Khyber Pakhtunkhwa (KPK), Pakistan from September, 2013 to June, 2014. A total of 300 apparently healthy individuals were sampled including 200 males and 100 females and the age of 1 – 60 years of age. They were categorized into four age groups. They were screened for HCV by rapid method and conformed by ELISA technique. Out of total 300 samples, 71 (23.6%) individuals including 50 (25%) males and 21 (21%) females were ELISA positive. The highest HCV infection was documented in age-group of 46-60 while lowest infection was found in age-group of 01-15 years. Second highest HCV infection was found in age-group of 31-45 while moderate infection was determined in 16-30 years of age-group. The main Risk factors were tattooing/piercing, dental surgery, general surgery, use of contaminated blades, re-use of syringes, drug addicts, and blood and organ donation. Lack of proper blood screening facilities in district Bannu and the lack of awareness about the possible routes of transmission of HCV are contributing a great deal towards the spread of infection among the population. Proper sterilization and screening procedures must be made to avoid a far bigger threat of more HCV infections in near future. Government should formulate laws and check the unqualified health care workers working in different parts of the district.

SERO-EPIDEMIOLOGY OF TOXOPLASMOSIS IN SUSPECTED FEMALE POPULATION RESIDING IN KHYBER PAKHTUNKHWA (PAKISTAN)**Alia Perveen, Ajmal Khan* and Syed Akram Shah***Department of Zoology, University of Peshawar, KP, Pakistan***E-mail: ajmalkhan399@hotmail.com*

Blood samples from 290 married women with obstetric history of abortion and still births were tested for the presence of IgG antibodies to *Toxoplasma gondii*, by using commercial indirect latex agglutination test. Out of 290 collected serum samples, 98 were found positive for *Toxoplasma* antibodies showing an overall incidence of 34%. Among them many subjects were with obstetric histories of abortion and pregnancy wastage; with symptoms of fever, headache and muscle pain. Many of the patients with positive antibody titre had a history of contact with cat or any other animal and soil, so role of cats or other animals in the spread of the disease cannot be ruled out. In Khyber Pakhtunkhwa meat consumed is generally well cooked so change of transmission by ingestion of *Toxoplasma* cysts in meat seem improbable. The findings of this study augmented the argument that *Toxoplasma gondii* infection plays an important role an abortion and pregnancy wastage.

**PREVALANCE OF HEPATITIS B VIRUS (HBV) IN DISTRICT BANNU KHYBER
PAKHTUNKHWA (KPK), PAKISTAN**

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Hepatitis means injury to the liver with inflammation of the liver cells. Studies indicate that in Pakistan, hepatitis B is a severe public health problem with increased morbidity and death. The rate of contact of hepatitis B is not known clearly in Pakistan, but incomplete data shows 35 to 38 percent prevalence with 4 percent being carriers and 32 percent having anti hepatitis B virus surface antibodies through natural conversion. The study was conducted in different areas of District Bannu, Khyber Pakhtunkhwa (KPK) Pakistan from September, 2013 to June, 2014. A total of 300 sample were collected, among which 172 (57.33) were males and 128 (42.66) are females. Only 78 were found positive for HBV both male and female individuals by rapid diagnostic test. But none of them were found positive for both HBV and HCV. Out of 78 positive individuals, 50 (29.06%) were males and 28 (21.8%) were females. Males were found to be more frequently infected as compared to females due to exposed to the risk factor. The positive samples were then confirmed by ELISA technique in which 59 were found positive and 19 negative, in which 38 (22.09%) were males and 21 (16.40%) were females. This study found that the knowledge about hepatitis B virus among males and females individuals is not enough and there are certain misconceptions regarding its mode of transmission through sharing room, touching etc which needs to be clarified.

**PREVALENCE OF HEPATITIS “C” VIRUS IN HEMODIALYSIS PATIENTS OF
KHYBER PAKHTUNKHWA, PAKISTAN**

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The objective of this study was to evaluate the prevalence of hepatitis ‘C’ virus (HCV) in hemodialysis patients of Khyber Pakhtunkhwa, Pakistan. For this purpose two main government hospitals *i.e.*, Government Lady Reading Hospital (LRH) and Khyber Teaching Hospital (KTH), Peshawar were selected. Statistical analysis of data was done by using Chi-square test (X^2 –test). Secondary data from both the hospitals was collected from August 2001 to December 2001, for 561 hemodialysis patients. Out of which, 317 were males and 244 were females; with age range 12-85 years (41.38 ± 15.29). The highest infectivity rate was recorded in age group 40-49 years. Overall prevalence of HCV in hospital data was recorded as 14.08%. Experimental data was collected from LRH, during January 2002 to March 2002. A total of 223 patients including 153 males and 70 females, with age range 8-80 years (44.82 ± 17.17) were studied. Of 223 hemodialyzed subjects, 31 were found positive for HCV showing a prevalence rate of 13.9%. The rate of HCV positivity was higher in age group 40-49 years.

PREVALENCE OF BETA THALASSEMIA IN BANNU DISTRICT, KPK, PAKISTAN**Muhammad Waseem Ullah Shah and Zia Ur Rahman Awan****Department of Zoology, Govt. Post Graduate College Banuu, Pakistan***Ziabiotech78@yahoo.com*

Beta-thalassemias are a group of hereditary blood disorders characterized by abnormalities in the synthesis of the beta chains of hemoglobin. Mutations in the *HBB* gene cause deficiencies in hemoglobin production, which can interfere with oxygen delivery resulting in wide range of disease severity. The present study was carried out at various hospitals of district Bannu. The aim of current study was to investigate the prevalence of β -thalassemia disease in hospital population related to age, consanguinity, blood group Hepatitis B virus and anti-Hepatitis C virus antibodies positivity. For this purpose, 223 patients were screened for the different parameters including specific type of β -thalassemia disease, their family history together with information about their siblings and offspring. Results demonstrated that males were comparatively more (56.95%) affected than females (43.04%). The highest representation of β -thalassemic patients (55.55%) was observed in < 3 years of age groups. The mean age of the patients was 5.7 year. Mean Hb of the patients was found to be 5.68 g/dl. The frequency of anti-Hepatitis C virus antibodies positivity was 9.41% among all β -thalassemic patients; however Hepatitis B virus frequency was absolutely zero %. In consanguine studies, outcome of cousin marriages result was higher (69.95%) than in unrelated (30.04%) outcomes. In conclusion, higher total consanguinity rate and inbreeding among thalassemic patients indicated genetic basis of the disease. However, higher anti-HCV positivity among patients reflects the poor facilities of transfusion.

EPIDEMIOLOGICAL ASPECTS OF CUTANEOUS LEISHMANIASIS IN PESHAWAR AND ADJACENT AREAS OF KHYBER PAKHTUNKHWA, PAKISTAN**Ajmal Khan*, Qaisar Jamal and S. Akram Shah***Leishmania Diagnostic & Drug Delivery Research Laboratory, Department of Zoology, University of Peshawar, KP, Pakistan***E-mail: ajmalkhan399@hotmail.com*

The present study was conducted from January 2013 to February 2014 to cover all the four seasons. The data of this descriptive cross-sectional study was analyzed and processed to determine the prevalence of cutaneous leishmaniasis in patients reported to *Leishmania* center/ Dermatology Unit Kuwait Teaching Hospital Peshawar, KP, Pakistan during this specific period; in correlation with region, locality, sex, age group, month and season. In this study, a total number of 4479 positive cases of CL were reported in KP and adjacent areas, of which 4269 (95.31 %) were local Pakistanis and 210 (4.69 %) were Afghan refugees. Among the locals, maximum cases were reported from two tehsils of Khyber Agency viz., Jamrud (41.09 %) and Bara (37.95 %). Out of 4479 positive cases, 2579 (57.58 %) were male, while females accounted for 1900 (42.42 %). The infection was more prevalent in the months of March, January and February; and the highest disease prevalence was in spring and winter, so that 34.75 % & 34.01 % of the disease cases had occurred in these two seasons respectively. The most frequent cases of CL were observed significantly in the age group of 0-9 years, followed by the age group of 10-19 years; while the least disease prevalence was in the people of age above 50 years. The analysis of the collected data and increased prevalence of CL revealed that Khyber Pakhtunkhwa and adjacent tribal areas are the

endemic foci of cutaneous leishmaniasis, caused by *Leishmania tropica* and *Leishmania major*; so health care observers should pay further attention on preventing the disease spread in the area.

**A REPORT ON PREVALENCE OF MALARIA INFECTION IN GENERAL
POPULATION OF BAJAUR AGENCY (FATA), PAKISTAN**

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This study was conducted from January 2013 to December of the same calendar year to cover all the four seasons. The data of this descriptive study was processed and analyzed to investigate the prevalence of malaria and its causative species in suspected patients reported to various laboratories of Agency Head Quarter Hospital Khar, Bajaur Agency. In this study, a total number of 9720 suspected individuals were examined, among which 1273 (13.1 %) were found to be positive for malaria infection with different *Plasmodium* species in their blood smears. Out of 1273 positive cases, 785 (61.7 %) were males and 488 (38.3 %) were females. The infection was more prevalent in November (21.4 %), December (21.1 %) & October (18 %); and was lowest in the month of April (6.7 %). The highest prevalence was recorded in autumn (36.8 %) & summer (30.3 %) and the lowest rate of infection was observed in spring (10.1 %). Majority of the malarial cases were due to *P. vivax*, which was the most common species; *P. falciparum* was less common species and mix cases of infection due to both *P. vivax* & *P. falciparum* were rare. Majority of the people with age group above 15 years were more infected (52.6 %). Such a highest incidence and prevalence of the infection revealed that malaria poses a great public health problem in the area, so Government and health authorities should pay attention on preventing and controlling the infection.

**PREVALENCE OF TUBERCULOSIS IN HUMAN POPULATION OF DISTRICT
PESHAWAR**

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The present study was designed to investigate the prevalence of tuberculosis in District Peshawar. A total of 1078 suspected patients were examined through sputum samples test for the diagnosis of TB. Out of 1078 suspected people, 884 (82 %) sputum samples result was positive where as 194 (17.9 %) sputum samples were found negative. Out of positive patients, 419 (47.3 %) were male and 465 (52.6 %) were female. The highest incidence was found in the age group of 15-29 years as 230 (26.01 %) and the lowest incidence was found in the age group of above 60 years as 149 (16.62 %). Both types of tuberculosis *i.e.* pulmonary tuberculosis and extra pulmonary tuberculosis were observed in this study. Pulmonary tuberculosis was less common than extra pulmonary tuberculosis. Out of positive cases, 351 (39.7 %) were having pulmonary tuberculosis and 533 (60.2 %) were having extra pulmonary tuberculosis. Most frequent incidence was observed in lower socio-economic class with 387 (43.77 %) as compare to middle class with 311 (35.2 %) and upper class with 186 cases (21.04 %). Based on such high incidence of TB in the area, more preventive measures are needed for controlling the disease.

**PREVALENCE OF MALARIAL DISEASE IN LOCAL POPULATION IN TEHSIL
BARIKOT, DISTRICT SWAT**

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During the present study prevalence of malarial disease were carried out in local population of Tehsil Barikot, District Swat. Blood samples were collected during May to November, 2014. A total of 997 individuals were screened for malarial test in tehsil Barikot including urban and rural area. A performa was designed including the past history of the individual *i.e* age, sex, education, domestic animal and socio-economic conditions. Samples were divided into four category of age *i.e* (1-15), (16-30), (31-45), (46-onward), gender wise, union council (Kota, Barikot, Shamoza and Ghalegi) and month wise. Thick and thin blood smear were prepared and examined under microscope. In a total of 997 samples, 204 (20.46%) were found positive, in which mostly under the age of 15 year (23.36%) and less cases were recorded in adult (46 and onward) which was 22.64%. Most of the positive cases were found in the month of November which are 24 out of 67 (35.82%) and less cases were noted in the month of May which are 12 out of 106 (11.32%). Most cases were found in U.C Kota 230 blood sample were collected out of 60 were positive (26.08%) and low prevalence in U.C Ghalaigai, a total of 320 sample, 42 were positive (13.12%). The Gender wise prevalence of malaria is more found in male than female. Male was 106 out of 385 (27.53%) and female 98 out of 612(16.01%). It is concluded that *P. vivax* is the most common in tehsil Barikot.

**PREVALANCE OF TUBERCULOSIS IN DISTRICT UPPER DIR KHYBER
PAKHTUNKHWA**

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The present study was carried out to investigate the prevalence of tuberculosis (TB) in sub division Sheringal, Dir proper, Barawal and Wari District Dir Upper in Khyber Pakhtunkwa (KP) province, Pakistan from (January 2013 to December 2013). During this survey I collect data from four tehsils of District Dir Upper. A total of laboratory reports of 380 patients were collected from four differents tehsils, in which 98(25.78%) were found positive and 282 (74.21%) were found negative tuberculosis patients. The higher incidence of tuberculosis was showed in tehsil sheringal 31(28.18%), and lowest rate of tuberculosis was found in tehsil wari 18 (22.5%). In Gender wise prevalence out of 98 positive cases 37 (22.42%) were male and 67 (28.37%) were female. This shows that the rate of tuberculosis is higher in female as compare to male. The higher infection of tuberculosis were found in the people of middle age from 13-30 (70.58%) and lowest infection are found in the children and adult about 8.57%. In month wise the higher prevalence was recorded in

the month of March (46.15%) while the lowest prevalence was found in the month of April showing (16.21%).

AN EPIDEMIOLOGICAL STUDY OF SCABIES IN HUMANS AT HARIPUR

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An epidemiological study of scabies was conducted at Haripur from Dec, 2002 to April 2003 to evaluate the prevalence and the important risk factors responsible for the spread of scabies. Surveys were carried out in six schools and a general population comprising 200 families. Out of a sample of 968 school children (1st-5th) 40 were detected as scabietic yielding a prevalence rate of 4.13%. Selecting 70 children as control, a case-control study was performed to assess the relative risk of scabies with respect to a variety of risk factors. Sleeping behavior (bed sharing) and the presence of head lice came out as a significant risk factors with relative risk of 3.0 and 2.44, respectively. Out of 200 families in general population, 81 were scabietic prevalence rate of 40.5%. Considering an individual as a unit, 109 cases were detected in 1193 individuals, exhibiting a prevalence rate of 9.13%. Significantly more common in females (10.4%) than males (7.9%), in lower socio-economic classes (13.8%) than the upper and middle classes (5.22%, 7.16%), living in katcha houses (23.6%) than living in puka houses (7.5%), having domestic animals at home (13.4%) without domestic animals (8.08%). No clear trend was indicated in the prevalence rate of scabies changing with educational level. The distribution of the number of cases per family followed a Poisson distribution, demonstrating that all the families surveyed were equally exposed to the risk of scabies. Various methods of treatment for scabies practiced by the people are discussed.

ASSESSMENT OF MICROALBUMINURIA AMONG DIABETIC PATIENTS VISITING SERVICES HOSPITAL LAHORE

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Diabetes is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism. Microalbuminuria is the release of albumin in urine. It is usually due to damage of podocyte cells in kidney caused by high level of glucose. The present study was performed for the assessment of microalbuminuria among Diabetic patients. 200 patients between the age of 30-60 years were included in this study (50 male diabetics, 50 female diabetics and 50 males and females in control groups). Demographic measurements such as weight, family history of diabetes or any other disease were taken of all the subjects. Blood samples were collected and serum HDL levels were assessed by chemistry analyzer. Fasting levels of blood sugar were measured by using glucometer. Hb1Ac levels were also measured. Urine samples were collected and microalbuminuria levels were assessed by ELIZA. The mean microalbuminuria levels in male diabetic subjects were higher than in female diabetic subjects. BSF was significantly correlated with microalbuminuria, HDL, Hb1Ac and weight. Males were slighter more susceptible to risk of nephropathy due to high level of

microalbuminuria than the females. Microalbuminuria is serious risk factor for further organ damage. Better glycemic control may reduce the nephropathy and cardio metabolic risk.

ASSESSMENT OF SERUM ALBUMIN AND CREATININE LEVELS IN HYPERTENSIVE PATIENTS VISITING SERVICES HOSPITAL, LAHORE

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Hypertension leads to the damaged organ as well as several illnesses such as renal failures aneurysm, heart failure, heart stroke or heart attack. The present study was conducted to assess the serum albumin and creatinine levels in the 260 subjects between the ages of 30-60 years. The study included 106 hypertensive females and 94 hypertensive males (Experimental group) and 30 non hypertensive males and 30 non hypertensive females (Control group). Demographic measurements such as weight, systolic and diastolic blood pressure and family history of hypertension or any other disease were recorded. Blood samples were collected and serum albumin and creatinine levels were assessed by chemistry analyzer. The statistical results showed that serum albumin levels were decreased in experimental groups as compared to the control groups in both males and females. A significant increase in serum creatinine levels has been observed in both hypertensive males and females as compared to control groups ($P > 0.05$). Increased Serum creatinine levels and lowered serum albumin levels in the hypertensive males and females indicated the risks associated with hypertension in these subjects and it may be useful in further diagnosis of cardiovascular or kidney diseases.

RISK FACTORS AND INCIDENCES ASSOCIATED WITH BREAST CANCER IN PAKISTAN

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Cancer is the abnormal growth of cells when it loses its cellular regularity. Body cancer forms may be benign or malignant. Mutations responsible for developing cancerous condition arise as germ-line or acquired. There is a versatility in cancers so among all of them breast cancer is the 2nd most death causing category in females. One in every nine women is captured by the breast cancer in Pakistan but its ratio is very low in males according to the one estimation its only one percent. There are various factors developing the category of breast cancer include socio-economic factors, genetics (BRCA1 & BRCA2), race, age, obesity, family history, previous medical history, hormones, reproductive history, life style, menstrual history, environment, Diet, exercise, etc. breast cancer is diagnosed by adopting among different strategies which are suitable to treat like Chemotherapy, hormone, therapy, Ct-scan, biopsy, MRI, chest-Xray, radiotherapy, self-screening surgery (mastectomy/lumpectomy). But the most important way is to defeat the breast cancer is self screening as well as by using certain veggies and developing awareness about this disease.

**MOLECULAR EPIDEMIOLOGY OF HBV GENOTYPES IN SOUTHERN BELT OF
KHYBER PAKHTUNKHWA, PAKISTAN**

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Hepatitis B virus (HBV) is the worldwide health problem and about 5% of the global population has been infected with HBV infection. Approximately 400 million peoples are chronic carriers. HBV genotypes in chronic HBV patients of the southern belt (Bannu, Dera Ismail Khan and Kohat) of Khyber Pakhtunkhwa Pakistan were identified in 2014. A total of 636 blood samples were collected (208 from Bannu, 197 from D.I. Khan and 231 from Kohat) from HBV suspected individuals of either sex, ranged from 1-70 years. All the collected samples from patients were screened with Immunochromatographic test (ICT), for HBsAg. The positive patients for HBsAg were processed for the detection of HBV DNA by nested PCR and HBV DNA positive patients were subjected for HBV genotyping by genotype specific PCR. Complete Blood Count (CBC) and Liver function tests (LFTs) were performed for every individual and were compared with particular HBV genotype. Data obtained was analyzed with statistical software SPSS. Of the total collected blood samples, 300 (47.2%) were found positive for HBsAg (36% males and 11.5% females). HBsAg positive patients were further processed for detection of HBV DNA by PCR, where 40% were found HBV DNA positive and were subjected to HBV genotyping by type specific PCR. Of the total HBV DNA positive samples, 245 (38.52%) were found with HBV specific genotype and 08 (1.3%) samples were not typed. Most predominant genotype (18.1%) of this study was the genotype A (Division-wise distribution was Bannu 6.3%, D.I. Khan 9.43% and Kohat 2.36%, followed by genotype A, 15% (Bannu 4.72%, D.I. Khan 1.57% and Kohat 8.65%). Co-infection of genotype A and D was found 3.5% (1.57% each from Bannu and Kohat and 2.36% from D.I. Khan). Genotype B, C, F and E was not detected in this study. Risk factors for HBV were sharing personal items, shaving in barbers shops, blood transfusion, dental risk, general surgery, history of injection, sexual contact with HBV positive partner and skin tattooing. This study shows that HBV is circulating in the study area with genotype G, the most prevalent in the southern belt of Khyber Pakhtunkhwa province. To stop the spread of this dreadful disease, Proper control measures should be taken in consideration.

**CURRENT STATUS OF PREVALENCE OF DIABETES MELLITUS IN SUKKUR
DISTRICT OF SINDH PROVINCE OF PAKISTAN**

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Diabetes mellitus is one of the most prevailing disease of world. The aim of this study is to evaluate the prevalence of diabetes mellitus in Sukkur district of Sindh, Pakistan. Six thousand four hundred seventy four (6474) blood samples from the individuals (males & females) of different age groups were taken to examine their blood glucose levels in fasting and random status. These individuals belonged to Sukkur city and nearby local areas. Approximately 38% of the cases (both

males and females) studied were found to have active diabetes, *i.e.*, raised blood glucose level of individuals, whereas 14% of the cases of studied were considered as at 'diabetic risk group'. It was also found that age groups 30 and above were the major victims of diabetes mellitus and the individuals of age 55 and above were at the high risk. The high risk of diabetes mellitus among these old age individuals (age 55 and above) could be because of the age factor and carelessness of eating habit. It was also observed that individuals hesitated to talk about their personal life, socioeconomic conditions, job-status, ancestral ailments, tension, anxiety etc. Some individuals were examined for either fasting, random blood glucose level, or both fasting and random blood glucose screening. Hence these hindrances made the task more difficult to find out the exact cause(s) and triggering factors of diabetes. It was also noted that there is a growing awareness of 'diabetes' in both males and females by showing great interest for measuring their blood glucose levels.

FACTORS ASSOCIATED WITH THE PREVALENCE OF ANEMIA IN UNIVERSITY STUDENTS

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Anemia is a condition characterized by a reduction in the red cell count or in the concentration of hemoglobin. Anemia is common in male and female of all ages, however it is particularly common in female adolescents and pregnant women. Several studies indicate the rise of anemia in Students, particularly university students. Various factors have been extensively studied to determine the association of risk factors in anemia. However, the subject is still understudied. In order to determine the factors responsible for anemia, I have carried out the detailed surveyed through a structured questionnaire. Our findings show that female students have significantly higher percentage ($P < 0.05$) of anemia than male students. Students who belonged to the urban areas were more anemic than students from rural areas. BMI less than 18.5 was significantly associated with anemia ($P < 0.05$) particularly in female students. In diet, the major factor was Milk and dairy product; the students who were taking milk after meal had significantly higher Odds of anemia ($P < 0.05$). In conclusion, higher prevalence was found in female students, the students who were living in urban areas, the students have lesser BMI and the students taking Milk after meals.

STUDY OF BEHAVIORAL CHANGES DUE TO OBSESSIVE-COMPULSIVE DISORDER IN THE POPULATION OF KARACHI

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Obsessive compulsive disorder is a psychiatric disease and it is consider as a world fourth long term disabling disease of anxiety disorder. Anxiety is characterize by worry, sleeplessness, shortness of breath, difficulty in concentrating on a particular work, tiring immediately etc. All these characters are responsible for changing the behavior of people. It is a developing disease in world and also in the population of Karachi. In this research work, study the behavior of different clinically diagnostic OCD patients. Total 149 patients of both sexes from different psychiatric

hospitals of Karachi of age 10-50 years were study. The duration of this study is from 2007-2012. OCD is clinically diagnosed on the basis of DSM-IV criteria (Diagnosis and statistical manual of mental disorder). Obsessions are recurrent thoughts and compulsions are repetitive action on the behalf of these thoughts. Therefore etiologies of OCD would be generalize which include genetic, neurobiological, psychoanalytical, behavioral and cognitive etiology. In 10-25 years age group 29% patients are included. In 26-50 years age group 50% patient are included. In 51-above year age group 20% patients are included this shows that the age group between 26-50 are more at risk of having OCD as compare to age group of 10-25 years. Therapies are use by two prospective behavioral and medicinal. There is 2% recovery in 10-25 year age group, in 26-50 years age group recovery is 3% and in 51-aboveyear age group the chance of recovery is 0.5%.

CONSUMPTION FREQUENCY OF GENETICALLY MODERATED FOOD AND INCREASED CANCER IN PAKISTAN

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Cancer will probably become an epidemic disease in the coming decades in Pakistan. Therefore, it is utmost necessary to combat with its social and medical causes. Large numbers of deaths were observed in most of the families, during last couple years. Therefore, in this study the authors are exploring the national data of cancer and common staple food of the society. In this connection survey of 50 cancer patients were done, in which income gender type of cancer, education, etc was asked. The social reasons of cancer are more vital. It has observed that the medically saved foods are costly as compared to common foods. Thus, the large number of people cannot afford it. Interviews of cancer patients revealed that it is most frequently present in the low and middle-income group. Apart from income groups, it is also found that awareness about use of safe food is also reasonably less in upper, middle and lower class. In this study, the list of medically safe food is given along with the rate list as the food in practice is also compiled the rate list.

PREVALENCE OF MALARIAL PARASITES (*P. VIVAX* AND *P. FALCIPARUM*) IN BLOOD OF HUMAN FEMALE AMONG RURAL AND URBAN POPULATION OF LAHORE IN 2014

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Malaria is a vector-borne disease caused by protozoan parasite of the genus *Plasmodium*. *Plasmodium vivax* and *Plasmodium falciparum* are prevalent in Pakistan. This study was undertaken to determine the prevalence of malarial parasites (*P. vivax* and *P. falciparum*) in blood of human female among rural and urban population of Lahore. Malarial parasites were identified in the blood slides of suspected patients of the disease from April to July. Out of 300 suspected cases of malaria, 7% were found to be positive for malarial parasite in blood smear slides. Out of positive cases, 90.5% were identified as *Plasmodium vivax* infection and 9.5% cases were identified as *Plasmodium falciparum*. It was observed that more cases were found in July. Moreover, the

prevalence of malaria was higher in the age group 18-27 years. It was also observed that malaria cause low blood pressure in 52.3% females suffered from malaria. It was also observed that prevalence of malaria was higher in rural areas and in areas where proper sewerage system was not available. As the income resources of middle class or lower class families were limited, mostly in the range of 1000-10,000 so they were unable to afford preventive measures like mosquito repellent lotion, insecticide treated bed nets etc. the present study revealed that the burden of malaria was high in females lived in rural areas of Lahore and more attention is required to overcome the prevalence of malaria.

MOLECULAR DETECTION OF HEPATITIS B VIRUS (HBV) AMONG VOLUNTARY ELISA POSITIVE BLOOD DONORS AT KHAIRPUR DISTRICT PAKISTAN

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Hepatitis B virus is considered as one of the most common viruses spreading through blood transfusion and organ transplants. This usually results in more considerable cases of disease and mortalities; so it is necessary to perform tests for viral infection in all blood donors. The present study aimed at highlighting the serological picture of blood donors and HBV suspected patients reported to the city hospital, Khairpur Mrs, Sindh, Pakistan. The present study was conducted on 1204 samples from 1300 blood donors and suspected patients reported to the City Hospital, Khaipur during August to December 2014. Virus was detected by two techniques: Preliminary cases were diagnosed by ELISA and the positive results were confirmed by PCR. ELISA test confirmed 930 (77.72%) as positive cases and 274 were negative. Furthermore, PCR test was performed to check the validity of 930 cases and 201 (21.61%) were found PCR positive. Comparison of PCR positive results with those of the ELISA positive indicates that the PCR techniques is more sensitive and reliable than the ELISA technique, as not all ELISA positive cases confirmed HBV infection.

RELATIONSHIP BETWEEN ERYTHROPOIETIN AND ANEMIA IN CHRONIC HEART FAILURE (CHF) PATIENTS

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Chronic Heart failure is inability of the heart to supply sufficient blood flow to meet the body's requirements. Damaged kidneys produce less amount of Erythropoietin; the primary factor for red blood cell production. Blood samples of 301 CHF patients were collected with mean age of 55.61 ± 0.79 years and disease duration ranged from 1-16 years. Data was analyzed on the basis of the age and disease duration of the patients. Among these patients, 42% were anemic (<12 g/dl in women and < 13g/dl in men). The ratio of normocytic normochromic anemia was most prevalent *i.e.* 56 patients in the anemic population of 88 samples for the evaluation of erythropoietin. Out of these, 47 patients had serum EPO less than minimum reference value, 30 patients had EPO within

normal range and 11 had higher levels of EPO. It is concluded that lower levels of EPO are conjoint in CHF patients due to anemia. Erythropoietin showed strong and indirect correlation with the degree of the severity of the disease.

STUDY OF CONGENITAL ADRENAL HYPERPLASIA DUE TO 21-HYDROXYLASE DEFICIENCY IN PEDIATRIC POPULATION

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Congenital adrenal hyperplasia (CAH) is a disorder of impaired adrenal steroidogenesis. The mutations in *CRY21A* leading to the deficiency of 21-hydroxylase (21OHD) is the most common cause of CAH. The aim of this research is to study the clinically affected phenotypes, demographics of 21OHD CAH pediatrics and elevated levels of 17 hydroxyprogesterone (17OHP) which serves as an effective biomarker of this disease. Out of this we also focused to find the most concerning factor affecting our present pediatric population. Medical records of 66 21OHD CAH pediatrics with age ranging from day 1 to 11 years during a one year period (2010-2011) including clinical features, 17OHP levels greater than 4ng/ml, karyotyping results, ultrasonographic findings of pelvic region were recorded. Patient data were analyzed using descriptive statistics. Out of 66 patients, 59 girls and 7 boys presented 2.7138 ± 4.68394 years as mean age of diagnosis, 145.4606 ± 109.94111 ng/ml as mean value of 17 hydroxyprogesterone levels. 46 (69.7%) patients showed parental cousin marriage, 29 (43.9%) patients were using medication with 3(4.5%) were surgically treated. 33 patients (50.0%) were of classical (saltwasting form), 23(34.8%) were of classical (simple virilizing form) and 10 (15.2%) were of Nonclassical form. Different frequencies of affected phenotypes were observed which included clitoral enlargement (33%), labial fusion (29%), vomiting and diarrhea (18%) hyperpigmentation of external genitalia (11%) and hypospadias (9%). In this study, CAH due to 21OHD contributed towards cases of ambiguous genitalia in females with higher frequency of salt wasting type and varying frequencies of affected phenotypes, High rate of parental cousin marriage is a concerning factor in the present population. For treatment goals, besides the early diagnosis and medication therapy, the issues of genetic counseling of families must be considered important. We can't predict anything about outcomes of completely virilised 21OHD CAH patients as we didn't follow them further.

4. MICROBIOLOGY

BACTERIAL CONTAMINATION OF VARIOUS EDIBLE VEGETABLES GROWN USING ORGANIC FERTILIZERS IN OSHIKHANDASS VILLAGE, GILGIT-BALTISTAN, PAKISTAN

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Bacteria are present everywhere, including on the surface of vegetables. At the time of consumption, these have the greatest potential for transmission to humans to cause infections. Use of raw vegetables without proper cooking pose high risk for human health. In Oshikhandass village of Gilgit-Baltistan, organic agriculture with use of human fecal manure as fertilizer is widely used, preferably on vegetables. This use of organic manure has led to concerns about contamination of vegetables with human pathogens. The present study was conducted in Oshikhandass village from May 2012 to October 2013. We randomly investigated bacterial load in 40 samples of organically grown lettuce (*Lactuca sativa*), tomatoes (*Lycopersicum esculentum*), mint (*Mentha longifolia*, coriander (*Coriandrum sativum*), and spring onion (*Allium cepa*). Vegetables samples were collected in sterile plastic containers from the fields of selected houses using sterilized disposable gloves; these were transported to laboratory and promptly processed for bacterial investigation. One gram of vegetable leaf was cut by sterilized scissors and rinsed with sterilized distilled water; this was serially diluted 10 folds. The highest three dilutions were taken to analyze the total microbial count using the standard plate count technique according to the WHO Manual for Laboratory Investigations of Acute Enteric Infections (WHO Manual CDD/83.3). The mean bacterial load on selected vegetable ranged from 4.67×10^5 - 8.64×10^5 Colony Forming Units (CFU) per gram. The highest bacterial load was found in lettuce (8.64×10^5), followed by coriander (8.10×10^5), mint (7.99×10^5), spring onion (5.57×10^5), and tomatoes (5.22×10^5). Five to seven groups of bacteria were found on all vegetables, with the percentage of samples contaminated shown in the Table 1.

Table I. Percentage of Vegetable Samples Contaminated with Bacteria

Vegetables	Lettuce	Tomatoes	Mint	Coriander	Spring onion
Sample (Nos)	40	40	40	40	40
Bacterial groups	7	7	6	5	6
<i>E. coli</i>	59.4	59.3	57.9	55.2	51.4
<i>P. aeruginosa</i>	54.5	60.6	55.2	51.0	42.3
<i>Salmonella</i>	28.5	26.2	30.5	33.5	30.1
<i>Enterococcus</i>	26.7	52.1	30.3	--	52.8
<i>Proteus</i>	22.4	27.2	--	--	33.3
<i>Shigella</i>	11.2	03.2	11.2	10.7	05.0
<i>S. aureus</i>	25.3	24.4	28.8	36.1	38.8

The high levels of *E. coli*, *P. aeruginosa*, *Proteus* and *Enterococcus* reflect fecal

contamination. It would be useful to know the types of *Salmonella* found to see if human pathogens such as *S. typhi* and paratyphi are present. It is of concern that *Shigella* is found, as low numbers of organisms are required to cause human disease.

ANTIMICROBIAL ACTIVITY OF GINGER, *ZINBIBER OFFICINALE* (ROSCOE, 1807)

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Antimicrobial activities of extract of zingiber officinale has been evaluated against the bacteria and fungi. The gram positive *Enterococcus faecalis* (ATTC 14506) and *Staphylococcus aureus* (ATCC 6538) and the gram negative included *Pseudomonas aeruginosa* (ATCC 14028). Methanolic, hot and cold water extract of plants were taken for antimicrobial assay through agar well diffusion method on inoculated mueller Hinton agar plates. The objective of these experimental efforts was to discover and explore the hidden, curative powers of these herbs that exist in nature as a tool to encounter causing organism. The maximum zone of inhibition 30.05mm of methanolic extract of Zingiber officinale was observed against staphylococcus aureus, 27.4mm against enterococcus faecalis, 20.2mm against *P. aeruginosa*, 30.3mm against salmonella typhi and 21.6 mm against Candida. While the maximum zone of inhibition of hot water extracts against *E. faecalis* is 20.6mm, 20.2mm against *Steph. uresus*, 20.3mm against *P. aeruginosa*, 21.6mm against typhi and 20.2mm against Candida. Based on the result obtained in the current study, it is concluded that plant extract of zingiber officinale have stronger and broad spectrum of antimicrobial activity against a number of pathogenic microorganisms.

INVESTIGATION OF BIOFILM FORMATION IN CLINICAL ISOLATES AND MONITORING INHIBITORY ACTIVITY OF MEDICINAL PLANT EXTRACTS

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This study aims at identifying biofilm forming microorganisms in clinical setting and checking the inhibitory effect of different medicinal plant extracts on them. Total 30 clinical samples were collected. Out of 30 samples, 15 samples were taken from patients suffering from urinary tract infections while, remaining 15 samples were taken from different types of wounds. On the basis of morphological variation, 13 urine and 12 wound strains were selected and were tested for their resistance against different antibiotics. 10 highly antibiotic resistant strains were selected for biochemical characterization. 3 strains were further characterized physiologically and genetically. According to the results, the strains turned out to be *Providencia stuartii*, *Shigella sonnei* and *Escherichia coli*. These strains were tested for biofilm formation using Test tube assay, Congo red assay and Liquid-interface coverslip assay. Strains showed significant biofilm formation by Test tube assay and Liquid-interface coverslip assay while, only *S. sonnei* gave positive result for biofilm formation in Congo red assay. Antibacterial activity of aqueous and methanolic extracts of three medicinal plants e.g. *Camellia sinensis* (Green tea), *Syzygium aromaticum* (Clove) and

Allium sativum (Garlic) was determined both individually and in combination against *P. stuartii*, *S. sonnei* and *E. coli* by agar well diffusion method. Aqueous extracts of green tea and garlic proved to be more effective in their antibacterial activity on studied strains as compared to methanolic ones, aqueous and methanolic extracts of clove were almost equally effective while, methanolic extract of garlic was least effective in its activity. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of these extracts were also determined. Moreover, these plant extracts were also tested for their antibiofilm activity against clinical isolates and proved to have significant inhibitory effect on biofilm formation thus, providing an alternative to treat various infections caused by these antibiotic resistant isolates.

SCREENING OF BIOLOGICAL ACTIVITIES OF *POLYGONUM AMPLEXICAULIS* (MASLOON) AS ANTIMICROBIAL AND ANTIOXIDANT AGENT

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The current study was undertaken to evaluate the *Polygonum amplexicaulis* extracts (rhizome and leaves) as antimicrobial and antioxidant agent against clinical bacterial pathogens. *Polygonum amplexicaulis* belongs to the family *Polygonaceae* that contains a large number of chemically complex and biologically active compounds. Both rhizome and leaves extracts were prepared through solvent extraction method using ethanol, methanol, acetone, dimethyl sulfoxide (DMSO) and diethyl ether solvents. These extracts were *in vitro* evaluated for antibacterial activity through agar well diffusion method. The tested pathogens were *Escherichia coli*, *Serratia marcescens*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes*. DMSO and acetone extracts of rhizome demonstrated significant inhibitory effect against majority of pathogens. All leaf extracts showed no antibacterial activity except diethyl ether extract. Four different concentrations (70%, 80%, 90% and 100%) were used to measure minimum inhibitory concentration of crude extracts of *P. amplexicaulis*. With 70% concentration maximum inhibition was shown by acetone and DMSO rhizome extract. The antibacterial potency of different antibiotics such as Ciproflaxin, Nalidixic acid, Tetracyclin, Kanamycin, Ampicillin, Penicillin G, Chloramphenicol and Streptomycin was also checked through agar disc diffusion method. Antioxidant activity of extracts was determined through ABTS⁺ and DPPH free radical scavenging methods, which also showed that acetone and DMSO extracts of rhizome and methanolic leaf extract have maximum scavenging potential. Phytochemical screening of both rhizome and leaves extracts of *P. amplexicaulis* showed the presence of various bioactive compounds. Among the screened phytochemicals, terpenoids, phenols and quinones were present in all extracts. The presence of antioxidant constituents, flavonoids and drug compounds were further confirmed by TLC-developed plates using 0.5% DPPH, KOH and AlCl₃ spray techniques. The spot screening against all tested pathogens through agar well diffusion method showed the significant use of *P. amplexicaulis* as antibacterial agent. Similarly TLC-bioautography against *E. coli*, *S. aureus* and *P. aeruginosa* by using MTT compound also indicated the antibacterial potential of *P. amplexicaulis* extracts.

PHYTOCHEMICAL SCREENING AND MICROBIOLOGICAL STUDIES OF *RUMEX HASTATUS*

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Rumex hastatus is a medicinal plant belonging to the family *Polygonaceae*. It is laxative, alterative, carminative and tonic and is being used in the remedy of various diseases. The current study was aimed to investigate the pharmacological, phytochemical and microbiological studies of the aerial parts (leaves and stem) of *Rumex hastatus*. For this purpose, antibacterial activity, antioxidant potential, phytochemical screening and the total contents of phenolic and flavonoids were evaluated. The antibacterial activity was performed against seven clinical bacterial pathogens viz., *Escherichia coli*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* through agar well diffusion method. It was observed that *S. aureus*, *S. pyogenes* and *S. epidermidis* were the most susceptible clinical pathogens to methanolic, ethanolic and acetonic extracts of *Rumex hastatus* with 9.0 ± 0.0 mm, 5.66 ± 0.57 mm and 6.66 ± 0.57 mm zone of inhibition. Similarly, the polar solvent fractions of *R. hastatus* such as acetone and methanol (70% and 60%) also showed the significant antioxidant potential by ABTS assay whereas DPPH assay showed 98% scavenging potential for methanolic and ethanolic fractions and 93% in case of acetone. The phytochemical screening gave the positive indication for the presence of bioactive compounds in all polar extracts like flavonoids, phenols, terpenoid, tannins, protein, carbohydrate, amino acids, phytosteroids, quinones and saponins. Thin layer chromatography (TLC) and TLC-developed plates also indicated the presence of flavonoids, bioactive compounds and antioxidant constituents. The spot screening against all tested pathogens through agar well diffusion method showed the significant use of *R. hastatus* as antibacterial agent. TLC-bioautography showed the significant antibacterial potential of *R. hastatus* which also indicating the presence of bioactive antimicrobial compounds in crude extracts of *R. hastatus*. Therefore current studies demonstrated that leaves and stems extracts of *R. hastatus* can be used as a good source of antibacterial and antioxidant agents due to the presence of phytochemical nutritional elements.

IDENTIFICATION OF NEW ANTI-MICROBIAL AGENTS AGAINST MULTIDRUG RESISTANT *STAPHYLOCOCCUS AUREUS*

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The phenomenon of development of resistance against a number of antibiotics that are structurally and functionally unrelated is termed as Multidrug Resistance (MDR). MDR has

emerged as the most challenging problem in the treatment of infectious diseases. The major reasons for development of resistance is the wide spread use of antibiotics for non-medicinal purposes, self-medication, non-judicial and prolonged consumption of antibiotics. This study was designed to screen libraries of fully characterized compounds against the MDR *Staphylococcus aureus* to identify the new and potent anti-microbial agents with the study of their mode of actions through flow cytometry. Microtiter alamar blue dye based spectroscopic procedure was used for initial screening. Five hundred and thirty eight (538) fully characterized compounds from various classes and sources were evaluated. Biscoumarin class of compounds of synthetic origin was identified as the potent anti-microbial agents against MDR *S. aureus*. The cytotoxicity of newly identified anti-microbial agents was evaluated against mouse fibroblast cell line (3T3). The new non-cytotoxic anti-microbial agents were then examined for the spectrum of activity and *ex-vivo* blood study as a step towards systemic implication.

BIOLOGICAL SULPHATE-REDUCTION USING FRUIT WASTES AS CARBON SOURCES

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Economical implication of dissimilatory sulphate-reducing bacteria (DSRB) has gained importance in the last three decades. The ultimate goal of metals'- and sulphate-loaded wastewaters' treatment by DSRB is directly coupled with the sulphate-reducing capacity of these bacteria. For the purpose, ten locally isolated SRB species were assessed in terms of better sulphate reduction performances using apple peelings, apple pomace, mango peelings and watermelon rind as economical carbon sources (growth substrates). Of all the fruit wastes employed in this study, watermelon rind appeared as a reasonably and equally good growth substrate for all the bacterial species. Maximally about 63% biological sulphate-reduction was observed using this substrate. While apple pomace served as an excellent carbon source only for *Desulfovibrio fructosovorans*-HAQ2 in reducing sulphate. Other nine bacterial species couldn't reduce sulphate significantly using this substrate. Biological sulphate reduction reached maximally up to 26% and 42% using apple and mango peelings, respectively as growth substrates. The findings of this study will be helpful in developing economical and environmental friendly bioremedial process (es).

COMPOSITION OF CYANOBACTERIA SPECIES FROM ARABIAN SEA

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Cyanobacteria are of great importance in marine food web as they contain substances of high biological value, such as carbohydrates, polyunsaturated fatty acids, proteins, pigments,

antioxidants, vitamins, minerals, etc. It is used either as a whole food or supplement food. Present work evaluated the nutritive quality of species of cyanobacteria, which were isolated, from the Arabian Sea and mass cultured. These included three filamentous species (*Leptolyngbya* sp., *Pseudoanabeana* sp. and *Pseudoanabeana* cf. *catenata*) and four unicellular species (*Chroococcidiopsis* sp.2, *Chroococcidiopsis* sp.3, *Chroococcidiopsis* sp.4 and *Chroococcidiopsis* sp.1). Nutritionally important major classes of natural constituents analysed include carbohydrates, proteins, lipids and mineral contents. Fatty acid profiles of derivatised lipid content were also obtained using GCMS. Dried mass obtained from mass cultures of cyanobacterial species were analysed for carbohydrate using Dubois *et al.* 1956 and showed total values ranging from 0.65 to 3.36%. Total lipid content was analysed using Folch *et al.* 1957, and found to range from 3.58 to 6.68%. Total proteins were also analysed using protocol of Lowry *et al.* 1951. A total of 11 different fatty acids were identified. Palmitic acid ME was observed in all species. Individually 5, 7, 9, 3, 2, 3 and 5 major fatty acids were found contributing in lipids of *Leptolyngbya* sp., *Pseudoanabeana* sp., *Pseudoanabeana* cf. *catenata*, *Chroococcidiopsis* sp.2, *Chroococcidiopsis* sp.3, *Chroococcidiopsis* sp.4, and *Chroococcidiopsis* sp.1, respectively. A study on mineral contents is also in progress.

MONITORING ROLE OF AI-2 PRODUCTION IN BIOFILM FORMATION BY MARINE MICROORGANISM

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This study aims to test marine microorganisms for their biofilm forming capability using the crystal violet *in vitro* microplate assay. We examined that whether patulin and penicillic acid, two well-known quorum sensing inhibitors (QSI) for human pathogen *Pseudomonas aeruginosa* PAO1, can prevent biofilm formation. This study reports, for the first time, the successful amplification of a putative *luxS* gene homologue in the marine microorganism *H. pacifica* ATCC 27122, as well as a log 3 fold expression of AI-2 during early and mid-exponential growth in marine nutrient broth batch cultures. Degenerated primers were designed based on the *luxS* protein sequence of ten gram-negative, \square^- , \square^- and \square^- proteobacteria, and used for *luxS* gene amplification in *H. pacifica*. AI-2 assays were carried out using the well-established *Vibrio harveyi* BB170 AI-2 bioluminescence assay. Effect of penicillic acid on AI-2 induction of *H. pacifica* showed strong inhibitory effect at non-growth inhibitory concentrations compared to patulin having adverse effect at the highest concentration (25 μ M) tested in our study. QSIs effect on biofilm forming capability of marine isolates was isolate specific. Detection of bioluminescence in the autoinducer bioassay and the presence of a putative *luxS* gene orthologue are biochemical and genetic evidence for the production of a signalling molecule(s) and provide a first step in characterizing *H. pacifica* quorum sensing. Our study emphasize that QSI compounds must be selected in the specific system in which they are to function and they cannot easily be transferred from one QS system to another.

IN VITRO INHIBITORY ACTIVITY OF HERBAL EXTRACTS AGAINST ORAL BIOFILMS

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Dental health issues, a serious problem in rural and urban populations of developing countries like Pakistan. Chemicals used to control oral health problems present several side effects and less effective to control biofilms hence the phytochemicals are good alternative of chemicals used. Hence the aim of this study is to assess the biofilm forming capacity of oral bacteria and the effect of herbal plant extracts on them. For this purpose, oral sample from 30 subjects at Punjab dental hospital, Lahore were collected. Out of 65 strains, 25 strains were purified on the basis of morphological variations. 10 highly resistant strains were characterized on basis of biochemical tests. Three strains were selected and characterized further by physiological and molecular characterization. Biofilm characterization was done for 3 strains [F₂₂ (*Bacillus cereus*), B₄ (*Enterobacter aerogenes*) and SpG₆ (*Micrococcus caseolyticus*)] following three methods *i.e.* Congo red, liquid interface coverslip method and test tube method. Selected strains were tested for their susceptibility to the aqueous and methanolic extracts of three (Garlic, Banana and Piper mint) plants in both planktonic and biofilm mode. The effect of plants in planktonic mode was tested by agar well diffusion method. Results showed presence of both gram positive and gram negative strains. Physiological characterization showed 37°C as optimum temperature for all strains, however, all strains showed best growth at pH 7 except *Enterobacter aerogenes* which showed best growth at pH 6. Biofilm formation by Congo red method resulted in black colonies for 3 strains which were further confirmed by cover slip assay after 24 hours and test tube method having maximum biofilm formation after 72 hours. Aqueous extract of garlic showed highest antibacterial activity for all strains. The methanolic extracts of plants showed more antibacterial effect than aqueous extracts except garlic. MIC was determined by broth dilution method. Biofilm formation reduced significantly by herbal plant extracts in all three strains. Our results suggested that these plant extracts played a critical role in the reduction of biofilm formation in both gram positive and the gram negative oral isolates, hence reducing the oral and systemic infection by these isolates.

BACTERICIDAL EFFECTS OF MEDICINAL PLANTS EXTRACTS AGAINST MONO CULTURE AND MIXED CULTURE BIOFILMS ISOLATES FROM FOOD INDUSTRIES

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Biofilm formed in food industrial premises is very common and of serious concern today in case of food hygiene. This study was carried out to evaluate the screening of food industrial biofilm forming bacteria and to evaluate the resistance of these isolates against commercial antibiotics and their susceptibility against medicinal plants. For this, bacterial isolates were collected from food industries. Antibiotic resistance of strains was checked and highly resistant strains were subjected to biochemical and physiological characterization indicated that these are mesophilic. Biofilm forming capacity was evaluated by different methods. Tube method indicated that 2F2, 1SH1, 7S2 form good biofilm after 72 hours from the time of inoculation. These strains form black colonies on Congo red media and showed biofilm formation by Air-liquid interface assay. Agar well diffusion assay was used to measure the antibacterial effect of aqueous and methanolic plant extracts of

Camellia sinensis, *Mentha piperita* and *Syzygium aromaticum* against selected strains in mono culture and mixed culture bacterial biofilms. The results showed that methanolic extracts inhibited bacterial growth with zone of inhibition ranging from 3.35 ± 0.494 to 11 ± 0.212 mm (ZI+S.D) in mono culture while 2.0 ± 0.0 to 20 ± 0.0 (ZI+S.D) mm in mixed culture. Whereas, aqueous extracts inhibited growth with zone of inhibition ranging from 2.2 ± 0.282 to 8.0 ± 0.01 mm (ZI+S.D) for mono culture and 2.0 to 5.25 ± 0.707 mm (ZI+S.D) in mixed bacterial cultures. Clove methanolic extract inhibit bacterial growth more strongly in case of combination of strains while all other aqueous or methanolic extracts inhibit bacterial growth more effectively against mono culture bacteria as compared to their effect on mixed cultures. The degree of antimicrobial activity of plant extracts tested can be put in the order: Clove > Green tea > Peppermint. Results have indicated that methanolic extracts are more effective to resist the growth of bacteria than aqueous extracts. Herbs and spices were commonly used in medicines since ancient times for treating various disorders exhibited antimicrobial activities against tested foodborne pathogens. These are proved to be a safe, effective and alternative way for treating the foodborne infections.

ANTIBIOTIC RESISTANT MICROORGANISMS ON THE SKIN OF HEALTHY FISHES.

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The following research was done to show the presence of antibiotic resistant bacteria upon the skin of healthy fishes, some collected alive from ponds and hatcheries. Relevant bacteriological broth and agar media were prepared under sterile conditions. Samples from fish skin were taken by means of a cotton swab and were inoculated in broth and later on agar plates for 24 hours. The growth observed is then purified. Biochemical tests and Gram staining were applied to identify the microorganisms. Antibiotic disc sensitivity test is then applied to identify the antibiotic resistant and antibiotic sensitive microbial species.

ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF PYRASULFOTOLE DEGRADING BACTERIA FROM SOIL

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The toxicity of herbicides and pesticides is one of the major environmental issues of the present day. This study was conducted with the aim of isolation of such bacteria that have capability to degrade such toxic compounds *i.e.* Pyrasulfotole herbicide from the environment. The specific compound *i.e.* Pyrasulfotole which belongs to a chemical class of pyrazoles was first introduced in North American and Australian markets, but nowadays usage of these herbicides is also increasing in Pakistan. Biodegradation of such compounds plays important role in removal of such silent killers from the environment and microorganisms play a vital role in biodegradation. The soil samples were collected from agricultural fields of Sindh and Punjab which were previously exposed to the herbicide, Pyrasulfotole. To achieve the goal of the present study, enrichment technique method has been used. The soil samples were treated with dose of pyrasulfotole (6 times at interval of 10 days). During the whole process the flask was placed in the shaking incubator. The treatment was given to enrich the soil with herbicide degrading bacteria.

These isolates were cultured on Msm (mineral salt media) plates and after growth these isolates were further purified on nutrient agar plates. The identification after morphological characterization and biochemical test shows two types of bacterial species which have capability to degrade Pyrasulfotole. The bacterial species *i.e. Micrococcus arborescens*. and *Brachybacterium* sp. found to be responsible for degradation of Pyrasulfotole. This study would also become a baseline study or an attempt for future research. The enzymes of these bacterial isolates may be further used in bioremediation or biodegradation techniques for degradation of pyrasulfotole from the environment.

5. MOLECULAR BIOLOGY

DNA EXTRACTION AND OPTIMIZATION OF PCR PROTOCOL FOR RAPID ANALYSIS IN *TOR PUTITORA*

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DNA was extracted from mahseer, *tor putitora* fishes from fresh and frozen muscles and the quality and quantity were analyzed. The fresh and freshly frozen tissue samples yielded higher quantity of DNA compared to long-term frozen tissues. The formalin and ethanol preserved mahseer samples showed difficulty in DNA extraction. The purpose of the study was to check the viability of extracting genomic DNA and to estimate the quality and quantity for RAPD analysis. RAPD – PCR protocol was optimized based on the concentration of reaction parameters. All parameters like concentration of DNA template, Magnesium Chloride, primers, Taq. Polymerase, dNTPs and annealing as well as denaturation temperature were successfully optimized for analysis of bands reproducibility and population diversity.

MITOCHONDRIAL DNA D-LOOP DIVERSITY AND EVOLUTIONARY RELATIONSHIP OF WILD PUNJAB URIAL (*OVIS VIGNEI PUNJABIENSIS*) WITH CLOSELY RELATED TAXA

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The taxonomy and systematics of wild sheep is complex with several classifications and revisions proposed on the basis of morphology, geographic distribution and chromosome number. Punjab Urial (*O. vignei punjabiensis*), an important sub-species of *O. vignei* is distributed in Pakistan and classified under vulnerable category, the taxonomic status of which remains uncertain. Analysis of mitochondrial DNA D-Loop of *O. vignei punjabiensis* revealed wide variations in the number and pattern of tandem repeats as compared to other Moufloniform wild sheep. The nucleotide and haplotype diversity was low to moderate. The average pairwise differences of Urial type sheep and other Moufloniform wild sheep populations was high indicating the distinctness of *O. vignei*. Bayesian and maximum likelihood phylogeny clearly established the divide between the lineages of *O. orientalis* and *O. vignei* populations. Also, the average pairwise differences was significantly higher ($P < 0.01$) between *O. vignei bochariensis* and *O. vignei punjabiensis*, despite both the populations being classified as sub-species of Urial type sheep. To compare *O. vignei punjabiensis* with Argaliform and Pachyceriform wild sheep, pairwise F_{ST} were utilized to perform

principal components analysis (PCA). PCA revealed clustering of Asiatic (*O. orientalis*), European (*O. musimon*) and Captive Mouflon type in one plane while *O. nivicola* and *O. canadensis* clustered separately. *O. ammon* and *O. vignei bochariensis* were observed to be clustering together while *O. vignei punjabiensis* was located distinctly. The tests for selective neutrality indicated purifying selection in Urial sheep while Asiatic Mouflon appears to have experienced a strong population bottleneck in the recent past. Considering the declining population trend and loss in genetic diversity due to possible genetic bottleneck events, there is an urgent need to implement strong conservation measures for *O. vignei punjabiensis* in the region.

CLONING AND EXPRESSION OF HUMAN INTERLEUKIN 15: A PROMISING CANDIDATE FOR CYTOKINE IMMUNOTHERAPY

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Recombinant cytokines have been employed successfully as potential therapeutic agent. Some cytokine therapies are already used as a part of clinical practice, ranging from early exploratory trials to well established therapies that have already received approval. Interleukin 15 is a pleiotropic cytokine having multiple roles in peripheral innate and adaptive immune cell function. It regulates the activation, proliferation and maturation of NK cells, T-cells, monocytes/macrophages and granulocytes, and the interactions between them thus acting as a bridge between innate and adaptive immune responses. Unraveling the biology of IL-15 has revealed some interesting surprises that may point toward some of the first therapeutic applications for this cytokine. In this study, the human interleukin 15 gene was isolated, amplified and ligated to a TA vector which was then transfected to a bacterial host, *E. coli Top10F'*. The sequence of cloned gene was confirmed and it showed 100% homology with the reported sequence. The confirmed gene was then subcloned in pET Expression system to study the IPTG induced expression of IL-15 gene. Positive expression was obtained for number of clones that showed 15 kd band of IL-15 in SDS-PAGE analysis, indicating the successful strain development that can be studied further to assess the potential therapeutic intervention of this cytokine in relevance to human diseases.

CLONING AND EXPRESSION OF AZURIN: A PROTEIN HAVING ANTITUMOR AND CELL PENETRATING ABILITY

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Cancer has become a wide spread disease around the globe and takes many lives every year. Different treatments are being practiced but all have potential side effects with somewhat less specificity towards target sites. *Pseudomonas aeruginosa* is known to secrete a protein azurin with special anti-cancer function. It has unique cell penetrating peptide comprising of 18 amino acids that has ability to enter cancer cells specifically. Reported function of Azurin is to stabilize p53 inside the tumor cells and induces apoptosis through Bax mediated cytochrome c release from mitochondria. At laboratory scale, we have made recombinant azurin through cloning rpTZ57R/T-azu vector into *E.coli* strain DH-5 α and subcloning rpET28-azu vvector into *E.coli* BL21-CodonPlus (DE3). High expression was ensured with IPTG induction at different concentrations

then optimized high expression level at 1mM concentration of IPTG for 5 hours. Purification has been done by using Ni²⁺ affinity chromatography. We have concluded that azurin can be a remarkable improvement in cancer therapeutics if it produces on a large scale. Azurin does not enter into the normal cells so it will prove a safe and secure treatment for patients and prevent them from hazardous anomalies.

CLONING, EXPRESSION AND CHARACTERIZATION OF MULTICOPPER OXIDASE (CueO) IN *KLEBSIELLA PNEUMONIAE* KW

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Multi-copper oxidase (CueO) is an enzyme in periplasmic membrane of bacteria and is thought to play its role in detoxification of copper by oxidizing Cu I to Cu II thus helping the cell survival. However, its exact role is yet to be elucidated by further studies. In this study, we have isolated, cloned and expressed the *cueO* gene of *Klebsiella pneumoniae* KW which is resident of highly contaminated water of local area. 1.6kb gene of the multi-copper oxidase of the above mentioned strain was cloned in DH5 α , and expressed in BL21 strain. Conditions of expression were optimized for various parameters including inducer (IPTG) concentration, time course after induction and temperature. The CueO enzyme is expressed as inclusion bodies at 37 °C. A considerable expression was achieved on 0.02mM IPTG while highest level of expression was found at 0.05mM IPTG concentration. On time course, it was found that expression of protein remained constant 8h onwards of induction. The transformed BL21 cells were grown at low temperature in order to achieve soluble expression of protein and it was found that highest expression achieved when cells were grown at 17 °C after heat treatment at 45 °C. The CueO protein was purified by Ni-affinity chromatography. Enzyme assays of CueO protein were carried out with phenolic substrates (2,6-Dimethoxyphenol (DMP) and Syringaldazine (SGZ)) to characterize its laccase activity. Kinetic studies gave the values of Km=0.2 μ M, Kcat=0.68 S⁻¹, Kcat/km=1.2s⁻¹ μ M⁻¹ for DMP, Km=0.25mM, Kcat=300 s⁻¹, Kcat/Km=1200S⁻¹mM⁻¹ for SGZ. There is further need to test the enzymatic activity towards various substrates and find its biotechnological applications in green chemistry.

PROSPECTS OF MOLECULAR BIOLOGY TECHNIQUES TO INVESTIGATE GENOMICS OF PAKISTANI DROMEDARY CAMEL

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Camel is a gifted animal for people living under harsh climatic conditions. This unique species possess wonderful physiology to perform efficiently in different environments by providing milk, meat, skin, wool, and draft power. Its enchanted characteristics attract the attention of genomic researchers to explore its genetics and genomics by applying cutting edge molecular biology techniques. Some of the latest techniques like investigation of important genes through conventional PCR, whole genome association genetics by GWAS, in-vitro gene expression studies

using RT-qPCR, multiplexed and next generation sequencing for SNP analysis through GBS (Genotyping by Sequencing) and studies for non-genetic contributors in its performance under epigenetics, may be applied to camel for better understanding of its genetic architecture. In Pakistan where we have more than 20 dromedary camel breeds the importance of such techniques is enhanced manifolds. In this context we have been done genomics work on indigenous camel breeds involving molecular genetic diversity, evolutionary history, heat tolerance, resistance to prions and immunity genes that came out with interesting results.

PROTEOMICS BASED APPROACHES FOR IDENTIFICATION OF DIFFERENTIALLY EXPRESSED PROTEINS IN COLORECTAL CANCER

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Colorectal cancer (CRC) accounts for 3rd most common cancer worldwide with an overall incidence rate of 75 males and 57 females for every 100,000 individuals in developed countries. Efforts to diagnose CRC at an early stage have largely been hampered due to non availability of stage specific protein biomarkers. In the present study, we have used proteomic based approaches (two dimension gel electrophoresis and MALDI-TOF mass spectrometry) to identify differentially expressed proteins in CRC. Surgically removed tumor and adjacent healthy tissue of CRC patients were collected from different hospitals of Lahore. A total of twenty tissue samples were collected and analyzed. Protein expression profile has been established for CRC and normal colonic mucosa after protein quantification. Protein profile was also compared by using ImageMaster 2D Platinum and Dymension softwares. ImageMaster 2D Platinum identified 17 spots that differed significantly ($P < 0.05$) with greater than 1.5 fold. These differentially expressed spots of interest were excised and subjected to MALDI-TOF MS analysis. The Peptide Mass Fingerprint produced by the MALDI-TOF MS was compared with the published databases of NCBInr and SWISS PROT and 9 out of 17 protein spots of interest were identified. Most prominent protein of interest that was elevated in expression was calreticulin that is chaperone protein. β -tropomyosin was identified as downregulated protein in CRC which is cytoskeletal protein and involved in cell migration during tumor metastasis. Further validation of these proteins by ELISA and western blot would likely enable us to identify potential protein candidates that may act as a protein biomarker. This work was partially supported by a grant from Higher Education Commission, Government of Pakistan.

DNA EXTRACTION, QUANTIFICATION AND PURITY ESTIMATION FROM FISH BLOOD AND MUSCLE SAMPLES

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The present study includes the process of DNA extraction from fish blood and muscle sample, and the differences between their extracted amount and the levels of purity. Blood samples were taken from live fishes collected from pond at SBKWU, Quetta. In this study, DNA was extracted from both blood and muscle samples, separately. The extracted DNA samples were run upon gel electrophoresis and PCR and were identified by using RFLP. Differences in quantity was

observed among the extracted DNA of both the samples as well as there was a difference observed in purity levels of extracted DNA, showing that DNA extracted from fish blood hold highest estimate of purity.

IDENTIFICATION OF PROTEIN BIOMARKERS IN PAPILLARY AND FOLLICULAR THYROID CANCERS THROUGH PROTEOMIC ANALYSIS

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She described that the surgically removed normal and pathological tissues of papillary and follicular thyroid carcinoma (PTC and FTC, respectively) were lysed and their proteins, lying in pH range of 3-10, were analyzed by two-dimensional gel electrophoresis (2-DE). Protein spots with 2-DE were visualized by staining with colloidal Coomassie stain. The protein profiles of tumor tissues were compared with their corresponding normal tissues, in both cases of thyroid cancer ImageMaster 2D Platinum softwares. Significantly different expressions of protein spots, having fold change greater than 1.5, were selected. Thirty-one spots thus selected were tryptic digested and subjected to MALDI-TOF-TOF analysis. The distinct proteins of interest, that were found to be under-expressed, included Peroxiredoxin-1 and Glutathione S-transferase in case of FTC, while no significant protein was observed in case of PTC. Both of these proteins have not been reported in relation to FTC.

MOLECULAR CHARACTERIZATION OF *TRYPANOSOMA* SPECIES FROM CHOLISTANI CAMEL, EQUINES, CATTLE, GOAT AND SHEEP

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Trypanosoma is the blood-dwelling protozoan parasites that cause a disease known as Trypanosomiasis which is very common in livestock species like cattle, sheep, goats, donkeys and camels. The objective of current study was to check prevalence and characterize the existing trypanosoma specie in animals of Cholistan region of Pakistan. Total 280 blood samples were harvested from camels (n=61), donkeys (n=52), cattle (n=52), goat (n=50) and sheep(n=60) in and around the Cholistan from different localities of Cholistan and Bahawalpur District from January to August 2014. The samples were subjected under different diagnostic techniques and so for as results shown 39 (13.9%) out of 280 samples are positive in thin layer microscopy. 145(51.7%) out of 280 are positive for PCV, similarly, 153(54.6%) out of 280 are positive for formal gel test. Further being confirmed by PCR using different primer sets *i.e.* TBR, TRYP 4 and ROTAT 1.2 under optimized conditions.

TRANSCRIPTIONAL PROFILING OF *cueR* - A merR LIKE TRANSCRIPTIONAL REGULATOR - IN RESPONSE TO SILVER IN *KLEBSIELLA PNEUMONIAE*

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Many fundamental aspects of bacterial cell physiology and pathogenesis are influenced by transition metal homeostasis. The intracellular concentration of essential metals or the presence of harmful elements is monitored by a set of transcriptional regulators that modulate the expression of factors that rapidly restore metal homeostasis. A large class of these metalloregulators belongs to the MerR family, a group of proteins that share similarity at the N-terminal DNA-binding domain. This study was carried out to explore gene *cueR* present in *Klebsiella pneumoniae* KW. CueR is a regulatory protein that regulates the expression of *cueO* and *copA* which are responsible for Cu⁺⁺/Ag⁺ resistance in members of Enterobacteriaceae. It is a MerR-like activator molecule for the transcription of genes. Determination of minimum inhibitory concentration (MIC) revealed that this bacterial strain is highly resistant to Ag⁺ upto 55 mM. Previously reported bacterial strains of family Enterobacteriaceae, did not show such a high MIC value against Ag⁺. *cueR* was cloned using pTZ57R vector in *E. coli* DH5 α . Later, it was sub-cloned in *E. coli* BL21C⁺- expression host-using pET 21 vector. Expression analysis of CueR protein was performed under IPTG induction. Protein was found mainly in soluble form as a thick band of protein was observed in supernatant. For purification expressed protein was subjected to ammonium sulphate precipitation. Protein largely appeared in 50% and 60% saturation levels of the salt. These fractions were combined to carry out dialysis. Transcriptional profiling of *cueR* in response to various concentrations of Ag⁺¹ (1 – 5 mM) was determined through real time PCR. Comparative quantification through Pfaffle method revealed that mRNA of *cueR* increases with increase in Ag⁺¹ concentration in a linear fashion.

DESCRIPTION AND DNA BARCODING OF THE INDIAN SILLAGO, *SILLAGO INDICA* (PERCIFORMES: SILLAGINIDAE) FROM THE COAST OF PAKISTAN

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Seventy-two specimens (139.2-192.4 mm SL) were obtained from fish market of Weifang, China, in March 2014, originally these specimens were captured in Pakistan and 34 muscle specimens collected from Karachi Fish Harbour in October 2014. Twenty-four morphological indices were counted, and measured; otolith morphology and swimming bladder were described and compared with *S. sihama*. The genomic DNA was isolated from muscle tissue by proteinase K digestion followed by a standard phenol-chloroform method. However, the swim bladder of *S. sihama*, the roots of two posterior extensions are adjacent and two posterior extensions are in close; the origin of the duct-like process is in front of the terminal of swim bladder and the joint of roots of two posterior extensions. The otoliths of *S. indica* are similar to *S. sihama* except the number of the distinct protuberances on the ventral edge and the gap or protuberance at the posterior end. The fragment of cytochrome oxidase subunit I (COI) gene of mitochondrial DNA was sequenced for phylogenetic analysis, the mean genetic distance within the species *S. indica* was 0.2%, net genetic

distances between *S. indica* and other six *Sillago* species ranged from 17.1% to 22.2%.

GENETIC STUDY OF MARINE ROCKFISH, *SEBASTESSCHLEGELII*

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In the present study, control region of mtDNA, AFLP markers and eight microsatellite DNA (SSR) markers were used to estimate the genetic diversity and population genetic differentiation of *S. schlegelii* collected from China and Japan. The results of the three markers were in accordance with each other. The genetic diversity of *S. schlegelii* was high according to the results of mtDNA, AFLP and SSR. No significant genealogical branches or clusters corresponding to sampling localities were detected by NJ and UPGMA tree. The results of AMOVA analysis and pairwise F_{ST} values showed no genetic divergence among different geographic populations and high gene flow was existed. The result of Structure showed all the populations of *S. schlegelii* shared one gene pool. Larval dispersal with the assistance of drifting seaweed and the current environmental factors may play an important role in shaping the contemporary phylogeographic pattern of *S. schlegelii*. The present study may be beneficial to population conservation and fisheries management to *S. schlegelii* and for species with the similar life history characters.

GENETIC VARIATIONS AMONG MAJOR CARPS OF INDUS RIVER ON THE BASIS OF RANDOMLY AMPLIFIED POLYMORPHIC DNA

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The progress of fish farming and fisheries management strategies mainly depends upon molecular systematics and genetic structure of culturable fish species. Major carps constitute an important group of fish with high diversity and intense commercial potential in many Southeast Asian countries including Pakistan, Iran, Bangladesh, Philippines and India. To study genetic relationship and diversity in morphological variants of major carps namely *Labeo rohita*, *L. calbasu*, *L. gonius*, *Gibelion catla* and *Cirrhinus mrigala* random amplified polymorphic DNA (RAPD) assay was evaluated. Twenty specimens belonging to each species were collected from different sites of Indus River at Taunsa Barrage, District Muzaffar Garh in Southern Punjab, Pakistan. Genomic DNA was isolated manually from fin tissues of fish. Eight arbitrary decamer primers were used to screen out species-specific RAPD markers among five species of major Carps. Highly reproducible and very distinct RAPD profiles with great degree of genetic variability were detected among species. All these primers show polymorphic as well as monomorphic bands on the basis of their loci. The RAPD depicted that *L. rohita*, *L. calbasu* and *L. gonius* are more closely related to each other than *G. catla* and *C. mrigala*. Genetically *L. calbasu* is more close to *L. rohita* than *L. gonius* and similarly *G. catla* is more close to *L. rohita*, *L. calbasu* and *L. gonius* than *C. mrigala*. *Cirrhinus mrigala* is genetically more different from other four species. This study

provided evidence that RAPD could be used for genetic differentiation of closely related species.

NEW RECORD AND MOLECULAR IDENTIFICATION OF *THALAMITA DANAЕ* STIMPSON, 1858 (DECAPODA: BRACHYURA: PORTUNIDAE) BASED ON FRESH MATERIAL FROM THE COASTAL WATERS, PAKISTAN

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Thalamita danae Stimpson, 1858, reported as a new record, based on an integrative taxonomy approach combining 16S partial sequence of mitochondrial DNA and morphological analyses was used to accurate identification of specimens. The morphological and molecular analysis provided the confirm evidence of *T. danae* in coastal waters of Pakistan. Results were confirmed by amplification of partial sequences of 16S mtDNA gene and sequence was searched for sequence similarity using BLASTn (Basic Local Alignment Tool), the result was showed 97% sequence similarity with partial sequences of *Thalamita danae* Stimpson 1858 with accession no: FJ152165, obtained from the gene bank indicated that mis identification of species does not occur. The obtained sequence was submitted to gene bank after confirmation of genetic and morphological similarity.

EXPRESSION OF *PSEUDOMONAS AERUGINOSA* MERCURY AND COPPER INDUCED METALLOTHIONEIN IN *E. COLI* BL 21 CODON PLUS

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Metallothioneins (MTs) play an important role in the detoxification of heavy metal ions such as Cu, Cd and Hg and found in a wide variety of organisms including bacteria, fungi as well as all eukaryotic plant and animal species. The bacteria *Pseudomonas aeruginosa* was isolated from the industrial area of Lahore Pakistan. PCR amplification clearly revealed presence of mtA gene encoding bacterial metallothionein responsible for metal sequestration and AAS analysis proved intracellular bioaccumulation of 26.5mg Hg/g and 32.3 mg Cu/g dry weight of cells. SDS-PAGE analysis confirmed mercury induced bacterial metallothionein with molecular weight 74 kDa, which corresponds to the predicted mtA gene. This *Pseudomonas aeruginosa* showed cross tolerance to cadmium, lead, arsenic and chromium along with resistance to multiple antibiotics. The *Pseudomonas aeruginosa* removed 92% of copper and 90% of mercury from the medium after 72 hrs of incubation.

6. PHYSIOLOGY

EFFECT OF KISSPEPTIN-10 ADMINISTRATION ON SERUM CORTISOL OF ADULT LABORATORY RATS

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Kisspeptin is a neurohormone involved in the regulation of reproduction, puberty onset and metabolism. It plays pivotal role in the regulation of Hypothalamic-Pituitary-Adrenal (HPA) axis. The activity of HPA axis is governed by the secretion of corticotrophic hormone releasing factor (CFR) and vasopressin (AVP) from hypothalamus, which activate pituitary corticotrophs to release ACTH. Secretion of glucocorticoids from adrenal cortex is in turn stimulated by ACTH. The study was conducted to evaluate the effect of KP-10 administration on serum cortisol of adult laboratory rats. Thirty five adult male rats were divided into seven groups. KP-10 was administered intraperitoneally to these rats in variable doses 10pg, 1ng, 1µg. Control group was administered with saline. Two separate groups were treated with hCG (50IU) and LH. An antagonist to KP receptor was applied to investigate the down regulation of of serum cortisol. One way ANOVA was applied to compare the results between groups. Results demonstrated no direct significant effect of KP-10 on cortisol concentration, although the levels appeared increased. In KP-234 treated group, cortisol concentration was non-significantly decreased. Overall LH and hCG treatment groups had levels comparable to control. Results show that intraperitoneal administration of different doses of KP-10 is not able to regulate serum cortisol concentration significantly. The Kisspeptin system has no direct link to HPA axis's working mechanism. It is concluded that regulation of basal levels in rats are not changed significantly by chronic administration of either KP-10 nor by LH and hCG. Their roles can be further studied by applying different routes of administration and by changing dosage concentration.

EFFECT OF *MENTHA ARVENSIS* L. (MINT) ON KIDNEY, ADRENAL AND LIVER OF MALE MICE

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Mentha arvensis L. is a distinct species of plants. It is used as antifungal, antifebrile, antibacterial, antipeptic ulcer agent, carminative, anti-spasmodic and is used to treat different diseases. It provides natural source of menthol. The research was conducted to investigate the effect of *Mentha arvensis* on kidney, adrenal and liver of male mice. Mice were divided into eight groups; four groups were sacrificed after 15 days while remaining groups were sacrificed after 30 days. Animals were administered low and chronic doses of extract *Mentha arvensis* through gavage. Negative control group were treated with DMSO. Blood samples were taken and serum was separated and stored. Serum samples were then analyzed for urea, creatinine and cortisol. Data were analyzed statistically through one-way ANOVA. $P < 0.05$ was considered statistically significant difference. A significant increase was observed in serum cortisol of mice treated with high dose of *Mentha arvensis* for 15 days as compared to 15 and 30 days treatment groups. A

significant decrease was observed in 30 days control group treated DMSO as compared to 15 days control group. No significant difference in serum creatinine levels of all treatment groups was observed. Histologically, changes including prominent disruption of cells were observed in liver, kidney and adrenal tissues of extract treated group. Increased hepatic nuclei, vacuolations and mononuclear infiltrations in hepatic tissues, damaged proximal tubules, increased space b/w collecting tubules, disrupted glomeruli, nephron architecture and epithelial cells in kidney tissues; whereas in adrenal tissues, hypotrophy, compact cell and cystic changes were observed. It is concluded that continuous administration of *Menthaarvensis* extract in mice for a month caused histomorphological changes in liver, kidney and adrenal tissues. Also significant increase was observed in cortisol levels of high dose treated groups.

THE EFFECT OF NIGELLA SATIVA OIL ON BLOOD LEVELS OF TRACE ELEMENTS IN MALE ALBINO RATS (SPRAGUE-DAWLEY)

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Trace metals are required in very small amounts for various but vital biological activities within the body. These microminerals mostly constitute the structural components of enzymes as cofactors. This study was designed to investigate the effect of *Nigella sativa* oil on trace element levels of whole blood in male albino rats. Fifteen adult male rats were divided into three groups. Two doses (2ml/kg and 1ml/kg b.w) of *Nigella sativa* oil was given orally for thirty days to Group I and II respectively, while Group III (vehicle) was administered with distilled water. *Nigella sativa* treatment caused significant increase ($P < 0.001$) in Ni concentration of both groups and Zn concentration of Group I in whole blood, while Mn, Cr concentrations of both treatment groups and Zn concentration of Group II were decreased in comparison to vehicle group ($p < 0.05$). No significant effect was observed in Pb, Mg and Co concentrations in blood as compared to vehicle group. It is concluded that *Nigella sativa* oil is involved in altering trace element balance in adult male rats.

EEEECT OF LONG-TERM ADMINISTRATION OF KISSPEPTIN ON ADULT MALE RAT TESTES AND ACESSORY SEX ORGANS

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This study was conducted to investigate long term effect of kisspeptin on testes and accessory sex gland of adult male rats, as this information is lacking to date. Kisspeptin was administered intraperitoneally to rats for continuous 12 days. A total of 40 Sprague- Dawley rats were divided into eight groups which included Group I: Control (0.9 % Saline), Group II: DMSO, Group III: hCG, Group IV: LH, Group V: 1 µg kisspeptin, Group VI: 1 ng kisspeptin, Group VII: 10 pgkisspeptin, Group VIII: 50 ng kisspeptin + 1 ng Kisspeptin. One way ANOVA was applied to statistically compare serum testosterone levels among experimental and with the control groups. Long term administration of kisspeptin led to non-significant decrease in serum testosterone levels

in kisspeptin treated groups as compared to the control groups, while in LH treated group there occurred significant increase ($P \leq 0.02$). In case of hCG, DMSO and saline treated groups testosterone concentration was not significantly increased. In contrast, 1 μ g kisspeptin, 1 ng kisspeptin and 10 pgkisspeptin, groups showed non-significant decrease in serum testosterone concentration. In 50 ng kisspeptin + 1 ng Kisspeptin group, serum testosterone concentration was increased as compared to 1 ng kisspeptin treated group but this increase was again non-significant. The histomorphological parameters of testes, seminal vesicle, prostate and epididymus were studied. The study demonstrated that long term kisspeptin administration to rats for 12 days results in degeneration of rat testes, seminal vesicles, prostate glands and epididymus. This observation was in line with parallel decrease in serum testosterone concentration in kisspeptin treated groups as compared to the control. Thus it appears as if long-term kisspeptin administration causes desensitization of the receptors located on these organs.

AN OVERVIEW OF DIABETES AND VITILIGO IN POPULATION OF KARACHI

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Diabetes & Vitiligo are probably the most common auto-immune diseases in population of Karachi. Diabetes is inability of pancreas to produce Insulin and maintaining blood glucose level while in Vitiligo melanocyte of skin destruct itself and loses melanin (pigment) of different parts of body. Patients were analyzed on the basis of their family history, age groups, therapeutics, rate of recovery and sensitivities are also recorded. During the present study, it is concluded that Auto-immune diseases are more likely to inherit because more than 60% of patients in vitiligo as well as diabetes mellitus having a positive family history of disease. Both types of diabetes are found among population of Karachi but type 2 is more commonly and abundantly found. No medicine could make pancreas to produce natural and effective insulin again but synthetic insulin could do the same work as well. 2% population of Karachi is infected with vitiligo. Many patients have developed sensitivity from sunlight, humidity, heat and foods like fish meat and eggs etc on the other almost equal number of patients were devoid of any sensitivity and leading a normal routine life.

AN INVESTIGATION ON PROTECTIVE EFFECT OF KISSPEPTIN-10 AGAINST INSULIN INDUCED HYPOGLYCEMIA IN ADULT MALE RHESUS MONKEYS (MACACA MULATTA)

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Insulin therapy in most of the diabetic patients is associated with hypoglycemia which may become lethal in severe condition. Various alternatives of regular insulin are prescribed for its treatment. Kisspeptin encoded by KISS1 gene is the neuropeptide which is mainly involved in puberty onset and reproduction. Since genes for kisspeptin and its receptor are not only expressed within the brain but are also widely distributed in peripheral organs including pancreas and liver. Furthermore, modulating role of kisspeptin in glucose metabolism has been suggested by in-vitro

studies. In current study, it was hypothesized that in-vivo administration of kisspeptin may attenuate the severity of hypoglycemia by down-regulating insulin secretion from islet cells. Different combinations of insulin (1IU/kg BW) and kisspeptin-10 (50µg) along with saline and insulin alone were intravenously administered to separate groups (n=3) of rhesus monkeys. Saphenous vein area was catheterized for dosing and sequential blood sampling. Pre-fast blood sample (0.5ml) was drawn the night before each separate experiment. Afterwards blood samples (0.5 ml) were taken at pre-dose, 30 min, 60 min, 90 min, 120 min, 150 min and 180 min intervals. Treatment with insulin alone led to significant decrease in blood glucose level. In contrast, combined treatment with insulin and kisspeptin-10 resulted in a significantly higher glucose level (*P=0.032) as compared to insulin alone treatment at 180 min. However, kisspeptin-pretreatment and post-treatment resulted in significant decrease of blood glucose levels. Although further intensive research is required for better evaluation, it can be safely concluded that there exists a potential for kisspeptin to be used as a therapeutic agent for treatment of insulin-induced hypoglycemia in diabetic patients.

EFFECT OF KISSPEPTIN-10 ON SERUM TESTOSTERONE LEVELS IN STALLION, DONKEY AND MULE

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This study was conducted to determine the response of serum testosterone in male equines following bolus doses of Kisspeptin, hCG and LH. Male animals of each equine species (stallions, donkeys and mules) were divided into four groups. Kisspeptin -10 was administered intravenously into the jugular vein of all animals. Group I: treated with 3 ml (0.95% saline); Group II: treated with 50 µg Kisspeptin; Group III: treated with 2500 IU hCG and, group IV treated with 400 µg LH. Serum testosterone levels among different treatment groups were compared through one way ANOVA, P<0.05 was taken as significant difference. Administration of Kisspeptin to all the three species *i.e.* stallions, donkeys and mules led to significant (P <0.001) increase in testosterone concentration at 240 min post dose as compared to the saline treated group. Upon LH administration a highly significant increase (P<0.001) in serum testosterone concentrations was noticeable at 240 min in stallions, donkeys and mules as compared to pre dose testosterone concentrations. In case of hCG treatment, the concentration of serum testosterone was also found significantly greater in stallions (P<0.05), in donkeys (P<0.01) and in mules (P<0.001) at 240 min post dose as compared to the pre-dose concentration. Administration of Kisspeptin and other reproduction related hormones to male equines causes significant increase in serum testosterone concentration demonstrating similar effect of all the peptides.

PREVALENCE OF ANEMIA AMONG PREGNANT WOMEN IN KARACHI

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Anemia is the most common nutritional deficiency disorder in the world. Approximately 1.3 billion people suffer from anemia. The most affected group is pregnant women. In pregnancy

the most common cause of anemia is iron deficiency. Approximately half of the population in developing countries is anemic. Prevalence of anemia among pregnant women has been investigated. The present study demonstrates 91% prevalence of anemia during pregnancy. Blood samples of 75 pregnant women were collected from March 2014 to November 2014. They belong to different ages, socio-economic status, educational level, trimesters and weight. Results show that 43% women were suffering from mild anemia, 35% women had moderate anemia and 23% women were severely anemic. 15% women of age 20-25 were anemic, 21.3% respondents of age 25-30 had low hemoglobin, 31% expected women of age 30-35 were anemic while 34% women of age 35-40 were highly susceptible to anemia. 85% of respondent who belongs to low and middle socio-economic status were anemic. The main reason is improper diet, high tea consumption and low intake of meat and iron rich food. The underweight and overweight women are susceptible to anemia. The minimum weight observed was 40kg and maximum weight was 93kg. The hemoglobin levels vary in trimesters. It was observed that anemia is more prevalent in women of low educational level than educated women. Women having miscarriage in their history is more likely to be anemic.

DEMAND AND SUPPLY OF NON INJECTED COW / BUFFALO MILK IN KARACHI

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The cows give milk at the desired time of owner by injected of *oxytocin*. It is saving the time of the milk producers by genetically moderate ways. A part from health of the cow, this injectively yield milk is also blame for vector of number of serious human deceases, *i.e.* breast cancer in women, etc. In this study it is tested that how many people are aware about the benefit for non-injected milk. Such awareness level was tested in 300 respondents in Karachi to access the demand safe milk. It has been observed that the debate on non genetically moderate food is remain under discussion in Karachi, but no proper effort has yet be taken to avoid it. The impact of such debates is also tested. For such tests the numbers of outlets are use as impact's tool. Hence, it is tried to find out the numbers of supplier of non-injected milk in Karachi. It is found that the demand of safe milk is present in two categories of respondents. Firstly in the highly educated people and secondly in people whose family member was suffered from the breast cancer. Where 90% respondents were found hopeless form the supply of such milk. The fresh cow milk is available in local markets at the price of Rs. 86/L, while no-injected cow fresh milk is having high prices of Rs.700/l.

DETERMINATION OF WHOLE BLOOD LEVELS OF ELECTROLYTES AND TRACE ELEMENTS IN PATIENTS WITH INTRACRANIAL NEOPLASMS

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Trace elements and essential electrolytes are essential for normal physiological functions. They are the structural components of cofactors / enzymes that function in immune system,

nutritional deficiencies, antioxidant defense and protection against chronic diseases. Altered levels of trace elements may lead to pathophysiological problems. The present study was designed to determine trace elements in the blood of patients with various intracranial neoplasms. After histopathology, patients were categorized into five groups mainly: oligodendroglioma, schwannoma, meningioma, astrocytoma and glioblastoma multiforme-IV. Blood samples were obtained and processed for atomic absorption spectrophotometry. Un-paired t-test was applied to compare diseased and healthy subjects. Statistical analyses revealed that in oligodendroglioma patient's the levels of Zn ($p < 0.00008$), Ni ($p < 0.0001$), Cu ($p < 0.01$) and Mg ($p < 0.0003$) were significantly lowered whereas Co ($p < 0.03$) and Mn ($p < 0.03$) were significantly elevated. No change in Pb, Cr and Fe level was observed. In schwannoma patients Zn ($p < 0.00006$), Cu ($p < 0.01$) and Mg ($p < 0.04$) levels were significantly decreased in blood however, Pb ($p < 0.03$) was significantly increased while insignificant change was noticeable in Mn, Co and Ni concentrations. Patients with meningioma had significantly lowered blood levels of Zn ($p < 0.001$) and Cu ($p < 0.01$) whereas, significant increase was observed with Cr ($p < 0.04$). Blood levels of Zn ($p < 0.00006$), Fe ($p < 0.01$), Ni ($p < 0.005$) and Cu ($p < 0.03$) were lowered significantly in glioblastoma multiforme patients whereas significant increase was observed in cobalt ($p < 0.02$), Pb ($p < 0.004$) and Cr ($p < 0.02$) levels. No change was found in Mn or Mg levels. Significantly decreased blood levels of Zn ($p < 0.0001$), Fe ($p < 0.007$) Ni ($p < 0.009$) and Mg ($p < 0.0001$) were observed in astrocytoma patients, significantly increased levels of manganese ($p < 0.01$) and lead (0.004) were present. No change was observed in Co, Cu and Cr levels. It is concluded that there is marked alteration of whole blood levels of essential elements in patients with various tumors.

EVALUATION OF POSSIBLE PROTECTIVE ROLE OF QUERCETIN ON BISPHENOL A INDUCED REPRODUCTIVE TOXICITY IN ADULT MALE SPRAGUE-DAWLEY RATS

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Bisphenol A is an environmental toxicant, which is used in various plastic products and food packing materials. It can leech from the walls of container to the food, and enters into the body. Bisphenol A has been observed to induce hazardous effects on reproductive health in animals and humans. Quercetin is an antioxidant substance for the prevention of many diseases. The present study was designed to evaluate the protective role of Quercetin on rat testes against bisphenol A. Twenty adult male Sprague Dawley rats (190-250g) were divided into four groups. First group served as control and was provided with saline. The second group of rats was given oral gavage of bisphenol A in normal saline. Third group served as a positive control and provided oral dose of Quercetin (50mg/kg). In fourth group, Quercetin (50mg/kg) was co-administered orally with bisphenol A (50mg/kg) in normal saline. All the treatments were carried out for 52 days. Bisphenol A treatment resulted in significant ($p < 0.05$) decrease in intratesticular testosterone concentrations, daily sperm production, efficiency of sperm production and number of spermatids/testis. Bisphenol A treatment also induced significant ($p < 0.05$) increase in the thiobarbituric acid reactive substance, whereas catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and glutathione reductase (GSR) activities were significantly ($p < 0.05$) decreased in testicular tissue of rat. Additionally bisphenol A treatment also significantly ($p < 0.05$) induced DNA damage. However, Quercetin treatment resulted in significant ($p < 0.05$) increase in intratesticular testosterone concentrations,

daily sperm production, efficiency of sperm production and number of spermatids/testis. Quercetin treatment also restored antioxidant defense system and decreased the levels of thiobarbituric acid reactive substance (TBARS) significantly ($p < 0.05$). Our results indicated that bisphenol A exerted its toxic effects on testis and Quercetin ameliorated toxic effects of bisphenol A on reproductive organs. Therefore, it was concluded that Quercetin can be used as a therapeutic drug against bisphenol A induced reproductive toxicity.

HORMONAL VARIATIONS IN FARMERS EXPOSED TO PESTICIDES

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The present study was undertaken to compare different hormone levels in farmers exposed to pesticides vs. non exposed ones. Two study groups were made having (n=20) in each group, A) not exposed to pesticides B) exposed to pesticides. Testosterone and thyroid stimulating hormone (TSH) levels were estimated with RIA kit and Eliza kit protocol respectively. The results shown a marked variation in hormone levels in both groups. Testosterone levels in group A ranged from 285.0 ng/dl- 472.0ng/dl, while in group B it was 123.3ng/dl-862.0 ng/dl. TSH quantified in group A ranged from 0.100 μ IU/ml-3.80 μ IU/ml while in group B is 0.400 μ IU/ml-2.600 μ IU/ml. Present study clearly indicate pesticides can cause endocrine disrupting effects because of their potential to modify the male hormone profile as a function of the type of pesticide used as well as the magnitude of exposure. Further researches on large samples are needed to confirm these findings and to obtain detailed information about mechanisms of toxicity.

7. TOXICOLOGY

GENO-TOXICOLOGICAL IMPACT OF WATERBORNE COBALT-LEAD-CADMIUM MIXTURE ON *CYPRINUS CARPIO*

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Goeno-toxicological impact of sub-lethal concentrations of cobalt-lead-cadmium mixture in terms of DNA damage were observed in erythrocytes of common carp, *Cyprinus carpio* by using alkaline version of comet assay. Heavy metals can increase DNA damage in a dose and time related manner. *Cyprinus carpio* was exposed to four sub-lethal concentrations of metals mixture for the duration of 30-days. Fish peripheral blood slides were prepared after 30-days of exposure for the assessment of DNA damage in terms of genetic damage index (GDI) and cumulative tail lengths of comets (CTL). Significantly maximum genetic damage index was observed due to 1/3rd of LC₅₀ exposure than that of positive control. Incidence of cumulative tail lengths of comets was also observed higher at 1/3rd of LC₅₀ exposure, followed by that of positive control, 1/4th of LC₅₀, 1/5th of LC₅₀ and 1/6th of LC₅₀ exposure.

HEPATOTOXIC AND HEMATOLOGIC EFFECTS OF BIFENTHRIN INSECTICIDE IN PIGEON (*COLUMBA LIVIA*)

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Over the last few decades, ecotoxicological impacts of pyrethroid insecticides have been gained attention by massive increase of their use in agriculture sector. The aim of present study was to assess the possible hepatotoxic and hematological effects of bifenthrin insecticide in avian species. To this, 36 Pigeons were (*Columba livia domestica*) randomly divided in three equal group (A, B & C). Bifenthrin 10 EC insecticide was orally administrated at a dose of 1/20 and 1/15 of LD50 (58mg. and 80mg./kg./day) for five weeks in test group A and B respectively whereas, group C birds kept as control. All birds supplied same quantity of food during investigation period. The assessment strategy of the present study used observation of clinical signs and stress related changes which were assessed by evaluating relative clinical symptoms, body weight, organ weight, liver histopathology and hematological investigations. A significant decrease ($P < 0.001$) in the body weight was recorded in both test group birds as compared to control. On exposure to insecticide treated birds demonstrated change in color of the liver with paleness to yellowish coloration. Histopathological examinations revealed that, enlarged hepatocytes increased sinusoidal spaces, vacuolation of hepatocytes in group A birds. While in group B, insecticide exposure exerted marked pathological changes in the liver like diffused vacuolation, congestion and deterioration of the hepatocytes, hypertrophy and hepatic fibrosis. Significant decrease in red blood cell counts RBCs, hemoglobin (Hb) and packed cell volume (PCV) of treated pigeons were observed in

comparison to their control. Significant changes in total and differential leucocytes counts (TLC & DLC) were also observed in treated birds. These results provide experimental evidence of ecotoxicology that bifenthrin insecticide induces hematological and histopathological changes in the birds even at low doses. So it is concluded that confined use of insecticides to minimize such hazardous effects to living organisms.

PESTICIDE IMPACT ON TISSUE PROTEIN OF FISH *OREOCHROMIS MOSUMBICUS*

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Indiscriminate use of pesticides in agriculture to control pests resulted in pollution. The pesticide pollution has become a threat to marine ecosystem as pesticides are extremely toxic to non-target organisms, like fish and affect fish health through impairment of metabolism, sometimes leading to mortality, adversely affecting the complex food-web and population dynamics. In the present study, an attempt has been made to investigate the acute toxicity of organophosphate pesticides (chlorpyrifos and malathion), synthetic pyrethroid pesticide (Cypermethrin) on total protein content of the fish (*Oreochromis mosumbicus*). For this regard fish is treated for 24 and 48 hrs with different concentrations of pesticides. The levels of total protein content showed a significant decrease for pesticide treated fish as compare to control. The most pronounced decrease was noticed in cypermethrin treated group followed by malathion and chlorpyrifos respectively. The present study reports metabolic dysfunction in response to pesticide toxicity in the fish. Pesticide acts as stress inducing agents which affect the functional state of tissues of the exposed organisms.

STUDY OF CONCENTRATION-DEPENDENT GENOTOXICITY OF COBALT TO *CATLA CATLA*

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The aim of the study was to evaluate the genotoxicity of cobalt to *Catla catla*. The concentration-dependent DNA damage in the peripheral blood erythrocytes of the fish was evaluated by using single cell gel electrophoresis. *Catla catla* (n=10) were exposed to five sub-lethal concentrations i.e., 1/3rd, 1/4th, 1/5th, 1/6th and 1/7th of LC₅₀, separately. After 14 days the peripheral blood erythrocytes were sampled for the assessment of genotoxicity in terms of percentage of damaged cells. Statistically significant (p<0.05) differences were observed among all concentrations. The DNA damage was observed to be concentration-dependent with significantly higher damage at 1/3rd of LC₅₀ (26.00±5.29%) while the percentage of damaged cells were least at 1/7th of LC₅₀ (2.00±0.22%) exposures of cobalt.

**CLINICO HEAMATOLOGICAL AND MUTAGENIC CHANGES INDUCED BY
ARSENIC AND COPPER SULPHATE IN ADULT BIRDS**

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The present experimental study was conducted to determine the clinico-hematological and mutagenic impacts induced by concurrent oral administration of arsenic and copper sulphate in adult male birds. After acclimatization a total of 28 adult male birds were randomly divided and kept in 7 groups each having four birds. All the experimental birds received arsenic and copper sulphate alone and in different combinations for 30 days. Blood samples were collected from each bird at days 10, 20 and 30 of the experiment. Various clinical signs like decreased feed intake, body weight, ruffled feather, depression, dullness, ocular discharge, open mouth breathing, diarrhea and pale comb were observed at higher levels of arsenic and copper sulphate. In treated birds the values of total erythrocytes counts, leukocyte counts, hemoglobin concentration and mean corpuscular hemoglobin concentration were significantly decreased while pack cell volume and mean corpuscular volume increased. Results showed that frequency of erythrocytes with micronuclei, blabbed, lobed, notched and cells with nuclear remnants was significantly increased. From the results of this study it can be concluded that arsenic and copper sulphate alone at higher levels and in combination even at lower levels poses serious clinico-hematological and mutagenic effects in avian species.

TOXICOLOGICAL ASSESSMENT OF ESSENTIAL OILS FROM RUTACEAE FAMILY

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Essential oils are produced as secondary metabolites by aromatic plants, predominantly belonging to families Apiaceae, Lamiaceae, Myrtaceae and Rutaceae. The family Rutaceae has great economic importance for its numerous edible fruits and essential oils. In the current study, essential oils of seven plants of the family Rutaceae including, *Aegle mameos*, *Murraya koenigii*, *Citrus reticulata blanco*, *Zanthoxylum armatum*, *Skimmia laureola*, *Murraya paniculata* and *Boenninghausenia albiflora* were used, for their toxicological assessment. These plants have already been reported beneficial in multiple ailments. That's why the present study focused on the toxicological assessment of essential oils from the plants. Seven groups of selected essential oil treated wistar rats were established against control group (n=5) which received water for 14 days; animals were offered feed and water *ad-libitum* and treated with essential oils (400mg/kg body weight). On day 15 all the animals were sacrificed and blood samples were stored for hematological and serological study. Hematological studies revealed a significant elevation in TEC

in animals treated with essential oils of *Murraya koenigii*, *Skimmia laureola* and *Boenninghausenia albiflora*, while an elevation in PCV and depletion in MCV was observed in animals treated with *Murraya paniculata* and *Boenninghausenia albiflora* respectively. Serological investigations demonstrated that LFT remained unaltered in all groups but a significant depletion in triglycerides and elevation in blood sodium level was observed in animals treated with essential oil of *Aegle marmelos* and *Citrus reticulata blanco*. *Zanthoxylum armatum* caused a significant increase in urea level. Treatment with *Skimmia laureola* and *Murraya paniculata* induced similar changes along with elevation in blood urea level. *Boenninghausenia albiflora* treatment caused a significant depletion in Triglyceride, HDL, and LDL level, and elevation in blood Urea and Sodium level. *Murraya koenigii* did not affect any serological parameter. The depletion in triglycerides is considered as beneficial and change in urea without alteration in creatinine is not considered as toxic output of any test material. So it could be concluded that all oils except *Boenninghausenia albiflora* can be considered safer for internal use with critical monitoring of sodium level. *Boenninghausenia albiflora* affected many markers including RBC, MCV, triglycerides, HDL, LDL, urea and sodium.

DNA FRAGMENTATION COMPARISON IN DIFFERENT FISH ORGAN AS POLLUTION ASSESSMENT TOOL

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Intense pollution being contributed by human is threatening irrigation and ground waters and also terrestrial and aquatic life. The present study was planned to assess the pollution level through percent DNA fragmentation in fish organs (kidney, liver, gills and muscle) of (*Wallago attu*, *Sperata sarwari*, *Valgarius valgarius*, and *Labeo rohita*) were collected from River Chanab as polluted and control samples from Fish Seed Hatchery, Satiana Road, Faisalabad. The impact of habitat on the quality of DNA and contribution of weight on %DNA fragmentation was also assessed. The fish samples were processed in the Research laboratory, GC University, Faisalabad. After morphometric measurements, each fish specimen was dissected to collect kidney, liver, gills and muscle. Percent DNA fragmentation was examined by using spectrophotometer. The different fish organs i.e. kidney, liver, gills and muscles analyses revealed that weight of kidneys, liver, gills, muscles within different weight categories, species and their interaction were highly significantly different ($P < 0.01$). Means of weight of kidneys, liver among different species showed that *W. attu*, *S. sarwari* were non-significantly ($P > 0.05$) different whereas *L. rohita* and *V. valgarius* were significantly different ($p < 0.05$). Whereas means of weight of muscles among different species were significantly different ($p < 0.05$). There was an increasing trend of %DNA Fragmentation in all the fish organs of different weight categories, whereas organs and their interaction showed highly significant effect ($P < 0.01$) compared with control samples where no DNA fragmentation was observed. The weight has a positive and highly significant ($P < 0.01$) correlation on %DNA fragmentation in kidney, liver, gills and muscle.

HISTOPATHOLOGICAL, HEMATOLOGICAL AND MOLECULAR STUDY IN LOCAL CARP (*CYPRINUS CARPIO*) EXPOSED TO ALONE AND COMBINED FIPRONIL AND BUPEROFEZIN**Aasia Bibi and Irfan Zia Qureshi***Laboratory of Animal and Human Physiology, Department of Animal Sciences, Quaid-i- Azam University 45320, Islamabad, Pakistan.*

Fipronil an insecticide which disrupts GABA receptors in the central nervous system causes the death of target insects, highly toxic to many aquatic species and alters the normal function of the endocrine, neuromuscular and reproductive systems. Buprofezin is insect growth regulator and disturbs the normal function of the endocrine, developmental and reproductive systems. The study was conducted to evaluate the toxic effects of buprofezin and fipronil alone and in combined form on hematological, histopathological, molecular and biochemical parameters in common carp (*Cyprinus carpio*). Sixty fishes were divided into four groups; three were treatment groups while one was control group. Fipronil alone group treated with dose of 400 g/L, Buprofezin alone group with 100 mg/L while combined group received 200 g/L fipronil and 50 mg/L buprofezin. After completion of 96 hrs blood samples were collected for complete blood picture, DNA extraction, smear preparation, serum separation and tissues for histomorphological study. One way ANOVA was applied to compare the results between groups. Results demonstrated significant effect of Fipronil on serum total protein, globulin and serum glucose concentrations ($P < 0.0001$, $P < 0.0001$, $P \square 0.002$), while no significant change was observed in albumin level. RBC counts and hematocrit percentage decreased significantly in all treatment groups ($P \square 0.0001$, $P \square 0.002$), hemoglobin concentration and platelet counts decreased significantly in fipronil alone and combined treatment groups ($P \square 0.001$, $P \square 0.01$), while WBC counts increased significantly in all treatment groups ($P \square 0.0001$). DNA damage was noticeable in buprofezin alone and combined treatment groups. Histological changes included nuclear degeneration, nuclear hypertrophy, cytoplasmic vacuolation and cellular hypertrophy, eosinophilic granules in cytoplasm, cellular atrophy, karyolysis, and melanomacrophages aggregate were observed in liver, kidney and gills of all treated group. The present study provides evidence that fipronil and buprofezin in both alone and combined states cause significant and physiologically relevant hematological, biochemical, molecular and histopathological changes in common carp.

GENOTOXIC SUSCEPTIBILITY AND BIOMARKER RESPONSES OF *LABEO ROHITA* IN POLLUTION SPECTRA**Bilal Hussain,^{1,2} Tayyaba Sultana,¹ Salma Sultana,¹ Shahid Nadeem³ and Shahid Mahboob^{1,4}**¹*Department of Zoology, Wildlife and Fisheries, Govt. College University, Faisalabad, Pakistan.*²*Department of Bioinformatics and Biotechnology, Govt. College University, Faisalabad, Pakistan.*³*Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan.*⁴*King Saud University, Riyadh, KSA.*

Comet assay and micronucleus assays with fish blood have shown to be practical approaches for genotoxicity testing and monitoring of water quality. These assays were used to assess DNA fragmentation in *Labeo rohita* collected from polluted areas of River Chenab. Cd, Cu, Mn, Zn, Pb, Cr, Sn and Hg were detected by atomic absorption spectrophotometry and found far more than the WHO permissible limits. Comet assay showed significant ($p < 0.05$) DNA damage in

Labeo rohita as 42.21±2.06%, 31.26±2.41% and 21.84±2.21 % DNA in comet tail, tail moment as 17.71±1.79, 10.30±1.78 and 7.81±1.56, olive moment as 13.58±1.306, 8.10±1.04 5.88±0.06, respectively from three different sites of the polluted area indicating significant dilution along the length of the river. Significant (p<0.05) differences were reported between polluted and farmed fish but non-significant (p>0.05) differences in farmed and upstream. Micronucleus assay showed similar findings of single micronucleus induction as 50.00±6.30, double 14.40±2.56 and nuclear abnormalities 150.00±2.92/ thousand cells. These high frequencies are the cause of reduction of 96% of population of this fish species in experimental area of the River Chenab. These findings infer that these novel fish DNA damage assays to detect genotoxicity could be used as expedient toxicity screening of aquatic environments. Due to sweeping extinction *Labeo rohita* showed the highest sensitivity for pollution and could be used as bioindicator and DNA fragmentation in this fish species as biomarker of pollution load.

**SHIELDING EFFECT OF WITHANIA SOMNIFERA (SOLANACEAE) ON
HEMATOLOGY AND SEROLOGICAL PROFILE OF FRESHWATER FISH *LABEO
ROHITA* AGAINST HEAVY METAL (MIXTURE) TOXICITY**

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The shielding effect of methanolic extract of *Withania somnifera* (Solanaceae) was analyzed against heavy metals induced toxicity in freshwater fish *Labeo rohita*. The metal mixture @ of 20mg/kg body weight was used to induce toxicity in fish of 400-500g body wt. The plant extract @ 200mg/kg and 400mg/kg body wt. was employed by dissolving in water on daily basis for a period of eight weeks. The hematology and serological profile of fish was analyzed. The fish exposed to metal mixture showed a significant decline in Hemoglobin (Hb), red blood cell count (RBCs), white blood count (WBCs) and packed cell volume (PCV). An increase in Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and acid phosphatase (APs) was observed. The present study verified the hypothesis of daily administration of WS plant extract against adverse effect of metal mixture intoxication in fish.

**RESPONSE OF ANTIOXIDANT ENZYMES OF FRESHWATER FISH *CHANNA
MARULIUS* AND *WALLAGO ATTU* TO ACUTE METAL (Cr, Cu, Cd and Pb)
EXPOSURES**

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Antioxidant systems are known to be sensitive to metal exposures and are suggested to use in predicting acute metal toxicity. In this study, several antioxidant enzymes, such as catalase

(CAT), superoxide dismutase (SOD) and Peroxidase (Px) were measured in the liver, kidney and gills of *Channa marulius* and *Wallago attu* exposed to (96 h) different concentrations of metals (Cr, Cu, Cd and Pb). Results indicated that superoxide dismutase activity increased with increasing concentrations of metals mixture. *Channa marulius* and *Wallago attu* liver, gills and kidney showed statistically significant differences for superoxide dismutase activity. Catalase activity in liver, kidney and gills of *Channa marulius* and *Wallago attu* were noted as 79.282 and 90.767 U/ml; 76.165 and 86.220 U/ml; 72.483 and 69.841 U/ml, respectively. The peroxidase activity in *Channa marulius* kidney was maximum followed by that of gills and liver. However, activity of peroxidase in liver, kidney and gills of *Wallago attu* were noted as 0.412, 0.261 and 0.238 U/ml, respectively. Results indicated that there were variations in responses of the enzymes to metal exposures, depending upon tissues and metals types. This study emphasized that the antioxidant enzymes are very sensitive to metals as their activities altered significantly, suggesting they could be helpful in predicting metal toxicity and useful as an early warning tool in natural monitoring studies.

CLINICO-BIOCHEMICAL CHANGES INDUCED BY ARSENIC AND COPPER SULPHATE IN ADULT BIRDS

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The present trial was conducted to determine the clinico-heamatological and physiological impacts induced by concurrent oral administration of arsenic and copper sulphate in adult male birds. After acclimatization a total of 28 adult male birds were divided randomly and kept in 7 groups of four birds each. For 30 days all the experimental birds received arsenic and copper sulphate alone and in different combinations. Blood samples were collected from each bird at days 10, 20 and 30 of the experiment. Various clinical signs like decreased feed intake, body weight, ruffled feather, depression, dullness, ocular discharge, open mouth breathing, diarrhea and pale comb were observed at higher levels of arsenic and copper sulphate. Physical parameters feed and BTW indicate that groups F and G showed significant higher ($P < 0.05$) values at day 10, 20, 30. Absolute weight of Liver testes, kidney, spleen, lungs trachea and thymus were higher in group F and G while proventricular relative weight was significantly ($P < 0.05$) higher in F and G groups. In biochemical parameters AST, TRIG, CHOL, Creatine-Kinase monobasic (CK-MB) showed significantly ($P < 0.05$) higher values in groups E, F and G at days 10, 20, and 30 for these parameters. ALP and MDA levels are significantly ($P < 0.05$) higher in D, E, F and G at days 10, 20 and 30. While groups G showed significantly ($P < 0.05$) increased levels at days 20 and 30 only in treated birds. From the results of this study it can be concluded that arsenic and copper sulphate alone at higher levels and in combination even at lower levels poses serious clinico-biochemical and physiological effects in avian species.

ANALYSIS OF HEAVY METALS IN RIVER SWAT KPK, KHYBER PAKHTOOKHWA, PAKISTAN

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In the present study the extent of heavy metals contamination such as iron, copper, lead, manganese and zinc in the water of river Swat has been assessed. Fresh water contaminations pose several serious risks to human health. River Swat serves as a main source of drinking water, agricultural activities and fishing in Swat and adjacent areas. Since there are no formal control measures of wastes into the river Swat, it is important to study the metals contaminant in it, thus assessing its suitability for domestic and agricultural use. In the present study different physico-chemical parameters and heavy metals in water in ten different sites of river Swat, Pakistan were evaluated. For this, the water samples were collected from each sampling station from January 2011 to January 2012. The heavy metals recorded in water are Cu (-0.05)–(0.02) ppm, Zn range 0.00–0.24 ppm, Pb (0.14)–(0.11) ppm, Mn (-0.02)–(0.01) ppm and Fe 0.65–0.02 ppm. The heavy metals concentration in water samples are below the permissible level set by WHO and FAO, which indicates that it is fairly suitable both for aquatic fauna and human use. The minor effect might be due to human made actions and the domestic sewage which were supplementary from time to time in the river and are altering the water quality of the river which were not suitable for fish and other aquatic life. Thus the fish species harvested in river Swat are safe for human consumption.

BIOCHEMICAL EFFECTS OF FENVALERATE ON THE FISH (*APHANIUS DISPAR*)

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Agricultural pesticides are well recognized as an economic means to control pests, but at the same time such chemicals are highly toxic to other species in the environment. Most of pesticides find their way into rivers, lakes and pond and eventually into the marine environment. The aim of this study was to assess the toxicity of fenvalerate (synthetic pyrethroid pesticide) on *Aphanius dispar* (killifish). The enzymes acetyl cholinesterase (AChE), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), acid phosphatase (ACP) and alkaline phosphatase (ALP) were used to assess the biochemical effect of fenvalerate in fish juveniles using enzyme analysis kit. The results depict both elevation and inhibition of enzymes in the fenvalerate treated fish as compared to control. Fenvalerate showed increases in the ALT and ALP activity, its values were significantly ($p < 0.05$) different from control value. While the activity of AChE levels decreased (92%) significantly ($p < 0.05$) and also AST activity levels decreased in fenvalerate treated fish by 84%. The activity of LDH and ACP decrease significantly ($p < 0.05$) by 92% and 97% respectively in fenvalerate treated fish. High sensitivity of marine organisms to these pollutants may have direct implications on the biodiversity including fish diversity.

STUDIES ON THE TOXIC EFFECT OF IRON, ZINC, LEAD AND NICKEL MIXTURE TO THE FISH, *CIRRHINA MRIGALA***Huma Naz^{1*}, Sajid Abdullah¹, Khalid Abbas¹, Wardah Hassan¹ and Moazama Batool²**¹*Department of Zoology, Wildlife & Fisheries, University of Agriculture, Faisalabad, Pakistan.*²*Department of Zoology, Government College Women University, Faisalabad, Pakistan.*

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Acute (96-hr LC₅₀ and lethal) toxicity test were conducted at constant water pH (7.5), temperature (30 °C) and hardness (225 mg L⁻¹) to study the effect of iron, lead, zinc and nickel on mixture on fish, *Cirrhinus mrigala*. The differences among replicates used for 96-hr LC₅₀ and lethal metals (iron, lead, zinc and nickel) mixture concentrations were showed statistically non-significant differences. The mean 96-hr LC₅₀ and lethal values were computed as 42.56±2.47 and 81.53±7.04 mg L⁻¹, respectively. However, the difference between LC₅₀ and lethal concentration was significantly different. The oxygen consumption by the fish increase as the metals mixture concentrations by the test medium and duration of exposure increased. Ammonia excretion by the fish increased concomitantly along with escalation in metals mixture concentrations of the test mediums. Sodium showed positive correlation with potassium and calcium while it had negatively significant correlation with magnesium. Potassium had positively significant relationships with calcium but negatively correlated with magnesium.

STUDIES ON 96-HR LC₅₀ AND LETHAL TOXICITY OF ALUMINIUM AND ZINC FOR *CIRRHINA MRIGALA***Shakeela Parveen, Muhammmad Javed and Khalid Abass***Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad*

The present experiments were performed to determine the tolerance limits of *Cirrhina mrigala* of aluminium and zinc in terms of 96-hr LC₅₀ and lethal concentrations. Toxicity tests were conducted in 70 liter glass aquaria under controlled laboratory conditions at constant water temperature (32°C), pH (7.25) and total hardness (250 mgL⁻¹). Ten fish were placed in each aquarium and tested against various concentrations of aluminium and zinc, separately, with three replications for each test dose. Constant air flow was maintained in each test medium. There existed significant difference between LC₅₀ and lethal concentrations of both aluminium and zinc for 150-day old *Cirrhina mrigala*. However, fish mortality was directly proportional to the metal concentration of the test media. The 96-hr LC₅₀ and lethal concentrations of aluminium for *Cirrhina mrigala* were determined as 76.31±4.14 and 145.47±2.25 mgL⁻¹, respectively. The 96-hr LC₅₀ and lethal concentrations of zinc for *Cirrhina mrigala* were computed as 85.83±1.21 and 162.66±9.07 mgL⁻¹, respectively. *Cirrhina mrigala* showed less sensitivity to zinc, in terms of 96-hr LC₅₀ and lethal concentrations than that of aluminium. Dissolved oxygen contents of the test media decreased significantly with an increase in metallic ion concentrations of both the test media of aluminium and zinc indicating more oxygen consumption by the fish due to metallic ion stress. Total ammonia and carbon dioxide contents of the test media increased significantly with the increase in metallic ion concentrations of the fish test media.

**ACUTE TOXICITY OF WATER-BORNE NICKEL AND MANGANESE MIXTURE TO
THE FISH *CTENOPHARYNGODON IDELLA***

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Acute toxicity tests were conducted to study the toxicity of nickel and manganese mixture for *Ctenopharyngodon idella* at constant water pH (7.5), temperature (30°C) and hardness (225 mg L⁻¹) during 96-hr exposure. The mean 96-hr LC₅₀ and lethal concentrations of metals mixture (Ni+Mn) were recorded as 63.24±2.93 and 121.44±8.23 mg L⁻¹, respectively for *Ctenopharyngodon idella*. There existed non-significant difference among three replicates used for acute toxicity tests. The difference between LC₅₀ and lethal concentrations was statistically different at p<0.05. The oxygen consumption by the fish increase and dissolved oxygen contents decreases in the test mediums as the metals mixture concentrations increased. Total ammonia excretion by the fish increased concomitantly along with increase in metals mixture concentrations of the test mediums. The correlation coefficients among magnesium, metal mixture concentrations, electrical conductivity and calcium were negatively significant.

**EFFECT OF INTRA PERITONEAL INJECTIONS OF EMULSIONS OF AFLATOXIN B₁
ON HEMATOLOGY, LIVER, KIDNEY AND INTESTINAL TISSUES OF *CATLA CATLA*
FINGERLINGS**

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The study was conducted to explore the effects of Aflatoxin B₁ by intraperitoneal administration and its effect on Histopathology of *Catla catla*. A total number of 25 healthy fish weighing 150 ±10 gm were collected and maintained in glass aquaria. The fish were given commercial fish ration (fish meal, maize gluten meal, soyabean meal, sunflower meal, rice polish, molasses) analyzed without detectable Aflatoxin B₁. Aflatoxin B₁ was administered intra peritoneally beneath the pelvic fin in each *Catla catla*. Dosages prepared were 0.09 ml Aflatoxin B₁/1cc distilled water, 0.180 ml/1.5 cc, 0.271 ml/2 cc, 0.361 ml/2.5 cc, and 0.451 ml/3 cc which was here after regarded as 10 ppb, 20ppb, 30ppb, 40ppb, 50ppb, respectively. Control group, however, did not receive any Aflatoxin B₁. Blood was collected from caudal vein, gills and heart previously rinsed with 2.7% ethylene diamine tetra acetic acid solution over 24, 48, 72, 96 and 120 hours. The results showed that Aflatoxin B₁ have exerted severe changes in the blood indices of *Catla catla*. Intraperitoneal injection of 0.09 ml/1cc to 0.451 ml/3cc resulted in reduction in

haemoglobin, red blood cells, ESR, total protein, albumen, globulin and their ratios. Values of these parameters remained non-significant in control group but significant when compared all the treatments among themselves or with the control group. The maximum total protein was observed in control group which gradually declined with increase in Aflatoxin dosage and/ or with increase in exposure time. Total protein and albumin concentrations significantly ($P < 0.05$) decreased in fish fed on feed with elevated levels of Aflatoxin B₁. Aflatoxin histopathology examinations showed no obvious lesions in the control. There were drastic changes in liver hepatocytes. There were fat vacuoles of variable shape and sizes in the cytoplasm of hepatocytes. The kidneys have swollen and are dark red in colour and friable. Necrotic changes are seen in the nucleus. The intestine was completely white with red spots on it. Intestine has broken integrity of mucosa. Epithelial cells can be seen undergoing degenerative changes. In conclusion, *Catla catla* is very susceptible to Aflatoxin intoxication. If injected intraperitoneally its toxicity spread immediately to each and every part of its body, hold control of each organ, destabilize, and disrupts its function changing its products or making of its own will. The cascade of events totally damage the functioning and viability of an organ ultimately succumbing the animal.

**ANALYSIS OF HEAVY METALS IN THE MUSCLES OF THREE FISH SPECIES
COLLECTED FROM THE FISH MARKET OF QUETTA CITY OF PROVINCE
BALOCHISTAN**

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The consumption of highly contaminated foods may prove to be lethal for the human being and could also bring some genetic disorders. Therefore, this study is focused on evaluating the trace metal levels in the muscle tissues of three commercially important edible fishes *i.e.*, catfish (*Sperata seeghala*), mullet fish (*Mugil incilis*) and major carp (*Catla catla*). Fish samples were purchased once a month from the local fish market of Quetta, Balochistan. The concentrations of heavy metals like Fe, Zn, Al, Mn, Cr, Co, Hg and Pb in muscle tissues of the fishes were detected by using atomic absorption spectrophotometric method. The basic aim of our study was to detect some useful and harmful metals elements in muscles of some edible fishes collected from the different environments *i.e.*, marine, fresh water and estuaries.

**EFFECTS OF IMIDACLOPRID ON THE DETOXIFYING ENZYMES OF SPIDER
NEOSCONA THEISI (ARANEAE: ARANEIDAE)**

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Organisms are generally well prepared to cope with chemical stressors by integrating available protective systems. Spiders as secondary consumers are especially exposed to elevated level of environmental toxins such as insecticides. The defense against toxic compounds can be established in increased activity of the enzymes by neutralization and detoxification. The laboratory

studies carried out to evaluate the sublethal effects of a neonicotinoid insecticide imidacloprid on the detoxifying enzymes of spider *Neoscona theisi*. Activity of acetylcholine esterase, carboxylesterase, glutathione-S-transferase was determined against the different concentrations of purified and formulated Imidacloprid. The results showed inhibition in acetylcholine esterase, activity in spiders treated with insecticide as compared to control groups. But high rate of inhibition was recorded in spiders treated with purified Imidacloprid as compared to formulation. However high quantity of carboxyl esterase and glutathione-S-transferase was recorded in both treated groups (formulated and purified) as compared to control group. It was concluded that these detoxifying enzymes can be used as biomarkers of insecticide toxicity in the spider species.

**EFFICIENCY OF DELTAMETHRIN, CYPERMETHRIN AND EXTRACTS OF
AZADIRACHTA INDICA AGAINST ADULTS OF COLEOPTERAN GRAIN PESTS**

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By knowing the effect of different insecticide on the mortality rate of insect, different strategies can be developed to control the invertebrate insect pest of stored grain products. In this research work three pesticides; Biosal (neem formulation), Deltamethrin (pyrethroid), and Cypermethrin (pyrethroid) were tested against three species of invertebrate grain pests *Tribolium castaneum* (Herbst), *Tribolium confusum*. Jacquelin du Val and *Sitophilus oryzae* (Linnaeus). Toxicity tests were carried out under laboratory conditions by keeping time duration of 24 hours after which LD₅₀s and LD₉₀s were calculated. Processed food commodities during storage and processing can be negatively affected by Bulk stored grain and stored-product insects, like *T. castaneum*, *T. confusum*. and other insects. *T. castaneum* is a cosmopolitan pest of stored foods and it is considered as the most destructive insect pests of stored grains and cereal products throughout the world. While in all facilities used for storage and processing of plant products rice weevil *S. oryzae* is an economic pest of stored products. Basil oil has insecticidal activity against *S. oryzae*. Neem seed extract, when treated with *T. castaneum* causes the harmful effects on its different life stages. *T. castaneum* and *T. confusum* were taken from infested grains while -the third species *S. oryzae* was taken from infested crops from PCSIR. These pests were treated with different pesticides *i.e.* Deltamethrin, Cypermethrin and neem formulation Biosal were used to find out 50% and 90% lethal doses with different concentrations. In these experiments *T. castaneum* was found to be more tolerance to all insecticides including Neem products while *S. oryzae* is very sensitive against all the tested insecticides.

**PESTICIDE EXPOSURE, RISK ASSESSMENT AND HEALTH PROBLEMS AMONG
PESTICIDES-EXPOSED WORKERS OF DISTRICT GUJRANWALA, PAKISTAN**

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In developing countries like Pakistan use of pesticide is high in agriculture sector to minimize pest infestation and increase crop production. Multiple exposures to pesticides for prolonged time period causes many clinical disorders *i.e.* dermatological, hepatic, nephritic,

respiratory and other clinical disorders in agriculture spray workers. This study aimed to determine correlation between pesticides exposure and alterations of hematological indices in spray worker. Questionnaire based data was collected from randomly selected two hundred and fifty workers. Data were analyzed for their personal hygiene, physical health and laboratory examinations. Majority of spray workers were males and belonged to age group 41-50. Hematological Indices in test group were affected severely whereas platelets count was high in few persons but Hb, MCV, MCH, TLC, RBC was low and neutrophil counts was significantly in normal range. High LFT and RFT values indicate prevalence of liver and kidney dysfunction respectively in insecticide applicator. The prevalence of hematological profile alteration seems to be correlated with the frequency of exposure to pesticide. The majority of insecticide applicators were affected with vomiting, nausea, headache, skin irritation and eye irritation. These symptoms seemed to be related to frequent insecticide exposure. The use of insecticides in the agriculture sector would likely to be threatening for human health directly and indirectly. This study concluded that indiscriminate use of pesticides in farm ing environments must be regularly assessed and farm workers must be trained for safe use of pesticides.

ACUTE TOXICITY OF PESTICIDES TO *OREOCHROMIS NILOTICUS*

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Pesticides are used in agriculture are effecting aquatic organisms may cause serious ecological and health hazards. The aim of present study was to investigate acute effects of Malathion, Endosulfan and Cypermethrine on fish, *Oreochromis niloticus* in terms of 96-hr LC50. Each pesticide was tested separately to check fish mortality rate and behavior in separate tanks having 60 liter water capacity. No feed was given during experimental period to avoid stress. Both 96-hr LC50 and lethal concentration of all pesticide varied significantly at $P < 0.05$. Although the observed significant difference among acute toxicity of three pesticides Malathion (32.70 $\mu\text{g/L-1}$) Cypermethrine (15.51 $\mu\text{g/L-1}$) and Endosulfan (6.74 $\mu\text{g/L-1}$). It was concluded that *Oreochromis niloticus* showed more sensitivity towards Endosulfan pesticide.

SECTION – I I

PESTS AND PEST CONTROL

STUDY ON DIFFERENT COMPONENTS OF RESISTANCE IN WHEAT GENOTYPES TO GREEN BUG (*SCHIZAPHIS GRAMINUM*) (RONDANI)

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The greenbug (*Schizaphis graminum*) (Aphididae: Homoptera) is among major wheat aphid species in Pakistan, and is reported as a wheat pest from most parts of the world. Host plant resistance is a strong pillar in Integrated Pest Management (IPM) program against various pests. Several greenbug resistant genes have been identified and introduced in commercial high yield cultivars of wheat to manage greenbug. Three tests were conducted to study resistance (Antixenosis, Antibiosis and Tolerance). During a preliminary test, only ten entries showed good response and were further tested for category of resistance. In Antixenosis test, greenbug showed no preference toward any of the tested genotypes. Antibiosis resistance were identified to greenbug in genotype 'MPT-V5', where minimum number of progeny (31.1 nymphs) were produced by greenbug and maximum time (8.5 days) was taken by the aphid to produce its first offspring. The percent dry weight change (DWT) was found maximum in 'MPT-V13' for both shoots (57.2) and roots (51.7), While, minimum percent dry weight change were found in 'MPT-V33', (shoots = 28.8 and roots = 22.7). The genotype 'PR-102' showed least tolerance with maximum Tolerance Index (shoots = 0.285 and roots = 0.247) and, genotype 'MPT-V33' was relatively tolerant among the tested genotypes with minimum Tolerance Index (shoots = 0.138 and roots = 0.109).

BIOLOGICAL CONTROL OF BERRY BUGS (HOMOPTERA: PENTATOMIDAE) WITH DIFFERENT PESTICIDES

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Three pesticides Biosal (neem extract), Cypermethrin and Deltamethrin were tested against three species of berry bugs *Halys fabricii* Fabricius, *Salixacaris sindellus* Ahmed and Kamaluddin, *Halys sulcatus*. (Thunberg). Regression models for toxicity of insecticide and test species were also developed to calculate the lethal concentrations after 24 hours of treatment LC_{50s} were calculated followed by LC_{90s} against test species. For the present work all the three bug species *i.e.* *S. sindellus*, *H. fabricii* and *H. sulcatus* were collected respectively from their host plants and brought to the laboratory. The toxicity test on bugs was carried out in the Department of Zoology, Jinnah University, Karachi. Specimens of all the species were taken more or less same age, size and weight. Botanical pesticide from neem tree Biosal, (neem formulation), Cypermethrin and Deltamethrin were used in toxicity tests. The tests were conducted in plastic jars with 10 insects in each jar having ratio of 5 males and 5 females. Each three species were treated with selected concentrations of pesticide. The mortality rate was recorded after 24 hours. The data was subjected

for calculating the LC_{50s} and LC_{90s} for tested insecticide by developing the regression models. Various types of bugs' species have been affecting the plants worldwide. Species of *hay lines* known as berry bugs, usually attacks berry, apple, and mulberry. Ahmed *et al.* (1997) studied the effect of RBa (neem extract) on the toxicity and nucleic acid contents in *H. dentatus* (= *H. fa bricii*); for the first time in Pakistan. Due to their continuous effects in food and environment, their negative impacts are felt and there is need to find alternatives which are effective in pest control. In Oregon, where *H halys* was first detected in 2004, the pest has become common in urban areas interspersed among high-value specialty crops that are known host plants. *H. halys* has been found in caneberry, winegrape, and hazelnut crops in Oregon's Willamette Valley, and it is beginning to cause crop injury in the region (Shearer and Wiman 2014). In this experiment *H. sulcatus* was found to be more tolerance to all tested insecticides including Neem product

DIFFERENTIAL FEEDING BEHAVIOUR OF RUSSIAN WHEAT APHID, *DIURAPHIS NOXIA*, BIOTYPES ON WHEAT AND BARLEY GENOTYPES MONITORED USING ELECTRICAL PENETRATION GRAPH RECORDING

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The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae), is globally a major pest of bread wheat, *Triticum aestivum* L. and barley, *Hordeum vulgare* L. Probing and feeding behaviour of *D. noxia* biotype 1 and 2 was measured on *D. noxia* resistant and susceptible genotypes of wheat and barley using waveforms produced during Direct Current Electrical Penetration Graph recording. The parameters related to the sieve element phase of feeding indicated significant differences among the genotypes and across biotypes, and differed by host plant species and plant resistant mechanism. Wheat 'H871' resistance to probing behaviour of both *D. noxia* biotypes appeared to be related to leaf cellular factors and sieve element factors. Barley resistance was found to be related to factors occurring in intercellular regions as well as the sieve element, as evidenced by significantly longer stay on resistant genotypes compare to susceptible genotypes by *D. noxia* spp.

CODLING MOTH (*CYDIA POMONLLA* L.) MANAGEMENT USING COMBINED TREATMENT OF INSECTICIDE, TRAP CATCHES AND DEGREE DAYS IN HIGH LAND QUETTA

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The present study was conducted in apple plot at Sariab, Quetta to evaluate the number and proper time of insecticides (Lorsban 40 EC, the Insect Growth Regulator (IGR), Match 50 EC and Talstar 10 EC) application against codling moth larvae during 2010 and 2011. The moth activity in the trial plot was monitored using pheromone (delta) traps. Degree days (°DD) was calculated to

examine their effect on flight activity of the moth using 10°C as lower developmental threshold (base temperature). Result of analyzed data showed highly significant differences between generations and treatments at P- value < 0.01 in 2010 and 2011. All the three products were found superior than control (un-treated) in reducing the percent infestation of codling moth in apple field. A significant difference was observed between Lorsban and Talstar for three timely sprays in 2010. Treatments with Lorsban at 80% petal fall gave efficient control than peak emergence and 5 moths trapped per week methods in both years of this study. Flight activity of first and second generation moth in pheromone trap was observed at 97.04 and 663.04 °DD in 2010 respectively. First flight of the successive two generations in 2011 was recorded at 97.66 and 707.93 °DD respectively. The overall population of moth captured in pheromone traps was higher in the first year than in the second year. This study has found that 3 to 4 timely sprays (combined with trap catches and °DD) per year effectively control the larval infestation of *C. pomonella* in field. The codling moth (Lepidoptera: Tortricidae) is well known as a common and world wide pest. The larvae feed on the fruit of a wide range of host plants including apple, pear, quince, and walnut. CM completes 2-3 generations annually, depending on locality and length of growing season. It is the major fruit-feeding pest in fruit growing regions of Balochistan. It is also a significant pest in the East, but has generally been managed by sprays used to control plum curculio and apple maggot. However, with the advent of trapping-based sprays for apple maggot, and a potential decrease in cover sprays, growers may begin to see more CM damage.

INSECTICIDAL ACTIVITY OF RED SEAWEEDS AGAINST PESTS

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Marine environment is the richest natural source, which provides food in form of fish, shellfish and seaweeds. Marine plants live in aggressive, competitive, exigent environment in many aspects which are entirely different from terrestrial plants, therefore, this situation demand the production of quite specific and potent active natural constituents in marine plants. Seaweeds are valuable source of bioactive natural metabolites, which are widely explored in traditional medicine and therapeutic applications, and as fertilizers and insecticides. More than 2400 natural products have been reported from seaweeds. Such botanicals, when used as pesticide, are found specific to the pests and are biodegradable and environment friendly. Certain natural constituents like terpenoides, acetogenins, phenols, tannins, etc. have strong insecticidal and repellent activity. As widespread use of chemical pesticide produces harmful or toxic effects on non-targeted organisms, kill beneficial insects, produce resistance among insects, reason for pest outbreaks and can produce toxicity and environment hazards. Nearly thousand species of insect are somehow related to stored commodities. In the present study insecticidal activity against pest was evaluated, and their antifeedant and larvicidal activity was observed using leaf disc (4 cm), impregnated in different concentration of extracts of four red seaweeds (Rhodophycota), collected from the Karachi coast. No-choice method with 24 hr observation was used. Briefly fresh leaves were collected, sorted, cleaned and extracts were prepared using Soxhlet extraction in solvents of varying polarity. The activity was also compared and related with other bio-pesticides.

EFFICIENCY OF DIFFERENT PESTICIDES FOR THE CONTROL OF COTTON JASSID

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The efficacy of six insecticides *i.e.*, Thiacloprid 48SC, Nitenpyram 10SL, Thiamethoxam 25WP, Acephate 75SP, Chlorfenpyr 360SC and Dimethoate 40EC were evaluated for jassid on MNH-886 variety of cotton sown in 2014 at Adaptive Research Farm, Vehari. The results showed that each treatment had significant difference in their effectiveness with one another. Nitenpyram 10SL was most effective 70 to 80% mortality of jassid population after 24 hours to 168 hours, when sprayed to cotton field crop, whereas, Thiacloprid 48SC, Acephate 75SP, Chlorfenpyr 360SC and Dimethoate 40EC were showed their controlling effect 30 to 50% against jassid population after 24 hours to 72 hours, but their efficacy was 20-25% after 168 hours. The jassid population mortality was noted less than 20% after 24 hours by applying Thiamethoxam 25 WS and same up to 72 hours. It is concluded that Nitenpyram 10SL is the most effective for the controlling of jassid on cotton crop among the above six tested insecticides.

EVALUATION OF VARIETAL RESISTANCE AND EFFICACY OF THREE INSECTICIDES AGAINST WHEAT APHID (*SHIZAPHIS GRAMINUM R.*) UNDER FIELD CONDITIONS

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A field trial was carried out to evaluate the varietal resistance in four wheat cultivars (Galaxy-2013, Punjab-2011, Millat-2011 and Sehar-2006) and efficacy of three synthetic insecticides (Pyrethrozine 50 WG @ 200 gm ha⁻¹, Imidacloprid 20 SL @ 625 ml ha⁻¹ and Lambda-cyhalothrin 2.5 EC @ 625 ml ha⁻¹) against wheat aphid (*Shizaphis graminum* Rond.). All wheat cultivars were attacked by aphid; however, Galaxy-2013 appeared to be the most resistant with minimum infestation *i.e.* 59.4 aphids per plant, during Mid-March when aphid population was at peak. Increase in temperature and relative humidity to a certain limit favored aphid multiplication; however, temperature above 29°C negatively affected aphid population. All insecticides significantly reduced aphid infestation; however, on numerical basis Imidacloprid 20SL appeared to be the most effective as it recorded minimum infestation (1.25 aphids per plant) and maximum percentage mortality *i.e.* 97.13%, fourteen days after application. Tenacity of imidacloprid was also justified by wheat grains analysis through HPLC technique for insecticides residual toxicity with maximum limit of detection *i.e.* 0.005 mg kg⁻¹. Maximum grain yield (5.58 t ha⁻¹) was recorded by Galaxy-2013 in plots treated with Lambda-Cyhalothrin. Nevertheless, highest yield losses were observed for Sehar-2006 (6%) as compared to control.

**CONSERVATION OF BIOLOGICAL CONTROL USING ENTOMPATHOGENIC FUNGI
AGAINST ACRIDID FAUNA FROM SINDH**

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Conservation biological control relies on modification of the environment to protect and encourage natural enemies that are already present within the system, thereby enhancing and improving their ability to control pest populations in a reliable way. At the present Entomopathogenic fungi includes *Aspergillus fumigates* Fresenius (1863) was tested in the colonies of Acridid maintained under laboratory condition. The acridid population was consist on 04 sub-families i-e Cyrtacanthacridinae, Calliptaminae, Eypreocnemidinae and Oxyinae were treated with the spore formulation of *A. fumigates*. It was found that this bio-pesticide gave significant result against reduction of acridid population in cages. Present study recommends that entomopathogens is widespread in the natural environment and cause infections in many pest species and it should be used at the commercial level. Present study provides the basic guideline for utilization of this.

**INVESTIGATION ON THE IMPORTANT PARASITIDS OF ORDER DIPTERA FROM
SINDH**

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Most important parasitoids of order Diptera was investigated from this region. It was found that amongst dipteran parasitoids families Tachinidae stand out as the most significant. All members of family Tachinidae attack on the larval stages on insects present in field usually they attack on pests of economic importance that cause significant damage to cultivated crops and forests. tachinds having variable general coloration i-e grayish, creamy along with black striped. Overall it was found that tachinds have been introduced and successfully established in field of biological control as bio-control agent.

**SUSCEPTIBILITY OF STORED SEMI-DRY AND DRY VARIETIES OF DATE-PALM
FRUIT OF SINDH TO THE INFESTATION BY *ORYZAEPHILUS SURINAMENSIS* (L.)
COLEOPTERAN PEST IN RELATION TO DATES CHEMICAL COMPOSITION**

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The date palm fruit is a very important source of nutrients and cash crop of Sindh, Pakistan. The climatic conditions of Sindh are ideal for cultivation of date palm. There are many varieties of date palm cultivated in Sindh but commercially important varieties are Kupro, Karbalian, Aseel, Fasly and Dadhi. During storage process these varieties are infested by Saw-tooth grain beetle

Oryzaephilus surinamensis (L.) which cause severe damage to these stored varieties consequently these could not export to advanced countries. During present study the infestation and feeding behavior of Saw-tooth grain beetle *Oryzaephilus surinamensis* (L.) was observed on Kupro, Karbalian, Aseel, Fasly (semi-dry dates) and Aseel and Dadhi (dry dates) in relation to sugar, moisture and ash contents. The whole study carried out in laboratory from October 2013 to April 2014. The maximum mean infestation of Saw-tooth grain beetle was recorded 40.42 Mean percentage on Kupro while the minimum mean infestation was recorded 22.85 on Aseel and Dadhi (dry dates). The highest sugar content and moisture percentage observed in Kupro 85.90% and 23%. Whereas lowest percentage was 65.50% and 10% in Aseel and Dadhi dry varieties. The mean infestation is positively correlated with sugar and moisture content. The maximum survival rate of Saw-tooth grain beetle was recorded 88% on Kupro and lowest survival rate 46.51% on Dadhi variety of date palm. Present study revealed that Kupro and Karbalian semi-dry varieties are more attractive to Saw-tooth grain beetle as compared to Aseel and Dadhi dry varieties. The reason of attraction was high sugar and moisture contents.

TEMPERATURE-TOXICITY RELATIONSHIP OF DIFFERENT INSECTICIDES IN HOUSE FLIES

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Mechanical transmission of pathogens by house flies, *Musca domestica*, is believed as the most effective route of disease transmission in humans. Although the use of insecticides as a preventive measure is common worldwide for the management of house flies, success of the measure could be compromised by the prevailing environmental temperature since it significantly affects toxicity of insecticides and thus their efficacy. Peaks of the house fly density and house fly-borne diseases are usually coincided and season specific, yet little is known about the season specific use of insecticides. To determine the temperature-toxicity relationship in house flies, the effect of post-bioassays temperature (range, 20-34°C) on the toxicity of seven insecticides from organophosphate (chlorpyrifos, profenofos), pyrethroid (cypermethrin, deltamethrin) and new chemical (emamectin benzoate, fipronil, spinosad) classes was evaluated by using a feeding bioassay method. From 20-34°C, the toxicities of chlorpyrifos, profenofos, emamectin and fipronil increased. Whereas, the toxicities of cypermethrin, deltamethrin and spinosad decreased. These findings suggest that for the reduction in house fly-borne diseases, house flies should be controlled with insecticides according to the prevailing environmental temperature.

FIELD EFFICACY OF DIFFERENT INSECTICIDES AGAINST MAIZE STEM BORER *CHILO PARTELLUS* SWINHAE (PYRALIDAE: LEPIDOPTERA)

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A field trial was carried out to evaluate the efficacy of four synthetic (Carbofuran 3G at 20 kg ha⁻¹, Cypermethrin 10EC at 625 ml ha⁻¹, Endosulfon 35EC at 1250 ml ha⁻¹ and triazophos 40EC at 2500 ml ha⁻¹) and one bio-insecticide (Emamectin benzoate 1.9% EC at 500 ml ha⁻¹) on hybrid

maize. All insecticides significantly reduced maize stem borer (MSB) infestation; however, on numerical basis Carbofuran 3G appeared to be the most effective insecticide against maize stem borer (MSB) as minimum infestation (0.66%) of MSB was recorded, 168 h after 2nd application. Number of dead hearts and damaged leaves were also significantly minimized by all insecticides. Moreover, Cypermethrin gave 100% control of larvae of MSB, 96 h after 2nd application. Likewise, larvae were completely controlled by Carbofuran and Endosulfan 168 h after 2nd application. Higher number of grains per cob (72%) and 1000-grain weight (24%) were recorded in insecticide treated plots as compared to control. Maximum grain yield (7.99 t ha⁻¹) was noticed in plots treated with Carbofuran. Thus, all synthetic-insecticides as well as bio-insecticide used in the study was effective against MSB and can be used in maize crop to control maize stem borer and increase grain yield.

ESTIMATION OF LIFE CYCLE AND FORAGING EFFICIENCY OF *PIERIS BRASSICAE* ON FOUR CROP PLANTS

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Photosynthesis is directly related with net primary production. The herbivores decrease photosynthesis and cause decreases in production. Lepidopterans are the main problem in loss of crop production. The economic importance of Lepidoptera is entirely due to their larval activities and chewing mouth parts. Quantitative analysis of consumption and utilization of host plants by insect herbivores is a commonly used tool in studies of plant-insect interactions. The cabbage butterfly *Pieris brassicae* is one of the most destructive insect pests, damaging various crops at all the stages ranging from seedling through flowering. The young caterpillars feed gregariously on leaves, resulting in defoliation of plants. So, *P. brassicae* was taken to know its response on four crop plants namely, brassica, cabbage, cauliflower and turnip. Larvae were provided with four pre-weighed crop plants termed as feed to estimate the life cycle and extent of foraging. The length and weight of larvae along with weight of feed was calculated on a daily bases and feed consumption was calculated by subtracting weight of water loss and initial weight from feed consumed. Cages were cleaned and larvae were given fresh pre-weighed feed on daily basis. This practice was continued until the larvae stopped feeding and converted into pupae. All the pupae were weighed and feeding index was calculated by dividing mean pupal weight with mean weight of leaf tissue consumed. After emergence adults were provided with fresh flowers and Cotton soaked with 20 % honey solution to check the longevity. Each trial was replicated three times. One-way analysis of variance with Tuckey contrast at 0.05 probability test by using Minitab was applied to check the effect of host plantation and consumption by larvae and also different life traits like length, duration of different life stages. *P. brassicae* was found to be a destructive pest species. Brassica was the most preferred diet of *P. brassicae* and it consumed 21.36±0.72g of brassica leaves during its entire larval duration. *P. brassicae* showed least consumption on turnip 8.22±0.60g with maximum larval duration of 17.67±0.47 days on brassica plants. Pupal weight of *P. brassicae* was also maximum (0.61±0.01g) when caterpillars were fed with brassica leaves followed by cauliflower (0.52±0.02g), cabbage (0.49±0.01g) and, finally, turnip (0.40±0.00g). Thus, pupal weight was directly correlated with the food consumption. A strong correlation among

pupal weight and percent adult emergence of *P. brassicae* was also observed. Percent adult emergence was maximum (90) along with pupal weight on brassica, followed by cauliflower (80), cabbage (80) and turnip (65). For larvae which fed on brassica, the adults attained a maximum body length of 45.45 ± 0.05 mm. Adult survival was also greater of 6.67 ± 0.47 days for the same crop. The quantification of damage caused by these polyphagous pests to different crops was helpful in assessing the total loss caused by these species as well as best preferred plants which could be used as an economical rearing source of these insects. Host plants, including brassica, cabbage, and cauliflower probably acted as reservoirs in the cropland for these pest species that could move to other crops to infest them. Successful management on such types of reservoir crops will be helpful in protecting other agricultural crops. However, to apply such types of strategies, there is a need for extending work on these pests to explore other possible hosts.

ESTIMATION OF DEVELOPMENTAL DURATION OF *MELANOSTOMA SCALARE* (DIPTERA: SYRPHIDAE) AND EXTENT OF PREDATION OF ITS LARVAE ALONG WITH PREDATORY PREFERENCE BY FEEDING ON FOUR SPECIES OF APHIDS IN PUNJAB, PAKISTAN.

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Aphid cause adequate reduction in yield and quality of crops. These cause the annual decrease in valuable cereal crops. Predatory interactions of (Syrphids: *Melanostoma scalare* for four aphid species: *Bravecoryne brassicae*, *Schizaphis graminum*, *Myzus persicae* and *Rhopalosiphum padi* present in the crop-system of Faisalabad, Pakistan was determined. The prey species were selected on the basis of their relative abundance from the sampled data of whole year. Hypothetical predator-prey interaction was estimated through regression analysis and highest interaction was found with *S. graminum* and *M. Persicae*. The predatory preference of *Melanostoma scalare* and impact on developmental period was examined by praying on different aphid species. All larval instars were evaluated on the basis of prey consumption under laboratory conditions. The experiment was conducted in laboratory at average temperature of $20 \pm 0.5^\circ\text{C}$, Relative humidity 60 ± 5 and Photoperiod of 16:8 h D: L. *S. graminum* was most preferred prey species with 504 ± 22.9 specimens consumed by larvae of *Melanostoma scalare*, followed by 495 ± 48.6 of *M. persicae*, 424 ± 24.6 *B. brassicae* and relatively less number of *R. padi* as 396 ± 10.03 specimens were consumed. Significant difference in predatory efficiency of *M. scalare* larvae on four aphid species was found. Shortest developmental duration of *Melanostoma scalare* larvae was on *Brevecoryne brassicae* for 9.25 ± 0.6 days and duration prolonged by feeding on *R. padi* 9.75 ± 0.35 *S. graminum* 9.83 ± 1.06 and maximum by *M. persicae* 10.3 ± 0.55 days. Predatory impact on aphids was most effective on *S. graminum*, due to their increased growth by more consumption, but significant predatory impact was found on other aphid species. Highest aphid predation was observed during 3rd larval instar of *Melanostoma scalare*. These estimated predatory impacts of this syrphid species will be supportive to efficiently control these aphid species by introducing the effective management strategies.

DEMONSTRATION AND EVALUATION OF THE YIELD POTENTIAL OF DIFFERENT INSECTICIDES AGAINST JASSID IN COTTON CROP UNDER THE AGRO ECOLOGICAL CONDITIONS OF POTOHAR ZONE

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Potohar plateau is a mountainous and rocky, covered with scrub forest, interspaced with flat lying plains. One of the major cash crops cultivated here is cotton. Jassid is an obnoxious insect pest of this locality. To manage this pest through different new chemicals a demonstration and evaluation trial of different insecticides (T₁ Control, T₂ Nytempyram (pyramid)10 SL@625ml/ha, T₃ Thiamethoxan 25WP (Actara)@60 gm/ha, T₄ Acephate 75WSP@825gm/ha, T₅ Dimethoate @ 250 ml/ha) were tested. It was found that Acephate 75 WSP @ 825 gm/ha successfully controlled jassid in an effective way and performed the best seed cotton yield due to more number of branches per plant, extra number of bolls per branch and heavier boll weight. So, it was concluded that Acephate should be preferably suggested to the farmers to manage jassid in this locality.

EVALUATION OF INSECTICIDAL POTENTIAL OF *EUCALYPTUS CAMALDULENSIS* AND *MELIA AZEDARACH* AGAINST RED RUST FLOUR BEETLE (*TRIBOLIUM CASTANEUM* HERBST)

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The red flour beetle, *Tribolium castaneum* (Herbst) is one of the severe pests of stored grain commodities. In the present studies the acetone extracts of two plant species of *Eucalyptus camaldulensis* and *Melia azedarach* were testified for their toxic and growth inhibitory action against larvae of *T. castaneum*. The plant extracts reported different responses at various exposure periods and concentrations. Highest insecticidal activity (4.68%) was achieved by plant extract of *E. camaldulensis* at maximum concentration (20.0%) after an interval of 72h. The minimum percent pupation (37.67%) and adult emergence (31.67%) was also checked by *E. camaldulensis*, while the extract of *M. azedarach* induced 51.33% and 48.67%, pupation and adult emergence respectively. Our study indicates that these plant extracts may be helpful for controlling the *T. castaneum* in stored grains.

**FIELD EVALUATION OF INSECTICIDES ON ONION THRIPS AND THEIR
SUBSEQUENT EFFECT ON BUD WORM INFESTATION IN SWAT VALLEY**

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Efficacy of three insecticides Lannate 40 SP (2.5g/lit H₂O), Karate 2.5 EC (3ml/lit H₂O) and Curacron 500 EC (4ml/lit H₂O) were tested for the control of onion pests (*Thrips tabaci*) and *Helicoverpa armigera* on onion crop in Agriculture Research Institute (ARI) during the growing season of 2011-2012. Evaluation of insecticides testing was based on average population of thrips and budworm; thousand grains weigh inflorescence/plant stalk/plant, umbel size, plant width, no. of florets/plant and stalk length. The insecticides were sprayed three times for the control of thrips and one time against budworm. Average populations of pests were recorded by counting number of alive pests on randomly selected plants 24, 48 and 72 hours intervals and on weekly basis. Results indicated that all insecticides gave significant control of both pests. However, Curacron500EC(4ml/litH₂O) proved best to keep minimum pests populations after every application as compared to other two insecticides and has least negative effect on vegetative and reproductive structure of onion bulb crop.

**COMPARATIVE TOXICITY OF IMIDACLOPRID AND PROFENOFOS AGAINST
PAPAYA MEALY BUG AT DIFFERENT pH SPRAY SOLUTIONS**

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The comparative toxicity of profenofos and imidacloprid is tested in our present research against papaya mealybug at different pH spray solutions by the help of two methods *i.e.*: Leaf dip method and Topical method. While determining the toxic effects of imidacloprid by topical method, the mortality of mealy bugs was recorded as 16.35%, 27.13%, 41.63%, 53.16% and 68.77% at 0.0004%, 0.0009%, 0.0195%, 0.0039% and 0.0078% concentrations, respectively whereas 10.33% mortality was observed in control, and 27.17%, 36.95%, 43.47%, 60.14% and 75.83% mortality was observed at 0.0004%, 0.0009%, 0.00195%, 0.0039% and 0.0078% concentrations, respectively in leaf dip method while 8% were found dead in control. During the determination of toxic effects of profenofos against papaya mealy bug, 30.6, 32.3, 36.6, 55.8, 51.6 and 86.6% mortality was recorded at 0.032%, 0.061%, 0.125%, 0.25%, 0.5% and 1%, respectively, whereas 16.6% were dead in control in topical method. In leaf dip method 12.45%, 24.90%, 36.62%, 56.41% and 82.78% mortality was observed at 0.03125%, 0.0625%, 0.125%, 0.25%, 0.5% and 9% were dead in control. LC₅₀ value for imidacloprid was 0.003% and 0.004% by leaf dip and topical method, respectively, and for profenofos was 0.245% and 0.372% in leaf dip and topical method, respectively. LC₉₀ value for imidacloprid was 0.009% in leaf dip method and 0.0104% in topical method whereas for profenofos LC₉₀ was 0.526% in leaf dip method while in topical method it was 1.018%. It was concluded that imidacloprid is more toxic than profenofos as lower concentration of imidacloprid is required to control the population of mealybugs as compared to profenofos. The mortality of mealybugs increases when pH is decreased of both tested solutions.

SUSCEPTIBILITY OF MANGO VARIETIES AGAINST FRUIT FLIES *BACTROCERA DORSALIS* (DIPTERIA- TIPHRITIDE)

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Fruit fly *Bactrocera dorsalis* is widely distributed throughout the Asia, mostly tropical and sub-tropical areas. It infested more than 250 host plant of fruit and vegetables. In Pakistan *Bactrocera dorsalis* case serious infestation in mango, citreous, guava and vegetables it causes 5-10% yield loss only in mango. Study of susceptibility of mango varieties against Fruit fly was carried out during 2014 in Laboratory conditions. Four Mango varieties (Beganpali, Sindhri, Sonara and Chunsa) were selected for study. During present study it is observed that all varieties were favorable to *Bactrocera dorsalis* to complete its all developmental stages. But infestation of fruit fly varied on all four varieties; the main reason was sugar content. The concentration of sugar attracts the fruit fly to attack.

INFESTATION OF *PECTINOPHORO GOSSYPIELLA* (PINK BOLLWORM) LEPIDOPTERA: NOCTUIDAE ON BT COTTON IN DISTRICT MATIARI, SINDH, PAKISTAN

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Cotton bollworm *Pectinophora gossypiella* (pink bollworm) is one of the major lepidopteron pests of the world and cause significant infestation throughout. In Pakistan especially in Sindh province the problem of Pink bollworm increases every year. Present study of carried out in two localities of Matiari District Halla and Saeedabd during 2014. BT cotton variety 602 was selected for experiment. The effect of the environmental factors (Humidity, Rainfall and Temperature) was also observed. During present study it is observed that the infestation of Pink bollworm started in June in both localities but it crosses the ETL from September to October. It is also observed that growers are not succeeding to get their final 3rd picking, which cause heavy loss of their yield. The problem of infestation of PBW is increased in BT a cotton varieties, especially BT 602. It is revealed that the increase ratio of infestation of PBW on BT and non BT cotton varieties which is a alarming situation not only for growers but also government.

EVALUATION OF DIFFERENT FOOD BAITS FOR KILL TRAPPING OF THE LESSER BANDICOOT RAT (*BANDICOTA BENGALENSIS*) IN FIELD CROPS OF POTHWAR AREA

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The lesser bandicoot rat (*Bandicota bengalensis*) has wide adaptation with rice-wheat-sugarcane cropping systems of Punjab, Sindh and Kheber Pukhtunkhawa and wheat-groundnut

cropping system of Pothwar area, thus inflicting heavy losses to these crops. Comparative efficacies of four food baits (onion, guava, potato and peanut butter smeared bread/Chapatti) were tested in multiple feeding tests for kill trapping of this rat at University Research Farm (Koont) located near Dhudial, Chakwal between October 2013 to July 2014 at the sowing, tillering, flowering and maturity stages of wheat, groundnut and millet crops. The results revealed that guava was the most preferred bait as compared to the rest of three, presumably due to particular taste and smell of the guava. The relative efficacies of all the four tested baits showed that guava scored the highest trapping success of 16.94 ± 1.42 percent, which was followed by peanut butter, potato and onion with trapping successes of 10.52 ± 1.30 , 7.82 ± 1.21 and 4.5 ± 1.10 percent, respectively. Crop stage/season-wise highest trapping success were achieved at maturity stages of the crops, presumably due to higher surface activity of the rat because of favorable climatic conditions, good shelter and food abundance. Moreover, the maturity stage of wheat crop coincides with spring breeding season and maturity stages of millet and groundnut match with monsoon/autumn breeding peak of the lesser bandicoot rat in Pothwar area. Among the four bait types the preferred order was guava > peanut butter > potato > onion.

TRANSMISSION OF AFLATOXICOGENIC FUNGUS IN WHEAT BY THE STORED GRAIN INSECT PESTS

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A number of insect pests have been reported to be associated with stored wheat, causing significant post-harvest losses. In addition, stored grain insect pests also play a significant role in the transmission of toxicogenic fungi by damaging the seed coat in stored wheat grains. An experiment was planned to assess the potential of the four stored wheat insect pests viz., *Cryptolestes ferrugineus* (Stephens), *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst), in the transmission of *Aspergillus flavus* (an aflatoxicogenic fungus) under laboratory conditions (25 ± 2 °C, $65 \pm 5\%$ RH). Wheat grains were inoculated with the spore suspension of the fungus and insects were introduced into the treated grains. A separate set of experiment was conducted for each stored insect species in five replicates. The results revealed that *R. dominica* and *T. castaneum* showed a highly significant correlation ($p < 0.01$) in the transmission of the fungus, whereas, rest of the two species showed non significant association. It is concluded that by managing the stored insect pests, the transmission of the fungus can be controlled in stored commodities.

PREDATORY POTENTIAL AND BIOLOGY OF GREEN LACEWING *CHRYSOPERLA CARNEA* (STEPHENS) (NEUROPTERA: CHRYSOPIDAE) ON SUGARCANE WHITEFLY

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Studies on predatory potential and biology of green lacewing *Chrysoperla carnea* (Stephes) were carried out on sugarcane whitefly, *Aleurolobus barodensis* under the laboratory condition at

26±2 °C and 65±5% R.H. The incubation period on sugar cane whitefly was recorded as 2.75±0.25 days. The larval period of 9±0.57 days with 89.75±1.25% of larval survival. The pupal period extended over 5.75±0.25 days with 81.7±1.65% survival to adult stage. Total life span was found to be 30±1.08 days for female and 22.50±1.08 days for male. *Chrysoperla carnea* female laid 322.50±4.78 eggs with 81±1.68% fertility. The predatory potential of green lacewing, *Chrysoperla carnea*, against sugarcane whitefly, *Aleurolobus barodensis* showed significant results. The maximum 115.25±4.98 nymph was consumed by the third instar with a consumption rate of 1.5018±0.039 nymphs/hour. *Chrysoperla cane* has found a promising controlling agent against sugarcane white fly.

CONTROL OF *TRIBOLIUM CASTANEUM* (RED FLOUR BEETLE) BY INERT AMMONIA UNDER LABORATORY CONDITION

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Tribolium castaneum (Red flour beetle) is a major pest in human stored grain food. Phosphine (Hydrogen phosphide) and methyl bromide are two most common insecticides that are used worldwide but the resistance of insect to phosphine insecticide has increased methyl bromide despite its high toxicity to store grain pest, it reduces seed germination and major contribution to ozone depletion. Inert ammonia has been synthesis from locally available chemicals (NH₄Cl + Na₂CO₃) that yielded a fruitful result to control the store grain pest. The adult *Tribolium castaneum* were taken with the help of camel brush from culturing jars the specimens were put into 5 desiccators. The infestation free rice grain was added. The chemicals ammonium chloride (NH₄) and Sodium bicarbonate (NaCO₃) were placed one petridish and treated with water. After 24 hours reading of each experiment were taken and LD₅₀ calculated.

PESTICIDAL ACTIVITY OF EXTRACTS OBTAINED FROM VILAYATI KEEKAR

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Prosopis juliflora Swartz DC is locally known in Pakistan as Vilayati Keekar. It is reported to contain pharmacologically active piperidine alkaloids. Mosquitoes are well known group of insects, acting as vector for many dreadful diseases such as Dengue fever is cause by *Aedes aegypti* L. Controlling these vectors by synthetic insecticides has resulted into environmental hazards

through development of insecticides resistance, accumulation of non-biodegradable chemicals in the ecosystem, biological magnification through the food chains and finally toxicity to non-target organisms, including human. In contrast, phytochemical insecticides are considered environmentally safe and biodegradable and comprise hundreds of secondary metabolites, which act synergistically as pesticide. Fresh leaves of Vilayati Keekar were collected, extracted and fractionated in solvents of varying polarity. The extracts and fractions were tested for their toxicity and repellency against 4th instar larvae and pupae of *A. aegypti* L. The LC₅₀ value, for toxicity was found to be 330, 1320, 550, 82.5, 60 and 66 ppm for methanol extract, aqueous, ethyl acetate, chloroform, benzene soluble and benzene insoluble fractions, respectively. The control and the check were < 5 %. The repellent activity of extracts and fractions was assayed against the adult mosquitoes by introducing hand method in front of 200 mosquitoes. Methanol, ethyl acetate and hexane extracts showed 90, 90 and 88 % repellency up to 10 hours respectively. Benzene soluble and insoluble fractions had the strongest repellent activity, with 82 and 80% repellency up to 10 hours. Chloroform, ethyl acetate, and aqueous fractions gave the repellency up to 8, 6, and 1 hours, respectively. The objective of the study is to find out new, cheap and available sources, which can lead to the development of natural pesticides.

PREVALENCE OF INSECT PESTS IN RICE FIELDS OF DISTRICT KAMBER SHAHDADKOT, SINDH

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This study describes geographical prevalence and spread of various insect pests on rice crop in district Kamber Shahdadkot which is one of the main rice growing area of Sindh in agricultural sector. Experimental work based on collection of different insect pests, their preservation in entomological laboratory, Identification methodology, statistical evaluation of collected species and recognition of their pest status as major or minor pest in said locality was carried out to meet valuable results. Study was carried out in months of September and October 2013, a favourably hot and warm period for growth of rice crop in district Kamber Shahdadkot. Many species of insect pests were collected such as Rice leaffolder *Cnaphalocrocis medinalis* Guenee, 185, Rice skipper *Pelopidas mathias* Fabricius 1798, Rice hairy caterpillars *Psalis pennatula* Fabricius 1793, Grasshoppers *Oxya velox* Fabricius 1787, Locust *Schistocerca gregaria* Forsskal 1775, Katydid *Conocephalus longipennis* Haan 1842, Rice Armyworm *Spodoptera litura* Fabricius 1775 whose invasion disclose their crop affinity, geographical distribution and climatic favour. Previous studies in Sindh and specially Punjab revealed presence of rice Stem borer as major pest that was not continuous finding in recent study due to effective use of chemical control methods and proper pest management procedures taken by the farmers to get rid of Stem borer while Leaffolders were observed as major pest along with group of other major and minor pests.

BIOLOGICAL CONTROL OF INSECT PESTS THROUGH ENTOMOPATHOGENIC NEMATODES IN PAKISTAN

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Insects are the most diverse species of animals living on earth. Herbivorous insects are responsible for destroying one fifth of the world's total crop production annually. Numerous insect pests on many different crops are being controlled by Entomopathogenic nematodes (EPN), including caterpillars, white grubs, root weevils, flea beetles, mintroot borer. EPNs have several important attributes that make them excellent competitors for biological control of soil insects. These nematodes can be produced by *in vivo* by baiting technique on insects and commercially by *in vitro* solid/liquid culturing. Utilizations of EPN has elevated intense interest and has been a growing concern globally mainly because of its potential efficiency. In Pakistan extensive work has been carried out on the isolation, distribution taxonomy, biology and efficacy of Entomopathogenic nematodes and as a result six new species viz., *Steinernemapa kistanense* Shahina *et al.*, (2001), *S. asiaticum* Anis *et al.*, (2002), *S. maqbooli* Shahina *et al.*, (2013), *S. bifurcatum* Shahina *et al.*, (2014) *S. balochiense* Shahina *et al.*, (2015) *Heterorhabditis pakistanense* Shahina *et al.*, (2015) were described while seven known species *S. abbasi*, *S. siamkayai*, *S. feltiae*, *S. carpocapsae*, *S. litrolae*, *H. indica* and *H. bacteriophora* were recovered for the first time from Pakistan. The genetic diversity and phylogenetic analysis of all isolates of *Steinernema* and *Heterorhabditis* were investigated on the basis of ITS-1-5.8S-ITS-2, 12S rDNA mitochondrial gene, D2D3 and 28S and got 160 accession number from NCBI USA Gene Bank and construct a genomic library. By the output of these researchers Intellectual Property Organization of Pakistan grant one International and twelve national patents. This paper highlights the mass production, and utilization of EPN as microbial bio-pesticide in bio-intensive pest management programme.

RESISTANCE CATEGORIES IN DIFFERENT LOCAL AND COMMERCIAL TRITICUM AESTIVUM CULTIVARS TO RHOPALOSIPHUM PADI

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The bird cherry-oat aphid (*Rhopalosiphum padi* L.) is a major pest of wheat (*Triticum aestivum* L.) crop in Pakistan, and many other countries around the globe. It causes significant production losses to the wheat crop in Pakistan. A number of genes from the conventional wheat cultivars that are resistant to the attack of *R. padi* have been incorporated in the commercial wheat cultivars so far to help manage the problem of *R. padi*. This study based on host plant resistance, one of the foundations of *R. padi* management programs. Thus, this laboratory study evaluates antixenosis, antibiosis and tolerance of commercial wheat cultivars (Pir Sabaq-2004, Atta-Habib, Saleem-2000, Serin-2010, Khyber-87, Sarhad-82, and Khushal-69) in three separate experiments. The selected wheat cultivars were tested for antixenosis, antibiosis and tolerance against the *R. padi* using the modified method of Flinn *et al.* (2001). Antibiosis resistance was identified to *R. padi* in

Serin-2010, where a minimum number of *R. padi* progeny 14.3 nymphs were produced in 11.4 days. The wheat cultivar (Atta Habib) was found to be significantly susceptible to *R. padi* attack as it produced the highest nymphs 26.1 in just 7 days. The other wheat cultivars (Pirsabaq-2004, Saleem-2000, Atta-Habib, Khushal-69, Sarhad-82 and Khyber-87) also did not show any antixenosis or tolerance to the *R. padi*. This study concludes that the wheat cultivar (Serin-2010) may show a superior source of resistance to *R. padi* attack in future breeding program.

NEED OF COMBINED ONLINE DATABASE FOR PLANT QUARANTINE PESTS

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Agriculture of Pakistan faced different pest problems time to time due to exotic organisms with favourable climate. Identifying pests (particular new emerging pests) require staff with specialised skills in all disciplines (Taxonomy and Biotechnology), which is only possible within large centralised laboratory facilities. Expertise in taxonomy, phytopathology and other fields in plant health are vital for sustaining sound phytosanitary issues also under threat. Sharing knowledge on regulated and non-regulated pests is necessary to manage a cost-effective and efficient plant health system in the context of expanding global trade. Unfortunately, there is no any effective combined online database in Pakistan. Currently, there is need of freely accessible combined online database which contains data on plant pathogenic quarantine pests which allow fast and accurate identification. Development of accurate identification tools for plant pests is vital to support trade of Pakistani commodities. This database will provide support for protective measures, against introduction of pests and their spread. The risk of introduction of pests into Pakistan is increasing because of the increase in import of commodities and origins of trade, introduction of new plants/crops, direct import through international travellers and the impact of climate change. At present, potato cyst nematode, potato tuber moth, fruit flies, mealybugs and other pests are major quarantine concern.

SELECTION OF SUITABLE PESTICIDES/INSECTICIDES FOR ERADICATION OF WEED FISH FROM FISH POND

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The aim of the study was to investigate the toxic effects of Dimethoate (T₁), Pyrethroidlambela cyhalothrin (T₂), Cypermethrin (T₃), Malathion (T₄) and Chillas (T₅) toxicity in the Nile tilapia (*Oreochromis niloticus*). The lethal concentration of pesticide/ insecticides viz. Dimethoate (T₁), Pyrethroidlambela cyhalothrin (T₂), Cypermethrin (T₃), Malathion (T₄), and Chillas (T₅), were applied on juvenile Tilapia mossambica to ascertain their efficacy in complete eradication of this fish. The fish was exposed to varying levels of the toxicant (0.5ppm, 1ppm, 1.5ppm, 2ppm) of all five chemicals using 48hrs static bioassay to determine the lethal concentration and lethal time for the different concentrations. Tilapia was very sensitive to

Dimethoate (T₁), Pyrethroidlambela cyhalothrin (T₂), Cypermethrin (T₃), Malathion (T₄) and Chillas (T₅) at 2ppm concentration at 48 hours of exposure. The mortality was found significantly higher in Pyrethroidlambela cyhalothrin (T₂) at 2ppm and at 1.5ppm 4.16±0.12, 4.16±0.72 respectively. Dimethoate (T₁), Cypermethrin (T₃), Malathion (T₄), and Chillas (T₅) also showed maximum mortality at 2ppm 4.15±0.027, 4.16±0.11, 4.0±0.23, 4.0±0.45 respectively. Physico-chemical parameters pH, temperature and Dissolved oxygen were monitored over 48 hours of exposure. Significant co-relation was observed between DO and increasing concentration of Malathion (T₄) 5.1±0.11 than in Chillas (T₅) 4.70±0.12 at 2ppm. pH was significant in Pyrethroidlambela cyhalothrin (T₂) 5.69±0.66 than in Cypermethrin (T₃) 5.30±0.64. The maximum mortality was observed in Pyrethroidlambela cyhalothrin (T₂) at different dosage (0.5ppm, 1ppm, 1.5ppm, 2ppm) when compared with all other pesticides.

EFFICACY OF *CHRYSOPERLA CARNEA* (STEPHENS) AGAINST SUCKING PESTS OF BRINJAL (*SOLANUM MELONGENA* L.)

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Brinjal (*Solanum Melongena* L.) is an important solanaceous vegetable crop in tropics and sub-tropics, having good source of nutrients, minerals and vitamins so attacked by several insect pests from planting till harvesting. Present field study was conducted against sucking pests by releases of generalist predator *Chrysoperla carnea*. Inundative releases of *C. carnea* were made weekly, to control sucking insect pests in twelve lines of Brinjal. The average infestation of whitefly and Jassid under untreated condition was not significantly different on all twelve Brinjal lines. The average infestation of whitefly was 2.5 to 9.1 and 0.5 to 4.25 per leaf during June-July (untreated control). The highest population of whitefly recorded was 16/ leaf in Brinjal line Adventa 319. The percent reduction in whitefly infestation in brinjal lines due to *C. carnea* inundative release was minimum (24.39) in Adventa 313 and was maximum (52.50) in Adventa 305, respectively. Average Jassid infestation recorded was 0.2 to 2.6 per leaf and 1.2 to 5.2 per leaf during June- July (untreated control). Minimum percent reduction in Jassid infestation recorded due to *C. carnea* release, was 26.8 in Adventa 303 whereas it was maximum (50.7) in Adventa 325.

EFFECT OF INSECTICIDE ON PREDATORY EFFICIENCY OF *OXYOPES JAVANUS* (ARANEAE: OXYOPIDAE)

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Present study was design to investigate the effect of insecticide, imidacloprid on predatory efficiency of agrobiont hunting spiders *Oxyopes javanus* against wheat aphid *Sitobian avenae*. To mimic the field conditions, experiments were performed in the mesocosm, which was created by placing a plastic sheet cage over an individually potted wheat plants (4-week old plants with six leaves). The top of the cage was covered with muslin cloth to facilitate ventilation. To assess sub

lethal doses of insecticide viz., LC₅, LC₂₀, and LC₃₀ bioassay was performed by applying insecticide on the dorsum of spiders. After application of sublethal doses of insecticides, spiders were exposed to different density of aphid. Logistic regression analysis revealed that *O. javanus* exhibited Type II functional response against *S. avenae* both in controlled and treated groups. The linear parameters was negative in all groups and proportion of prey killed decreased as the prey density increased. Data showed significant decrease in the predation rate of *O. javanus* treated with insecticide as compared to control. However, at low prey density, no significant difference in mortality of aphid was recorded at different concentrations of insecticides. At high prey densities, response of spider varied according to concentration of insecticide. This study showed that insecticide decrease the predatory potential of surviving spiders and this decrease is directly related with exposed quantity of insecticide. To use *O. javanus* in the agro ecosystem as biological control agents, further information are required about the factors that affect their predation pressure on pest in the agroecosystem.

APPLICATION OF ETHANOLIC LEAF EXTRACT OF PLANT *GLIRICIDIA SEPIUM* (JACQ.) AND DIELDRIN TO CONTROL THE DAMAGES OF SUBTERRANEAN TERMITES

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The present work was carried out to promote indigenous pesticides for the control of subterranean termites. Ethanolic leaf extract of *Gliricidia sepium* (Jacq.) and Dieldrin were prepared in different concentrations i.e. 0.5%, 01% & 1.5% respectively. Stakes treated with high concentration i.e. 1.5% of ethanolic leaf extract of *G. sepium* showed highly effective against termites, with concentration of 01% were found damaged by termites, whereas stakes treated with 0.5 were severely damaged by termites in one year, and those stakes which were treated with Dieldrin, remained un attacked by termites up to one year. Controlled stakes were severely damaged by termites within six months. The damaging activity is the most outstanding features of termites. (Ibrahim *et al.*, 2012). Most of the species attack on crops and cause serious damages (Ahmed *et al.*, 2006), due to which the crops are unable to survive properly (Robinson, 2005). Organochlorine pesticide was banned as termiticides (Ware, 2000), due to its toxic effects on the environment which were harmful for human health and other organisms (Walker and Newton, 1998). In Pakistan the termites are usually controlled by the applications of insecticides (Ahmed *et al.*, 2007). Research on *G. sepium* for the first time in Pakistan is being acknowledged at PCSIR labs complex, Karachi (Nazli *et al.*, 2008). This plant is being utilized for fuel timber, living fences, green mulch, animals nourish, gloom and as support plant. (Csurhes and Edwards, 1998). Leaves of *Gliricidia* plant were collected from Coastal Agriculture Research Station, SARC, PARC, Karachi and its fresh dried leaves were prepared in ethanol and used to control termites population. Wooden stakes were of a particular size were treated in different concentrations of prepared leaf extract and Dieldrin as termiticides. The treated and controlled stakes were fixed in termites' infested soil. The data was subjected to analyze the \pm standard error of mean and standard deviation calculated by SPSS ver-17 program to observe the difference between each treatment. The residual toxic effects of ethanolic leaf extract of *G. sepium* shows the termites repelling properties and treated stakes were safe against decaying in the soil while controlled samples were severely damaged or decayed

within one year. This observation shows that ethanolic leaf extract of *G. sepium* have termiticidal activity.

**EFFECTIVENESS OF DIFFERENT INSECTICIDES FOR THE CONTROL OF
WHITEBACKED PLANTHOPPER (*SOGATELLA FURCIFERA* HORVATH) UNDER
FIELD CONDITIONS IN THE PUNJAB, PAKISTAN**

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Planthoppers are considered very destructive pests of rice in Pakistan. Amongst planthoppers, Whitebacked planthopper (WBPH) is one of the important sucking pest insect of rice crop and it can cause significant yield loss. A field trial was conducted at Rice Research institute, Kala Shah Kaku to check the efficacy of different insecticides for the chemical control of whitebacked planthopper. Seven insecticides viz., Proaxis 60SC (gamma-cyhalothrin), Regent 80WG (fipronil), Pronto 70WG (imidachloprid), Virtako 40WG (thiamethoxam +chlorantraniliprole), Karate 2.5EC (lambda-cyhalothrin), Confidor 70WG (imidachloprid), Padan 4G (cartap hydrochloride) were applied at economic threshold level(ETL) and observations were recorded 24 hours, 72 hours and 7 days after insecticide application. The experiment was laid out in RCBD with eight treatments including a control, replicated thrice. Results indicated that all the test insecticides provided significantly better control as compared to untreated check. Confidor with 5.33 post treatment WBPH population was most effective after 24 hours followed by Proaxis (6.00), Pronto (6.33), Virtako (7.00), Karate (7.00) and Padan (12.33). After 72 hours least WBPH population (1.67) was observed in case of Confidor followed by Karate (2.00), Virtako (2.33), Regent (2.33), Pronto (2.67), Proaxis (3.33) and Padan (4.00). After 7 days significantly lowest population of target pest (1.33) was recorded in case of Padan and Confidor followed by Karate (1.67), Pronto (1.67), Regent(1.67) and Proaxis (2.00).

**STUDY ON POPULATION DYNAMICS OF URBAN RATS IN FOOD-BASED
INDUSTRIES IN KARACHI**

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Rats are present virtually in every area where there is food and shelter. The food based industries in Karachi are also facing the same issue. Rats can cause damage in variety of ways. They spread disease; contaminate food, cause damage (machinery, cables and sophisticated and expensive equipment), loss in customers and profits, risk of prosecution and closure. In order to determine population dynamics and species complex of commensal rats in different food based industries of Karachi, snap trapping was conducted. Three food based industries at different locations in Karachi were selected. Snap trapping was conducted on fortnightly basis in facilities (Production, Storage, Outside, Offices, Workshops and Canteens) of selected industries. The data was analyzed through factorial ANOVA, integrating species population in different facilities. The

data was pooled on seasonal basis to determine overall population dynamics with respect to seasons. Overall trap index revealed occurrence of three commensal rat species in the food based industries in Karachi namely *Rattus rattus*, *Rattus norvegicus* and *Mus musculus*. *R. norvegicus* being pre-dominant species. Seasonal trapping revealed the population fluctuation in different seasons. Fluctuation in rat's specie population is mainly dependent on availability of food, shelter and variation in temperature. No significant variation in damage and population during different seasons was observed. Thus, the rodent management practices must be applied throughout the year to overcome the losses caused by rats.

TOXICITY OF PHOSPHINE AGAINST RESISTANT AND SUSCEPTIBLE POPULATIONS OF *TROGODERMA GRANARIUM*

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Khapra beetle, *Trogoderma granarium* is one of the most notorious pests of stored grains. When world population is increasing faster than ever, one third of potential food supply is lost to pest each year. This study was aimed to evaluate the toxicity of phosphine to 4th, 6th instar larvae and adult beetle in phosphine resistant populations viz., Gujranwala, Mandi Bahauddin I (M.B.Din I), Mandi Bahauddin II (M.B.Din II), Gujrat and Sargodha and a susceptible population of *T. granarium*. The 4th, 6th instar larvae and adult beetles of *T. granarium* were exposed to different sub lethal concentrations of phosphine for 20 hours. Gujranwala population was found to be more resistant to phosphine (LC₅₀ 15.8, 13.5 and 12.6ppm) in 4th, 6th instar larvae and adult beetles, respectively whereas Sargodha population was least resistant (11.6, 10.4, 8.2ppm) in 4th, 6th instar larvae and adult beetles, respectively. Gujranwala population required 113.51, 128.81 and 168.1% more phosphine concentration in 4th, 6th instar larvae and adult beetles, respectively with reference to susceptible population of *T. granarium*. The various insect populations based on LC₅₀ of phosphine is graded as Gujranwala > M.B.Din I > Gujrat > M.B.Din II > Sargodha > control. The LC₅₀ values of phosphine against adult is always less than of 6th instar larvae and likewise LC₅₀ of phosphine against 6th instar larvae is always less compared to 4th instar larvae in all populations. The 4th instar of Gujranwala required 17.03 and 25.39% more phosphine than 6th instar larvae and adult beetle, respectively. Based on LC₅₀ data, gradation followed by developmental stages is 4th instar larvae > 6th instar larvae > adult. Adult insects seem to be more sensitive to phosphine compared to 6th and 4th instar larvae.

OBSERVATIONS ON REPRODUCTIVE BIOLOGY AND POST-NATAL DEVELOPMENT OF CAPTIVE *NESOKIA* SP. INFESTING DATE-PALM ORCHARDS OF NOK-KUNDI, BALOCHISTAN

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The rodent species of genus *Nesokia* might have' migrated to Pakistan from Iranian Balochistan and infested thousands of date-palm trees at Nok-kundi and Mashkale in Balochistan

and inflicting the colossal loss of millions of rupees. The rats attack the sucker plants first and feed on the roots, leaves, branches and stem at the base and then make tunnel in the stem and feed on the sweet part (pith) of the tree. As a result, damaged trees begin to gradually dry, resulting incomplete kill. To control this rat, detail knowledge of its biology was lacking. To fill this lacuna, some observations on the post-natal development and reproductive patterns were recorded. The minimum interval between births was recorded 23 days. The litter size varied from 1-5 young and litter of 4 young occurred more frequently. The average liter size was 3.45 ± 0.31 young/female. The annual rate of reproduction was 35 young/female. Parturition of sibling pair of known age produced first litter at an age of 121 days. Assuming a 5 days' estrus cycle in this species and considering 23 days gestation period, the mean age of reproductive maturity was calculated 93 days. The minimum age of perforation of the vagina in young female was observed at the age of 92 days. Male were scrotal at the age of 34-45 days. Eyes were opened between. days 21-25. Response to auditory stimuli was first observed on day 20-21. The spots of teats appeared on day 12 and remained visible upto the end of third week before being covered by hairs. Incisors eruption started from day 8 and completed on day 12. Weaning was recorded till day 27. The pest species of rat causing infestation to date-palm is semi-fossorial and efficient climber like roof rat (*Rattus rattus*) and has high cannibalistic behavior similar to lesser bandicoot rat (*Bandicota bengalensis*). Face washing, scratching and haunch sitting were observed on day 18 while social grooming was noted on day 25. The mass of young at birth was 5.4 g, head body length 47.1 mm, tail length 18.1 mm, hind foot length 8.7 mm and ear length 1.8 mm. Maximum weight of male *Nesokia*, sp was recorded 496 g, while females gained the weight upto 339.0 g.

EFFECT OF BIOCONTROL AGENT *CHRYSOPERLA CARNEA* STEPHENS ON THE SUPPRESSION OF SUCKING INSECT PESTS; *BEMISIA TABACI* GENADIUS (WHITEFLY) AND *AMRASCA DEVASTANS* DIST. (JASSID) IN *ABELMOSCHUS ESCULENTUS* L. (OKRA).

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Studies were carried out to find out the effect of inundative releases of *Chrysoperla carnea* on the infestation of sucking insect pests in Okra varieties during 2013. Nine varieties of Okra were tested for the infestation of jassid and whitefly for the entire crop period. The average infestation of whitefly and jassid under treated and untreated conditions was significantly different on all nine Okra varieties. It was found that mean number of whitefly on Okra varieties increased steadily from second week of June till the end of August and afterwards the infestation declined. Average number of whitefly recorded was 11.44-16.35 per leaf from June to August under untreated control. Maximum mean percent reduction in whitefly infestation due to release of *C. carnea* was recorded in Sabz pari (40.3), and minimum was found in Okra7100 (14.10). Infestation of jassid on Okra cultivars started increasing from second week of June, reached maximum in the third week of July and declined afterwards. Average number of jassid recorded under untreated conditions varied from 4.03 to 33.7 per leaf. Mean percent reduction in jassid infestation in IKOH 4510 (73.5) was highest, whereas, minimum was found in Parbhani karanti (51.1).

***ECHINOCHASMUS SWABIENSIS* N. SP (TREMATODA: ECHINOCHASMINAE
ODHNER, 1910) FROM THE BLACK KITE *MILVUS MIGRANS* IN SWABI, KP,
PAKISTAN**

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Genus *Echinochasmus* Dietz, 1909 syn. of *Monilifer* Dietz 1909, Partim, *Heterochinostomum* Odhner, 1910 and *Velamentophorus* Mendheim, 1940 is a well-established genus of trematodes of birds characterized by strong head collar with a single interrupted row of spine, testes usually large as compared to the body size and uterus short with few winding and large eggs. During studies on trematodes of birds, new species *Echinochasmus swabiensis* n. sp are described. Description is based on a single polished and permanently mounted specimen. Body is small and spinose. Head collar bears 24 spines in a single interrupted row. Oral sucker is terminal, rounded and slightly protruding. Prepharynx is absent. Pharynx is rounded to somewhat oval followed by short esophagus. Acetabulum is very large as compared to oral sucker and Suckers width ratio is 1:4.6. Testes are large, median, tandem, subequal, somewhat rectangular in shape and nearly contiguous. Anterior testis is slightly wider than long while posterior testis is slightly elongated than broad. Cirrus pouch is small, just above the acetabulum. Ovary is small, lateral and oval in shape and situated between ventral sucker and anterior testis. Vitellaria are composed of large follicles, commencing at nearly the middle of the acetabulum, extending backward in lateral fields and meet behind the posterior testis. Uterus is short with few winding, having nine to ten large eggs. The present species is different from other related species described from birds in different parts of the world as well as from the species recorded from birds in Pakistan, including *E. mohiuddini*, *E. passerii*, *E. jamshorensis* and *E. atrae*.

**FEEDING DETERRENCE OF SOME ADDITIVE PLANT POWDERS AGAINST
TRIBOLIUM CASTANEUM (RED FLOUR BEETLE) INFESTING RODENT POISON
BAITS**

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The anticoagulants are being used for rodent control since last three decades. Rat baits containing anticoagulants become infested by insect pests such as, red-flour beetle, *Tribolium castaneum*. Materials used for bait formation and as taste additives to attract rodents may be more attractive for the insect pests. Moreover, stored food commodities may become infested with insects introduced by anticoagulants. Some plants have feeding deterrence for insect pests with out affecting the palatability of poison bait for rodents. In this regard, the effect of five plants powder (Seeds of *Azadirachta indica* (neem), roots of *Valeriana officinalis* (balchar), rhizomes of *Acorus calamus* (sweetflag), rhizomes of *Curcuma longa* (turmeric) and rhizomes of *Saussurea lappa* (kuth)) were studied for their feeding deterrence (anti-feedant) activities against *Tribolium castaneum*. For testing plants, wheat flour, broken rice, taste additive (egg), poison and plant powders were mixed in a ratio accordingly. Plant powders were tested in three (2.00%, 1.00% and

0.50%) doses, in comparison with control (plain bait). For the purpose Petri dishes (9 cm diameter, 1.5 cm deep) were divided by marker on the outside in equal halves. One half was filled by plain bait whereas the other half was filled by treated (50 g in each) by using a hard board partition. The partition was removed and fifty *Tribolium castaneum* (starved for 24 hours) were released in the middle of the Petri dish (providing option for the selection of treated or un-treated flour). After one hour and six hours (at 0900 and 1500 hours) the settled insects were counted for five consecutive days. All the tests were replicated for five times. The repellency was observed continuously up to the second, fourth and eighth weeks; however fresh insects were used in all the tests, using the same treated and untreated (control) bait. Percentage repellency was calculated by deducting the percentage of insect on the treated half from the insects on the untreated half. Evaluation of weekly repellency of all the plants was carried out by comparing the results statistically. Neem (*Azadirachta indica*) seed powder proved it the best feeding deterrent additive for the rodent poison baits. Neem gave 100% deterrence up till the 8th week followed by kuth, which gave 78.65% deterrence up till the 4th week that gradually decreases to 62.98% in the 8th week; sweet flag gave 58% deterrence that decreased to 48% in the 8th week. Turmeric proved it a good antifeedant by giving 67.89% deterrence up till the 8th week. Valerian could not prove it a feeding deterrent at all. In these studies neem was found the best feeding deterrent for the beetle; however there is a need to discover more plant products additives as feeding deterrent to protect rodent poison baits from insect invasion.

DIAGNOSIS OF DNA DAMAGE IN PERIPHERAL BLOOD ERYTHROCYTES OF FISH DURING CHRONIC EXPOSURE OF ZINC

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Various agricultural and industrial chemicals containing heavy metals are contaminating the aquatic environments adversely. Metals are biologically non-degradable contaminants that can cause toxicity in aquatic animals through oxidative damage to membrane lipids, DNA and proteins. The present study was conducted to determine the zinc induced genotoxic damage in peripheral blood erythrocytes of 150-day old fish, *Labeo rohita* using Comet assay and micronucleus test under controlled laboratory conditions. After 30-day exposure of fish to four different sub-lethal concentrations viz. 17%, 25%, 33% and 50% of LC₅₀ of zinc, separately, peripheral blood of exposed fish was examined for damaged nuclei (%), genetic damage index (GDI), cumulative tail length (µm) micronuclei frequency (%) and frequency of other nuclear abnormalities. Chronic exposure of zinc to *Labeo rohita* induced DNA damage in peripheral erythrocytes of fish that varied significantly (p<0.05) with exposure concentration. The DNA damage caused even by the lowest concentration of zinc was significantly (p<0.05) higher than that recorded in negative control group. Significantly higher genetic damage in terms of percentage of damaged nuclei (78.67±1.15%), genetic damage index (2.47±0.03) and cumulative tail length of comets (222.70±1.11 µm) was recorded at 50% of zinc LC₅₀ exposure to the fish while this damage was significantly minimum at 17% zinc LC₅₀. Significantly higher micronuclei frequency of 33.88±2.51% was observed at 50% of zinc LC₅₀ while it remained significantly least (14.36±1.02%) due to 17% zinc LC₅₀. This study reveals that both Comet assay and micronucleus test can be used as useful tools for the determination of genotoxic effects of metals on fish.

SECTION – I I I

E N T O M O L O G Y

**INFESTATION OF *FELICOLA SUBROSTRATUS* (BURMEISTER, 1838)
(TRICHODECTIDAE: ISCHNOCERA: PHTHIRAPTERA) ON *FELIS SILVESTRIS*
CATUS L. (FELIDAE: CARNIVORA: MAMMALIA) FROM KARACHI, PAKISTAN.**

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The domestic cat, *Felis silvestris catus* L. (Felidae: Carnivora: Mammalia) is a most common pet and has fabulous human social interaction throughout the world. In the present study, 86 cats in different areas and streets of Karachi were examined for their chewing lice. It was observed that cats in poor health condition were more infested with both nymphs and adults of *Felicola subrostratus* (Burmeister, 1818) (Trichodectidae: Ischnocera: Phthiraptera). The rate of infestation was reported medium to high in areas of poor societies which is recorded 40-100% whereas the areas with good, educated societies the cats were infested with the rate of nil and very low to low-medium i.e., 0-37% was observed. It is also observed during the present study, that *F. subrostratus* is highly successful species and growing rapidly between its host in a common locality with similar ecological and unhygienic conditions. It is the first record of this louse species from cats in the region.

**BUTTERFLY FAUNA (RHOPALOCERA: LEPIDOPTERA) OF HYDERABAD REGION,
SINDH, PAKISTAN**

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Butterflies are very beautiful and attractive insects, because their wings are covered with scales of different bright colours. They are economically very important, adults are good pollinators and larvae (caterpillars) of some species are injurious to many crops, vegetables and fruits. Present study was carried out during 2014 from Hyderabad region. During the study period, a total of 1310 specimens were collected with the help of insect net from the flowering plants, agricultural crops, vegetables and fruit farms of Hyderabad region. Fifteen species were identified that belong to seven genera and four families i.e. Danaidae, Papilionidae, Pieridae and Nymphalidae were recorded. Out of fifteen species, two species *Danaus chrysippus* (Cramer) and *Danaus genutia* (Linnaeus) belong to family Danaidae; two species *Papilio demolus* (Linnaeus) and *Pachilopta aristolochiae* (Fabricius) belong to family Papilionidae; four species *Junonia orithya* (Linnaeus), *Junonia lemonias* (Linnaeus), *Junonia almana* (Linnaeus) and *Hypolimnas misippus* (Linnaeus) belong to family Nymphalidae; seven species *Anapheis aurota* (Linnaeus), *Eurema hecabe simulata* (Moore), *Colotis amata* (Fabricius), *Colotis etrida* (Butler), *Catopsila pomana*

(Fabricius), *Colotis danae danae* (Fabricius) and *Colotis vestalis* (Butler) belong to family Pieridae. The aim of present study was to explore the fauna of butterfly of Hyderabad region, Sindh, Pakistan.

KEY TO THE ACRIDIDAE (ORTHOPTERA) FROM PUNJAB

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Many species of the Acrididae is considered most of important pest of many agricultural crops in Punjab but still their correct identification has not been done. At the present an attempt has been made and keys were prepared which based on the easily morphological characters for the sub-families, genera and species of the Acrididae.

STUDIES ON SOUND PRODUCING ORGANS BY USING SCANNING ELECTRON MICROSCOPY (SEM) OF *SVERCACHETA* SP. AND *BRACHYTRYPES PORTENTOSUS* (LICHTENTEN) (GRYLLIDAE: GRYLLINAE) WITH REFERENCE TO THEIR SYSTEMATIC RELATIONSHIPS

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The specimens of *Svercacheta* sp. and *B. portentosus* (Lichteinten) are described on the basis of their sound producing structures *i.e.* tegmina, plectrum, stridulatory file and teeth, for the first time in Pakistan. Specimens were collected from Quetta in Baluchistan and Dir, in Khyber Pakhtoon Khaw were subjected to Scanning technique of Electron Microscopy following the techniques of David *et al.* (2003) to study these structures. The pars stridulum pattern, the stridulatory file and the number of teeth on file appear as important characters to identify males of the presented species from other related taxa found in Pakistan. The specimens were boiled for a few minutes, and then the right tegmen was detached from the specimen, placed on a slide and covered with a clean coverslip for photography by using Nikon Cool Pix 5400 digital camera after placing it under Nikon SMZ 800 Binocular. Then the tegmen was mounted on a stub, placed to coat with auto coater into JEOL model no. JFC-1500 Japan having gold target, which coated up to 300⁰A, then scanned with Scanning Electron Microscope, JEOL Japan model no. JSM 6380A and studied from the ventral region as SEM pictures of the file, at Centralized Science Laboratory, University of Karachi, Karachi. The structures of the tegmina of different species used for acoustic signals, having different sound producing organs (Walker and Carlysle, 1975), were found to be very important taxonomic characters. Allard (1910) recognized for the first time the geographical variation in the sounds of field crickets. At the beginning of the 20th century the taxonomists of this group recognized the differences in the calling notes of the males, which are being used to attract the conspecific female partners. In the singing crickets, unidentified species can be recognized by their song differences (Davis, 1922; Fulton, 1930; Pringle, 1955; Thomas and Alexander, 1957). Stridulation is considered as a significant character for determining different species. Number of stridulatory pegs, length of file, size and distance of teeth of file appear to be associated with

stridulation (Alexander and Thomas, 1959; Alexander and Bigelow, 1960; Walker, 1962; 1963). Diversity of stridulatory organs, signals and behavior solve the problems of origin and evolution of species in Ensifera (Desutter-Grandcolas, 2002). It is concluded from the present results and those reported in the literature to-date that most taxa above and below subfamilies level in crickets could be identified on the basis of file teeth structures.

PROPAGATION OF MELANOGASTER AND NON- MELANOGASTER SPECIES OF DROSOPHILA CAPTURED FROM FIVE CITIES OF PAKISTAN

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Although climatic conditions of Pakistan are suitable for propagation of *Drosophila* species yet there is a big lapse in using *Drosophila* as an experimental model. Out of 1579 globally reported species of *Drosophila*, 275 species have been found in India while only 20 have been reported from Pakistan. A study was carried out from October 2013 to November 2014 to propagate *Drosophila* species in laboratory to build up the confidence in Pakistani biologists in choosing it as a model organism. The *Drosophila* flies were captured from wild by using suitable baits. During the period of study 79 cultures were propagated by placing single female fly in porridge, sugar and yeast medium at 25°C. After 8-20 days, depending upon type of species, their progeny emerged out. Morphological features were studied for their identification and authentic key guides were used for further confirmation. The cities included in the study were Rabwah, Karachi, Murree, Barian and Nathiagali. *D. melanogaster* and *D. immigrans* were found in all above mentioned localities. *D. busckii* and *D. trilineata* were found in Rabwah only, *D. takahashii* and *D. ananassae* in Karachi and Rabwah, while *D. jambilina* in Murree as well as in Karachi and Rabwah. All these species are reported for the first time in these cities of Pakistan, except Rabwah; from where only *D. busckii* and *D. trilineata* were reported for the first time, while the rest 5 species have previously been reported. Out of 79 propagated cultures 47 consist of *D. melanogaster*, 29 were non- *melanogaster* while 3 cultures belong to another genus *Zaprionus* of family Drosophilidae. The distribution of kinds of the species found in different regions is the matter of further research.

TAXONOMIC STATUS OF ACRIDIDAE (ORTHOPTERA) FROM NARA DESERT KHAIRPUR

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Keeping in view the importance of diversity of desert grasshoppers, present investigation has been carried out on the taxonomy of Acridid grasshoppers from Nara desert. The specimens were collected from various localities of Nara desert. The material was sorted out into 09 genera including 12 species i.e. *Spathosternum prasiniferum* (Krauss, 1877), *Heteracris littoralis* (Rambur, 1839), *Aiolopus thalassinus thalassinus* (Fabricius, 1781), *Hilethera aeolopoides*

(Uvarov, 1923), *Trilophidia annulata* (Thunberg, 1815), *Acrotylus humberlinus* (Saussure, 1884), *Sphingonotus rubescens rubescens* (Walker, 1870), *S. savignyi* (Saussure, 1884), *S. theodori theodori* (Uvarov 1924), *Aulacothrus luteipis* (Walker, 1871), *Ochrilidia geniculata* (Bolivar, 1913), *O. gracilis gracilis* (Krauss, 1902). In addition to this, for identification and characterization simplified taxonomic keys were also prepared. Hopefully, this study will be beneficial to explore the diversity of desert fauna.

IMPACT OF SOME WINTERING BIRDS ON THE INSECT'S POPULATION FROM SINDH

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During the winter an estimated population of million of migratory birds prefer to stay on various wetlands of Sindh. Present investigation has been made to investigate the impact of wintering birds such as Coots, Moorhens and Gallinules on insects population i-e mustard aphid (*Lipaphis erysimi*) Kaltenbach, mustard sawfly (*Athalia proxima*) Klug and painted bug (*Bagrada picta*) (Fabricius). Furthermore, it has been noticed that the predator are very helpful in controlling the population of various insects' pests by feeding on these insects. It can be achieved by providing conditions that will facilitate the natural enemies to increase their population. These biological agent or natural enemies are the main source of decreasing the population of harmful insect pests. Present investigation may led to the control planning for pest species.

EFFECT OF FOOD PLANTS ON THE DEVELOPMENT AND SURVIVAL RATE OF ACROTYLUS HUBERTIANUS SAUSSURE (OEDIPODINAE: ACRIDIDAE: ORTHOPTERA), FROM MATIARI DISTRICT

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Acrotylus humberlinus Saussure is a major agriculture pest in Matiari district. This species consumes a wide variety of food plants from different families. During present study 05 food plants i.e. *Zea mays* (Maize), *Saccharum officinarum* (Sugarcane), *Sorghum bicolor* (Jowar), *Cynodon dactylon* (Grass) and *Brassica oleracea* (Cabbage) belonging to Poaceae and Brassicaceae were tested against this pest. During the present study it was observed that *A. humberlinus* nymph and adult accepted all plants species for feeding. The growth rate of *A. humberlinus* was found significantly high i-e (41.20±0.51 days), (39.35±12.50 days) and (39.29±0.58 days) on *Z. mays*, *S. officinarum* and *B. oleracea* respectively, while it was reported 33.28 ± 0.79 days and 32.8 ± 0.40 days on *C. dactylon* and *S. bicolor* respectively. However, survivability of *A. humberlinus* fed on these plants did not differ remarkably, although their survivability was higher on *S. officinarum* followed by *B. oleracea* and *Z. mays*. The present study has been carried out for the first time hopefully it will boost fresh biological literature regarding area of proposed research.

**PRELIMINARY STUDY ON TAXONOMY OF HEXACENTRINAE KARNY, 1925
(ORTHOPTERA: TETTIGONIIDAE)**

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The present study is based on collections containing members of the little studied subfamily Hexacentrinae Karny, 1925. A single genus *Hexacentrus* Serville, 1831 with 02 species i-e *Hexacentrus unicolor* Serville, 1931 and *Hexacentrus pusillus* Redtenbacher, 1891 were came in collection during the 2013. Hexacentrinae may be a sister group of Conocephalinae due to reason that majority of representatives of subfamilies Hexacentrinae and Conocephalinae having significant similarities in their venation of hind wings beside this, its predatory mode of life make the association with other subfamilies such as Tympanophorinae Listroscolidinae, and Saginae, it was found that all the species of *Hexacentrus* live in the thick vegetation at the present I have reported single male and female of this subfamily hopefully, my further study with more material will confirm this reality.

**ON THE INCIDENCE OF THE DESERT LOCUST *SCHISTOCERCA GREGARIA*
(FORSKAL) (ORTHOPTERA: ACRIDIDAE: CYRTACANTHACRIDINAE)**

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The desert locust, *Schistocerca gregaria* (Forsk.) is the representative of the genus *Schistocerca* Stal and one of the most notorious pests of agricultural crops. A total of 52 specimens came in collection from Thar Desert. A single genus with a single species i-e: *Schistocerca gregaria* (Forsk.) has been reported from studied area it incidences at locality level was highlighted it was also observed that these pests form huge swarms, and causes severe damage to many agricultural crops. However, these are initial findings further investigations are in progress.

**A NEW FRUIT FLY SPECIES, *BACTROCERA ASHFAQI* WITHIN THE GENUS
BACTROCERA MACQUART (DIPTERA: TEPHRITIDAE: DACINAE) FOUND IN
PAKISTAN.**

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Fruit flies of the family Tephritidae are the most damaging insect pests of economic importance infesting almost all kinds of fruits. This paper covers existing familiarity on the

occurrence of a new fruit fly species *Bactrocera ashfaqi* among genus *Bactrocera* Macquart (Diptera: Tephritidae: Dacinae) detected in Pakistan. Current data show that *B. ashfaqi* is principally originating alongside of the few hilly areas of country and its host fruits are persimmon (*Diospyros kaki*) and pear (*Pyrus communis*). The species confirmed as newly occurring in the locality is briefly compared to that of other already known species recorded from various regions. The information on host plants, location of type specimens and geographic distribution records for the new species is given. A complete list and key are presented to the species recorded for the genus *Bactrocera* Macquart from the Pakistan. Additionally, the references consulted in the compilation of the information are added and listed for placing the collected specimen in the subfamily Dacinae. The existence of *B. ashfaqi* in currently known localities can be attributed to the biodiversity of host plants and the constant availability of fruits in these afforests. Further behavioral and ecological studies are needed to prepare and implement integrated methods in order to better control of this new economical important pest.

STUDIES ON MORPHOLOGY AND PHALLIC COMPLEX OF *OEDALEUS SENEGALENSIS* KRAUSS (ORTHOPTERA: ACRIDOIDEA) FROM PAKISTAN

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The genus *Oedaleus* Fieber, (1853) were considered economically pest. In present work *Oedaleus senegalensis* specimens has been collected from the cultivated fields, semi arid areas, along the sides of standing crops and rocky areas. This species have been found and that were described with four illustrations of phallic complex and description also provided. The epiphallus bridge shaped, bridge comparatively wider, thickening and slightly curved. Anterior projections well marked, finger like with acute rounded boundaries. This investigation was taken to understand the structural pattern of Phallic complex to update new research workers.

STUDY ON THE BIOLOGY AND ECOLOGY OF *POEKILOCERUS PICTUS* FABRICIUS (ORTHOPTERA: PYRGOMORPHIDAE)

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Akk grass hopper, *Poekilocerus pictus* Fabricius is also regarded as mount road grasshopper due to its bright coloration. Among such diversify group of grasshopper Akk grasshopper is very common insect occurring in Pakistan, India and Afghanistan. *P. pictus* is usually present in small number, its population occasionally may increase segregatly to the extent of an outbreak and causes severe economic damage to crops after its normal host plants has been defoliated and depleted. At the present its biology along with its association with *Calotropis procera* was studied.

PREVALENCE OF GRASSHOPPERS (OEDIPODINAE: ORTHOPTERA) FROM THAR DESERT

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Thar Desert is considered as the seventh largest desert on planet earth and third in continent Asia. Perhaps, the land of desert constitutes a number of insect fauna but this region was still untouched due to harsh geographical condition. During the present study an attempt has been made to collect the grasshoppers' fauna. At the present 213 specimens were collected and sorted out into different subfamilies and specimens of Oedipodinae were confirmed i.e. *Acrotylus humbertianus* Saussure, 1884 and *Acrotylus longipes* (Charpentier, 1845) while remaining are in the process of identification.

TAXONOMY OF THE GENUS *DECTICUS* SERVILLE, 1831 (DECTICINAE: TETTIGONIIDAE: ORTHOPTERA) FROM PAKISTAN

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The recent growing interest on the *Decticus* Serville, 1831 fauna of Pakistan in particular, has enabled the discovery of two species i.e. *Decticus verrucivorus* (Linnaeus, 1758) and *Decticus albifrons* (Fabricius, 1775). *Decticus* species are having significant biometrical and morphological differences with each other. In *Decticus albifrons* genital plate transverse with thickened posterior margin with small median notch while male cerci in *D. verrucivorus* restrain with denticles near middle. All morphological differences were highlighted by illustration and photographs. Furthermore, the species of *Decticus* are having jumping capacity when approached. Collection of these two species from Pakistan constructed new records. This study was supported by grants received from Higher Education Commission Pakistan under Indigenous PhD Fellowship for 5000, Scholars Phase-II.

A LABORATORY BASED STUDY ON REARING OF RICE GRASSHOPPER *OXYA HYL A HYL A* (SERVILLE) (OXYINAE: ACRIDIDAE: ORTHOPTERA)

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Oxya hyla hyla (Serville) is small rice grasshopper and it is widely distributed in Sindh. It's breeding capability, growth rate and survivability was noted under laboratory condition during the year 2014. It was observed that copulation time of insect was noted 48 ± 7.53 minutes and total No. of mating was noted 2.2 ± 8.3 . Beside this, it was observed that female deposit 2.2 ± 0.8 egg pods during entire life however, total No. of eggs per pod was counted 26.16 ± 5.11 . It was very interesting to note that male could not survive after mating process however female die after

completing her all oviposition process. The present investigation has been carried out for the first time.

**PREVALENCE OF IMMATURE STAGES OF SUBFAMILY GOMPHOCERINAE
(ACRIDOIDEA: ORTHOPTERA)**

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In the result of an extensive survey a total 863 specimens (immature) of 04 species of Gomphocerinae were collected from various districts of Sindh, the overall percentage of Gomphocerinae species were reported 66.66% for *Ochrilidia gracilis gracilis* (Krauss, 1902), 51.22% for *Ochrilidia geniculata* (Bolivar, 1913), 46.96% for *Aulacobothrus lutipes lutipes* (Walker, 1871) and least percentage i.e. 36.26% for *Aulacobothrus infernus* (Bolívar, 1902) was calculated. Beside this, our field observation showed that these species cause serious threat to all agricultural crops available in their vicinity.

**PRELIMINARY STUDIES ON THE GRYLLIDAE BOLÍVAR, 1878 (ORTHOPTERA)
FROM THAR DESERT**

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The insects belonging to the gryllidae are considered as the severe pest of the agriculture crops. At the present 77 specimens of gryllidae was collected. The collected material was sorted out into 04 species and only single species i.e. *Gryllopsis virgulata* Chopard, 1935 was identified, while remaining are in process of identification.

**STUDY ON THE BIOLOGY OF *SCELIO HIEROGLYPHI* TIMBERLAKE
(HYMENOPTERA: PLATYGASTRIDAE)**

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The most charismatic species *Scelio hieroglyphi* Timberlake of the genus *Scelio* Latreille was studied during the present investigation. It was found that there were two larval instars and first larva is teleaform in that it is constricted into unsegmented cephalothoracic and abdominal region. However, its mandibles were found large, curved and sharply pointed and used to kill other larvae of *Scelio* in the same host egg or to destroy the host embryo. Hopefully, present finding will make use of biodiversity informatics standards to reference supporting data.

REVIEW OF GENUS *SALIXOCORIS* AHMED AND ABBASI 1974 (HEMIPTERA, PENTATOMIDAE, HALYINI) OF PAKISTAN**Abdul Manan Shaikh,¹ Nasreen Memon,² Mansoor Ali Shah² and Nadir Ali Birmani²**¹*Department of Zoology, Shah Abdul Latif University, Khairpur, Sindh, Pakistan*²*Department of Zoology University of Sindh, Jamshoro, Sindh, Pakistan*Email: amanan.shaikh@salu.edu.pk

Ahmad and Abbasi (1974) described genus *Salixocoris* in tribe Halyini from Peshawar, NWFP, Pakistan with type species *S. peshawarensis*. Ahmad and Kamaluddin (1978) added one more species *S. sindellus* from Hyderabad, Sindh, Pakistan. Memon and Manan (2004) also added a *S. excavates* from Sindh, Pakistan. Presently addition of a new species to this genus from different localities of Pakistan is discussed and a key of all four species prepared.

FIRST RECORD AND REDESCRIPTION OF PARAGUSHAEMORRHUSMEIGEN (DIPTERA: SYRPHIDAE) FROM BALOCHISTAN, PAKISTAN**Juma Khan Turk, Nasreen Memon, Bhojoo, Mal, Attaullah Ansari and Dildar Ali Solangi***Department of Zoology, University of Sindh, Jamshoro-76080, Pakistan*

ParagushaemorrhousMeigen is a species of Hover fly belonging to family Syrphidae of order Diptera. This is a small sized hover fly commonly called syrphid fly. This species is beneficial because adults play very important role in pollination and its larvae are predators used in biological control. The specimens were collected from different localities of Quetta, Balochistan and identification was done with the help of available literature. This species is redescribed here on the basis of morphological characters, male terminalia, male and female external genitalia. This syrphid fly has been reported for the first time from Balochistan, Pakistan.

PREVALENCE OF DENGUE FEVER MOSQUITO *Aedes Aegypti* (DIPTER: CULICIDAE) IN HYDERABAD DISTRICT**Attaullah Ansari, Nasreen Memon, Mansoor Ali Shah, Bhojoo Mal, Shakeel Ahmed Memon and Juma Khan***Department of Zoology, University of Sindh, Jamshoro-76080, Pakistan*

Mosquitoes are the best known group of insects because of their importance to men as vectors of some of the most distressing human diseases such as Dengue, Malaria, West Nile Fever and Yellow Fever. Dengue fever is the most rapidly spreading viral disease transmitted to human being by the bite of female *Aedes* mosquitoes. This study was conducted during dry (January 2011 to June 2011) and wet seasons (July- November 2011) in four localities of Hyderabad (Qasimabad, Latifabad, Hyderabad city & Hyderabad rural), specially focusing in those areas where dengue cases were reported. The mosquitoes were sampled with the help of sucking glass tube and insect hand net; in addition the data of dengue patients were collected from Liaquat University Hospital Hyderabad. During dry season (January to June 2011) the rain fall was minimum which left negative effect on the population of *Aedes aegypti* and its population was less and about 77 specimens (55 larvae and 22 adults) were collected from different areas of three localities of

Hyderabad i.e. Hyderabad city, Latifabad and Qasimabad. On other hand during wet season (July–November 2011) the rainfall was high in all studied localities which left positive effect on the population of *Aedes aegypti* and its population was high and total 167 specimens (106 larvae and 61 adults) were collected from different areas of all four localities of Hyderabad. This is the first record of *Aedes aegypti* mosquitoes from Hyderabad. A total of 97 confirm dengue cases were reported in the year 2011, the peak season started after monsoon period in the month of September in which 47 cases were reported followed by October 44 and in November 2 cases were reported.

LEPIDOPTERA DIVERSITY IN TOLIPIR NATIONAL PARK, AZAD JAMMU AND KASHMIR, PAKISTAN

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Study conducted in summer, 2014 on Lepidoptera diversity over some 52 km² Tolipir National Park using line transect sampling and recorded 20 butterfly species belonging to two families. Various diversity indices, i.e. Simpson Index (0.09), Shannon Winner Index (3.05), Berger-Parker Dominance Index (0.06), Margalef's Index (3.27), Menhinick Index (0.89), Buzas and Gibson's Index (0.96) were also calculated. The Lepidoptera diversity decreased along elevation gradient, % plant cover and temperature (1,367 – 2,617 m asl). We assume that focusing the conservation practices upon these species will promote the preservation of a wide range of organisms inhabiting Tolipir National park

POPULATION FLUCTUATION OF LEAF MINER, *ACROCERCOPS SYNGRAMMA* (MEYRICK) LEPIDOPTERA: GRACILLARIIDAE ON DIFFERENT MANGO VARIETIES AT DISTRICT KHAIRPUR

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The present study was carried out on mango farmer's field about 15 years old located at taluka Kingri, district: Khairpur, during, 2014. Five prominent mango varieties namely; Sindhri, Langra, Fajri, Chaunca, Siroli were kept under observation in summer season from 20, Aug - 1st, November on twice per weekly basis. The experiment was conducted on five treatments and replicated three times on randomize complete block design (RCBD) to ensure the pest population fluctuation and leaf damage percent per plant. Therefore, five severely damaged shoots were observed from one side thus, twenty shoots for single plant from north, east, west and south sides, respectively. The samples for the further confirmation were brought under Entomology laboratory conditions, Department of Zoology, SALU – Khairpur. The insecticide control measures were prohibited under the experimental field. Only the naturally population was kept under observation.

The results of study showed that maximum mean population of leaf miner, *Acrocercops syngamma* was observed on Chaunca 44.87±1.27 followed by Fajir 43.87±0.58, Sindhri 43.07±1.26 and Langra 38.31±1.03 on different mango varieties whereas; the minimum damage percent was observed on Langra 13.50% followed by Siroli 13.69%, Sindhri 16.04%, Fajiri 16.11% and Chaunca 16.33%, respectively. The analysis of variances showed the significant difference among all varieties (P<0.001). It is concluded that the heavily infestation of leaf miner pest was observed on Chanuca and the lesser on Langra mango varieties.

SOME STUDIES ON THE EFFECT OF MOISTURE CONTENTS OF *CRATEVA ADANSONII* (BARNA) AND *POPULUS DELTOIDES* (POPLAR) ON THE FEEDING OF SUBTERRANEAN TERMITES

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The moisture contents of wood and temperature play very crucial role in deciding the food and wood preference by the subterranean termites. A study was conducted to determine the role of moisture contents of the wood of *Crataeva adansonii* (barna) and *Populus deltoides* (poplar) in the feeding of subterranean termites and wood degradation by them. The wooden logs of *Crataeva adansonii* (barna) and *Populus deltoides* (poplar) were divided into small blocks of dimension 13x5x2 cm³. Then different treatments were made by sun drying and oven drying of wood at 80° C and 100° C temperatures. Water reabsorbance was checked at the intervals of 5th, 10th, 15th, 20th and 25th day and then blocks were exposed to the termites and percent weight loss was calculated. The results obtained showed that moisture absorbance was more in sap wood than the heart wood of barna and poplar. Poplar wood absorbed more water as compared to barna. Oven dried wood at 100° C absorbed maximum moisture in seasoned wood. In the seasoning of wood experiment the oven dry wood was more resistant to termites as compare to sun drying of wood as less consumption of wood was observed in oven dried woods. Maximum damage was in control treatments. Oven dried (100° C) woods of barna and poplar showed less damage than all other treatments giving the conclusion that as duration and level of oven drying increases, the damage to the wood decreases. The sap wood of barna as well as poplar was more preferred as compared to their heart wood. The poplar wood on overall basis was more preferred by subterranean termites than barna wood.

SPIDER FAUNA OF RICE CROPS IN DISTRICT DADU, SINDH, PAKISTAN

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Present study was conducted during the year 2014. To identified the spider fauna of rice in district Dadu. Result of surveyed areas showed the presence of five families namely Araneidae, Sparassidae, Lycosidae, Tetragnathidae and Oxyopidae. Families were identified on the basis of taxonomical keys.

HOST FEEDING PREFERENCE OF *CULEX QUINQUEFASCIATUS*. SAY OF SUB FAMILY CULICINAE IN DISTRICT DADU SINDH PAKISTAN**Nosheen Jehajo, Nasreen Memon and Naheed Shah***Department of Zoology University of Sindh, Jamshoro*

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Culex quinquefasciatus commonly known as the southern house mosquito. Mosquitoes were collected throughout the year during 2013 with light traps and by bait collection through aspirator. The total mosquitoes collection was show that *C. quinquefasciatus* was more predominant (90%) over the other species present and have anthropophilic in nature.

DISTRIBUTION OF SAND FLY IN THE PUNJAB WITH SPECIAL REFERENCE TO CLIMATE CHANGE IN PAKISTAN**Ayesha Akram¹, Abdul Qadir^{1*}, Hafiz Azhar Ali Khan², Hassan Ali³ and Arshed Makhdoom Sabir⁴**¹*College of Earth & Environmental Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore*²*Institute of Agricultural Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore*³*Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan*⁴*Rice Research Intitute Kala Shah Kako, Punjab, Pakistan*

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The aim of current research was to examine the distribution of sand fly with reference to climate change and knowledge, Attitude and practices related to diseases caused by sand fly. It was hypothesized that there is a relation between climatic change and dispersal of sand fly. Leishmaniasis is a vector-borne disease caused by sand fly. Success of any disease control program depends upon the knowledge, attitude and practices of the community. For this purpose a KAP study was conducted during December to March 2014 in the five cities; Multan, Bahawalpur, Faisalabad, Jhang and Lahore, Pakistan. About 250 people of these were interviewed through convenient sampling method. The data was analyzed using descriptive analysis. The results indicated that a significant number of people (68.4%) were not aware about sand fly and the diseases caused by this vector. Moreover, the extents of people (73.2%) consider that climate change has effect on the transmission of diseases. So in present study the current and future distribution of san fly in different climate scenarios is predicted using maximum entropy model. The findings indicated that Precipitation of Driest Quarter (Bio17) and Mean Diurnal Temperature Range (Mean of monthly) (Bio 2) provided the most useful information for Leishmaniasis, these variables were the top two predictors in the Maxent model with contributions of 16% and 15.8% respectively. Averaged future predictions from bioclimatic models of 2050 showed decrease in the spread in Baluchistan while altitudinal shift in the areas of KPK, Punjab and Gilgit-Baltistan Provinces. So the findings of current research revealed that climate change had impact on the distribution of sand fly.

MORPHOMETRIC CHARACTERISTICS OF *APIS DORSATA* FROM FAISALABAD DISTRICT

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Apart from *A. dorsata* is found throughout Pakistan. Present research provide latest information about morph-metric variations in honey bees from Pakistan, based on 40 worker bees samples collected from each hive of ten different areas (Marzipura, Chak 279 Rb, Chak 29 Jb, Shakot, Bhawana, G M Abad, Chak 30 Jb, Pansara, Chak 32Jb and Chak 33 Jb of Faisalabad district, Pakistan. It has been found that body width (4mm), number of simple eyes (3), number of sub segments of antenna (10), length of antennal filament (3mm), number of abdominal segments (6) were exactly same in all bees samples collected from Faisalabad district. Similarly, there was no divergence found among length of hind wing (8mm), width of hind wing (2mm), length of fore wing (13mm), width of fore wing (4mm), length of foreleg (9mm), length of middle leg (10mm), length of hind leg (10mm), length of tibia (4mm), length of femur (3mm), length of basitarsus (3mm), width of basitarsus (1mm), length of 2nd tergite (8mm), width of 2nd tergite (2mm), length of 3rd tergite (9mm), width of 3rd tergite (3mm), length of 4th tergite (9mm), width of 4th tergite (2mm) and in the color of marginal setae on the hind leg tibia of all *A.dorsata* collected from district Faisalabad. However, the cubital index of fore wing and number of humuli showed variation among these populations.

A SURVIVAL RATE COMPARISON AMONG THREE *PARDOSA* SPECIES OF LYCOSID SPIDERS UNDER LABORATORY CONDITIONS

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The survival rate of three *Pardosa* species (*P. birmanica*, *P. leucopalpis* and *P. oakleyi*) was studied under laboratory conditions. The spiders were reared in clear perplex cages on artificial diet. The cotton swab was soaked in modified diet and kept in the cage. The diet was changed after regular interval (24 hours). After mating ten female spiders were selected for research in separate cages. On hatching newly born spiders lings were climbed on the mother back for 4 days after that they were separated from the mother's back. A group of seventy spider's lings was separated randomly from all the cages for research and to prevent cannibalism, each spider-ling was introduced into an isolated cage. Daily observations were noted on each cage. The first molting stage was spent on the mother's back. The selected *Pardosa* species exhibited eight instars to become adult. After the emergence of egg 60% survival rate was observed durring the 1st molting stage of *P. birmanica* 61%, 70%, 68%, 83%, 77%, 79%, 80% of survival rate was observed during the 2nd, 3rd, 4th, 5th, 6th, 7th and 8th. The highest survival rate of *P. birmanica* were observed

at 8th instar level. The survival rate of *P. leucopalpis* was observed at each instar level and indicated that 45 % survival rate was observed at 1st molting stage, 50%, 56%, 60%, 75%, 83%, 80%, 81%, was observed during the 2nd, 3rd, 4th, 5th, 6th, 7th and 8th instars. The *P. oakleyi* had 47% survival rate at 1st molting stage, 47%, 53%, 60%, 65%, 70%, 74%, 71%, 75% survival rate was observed during the 2nd, 3rd, 4th, 5th, 6th, 7th and 8th instar level. The percent survival of male and female of three selected *Pardosa* species was also determined.

GENUS *NEOSEIULUS* HUGHES (ACARI: PHYTOSEIIDAE), NEW RECORDS AND NEW SPECIES FROM PAKISTAN

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Family Phytoseiidae comprises of diverse group of predatory mites known to the world as the most promising biocontrol agents regulating the population of sucking insects and mites pest. Genus *Neoseiulus* Hughes is one of the major concerning groups within family Phytoseiidae to be used as natural enemies. This study describes two new records, *Neoseiulus barkeri* Hughes and *Neoseiulus paspalivorus* De Leon for the first time from Pakistan. A new species *Neoseiulus reticulatus* n.sp. is also described here thus making total 36 species from this country. A key to the 36 valid species from Pakistan is also described.

SPECIES COMPOSITION OF THE TRIBES COTESIINI AND APANTELINI (MICRGASTRINAE: BRACONIDAE: HYMENOPTERA) OF THE NWFP.

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Studies were conducted to record the species composition of the two tribes of microgastrinae (Braconidae) viz. Apantelini & Cotesiini of the NWFP. Two genera i.e. *Apanteles* and *Dolichogenidea* were recorded in the tribes Apantelini. Genus *Apanteles* was found to be more diverse with eight species. The species recorded are *calycinae*, *malakanderi*, *agilis*, *harooni*, *significance*, *peshawarensis*, *carinatus* and *parasae*. Out of these species *malakanderi*, *harooni*, *peshawarensis* and *cainatus* are new to the science. Genus *Dolichogenidea* is represented by one species i.e. *lactea*. In tribes Cotesiini two genera viz. *Cotesia* and *Glyptapanteles* were recorded. Genus *Cotesia* is represented by six species including *tibialis*, *qadeeri*, *lycophron*, *flavipes*, *carinatus* and *irfani*. Species *carinatus* and *irfani* are reported as new to science. Only one species *maculitarsus* were found in *Glyptapanteles*. Overall, a total of 16 species are reported in the two tribes in the NWFP. Descriptions and keys have been prepared for the identification of the reported species. Information is provided on distribution and seasonal pattern of flight in the NWFP. Illustrations of important diagnostic characters have also been provided.

**DIVERSITY AND RELATIVE ABUNDANCE OF ORDERS COLEOPTERA AND
DIPTERA AMONG RICE, WHEAT AND MAIZE CROPS OF CENTRAL PUNJAB**

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Cereals are major food source of Pakistan and About 90% of the grains available for the consumption are consisting of cereals. Impact of insects is the principle factor for ideal outcomes and half of the world's recognized animal species are insects. Hence research on their occurrence is pre-requisite for future ideology. Coleoptera is the biggest order playing vital role to accomplish the pollination, decomposition, spread of seeds, cycling of nutrients in various agro-ecosystem. Whereas, Dipteran act as a vector of many diseases. Hence for future concern, diversity of both orders was recorded among maize, rice and wheat in central Punjab from September 2013 through April 2014. Successive sampling was done in different areas those were located around the city of Faisalabad by using different sampling methods like hand picking, forceps and netting. The collected specimens were preserved, labeled and identified properly in the laboratory. Data was analyzed statistically by using Shannon-Weiner diversity index and maximum diversity was recorded in rice fields and low in wheat fields and order Diptera was higher in all fields as compared to Coleoptera. Overall specimens of Coleoptera and Diptera collected were 2206, in which 1820 specimens from wheat, 167 from rice and 219 from maize. Order Coleoptera consisted of 6 species, whereas 11 species of Diptera were recorded from the three crops. Total species identified were 42 of which 9 were identified from wheat, 28 from rice and 18 from maize. *Coccinella septempunctata* (1589) belonging to order Coleoptera was the most abundantly found species. However maximum diversity was found in the rice crop.

**TAXONOMIC STUDY OF WASPS SPECIES (HYMENOPTERA: VESPIDAE) FROM
HAZARA REGION, KHYBER PAKHTUNKHWA, PAKISTAN**

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This study was based on the random collection of wasps species from three Districts of Hazara Region, Khyber Pakhtunkhwa, Pakistan. In the present research work family Vespidae under the three subfamilies Eumeninae, Polistinae and Vespinae were studied. A total of 7430 wasps specimens were collected during their active season March to October of three consecutive years from 2011 to 2013. Identification up to lowest possible taxa were made with the help of latest available literature at National Insect Museum (NIM), National Agriculture Research Center (NARC), Islamabad and in Richard Gilder Graduate School, American Museum of Natural History, New York, USA. Identification yielding nineteen (19) species within eight (08) genera of three subfamilies Eumeninae, Polistinae and Vespinae of family Vespidae. Among nineteen (19) species

four (04) species were recorded for the first time from Pakistan, nine (09) species were new to Hazara Region, Pakistan. Wasps of these subfamilies are social and semi social. The wasps were collected. For DNA extraction the collected specimens were preserved in 95% ethanol. Molecular study of the collected 19 species of the three subfamily of Eumeninae, Polistinae and Vespinae were studied with eight luci, in which two genes Pol, Wg were nuclear and six genes 12s, 16s, 28s, Col1, Cyt B and Jerry were mitochondrial. Phylogenetic relation were analyzed by using geneious and MAFFT software available online.

LIFE CYCLE OF *TRIBOLIUM CASTANEUM* (RED FLOUR BEETLE) IN DIFFERENT FOOD HABITAT UNDER LABORATORY CONDITION

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Tribolium castaneum (Red flour beetles) are very destructive to stored grain product in Pakistan. Present study was conducted to observe the food preference for growth at different stages in *Tribolium castaneum* on two different flour food media, *Triticum indicum* (Wheat), *Oryza sativa* (Rice) under laboratory condition. Collection of flour beetles from store bin and guni bags of shop and house and reared in lab. Material was studied and compared with present literature. Larval and pupal developmental time faster on *Triticum indicum* and slower in *Oryza sativa*. There were good differences in the growth and life cycle in both media. Morphologies and behavior of different instar were significantly studied.

DIVERSITY OF DRAGONFLIES DWELLERS OF THE UPPER SIREN VALLEY IN MANSEHRA

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The present work is aimed to study diversity of dragonflies species encountered in the upper Siren valley in Mansehra. This study has been carried out in ten stations for two consecutive years 2012-2013. The dragonflies were investigated by monitoring numerical, locality abundance, and periodical abundance diversity. The numerical diversity showed twelve dragonflies species. A total of 300 specimens were collected from the study area. Which yielding, 12 species, 7 genera and 2 families, Libellulidae and Gomphidae the identified species were Spine-legged Redbolt, *Rhodothemis rufa* (Rambur, 1842), Black tailed skimmer, *Orthetrum cancellatum* (Linnaeus, 1758), Blue or Black percher, *Diplacodes Lefebvrei* (Rambur, 1842) Common red skimmer, *Orthetrum pruinosum neglectum* (Rambur, 1842), Clubtails, *Onychogomphus bistrigatus* (Selys, 1854), Ground skimmer, *Diplacodes trivialis* (Rambur, 1842), Aeshna minuta, *Palpopleura sexmaculata* (Fabricius, 1787), Blue Marsh Hawk, *Orthetrum glaucaum*, (Brauer, 1865), Common skimmer, *Sympetrum decoloratum* (Selys, 1884), Eastern Least Clubtail, *Stylogomphus albistylus*, Triangle Skimmer, *Orthetrum triangulare triangulare* (Selys, 1878), *Sympetrum commixtum* (Selys, 1884). The most encountered species found was *Orthetrum pruinosum neglectum* whose 55 specimens

were collected. The least encountered species was *Orthetrum triangulare triangulare*, 42 followed by *Rhodothemis rufa*, 39. The Seran Valley is rich in insect biodiversity, the dragonflies fauna of this valley needs to be further explored and similar surveys on large scales are recommended to fully evaluate the dragonfly fauna of District Mansehra.

THE INFLUENCE OF WINTER WHEAT VEGETATION AND CLIMATIC FACTORS ON THE BIODIVERSITY AND POPULATION DYNAMICS OF THE SPIDER'S FAUNA

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Spiders are natural predators of almost all type of insect pests and wheat is a pivotal crop of Pakistan, it may encounter by various insect pests that ultimately results in reduction of its total yield. Various species of diverse fauna are inhabitant of Wheat fields. Hence, as a part of investigation an extensive survey of spider fauna was carried out throughout the growth period of winter wheat for both ground and foliage species. Sampling was done for every week from December through April during 2008 and 2009. A total of 1209 specimens belonging to seven families, 20 genera and 55 species were recorded. Lycosidae was the most dominant family with 21 species (38%) whereas clubionidae comprised single species (1.8%) with least in number. Maximum spiders were collected in the month of March (151) followed by February (143) and April (128) during 2008. During 2009, maximum spiders were captured in March (169) through January (156). March was found enriched with spider's diversity and the highest evenness was recorded during December. Species composition and abundance was varied during the investigation period due to the climatic conditions and the early developmental stages of wheat that improved gradually at maturation /ripening stage and then dropped again. The aim is to find and identify the best factors, impact on species prevailing with the age of crop in order to save and enhance the crop yield.

STUDIES ON SOME FLIGHTLESS GENERA OF ACRIDIDAE AND DERICORYTHIDAE (ACRIDOIDEA , ORTHOPTERA) OF PAKISTAN

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The flightless genera belonging to families of Acrididae and Dericorythidae of Acrididoidea collected from the mountainous area of Qutta and Zhob area of Baluchistan and Khyber Pakhtunkhwa. From the collected specimens following species are identified; *Parancophoma kashmericum* Miskcheuko, *P.minutum* Mishchenko of subfamily Paraconoplyminae *kabuha balucha* Uvaro *Kabula* sp. of subfamily Teratodinae of family Acrididae and *Conophoma* sp. of subfamily *Conophyminae* family Dericorythidae. The female of *Conophyma minutum* is described for the first time.

COLLECTION OF INFESTED GUAVA FRUITS IN HYDERABAD DISTRICT FOR ANALYSIS THE PARASITOID UNDER LABORATORY CONDITIONS

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Studies were carried out at the Nuclear Institute of Agriculture (NIA) Tandojam for consecutive three years (2011-2014) to assess the infestation of fruit flies *Bactrocera zonata*, *B. dorsalis* and parasitization of larval cum pupal parasitoid *Tribliographa daci*. The guava fruits under the infestation of *B. zonata* and *B. dorsalis* were collected and stored to examine the *T. daci* parasitization. Regardless the sex of fruit flies, their infestation as well as parasitization of the parasitoid were affected significantly ($P < 0.05$) by months of the year; while non-significant ($P > 0.05$) influence of the years of study was observed. Out of total monthly mean pupal number (192.01±17.52) with mean pupal weight of 1.88±0.13, the total emerged *B. zonata* pupae were 118.07±11.14 including 15.01±1.20 male, 101.61±10.36 female, 1.44±0.07 deform and the percentage of pupal emergence was 60.61±1.49%. The *B. zonata* pupal emergence was higher i.e. 70.32±0.87, 70.37±0.22 and 70.32±0.65% in the month of May during 2011-12, 2012-13 and 2013-14, respectively; while *B. zonata* pupal emergence was lowest in December during all the three years of study. The total emerged *B. dorsalis* pupae were 7.67±0.58 including 1.67±0.07 male, 5.53±0.53 female, 0.46±0.02 deform and the percentage of *B. dorsalis* pupal emergence was 4.41±0.18%. The *B. dorsalis* pupal emergence was higher i.e. 6.66±0.62, 6.43±0.19 and 6.61±0.37% in the month of January during 2011-12, 2012-13 and 2013-14, respectively; while *B. dorsalis* pupal emergence was lowest in May. The total emerged *T. daci* pupae were 51.19±6.18 including 8.61±0.71 male, 42.58±5.69 female, and the percentage of *T. daci* emergence was 23.83±1.18%. The *T. daci* pupal emergence was higher in August i.e. 31.15±0.54, 31.35±0.22 and 32.57±0.48% and in November 31.79±1.06, 32.72±1.01 and 32.71±1.04% during 2011-12, 2012-13 and 2013-14, respectively; while *T. daci* pupal emergence was lowest in February. This indicates that *B. zonata* pupal emergence was higher in the months of high temperature and lower in the lower temperature; while *B. dorsalis* emergence was higher in months of low temperature and lower in months of high temperature. However, *T. daci* parasitization did not show a linear association with the temperature variation.

**PROLONGED COLD STORAGE OF SEVEN SPOTTED LADYBIRD BEETLE
COCCINELLA SEPTEMPUNCTATA (COLEOPTERA; COCCINELLIDAE): AN
EFFICIENT TOOL IN MASS REARING**

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Most ladybird beetles do not fly if the temperature drops below 55°F (13°C) and hence spend the cooler months in diapause, an insect analogue to hibernation. This phenomenon can be a

useful tool to enhance the availability of these useful natural enemies by storing them at low temperatures. Present studies were designed to enhance mass rearing techniques of seven spotted ladybird beetle *Coccinella septempunctata* (Coccinellidae; Coleoptera) by studying long term cold storage technique to facilitate their field application. Cold storage studies showed that beetles can be stored on average up to 27 weeks at 4°C but the survival rate decreases with time. Also, water feeding during cold storage could be a crucial factor. Water feeding at weekly intervals is effective for short term storage as it increases the survival rate. However, for long term storage water feeding at long intervals is recommended so that there is minimum disturbance.

SPECIES DIVERSITY AND DISTRIBUTIONAL PATTERN OF COCKROACHES IN LAHORE, PAKISTAN

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Cockroaches are found as most common urban pests of tropical countries in world, prompting economic and serious health risk problem for humans by carrying microbes and allergens, acting as vector for various pathogens of disease. The present study on cockroach species composition, diversity, abundance and richness was conducted from April 2013 to March 2014 in various human dwelling localities of urban area of district Lahore, Pakistan. Cockroaches were collected randomly by hand, food baited traps and sticky traps throughout the year. Study area includes different residential areas, hospitals, Shopping malls/stores and institutes. Total 4 species were collected and identified from the study site include *Periplaneta americana*, *Blattella germanica*, *Blatta orientalis*, and *Blatta lateralis*. *B. germanica* was found as the most dominant indoor species with highest diversity indices in study areas. Overall cockroach species diversity was highest in July–September with Simpson index of diversity 0.6547 and Shannon index -0.51389 followed by October–December with diversity indices of 0.6521 (Simpson index of diversity) and -0.51187 (Shannon index). *P. americana* as outdoor species was found second broadly distributed in the study area. Residential areas and hospitals are highly infested with *B. germanica* followed by *P. americana*. Population index of *B. germanica* for hospitals was doubled than residential areas. *B. orientalis* and *B. lateralis* are intermediately distributed in residential areas and narrowly distributed in hospitals. *B. lateralis* is observed by pest management professionals as displacing *B. orientalis* in outdoor habitat through competing with its habitat and food sources.

IDENTIFICATION OF CRICKET SPECIES (ORTHOPTERA: GRYLLIDAE) ON THE BASIS OF MALE SONG PATTERNS AND SOUND PRODUCING ORGANS

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In the present work, different genera and species of family Gryllidae were identified on the basis of acoustic characters i.e. male song features, and morphology of sound producing organs, i.e. pars stridens, tegmina, and plectrum. These characters were successfully used to clearly differentiate different sibling species which were considered earlier as a single species and their

identification was confirmed without any difficulty and with ultimate satisfaction. Until the beginning of 20th century the cricket species were identified worldwide by using their external morphological characters including those of their external male and female genitalia, but the taxonomists were not satisfied with using these characters. Allard (1910) was the first researcher who recognized for the first time geographical variations in the sounds of field crickets. Fulton (1932) was the first natural historian who used male calling song to separate cricket species including sibling species, which were previously considered as a single species. When the new technologies of sound recording became available acoustic characters are being used as taxonomic characters, and is being considered as the most reliable and useful characters for their identification. Stridulatory organs are now also considered as important taxonomic characters for the recognition of cricket species especially when electron microscope giving high powered resolution became available. In stridulum, different structures were studied, i.e. particular numbers of teeth, density and structure of teeth, and length of file. For the present work crickets were collected from different areas of Pakistan, usually at night. Songs of males were recorded with the help of Steinberg H4n Cubase LE4 Bundled Handy recorder. After recording, songs were sliced with the help of Audacity 1.3 Beta (Unicoded) software. These sounds were studied and analyzed by the use of software Matlab version 7.12.0.635 (R2011a) 32-bit (Win 32) on March 18,2011, with License No. 161052. After sound recording and preservation, the specimens were softened in boiling water for detaching the right fore wing, for taking photograph by using Nikon Cool Pix ~400 digital camera after placing it under Nikon SMZ 800 Binocular. Morphological study of file teeth, plectrum, and microtracheae were analyzed with the help of Scanning Electron Microscope. SEM photographs were further analyzed by using the software of Coral Draw 13, Coral Inc. 2005. The results were compared with the descriptions of male calling songs and those of sound producing organs of closely related taxa given in the literature.

SPECIES COMPOSITION OF ANTS RECORDED FROM DISTRICT MANSEHRA

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Present study highlights species composition of Formicidae fauna of district Mansehra. Detailed surveys were carried out in eighteen localities. As a whole record of 30 species identified under 18 genera of 3 subfamilies is reported herein. Among these, subfamily Myrmicinae was dominant with 22 species and 12 genera followed by subfamily Camponotinae having 7 species and 5 genera while a single species of subfamily Ponerinae has been recorded. In order to provide updated information help was also taken from recently published literature on Ants fauna of Pakistan and housed collection of family Formicidae in National Insect Museum.

EFFECT OF ANCHORS ON WEB STRUCTURE OF *NEOSCONA THEISI*

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The present study was conducted to examine the plasticity in the web of *Neoscona theisi* in three different complexities of vegetation to test the influence of available space on web structure. This was done by integrating field experiments. The plots were randomly selected and converted

into three treatment groups having different number of plants (group-I = 900 plants/m², group-II = 700 plants/m² and group-III = 450 plants/m²). Vegetation complexity was checked by throwing wooden stick in vegetation and counting the number of plants touching the stick. Each experimental group was consisted of 10 replicate plots. Each plot was separated from the other by four meter distance to restrict the spiders to travel from one plot to the other. In each plot five adult female spiders after starvation of four days were placed. When the spiders formed webs, different measurements viz., web size, mesh height, capture area and capture thread length were recorded. The spiders exposed to dense vegetation (T₁), formed smaller webs compared to less dense plots (T₂ and T₃). Web parameters i.e., web size, mesh height, capture area and capture thread length were gradually decreased with the density of the vegetation. The study suggested that the available space between anchor points plays crucial role on web size and web geometry of the spider.

DIVERSITY OF BUTTERFLIES IN BAHAWALPUR

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The objective of present study was to document butterfly species from area of Bahawalpur. Total 163 specimens were collected from different habitats from December 2013 to May 2014 and identified by keys. Total four families and 10 species were documented in study area. Family Nymphalidae found to contain *Danaus chrysippus* and *Junonia almana*. Species of family Pieridae were *Colotis etrida*, *Colotis amata*, *Belenois aurotas*, *Neophasia menapia*, *Colias philodice*. Species of family Lycaenidae are *Taracus callinara* and *Euchrysops cnejus*. Family Papilionidae represent only one specie *Papilio demoleus*, among them *Neophasia menapia* (family Pieridae) followed by *Danaus chrysippus* (family Nymphalidae).

SECTION – IV

PARASITOLOGY

DIPLOTRIAENAE SP. FROM COMMON MYNA (*ACRIDOTHERES TRISTIS*) IN JOHI DISTRICT SINDH, PAKISTAN.**Syed Ali Taqi Shah, Riddain Imtiaz and Sanjota Nirmal Das***Department of Zoology, University of Sindh, Jamshoro,
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Seven Birds common *Acridotheres Tristis* were purchased from District Johi. The birds were examined in the parasitological laboratory for collection of helminthes parasites seven out of five mynas were found infected with nematodes. The nematodes were found in a bunch form in the body cavity. The nematodes studied alive under binocular later, there were fixed in 70% alcohol and stored in a mixture of glycerin and alcohol 70% a detailed studied was conducted. The specimen were identified as belonging to the genus *Diplotriana* (Railliet *et* Henry 1905) 20 males and 12 females specimens were collected and studied in detail. Male: Long and slender worm cuticle slightly striated anterior end comparatively more attenuated and attains with gradually mouth opening simple, without lips surrounded by minute lateral and medium pairs of minute papillae. Esophagus is long ending into club shaped structure tail ends into broadly ended and Candle alae are absent. Female: Body is longer shorter than males. Body cuticle slightly striated. Mouth structure rather same as in male with esophagus trident prominent structure. Genital opening is anterior body region. Eggs double welled thick shelled oval in shaped.

EPIDEMIOLOGY OF *PLASMODIUM* PARASITEMIA IN GENERAL POPULATION OF BANNU DISTRICT KHYBER PAKHTUNKHWA (KPK), PAKISTAN**Muhammad Ilyas Khan and Zia Ur Rahman Awan****Department of Zoology, Govt. Post Graduate College Banuu, Pakistan
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Malaria is one of the global public health problems and imposes a major burden on health in under developed countries of world. Half of world population is at risk of malaria with an estimated 250 million clinical cases and nearly one million deaths were reported. Malaria is a leading cause of morbidity and mortality in the developing world, especially in the sub Saharan Africa where the transmission rate is highest and there it is considered as a major obstruction to economic development. A parasitological survey was conducted to know the epidemiology of malaria and their causes in different conditions. Total four hundred and forty (440) blood samples were randomly collected in different localities of district Bannu from both males and females population of varying age group (1- >50) from the months of September 2013 to June 2014. Malaria was highly prevalent 131/440 (29.77 % microscopy) and 97/440 (22.04% RDT) in the studied area. *P. vivax* was found to be more prevalent both by Microscopy 116/131 (88.54%) and RDT 61/97 (62.3 %). Males of varying age groups were highly susceptible to malaria infection 80/235 (34.04 % by microscopy) and 59/246 (23.98% by RDT_s) than females of varying age groups 51/205 (24.87 % by microscopy) and 38/194 (19.58% by RDT_s). Both diagnostic results

showed that highest cases were found in a sector A, while the lowest prevalence of malaria infection was found in a sector C.

RAILLIETINA PASSERINA N. SP. (DAVAINEIDAE: CESTODA) FROM THE INTESTINE OF HOUSE SPARROW *PASSER DOMESTICUS* (PASSERIDAE: PASSERIFORMES) FROM HYDERABAD, SINDH, PAKISTAN

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During the examination of 29 House Sparrows (*Passer domesticus*) from Sindh, Pakistan, two specimens of genus *Raillietina* Fuhrmann, 1920 (Davaineidae: Cestoda) were collected from intestine of a single host and identified as *R. passerina* n. sp. The specimens were flattened under slight cover glass, fixed in formal-alcohol-acetic acid, stained with Borax carmine, dehydrated in graded series of ethanol, cleared in clove oil and xylol and mounted in Canada balsam. Diagrams were made with the help of camera Lucida. The specimens differ from its congeners in scolex and diameter of suckers, mature and immature segments, and number of hooks present in a single row, testes and ovary size. Presences of Mehli's gland, vitelline gland, seminal receptacle as well as number of eggs are further distinguishing features of the new species. The species name refers to the generic name of the host.

RECORDS OF CHEWING LICE (PHTHIRAPTERA: INSECTA) FROM BIRDS OF FAMILIES CACATUIDAE AND PSITTACULIDAE (PSITTACIFORMES: AVES) FROM SINDH REGION, PAKISTAN

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Birds belong to families Psittaculidae and Cacatuidae (Psittaciformes: Aves) were examined for their chewing lice (Phthiraptera) from different regions of Sindh, mainly Karachi and Hyderabad. It is the first taxonomical study of chewing lice on parrots of Sindh region. The lice belonging to suborder Amblycera included three species of family Menoponidae, were *Afrimeneopon waar* (Eichler, 1947), *Franciscoloa psittacus* sp. n. and *Colpocephalum pilgrim* Price, 1967 from Budgerigars, *Melopsittacus undulatus* Shaw (Psittaculidae), and suborder Ischnocera included four species of family Philopteridae, were *Neopsittaconirmus lybartota* (Ansari, 1947) from *Psittacula krameri* (Scopoli) (Psittaculidae), *N. chandabani* (Ansari, 1947) from *Psittacula eupatria* (L.) (Psittaculidae), *N. clayae* Guimaraes, 1974 from *Probosciger aterrimus* (Gmelin) (Cacatuidae), *Neopsittaconirmus* sp. from *Nymphicus hollandicus* (Kerr) (Cacatuidae) and *Echinophlopterus alexanderius* sp. n. from *Psittacula alexanderi* (L.) and *P. krameri* (Scopoli) (Psittaculidae). In the present study, all the chewing lice species were newly recorded from the region except of *A. waar*; genus *Franciscoloa* and *Colpocephalum* were found first time on the present host species; the genus *Franciscoloa* was named for its host family name; the new species of *E. alexanderius* was named for its host species from which it was being first collected.

**CERCOCOTYLA ATTHISI N. SP. (DIPLOSTOMATIDAE: STRIGEIDIDA: TREMATODA)
FROM THE INTESTINE OF COMMON EURASIAN KINGFISHER *ALCEDO ATTHIS*
(ALCEDINIDAE: CORACIIFORMES) FROM MATIARI, SINDH, PAKISTAN**

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A new species *Cercocotyla atthisi* n. sp. is described from the intestine of Common Eurasian Kingfisher *Alcedo atthis* (Lin.) (Alcedinidae: Coraciiformes) collected from Matiari, Sindh Pakistan. On the basis of diagnostic differences between present species and its congeners in having large, tripartite body, midbody with plications on its surface and testes of smaller size, a new species *C. atthisi* is proposed. The name of the species refers to the specific name of host. *Alcedo atthis* is new host record for the genus *Cercocotyla*; however this genus is being reported for the first time from Pakistan.

ABOUT SOME ANTIHELMINTHICS USED FOR NEMATODES OF GOATS

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Chemical composition of anthelmintics used for goat nematode infections is described here used recently in Pakistan and other countries. These includes Thiabendazole, Mebendazole, Fenbendazole, Oxfendazole, Oxibendazole, Albendazole, Febantel, Haloxon, Levamisole, Morantel, Ivermectin, Valbazen, Levamisole, Dextomax, Benzimidazole. Some of these are effective for human nematodes. Types of goats in Pakistan is also mentioned.

ACANTHOCEPHALA OF BIRDS: A REVIEW UPDATE

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A review update of Acanthocephala of birds is given. Various parasites of birds are listed Acanthocephala of birds from India and Pakistan is reported. Diseases of birds and economic importance is described. This information provides a comprehensive update of Acanthocephala of birds and will be useful for students and researchers in this field.

**PREVALENCE OF HELMINTH PARASITIC INFECTIONS IN MICE (*MUS MUSCULUS*)
IN DIFFERENT AGRICULTURAL FIELDS OF DISTRICT SWAT, PAKISTAN**

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During the study a total of 81 mice were trapped from different agricultural fields of district Swat, Pakistan. The viscera were collected after autopsy examination of mice, and preserved in 70% alcohol and then brought to VPCI, SARC/PARC, Medical Zoology Laboratory for helminths investigation. The viscera were then opened and the organs like stomach, small intestine, large intestine, liver and lungs were opened longitudinally. These organs were found to be infected with helminth parasites: Nematodes and Cestodes. Nematodes (Round worms) were isolated and placed in a petri dish containing normal saline solution, then preserved in 70% alcohol and glycerin solution for detail microscopic examination while the Cestodes were slightly pressed between two glass-slides, kept in FAA solution for 24-hours, for fixation then de-hydrated with graded series of alcohol, stained with Mayer's Carmalum, cleared with clove oil, rinsed with xylene and mounted with Canada balsam. The overall prevalence in order to their intensity is as: *Syphacia* sp. 5 (6.17%), *Heterakis* sp. 4 (4.93%), *Aspicularis* sp. 3 (3.70%), *Hymenolepis diminuta* 3 (3.70%), *H.swatensis* sp.n. 1 (1.20%) were reported. No considerable difference in the prevalence of parasites was noted b/w areas, crops, crop stages and sex of the host while adults were found more infected than sub-adults.

**PREVALENCE OF CHEWING LICE SPECIES (PHTHIRAPTERA: INSECTA) ON
COMMON DUCK, *ANAS PLATYRHYNCHOS* (ANSERIFORMES: AVES) IN KARACHI
REGION, PAKISTAN**

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The chewing lice (Phthiraptera: Insecta) were examined for their population and rate of infestation from common ducks, *Anas platyrhynchos* (Anseriformes: Aves) in Karachi region during the year 2012-2013. 16 ducks have been observed in different localities of Karachi. Their chewing lice have been collected, mounted in microscopic slides and identified using latest literature. Four species of chewing lice were reported during the survey, with their population density on host body. The data were taken from every locality by random collection method. The chewing lice species were recorded with their prevalence were *Holomenopon leucoxanthum* (Burmeister, 1838) = 52.61% with its highest population density; *Anaticola crassicornis* (Scopoli, 1763) = 22.85%, *H. fatemae* Naz and Rizvi, 2012 = 18.86%, *Ardeicola gaibagla* Ansari, 1947 = 4.85%, and *Trinoton querquedulae* (Linnaeus, 1758) = 0.8% with the least density of lice population on birds. During the survey, *H. leucoxanthum* was recorded as new from Pakistan, however all species were newly recorded from Sindh region, Pakistan.

COMPARISON OF INFECTION RATE BETWEEN GULFAM (*CYPRINUS CARPIO*) AND OTHER CARP FISHES CAUSED BY ECTOPARASITES FROM CARP FISH HATCHERY DISTRICT BADIN, SINDH, PAKISTAN

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During the research period from August 2011 to July 2012 to find out magnitude of parasitic infection in different fish species cultured at carp fish hatchery district Badin with special reference to Gulfam (*Cyprinus carpio*) carp fish. A sample of 258 specimens of fishes was examined for parasitic infection. The present study shows the comparison of Gulfam (*Cyprinus carpio*) fish with other Carp fishes. Out of 82 Gulfam fish (*Cyprinus carpio*) 54 was found infected which is (66%). Out 40 Rohu (*Labio rohita*) 20 was found infected which is (50%), out of 20 Black Rohu (*Labeo calbasu*) 8 was found infected which is (40%). Out of 20 Cereah (*Labio gonius*) 7 was found infected which is (36%). Out of 16 Groj (*Mastacembelus armatus*) fish 04 was found infected which is, (25%). Out of 30 Shakur (*Channa punctatus*) 12 fishes found to be infected which is, (30%), Out of 20 Shakur (*Channa marulus*) 5 was found infected which is (25%), out of 12 Shakur (*Channastraitus*) 4 was found infected which is (25%), out of 18 Morakhi (*Cirrhinus mrigala*) 3 was found infected which is, (11%) Highest infection was observed in Gulfam (*Cyprinus carpio*) and lowest infection was observed in Morakhi (*Cirrhinus mrigala*).

DETECTION OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX* BY POLYMERASE CHAIN REACTION IN KARACHI-PAKISTAN

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Malaria is the world's worst public health problem, at the current situation, more people are ill with malaria than any other disease and the numbers of infected peoples are increasing day by day. It is one of the major health challenges of the poorest and developing countries throughout the world. It is effecting on the behavior, socioeconomic status and distribution of human population. An epidemiological and comparative study for malarial parasites was conducted in Karachi-Pakistan. The investigation based on finding and particular identification of malaria parasites in blood films made from a sample of peripheral blood and the finding of species specific parasite DNA in a blood samples, using a PCR method for the comparison. The routine microscopic examination for identification of different *Plasmodium* species is very valuable and cheap but this method is laborious and time-consuming, due to advancement in the techniques, an alternative diagnostic method such as PCR, has been successfully used for the detection of malarial parasites that is more sensitive and specific and especially is the best in low paracitemia cases. For the determination of high frequency of *Plasmodium* species comparison of diagnosis was done by microscope and circumsporozoite gene extracted from the blood samples which were collected from 100 patients between the age of 5-65 males and females having high temperature and severe headache complains, residing in five different districts of Karachi -Pakistan during one year. The blood was taken directly onto a slide from a finger. Microscopic test (oil immersion objective at 1000 x magnification) was done with Giemsa-stained thick and thin blood smears whereas PCR

was performed on dried blood removed from slides and filter papers and used for species-specific parasite deoxyribonucleic acid (DNA) amplification by the polymerase chain reaction (PCR). Out of 100 Giemsa-stained slides 54 were positive for malaria parasites in which 31 and 19 were identified as a *Plasmodium vivax* and *Plasmodium falciparum* infections, respectively, whereas 6 were having mixed *P. vivax* and *P. falciparum* infections by microscopy and 4 more mixed infections were found by PCR methods. The present investigation revealed that, those areas where transmission of both *P. vivax* and *P. falciparum* occurs, nested PCR detection of malaria parasites can be a very useful complement to microscopically diagnosis, in microscopic examination, false positives does occur, generally as a result of poor staining techniques. Identification of the species of malaria involved is still necessary. PCR using appropriate primers is highly specific. This procedure can be valuable in determining the existence of mixed infections missed by microscopy. The sensitivity and specificity of PCR for detection of *P. vivax* and *P. falciparum* malaria was higher than that of microscopy.

HISTOPATHOLOGY OF INTESTINE OF FRESHWATER FISH *CHANNA PUNCTATUS* INFECTED WITH ACANTHOCEPHALAN PARASITES FROM DISTRICT BADIN SINDH, PAKISTAN

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This study was conducted to know the prevalence and pathological changes in the intestine of fresh water fish *Channa punctatus* naturally infected with acanthocephalan parasites. During present investigations 52 fishes (*Channa punctatus*) were examined. The parasites collected from the intestine were preserved, stained and mounted by using standard techniques. For the histopathological investigation tissue samples from infected intestine were fixed in Bouin's fluid for 24 hours using routine histological techniques, 6-8 micron thick sections were prepared and stained with hematoxylin and eosin finally mounted in Canada balsam. The histopathology of intestine revealed distortion of glands, vacuolation, distortion and separation of muscular layer. Disintegration of villi with erosion, hyperplasia of intestinal villi and lamina propria. Architectural disintegration of tunica mucosa and goblet cells were also noticed from the experimental fish.

PREVALENCE OF HELMINTH PARASITIC INFECTIONS IN INLAND FISHES OF SINDH.

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Helminth parasites of fish from inland waters in Pakistan have been studied by very few workers Bilqees *et al.*, 1996, 1997, Bilqees and Fatima, 2000, Naich and Bilqees, 2002, but reports on the prevalence of parasites and parasitic diseases are scarce, Bilqees, 1990, Khan *et al.*, 1991,

Bilquees, 1997, Naich and Bilquees, 1997, Ghazi *et al.*, 2006 and Ayaz *et al.* 2013 Present studies are an addition to the same in Pakistan. Common edible fishes were either collected through netting or purchased randomly each month from fish markets of Thatta, Gharo, Landhi/Quaidabad and West Wharf Road, Karachi where fishes are brought for export purposes, the main source being Kalri lake, Thatta. The fishes procured were *Labeo rohita* (Rohu), *Catla catla* (Thaila), *Cyprinus carpio* (Gulfam), *Ctenopharyngodon idella* (Grass carp), *Cirrhinus mrigala* (Morakhi), *Channa* (*Ophiocephalus striatus*, *Channa* (*O.*)*marulius*, (Soul), *Mastacembelus armatus* (Eel), *M. pancalus*, *Mystus seenghala*, *M. cavasius*, *M. tengara*, *Wallago attu* (Malli), *Hilsa ilisha* (Palla) *Rita rita*, *Tilapia* sp. *Heteropneustus fossilis* and *Notopterus notopterus*. The parasites recovered during prevalence studies are: Nematodes: *Rhabdochona bilqueesae* sp. n., *Cucullanus pseudoannulatus* Rehana and Bilquees, 1986; *Procamallanus* sp. *Raphidascaroides* sp. and *Larval nematodes*. Acanthocephala: *Neoechinorhynchus nawazi* sp.n., *Acanthosentis sindhensis* sp.n., *Pallisentis* sp. Trematoda: *Genarchopsis heckmanni* sp.n., *Eocreadium sindhensis* sp.n., *Clinostomum* (metacercaria), *Isoparorchis hypselobagri* (Billet, 1898), *Phyllodistomum ritai Khan*, 1985 Cestoda: *Gangesia bengalensis* (Southwell, 1913) Verma, 1928; *Senga lucknowensis* (Johri, 1956) Fernando and Furtado, 1963; Rehana and Bilquees, 1979 ; *Amphilina kalriai* sp.n.

The parasites were process according to the standard procedures for further detailed study. Total *Labeo rohita* examined during the period (2005-2009) were 107 out of which 5 were found infected, prevalence rate was 4.67%. Total *Channa* (*O.*) *striatus* examined during the period were 139, out of which 20 fishes were found infected and hence prevalence rate was 14.38%. Total *Mystus cavasius* examined were 28, out of which 4 fishes were found infected, prevalence rate was 14.28%. Total *M. seenghala* examined were 70, out of which 6 were found infected and prevalence rate was 8.57%. *Wallago attu*, total fishes examined were 122, out of which 20 were found infected and the prevalence rate was 9.83%. Total *Cirrhinus mrigala* examined were 79, out of which 6 were found infected, prevalence rate was 7.59%. Only 05 *Heteropneustus fossilis* were examined out of which 2 were found infected. Prevalence rate was 40%. *Tilapia* sp. examined were 55 out of which 9 were found infected. Prevalence was 4.95%. *Mastacembelus armatus*, total examined were 28 out of which 01 was found infected, prevalence rate was 3.57%. *Rita rita* examined were 25 and 3 were found infected. Prevalence rate was 12%. Infection was absent in the following fishes. *Hilsa illisha*, *Ctenopharyngodon idella*; *Notopterus notopterus*; *Cyprinus carpio*, *Channa* (*O.*) *marrulius* and *Masacembelus* sp. Additionally 351 *Channa* (*O.*) *striatus* were examined for prevalence study of *Pallisentis* sp. and 597 *Rita rita* were examined for prevalence of a common trematode parasite *Phyllodistomum* sp.

FREQUENCY OF CUTANEOUS LEISHMANIASIS AMONG PATIENTS ATTENDING TERTIARY HOSPITALS IN PESHAWAR, PAKISTAN

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Cutaneous leishmaniasis (CL) is increasing tremendously and is becoming endemic in many regions of Pakistan. The bordering areas along Afghanistan have constituted an endemic belt that had invaded the neighboring urban and rural areas. It was pointed out by different studies that the disease is dramatically spreading in the country and if the preventive measures are not taken, it would be a serious public health problem in the country. In this study, a total of 67 suspected

patients attending tertiary hospitals in Peshawar from areas other than Jamrud (Khyber agency) were included. Microscopy smeared slides of all the suspected patients showed positive results. Both isolated and intra-macrophage forms of *Leishmania* amastigotes were seen in all the sildes. This survey was conducted after the outbreaks of cutaneous leishmaniasis in some areas of Peshawar and aimed at the prevalence of CL in Peshawar and surrounding areas. Among 67 smear positive patients for CL, 43 individuals were male and 24 were Female; showing higher prevalence among males. The infection was more prevalent among Pakistanis 63 (94.03%) than the afghan refugees 4 (5.97%). Investigating the CL infection by age it was more prevalent among age group 11-20 years. We also investigated the duration of CL lesions, the maximum number of CL ulcer was found with duration of 3-4 months with a prevalence of 43.28%. Lesions as 5-6 months or more than 6 months showed less prevalence. By counting the number of lesions, majority (89.55%) of the subjects were having 1-2 lesions. Dry type lesions were more prevalent (85.07%) as compared to wet type lesions. Our study shows that the disease, once endemic in Balochistan, has become now highly prevalent in non-endemic areas of Pakistan, like Peshawar and its surrounding ares viz., Matani, Charsadda, Nizampur, Baghwanan and Nowshehra.

**TANAISIA SWABIENSIS SP.N (TREMATODA: EUCOTYLIDAE SKRJABIN, 1924)
FROM THE KIDNEY OF *CORVUS SPLENDE* IN DISTRICT SWABI, KP,
PAKISTAN**

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The genus *Tanaisia* Skrjabin, 1924 is a well-established genus accommodating the species of trematodes collected from the kidney tubules of the birds. A new species of the genus *Tanaisia* Skrjabin, 1924 is described here from the kidney of common crow (*Corvus splendens*) in district Swabi, KP, Pakistan, and named *Tanaisia swabiensis* n.sp. Observations are based upon nine stained and permanently mounted specimens. The new species are characterized by elongated, tapered anterior end and broadly rounded posterior end with small spines on the tegument. Oral sucker is transversely oval and terminal in position. Prepharynx is absent. Pharynx is muscular, bulbous, partially dorsal to oral sucker. Esophagus is invisible and ventral sucker is lacking. Intestinal ceca are extending backward and fuse together to form complete arch at the distance of 0.35-0.40 from posterior extremity. Testes are symmetrical, juxtaposed, irregularly in shape, but not strongly finger like lobes, elongated vertically and intercecal. Seminal vesicle is above and at left side to ovary. Ovary is elongated transversely, sub median, situated just above the right testis and is oval shape. Vitelline follicles forming two narrow bands commencing at the level of the middle of the testes and vitelline duct leading from anterior part of vitelline gland, passing anteriorly to small, median, postovarian vitelline reservoir. Uterus occupying almost whole body, touching the body wall of the worm (except at the vitellarial region), containing smooth, symmetrically oval, elongated, dark brown operculated eggs. The present species is different from all other described species in general body shape and size, shape, size and position of internal organs as well as in size and shape of eggs. It is also different from *T. karachiensis* Begum *et al.*, 1997 having pointed spines instead of scales, ovary is oval instead of lobes, vitellaria are commencing at the level of the middle of the testes instead at the level of the ovary and post vitellarian space is 0.8-1.03 at one side while 0.85-1.2 at the other side instead of 0.59-0.72 in *T. karachiensis*.

**TOXOPLASMOSIS AS A ZOONOTIC HAZARD TO HUMAN HEALTH BY CATS IN
PARACHINAR AND PESHAWAR REGIONS OF PAKISTAN**

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A total of 170 cat's faecal samples (120 samples from parachinar and 50 samples from Hayatabad area of Peshawar) were collected to investigate the prevalence of *Toxoplasma gondii* oocysts. Out of 170 collected samples, 47 were found positive for *Toxoplasma gondii* oocysts showing an overall prevalence of 27.6%. Of 120 samples collected from Parachinar, 36 were found positive for oocysts of *Toxoplasma gondii* giving prevalence rate of 30%; where as of the 50 samples collected from Hayatabad area of Peshawar, 11 were found positive for oocysts of *Toxoplasma gondii* showing prevalence rate of 22%. The results of this study suggest that Toxoplasmosis is very common in cats so they augment the argument that *Toxoplasma gondii* infection plays an important role in abortion and pregnancy wastage.

**PREVALENCE OF NEMATODES & CESTODES IN THE CHICKENS OF DISTRICTS
MARDAN AND SWABI, KPK, PAKISTAN**

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The study was aimed to examine the prevalence of helminth parasites of chicken in District Mardan and Swabi. A total of 52 domestic and farm chickens were analysed by intestinal scraping method. The total prevalence of helminthes in chickens was 78.84%. The infection of cestodes (55.76) was high as compared to nematodes (19.23%). Prevalence of *Raillietina echinobothrida* was 62.22% and of *Heterakis gallinarum* was 17.77% while that of double infections was 3.84% in domestic chickens. In farm chickens *Heterakis gallinarum* was observed 28.57% and *Raillietina echinobothrida* was 14.28% while no double infection were found. The study suggested that above mentioned helminths could be a major damage to the health of domestic and farms chicken and results in the lesser production of chickens in the area of study.

***LYPEROSOMUM (LYPEROSOMUM) PAKISTANENSIS* N. SP (TREMATODA:
DICROCOELIIDAE, ODHNER, 1910) FROM *CORVUS SPLENDENS* IN DIR LOWER,
KP, PAKISTAN**

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A new species of genus *Lyperosomum* Looss, 1899 syn. of *Oswaldoia* Travassos, 1920, and *Paralutztrema* Faust, 196 is described here during the survey of trematodes from crow *Corvus*

splendens collected from district Dir Lower, KP, Pakistan. The species *lyperosomum* (*lyperosomum*) *pakistanensis* n. sp is different from other related species described from different parts of the world. Description is based on ten permanently mounted specimens. Body is elongate, fusiform, lanceolate, dorsoventrally flattened with maximum width at the level of posterior testis. Oral sucker is oval, subterminal and smaller than acetabulum. Prepharynx is absent while pharynx is well developed, small, globular and partially dorsal to oral sucker, followed by esophagus. Intestinal bifurcation is closed to acetabulum than pharynx. Acetabulum is submedian, elongated oval and larger than oral sucker. Testes are two, oval, oblique in position and separated by uterine coil from each other as well as from ovary. Cirrus sac is flask-shape and is situated a little distance above the acetabulum. Genital pore is submedian to lateral in position, just posterior to pharynx. Ovary is nearly round, submedian to median in position while seminal receptacle is round to oval, situated posterolateral to ovary. Vitellaria are follicular, extending from the posterior border of anterior testis at one side and anterior border of posterior testis from other side. Mehlis gland is not visible due to dense by uterus. Uterus is consisting of ascending and descending coils, having numerous eggs. The present species has close resemblance with *L. malaysiae*, but has maximum width at the level of posterior testis while that of *L. malaysiae* at acetabular level. Moreover cecal bifurcation is close to acetabulum than oral sucker while close to oral sucker in *L. malaysiae*. The new species is also resembles with *L. megacotylosum* but has maximum width just posterior to ovary; cecal bifurcation is closer to oral sucker than ventral sucker and genital pore is median and inbetween the pharynx and acetabulum. The new species name refers to the country of the host.

**NEW HOST RECORD FOR THE GENUS *STRONGYLOIDES* GRASSI, 1879
(STRONGYLOIDAE: NEMATODA) FROM *PASSER DOMESTICUS* (PASSERIDAE:
PASSERIFORMES) IN HYDERABAD, SINDH, PAKISTAN.**

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A single female nematode specimen of the genus *Strongyloides* Grassi, 1879 (Strongyloidae: Nematoda) is collected from gizzard of *Passer domesticus*. The present specimen has close resemblance with *S. avium* Cram, 1929 in all essential features but differs in body length. *Passer domesticus* is a new host record for the genus *Strongyloides* Grassi, 1879. However, this genus is being reported for the second time from Pakistan.

**A NEW DICROCOELIID TREMATODE *CONSPICUUM PAKISTANENSIS* N.SP.
(DIGENEA: DICROCOELIIDAE) FROM BANK MYNA *ACRIDOTHERES GINGINIANUS*
(PASSERIFORMES: STURNIDAE) OF KHAIRPUR, SINDH, PAKISTAN.**

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In result of ongoing helminthological studies of Bank Myna *Acridotheres ginginianus* (Passeriformes: Sturnidae) of Khairpur, Sindh, Pakistan, a total of 45 birds were examined. During

examination of gut contents and visceral organs, 5 live Dicrocoeliid trematodes belonging to genus *Conspicuum* Bhalerao, 1936 were recovered from gallbladder. These trematodes differ from their congeners in having conical body with maximum width at testicular level, ventral sucker larger, testes larger situated posterior or posterolateral to ventral sucker, ovary submedian, pre-equatorial situated behind ventral sucker, cirrus sac elongated between pharynx and intestinal bifurcation, vitellaria form small follicles commencing from level of testes or ventral sucker extend up to posterior half of body. On basis of diagnostic differences between present and previously described species, a new species *Conspicuum pakistanensis* is proposed. Name of new species refers to name of the country. However this genus is being reported for the first time from Pakistan and Bank Myna *Acridotheres ginginianus* is a new host record for the genus *Conspicuum* Bhalerao, 1936.

**A NEW NEMATODE, *DIPLOTRIAENA BIDENTI* N. SP. (FILARIIDAE: NEMATODA)
FROM THE INTESTINE OF *PASSER DOMESTICUS* (PASSERIDAE: PASSERIFORMES)
IN JAMSHORO, SINDH, PAKISTAN**

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A new species of nematode *Diplotriaena bidenti* n. sp. (Filariidae: Nematoda) is described and illustrated from the intestine of House Sparrow *Passer domesticus* (Passeridae: Passeriformes) collected from Jamshoro, Sindh, Pakistan. The new species differs from other members of the genus in morphometry of body, spicules, eggs, oesophagus and vulva. Presence of caudal papillae and two-forked chitinous structure in female species also distinguish *D. bidenti* n. sp., from other species of the same genus. The above mentioned facts suggest that the present nematode is new to science, hence named as *Diplotriaena bidenti*. The new species is named after two-forked chitinous structure.

***CENTRORHYNCHUS DADUI* N.SP. (ACANTHOCEPHALA: CENTRORHYNCHIDAE) IN
HOUSE CROW *CORVUS SPLENDENS* (PASSERIFORMES: CORVIDAE) IN SINDH,
PAKISTAN.**

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During current studies on the helminth parasites of House Crow *Corvus splendens*, a total of 11 hosts were collected from Dadu district of Sindh Province. During examination of gut contents and visceral organs, a total of 07 acanthocephala belonging to the genus *Centrorhynchus* Lühe, 1911 were collected from intestine of two hosts. Present species differ from its congeners in body shape and size, number of longitudinal rows of hooks, number of hooks in each row, size of hooks and size and shape of different organs. On the basis of differentiating diagnostic characteristics between present species and its close allies, a new species *C. dadui* is proposed. The name of new species refers to the locality of the host bird.

STUDIES ON GROWTH TRAITS AND PARASITIC VARIATIONS AT DIFFERENT LIFE HISTORY STAGES IN PEAFOWL, *PAVO CRISTATUS* IN CAPTIVITY

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Present study on biological traits and parasitic variations at different life history stages in peafowls in captivity was conducted at Poultry, Zoo and Wildlife Facilities, Governor of the Punjab House, Lahore. The eggs of Indian peafowls were collected and each egg was weighed and its length and breadth was taken. Peafowl eggs were divided into three egg weight groups and were classified as light (68-80g), medium (81-90g) and heavy weight (91-100g) eggs. Eggs were incubated under standard conditions of incubation. After hatching, 30 chicks selected; 10 from each category, each hatched chick was weighed and wing length and wing span were measured at the initiation of the experiment and thereafter the increase in chick weight, wing length and wing span were taken on weekly basis. A significant relationship between egg weight and hatched chick weight was observed in peafowls (*Pavo cristatus*) from day old chick to 6-month stage. The fecal parasites isolated from peafowls include *Ascaridia* spp., *Heterakis* spp, *Eimeria* spp, *Capillaria* spp, *Isospora* spp and *Syngamus* spp and their prevalence was 12%, 11%, 9%, 4%, 2% and 1%, respectively. Blood parasites include *Leukocytosons*, *Plasmodium Haemoproteus* and their prevalence was 9.2%, 10.83% and 4.17%, respectively. Mallophaga was the only ectoparasite genus identified from peafowls during present study.

**DESCRIPTION OF NEW TREMATODE *PARATANAISIA MOHAMMADISHAQI* N.SP
(TREMATODE: EUCOTYLIDAE) IN MALLARD *ANAS PLATYRHYNCHOS*
(ANSERIFORMES: ANATIDAE) OF SINDH, PAKISTAN.**

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In result of ongoing helminthological studies of Mallard *Anas platyrhynchos* of Kamber-Shahdaddkot District of Sindh province, Pakistan, a total of 57 birds were captured from different localities of study area and brought to the parasitology laboratory of the department of Zoology, Shah Abdul Latif University, Khairpur. During examination of gut contents and visceral organs, a total of 3 specimens belonging to genus *Parataniaisia* Teixeira de Freitas, 1959 were collected from kidneys of host bird. Present species differ from its congeners in having different body shape, size and shape of different organs and other morphological characteristics. On the basis of major diagnostic differences between present species and its close allies, a new species *Parataniaisia mohammadishaqi* is proposed. This genus is being reported for the first time from Pakistan and *Anas platyrhynchos* is new host record for the genus *Parataniaisia* Teixeira de Freitas, 1959.

**ACANTHOCEPHALLUS MUNIFI N.SP. (ACANTHOCEPHALA: ECHINORHYNCHIDAE)
IN JUNGLE BABBLER *TURDOIDES STRIATA* (PASSERIFORMES: LEIOTHRICHIDAE)
IN KHAIRPUR DISTRICT, SINDH, PAKISTAN.**

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During current studies on the helminth parasites of birds of Khairpur district, a total of 11 Jungle babbler were collected from different localities. During helminthological examination of gut contents, a total of 05 acanthocephala belonging to the genus *Acanthocephallus* Koelreuther, 1771 were collected from intestine the hosts. Current species differs from its close allies in shape and size of body; lemnisci larger than proboscis receptacle; number of longitudinal rows of hooks, number of hooks in each row; proboscis swollen at the base; largest size of testes which are tandem, overlapped; shape and size of other organs. On the basis of these differentiating characteristics between present species and its close allies, a new species *Acanthocephallus munifi* is proposed. Name of new species refers to Muhammad Munif Khan, the Professor of Parasitology.

**HELMINTH PARASITES OF DOMESTIC CHICKEN *GALLUS DOMESTICUS* IN
DISTRICT KHAIRPUR, SINDH, PAKISTAN.**

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A study was conducted to investigate the helminth parasites of *Gallus domesticus* in district Khairpur. For this purpose 35 live hosts were collected from different localities of rural areas of study area and brought to the Parasitology Laboratory of Department of Zoology, Shah Abdul Latif University, Khairpur. During examination of gut contents and visceral organs all birds were infected with cestodes (80%) and Nematodes (75%). Cestodes were categorized in three genera consisting of *Cotugnia* (80%), *Raillentina* (75%) and *Choanotania* (1%). Nematodes were categorized into two genera *Subulura* (100%) and *Ascaridia* (1%).

**THAPAROTREMA PEDICELLATUM (VERMA 1927) IN *RITA RITA* (SILURIFORMES:
BAGRIDAE) OF SINDH, PAKISTAN**

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During current studies on helminth parasites of freshwater fish *Rita rita* of Jamshoro district, a total of 25 hosts were examined. Live fishes were collected from different habitats of

study area and brought to Parasitology Laboratory of Department of Zoology. Fishes were dissected and viscera separated in Petri dishes and examined under dissecting microscope. During helminthic examination, only 05 specimens belonging to genus *Thaparotrema* Gupta, 1955 were collected from gallbladder of fishes. Methods described by Garcia and Ash (1979) and Schmidt (1988) were followed for collection and preparation of trematodes for detailed study. Diagrams were made with the aid of camera Lucida. Present specimens have close resemblance with *Thaparotrema pedicellatum* (Verma, 1927) in all essential features and identified as such. Previously this species is reported from intestine of *Rita rita* in India whereas present one is recorded from gallbladder of *Rita rita*. This is first record of genus *Thaparotrema* Gupta, 1955 from Pakistan.

TREMATODE OF INDUS VALLEY BULLFROG *HOPLOBATRACHUS TIGERINUS* (DAUDIN, 1803) IN DISTRICT UMERKOT, SINDH, PAKISTAN.

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During current studies on helminth parasites of Indus Valley Bullfrog *Hoplobatrachus tigerinus* (Daudin, 1803), a total of 2 hosts were collected from different localities of district Umerkot, Sindh, Pakistan. During examination of gut contents and visceral organs, the trematodes belonging to the genus *Laiogonimus* Vercammen-Grandjean, 190 and *Ganeo* Klein, 1905 were collected. Among these, the genus *Laiogonimus* Vercammen-Grandjean, 190 is being reported for the first time from Pakistan.

COMPARATIVE STUDIES ON GROWTH PERFORMANCE, PARASITIC VARIATIONS AND REPRODUCTIVE TRAITS IN DOMESTIC AND WILD PIGEONS

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The eggs of domestic and wild pigeons were collected from Captive Breeding Facilities for Birds, Ravi Campus, University of Veterinary and Animal Sciences, Lahore. Each egg was weighed and its length and breadth were taken. On weight basis the eggs were divided into three weight categories and were classified as light, medium and heavy weight egg categories. Chick weight in domestic and wild pigeons varied significantly between heavy, medium and light weight egg categories from 1st week to 6-month stage. The prevalence of *Ascaridia* spp. was 51%, *Giardia* spp. 12%, *Eimaria* spp. 49%, *Capillaria* spp. 21.2% and *Isospora* spp. 7.5% in fecal sample while in blood samples the prevalence of *Leukocytozons* spp. was 25 %, *Plasmodium* 75.8 %, *Haemoproteus* 85 %, *Trypanosoma* spp. 3.3 % and *Micrifilariae* spp. was 7.5 % in domestic pigeons. Similarly, endoparasitic variations in wild pigeons were observed, the prevalence of *Ascaridia* spp. was 76%, *Giardia* spp. 16.2%, *Eimaria* spp. 40%, *Capillaria* spp. 15.8% and *Isospora* spp. was 5% in fecal sample while prevalence of *Leukocytozons* spp. was 25 %, *Plasmodium* spp. 75.8 %, *Haemoproteus* spp. 85 %, *Trypanosoma* spp. 3.3 % and *Micrifilariae* spp. was 7.5 %.

PREVALENCE OF PARASITES IN RING NECKED PHEASANTS (*PHASIANUS COLCHICUS*) IN CAPTIVITY

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Fecal and blood samples of ring necked pheasants *Phasianus colchicus* were analyzed to record the parasitic prevalence in these pheasants. A total of 1000 samples, 500 blood and 500 fecal samples were collected from Captive Breeding Facilities for Birds, Department of Wildlife and Ecology, Ravi Campus, University of Veterinary and Animal Sciences, Lahore. Parasitic genera identified from blood samples of *P. colchicus* include *Leukocytozoon*, *Plasmodium* and *Haemoproteus*. Prevalence of *Leukocytozoon* was 16 % while the prevalence of *Haemoproteus* was 14.3%. Parasitic genera identified from fecal samples of *P. colchicus* include *Eimeria*, *Isospora*, *Trichomonas* and *Giardia*. Eggs of five species of nematodes viz. *Capillaria*, *Syngamus trachea* and *Ascaridia*, *Heterakis isolonche* and *Heterakis gallinarum* were also identified from the fecal samples. The ectoparasites include one species of burrowing mite *Knemidocoptes mutans* and two species of chewing lice i.e. *Amyrsidea perdicis* and *Lipeurus maculosus*.

COMPARATIVE STUDY OF ECTO-PARASITES IN BLUE ROCK PIGEON (*COLUMBA LIVIA*) AND DOMESTIC PIGEON (*COLUMBA LIVIA DOMESTICA*)

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The present study was conducted to record the prevalence of ecto-parasites (chewing lice) in Blue Rock Pigeon (*Columba livia*) and Domestic Pigeon (*Columba livia domestica*) in captivity at Jallo Wildlife Park, Lahore. For this purpose, 116 specimens (63 adult male and 53 adult females) of Blue Rock Pigeon and 46 birds (30 male and 16 females) of Domestic Pigeons were randomly examined. During study ecto-parasites were collected, preserved, stained and identified. All the specimens were found infested with different species of ecto-parasites. In Blue Rock Pigeon, total 2898 lice (1194 female, 1324 male, 380 nymph) were identified with prevalence of female lice (41.20%), male with 45.72% and nymph with 13.11%. Whereas, 1622 lice (705 female, 853 male, 64 nymph) pertaining to 19 species, with prevalence 100% (female lice 43.46%, male with 52.59% and nymph with 3.94%) in Domestic Pigeon. Utmost prevalence of lice was for *Columbicula Columbae* with prevalence of (88.42%) and (80.64%) in Blue Rock Pigeon and Domestic Pigeon, respectively. Average number of lice, were maximum in female as compared to male. It was concluded that to sustain the population for future concern, sanitary and phyto-sanitary measures should be taken as the prevalence was accelerating among females.

IN VITRO AND IN VIVO EVALUATION OF ABAMECTIN AND AQUEOUS EXTRACTS (LEMON GRASS, MORINGA) AGAINST ROOT KNOT NEMATODE ON EGGPLANT

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Present study was conducted to assess the potential of abamectin and aqueous extracts of lemon grass and moringa against root knot nematode on eggplant. Plant extracts and abamectin were tested both in vivo and in vitro. Different concentrations of plant extracts (5%, 10%, 15%) and abamectin (0.2%, 0.4%, 0.8%) were applied to evaluate the effectiveness on egg hatching, juveniles mortality, plant growth and on nematode reproduction at various time intervals. Each treatment was replicated four times under completely randomized design. Maximum mortality of juveniles and minimum hatching was observed at 0.8%, 15% concentration of abamectin and plant extracts respectively. Exposure time also affected mortality and hatching percentages. Abamectin was more effective in reducing nematode population under field conditions as compared to plant extracts. Plant extracts at 15% concentration showed significant reduction in nematode reproduction. Abamectin greatly reduced number of egg masses, females and galls per root system. So, it could be successfully used to control nematode population.

PREVALENCE OF FISH-BORNE ZOONOTIC ECTOPARASITES IN FISH FAUNA OF DIFFERENT DHANDS OF BALLOKI HEADWORKS, PAKISTAN

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A number of parasites are responsible for the fish-borne parasitic zoonoses especially trematodes, cestodes, nematodes and protozoans that infect large number of humans around the world especially in poor countries. However, the status of the human host immune system plays a vital role in the severity of the disease. The objective of the present investigation was to throw light on infectious protozoans, trematodes and copepods of fish fauna at different landing sites of Balloki Headworks during summer season. Three herbivorous (*Labeo rohita*, *Catla catla* & *Cirrhinus mrigala*) and two carnivorous (*Wallago attu* and *Aorichthys aor*) fish species were observed. Three hundred and fifty samples were collected live or freshly dead and were subjected to parasites examinations in laboratory. The study reveals the existence of 12 species of parasites including: five species of protozoa (*Chilodonella*, *trichodina* *Ichthyophthirius multifiliis*, *Ichthyobodo* and *Apiosoma*), two species of monogenean trematodes (*D. minutus* and *Gyrodactylus elegans*) and five species of crustaceans (*Argulus foliaceus*, *Lernaea cyprinacea*, *Lernaea polymorpha*, *Lernaea oryzophila*, and *Lernaea lophiara*). The ectoparasitic infestation of protozoan, trematodes and copepods was 25.01%, 13.87% 61.12%, respectively. Most common parasite was *Lernaea* on *Catla catla* was observed as most prevalent species for the parasites whereas, *Aorichthys aor* showed lowest infestation. Highest parasitic load was recorded at Khanki Nagar landing site and lowest was observed at Syedahawala landing site.

STUDY OF HELMINTHS AND COMPARATIVE EFFICACY OF PYRENTAL PAMOATE AND ALBENDAZOL IN GEESE

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Geese are waterfowls reared not only because these are used as a meat source but also an economic source for breeders. There are several factors leading to the large scale mortality of geese among them the helminths are reported to cause significant lesions in wild geese. Different medications are in practice against the helminths. In this study infection rate of helminths and comparative efficacy of Albendazol and Pyrental Pamoate was determined in geese. In experiment 40 (n=40) common geese from Jallo Park, Lahore Pakistan, were obtained and their fecal samples were collected for determination of their helminth load. Among geese; unaffected were placed in group A (n=10), while the affected were grouped B (n=15) and C (n=15). Group B and C were treated with 0.1ml/kg BW Albendazol and 0.1ml/kg BW Pyrental Pamoate respectively. Chemotherapeutic trial continued for 10 days and fecal helminthic egg counts were carried out at day 3rd, 7th, & 10th. Fecal samples collected after medication was analyzed. Group 'B' administered with albendazol at post medication i.e. day 3rd, 7th and 10th showed 42.5%, 66.9% and 88.9% reduction in EPG respectively. Whereas in case of Pyrental Pamoate percentage reduction in EPG were 30.8%, 49.7% and 74.2% at day 3rd, 7th and 10th in Group C respectively. Post-medication EPG showed that in treatment group with 0.1ml/kg BW Albendazol percentage EPG was significantly lower as compare to 0.1ml/kg BW of Pyrental Pamoate.

ROLE OF MARINE NEMATODES IN ENVIRONMENTAL MONITORING ASSESSMENT

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Marine nematodes are the most abundant and diverse metazoan in sea and play a significant role in the marine environment. The Arabian Sea, touching the large coastal area of Pakistan, has tremendous untapped information on marine nematodes fauna which is the dominant group of the meiofauna. Nematoda is the group which varies in senility to pollutants and environmental perturbation, therefore presence of a specific nematode species in high population indicates specific type of pollution. Despite pollution large numbers of bacterial feeder nematode genera were present in high percentage. This study indicates that there is no effect of industrial wastes containing heavy metals and other pollutants on nematode assemblages on the dumping areas and nematodes have the ability to tolerate heavy metals and other pollutants. As a group they are more resistant to different forms of pollution than other benthic groups. So the use of nematode in monitoring the sea pollution thus required at least some knowledge of nematode systematic as the response of different species is very different. Considerable awareness has been generated in the recent past towards free-living marine nematodes and particularly to the species of the Arabian Sea of Pakistan. So far a total of 120 species of free-living marine nematodes belonging to 78 genera, 36 families, 6

suborders and four orders have been reported from Pakistan. Out of these 30 species are new to science while 90 species have been reported from Pakistan. During the recent analysis of meiobenthic structure in relation to pollution and their systematic studies a new species of marine nematode *Metoncholaimus siddiqii* n. sp., has been encountered which is described and illustrated.

MULTIPLE ANTHELMINTIC RESISTANCE ACQUIRED BY NEMATODES IN AWASSI SHEEP BREED IN SUB-TROPICAL POTHWAR AREA, PAKISTAN

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The study was conducted to detect multiple anthelmintic resistances acquired by nematodes in Awassi sheep by using three major anthelmintics with different combinations. Sixty Awassi ewes were randomly divided in to four groups on basis of faecal egg count (EPG), each group comprised of 15 ewes. Group-1 was served as non-treated control, Group-2 was treated with albendazole through oral drench at 0.75 mg /lb of body weight while Group-3 was treated with mixture of (levamisole +ivermectin) through subcutaneous injection at a dose of 1 ml/50 lb +0.5 ml / 55lb of body weight, and group 4 was treated subcutaneously with ivermectin having dose of 1 ml / 110 lb by body weight. The efficacies for albendazole, mixture of levamisole + ivermectin and ivermectin were 51.0%, 82.0% and 82.8% respectively. It has been observed that the reduction in EPG was increased following post-treatment period however there is no significantly increase in body weight and significantly increase in PCV, Hb, TEC and decrease in TLC count with increased in TEC throughout the trial. It was concluded that different factors have contributed in the development anthelmintic resistance including solely prolong dependence on anthelmintic usage, under dosing without good understanding of the principle of anthelmintic treatment strategy. There is need of tangible efforts to control the spread of anthelmintic resistance in the areas.

CLINOSTOMUM AWADHI LEIDY, 1856 IN PHALACROCORAX NIGER OF SANGHAR SINDH, PAKISTAN

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Twenty six trematodes belonging to genus *Clinostomum* were collected from the esophagus of Little Cormorant *Phalacrocorax niger* from Sanghar, Sindh, Pakistan. The present specimens differ from its congeners in having body shape, vitellaria, location of testes and ovary, cirrus shape and position. Species name refers to the locality of the host collected from.

HELMINTH PERASITES OF *CLUPISOMA NAZIRI* AT RIVER KABUL, NOWSHERA, PAKISTAN

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A study of the helminth fauna of *Clupisoma naziri* at River Kabul, Nowshera was conducted from March to August, 2013. A total of 183 fish specimens were captured and infection rate was observed in gills, liver and intestinal region. Overall eight species belonging to three genera of helminth i.e. Trematode, Cestode and Nematode were found. One species of trematode parasites i.e. *Clinostomum complanatum* (liver fluke) in liver, two species of cestodes (tape worms) i.e. *Trianophorus nodulosus* and *Diphyllobothrium sebgo* in gills and five species of nematodes (round worms) i.e. *Contracaecum spiculligerum*, *Eustrongylides excisus*, *Paracuararia adunca*, *Raphidacaris acus* and *Spiroxys chelodinae* in intestinal region were observed. Results show that *Clupisoma naziri* was infected more in intestinal region by nematodes as compared to gills and liver by cestode and trematode respectively. The infection rate was 80% among collected specimens. It is concluded that the fish in natural environment are suffering with heavy parasitic infection and this might be one reason for constant population decline of this species in River Kabul.

FIELD EVALUATION OF FAMACHA[®] EYE MATCHING CHART IN HAIRY GOATS OF SUB-TROPICAL PUNJAB, PAKISTAN

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This study was aimed to evaluate the efficiency of FAMACHA[®] eye matching chart system for treatment of clinically identifying individual goats to conserve the efficacy of major anthelmintic groups. This investigation was carried at Livestock Production Research Institute, Kharimurat (LPRI) Pakistan. Prior to start of treatment 5000 L₃ larva of different gastrointestinal nematodes were given orally to each animal. In this trial three anthelmintics drugs viz, albendazole, levamisol and ivermectin were evaluated by screening through FAMACHA[®] chart. Sixty hairy goats (Jattal) from local goats breed were selected for this purpose. The goats were randomly divided into four groups based on equal egg per grams (EPG). Among these one group were served as untreated (Control 1) while the other three groups (2 3 and 4) were treated with albendazole, levamisol and ivermectin, respectively. The blood samples withdrawn from jugular vein in EDTA coated with vacutainers, while faecal sample were directly collected from rectum. Each category of FAMACHA[®] chart was correlated with hematocrit (Ht) values (chosen as the, "golden strander" of anemia). The live weights (LWT), blood packed cell volume (PCV), faecal egg count (FEC), total erythrocyte count (TEC), total leucocyte count (TLC) and hemogloubin were also monitored according to experimental design. The data was subjected to statistical analysis like pair T test and Pearson correlation among packed cell volume, FAMACHA[®] chart value and faecal egg count. The live weight (LWT), blood packed cell volume (PCV), hemoglobin level and total erythrocyte count (TEC) were increased ($p < 0.05$) significantly in treated groups and decreased ($p > 0.05$)

significantly in control group. Faecal egg counts (FEC) and total leucocyte count (TLC) were decreased ($P < 0.05$) significantly in treated groups but in control group increased ($p < 0.05$) significantly. FAMACHA[®] values were negatively correlated with packed cell volume and were significant ($p < 0.05$). The percentage of goats recommended for treatment decreased from 85% for eye score of 3, 4 and 5 to 23.3% for eye scores of 4 and 5. The results indicated that through the evaluation of FAMACHA[®] eye matching chart system; conservation of anthelmintic drugs could be achieved.

***ECHINOSTOMA GARZETTI* SP.N (DIGenea : ECHINOSTOMATIDAE) IN THE LITTLE EGRET *EGRETTA GARZETTA* IN SINDH, PAKISTAN**

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During a survey of Helminth Parasitic infections in *Egretta garzetta* Little egret, shot down from the District, Jamshoro, for collection of internal Helminth parasites, fifteen hosts were examined at random intervals, out of which twelve were found infected with twenty five trematodes, these specimens were recovered from the small intestine of the host. The specimens were mounted permanently according to the standard procedures. A detail study was conducted and a new species *Echinostoma garzetti* sp.n is proposed belonging to the genus *Echinostoma* (Rudolphi, 1809) from the small intestine of *Egretta garzetta* in Sindh, Pakistan. The new species is characterized by having: smaller oral sucker, smaller prepharynx and esophagus. Number of collar spines (which are 47 in number), cirrus sac being kidney shaped with thick walls, testes larger in size and bilobed with rough indentations, therefore a new species *Echinostoma garzetta* sp. n. is proposed. The species is named referring to the host bird.

TO STUDY THE PREVALENCE OF *BLASTOCYSTIS* SPECIES FROM DIFFERENT ANIMAL HOSTS IN CAPTIVITY

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Blastocystis sp. is an anaerobic enteric protozoan organism harboring the intestinal tract of a wide variety of animals, including humans which can be symptomatic or asymptomatic. A total number of 35 fecal samples of animals from 2 different zoological gardens *i.e.* Karachi Zoological Garden and The Wildlife Experience Centre were investigated for the presence of *Blastocystis* sp. in this study using iodine stain by light microscopy. Out of 35 fecal samples 28 (80%) indicated the presence of the parasite while others were free of the species. However, the 25 fecal samples from Karachi Zoo demonstrated, 21 (84%) infested animals while the 10 samples from The

Wildlife Experience Centre showed 7 (70%) animals infested with *Blastocystis* sp. The presence of intestinal helminthes, protozoans and nematodes was also observed and all the fecal samples were heavily infested with a wide range of organisms. This study is the initial step to investigate the presence of *Blastocystis* sp. which could serve as a helpful research to eliminate the risk of pathogenicity of the organism in order to avoid transmission of Blastocystis infection to other animals and human beings which are in close contact with the animals. This could be achieved by ensuring better cleaning, feeding, caging conditions and regular monitoring of the animals for the detection of *Blastocystis* sp.

TISSUE DAMAGE IN FISH SKIN OF *JOHNIUS DUSSUMIERI* INFECTED WITH *ICHTHYOPHTHIRIUS* SP. FROM KARACHI COAST

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Tissue damage observations are made on the skin infection of the fish *Johnius dussumieri* infected with *Ichthyophthirius* sp. For this purpose histological sections were prepared by usual technique, stained with haematoxylin and eosin, dehydrated in graded series of alcohols, cleared in clove oil and xylene and mounted permanently in Canada balsam. Photographs were prepared with Nikon (Optiphot-2) photomicroscope using a Fuji colour film. This infection severely damaged the skin tissue, common findings were erosion of outer surface, dislocation of muscle fiber and shrinkage and atrophy which produced large spaces in between the muscles fibers. Hyaline degeneration of muscles fiber was also prominent in some sections. Muscle fibers were severely damaged and fragmented.

***KNIPOWITSCHIATREMA PAKISTANENSIS* SP.N (TREMATODA:HETEROPHYIDAE)
AND A NEW HOST RECORD *STERNULA ALBIFRONS* (LITTLE TERN) IN
HYDERABAD, SINDH, PAKISTAN**

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During an investigation on helminth parasites of birds five *Sternula albifrons* (Little tern) were purchased from District Hyderabad, Sindh, Pakistan. The birds were anaesthetized, autopsied and examined for helminth parasitic infections. Out of five two were found infected with two specimens. The worms were mounted permanently according to standard procedure for further detail study and identified as belonging to genus *Knipowitschiatrema* (Issaitschikow 1927) *pakistanensis* sp.n. The new species is characterized by having: Body sub cylindrical, the oral

sucker is terminal, smaller than acetabulum, Pre-pharynx is fairly long, esophagus long, acetabulum muscular, much larger than oral sucker, seminal vesicle large, flask shaped, quite voluminous in size, lie just above the ovary, with genital opening below the acetabulum, ovary small, rounded, pre-testicular, testes tandem post-equatorial, anterior testis roughly spherical, posterior testis is oval, larger than anterior testis, both testes larger than the ovary, vitelline follicles arranged laterally in the posterior region of the body below the testes, uterus fills the space in posterior extremity, passes between the testes, ovary, and seminal vesicle and opens into genital pore below the acetabulum, eggs are smaller and thin shelled. A new species *Knipowitschiatrema pakistanensis* is therefore proposed. This genus is reported first time from new host and new locality.

***PSEUDOCHAUHANEA FORSTERI* N.SP. (MONOGENEA: CHAUHANEIDAE LABEDEV, 1972) FROM THE FISH *SPHYRAENAE FORSTERI* OF KARACHI COAST, PAKISTAN**

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Pseudochauhanea forsteri a new monogenea is reported here from the gills of fish *Sphyraena forsteri*. The new monogenean species characterized by elongated, anteriorly tapered body. Head truncate at apex with terminal mouth, globular pharynx. Esophagus bifurcating in front of vagina. Intestinal limbs subdivided into lateral branches. Numerous rounded testes. Ovary is in the middle fifth of the body far anterior to testes. Spindle shape uterus. Vagina bell-shaped, haptor in V-shaped, asymmetrical without terminal anchors. Clapms are unequal on both sides, 31-37 on right side, 14-20 on left side. The name *Pseudochauhanea forsteri* refers to the host specie.

MALARIA AND INTESTINAL HELMINTHES CO-INFECTION AMONG SCHOOL CHILDREN IN KARACHI

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This study aimed to the study evidence on the extent of malaria and soil-transmitted intestinal helminth (STH) co-infections in children of 5 primary school belonging from Maripur, Karachi namely PMA, PAF, Maripur Govt. School, Sheema Public School and Baharia Foundation during January to December 2013. Stool, urine and blood samples of 770 students were collected for observing the effect of Malaria, Hook Worm and Round Worm on prevalence of Hemoglobin concentration and anemia condition. Fresh blood, urine and stool samples were collected from primary school students aged 5-11 years. Stool samples were examined for round worm and hook worm using the Kato Katz technique (WHO 1994). Urine samples were examined for round worm eggs in 10 ml urine according to Nucleopore ® filtration method (WHO; 1991). Thick blood smear were prepared, stained with Gimsa and examined microscopically for malaria parasites at 100x magnification. Hemoglobin concentration was determined using a portable digital hemocue machine. (Hemocue ® Agelholm, Sweden). The children who were not infected with any parasite showed 27.2% anemia while the children co-infected with plasmodium and helminthes found 60% anemia. Prevalence of anemia was specially associated with malaria, while helminth co-infected with malaria show high intensity of anemia and low Hb level because anemia is inversely proportional to Hb level.

MANAGEMENT OF NEMATODES ASSOCIATED WITH WALNUT (*JUGLANS REGIA* L.) BY USING POULTRY MANURE AND CARBOFURAN

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Poultry manure and carbofuran were applied for their nematicidal nature on walnut tree affected by stilet bearing nematodes in District Abbottabad of Hazara Division from May 2012 to March 2013. Poultry manure was used at the ratio of 8 kg/ tree while carbofuran was used at the ratio of 150 gm/ tree. Carbofuran was used for comparison. Untreated trees were kept for comparison with the treated one. Both poultry manure and carbofuran were applied around the tree trunk. Soil samples collected at 3,6 and 12 month were processed by using Baermann funnel technique. Nematode population was noted under stereoscopic microscope and compared with the untreated one. Both poultry manure and carbofuran effectively controlled the *Helicotylenchus pseudorobustus*, *Psilenchus hilarulus* and nematode population. The recorded data was subjected to analysis of variance (ANOV A) and histograms were prepared.

STUDY OF BEHAVIORAL ASPECTS, FEEDING HABIT AND PREVALENCE OF INTESTINAL HELMINTHS IN BENGAL TIGER (*PANTHERA TIGRIS TIGRIS*) KEPT IN CAPTIVITY AT KARACHI ZOOLOGICAL GARDEN, KARACHI.

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It is important for us to understand the behavior of captive wild animals for their welfare. Animals kept in the captive environments of zoological parks are usually not provided with opportunity to conduct natural behaviors due to spatial constraints and negative public reaction which develops abnormal behaviors. Bengal Tigers (*Panthera tigris tigris*) present a difficult case, they have large home ranges in the wild and predatory hunting behaviors that are difficult to provide for in captivity. This research study covers three aspects of Bengal tiger's life from April 2014- December 2014 at Karachi zoological garden. The first portion covers behavioral study which concluded that the stereotypic behavior is clearly influenced by the absence of vegetation, small sized pool, and small sized enclosures. Above all this pacing is directly affected by environmental enrichment and can be lowered by increasing enrichments. This study reveals that high enrichment level decreases pacing up to 32%. This research study also displays overall expenditure of time on different behaviors by tigers during summer and winter season. Results show that Bengal tigers kept in captivity spent 73% of their time on resting which is due to boredom or unavailability of space. On the basis of time expenditure results, it was concluded that older tigers are more inactive than younger ones. These results suggest that captive tigers should be housed in more complex and natural environment. Second part of study explains the feeding habit of Bengal tigers in captivity. This part of study suggests that tiger should be fed with large part of carcass or whole parts with bones. Food can be used as novel enrichment item. Placement of

feeding poles in enclosures is recommended as this will help in exercise and also increases the appetite of animal. The third part of study discusses prevalence of gastro intestinal helminths in captive tigers. Faecal sample of two tigers were examined for intestinal helminthic infection at Karachi zoological garden. EPG value for male tiger ranges from 400-1800 and the value for female ranges from 200-1600. This study suggests the regular examination of animals for the prevention.

A SURVEY OF AVIAN PHTHIRAPTERA (CHEWING LICE) FROM HYDERABAD, SINDH, PAKISTAN

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Chewing lice (Phthiraptera: Amblycera, Ischnocera) have been reviewed on common birds of Hyderabad during 2013-2014. Both suborders Amblycera, including family Menoponidae and suborder Ischnocera with the only avian family Philopteridae were reported from different localities of Hyderabad. All the species were collected, identified by microscopy and latest literature. It is the first chewing lice survey that has been carried out in the region, which reported six species of Menoponidae and seven species of Philopteridae. The menoponid species are *Colpocephalum turbinatum* Denny, 1842, *Hohorstiella lata* (Piaget, 1880) and *H. modesta* (Ansari, 1951) were found on Pigeons and doves, *Menacanthus eurysternus* (Burmeister, 1838) on House sparrow, Common Myna, Bank Myna, *Menacanthus pallidulus* (Neumann, 1912), *M. stramineus* (Nitzsch, 1818) and *Menopon gallinae* (Linnaeus, 1758) found on domestic and wild fowls and *Myrsidea splendidicola* Klockenhoff, 19783 on House crow. In these chewing lice, *M. eurysternus* is first time recorded from common myna, *Acridotheres tristis* from the region of Hyderabad. The philopterid species are *Anaticola crassicornis* (Scopoli, 1763) from Ducks and Greylag Goose, *Brueelia nebulosa* (Burmeister, 1838) from Common Myna, *B. saliemii* Ansari, 1957 from House Crow, *Campanulotes compar* (Burmeister, 1838), *Columbicola columbae* (Linnaeus, 1758) and *C. tschulyschman* Eichler, 1942 from Pigeons, *Goniodes dissimilis* Denny, 1842 and *Lipeurus tropicalis* Peters, 1931 from Domestic fowls. In these chewing lice, all species are recorded for the first time from Hyderabad.

STYLET BEARING NEMATODES ASSOCIATED WITH WALNUT (*JUGLANS REGIA* L.) IN DISTRICT ABBOTT ABAD

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Identification of stylet bearing nematodes associated with walnut *Uuglans regia* L.) in different localities namely Baghnotar, Nathiagali, Kakul, Bandi Pulah, Gulistan colony, Sheikh ul

Bandi and Ihanghi of District Abbottabad, KPK, Pakistan have been presented. Soil samples were collected from the base of the walnut tree and were processed by using Bearmann funnel technique. Specimens collected were killed by heat and then fixed in T AF and later transferred to 1.25 glycerin for permanent mounting. Nine species of nematodes *Tylenchus* sp., *Psilenchus hilarulus*, *Meloidogyne* larvae, *Aphelenchus* «*venae*, *Tylenchus* juveniles, *Filenchus sheri*, *Pratylenchus thornei*, *Helicotylenchus pseudavobustus* and *Helicotylenchus dihystra* have been identified from these localities. Most commonly found nematode species were *Aphelenchus avenae*, *Psilenchus hilarulus*, *Tylenchus* spp. and *Pratylenchus thornei*.

RECORDS OF CHEWING LICE (PHTHIRAPTERA: INSECTA) FROM BIRDS OF FAMILIES CACATUIDAE AND PSITTACULIDAE (PSITTACIFORMES: AVES) FROM SINDH PAKISTAN

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Birds belong to families Psittaculidae and Cacatuidae (Psittaciformes: Aves) were examined for their chewing lice (Phthiraptera) from different regions of Sindh, mainly Karachi and Hyderabad. It is the first taxonomical study of chewing lice on parrots of Sindh region. The lice belonging to suborder Amblycera included three species of family Menoponidae, were *Afrimeneopon waar* (Eichler, 1947), *Franciscoloa psittacus* sp. n. and *Colpocephalum pilgrim* Price, 1967 from Budgerigars, *Melopsittacus undulatus* Shaw (Psittaculidae), and suborder Ischnocera included four species of family Philopteridae, were *Neopsittaconirmus lybartota* (Ansari, 1947) from *Psittacula krameri* (Scopoli) (Psittaculidae), *N chandabani* (Ansari, 1947) from *Psittacula eupatria* (L.) (Psittaculidae), *N clayae* Guimaraes, 1974 from *Probosciger aterrimus* (Gmelin) (Cacatuidae), *Neopsittaconirmus* sp. from *Nymphicus hollandicus* (Kerr) (Cacatuidae) and *Echinophlopterus alexanderius* sp. n. from *Psittacula alexanderi* (L.) and *P. krameri* (Scopoli) (Psittaculidae). In the present study, all the chewing lice species were newly recorded from the region except of *A. waar*; genus *Franciscoloa* and *Colpocephalum* were found first time on the present host species; the genus *Franciscoloa* was named for its host family name; the new species of *E. alexanderius* was named for its host species from which it was being first collected.

SECTION - V**FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY,
MARINE BIOLOGY****1. ECOLOGY AND ENVIRONMENTAL POLLUTION****HABITAT PREFERENCES OF VARIOUS MAMMALIAN SPECIES INHABITING
RIVER CHENAB, PAKISTAN**

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Present research was conducted to assess the anthropogenic impacts on mammalian diversity around river Chenab, Pakistan. The mammalian diversity was recorded along forested landscapes, cultivated plantations, peri-urban and urban areas. The data on diversity and distribution of various mammalian species of the study area was collected through direct count method viz. personal observations and sound records; and indirect count methods viz. presences of carcasses, fecal pellet, pug marks and meeting with local communities. The habitat preferences of large, medium and small mammals varied significantly. A decline in mammalian diversity was observed from forest habitat to urban landscapes. Indian wild boar, Asiatic jackal, Indian fox and long eared desert hedgehog preferred forested areas while various mice and rat species preferred human habitations. Similarly, few species like squirrels and jird preferred cultivated areas. It can be concluded from present study that many of the mammalian species area habitat specific and corridors and connections between different landscapes are important for the conservation of mammalian diversity.

**ANALYSIS OF MICRO-NUTRIENTS IN SOIL OF UPLAND AND LOWLAND
ENVIRONMENTS**

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Soil and water degradation puts crucial impact on the global environment and human health. The paper focuses on the principal chemical and physical attributes serving as indicator of soil and water quality of the grass land and wet land of Makra meadows and Kallar Kahar, Pakistan. Practiced indicators considered in soil analysis at three different depth and gradients of soil embrace EC, pH, organic matter %, phosphorus, potassium, and texture and percentage

saturation while the water quality assessment comprised of electrical conductivity, Ca⁺⁺ & Mg⁺⁺, Na⁺, Cl⁻, carbonates, bicarbonates, residual sodium carbonate (RSC) and sodium absorption ratio (SAR). The soil and water of Makra meadows was acidic in nature while that of Kallar Kahar was alkaline. Soil texture was found to be holding a major control over organic matter dynamics. Soil and water data is presented to provide assistance to site managers and policy makers.

THE INFLUENCE OF CROP PRACTICES ON THE BEHAVIOR OF EARTHWORM POPULATIONS IN THE SELECTED FIELDS AND THEIR EFFECT IN THE SOIL FERTILITY

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The influence of crop practices, the use of fertilizers and pesticides showed affects upon earthworm's diversity and abundance. A total of 300 samples of earthworm specimens were collected from crops like Sugarcane, Berseem, Wheat, Tobacco and vegetables such as Carrot, Garlic Onion and Pea and a total of 179 specimens of three species, belonging to two genera viz, *Pheretima hawayana* (58.66%), *P. posthuma* (39.66%) and *Microscolex phosphorus* (1.67) were harbored during the two transitory phases of seasons. Maximum number of earthworms was found in the Garlic field (20.67%) and least in number was harboured from Tobacco fields (8.93%). The environmental, physicochemical factors along with crop practices showed profound affects upon the diversity and abundance of earthworms. Electric conductivity of the soil is indirectly related to the number of earthworms (p<0.01). When the electric conductivity increases the number of earthworms decreases and at pH 7.7, the number of earthworms decreases in Berseem field. The high concentration of phosphorous and potassium in the soil was favourable to the survival of earthworms in September. Carbon, nitrogen and carbon/nitrogen was also have the influence on the earthworm species (p<0.01). In the selected fields, increase in agricultural practices, increasing trend to use the pesticides and fertilizers to minimize the earthworms diversity and abundance.

AMBIENT CONCENTRATIONS OF PARTICULATE MATTER AT HIGH ALTITUDE TOURIST RESORTS IN PAKISTAN AND CHINA

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Particulate matter exhibits different behaviour with change in altitude and variation in meteorological factors. A comparative analysis was carried out to monitor levels of air-borne particulate matter (PM) at higher elevation (above 3000 m) in both China and Pakistan. The locations selected were tourist spots i.e. Makra meadows, Payee in Shogran, Pakistan (3089 m) and Lang Mu Si, Ruo Er Gai, Tibet in China (3300m). Real time monitoring of PM was carried out at

both sites using DustTrak DRX (model 8533, TSI Inc.) for 24 hours each. In Pakistan, the respective average values for PM₁, PM_{2.5}, PM₄, PM₁₀ and PM_{Total} were recorded to be 405.17, 406.82, 410.05, 412.27, and 414.82 µg/m³ while in China the pollutant levels were considerably lower with respective averages for above mentioned parameters monitored to be 73.27, 80.38, 85.39, and 110.23 µg/m³. Trans-boundary transport of pollutants often results in accumulation of pollutants at high elevations. There is scarcity of data regarding the behaviour adopted by particulate matter at high altitudes and much work needs to be conducted for a more thorough understanding.

PARTICULATE MATTER CONCENTRATION VARIATIONS AT DIFFERENT ALTITUDINAL RANGES WITH ENVIRONMENTAL DYNAMICS

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Ambient air quality is defined not only by the source strength but a variety of meteorological parameters as well. In the current study, ambient concentrations of PM along with temperature and relative humidity levels were monitored at seven different locations of Pakistan. DustTrak DRX (Model 8533, TSI Inc.) was employed for twenty four hours real time monitoring of particulate matter at the selected sites. A considerable variation was observed in 24 hour trend of PM at different locations owing to variation in meteorological conditions due to different altitudes and seasons, and natural and anthropogenic sources in the vicinity. The highest average concentrations of 405.17 µg/m³ were observed at highest elevation (Makra peak, Shogran, 3089 m) while lowest averages (102.47 µg/m³) were obtained at seaside (Hawks Bay, Karachi, 0 m). Correlation factor was determined for PM and meteorological parameters at each location with varying results. More research needs to be conducted to have a more comprehensive knowledge about the physical parameters controlling particulate dispersal at different heights within a country.

PREVALENCE AND HABITATS OF CARNIVORE SPECIES IN RIVERINE FOREST OF DERA GHAZI KHAN FOREST SUB-DIVISION

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A study was conducted in riverine forest of Dera Ghazi Khan Forest sub-division during July 2013 to August 2014. Seasonal surveys (n=84) were conducted in seven selected sites in the study area, three surveys at each site in each season covering 1400 km² area. Direct observations as

well as indirect signs methods were employed on 52 fixed width line transects which were surveyed on foot and motor bike for observing foot prints, scats, denning / burrowing sites and dead animals. Local inhabitants were interviewed for gathering information about carnivores using a predesigned questionnaire and reference photographs. A field data sheet was used for each transect to record species signs and habitat type where these signs were recorded. Presence of five carnivore species was confirmed in the study area which included Asiatic Jackal (*Canis aureus*) was present in all study sites, Jungle Cat (*Felis chaus*) and Small Indian Mongoose (*Herpestes javanicus*) in six study sites while Bengal Fox (*Vulpes bengalensis*) and Smooth-coated Otter (*Lutrogale perspicillata*) were present in three study sites each. Maximum sign density was of Asiatic Jackal (0.0735), followed by Jungle Cat (0.0607), Small Indian Mongoose (0.0185), Bengal Fox (0.0050) and Smooth Coated Otter (0.0021). Seven habitat types were identified in the study area with highest species diversity in Grassland and Shrub land (five spp) followed by Forest and Crop land (four spp); three spp in Barren land and one species for Marsh land and Human settlements each. Marsh land, grassland and human habitation habitat were found in all study sites; shrub land, crop land and barren land in three sites while Forest land in five sites. Sign observation points were plotted on map using GIS tool. Results showed that increasing human pressure, livestock grazing and change in land use pattern have adverse affect on distribution of these carnivore species.

A STUDY OF AMPHIBIAN ENVIRONMENT IN DISTRICT JAMSHORO, SINDH-PAKISTAN

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Amphibians are highly aquatic hence water has great impact on their development and survival. Water contamination not only affects the eggs and larvae but the adult amphibians also because of their respiratory skin. Thus environmental pollution is one of the main causes behind mortality of these animals. Unfavorable environment contributes to their declination hence present study was conducted from the year 2011 to 2013 in District Jamshoro (11,517 km²) where existence of four anuran species: *Hoplobatrachus tigerinus*, *Euphlyctis cyanophlyctis*, *Allopa hazarensis* and *Bufo stomaticus* were already confirmed. The amphibian environment was investigated through some main Physico-chemical parameters including pH, Electric conductivity, Total dissolved solids, hardness, alkalinity, sulphate and phosphate by using instruments, procedures, chemicals and reagent of analytical grade. The results of present study revealed highly polluted status of whole amphibian ambient due to extreme level all the analyzed parameters which may make environment intolerable for amphibian fauna in the study area. Only pH value was persistently normal during entire range of study period. Monthly changes in water quality remained constant; volume of all the parameters rose to uppermost level during July and decreased to least value in November each year of analysis. This environmental condition requires urgent implementation of management plans to save amphibian fauna from being deteriorated in District Jamshoro.

ROLE OF EDTA IN ENHANCING ROOT TO SHOOT METAL TRANSLOCATION AND ASSOCIATED LEACHING HAZARD IN *Pennisetum glaucum* AND *Sorghum bicolor*

Khadija Rafiq

The research work was conducted to investigate the role of plant species for phytoextraction of metals from contaminated soil and to examine the effectiveness of EDTA in enhancing metal mobility and bioavailability along with taking into consideration the associated potential risk of ground water contamination. *Pennisetum glaucum* and *Sorghum bicolor* were selected for the chelate assisted phytoextraction experiments. EDTA was applied after plant establishment in soil, artificially spiked with the salts of heavy metals (Cu, Cd and Cr). Estimation of leached metal content was done by collection of leachates at weekly intervals after EDTA treatment. Samples were subjected to acid digestion and quantification of heavy metals was carried out by Atomic Absorption Spectrophotometer. Analysis of metal content in the plants revealed the greater accumulation of metals in roots than shoots. Metal extraction efficiency of *Sorghum bicolor* proved to be better as compared to that of *Pennisetum glaucum*. The study showed that EDTA increased the metal mobility in plants as well as in leachates. EDTA causes excessive leaching of metals which poses a possible menace of ground water contamination. Consequently low dosage of EDTA is recommended to minimize the risk of ground water contamination.

MICROBIAL LEVELS IN INDOOR MICRO-ENVIRONMENTS DURING ACTIVITY AND NON-ACTIVITY PERIODS IN RESIDENTIAL HOUSES OF LAHORE, PAKISTAN

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Air borne micro-organisms can pose a serious health risk to the human health. Level of suspended bacteria present in the surrounding air is affected by a variety of environmental factors as well as type of activities being carried out at any specific time. The present study was conducted as a part of a larger study and consisted of monitoring of air-borne levels of bacteria in indoor micro-environments of five residential houses of Lahore. Agar coated petri plates were exposed face upwards for twenty minutes each in the kitchens and living rooms during activity and non-activity periods in a day. The colony forming units per cubic meter (cfu/m³) in kitchens during the activity time ranged between 1022.01 cfu/m³ – 4481.13 cfu/m³. In the living rooms, these levels ranged from 1179.24 cfu/m³ to 3183.96 cfu/m³. In the absence of any major activity the cfu/m³ fell to considerably lower levels. It was observed that colony forming units of bacterial species increased during activity times in both micro-environments and decreased when there was minimum activity in the rooms. The predominant species included *Micrococcus* spp., *Staphylococcus* spp., and *Bacillus* spp. which are a common constituent of the indoor environment and are known to be opportunistic pathogens as well.

COMPARATIVE AMBIENT AND INDOOR PARTICULATE MATTER ANALYSIS OF OPERATION THEATRES OF GOVERNMENT AND PRIVATE (TRUST) HOSPITAL OF LAHORE, PAKISTAN

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Air pollution is a major distress in Pakistan where both indoor and outdoor pollutants baffles certain risks to the community health. The accretion of particulate matter (PM) in operation theatres of hospitals has not yet been studied comprehensively in Pakistan. The purpose of this research was to testify the degree of isolation of the air of operation theatres from ambient air. Monitoring of both indoor (Operation theatre) and outdoor concentrations for the comparative analysis of the data and determination of PM levels indoors in both activity and non-activity time spans in operation theatres of Services (government) and Shalamar (private) hospital, Lahore, using a direct reading instrument, DUSTTRAK Aerosol Monitor (TSI Model 8520) and DRX Aerosol Monitor (TSI Model 8533). The highest concentration of PM was observed in Orthopedic operation theatre of Services hospital during working hours having average concentration of 756.74 ± 539.66 , 809.4 ± 584.28 , 823.72 ± 585.05 , 875.19 ± 585.87 and $969.58 \pm 581.16 \mu\text{g}/\text{m}^3$ of PM_{10} , $\text{PM}_{2.5}$, PM_4 , PM_{10} and PM_{total} respectively while the $\text{PM}_{2.5}$ average concentration in outdoor was $294.021 \mu\text{g}/\text{m}^3$. The minimum average PM concentration was sampled in the Orthopedic Operation theatre of Shalamar hospital during working hours 17.78 ± 7.56 , 18.7 ± 8.12 , 19.86 ± 8.53 , 25.73 ± 8.85 and $39.47 \pm 9.35 \mu\text{g}/\text{m}^3$ for PM_1 , $\text{PM}_{2.5}$, PM_4 , PM_{10} and PM_{total} respectively which is probably because of Laminar Flow system that has air regulating devices in supply and return air ducts. Factors such as ventilation system, building age, possible sources of infiltration, number of people present in the Operating area seems to actively participate in determining the PM concentration in Operation theatre.

EFFECT OF NITROGENOUS FERTILIZERS ON TADPOLE SURVIVAL OF COMMON SKITTERING FROG (*EUPHLYCTIS CAYANOPHLYCTIS*)

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Amphibians are more sensitive to chemical stressors such as fertilizers, heavy metals and pesticides. The consequences of nitrate pollution range from gross toxicity to subtle changes in physiology and development in amphibians. Data on effects of fertilizers on anurans are lacking in Pakistan. The present study was therefore conducted to investigate the effects of ammonium nitrate on tadpole survival of Common Skittering frog (*Euphlyctis cyanophlyctis*). Tadpoles (spawns) of this frog were collected from seasonal shallow water ponds formed during rainy season in the ponds of Rawalpindi and Islamabad cities. The tadpoles were collected with dip nets and transferred into buckets filled with water from the same pond. The buckets were kept for 24 hours in the laboratory. Nitrate solutions. Five nominal concentration of ammonium nitrate from granular

ammonium nitrate viz. 1000, 500, 250, 125 and 62.5 mg/L nitrate (pH ranging from 7.37 to 7.41) were prepared. Each concentration was replicated thrice. A batch of ten tadpoles was treated with five concentrations, and was compared with a batch of ten untreated (control) tadpoles. The tadpole survivorship was calculated as number of metamorphosed tadpoles/ total number of tadpoles initially introduced*100. The number of tadpoles across replications and treatments were compared using one-way ANOVA. The relationship between number of tadpoles and concentrations was examined using linear regression. The mean tadpole survival when treated with the different concentrations of ammonium nitrate i.e. 62.5, 125, 250, 500 and 1000 mg/L was 83 %, 78 %, 59 %, 37 % and 0 %, respectively. The mean tadpole survival differed significantly among the studied concentrations ($F= 77.11$ (5, 96), $p < 0.05$). The linear regression showed that the relationship between tadpole survival and concentrations of ammonium nitrate was significant ($R^2= 0.92$, $F(1, 22) = 273.95$, $P < 0.05$). However, no malformations in the metamorphosed frogs were recorded. It is concluded that nitrates in higher concentration may affect anuran species in croplands with extensive nitrogen-based fertilizer use. It is suggested that detailed studies be carried out on the monitoring of nitrate levels in the croplands and that field data be collected to understand how nitrate pollution affect anurans in the croplands.

2. FRESHWATER BIOLOGY AND FISHERIES

PHYSIOCHEMICAL ANALYSIS OF VARIOUS POND WATER BODIES FOUND IN FOUR DISTRICTS OF PROVINCE BALOCHISTAN, PAKISTAN.

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Present study includes the analysis of different physio-chemical properties including color, texture, salinity, temperature, dissolved oxygen (DO), pH, Electrical conductivity and the metal contents (Zn, Ni, Cd, Pb, Mn, Cu) of fish pond during the period from August 2014 to December, 2014. The pond water samples were collected from the four different districts of Balochistan province including District Sibi (City Sibi, Pond Location Circuit House), District Bolan (Village Mithri), District Jaffarabad (Village Hajji Yaqoob Khan Pandrani) and District Nasirabad (Village Jumma Khan Umrani). Balochistan is the largest province by area forming around 44% of the Pakistan's over-all land mass, and is smallest in position of population. All these ponds were built especially according to economic considerations except the pond of district Sibi (Circuit House) which is used as an ornamental pond. Thus, our present work will provide useful information's to the aqua culturists to improve the ecological setup in a fish pond, evading trauma related diseases, parasitic harms and eventually generating aquatic beings more efficiently. Furthermore, such study may also have an important aspect as for determining the growth rate and productivity of fishes.

DETERMINATION OF WATER QUALITY AND ESTIMATION OF HEAVY METALS IN WATER OF NULLAH LAI, RAWALPINDI/ ISLAMABAD PAKISTAN.

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Water samples from 11 different locations of Nullh Lai in metropolitan city of Rawalpindi and Islamabad were collected and analysed for physical Parameters viz, colour, temperature, pH, odour, flowrate, were measured and chemical parameters viz, total hardness, Electrical conductivity (ECT), alkalinity, chlorides, nitrites, sulphates, were analysed, High concentration of pH, EC., Hardness Chlorides, and Nitrite were detected at F/7 and new Katariyan Bridge. Heavy metals Zn, Cd, Cu, Pb, As, Co, Mn, and Ni were measured. A site PNK was without load of pollution because it was starting point of nullah lai and a natural stream. In downstream of nullah high concentration of all parameters were found as compared to upstream. The study conclude that water quality of nullah lai is polluted and most results are above permissible limits. The city sewage discharge, urban run off, dumping of waste material and industrial waste effects the water quality

of nullah. The results suggest urgent need wise management of human activities in catchment of nullah lai.

ASSESSMENT OF WATER QUALITY PARAMETERS IN RIVER KABUL KHYBER PAKHTOONKHTWA, PAKISTAN

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Seasonal variations in water quality of River Kabul were analyzed in Khyber Pakhtunkhawa (KPK) province of Pakistan. The water quality was studied at nine sites at upstream and downstream in Kabul River during 2010 to 2011. Physico-chemical parameters of the samples were measured. The study revealed that the contamination level in River Kabul is rising because of discharge of industrial discharges and domestic waste directly into the river. Water parameters measured included chemical oxygen demand (COD), dissolve oxygen (DO), pH, temperature, electrical conductivity (EC), total dissolved solids (TDS), total suspended solids (TSS), total hardness (TH), total phosphorus (TP), total (nitrate + nitrite) nitrogen (TON), SO₄²⁻, Cl. The mean values of the measured parameters were compared with National Standard for Drinking Water Quality (NSDWQ) and U.S. Environmental Protection Agency (USEPA) standards. The result revealed that E.C values of all sites were falls above the standards values and DO values values of all sites except site nine were below the standards values.

PHYSICAL AND CHEMICAL LIMNOLOGY OF BANJOSA LAKE POONCH AZAD KASHMIR (PAKSITAN)

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Present work deals with the analysis of Physico-Chemical parameters of water samples of "Banjosa Lake", which is situated 20 Kilometer from the city of Rawalakot in District Poonch of Azad Kashmir, Pakistan during 2014. The Physico-Chemical parameters like water temperature, turbidity, pH, Conductivity, TDS, Salinity, Bicarbonates, Hardness, Chloride, Sulphate, Nitrate, DO, BOD, COD, Na, Ca, Mg were studied. The mean value of these parameters were found to be 23.9°C, 0.02, 8.09, 229ms/cm, 117mg/L, 0.00mg/L, 123mg/L, 97.5mg/L, 8.12mg/L, 29.62mg/L, 0.014mg/L, 0.33g/L, 6.30mg/L, 13.5mg/L, 38.25mg/L, 21.5mg/L, 16mg/L, 10mg/L. metal concentration were also determined Fe, Cu, Zn, Ni, Pb, Cd, Co. the value of these parameters were found to be 0.003mg/l, 0.001mg/L, 0.001mg/L, 0.013mg/L, 0.002mg/L, 0.001mg/L, 0.010mg/L, 0.40mg/L, respectively after the assessment it is concluded that the water of lake is suitable for all types of life and is free from pollution

SOME OBSERVATIONS ON THE DISTRIBUTION AND ABUNDANCE OF TALANG QUEENFISH (*SCOMBEROIDES COMMERSONIANUS*) IN THE OFFSHORE WATERS OF PAKISTAN

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Talang queenfish (*Scomberoides commersonianus*) is an commercially important fish belonging to Family Carangidae which is caught mainly by pelagic gillnets along the coast of Pakistan. In the offshore fisheries, Talang queenfish is considered to be the most important bycatch species. Data collected from October 2012 to December 2014 revealed that this species is predominantly found during August to October with peak in September and minor increase in catch during April and May. Its main fishing grounds are located between 50 to 200 m depth all along the coastline. Its size ranges between 50 and 85 cm with 70-75 cm as dominating size cohort. Based on the size frequency data various population parameters were studied which indicates that its stocks are still healthy. Juveniles of about 30 to 35 cm are also caught in large quantities in the shallow coastal waters by seine net. It is feared that if juveniles are continued to be caught in shallow waters, it will lead to depletion of the stocks in the offshore waters.

PRESENT STATUS AND PRODUCTION OF AN INVASIVE CLARID CATFISH *CLARIAS BATRACHUS* (LINN., 1758) IN FRESHWATER BODIES OF THATTA DISTRICT

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Catfish is a significant group of fish in the world. *Clarias batrachus* (walking cat fish or Magur) is commercially important as well as indispensable aquaculture species in many Asian countries. In Pakistan *C. batrachus* was first time reported from Thatta (Baloch and Jafri, 2004). It is commercial fish and fetch high price at market. A monthly survey from January to December, 2012 in Thatta district was undertaken for habitat identification and production of *C. batrachus*. The result reveals that *C. batrachus* occurs in Indus River, Keenjher Lake, K. B feeder canal; small water bodies connected to Indus River and private fish farms. The main markets of *C. batrachus* were include Thatta, Chuhar Jamali, Jati, Mirpur Sakro, Sajawl and Gharo. Fish production showed that Thatta ranked first in terms of production of *C. batrachus*. Annual production was estimated for Thatta (5.39mt), Chuhar Jamali (3.7mt), Jati (2.76mt), Sakro (1.59mt) Sujawal (1.06mt), and Gharo (0.99mt), making a total production of *C. batrachus* in Thatta district 15.49mt tones in 2012. Length-weight equations were calculated separately for male, female and combine populations are as follows,

$$\begin{aligned} \text{Log } W &= -1.3825 + 2.4779 \times \text{log } L \text{ (Male)} \\ \text{Log } W &= -0.4411 + 1.8863 \times \text{log } L \text{ (Female)} \\ \text{Log } W &= -1.3825 + 2.4779 \times \text{log } L \text{ (Combine)} \end{aligned}$$

The gut contents' revealed that shrimps, insects, snails, debris, and fish flesh are common diet of *C. batrachus*. The stomachs of fish mostly contained insects (water bugs) and debris, that shows

carnivorous and insectivorous feeding habits. More than 90% of fish stomachs were filled with food during January and about 80% 75% in February. In March and April about 75% while from May to September 10% food was found probably due to development of gonads during that period. In November 80% and December more than 90% stomachs were found filled. *Clarias batrachus* is an exotic species occurring in Thatta area with an annual production of 15.49 mt (2012). It was noted that it is a carnivorous species mostly feeding on water bugs.

STUDY OF THE PHYSIOCHEMICAL PROPERTIES OF SOIL IN FISH POND AT CIRCUIT HOUSE, DISTRICT SIBI, BALOCHISTAN.

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The study was conducted to detect physiochemical properties and Metals concentration in soil of Circuit house Sibi pond. An aquaculture system is balanced by Pond soil because soil properties play a vital role in growth and survival of aquatic organisms. The pond soil can use as a bumper to the aquatic ecosystem and it serves as a biological filter through the adsorption of fish excretions, algal metabolites and organic residues of feed. Pond soil provides all the important nutrients with water. Pond soil samples were collected from aqua pond of district Sibi areas. The pH, TDS, EC and metals concentration (Zn, Ni, Cu, Cd, Mn, Pd, Na, K, Ca) by Atomic absorption spectroscopy was determined. The pH of pond soil is range from 9.1-9.3 ppt/ml with an average value 9.2 ppt/ml, total dissolved solids is range from 173.3-175.3mg/L with an average value 174.6 mg/L, electrical conductivity in range from 289-290 μ s with average value 290 μ s. Therefore, our present study would be helpful to understand the quality of soil in a fish pond and its impact on all aquatic biota found in fish pond.

HISTORICAL PERSPECTIVE OF SHARK FISHERIES OF PAKISTAN

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Sharks fishing is known to be one of the oldest practice of the fishermen of the coastal areas of Sindh and Balochistan. By middle of 1800's, a substantially well organized fishery for shark was in existence which involved processing and export of shark fins and meat. A well diversified shark fauna was known from the northern Arabian Sea which was harvested through gillnets and line gears. Aimed handline shark fishery using livebait was based mainly in Karachi, Ormara, Pasni, Gwader and Jiwani. Large sharks such as shirtfin mako (*Isurus oxyrinchus*), tiger

shark (*Galeocerdo cuvier*), bull shark (*Carcharhinus leucas*), pelagic thresher (*Alopias pelagicus*) bigeye thresher (*Alopias superciliosus*), oceanic whitetip shark (*Carcharhinus longimanus*), scalloped hammerhead sharks (*Sphyrna lewini*) and great hammerhead (*Sphyrna mokarran*) were dominating among the large shark whereas among the small sharks, milk shark (*Rhizoprionodon acutus*), grey sharpnose shark (*Rhizoprionodon oligolinx*) and spadenose shark (*Scoliodon laticaudus*) were observed to be dominating the catch. Blacktail reef shark (*Carcharhinus amblyrhynchos*), pigeye shark (*Carcharhinus amboinensis*), silky shark (*Carcharhinus falciformis*), blacktip shark (*Carcharhinus limbatus*), blacktip reef shark (*Carcharhinus melanopterus*) and sicklefin lemon shark (*Negaprion acutidens*) are common among medium sized sharks. Fleet engaged in shark fishing increased during 1988 and 1993 which resulted in major decrease in the catches. It is estimated that at present about 6,000 m. tons (2013 data) whereas at one stage shark landings was estimated to be around 35,000 m. tons (1999 data). Shark meat is consumed in Pakistan whereas shark fins and meat (in salted dried) is exported. In the paper reasons for decreases in landings, change in species composition and ecosystem impacts are discussed.

GROWTH PERFORMANCE OF *CATLA CATLA* AND *LABEO ROHITA* IN POULTRY MANURE FERTILIZED POND

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This experiment was conducted to assess the impact of poultry droppings fertilization on the growth performance of *Catla catla* and *Labeo rohita*. An earthen pond of one acre was stocked with *Catla catla* and *Labeo rohita* with stocking densities of 400 and 800, respectively. On the basis of nitrogen content, the poultry droppings were added to pond water, on daily basis, at the rate of 0.17g N/100g fish weight. Random samples of both fish species were captured fortnightly for growth analyses. The growth parameters which were examined during this study include increase in weight, fork and total lengths, length-weight relationship, condition factor and net fish yield. At harvesting, distinct weight gains were observed in both fish species. The weight-lengths relationship of both species was found to be positive and highly significant. However, the growth of *Catla catla* was found to be significantly higher than *Labeo rohita* under the poultry manure integrated pond.

EFFECTS OF POULTRY MANURE FERTILIZER ON PHYSICO-CHEMISTRY AND PLANKTONIC PRODUCTIVITY OF FISH POND WATER

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This experiment was conducted to monitor the effects of poultry manure, as fertilizer on the physico-chemistry and planktonic productivity of fish pond water during twelve fortnights (six months). One acre pond was stocked with 2000 fingerlings of five fish species with stocking ratio

of 40:20:15:15:10 for *Labeo rohita*, *Catla catla*, *Cirrhina mrigala*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*, respectively. Pond water was fertilized with poultry droppings at the rate of 0.17 g nitrogen per 100g fish weight on daily basis. During this study period, the physico-chemical variables viz. pH, temperature, dissolved oxygen, electrical conductivity, total hardness, calcium, magnesium, total alkalinity, carbonates, bicarbonates, ammonia, nitrates, phosphates, chlorides, sodium, potassium and dry weight of planktonic biomass was monitored on fortnightly basis. Under poultry manure fertilization, all physico-chemical parameters showed significant differences among their means. Planktonic biomass found to be dependent upon a number of parameters. With some parameters it showed positively significant correlation like pH while with others planktonic biomass showed negative but significant correlation.

STUDIES ON PRE AND POST OVULATORY STAGES IN *OREOCHROMIS MOSSAMBICUS* (TILAPIA)

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An attempt was made to study the ovulatory stages in *Oreochromis mossambicus* (Tilapia). These fishes were collected from adjoining waters of Jamshoro Sindh, from early August to early December by 20 days interval. Their mean body weight recorded as 44.2 ± 8.0 . After the histological examination, it was observed that ovaries of the fish collected in early August contains stem cells in ovigerous folds, that developed, matured and released from the ovary. As the first batch released, second batch was noted by appearance of stem cells in ovigerous folds. The second batch followed by third batch and so on. Hence, *Oreochromis mossambicus* is a multi spawner fish, that breeds in clutches and ova are released in batches.

BYCATCH OF COMMERCIAL FISHING PRACTICES: A MAJOR THREAT TO THE MARINE BIODIVERSITY IN PAKISTAN

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Aluterus monoceros (Linnaeus, 1758) is a circumglobal fish belonging to family Monacanthidae which is found in coastal and offshore waters of Pakistan. It is mainly caught by gillnets which are being operated in neritic and offshore waters. Its commercial harvesting and export was started in 2004 and since then it is exported in frozen gill and gutted form to Japan, Korea and China. Although records of its landings is not made but it is estimated that its annual landings in Pakistan is about 2,500 m. tons. This species is harvested throughout the year but main fishing seasons are from September to January with peak in October with a minor increase in catches during May. Its size ranges between 25 and 70 cm with size class 45 to 50 cm dominating in the catch. Using length frequency data collected from January 2013 to December 2014, population parameters were calculated. The species was found to feed on a variety of invertebrates and fishes. It breeds in winter (October and January).

EFFECTS OF HUMAN ACTIVITIES ON WATER QUALITY AND FISH FAUNA OF RIVER CHENAB IN BAJWAT, PAKISTAN

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The present study was designed to highlight the deleterious effects of human activities on water quality and fish fauna of river Chenab and its tributaries in Bajwat, Sialkot. For this purpose, water samples and fish samples were collected along with habitat quality parameters at 6 sampling locations on seasonal basis from March, 2013 to June, 2014. Water samples were analyzed for 15 water quality parameters. Hierarchical Agglomerative Cluster Analysis (HACA) classified study area into three different classes of sites: least impaired, impaired and moderately impaired regions on the basis of variations in water quality parameters. Discriminant Function Analysis (DFA) identified 3 water quality parameters *viz.*, COD, TDS and Zn which showed significant spatial variations, whereas, major seasonal variations were observed in stream flow, temperature, EC, pH, nitrate, cadmium and DO. Factor Analysis (FA) identified the five main sources of contamination such as municipal waste, agricultural, urban runoff, parent rock material, Hydrological modifications and recreational Activities. A total of 776 individuals of 32 fish species belonging to 13 families and 19 genera were recorded in sampling seasons (spring, post monsoon and pre monsoon) from river Chenab and its tributaries. Highest diversity indices were recorded from downstream of river Chenab. The highest species richness was noted in post monsoon after rainy season, whereas, lowest in spring season before rainy season. The results indicate that water quality and fish fauna of Jammu Tavi facing degradation due to municipal sewage, agricultural practices, water diversions and trans-boundary movement of pollutants in the catchment area. The findings of this study can be helpful for future management of other streams and rivers in the same eco-region.

LIMNOLOGICAL STUDY RELATED TO PHYCICO-CHEMICAL CHARACTERISTICS OF WATER IN BAGHSAR LAKE DISTRICT BHIMBER AZAD JAMMU AND KASHMIR

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Lakes are most diverse productive and interactive ecosystems in the world. Present study was conducted on bagsar lake which is located in Bagsar village, Tehsil Samahni, District Bhimber Azad Jammu & Kashmir. The lake water indicated average pH 6.86, Turbidity 2.62 NTU, Conductivity 453.45µm/cm, Hardness 165mg/L. Lak water showed low dissolved oxygen (DO) 3.43mg/L and slightly high concentration of chemical oxygen demand (COD) 65mg/L. Other parameters like hardness, chloride, bicarbonate, nitrate, nitrate and metal concentrations were also analyzed. Lake area is reducing so protection is needed.

USE OF PHYTASE FOR IMPROVING GROWTH PERFORMANCE AND MINERAL DIGESTIBILITY OF *LABEO ROHITA* FINGERLINGS FED ON SUNFLOWER MEAL BASED DIET

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The present study (60-day) was conducted to evaluate the efficacy of phytase supplementation on growth performance and mineral digestibility of *Labeo rohita* fingerlings. Test diet was made by replacing 30% of reference diet with sunflower meal as test ingredient. Seven sunflower meal based test diets were prepared by spraying graded levels of phytase (0, 250, 500, 750, 1000, 1250 and 1500 FTU kg⁻¹) to the basal diet. Chromic oxide was used as inert marker in the diets to assess the minerals digestibility. The results of our study showed higher growth performance of fingerlings in response to phytase supplementation. Maximum performance was obtained by the fingerlings fed on test diet having 750 FTU kg⁻¹ level of phytase supplementation. Similarly, mineral digestibility was also improved by phytase supplementation at 750 FTU kg⁻¹ level. It was concluded that the phytase supplementation to sunflower meal based diet at 750 FTU kg⁻¹ level is optimal to release adequate chelated minerals for maximum growth performance of *L. rohita* fingerlings. These results also suggest that phytase supplementation in plant based diets may decrease the need for supplementing minerals, which will reduce the cost of fish feed and minerals discharge through feces into the aquatic ecosystem resulting in environment friendly aquaculture.

FORAGE RATIO AND FEEDING BEHAVIOR OF A FRESHWATER FISH *SALMOSTOMA BACAILA* OCCURRING IN PUBLIC WATERS IN SINDH, PAKISTAN

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Salmostoma bacaila belonging to family Cyprinidae, usually inhabits in shallow meso-eutrophic stagnant waters as well as in slow running streams. It is a surface feeder and regarded as omnivorous fish. Eighty specimens of different age and size groups were collected and studied from Jamshoro, Hyderabad and Sukkur districts during 2014. The study was carried out for mouth gape, gill rakers, stomach content and feeding behavior of the fish. The average of mouth gape of fish 1.5±1.272 cm gill raker length 3.8±2.545 mm and stomach contain 57% larvae (semi-digested), 23% zooplankton, 10% phytoplankton and 10% miscellany of mud and plant debris were observed. The study indicated that *Salmostoma bacaila* is active useful larvaevorous fish in feeding habit and can be used for the biological control on mosquito larvae.

STUDIES ON GROWTH, SURVIVAL AND GUT MICROBIAL LOAD OF SILVER CARP, *HYPOPHthalmICTHYS MOLITRIX* FINGERLINGS FED WITH DIETS CONTAINING PROBIOTICS

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The success and failure of fish culture depends on the early growth stages of fish. The present study was designed with aim to investigate the effect of probiotics on growth rate, survival rate and gut microbial load of silver carp, *Hypophthalmichthys molitrix* fingerlings with 90 days experimental trial. In this experimental trial, the probiotics were used at two different levels (3% and 7%). For this purpose, three earthen ponds were selected. Twenty fingerlings were randomly selected and stocked in each pond. Fish in pond 1 (T₁) control group were given with diet without any probiotics. In pond 2 (T₂) fish were fed with a diet having 3% probiotics while in pond 3 (T₃) fish were fed with diet containing 7% probiotics. Fish fingerlings were fed with experimental diet on daily basis. Physico-chemical parameters were kept according to fish growth and survival requirements. Growth parameters of Silver carp fingerlings (weight gain, FCR, SGR) were measured on weekly basis throughout experimental trial. The data obtained was subjected to statistical analysis of variance and DMR. The study results showed that all the diets containing different levels of probiotics significantly ($P < 0.05$) improved silver carp growth rate and feed utilization compared to the control diet. However, 7% probiotics concentration showed the best result regarding total weight gain, daily growth rate and relative growth rate.

A COMPARATIVE STUDY ON THE MORPHOLOGICAL ASPECTS OF SCALES OF LOCAL FISH SPECIES

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The study was conducted to study the scales of eight freshwater fishes i.e., rohu, common carp, mori, silver carp, bighead carp, grass carp and subzeg. Scales were removed from selected regions of the fish body i.e., from snout to tail region. They were air dried and cleaned with a dilute solution of laboratory grade detergent, stained with eosin and finally mounted between a pair of glass slides and examined under microscope and photographed. The digital images of fish scales were identified according to their characteristics. Six characteristics of scale were studied and data were recorded. For comparison of shape variations statistically, Principal Component Analysis (PCA) was used. Images were binarized using SHAPE software. Chain code data was then subjected to EFA to obtain normalized Elliptical Fourier shape descriptive/coefficients, which were then subjected to PCA, Box and Whisker Plot and Kruskal-Wallis Test. Results showed that scales in all eight species were cycloid. Shapes of scales were highly varied. They were rectangular, square, pentagonal, hexagonal, oblong, round, triangular or oval. The shape of margin varied from smooth to round, tongue like or triangular. Two types of radii were observed i.e., primary and secondary except that no secondary radii were found in tilapia. A central canal was present on the scales removed from the lateral line. In all fish species, scales collected from region C showed the

highest number of scales in all the fish species. PCA-1 showed the greatest percentage of variance as well. Box plots showed the close and distant relationship among the scales of various selected region. Scales with similar shapes had scores that were plotted close together while those that differ were plotted in the form of points according to the scatter plot. Scale shapes were variable and species specific. Environmental influences might affect fish scales morphology defining fish stocks characterized by phenotypic differences. Greater number of radii's is related to better nutrition and its number only depends on the location of the scale on the fish body. The present study demonstrates that scale characters can provide useful taxonomic information on the morphological difference inter or intraspecifically.

REPRODUCTIVE ASPECTS OF *GLOSSOGOBIOUS GIURIS* (HAMILTON) IN MANCHAR LAKE, SINDH, SOUTHERN PAKISTAN

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Glossogobius giuris (Hamilton) belong to order Perciformes and family Gobiidae, it is one the important goby fish, locally called as Gullo or Gup gullo. *Glossogobius giuris* has tolerance for high range of salinity thus can found from freshwater to estuarine water. It is widely distributed in the freshwaters and estuaries of Pakistan, India, Bangladesh, Burma and Far East (Bhuiyan, 1964). Knowledge about reproduction of a fish is important for the management of conservation as well as to for the hatchery management. Manchar lake is the largest lake in Pakistan, it is shallow and polluted lake situated in district Jamshoro. Fish samples were collected from fisherman catch over the period of one year from January 2010 to December 2010. Chilled specimens were brought to the laboratory where all the observations were made. Gonado-somatic index (GSI) was used to determine the reproductive season of fish using following formula $GSI = (GW \times 100) / BW$; where GW gonadal weight and BW is the body weight; Fecundity estimations were made with following formula; $F = (GW/L) \times$ average of three sub-samples where F fecundity, GW gonadal weight and L is Length of ovary. The fish has distinct sexual dimorphism, males were with straight, thin and pointed genital papilla while females were with short fleshy and circular genital papilla. Minimum size of female carrying mature eggs was 22.1 cm and its ovary weight was 0.81 g. Gonado-somatic index (GSI) of *G giuris* starts to increase in month of February it peaks in the month of March it starts to decrease after June and reaches to minimum in September, suggesting that the fish has prolonged spawning periods starting from February and ending in September. Maximum and minimum fecundity recorded was 38565.09 and 10567.32 from the fish with total length 23.8 and 10.6 cm respectively.

GROWTH PERFORMANCE AND METALS RETENTION ABILITIES OF FIVE METAL STRESSED FISH SPECIES REARED IN EARTHEN PONDS

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Five fish species viz. *Catla catla*, *Cirrhina mrigala*, *Ctenopharyngodon idella*, *Labeo rohita* and *Hypophthalmichthys molitrix* were stressed with Mn+Zn+Pb mixture, on the basis of

their 1/3rd of LC₅₀, for one month under controlled laboratory conditions. After stress, all the five fish species were grown in earthen ponds holding metals deprived water under composite culture conditions for 210 days. The post-stress of metals mixture caused significantly lesser weights, fork and total lengths, feed conversion ratios and condition factor to all the five fish species when compared with control (un-stressed fish). Treated five fish species showed non-significant differences for their final average weights while increase in fork and total lengths, condition factor values and feed conversion ratios showed significant variability. Metals mixture stress to the fish caused significantly higher retention of Mn, Zn and Pb in fish body at final harvest. Both at stocking and final harvest, fish kidney and liver had significantly higher metals than the other organs. However, fish bones contained significantly least metals. In treated fish the overall retentions of all three metals varied significantly as kidney>liver>skin>muscle>fins>gills>bones while control fish showed metals retention pattern of kidney>liver>fins>skin>gills>bones. At final harvest, *Cirrhina mrigala* had an average higher metallic ion concentration of 140.06±207.66µgg⁻¹ with statistically non-significant differences with other species of fish, except *Hypophthalmichthys molitrix* that contained 120.12±189.98µgg⁻¹ metals in its body. Pb showed significantly higher retention ability in fish body, followed by Zn and Mn. After 210 days of rearing, all the five treated fish species exhibited significant depuration of all metals from their bodies that followed the order Zn>Mn>Pb.

**MORPHOMETRIC AND MERISTIC CHARACTERISTICS OF INDIAN MAJOR CARP,
*CIRRHINUS MRIGALA***

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The aim of present study was to check the variation between wild and cultured fresh water fish *Cirrhinus mrigala* on the basis of morphometric and meristic parameters. For this proposed research work fish specimens were collected from two wild sources, river Chenab and river Ravi and one cultured source, ponds of fisheries research farms, University of Agriculture, Faisalabad. Total 120 fish samples were collected from the three selected locations. To check phenotypic variation between wild and cultured populations of *C. mrigala*, 15 morphometric characters (Total length, Fork length, Standard length, Head length, Head width, Dorsal fin length, Dorsal fin base, Pectoral fin length, Pectoral fin base, Pelvic fin length, Pelvic fin base, Anal fin length, Anal fin base, Caudal fin length and Caudal fin base) and 6 meristic characters (Dorsal fin rays, Pectoral fin rays, Pelvic fin rays, Anal fin rays, Caudal fin rays and Lateral line scales) were studied. Univariate analysis was used to analyze the morphometric and meristic data of present study. The inferences of present research work showed that out of 15 morphometric characters 11 characters were significantly different (P<0.01) in Chenab and Ravi populations, 8 characters were significantly different (P<0.01) in Ravi and UAF populations and all the characters were significantly different (P<0.01) in Chenab and UAF populations. Out of 6 meristic characters 3 characters showed significant difference (P<0.01) among Ravi and UAF populations and 3 characters showed significant difference (P<0.01) among Chenab and UAF populations whereas all the meristic characters were similar (P<0.05) in Chenab and Ravi populations. The present study contributes baseline biological information that is expected to be helpful in facilitating the development of management strategies in relation to the fishery and conservation of *C. mrigala* populations in the selected locations.

**EVALUATION OF DIFFERENT STOCKING RATIOS (*CHANNA MARULIUS*:
OREOCHROMIS MOSSAMBICUS) ON THE GROWTH PERFORMANCE OF *CHANNA*
MARULIUS IN FERTILIZED PONDS**

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To evaluate the effect of different stocking ratios of *Channa marulius* with *Oreochromis mossambicus*, fishes were stocked in three earthen ponds designated as T1, T2 and T3 respectively. Ponds were fertilized with organic (cow dung) and inorganic fertilizer (nitrophos) @ 2gN/100g of a wet fish body weight daily. Ponds were stocked with 1:10, 1:20 and 1:30 ratios of *Channa marulius* with *O. mossambicus* in T1, T2 and T3 respectively. *C. marulius* were added @30 fish/pond. The cultured fish stock were captured randomly and their body weight and total lengths were measured fortnightly. After obtaining the data, the fish were released back to their respective ponds. The results of present results revealed that *C. marulius* showed best growth performance in T₃ (predator-prey stocking ratio 1:30) of *C. marulius* versus *O. mossambicus* was used. The maximum final average weight in T3 was 1117.5±9.12. The maximum weight gain of *C. marulius* was perhaps on account of sufficient supply of *Tilapia* in T3. It was followed by T2 in which maximum final average weight was 1025.6 ± 0.56 (predator-prey stocking ratio 1:20). The minimum final average weight was observed in T1 in which 969.5± 0.70 (predator-prey stocking ratio 1:10) was calculated.

**IDENTIFICATION OF FISH FAUNA OF SARKI LAWAGHER DAM KARAK KPK,
PAKISTAN**

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This discussion is emphasized on the identification of fish fauna in the water of Sarki Dam. The preliminary survey of Ichthyofauna of Sarki dam was carried out during February, 2011 to April 2011. A total of 108 fishes were collected and identified from of the dam. Fishes collected and identified belongs to 3 order, 5 Families, 12 Genera and 13 species. In the present survey cyprinidae family was the richest Family of Sarki dam Karak, which is represented by 7 species i.e. *Puntinus ticto*, *Barilius vagra*, *Cirrhinus mrigalla*, *Hypothalmactus molitrix*, *Labeo caeruleus*, *Cyprinus varpio* and *Ctenopharyngodon idella*. Family Schilbeidae and Siluridae was represented by only one species *Clupisoma naziri* and *Wallago attu*, while Family Begridae and Channadae represented by two species i.e., *Sperata salwari*, *Mastacembelus bleekeri* and *Channa gachua*, *Channa punctatus* respectively.

**IDENTIFICATION OF FISH FAUNA IN RIVER SWAT AT DISTRICT CHARSADDA,
KPK, PAKISTAN**

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The preliminary survey of Ichthyofauna of River Swat at District Charsadda was carried out from October, 2011 to March 2012. A total of 71 fish were collected and identified from of the River Swat. Fish collected and identified belongs to 4 Order, 5 Families, 8 Genera and 8 Species. In the present survey Cyprinidae Family was the richest Family of River Swat at District Charsadda, which is represented by 3 Species *i.e.*, *Hypophthalmichthys molitrix*, (Voleviennus, 1884) *Cyprinus carpio* (Linnaeus, 1758) and *Schizothorax plagostomus*, (Heekel, 1838) while Family Siluridae, Mystacembeledae, Channidae and Bagridae were represented by only one species: *Wallago attu*, (Bloch and Schacider, 1801); *Mystacembelus armatus*, (Lacepede, 1800); *Gara gotyla* (Gray, 1830); *Channa gachua* (Hamilton, 1822) and *Rita rita* (Hamilton, 1822) respectively.

**WHOLE BODY PROXIMATE COMPOSITION AND MINERALS STATUS OF ROHU
(*LABEO ROHITA*) JUVENILES FED AN ACIDIFIED PHYTASE PRE-TREATED
SOYBEAN MEAL BASED DIET**

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The present project was designed to evaluate the whole body proximate composition and minerals status of rohu (*Labeo rohita*) juveniles fed an acidified phytase pre-treated soybean meal based diet. Experimental diets were supplemented with phytase (0 FTU/kg and 1000 FTU/kg) and citric acid (0% and 2%) in 2² factorial arrangement under completely randomized design. The test diets were fed to fish at the rate of 2% of live wet weight. After the completion of trial, five fish from each replicate was taken for whole body proximate composition and minerals analysis. Dry matter, crude protein, ether extract and ash were significantly (p<0.05) increased by citric acid supplementation in whole body of *L. rohita* juveniles. Similarly, pre-treatment of phytase also resulted in improved (p<0.05) whole body contents of these nutritional attributes. However, the combination of phytase and citric acid did not reveal any significant interaction on whole body proximate analysis. Citric acid supplementation increased (p<0.05) the whole body minerals including Ca, Mg, Cu, Zn, Mn, Fe, P, Na and K by 10%, 31%, 14%, 9%, 19%, 20%, 24%, 28% and 14% respectively in the *Labeo rohita* juveniles. Supplementation of PHY also improved (p<0.05) the whole body mineralization. The combination of PHY and CA supplementation significantly (p<0.05) enhanced the contents of these minerals in the juvenile's whole body.

**NUTRIENT DIGESTIBILITY PERFORMANCE BY ROHU (*LABEO ROHITA*)
JUVENILES FED ACIDIFIED AND PHYTASE PRE-TREATED SUNFLOWER MEAL
BASED DIET**

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Present research work was conducted to investigate the effects of microbial phytase and citric acid supplementations on nutrient digestibility performance by rohu (*Labeo rohita*) juveniles fed sunflower meal based diet. Basal diet was supplemented with two levels of phytase (0 FTU/kg and 1000 FTU/kg) and each level of this phytase supplemented diet was further supplemented with two levels of citric acid (0% and 2%). Chromic oxide (1%), as an inert marker, was added in the diet to determine the nutrient digestibility. Results showed that digestibilities of dry matter, crude protein and ether extract were significantly ($p < 0.05$) enhanced by citric acid supplementation. Similarly, phytase pretreatment also resulted in improved ($p < 0.05$) digestibilities of dry matter, crude protein and ether extract. However, their combination did not show any significant ($p > 0.05$) interaction for these nutritional attributes. Also, citric acid and phytase supplementation improved ($p < 0.05$) the digestibilities of P, Na, K, Ca, Mg, Cu, Zn, Mn and Fe. Nevertheless, addition of both the supplements (citric acid and phytase) simultaneously did not show any interaction for the digestibility of these minerals. Hence, it is concluded that PHY (1000 FTU/kg) and CA (2%) supplementation to sunflower meal based diet improved the nutrient digestibility in *Labeo rohita* juveniles.

**COMPARATIVE MICROBIOLOGICAL QUALITY ASSESSMENT IN ROHU (*LABEO
ROHITA*, HAMILTON) COLLECTED FROM DIFFERENT SOURCES**

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The quality assurance of fish meat is the reflection of microbiological, physical and chemical characteristics during the handling and fish storage. Food security is a complex issue where fish and fishery product are generally considered as high-risk commodity of pathogens natural toxins and other possible contaminants. The study was conducted to determine the bacterial load of *Labeo rohita* of collected from different sources. Some parameters i.e. (APC), Total Coliform (T.C), (F.C) and *E. coli* were determined. *Labeo rohita* was selected for experiment because of popular fish consumed in Punjab-Pakistan due to its good taste. Fish sample were collected from different water bodies of Punjab i.e. Fish Farm Complex, UVAS, Ravi Campus ,

Shahdra bridge, Lahore on River Ravi, and Bhaati Fish Market, Lahore. During this exploration or analysis result was analyzed that highest APC was calculated in River Ravi (2.33×10^6). In Ravi Campus fish farm, APC was 2.48×10^4 while Lowest APC was calculated in Bhaati Fish Market (1.86×10^6). The fecal coliform in River Ravi (210MPN/g), Ravi Campus Fish Farm (9.1MPN/g), Bhaati Fish Market (75 MPN/g) were observed. *E. coli* was observed in River Ravi and it showed +ve results while Fish Market have showed both +ve and -ve results. Lowest quantity of *E. coli* was observed in Ravi Campus fish form showed all -ve results. Fish of River Ravi was highly contaminated by microbes, because there were no any sanitary measures. Fish samples from fish market were less contaminated as compared to River Ravi. There were also chances of microbial attack due to rough handling and poor hygienic conditions. Fish from the Ravi Campus farms were no contamination and microbial attack.

CITRIC ACID AND PHYTASE SUPPLEMENTATIONS IMPROVE BODY PROXIMATE COMPOSITION AND MINERALIZATION IN *LABEO ROHITA* JUVENILES FED CORN-GLUTEN MEAL BASED DIET

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The present research work was conducted to study the synergistic effect of phytase and citric acid on body proximate analysis and mineralization of *Labeo rohita* juveniles fed corn-gluten meal based diet. Two citric acid levels (0% and 2%) and two phytase levels (0 FTU/kg and 1000 FTU/kg) were used to formulate four experimental diets namely CGM1 (0 FTU/kg phytase and 0% citric acid), CGM2 (0 FTU/kg phytase and 2% citric acid), CGM3 (1000 FTU/kg phytase and 0% citric acid) and CGM4 (1000 FTU/kg phytase and 2% citric acid). During feeding trial fish were fed twice a day at 4% of live wet weight. At the end of the experiment, five fish from each replicate was taken and proximal analysis and mineral profile of whole body of juveniles were estimated. Increased ($p < 0.05$) dry matter, crude protein and crude ash while decreased ($p < 0.05$) ether extract were observed in response to citric acid and phytase supplementations. However, maximum improvement in dry matter and crude protein were observed in CGM4 diet containing both (citric acid and phytase) the supplements. Similarly, improved body mineralization was also observed in juveniles fed on citric acid and phytase treated diets as compared to control diet.

USE OF BLACK PEPPER SEEDS AS GROWTH ENHANCER IN *LABEO ROHITA*

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Nutritionists are continuously working on the use of natural products as growth promoters for their respective animal feed. This is due to their least side effects which are imminent by the use of various chemicals. Fish diet also needs some breakthrough in finding such natural growth promoters. Black pepper seed have natural flavor that could be explored as a feed additive to

enhance growth, disease resistance and survival in fish. In the present study, we studied the effect of graded levels of black pepper seed (BPS) on growth performance, feed and nutrient utilization, body composition in *Labeo rohita*. The trial was conducted in four cemented rectangular tanks. Each tank was further sub-divided into three replicates having dimensions 2.896 x 0.762 x 0.914 (length x width x depth). There were 12 fish (*Labeo rohita*) stocked (with pre-recorded their morphometric) in every replicate with average weight of 9.9 grams. The fish feed was formulated, prepared, pelleted and dried having 30% CP level. It was further categorized into experimental feed on the basis of BPS *i.e.* 0.0, 0.5, 1.0, and 2.0 % of BPS as feed additive. The feed was dispensed twice a day, six days a week, and for 60 days. The Physico-chemical parameters were maintained and recorded on daily basis. Each fish was caught every fortnightly and its weight and length was recorded. Fish fed with 0.5% (BPS) exhibited significantly higher ($P<0.05$) SGR, feed conversion ratio (FCR), % weight gain. Fish fed 0.5% BPS had significantly higher ($P<0.05$) protein, fat, ash, fiber contents. Anyhow, no significant difference ($P>0.05$) was found in Phosphorus contents. Therefore, it is recommended to use black pepper seeds in feed as growth enhancer in fish at commercial scale @ 0.5% BPS.

**CHEMICAL COMPOSITION AND MINERAL PROFILE OF *CHANNA MARULIUS*,
WALLAGO ATTU AND *AORICHTHYS AOR SARWARI* INHIBITING RIVER INDUS IN
MIANWALI DISTRICT**

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Fish is a food of excellent nutritional value, providing high quality protein and a wide variety of vitamins and minerals, including vitamins A and D, phosphorus, magnesium, selenium, and iodine. The chemical and mineral composition of fish musculature reflects its nutritional value and physical condition, it fluctuates extensively from species to species and within the same species in different weight categories depending upon feeding habits. The carnivorous fish species are thought to be the most evident source of protein due to their feeding habits. This study was carried out to investigate the chemical composition and mineral profile of three carnivorous fish species (*Channa marulius*, *Wallago attu* and *Aorichthys aor sarwari*) inhabiting the River Indus in Mianwali District, which are indigenous and widely consumed in the study area. The study followed a 3 x 3 factorial arrangement by involving 3 fish species, each with 3 weight categories ($W1\leq 1.0$, $W2\leq 1.5$ and $W3\leq 2.0$ Kg). Fishing was performed with the help of local fishermen. Thirty samples of each fish species were selected from three weight categories on the basis of their routine weights at which they are caught and sold in the study area. The chemical composition of fish muscles was assessed by following the standard methods of Association of Official Analytical Chemists (AOAC). Selected minerals (Ca, K, Mg, Na, P, Cr, Fe, Mn, Ni, Zn, Hg and Pb) in fish muscles were analysed by a Varian Vista-MPX CCD simultaneous ICP-AES (Varian Inc, Australia) machine. Mineral concentrations were expressed as mg kg^{-1} wet weight. Minitab 16 software was used to test the main effects of fish species, weights and their interaction for each mineral at $P<0.05$. The mean mineral contents in fish muscles were also compared with the International permissible levels for food fish. It was investigated that crude protein contents were 76.16, 79.60 and 81.3% in *Channa marulius*, *wallago attu* and *Aorichthys aor sarwari*, respectively. The highest fat contents were reported in *Chana marulius* (17.20%) while ash was

reported as 4.46, 3.31 and 2.84% in *wallago attu*, *Channa marulius* and *Aorichthys aor sarwari*, respectively. The highest amount of carbohydrates was found in *Channa marulius* (3.34%) and lowest in *Aorichthys aor sarwari* (1.75%). Generally metal concentrations increased with increased weight of fish. Cd and Cu were not detected in any of the analysed fish species. Mineral concentrations in fish species were within the limits of international standards for food fish except Mn, Cr, Hg and Pb. In this study significant differences were observed in the chemical composition and mineral profile of fishes having same trophic level. Smaller fishes were high in protein contents and low in fats and minerals, so these should be preferred by the consumers. As toxic minerals exceeded the permissible levels of food fish so the consumption of these fish of study area is a point of concern for human health. This study suggests that it is time to take preventive measure to reduce the bio magnification of heavy metals in aquatic organisms.

FECUNDITY OF THE ASIAN STINGING CATFISH *HETEROPNEUSTES FOSSILIS* (BLOCH) FROM MARSHES OF THE RIVER CHENAB NEAR HEAD MARALA, PAKISTAN

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The fecundity of *Heteropneustes fossilis* was studied by gravimetric method. Ovaries were weighed to the nearest mg. Then a part (75–100 mg) was cut from their mid points, weighed and processed for separating and counting the eggs. The mean number of eggs was 11716.04 for a fish with mean total length 18.8 cm, mean total weight of 78.04 g and mean relative fecundity 143.18 ova/g b.w. Absolute fecundity showed almost linear relationship to total length and total weight. The relative fecundity varied considerably. Relation of fecundity to size revealed that the larger fish were more fecund than the smaller ones.

AGGLOMERATIVE HIERARCHICAL CLUSTERING BASED ON MORPHOMETRIC PARAMETERS OF THE POPULATIONS OF *LABEO ROHITA*

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Labeo rohita populations from five geographical locations from the hatchery and Riverine system of Punjab-Pakistan were studied for the clustering on the basis of similarities and differences based on morphometric parameters within the species. Agglomerative Hierarchical

Clustering (AHC) was done by using Pearson Correlation Coefficient and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) as Agglomeration method by XLSTAT 2012 version 1.02. A dendrogram with the data on the morphometrics of the representative samples of each site divided the populations of *Labeo rohita* in to five major clusters or classes. The variance decomposition for the optimal classification values remained as, 19.24% for within class variation while 80.76% for the between class differences. The representative central objects of the each class, the distances between the class centroids and also distance between the central objects of the classes were generated by the analysis. A measurable distinction between the classes of the populations of the *Labeo rohita* was indicated in this study which determined the impacts of changing environment and other possible factors influencing the variation level among the populations of the same species.

ROLE OF GENETIC MARKERS IN FISHERIES AND AQUACULTURE

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DNA markers have been reliably utilized to investigate different aspects of fisheries and aquaculture. DNA markers innovations have been played an effective role in evolutionary biology, preservation genetics, molecular ecology and population genetics. Popular genetic markers in the aquaculture community include allozymes, mitochondrial DNA, RFLP, RAPD, AFLP, microsatellite and SNP markers. DNA markers have made rapid improvement in aquaculture including investigation of inbreeding and genetic variability, species and strain recognition, parentage assignments and the construction of high-resolution genetic linkage maps. Our goal is to encourage researchers to evaluate the molecular markers. They need to deploy and shift their thinking away from analyses of stock structure towards more aggressive pursuit of questions related to genome structure and function.

ZOONOSIS AND WILD FISHERIES RESOURCES: *AEROMONAS SOBRIA* AS THE CAUSATIVE AGENT OF HEMORRHAGIC SEPTICEMIA IN WILD FISHERY RESOURCES, PAKISTAN

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Motile *Aeromonas* Septicemia (MAS) is responsible for fish diseases and millions of dollars of economic losses. Among *Aeromonas* species, *Aeromonas sobria* is supposed to be the major causative agent of hemorrhagic septicemia in wild and farm fish resources. Keeping in view the economic importance of *A. sobria* as the causative agent of fish diseases, the present study was designed for the isolation, characterization and antibiotic sensitivity of the causative agent of hemorrhagic septicemia in wild fisheries resources of Potohar region, Pakistan. Eighty samples of

fish from rivers, lakes and dams with clinical signs of hemorrhagic septicemia were collected during winter season (2010-2011). Characterization of isolates was completed by biochemical and Analytical Profile Index (API 20NE) tests. All sixty four isolates were identified as *A. sobria*. Antibiotic sensitivity of *A. sobria* was determined against 10 antibiotics. *A. sobria* was found resistant to chloramphenicol, cotrimoxazole, norfloxacin, ofloxacin and gentamycin but sensitive to amoxicillin, azithromycin, ceftiofur, erythromycin and penicillin. Present study provides the first evidence of presence of antibiotic resistant bacteria in Pakistani wild fisheries resources affected with hemorrhagic septicemia. These antibiotic resistant bacterial strains can be transmitted to humans by these aquatic products and by-products. Immediate attention should be given for the affective control of this disease in wild fisheries resources to minimize the risk of transmission of diseases in human and fresh water resources of Pakistan.

COMPARISON ON BIOMETRICS, SIZE AT SEXUAL MATURITY AND NATURAL MORTALITY OF TWO ENDANGERED LOACH (BOTIA DARIO AND B. LOHACHATA) FROM THE GANGES RIVER (NW BANGLADESH)

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The endangered loach *Botia dario* (Hamilton, 1822) and *B. lohachata* (Chaudhuri, 1912) are important riverine freshwater fish species of the family Cyprinidae and distributed in the Indian sub-continent including Bangladesh, Bhutan, India, Nepal and Pakistan. This study compares the biometric relationships including length-weight relationship (LWRs), length-length relationship (LLRs), condition factors (allometric condition, K_A ; Fultons condition, K_F ; relative condition, K_R), relative weight (W_R), form factor ($a_{3,0}$), size at sexual maturity (L_m) and natural mortality (M_w) between two endangered loach *B. dario* and *B. lohachata* in the Ganges River, northwestern Bangladesh. Samples (*B. dario* = 142 and *B. lohachata* = 110) were collected from the Ganges River using different types of fishing gears during July 2013 to December 2014. Individual lengths including total length (TL), fork length (FL) and standard length (SL) were measured to the nearest 0.01 cm, and total body weight (BW) was measured using an electronic balance with 0.01 g accuracy. The LWR was calculated using the equation: $W = a \times L^b$, where W is the body weight (g) and L is the total length (cm). The TL was ranged from 5.59-12.87 cm (BW= 3.40-27.87 g) for *B. dario* and 3.70-6.80 cm (BW= 0.71-2.88 g) for *B. lohachata*. The TL of *B. dario* (mean \pm SD = 7.91 \pm 1.78) was significantly higher than that of *B. lohachata* (mean \pm SD = 5.32 \pm 0.62) ($P < 0.001$). Also, BW was significantly different between the species ($P < 0.001$). The calculated allometric coefficient (b) indicated negative allometric growth in both species ($b < 3.00$, $P < 0.01$) in the Ganges River. The slopes (b) of LWRs were not significantly difference between the species ($F = 0.089$, $p = 0.765$), but the intercepts ($\ln a$) showed significant differences ($F = 4768.94$, $P < 0.001$). All LWRs were highly significant ($P < 0.01$), with r^2 values ≥ 0.950 . In addition, all LLRs were highly significant ($P < 0.001$), with r^2 values ≥ 0.970 for both species. All the condition factors (K_A , K_F and K_R) were significantly different between two loaches ($P < 0.001$).

The W_R was not significantly different from 100 for *B. dario* (median = 99.86, $p=0.527$) and *B. lohachata* (median = 100.30, $P = 0.609$), indicate the balance populations in presence of prey and predator in the Ganges River. The $a_{3,0}$ of *B. dario* and *B. lohachata* were 0.0104 and 0.0052, respectively. The L_m for *B. dario* and *B. lohachata* were 7.90 cm ($CL_{50\%} = 6.30 - 9.90$ cm) and 4.40 cm ($CL_{50\%} = 3.60 - 5.40$ cm), respectively. In addition the M_W for *B. dario* and *B. lohachata* was 1.77 and 1.96 per year. The results of this study would be very effective for fisheries managers, fish biologists and conservationists to initiate early management strategies and regulations for the sustainable conservation of this two threatened species in the Ganges River and surrounding ecosystem.

EFFECT OF VARIOUS FISH PROCESSING TECHNIQUES ON THE NUTRITIONAL COMPOSITION OF *PANGASIU* *PANGASIU* FISH STORED UNDER ROOM ENVIRONMENT

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The effects of three different traditional processing methods (smoking, frying, and salting) on Proximate composition of *Pangasius pangasius* fish stored under ambient room condition were determined. *Pangasius* fish fillets were procured from Hyperstar Cash and Carry Center, Lahore to determine the proximate properties of the raw fish, smoked fish, fried fish, salted fish and sun drying before and after storage at room conditions. The nutritional parameters studied were moisture, crude protein, crude fat, ash, crude fiber, and nitrogen-free extract contents. The results indicated that traditional processing methods have significant effects on the proximate composition of *pangasius* fish. Mean moisture (5.94, 2.90, 5.32, 5.09, 5.22%) crude protein (44.92, 50.26, 57.30, 52.27, 55.93%) crude fat (13.44, 11.71, 12.22, 9.12, 9.38%), ash (24.37, 26.49, 22.78, 15.49, 18.33%) dry matter (94.06, 97.10, 94.68, 94.91, 94.78%) of raw fish, smoked fish, fried fish, salted fish and sun drying fish were respectively. The changes in all the parameters after processing were found significant at ($P<0.05$) for the four traditional processing techniques. During the storage under room conditions for 15 days, changes in all studied parameters were significant ($P<0.05$).

RELATIVE DIVERSITY AND THREATS TO COMMERCIALY IMPORTANT FISHES OF THE RIVER RAVI, PAKISTAN

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The present study was conducted for consecutive three years (2011–2013) to assess the relative diversity of commercially important fish fauna of the river Ravi. The fishes were sampled

from six sites of anthropogenically affected segment of the Ravi flowing along the Jhok Reserve Forest. The diversity, evenness and richness of twenty two native and exotic fish species belonging to nine families were calculated. Statistical analysis revealed that diversity indices were decreased gradually. In addition, stressed populations of *Xenentodon cancila*, *Channa marulius*, *Macrognathus pancalus* and *Bagarius bagarius* were observed among the rich populations of *Mystus cavasius*, *Channa punctata*, *Oreochromis aureus*, *Labeo rohita*, *Catla catla* and *Wallago attu*. The study necessitates the implication of proper conservatory measures and therefore, suggests future studies on pollution impacts and breeding biology of the said stressed fish species of the Ravi.

LIFE HISTORY TRAITS OF ENDANGERED HALFBEAK *DERMOGENYS PUSILLA* IN THE GANGES RIVER, NORTHWESTERN BANGLADESH

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The wrestling halfbeak *Dermogenys pusilla* (Khul & van Hasselt, 1823) is nutritionally important fish found in fresh-, brackish- and marine-waters. This fish is distributed in Asian countries including Bangladesh, India, Indonesia, Myanmar and Thailand. But the natural populations are decreasing due to over exploitation, habitat loss and other ecological changes to their habitat and categorized as endangered in Bangladesh. The present study describes the first complete and comprehensive description on life-history traits of *D. pusilla* including sex ratio, length-frequency distributions (LFDs), length-weight relationships (LWRs), length-length relationships (LLRs), condition factors (allometric, K_A ; Fulton's, K_F ; relative, K_R), relative weight (W_R), form factor ($a_{3.0}$), size at sexual maturity (L_m) and fecundity (F_T) in the Ganges River, northwestern Bangladesh. Samples were collected using different traditional fishing gears during January 2013 to December 2014. In addition, a total of 36 mature female were randomly selected for the estimation of fecundity. For each individual, a total of sixteen lengths were measured to the nearest 0.01 cm and total body weight (BW) was measured with 0.01 g accuracy. A total of 75 individuals were hardly collected, where 46.67% were male and 53.33% were female. The overall sex ratio (male: female = 1:14) did not differ significantly from the expected ratio 1:1 ($df = 1$, $P > 0.05$). The TL was ranged from 6.60-16.10 cm (BW = 1.20-10.86 g) for males and 7.5-15.5 cm (= 1.60- BW 10.50 g) for females. The BW showed significant difference between the sexes ($P < 0.001$). All LWRs of males and females were highly significant with r^2 values ≥ 0.943 . There is no significant difference for LLRs (16 lengths) between male and female populations, but relationships were highly significant with $r^2 > 0.940$. The condition factors including (K_A , K_F and K_R) were significantly different between the sexes ($P < 0.001$). The W_R shows no significant difference from 100 for male and female ($p=0.405$) indicating a balance population in the presence of prey and predator in the Ganges River. Form factor ($a_{3.0}$) was 0.0025 and 0.0024 for male and female respectively. In addition, the L_m was 9.70 cm ($CL_{50\%} = 7.72$ -12.20 cm) in TL for males and 9.40 cm ($CL_{50\%} = 7.50$ -1.80) in TL for females. Total fecundity was varied

from 620-1544 with a mean value 1125 ± 248 (BW = 4.10-10.50 g) in our study. The results of this study would be very effective for the population assessment in the Ganges River ecosystem and would be useful for fisheries managers, biologists and conservationists to initiate early management strategies and regulations for the sustainable conservation of the remaining stocks of this endangered halfbeak in Bangladesh and neighboring countries.

SPECIES COMPOSITION AND SEASONAL ABUNDANCE OF FINFISHES IN CREEK SYSTEMS OF INDUS DELTA, SINDH, PAKISTAN

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This study was conducted to evaluate the finfish species composition and seasonal abundance in the creek areas of Indus Delta, Sindh, Pakistan. Monthly fish samples and selected environmental parameters were collected during February 2010 to May 2011 at two selected locations of Ketu Bandar creek system. The information revealed eighty-four finfish species, corresponding to 2274 individuals in 60 genera and 29 families weighing 217kg. Most speciose family at the study sites were Mugilidae (9 species) followed by Sciaenidae (8 species) and Ariidae (7 species). Two families viz., Clupeidae and Sparidae, each consisted of 5 species. Each of the three families viz., Cynoglossidae, Engraulidae and Platycephalidae, had 4 species; rest of the 21 families were represented by 1-3 species. There was no significant correlation observed among the environmental parameters except between temperature and dissolved oxygen (Pearson correlation 0.52, $p < 0.03$, $r^2 = 0.27$). The Shannon-weiner diversity index (H) and evenness (e^H/S) was analysed for month-wise collection and two sampling locations. The diversity of collected fishes was generally much higher in the Wide Creek as compared to the Small Creek area, except in April 2011. The maximum evenness of the sampling months was found in December 2010, as recorded number of species was lowest and the specimens were evenly distributed among the species. Generally, uniform evenness values were encountered in the Small Creek as oppose to fluctuating values in the Wide Creek area. The study concluded that the Indus Delta creek system is a highly productive zone in terms of high fish diversity and biomass. Threats like indiscriminate fishing and low availability of freshwater appear to have profound affect on Indus delta ecosystem.

THE EFFECT OF DIFFERENT STOCKING DENSITIES ON GROWTH, PRODUCTION AND SURVIVAL RATE OF PANGAS (*PANGASIU HYPOPHthalmus*) FISH IN CEMENTED TANKS AT FISH HATCHERY CHILYA THATTA, SINDH-PAKISTAN.

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The effect of stocking density on growth performance, production and survival of Pangas, *Pangasius hypophthalmus* were evaluated in cemented tanks. Fry of Pangas (1.52 ± 0.03 cm in length and 1.08 ± 0.02 g in weight) respectively were stocked into cemented tanks measuring 15 x

6 x 3 ft. Three treatments with two replicates were used: T₁-100; T₂-150 and T₃-200 fry/ tank. Pangas fry were fed twice daily with formulated feed 35 % protein at 10%, 5%, and 3% body weight for the first, second, and third month, respectively. After 90 days, the Highest growth performances (determined in terms of average weight) were recorded in T₁ (27.5±2.5 g) and T₂ (22.4±2.8 g) while T₃ (18.2±3.5g) recorded the smallest growth. Production differed significantly among treatments (P<0.05). Feed conversion ratio (FCR) of 1.0, 1.02 and 1.05 in T₁, T₂ and T₃, respectively were not significantly different (P>0.05). Survival was significantly different among treatments (P<0.01). Highest survival (100%) was attained in T₁ with lower stocking density, followed by T₂ (96%) and T₃ (90%). Survival was greatly influenced by the stocking densities in all treatments. The water quality parameters and their monthly fluctuations recorded throughout the study period were found within the suitable ranges for the fish culture such as temperature 27.0 to 28.7 °C, dissolved oxygen 5.7 to 6.2 mg/lit, pH 7.2 to 7.5, Ammonia from 0.45 to 0.51mg/L, Hardness 105 to 110 ppm and Nitrite 0.152 to 0.161 mg/L.

**EFFECT OF DIFFERENT FEEDS ON GROWTH AND SURVIVAL OF SNAKEHEAD
CHANNA MARULUS IN PLASTIC TANKS**

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The present study was conducted over a period of 48 days during October to November 2014 to observe the effect of different feeds on growth and survival of snakehead (*Channa marulius*) reared in plastic tanks having 40 liter water capacity each at the laboratory of Fresh Water Biology Department, University of Sindh. Juveniles mean length and weight (10.63 ±0.98 cm, 9.03±1.4g), stocked after acclimatizing respectively. Three treatments with two replicates were used: T1-Squid feed; T2- Tubifex feed and T3-Pellet feed. Fry were fed twice a daily with 5% of total biomass. After 48 days, the highest growth were determined in T1 (15 ± 0.33 cm and 15.8±0.0 g length and weight), T3 (14.13±0.75 cm and 13.35±0.66 g length and weight) while in T2 lowest growth (11.81 ± 0.95 cm and 10.47±1.18 g length and weight) was recorded. Average daily weight gain (ADWG) was recorded T1-0.35, T2 – 0.22 and 0.28 in T3 which is significantly different among treatment (P>0.05). Specific growth rate (SGR) was 1.16, 0.96 and 1.11 in T1, T2 and T3 respectively which is significantly different among treatments (P<0.01). Condition factor was T1- 0.61, T2- 0.72 and T3-0.62 which is significantly different among treatments (P<0.01). Feed conversion ratio of 0.4, 0.4 and 0.4 in T1, T2 and T3, respectively not significantly different (P>0.05). Survival was significantly different among treatments (P<0.01). Highest survival (90%) was attained in T1 feed on squid feed followed by T2 (50%) and T3 (80%). Water quality parameters recorded throughout the study period were found within the ranges for fish culture such as temperature 25.2 ± 0.20 °C, dissolved oxygen 5.2 ± 0.11mg/L, pH 6.9 ± 0.15, ammonia from 0.48 ± 0.02 1mg/L and hardness 118±2.4 mg/L. Highest growth achieved on squid feed and on pellet feed growth is also satisfactory on the basis of these results, it is recommend that in mass culture of this species on pellet feed give more benefit and reduced the coast.

EFFECT OF DIFFERENT PROTEIN LEVELS OF ARTIFICIAL FEED ON GROWTH, FEED CONVERSION AND BODY COMPOSITION IN GMT TILAPIA FRY**Daniel Masood, Noor Khan, Sadaf Aman, Usman Atique, M.Asif Raza, Mirza Abrar Manzoor and Mati Ullah Khan***Department of Fisheries & Aquaculture, University of Veterinary and Animal Sciences, Lahore, Pakistan*

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Tilapia culture across globe is taking its firm roots in the present aquaculture scenario. In the present study a 120 days feeding trial was conducted to determine the effect of different protein levels of locally formulated artificial feed on the growth performance and survival of GMT strain reared in HAPAS made from Bolton cloth. Three types of feeds designated as T1 (30% CP), T2 (35% CP) and T3 (38% CP) were manually dispensed twice a day for 5 days a week. Results of the study indicated significant differences of growth among T1 and T3. Non-significant differences were observed among T1 and T2, and T2 and T3 of SGR as it was observed as T1 0.40 ± 0.04^a , T2 0.42 ± 0.02^a , T3 0.47 ± 0.01^a . FCR was 2.87 ± 0.28^a , 2.73 ± 0.12^a , 2.56 ± 0.03^a and were also found to be non-significant. Non-significant RGR values were observed as 1.59 ± 0.73^a , 2.26 ± 0.17^a and 2.67 ± 0.01^a . Proximate analysis of fish whole body reveals that protein, fats, fiber and phosphorous shows non-significant difference. In case of moisture, T3 shows significant difference than T1 and T2. 4.43 ± 1.90^b , 5.13 ± 0.09^b , 9.17 ± 0.81^a and it was observed that ash in T2 and T3 shows significant difference while T1 showed non-significant difference with both the treatments of T2 and T3 like 10.68 ± 0.03^{ab} , 8.6 ± 0.02^b , 11.31 ± 1.1^a respectively. It was, therefore, concluded that 38% CP feed when given to GMT, shows significantly higher growth increments in GMT.

FOOD AND FEEDING HABIT OF CAT FISH (*SPERATA SEENGHALA*) COLLECTED FROM RIVER INDUS OF DISTRICT SUKKAR, PAKISTAN.**Rukhsana Bibi,¹ Mussarat bi bi,¹ Hina gul Gharsheen,¹ Zubia Masood¹ and MussatUl-Ain²**¹*Department of Zoology, Sardar Bahadur Khan Women University, Quetta, Balochistan*²*Department of Zoology, University of Karachi-75270.*

The present study was carried out on the food and feeding habit of cat fish (*Sperata seenghala*) that was collected from the Sukkar district of Pakistan. A total of 30 sample of the cat fish were collected from the landing sites of river Indus during the period from August 2014 to December 2014. In this study, the gut was removed and length of the stomach was measured. The length of stomach was 7.5cm. While the size of stomach was greater than the size of intestine. The content of both stomach and intestine was removed and preserved in 4% formalin. The content were analyzed under the microscope which was consist of small fishes, their scales, eggs, crustaceans, molluscs shells, but the crustaceans were dominated in the whole content mostly in the stomach. As fishes can be classified into different groups on the basis of their feeding habits i.e., the limnivores, carnivores, herbivores and detritivores, therefore, the result of the present investigation revealed that catfish can be placed in carnivore group according to its feeding habits or diet.

ROLE OF FISHERIES IN INCOME GENERATION IN POOR POPULATION OF SINDH

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The purpose of this paper is to examine the income generation possibilities of poor population of Sindh, who are engaged in Fisheries industry. Since in coastal areas of Sindh the living population is very poor and even lives below the poverty line. Therefore, the role of Fisheries Industry is many fold in this context because the role of Fisheries are very imperative and vital in order to generate income opportunities to the masses of population who are engaged with this industry. Our coastal belt is 1075 km long and is ready to serve for job for everyone, where Sindh Province is containing half of it approximately. Therefore, most of the poor rushed to coastal belt and inland watercourses. Human settlements are very old practice across the water resources. The Pakistani Fisheries is also accommodating rich and industrial personnel. The Fisheries Industry is not required any type of mandatory registration and investment. There are 35 segments of this industry and every segment is having very large opportunities for poor. It is also observed that this industry is labor-intensive industry. Poor can increase their income in many fold by increasing their efforts. Every segment is having 10-15 portions. Like the segment of aquarium Fisheries is having 16 portions; 1) Aquarium fish collecting Fishermen; 2) Middlemen; 3) Short-term Stockers; 4) Long-term stockers; 5) Accessories Retailers; 6) accessories Wholesalers; 7) Accessories Khaipias; 8) Exporters; 9), Importers; 10) Medicine Maker; 11) Food Maker; 12) Glass Seller; 13) Aquarium Making product Seller; 14) Carpenters; 15) Toys Makers; 16) Electricians; 17) Researchers; 18) Accessories Repairers. All of these 18 portions are for fresh water aquariums fishes. Similarly, 16 portions are also present for Marine trade. 35 segments will definitely contain 525 portions. It is found that the title of every portion reveals that it is containing income generation probabilities for poor of Sindh Province. So, in this way the Fisheries industry not only providing new job opportunities in this area but also plays a vital role to reduce the poverty. The results of the study concluded that the Fisheries' industry across the coastal belt of Sind Province provides many opportunities of employment generation to the poor people of the area, besides that Fisheries' industry also help the government in order to reduce the poverty level in the coastal areas of Sindh Province and inland fisheries.

FISH LANDING AND HEALTH STATUS OF FISHERMEN'S FAMILIES IN KARACHI

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Fish landing in Karachi is not increasing with the proportion to increase in the number of fishing gears and fishing boats. A part from other reasons the health of Fishermen's families are also very important in this regards. Some other studies also report that Fishermen's families are having one sick member in every month. Therefore, this study found that the sick member compel angler to take off from the job for 4 days in a month. These 4 days off is also fall impact on fish landing in many ways? Female family members are largely found malnourishment. Out of sample

of 300 women, 250 found with HB of less than 10. In turn, male members will have participated in the home affairs. That is also a one cause of losing concentration of Fishermen from work.

**COMPARISON ON MORPHOMETRIC RELATIONSHIPS OF THE POOL BARB
PUNTIUS SOPHORE IN TWO DIFFERENT ECOSYSTEMS IN THE NORTHWESTERN
BANGLADESH**

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The Pool barb, *Puntius sophore* is one of the small indigenous fish species of the family Cyprinidae, which is an excellent source of macro and micro-nutrients that can play an important role in the elimination of malnutrition problem in Bangladesh. It inhabits in fresh water and brackish water ecosystem and distributed in Asian countries including Afghanistan, Bangladesh, China, India, Myanmar, Nepal, India and Pakistan. The present study describes the comparative study on length-frequency distributions (LFDs), length-weight relationships (LWRs), length-length relationships (LLRs), condition factors (Allometric, K_A ; Fulton's, K_F ; Relative condition, K_R ; Relative weight, W_R) and form factor ($a_{3,0}$) of *P. sophore* in two different ecosystems such as Ganges River, and a L-shaped earthen Pond, northwestern Bangladesh. Sampling was conducted using traditional fishing gears including cast net, square lift net and conical trap during July 2013 to December 2014. All the lengths including total length (TL), fork length (FL), and standard length (SL) were measured to the nearest 0.01 cm using digital slide calipers, and total body weight (BW) was measured using an electronic balance with 0.001 g accuracy. The LWR was calculated using the expression: $W = aL^b$, where the W is the body weight (BW in g), L the total length (TL in cm), a and b are the parameters of the regression. Fulton's condition factor (K_F) was calculated using the equation: $K_F = 100 \times (W/L^3)$, where W is the total body weight (BW, g) and L is the total length (TL, cm). A total of 185 individuals (from Ganges River = 135 and from L-shaped Pond = 50) ranging from 5.20 cm to 10.00 cm TL and 1.86 g to 15.88 g BW were analyzed in this study. The results showed significant differences in the LFDs between River and Pond ($P < 0.001$). In addition, BW of Pond was significantly higher ($P < 0.001$) than that for River. The Fulton's condition factor (K_F) showed significant variations between populations ($P < 0.001$), but the relative weight (WR) showed no significant variations ($P = 0.959$) during the study. However, W_R was significantly different from 100 for both populations ($P < 0.01$), indicating an imbalance habitat with food availability relative to the presence of predators for this pool barb in the Ganges River and L-shaped pond. The calculated form factor ($a_{3,0}$) was 0.0163, and 0.0229 in the Ganges River and earthen Pond for *P. sophore* suggesting that, this fish can be classified as relatively elongate which is characteristic of many riverine fishes. To the best of the knowledge, this study reports the first complete and comprehensive description of all these parameters of *P. sophore* with consideration two different ecosystems in Bangladesh, which would be very effective for sustainable conservation of the remaining stocks of this species within the Ganges River and pond ecosystem.

EXPLORATION OF FISHERIES TRADE BETWEEN PAKISTAN AND LITHUANIA

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The purpose of this paper is to identify and evaluate the opportunities of fisheries trade between Pakistan and Lithuania. It is identified that 8 species of edible fish, 3 species of crabs, and 3 species of shrimps of Pakistan is having export demand. On the contrary, the import from Lithuania is very costly due to air freight and high price. The price evaluation of above 14 species was compared with Pakistani relative prices and it was regressed with Lithuanian relative prices. It is found that there is 80% profit margin for Pakistani exporters. Moreover, the 14 Pakistani species of sea animals are not available in Lithuania. The sample size was 50 respondents (fish traders) and the survey was conducted from the respondents of both the countries likewise. The model run for the research paper was regression analysis. With the respected search, it was found that with the proceeded survey the result came out positive i.e. 80% profit margin. The findings from this research paper many come handy in the fishing industry. The results of the study concluded that there are many opportunities of trade in Fisheries industry between Pakistan and Lithuania. The trade between both the Countries is very much positive and profitable. Some fisheries products of Lithuania can also be bartered with Pakistan.

MODELING THE GROWTH OF CRITICALLY ENDANGERED *CLUPISOMA GARUA* IN THE GANGES RIVER, (NW BANGLADESH) USING MULTIPLE FUNCTIONS

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The Garua Bachcha, *Clupisoma garua* (Hamilton, 1822), a freshwater fish, widely distributed in the Indian Sub-continent including Bangladesh, India, Nepal and Pakistan. This fish is locally known as Ghaura, Muribacha in Bangladesh and Chel-lee, Dhon-gu-nu, Ka-raad in Pakistan. It is commercially important for both subsistence and artisanal fisheries in Bangladesh. However, wild populations of *C. garua* are declining rapidly due to overfishing, habitat loss, and ecological changes to its natural habitat and subsequently, it is categorized as critically endangered in Bangladeshi waters. The growth pattern of male and female *C. garua* was estimated separately using the monthly length-frequency distributions through multiple functions. In total, 1200 specimens ranged from 3.70 cm to 23.55 cm in total length (TL) were collected from the Ganges River, northwestern (NW) Bangladesh from August 2011 to July 2012. Birth-date was determined by means of monthly mean gonadosomatic index (GSI) and was assigned to the month when the peak of mean GSI occurred. The age of each cohort was estimated in months from the assigned birth-date to each sampling month. Growth patterns for both males and females were modeled by fitting three different equations [e.g., von Bertalanffy equation, $L_t = L_\infty(1 - \exp(-k(t - t_0)))$];

Gompertz equation, $L_t = L_\infty \exp(-\exp(-k(t - t_0)))$; and Robertson equation, $L_t = L_\infty (1 + \exp(-k(t - t_0)))$ between the mean total lengths (TL) and corresponding ages. The best fitting model among these three equations was selected on the basis of Akaike's information criterion (AIC). TL-frequency distribution demonstrated significant difference ($P < 0.001$) between sexes. The sex ratio was found to be significantly differed from the expected value of 1:1 in favor of males. A birth date was assigned to the month, when the peak mean GSI occurred which was found in April for *C. garua* during the present study. Results revealed that the growth of *C. garua* was best described by the von Bertalanffy model as $L_t = 29.69 (1 - \exp(-0.157(t - 1.718)))$ for males and $L_t = 31.71 (1 - \exp(-0.161(t - 2.274)))$ for females based on the AIC, where L_t is the total length (cm) at age t (month). Females grew faster than males, and their absolute growth was larger than that of males at any age. The von Bertalanffy growth model, in terms of body weight (BW), provided equations as $W_t = 136.76 (1 - \exp(-0.021(t - 4.186)))^{2.699}$ for males and $W_t = 150.02 (1 - \exp(-0.033(t + 0.915)))^{2.637}$ for females, where W_t is the body weight (g) at age t (month). Furthermore, the calculated growth-performance indices were $\phi' = \log_{10} (0.157 + 2 \log_{10} (29.69))$ for males and $\phi' = \log_{10} (0.161 + 2 \log_{10} (31.71))$ for females. The overall growth-performance index (ϕ') was higher for females (2.21) than males (2.14). In addition, the longevity of male *C. garua* was estimated as 19.11 months and that for female as 18.64 months. The size at sexual maturity of male and female *C. garua* was estimated as 13.40 cm TL and 13.70 cm TL, respectively. Parameters of length-weight relationships between TL (cm) and BW (g) showed negative allometric growth in both males and females. These growth parameters would be useful for stock assessment of this species in the Ganges River. In addition, the results of this study would be an effective tool for fishery biologists, managers and conservationists to initiate early management strategies and regulations for the sustainable conservation of the remaining stocks of this species in the Ganges River and surrounding ecosystems.

EFFECT OF STOCKING DENSITY ON ADULT TURBOT IS GROWTH, PHYSIOLOGY AND WATER QUALITY IN RECIRCULATING AQUACULTURE SYSTEM

Gao Chen-Rin and Lei Ji-Lin

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To explore the effect of density on the growth performance, physiology of adult turbot and water quality in the recirculating aquaculture systems, we divided the turbot (average weight: 589.9 ± 44.65 g) into three groups and reared the fish at three stocking densities: A low density (14.30 kg/m^2), B medium density (20.49 kg/m^2); C high density (31.32 kg/m^2). The culture duration had been for 120 days. At the same time, the parameters including weight distributing, feed conversion ratio, blood lysozyme level of turbot and TAN, $\text{NO}_2^- \text{N}$, COD of the water were also detected. The results showed that the final stocking densities of A, B and C reached 30.09 kg/m^2 , 41.30 kg/m^2 , 60.07 kg/m^2 respectively and the survival rate of each experiment group reached at 95% or above. It was also found that stocking density did not significantly affect the survival rate of turbot. The turbot in high density showed lower growth rate at the beginning of the research. The uniformity of weight distributing within experiment groups decreased with increased density. When the experiment has been done for 5 days, the bacteriolytic enzyme levels of high density group of turbot were elevated, but decreased after 20 days and significantly lower than that

of the low-density group after 40 days. Stocking density had significant influence on feed conversion ratio, and the turbot in high density showed higher feed conversion ratio. At the fifth day after crowding blood lysozyme level of the turbot in group C increased little, but it decreased markedly after 40 days in comparison with control group ($P < 0.01$). During the research, the recirculating aquaculture systems were stable and stocking density had significant influence on the TAN, NO_2^- , N , COD ($P < 0.05$), however, these several parameters of the high-density group did not exceed the highest concentration level acquired in water quality standards. With the rise of stocking density, various water quality parameters were significantly higher. Based on these experiment results, this experiment will provide references for the land-based recirculation systems of turbot culture.

STUDY OF SCALES OF MUGIL FISH *MUGIL CEPHALUS* BY SCANNING ELECTRON MICROSCOPY.

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Scales have numerous hidden information that can be helpful for fish identification such as circuli, annuli, radii. A traditional approach has been made for studying scales of *Mugil cephalus*. In the present study 10 small fish samples of Mugilidae family species *Mugil cephalus* were sampled and purchased from market of joint road Quetta. *Mugil cephalus* is important food fish species in the mullet family mugilidae. Fish ranges in length from 12½ cm to 17½ cm. The scale sample were taken from following region of fish such as HS (head scales), CS (caudal scales) and TRS (transfer row scales). Scales have been subjected to scanning electron microscopy (SEM) for detail structure.

EFFECT OF GARLIC EXTRACT ON THE SHELF LIFE, PROXIMATE ANALYSIS AND ORGANOLEPTIC OF SMOKED COMMON CARP (*CYPRINUS CARPIO*)

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Fish is an extremely perishable food commodity, that subject to post harvest losses ranging from bacterial and autolytic spoilage resultantly cause fish to lose its organoleptic qualities, and generally unacceptable for human consumption. It is this perishability of fish that makes it to be processed into fish based products, such as smoked, canned fish, fishmeal and fish burgers. The study was conducted to assess the effect of garlic extract on the shelf life and nutritional value of smoked Common carp (*Cyprinus carpio*) stored at 30°C was determined for the period of month. The fish was purchased from the Metro Cash and Carry Center, Lahore. Biochemical analysis, organoleptic study and microbiological were determined to find out the quality changes, and shelf

life of experimental sample. Proximate composition, TBA and TMA were found statistically significant ($P \leq 0.05$), in the traditional smoked fish *Cyprinus carpio* during the storage period. Garlic extracted fish samples have increased percentage of crude protein (51.27%), crude fat (14.07%), ash contents (20.66%), moisture (6.20%) and the dry matter (94.30%) while the control samples have less percentage of values crude protein (49.26%), crude fat (14.15%), ash contents (14.05%), moisture (5.63%) and the dry matter (93.80%). The lowest TBA (0.10 mg MDA/kg), and TMA (2.52 mg N/100 g) values were recorded in 10% garlic extract samples, while the highest TBA (2.02 mg MDA/kg) and TMA (16.02 mg N/100 g) occurred in the control samples. The organoleptic study results showed 10% garlic extracted samples had the best overall acceptability as compared to the control fish samples after 15 days of storage.

DIVERSITY AND DISTRIBUTION OF FISH FAUNA OF INDUS RIVER AT TAUNSA BARRAGE, SOUTHERN PUNJAB, PAKISTAN

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Present study was conducted to determine the diversity of fish fauna of Indus River at Taunsa Barrage, Southern Punjab Pakistan. For this purpose sampling was conducted from October, 2013 to August, 2014 at eight sampling sites to assess the fish diversity and distribution pattern. A total of 2249 fish specimens were collected by using variety of fishnets and identified to species level utilizing standard key on the basis of morphometric characters. Total specimens were represented by seventy species belonging to 43 genera and 17 families. Of the recorded species, thirty two were commercially important. Data analysis revealed that river contain high diversity of fish (species richness 70, Shannon's index=3.66; Simpson-D=0.96; Evenness = 0.55). The values of following indices as Chao-1 (70.75), Chao 2 (70.8), Jackknife 1 (72.9), Jackknife 2 (71.9) and Bootstrap (70.17) showed complete sampling of the area as number of missing species was very few. Two new species *Gonialosa manmina* and *Rita macracanthus* were also recorded from collected specimens. The study further indicates that the population of many endemic and commercially important fish species has declined while three exotic species (*Oreochromis mossambicus*, *Oreochromis aureus* and *Oreochromis niloticus*) has increased in the sampling area. Similarly, the abundance of exotic common carp (*Cyprinus carpio*) is also high in the Indus River.

EFFECT OF STOCKING DENSITY ON ADULT TURBOT IS GROWTH, PHYSIOLOGY AND WATER QUALITY IN RECIRCULATING AQUACULTURE SYSTEM

Gao Chen-Rin and Lei Ji-Lin

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To explore the effect of density on the growth performance, physiology of adult turbot and water quality in the recirculating aquaculture systems, we divided the turbot (average weight: 589.9 ± 44.65 g) into three groups and reared the fish at three stocking densities: A low

density(14.30kg/m²) B medium density (20.49kg/m²)° C high density(31.32kg/m²). The culture duration had been for 120 days. At the same time, the parameters including weight distributing , feed conversion ratio, blood lysozyme level of turbot and TAN, NO₂⁻N , COD of the water were also detected. The results showed that the final stocking densities of A, B and C reached 30.09kg/m²□41.30kg/m²□60.07kg/m² respectively□and the survival rate of each experiment group reached at 95% or above. It was also found that stocking density did not significantly affect the survival rate of turbot. The turbot in high density showed lower growth rate at the beginning of the research. The uniformity of weight distributing within experiment groups decreased with increased density. When the experiment has been done for 5 days, the bacteriolytic enzyme levels of high density group of turbot were elevated, but decreased after 20 days and significantly lower than that of the low-density group after 40 days. Stocking density had significant influence on feed conversion ratio, and the turbot in high density showed higher feed conversion ratio. At the fifth day after crowding□blood lysozyme level of the turbot in group C increased little, but it decreased markedly after 40 days in comparison with control group(P<0.01). During the research, the recirculating aquaculture systems were stable and stocking density had significant influence on the TAN□NO₂⁻N□COD (P<0.05), however, these several parameters of the high-density group did not exceed the highest concentration level acquired in water quality standards. With the rise of stocking density, various water quality parameters were significantly higher. Based on these experiment results, this experiment will provide references for the land—based recirculation systems of turbot culture.

BILE CONTENTS FROM COMMERCIALY AVAILABLE EDIBLE FISH

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In non-mammalian vertebrates, such as fish and reptiles bile alcohols are formed, whereas invertebrates do not produce bile acids and bile alcohols. Bile salts are produced by almost every class of vertebrate animals and remarkable diversity in their structure has been revealed across species. Study of bile salts and acids has led to certain significant facts indicating the existence of molecular evolution in the basic C₂₇ skeleton of Cholesterol. Most of the compounds isolated from the bile are found to contain C₂₄ to C₂₇ skeletons. C₂₆ alcohols and acids are also produced by the oxidation of the side chain of Cholesterol. Bile from gallbladders of *Muraenesox bagio* (Bam), *Pomadasys argenteus* (Dother), *Labeo rohita* (Rohu), *Arius platystomus* (Singhara), *Arius tenuispinis* (Khagga), *Pomadasys commersonni* (Holoola), and *Kishinoella tonggol* (Dawan) were extracted, derivatised and analysed by GC-MS for identification of bile acids and bile alcohols. Cholic acid and chenodeoxycholic acid were found as major bile acids in all species with

cholesterol as major bile alcohol. *Muraenesox bagio* was also found to contain 3 α ,7 α ,12 α -trihydroxy-23-cholestenoic acid and 5 α -anhydrocyprinol. Other bile acids and bile alcohols identified in *Labeo rohita* were allodeoxycholic acid, 12-oxo-3 α -hydroxycholanic acid, 3 α ,7 α ,12 α -trihydroxy-24-cholestenoic acid, 5 α - and 5 β -anhydrocyprinol and 5 β -Homocholane-3 α ,7 α ,12 α ,25-tetrol. Bile acids identified in *Arius platystomus* were allochenodeoxycholic acid, allodeoxycholic acid, 3 α ,7 α ,12 α -trihydroxy-24-methyl-5 β -cholestane-26-oic acid, and 3 α ,7 α ,12 α ,24-tetrahydroxy-5 α -cholestane-26-oic acid. *Kishinoella tonggol* was found to contain 3 α ,7 α ,12 α -trihydroxy-5 α -cholestanoic acid and 3 α ,7 α ,12 α -trihydroxy-5 β -cholestanoic acid. Bile alcohol 5 β -cyprinol was present in significant amounts with 5 β -cholestane-3 α ,7 α ,12 α ,24-tetrol being the other contributors in the bile of *Kishinoella tonggol*. To conclude 10 different bile acids and 5 different bile alcohols were identified from gallbladder bile of 7 different fish.

GROWTH PATTERNS OF METALS MIXTURE STRESSED FISH UNDER SEMI-INTENSIVE POND CULTURE SYSTEMS

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The project work was focussed on the growth performance of metals mixture stressed fish in earthen ponds. The effects of sub-lethal concentrations of metals mixture (Zn+Pb+Mn) on the growth performance of *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, *Cirrhina mrigala*, *Catla catla* and *Labeo rohita* was elucidated in earthen ponds. The growth of fish was monitored in terms of increase in wet weights (g), fork and total lengths (mm), condition factors, feed conversion ratios and specific growth rates that were compared with the control (un-stressed) fish. Fish were grown in ponds under composite culture system for a period of 210 days. Fortnightly data on all the growth parameters, as mentioned above, were collected and analyzed statistically. The present study reveals that Zn+Pb+Mn stress to the fish caused significant effects on the growth response of all the five fish species. After 210-day experimental duration, the treated fish attained significantly lower weights as compared to the control. However, the fish growth in terms of weight followed the order *Cirrhina mrigala* < *Labeo rohita* < *Catla catla* < *Ctenopharyngodon idella* < *Hypophthalmichthys molitrix*. The increase in fork and total lengths of five treated and control fish species exhibited statistically significant differences. Among all five fish species, *Cirrhina mrigala* gained significantly higher fork and total length increments. All the control fish species showed statistically ($p < 0.05$) lower condition factor values as compared to the treated fish species. However, among the five fish species, *Hypophthalmichthys molitrix* exhibited significantly higher condition factor as compared to other fish species. Highly significant differences were recorded for feed conversion ratios of treated and control ponds. Feed conversion ratios of ponds varied significantly ($p < 0.05$) among various fortnights of the study period. There existed statistically significant differences for specific growth rates of stressed and control fish. The differences among five fish species varied significantly also. However, the control fish showed significantly better specific growth rate than that of Zn+Pb+Mn stressed fish.

INFLUENCE OF TRADITIONAL SMOKE-DRYING TEMPERATURE AND DURATION ON THE BIOCHEMICAL ANALYSIS OF ROUND SOLE FISH (*SOLEA SOLEA*)

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Seafood has traditionally been an essential constituent of people's diet in many countries. To extend the shelf life, fish preserved by freezing, sun drying, salting, canning and smoking techniques. About 25 to 30% of the world fish catch is consumed in the dried, salted, smoked form or with combination of two techniques. It has been observed that different processing and drying methods have different effects on the nutritional compositions of fish. The aim of this research was to assess traditional smoke-drying temperature and durations on the biochemical analysis of round sole fish (*Solea solea*). Fish samples were purchased from Metro Cash and Carry Centre, Lahore-Pakistan. There were three temperature ranges (60, 70 and 80°C) and three time intervals (6, 9 and 12 hours) using the oven drying. The biochemical analysis of fresh and oven-dried fish was performed. The temperature and time intervals have significant effect on the nutritional status and quality of smoke dried fish. The results showed that the crude protein was 54.35, 53.33 and 52.93%, at. Crude fat 13.86%, 12.08% and 11.02%, the ash contents 26.64%, 24.24% and 22.91%, moisture contents were 7.81%, 6.21%, and 5.97% and the dry matter 92.19%, 93.29% and 94.03% at the temperature of 60, 70 and 80°C respectively. It is concluded from the results that the best smoked fish in terms of nutrient and organoleptic parameters were the products dried at 70°C for 12 hours and 80°C for 9 hours.

3. MARINE BIOLOGY

OCCURRENCE AND ABUNDANCE OF COMMERCIALY IMPORTANT FISH SPECIES OF FAMILY CLUPEIDAE AND ENGRAULIDAE IN THE MIANI HOR, SONMIANI BAY, BALOCHISTAN

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Sonmiani bay, Balochistan is located at the latitude of 25° 27' 431" North, and the longitude of 66° 33' 700" East approximately. A large shallow sea bay, Miani Hor with extensive mangrove swamps and intertidal mud flats is important for shelter and large compositions of waterbirds, fin fish, shrimp and crabs. These fishery items are consumed locally, and brought to market. The present research has been undertaken to document the occurrence and abundance of commercially important species of two families (Clupeidae and Engraulidae) inhabiting in the Miani Hor lagoon. For this purpose monthly samples were collected by the commercial fishing boats over a year from selected stations. Fauna were collected by means of different gears (gill net, cast net, beach seine and wire net) used to get well representative samples of different niches. Among the species of Clupeidae and Engraulidae, a total of 23 species of these families was identified in the catch samples. Family Clupeidae found dominant with 15 species in which *Hilsa kelee*, *Ilisha megaloptera* and *Escualosa thoracata* found abundant in most of the year, whereas Engraulidae family ranked second with 8 species among those the *Thryssa setirostris*, *T. vitrirostris* and *T. malabarica* found in abundance. The species diversity, biomass distribution and catch sizes varied with the months and showed a seasonal pattern in lagoon water. The change in salinity and temperature was observed as the main factor for change of species diversity in the lagoon water.

DISTRIBUTION AND ABUNDANCE OF MAJOR GROUPS OF ZOOPLANKTON WITH RESPECT TO PHYSICO-CHEMICAL PARAMETERS, IN THE COASTAL WATERS OF KARACHI, PAKISTAN

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The studies on zooplankton population structure, distribution and abundance are scarce from Pakistani waters particularly from the near-shore waters off of Karachi. Most of the previous work is done on a few individual groups and species of zooplankton. The present study was proposed with an objective to assess the distribution and abundance of zooplankton from near-shore waters off of Karachi and also to correlate quantitative data with water quality parameters. This study showed higher population diversity of zooplankton groups. The seasonal abundance of zooplankton groups varied from station; the peak zooplankton abundance occurred in April (16880 individuals/m³) at St. 1 and the lowest abundance was recorded in July (954 individuals/m³) at St.4. In general, copepods (highest 66435 individuals/m³ at St. 4 and lowest 58269 individuals/m³ at St.2

always maintained dominance in the zooplankton population. Water parameters were variable through the year and showed low correlation with abundance data. The zooplankton population appears to be regulated by other factors (such as grazing pressure, etc.) and the water parameters had little contribution in controlling zooplankton population. For example, chlorophyll content (primary production) showed only weak positive correlation ($r=0.170$) with zooplankton abundance. The study area showed relatively stable environmental conditions and high zooplankton population which is beneficial for fisheries production.

INFLUENCE OF PHYSICOCHEMICAL CHARACTERISTICS OF SEDIMENT ON METAL ACCUMULATION IN DOTILLID CRAB, *ILYOPLAX FRATER* AT DIFFERENT COASTAL AREAS OF KARACHI, PAKISTAN

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The current study aimed to investigate the heavy metal accumulation in deposit feeder crab, *Ilyoplax frater*, with relation to physicochemical properties of sediment from coastal areas of Karachi. Sediment and crab samples were collected during Oct. and Nov. 2011 from intertidal mangrove areas of Korangi Creek (KC), Port Qasim (PQ) and RatoKot (RK), Karachi. The order of heavy metal accumulation in crabs observed as $Fe > Ni > Pb > Cu > Co > Zn > Cr > Cd$ at KC, however, similar order of heavy metal meditations at PQ and RK were examined as $Fe > Co > Cu > Zn > Ni > Pb > Cr > Cd$. Results disclose that all metals varied significantly ($p < 0.01$) in sediment and *I. frater*. Significant variations ($p < 0.05$) among the sites were observed in grain size, pH and both were detected most influencing parameters on metal meditation in *I. frater*. Regression analysis indicated that the Pb concentration in crab was linearly correlated with Pb in sediment ($R^2 = 0.986$). While Ni ($R^2 = 0.676$) and Cd ($R^2 = 0.891$) were observed negative association with exposure concentration. Accumulation factor (AF) of Co, Zn, Pb, Cu and Cd recognized greater than 1.0 and signify that these crabs may act as a potential indicator for these metals. The current study reveals the ecological significance of deposit feeder crab, *I. frater*, as good accumulator of metal pollution in intertidal areas of Karachi coast.

SOME BIOCHEMICAL VARIATIONS AMONG SPECIES OF FAMILY DOTILLIDAE (BRACHYURA, OCYPODOIDEA) FOUND ALONG THE COAST OF PAKISTAN

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Crabs of family Dotillidae are small Brachyuran detritivorous crabs, inhabitant of sandy or muddy shores. They construct burrows during high tide and emerge as the tide descends, to perform surface activities. From Pakistan 5 species from three genera i.e. *Dotilla*, *Scopimera* and *Ilyoplax* have been reported. Current study aimed to infer biochemical relationships among species of Dotillid crabs using polyacrylamide gel electrophoresis. Interspecific biochemical similarity obtained using Jaccard coefficient for *Dotilla blandfordi* and *Scopimera crabricauda* is 0.50, for

Scopimera crabricauda and *Ilyoplax frater* is 0.57, *Dotilla blandfordi* and *Ilyoplax frater* has a coefficient value 0.42. The species of genus *Ilyoplax* showed a coefficient of 0.58-0.62.

**LABORATORY BIOASSAY FOR TRIBUTYLTIN TOXICITY IN TWO SPECIES OF
THAIS (GASTROPODA: THAIDIDAE)**

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Imposex is a genital disorder characterized by imposition of male sexual characteristics in female gastropods mainly due to exposure to tributyltin (TBT). This compound (TBT) is used as biocidal agent in antifouling paints and applied on the ship hulls and other marine submerged structures such as fishing gears, aquaculture installations and buoys. In the present study TBT bioassay experiments were carried out to determine imposex inductive and endocrine disruptive effect of TBT in two different species of neogastropods of genus *Thais*. In this bioassay experiment normal specimens of *T. bufo* and *T. rudolphi* were exposed to three different concentrations (100, 500 and 1000ng/L) of TBTCI for four weeks in laboratory. At the end of experiment specimens were morphologically analyzed and level of free testosterone was determined by radioimmunoassay (RIA). TBT body burden was estimated by gas chromatograph coupled with a flame photometric detector (GC-FPD). In *T. bufo* and *T. rudolphi* exposed to 500 and 1000 ng l⁻¹ of TBT **a** and **b** type of imposex stages developed, however, in 100 ng l⁻¹ and control groups showed no imposex condition. The exposure to TBT caused the elevation of free testosterone level in imposex females as compared to normal females. Bioaccumulation of TBT and its degradative products (DBT and MBT) in whole body tissue were also found to be dose dependent and exposure to higher dose of TBTCI showed high mortality as compared to control group. These observations indicate that the TBT act as potential imposex inducer and endocrine disruptor in the targeted gastropod species and these species can be used as sensitive bio-monitoring tool for the detection of TBT contamination.

**ON A NEW RECORD OF THE GENUS *UCA* (DECAPODA, BRACHYURAN,
OCYPODIDAE) FOUND ALONG THE COAST OF PAKISTAN**

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This is the first record from Pakistan of *Uca* (*Austruca*) *iranica* Pretzmann, 1971, a species that occurs in mangrove and non mangrove areas. *U. iranica* appears to be one of the frequently

and abundantly distributed, broad front *Uca* species on the coast of Pakistan. This species was previously reported from the Gulf of Oman and the Persian Gulf. After this report the four species of *Uca* known from Pakistan have been confirmed.

BIOCHEMICAL COMPOSITION OF THREE EDIBLE CRAB SPECIES OF FAMILY PORTUNIDAE FOUND IN COASTAL WATERS OF PAKISTAN

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This study was conducted to assess the nutritional quality of the tissues of edible crabs (Family: Portunidae) found in Pakistan coastal waters. In recent years crabs have already become commercially important seafood species worldwide that's why crab is sold at high market prices. In present study the biochemical composition like lipid, protein and carbohydrate of three species of family Portunidae: *S. olivaceae*, *Portunus pelagicus* and *P. sanguinolentus* were selected for study. The results of the present study showed the variations in total lipid concentration in edible tissues of three species. The maximum lipid concentration was observed in *Portunus pelagicus* (1.72 ± 0.12 mg/g), in *Scylla olivaceae* (1.71 ± 0.445 mg/g) and minimum (1.43 ± 0.245 mg/g) was noticed in *P. sanguinolentus*. The difference in percent concentration was observed between the species with highest in *Portunus pelagicus* (8.60%), in *Scylla olivaceae* (8.58%) and was lowest in *P. sanguinolentus* 7.17%. The percent composition of protein and carbohydrate also varied among the species.

DIVERSITY AND STRUCTURE OF MICROZOOPLANKTON COMMUNITY AT THE JUNCTION OF SINDH AND BALOCHISTAN COAST, PAKISTAN

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Microzooplankton play a significant role in the marine pelagic food web, mediating energy from primary producers to the higher trophic levels. The diversity and structure of microzooplankton community was assessed during a survey along a transect at the junction of Sindh and Balochistan coast in the northern Arabian Sea, Pakistan. The samples collected at three stations revealed the presence of a diverse microzooplankton (heterotrophic planktonic organisms) community. A total of 36 microzooplankton taxa were recorded with ciliates being the most abundant group during this study. Twenty one species of ciliates were recorded from 15 genera. The bloom forming red tide ciliate *Mesodinium rubrum* was the predominant species at all three stations followed by other naked ciliates *Leegaardiella ovalis* and *Strombidium emergens*. Dinoflagellates were represented only nine species from 8 genera. *Gyrodinium spirale* was the abundant and common heterotrophic dinoflagellates species at all three stations, while other dinoflagellates were scarce at station II and III. Total microzooplankton mean abundance ranged from 650 to 2500 cells l⁻¹. Ciliates were most abundant ranging between 600 to 1800 cells l⁻¹. Dinoflagellates were the second abundant form of heterotrophic microzooplankton with population ranging from 50 to 700 cells l⁻¹. A clearly increasing gradient in both abundance and diversity of microzooplankton was observed at station I.

Further studies are suggested to assess the seasonal trend of the microzooplankton population, and to determine the ecological importance of these species, particularly with respect to harmful algal bloom and their potential impact on human health.

THE ANALYSIS OF STOMACH CONTENTS OF A JUVENILE WHALE SHARK

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The whale shark *Rhincodon typus* Smith, 1828 are known to target plankton and small nekton as food sources. The whale shark often entangled in the long fishing gillnets and trawl nets in the economic exclusive zone (EEZ) of Pakistan. On 31st January 2013, a juvenile whale shark was caught accidentally by trawl net in the area between Miani Hor and the Hingol river mouth, at a depth of about 80 meters. The specimen was an immature female of 6.8 meters length and weight about 2.47 tonnes. The analysis of stomach contents of this whale shark was documented. The presence of wide range of prey items were found in the stomach of whale shark including copepods, larger nektonic planktons, crustaceans, crab larvae, small bait fish and cephalopods. This study showed that WS preferred to prey on larval fishes and zooplankton species. The feed analysis of stomach contents further suggest that juveniles of largest whale shark of the world, *Rhincodon typus* come into the coastal waters of Pakistan during the winter season, due to the warm and temperate waters and availability of plenty food in the area.

BENTHIC MACRO-INVERTEBRATE COMMUNITY COMPOSITION AND ITS RELATIONSHIP WITH BIOLOGICAL AND PHYSICO-CHEMICAL PROPERTIES OF SEDIMENT, WITHIN MANGROVE FOREST OF INDUS DELTA (KETI-BUNDER), PAKISTAN

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The macro-benthos is the stable residents of the mangrove forest. The distribution, abundance and diversity of macro-benthos depends on various properties of the sediments, such as porosity and permeability of the soil, grain-size, water contents, organic contents, salinity, dissolved oxygen of pore water, etc. Mangrove ecosystem in the Indus delta has not been studied with respect to its fauna and sediment properties. Therefore the present study was carried out to describe the diversity and abundance of fauna, and sediment and pore-water properties. The study was carried out at 12 distinct sites on the basis of forest structures and biological properties. The variations in the assemblages of macro fauna and their bio-dependent properties of the sediments at various special scales have been measured. Data was taken during May and June in 2005 using quadrates method. The properties measured at different sites appeared to have significant variations. PCA ordination of physico-chemical and biological characteristics of sediments (temperature, water contents, organic content, chlorophyll, density and size of pneumatophores, density and diameter of crab burrows) and pore water characters (pH, Eh, salinity, temperature; and nutrients: ammonium, nitrates, nitrites, phosphates) clustered the 12 sites in four groups. Cluster appeared to be controlled by pneumatophores density, burrow density, chlorophyll content and

redox potential recorded at these sites. The different sites appear to provide distinctive resources that are exploited by different groups of benthic fauna. Variable sediment features appear to influence the distribution of benthic fauna and also indicate diversity of habitats in the Indus delta region.

MEAT YIELD IN CORAL CRAB *CHARYBDIS FERIATUS* (LINNAEUS, 1758) FROM COASTAL WATERS OF KARACHI, PAKISTAN

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Meat yield is depending upon the size as well as sex (male and female) of crabs. For the estimation of meat yield crabs (*Charybdis feriatatus*) were collected twice in a month from commercial landings at Korangi Fish Harbour (KFH), Karachi, from May 2004 to April 2006. A total of 2,882 crabs were collected during present study. For the estimation of meat content, different body parts (thorax, chela and periopods (legs)) was detached manually from *C. feriatatus* body for the analysis of eat content. The weight of meat was recorded separately for thorax, chelae and periopods of each crab respectively. In male total weight of meat varied from 13.8 g to 428 g while in female total meat varied from 5.5 g to 182 g. Male and female thorax contain high meat content among various body parts 60.65 % and 63.5 % in male and female respectively, followed by chelae (26.4 % in male and 24 % in female) and periopods (12.9 % in male and 12.5 % female), respectively. The high percent (%) of meat yield in the *Charybdis feriatatus*, compared to other brachyurans could be due to morphology. The data will help to improve the knowledge and importance of *Charybdis feriatatus* one of the edible crab in Pakistan.

GENOTOXIC EFFECT OF PESTICIDES ON GILL TISSUES OF GREEN-LIPPED MUSSEL (*PERNA VIRIDIS*)

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The marine ecosystem is constantly threatened by wide variety of anthropogenic substances arising as a result of hazardous chemicals from industries, agricultural sources, sewage disposal, heavy metal, oil, petroleum etc. Pakistan is an agricultural country therefore pesticides are widely used on its cropland. The present study is carried out to assess the Micronucleus (MN) frequency in the gill tissues of green mussel (*Perna viridis*) after exposure to different concentrations of organophosphate pesticides (chlorpyrifos, malathion), synthetic pyrethroid pesticide (cypermethrin, lambda-cyhalothrin) and herbicide (bucril). MN is considered as the marker of cytogenetic damage, appearing after the impact of genotoxic compound. The MN frequencies of the pesticides treated mussels are observed to increase significantly ($p < 0.05$) in a dose-dependent relationship at all exposure periods as compare to control. The highest MN frequencies were recorded after cypermethrin exposure on twelfth day (10, 11.5 and 13.5‰ for 0.5 ppm, 1 ppm and 1.5 ppm concentrations respectively) in gill tissue. The genotoxicity of pesticides on *Perna viridis* in this study is found to be in the order of cypermethrin, chlorpyrifos, malathion, lambda-cyhalothrin and bucril, in gill tissues.

OLFACTORY MORPHOLOGY OF FIVE PERCIFORM FISHES OF PAKISTAN**Gul-e-Hina Kaleem and Ghazala Siddiqui***Centre of Excellence in Marine Biology, University of Karachi, Karachi*

The olfactory systems of five marine perciform fishes including *Rhadosargus sarba*, *Epinephelus diacanthus*, *Nemipterus japonicus*, *Sphyrna obtusata* and *Pomadasys kaakan* belonging to family Sparidae, Serranidae, Nemipteridae, Sphyrnidae and Haemulidae, respectively were studied. The morphological structure and variations regarding structure, size and position of nostril, size of olfactory chamber, olfactory rosette structure, size and arrangement of lamellae and presence of nasal sacs etc. The targeted species had valvular lips separated by a nasal bridge. The nostrils of *N. japonicus* and *E. diacanthus* were round and nearly of same diameter, separated by small epidermal bridge. In *N. japonica* and *R. sarba* the rosette was oval and in *E. diacanthus*, the rosette was found. In *S. obtusata*, the anterior opening was small and round whereas, the posterior nostril was large and slit-like with oval and slightly bean shaped rosette. Except for *S. obtusata*, all species had prominent nasal flap at the anterior opening. The number of lamellae differed with the standard length and varied among the pair of rosette in all species. The morphological variations in olfactory organs indicate their species specific role in biological functions and chemoreception.

EVALUATION OF SEASONAL, SEX AND SIZE WISE FEEDING HABITS IN SCOMBEROIDES COMMERSOHNIANUS AND S. TOL (FAM: CARANGIDAE) IN THE NORTHERN ARABIAN SEA COAST OF PAKISTAN**Nazia Qamar and Sher Khan Panhwar****Center of Excellence in Marine Biology, University of Karachi, Karachi, Pakistan.**Email: sk.panhwar@uok.edu.pk

Random samples of *S. commersonianus* and *S. tol* were collected between July 2013 to June 2014 with different fishing gears (Purse seines, gill nets, pelagic trawl and beach seines) from the commercial catches from Karachi fish harbour. Some 435 specimen of *S. commersonianus* percentages 69.20 males and 30.80 females and 353 individuals 37.39 males and 62.61 females of *Scomberoides tol* were used to estimate feeding habits. The individuals of *S. commersonianus* measuring 18-130cm, weighing 30-16100g and *S. tol* ranging 19-68 and weighing 34-1700g were recorded. Each stomach was analyzed and quantified gravimetrically with percentage of occurrence, numerical and weight composition method. Both of the species observed as carnivore predator mainly feeds on *Acetes* spp. clupeid and Trichiurid species. Overall assessment of feeding in *S. commersonianus* revealed that it prefer fishes Teleosts (70.56%), Digested matter (15.45%), Crustacean (10.16%), Molluscs (3.17%) and Coelenterates (0.64%), whereas *S. tol* food items composition was Teleosts (47.46%), Crustaceans (40.71%), digested matter (8.55%), Molluscs (2.68%), and Polychaete (0.51%). The index of relative importance (IRI) and index of absolute importance (AI) revealed noticeable variations in size and season observed in both of the species. The diet composition did not significantly differ between the sexes. This study has demonstrated ecological role of two important carangids in the food web in the Northern Arabian Sea coast of Pakistan.

POLYCHAETES LARVAE AND THEIR DEVELOPMENT**Syeda Nasreen¹ and Safia Mushtaq²**¹*Department of Zoology, University of Karachi, Karachi*²*Centre of Excellence in Marine Biology, University of Karachi, Karachi*

The diversity of polychaete larvae in the form of developmental patterns has been attracted in the world except Pakistan. Early stages of polychaetes have been known since the late nineteenth century. The study has been designed and conducted to fill the gap in Pakistan. It involves extensive collection of zooplankton from the coastal waters of Karachi, for the period of two years, which play a vital role in marine ecosystem, act as a primary or intermediate consumer although they have a very brief planktonic existence. polychaetes Larvae are chiefly marked by cilia & pigmentation. The benthic juvenile secretes a mucoide tube & begins to burrow into the calcareous substratum. A number of identified & unidentified polychaetes larvae are reported from the coast of Karachi.

DIVERSITY OF ASSOCIATED COMMUNITIES WITH MARINE SPONGE INHABITING COASTAL WATER OF KARACHI, PAKISTAN**Hina Jabeen, Seema Shafique, Zaib-un-Nisa Burhan and Pirzada J.A. Siddiqui***Centre of Excellence in Marine Biology, University of Karachi, Karachi-75270, Pakistan.*Email: seema.shafique@uok.edu.pk

Sponges are sessile, incipient and diploblastic metazoans that abundantly found in marine subtidal zones worldwide from tropical to polar waters. They play a crucial role in benthic food webs because of their ability to support several benthic microbial communities as symbiont. In order to study the associated communities with marine sponge sampling was done during Jan 2013 – Dec 2013. The collected specimen washed in the field and the retained water (200 ml) passed through sieve (1mm) and preserved in 4% formalin solution. In the laboratory preserved sample observed under stereo-microscope. A total of 129 species of microbes, unicellular algae, protists, rotifers, larvae of polychaetes, decapods, cirripedes, copepods, amphipods as well as several families of insects and some unidentified eggs of fishes and amphibians were reported. Studies of communities in relation to diversity showed that diatoms were dominant taxa as symbiotic phytoplankton and among zooplankton, crustaceans (decapods, cirripedes, copepods, amphipods and isopods) and polychaetes larvae were abundantly found.

A NEW RECORD OF *AMPELISCA SCABRIPES* WALKER, 1904 (CRUSTACEA: AMPHIPODA: AMPELISCIDAE) FROM THE NORTHERN ARABIA SEA**Hala Bano¹ and Quddusi B. Kazmi²**¹*Govt. College for Women Shakra-e-Liaquat Karachi*²*Marine Reference Collection Centre, University of Karachi, Karachi, Pakistan.*hala.bano@live.com and qbkazmi@yahoo.com

The *Ampelisca scabripes* Walker, 1904 is a new record from the Pakistani territorial waters. The species belongs to the family Ampeliscidae. The material was collected from Gawader near the Balochistan coast. The species is illustrated and described in detail.

SEX RATIO AND GONADOSOMATIC INDEX OF *TERAPON JARBUA* (FORSSKAL, 1775) FROM PAKISTAN COAST**Musarratulain¹ and Zubia Masood²**¹*Department of Zoology, University of Karachi, Karachi-75270, Pakistan*²*Department of Zoology, Sardar Bahadur Khan Women University Quetta Balochistan*

Management and control of fisheries and aquaculture can be made easy by the studies on reproductive biology of fishes. In the present study sex ratio and gonadosomatic index of *Terapon jarbua* (Forsskal, 1775) were estimated to find out the appearance of male & female and the spawning season of that fish. 426 specimens of *Terapon jarbua* were collected from Karachi fish harbour, during January 2014 to December 2014. Out of that collection 207 were male and 219 were female; showing the sex ratio 1:1.05 (M:F). The values of gonadosomatic index ranged from 0.47 to 1.60 for males and 0.90 to 3.86 for females. During the study the higher values of gonadosomatic index in both sexes were observed in April and July, showing the peak spawning season of *T. jarbua*.

INCREASING TRENDS IN CAPTURE OF JUVENILES: A RED SIGNAL FOR FISHERY RESOURCES!**Sher Khan Panhwar, Noreen Farooq, Nazia Qamar, Shahnaz Jahangir and Khawar Paraveez Awan***Center of Excellence in Marine Biology, University of Karachi, 75270.*Email: sk.panhwar@uok.edu.pk

In this study, some 562 individuals belonging to the (38) families and (67) species were procured from August 2014 to January 2015. The samples were collected from four fish landing sites of Zero point Badin (ZPB), Hyderi fish jetty (HFJ), Clifton Karachi (CFK) and Karachi Fish Harbour (KFH). The fishes mainly caught with purse seines, gill nets, pelagic and demersal trawls and beach seines. The composition of fish families by weight from the KFH was categorically Platycephalidae 20%, Engraulidae and plotosidae 12% respectively; from HFJ, family Sciaenidae comprises of 26%, Dasyatidae 23% and Muraenesocidae 13%; from ZPB, Gobiidae 31%, Soleidae 14% and Polynemidae and Sciaenidae 12%; and from CFK, Clupeidae 49%, Penaeidae 28% and Trichiuridae 10%. The Shannon Weiner diversity index H' was estimated for four sampling sites, the highest ($H'=3.29$) and the lowest ($H'=1.93$) diversity for KFH and CFK respectively. Estimated species richness indicated highest $R=8.49$ from HFJ followed by KFH, $R=5.24$ and the lowest $R=2.28$ from CFK. An average 40t/day trash has been recorded from nine fish landing sites in Karachi with an average 15t/day have been landed at KFH. Surprisingly, there is a general assumption that the fish species which does not attain an ideal size at maximum age or not preferred for food consumption are often went into trash. With this question in mind, this study has demonstrated that such assumption is unreasonable and increasing trends in capture of juveniles are deteriorating. It is proposed that stringent actions are needed to prevent capture of undersize populations of important species would be better for the sustainability of food web and exploitation of the fishery resources.

OCCURRENCE AND ABUNDANCE OF MACROBENTHIC FAUNA IN MANGROVE SWAMPS OF SANDSPIT BACKWATERS, KARACHI, PAKISTAN

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In present study occurrence and abundance of macrobenthic fauna has been carried out during the period of Jan 2006-2007 with the aim of studying the macrofaunal composition and seasonal changes along the three transects from Sandspit backwaters. Sandspit backwaters lies about 18 km south-west of Karachi city and contain shallow tidal lagoons, inter-tidal mudflats and 400 hectares mangrove swamps. The western side is comprised of open sandy beach which stretches about 20 km along the Arabian Sea coast .The area is densely populated by *Avicennia marina*. Sediments was collected using box corer from the mangrove swamps intertidally from three different transects for studying the macrobenthic composition. Diverse assemblages of four abundant faunal groups were found during the study. A total of 78 species of macrobenthic invertebrates were recorded. Four major faunal groups are being discussed. Gastropods were comprised of 43 species (55%) and was the most dominated taxa, followed by bivalves, 12 species (15.38 %) and crustaceans, 12 species (15.38) which were equally represented. Polychaetes consisted of 8 species (10.26 %) and the fourth group included under Others have 4 species (5.12%) consisting of nematodes, sand dollars, sea anemones and insect larvae were observed. *Cerithidea cingulata* was found to be the most abundant animal in the backwaters. Seasonal variation among the transects and the intertidal fauna is discussed.

PARENTAL CRUSTACEAN FEMALES OF LARVAE USED FOR MORPHOLOGICAL ANALYSES OF LABORATORY HATCHED DEVELOPMENTAL STAGES AND PLANKTONIC CRUSTACEAN STAGES IN PAKISTANI WATERS

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The taxonomic composition of crustacean larvae is from 59 mother crustacean species collected from the intertidal region of coast of Pakistan and reared in the laboratory. Identification and laboratory rearing of stages obtained from the plankton samples was also carried out. The largest number reared family is of Alpheidae and most specious group in the plankton is the Brachyura. The zoea 1 was the most available stage. New records for the area through updated taxonomy are 18 mother species. New development larval stages of 37 crustacean species are for the first time cultured in the laboratory in Pakistan and outside Pakistan. No such information is present in any part of the world. Plankton- caught larvae reared in the laboratory are of *Metapenaeus stebbingi* and *Panulirus polyphagus*. Comparison of planktonic and laboratory reared larvae showed field collected larvae larger than laboratory cultured, and they have more setae as compared to laboratory cultured larvae .

DISTRIBUTION AND ABUNDANCE OF FRESHWATER TURTLE SPECIES OF POTHWAR PLATEAU, PAKISTAN

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Freshwater turtles form major biodiversity component of aquatic ecosystems, and also serve as keystone species benefiting other animals and plants. However, scientific studies on their conservation and management are scanty. In the current study, we investigated the distribution and abundance of freshwater turtle species from two districts (Rawalpindi and Chakwal) of the Pothwar Plateau. To find out the distribution of various freshwater turtle species in the study area, major water bodies were surveyed, observations were recorded by visual method and by using cast net. The species were identified by matching with the published identification keys. Abundance of turtle species was estimated by direct count as well as by "Capture, Mark, and Recapture method. Results showed a total of four species of freshwater turtles in the study area including Indus mud turtle (*Lissemys punctata*; 45%), Indian softshell turtle (*Nilssonina gangeticus*; 23.3%), Brown River turtle (*Pangshura smithii*; 25.3%) and Indian roofed turtle (*Pangshura tecta*; (6.1%). *Lissemys punctata* was found abundant, *Nilssonina gangeticus* and *Pangshura smithii* more common and *Pangshura tecta* least common in the study area.

A GIS BASED ANALYSIS OF ESSENTIAL CREEK HABITATS FOR THE EVALUATION OF COMMON MARINE SPECIES AT THE COAST OF SINDH, PAKISTAN

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Pakistan has resourceful coastline of 1,100 Km long, which is having a great potential of marine resources including marine fishes, prawns and crabs. For the evaluation of common marine species, creeks area of Sindh coast has been selected as a study area. Most of these creeks are dominated by mangrove forest. The main objective of this study was to develop a GIS-based map and analyze the relative importance of various habitat types in supporting marine species populations at various life history stages. Major habitats include shrimp stocks, juveniles of selected common fish species and coastal small pelagic fish. Based on total catch six most abundant marine species *Penaeus indicus*, *Acanthopagrus latus*, *Johnius belangerii*, *Stolephorus indicus*, *Charybdis sp.* and *Escualosa thoracata* have been selected. The spatial distribution of marine species has significant correlation with the adjoining environment of the creeks, which has been evaluated in this paper with SRS (Satellite Remote Sensing) techniques. Furthermore, Habitat Suitability Index (HSI) is developed to map habitat quality of species through bathymetric, catch and environmental datasets. Based on the GIS analysis the significant relationship has been observed between environmental data and catch data which would provide a baseline for the evaluation of fish resources in Pakistan.

ON SUBADULT STAGE OF *CHARYBDIS*, ZOEIA I AND CRABLET OF *SCYLLA* FROM PAKISTANI COASTAL WATERS (CRUSTACEA: DECAPODA: PORTUNIDAE)

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The subadult stages of decapods are as subject to evolutionary adaptations as adults. The extent to which post larval stages basic morphology is altered is not yet fully evaluated in decapods. But in some cases study of these stages has clarified some systematic problems. Certain appendages and morphological features have proven to be highly useful in discriminating species, due to their particularities. Many species of decapods are commercially consumed. Of these the family Portunidae is a distinctive well represented group of marine crabs. Few studies on the life cycles of Portunidae have been carried in Pakistan but no one on post larval stages. The report on two sub adult portunids described in this paper is being presented from Pakistan with a hope that interest in their systematic utility will be forthcoming. The subadult and instar of porunids and larvae (first zoea) are illustrated and described. But due to small sample this study should be regarded as rather tentative.

USING *DONAX* SP. AS BIOINDICATOR TO COMPARE MORPHOLOGICAL CHARACTERISTICS AND ENVIRONMENTAL HEALTH OF TWO SANDY BEACHES OF KARACHI COAST

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A total of 32 samples were collected in duplicate from 04 Stations at Sandspit and Clifton (S1, S2, C1 & C2) in pre-monsoon (March) & during South West monsoon (April-August) period. A total of 3446 organisms were reported from Clifton and 22 from sandspit during studies period. Donax was most abundant in Pre Monsoon season at both sites and scarce in late SW Monsoon season. The maximum fraction of sediment texture consists of fine sand at both sites. The total abundance and species richness is relatively higher at Clifton. Donax sp. was the most abundant species at Clifton mainly at sea view which is relatively more disturbed site. The present study revealed that the greater variability in relative abundance of Donax sp. at Sandspit and Clifton is influenced by the magnitude of pollution and presence of high organic matter in sediments rather than morphological characteristics of sediment.

MEASURING RISK OF GREEN TURTLE HABITAT THROUGH GEOSPATIAL INDICATORS: A CASE STUDY OF HAWKS BAY BEACH

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Hawks Bay beach is amongst one of the famous marine turtle nesting beaches of Pakistan. It has significant geographical characteristics, which provides suitable nesting grounds for the

marine turtles. Green turtles (*Chelonia mydas*) nesting activity on this sandy beach is highly frequent throughout the year. Their nesting habitats are under many severe threats. Most of them are human induced. Satellite remote sensing techniques will be used for the evaluation of risks of the nesting habitat of Green turtles. It will be used to examine the factors for the depletion of nesting sites at the ground. Furthermore, the assessment through GIS techniques will be help full to quantify the nesting pits. This study will be helpful to monitor the marine turtle species activities through successive techniques, which would help to protect these unique creatures.

ABUNDANCE, BREEDING AND GROWTH OF THE OCYPODIDE CRAB *DOTILLA BLANFORDI* ON KARACHI COAST.

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The Ocypodidae family posses most familiar amphibious and terrestrial crabs like fiddler crabs and ghost crabs. The crabs have a significant role in detritus formation nutrients recycling and dynamics of the ecosystem together with many Annelids and nematodes living in the sediment. The samples of Ocypodid crab *Dotilla blanfordi* were randomly collected on monthly intervals for the period of March 2007 to May 2008 from supra tidal zone of two different shores of Karachi coast i.e., Clifton and Korangi Creek. Sampling of crabs was determined by quadrature method. Sex ratio, population structure and ecology of these crabs were determined. For statistically analyzing the data 100 samples were taken randomly in each month for each species from the sample collected in ten quadrates from each sites and measured their carapace length (CL), carapace width (CW), chela length (ChL), abdominal length (AbL) and abdominal width (AbW). According to CW different size classes were made for each species sampled from all three studied sites. Significant seasonal variations were observed in the abundance of total crabs sampled at Clifton and Korangi Creek of Karachi Coast. Population structures of *Dotilla blanfordi* at all studied beaches were studied. The growth relationships between CL, CW, and AbW in all six species were also studied at Clifton and Korangi Creek.

INTERTIDAL MOLLUSCS FROM THE SANDY BEACH OF CLIFTON, KARACHI COAST

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The present study deals with the seasonal variation, species composition and diversity of molluscs (gastropods and bivalves) occurring on sandy beach of Clifton during thirteen months, using quadrature sampling method. Standard laboratory procedures were used to determine the total numbers total weight (animal+ shell weight), and dry tissue weight. A total of fort eight species of gastropods and twenty seven bivalve were collected during the present study. A greater number of animals were collected in the winter months than in the summer. Indices of diversity, richness, evenness and margalef index were computed. The highest values of Shannon Weiner diversity index, species richness, species evenness and margalef index were noted in winter season. Values of species diversity indices showed seasonal variation.

AN OVERVIEW OF POTENTIALLY OCCURRENCE OF HARMFUL ALGAL BLOOMS (DINOPHYCEA), SEASONAL DISTRIBUTION AND ENVIRONMENTAL EFFECTS IN KARACHI COASTAL WATERS OF PAKISTAN

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An emerging threat to the marine ecosystem is from the harmful algal blooms or red tides that caused sea food poisoning of fish, birds and shellfish. Some of these species produces potent neurotoxins. Harmful algal blooms are presents throughout the coastal areas of Arabian sea and some of them are associated with massive fish killings along gulf regions. *Noctiluca scintillan* is the most frequent HAB in Pakistan waters. Very little information about the microbial communities in Pakistani regions, the occurrence of some of harmful algal species which can have serious human health consequences (sea food poisoning syndrome), impact on fisheries potential, desalination plants but also reflect environmental pollution or even climate change. The phytoplankton samples were collected from Manora Channel during May 2002-July 2003 and examined under light microscopy by using Utermohl methods. Taxonomic techniques (Light, scanning and fluorescence microscopy) were used to critical examination the morphological characters of the planktonic community. A number of the toxic and harmful algal species were identified first time in this region including, *Gambierdiscus*, *Cochlodinium fulvescense*, *Prorocentrum faustaie*, and *Prorocentrum*, *Alexandrium*, *Dinophysis*. Only *Gyrodinium* sp displayed very high abundance (48,166 cells/L) for the first time in this area during October 2002. This species also reported in same range of the other Gymnodinales species which have caused fish-killing and damaged aquaculture industries in Gulf regions during past years. The abundance of other dinoflagellate species such as *Ceratium furca*, *C. fusus*, *Protoperidinium steinii*, *Pyrophacus steinii* and *Scrippsiella trochoidea*, *Cochlodinium fulvescense*, *Prorocentrum* species and toxic producing species such as *Alexandrium ostenfeldii*, *Gonyaulax spinifera*, *A. tamarensis* and *Dinophysis caudata* were recorded from 100 cells/L to 6000 cells/L. These species showed significant positive correlation with chlorophyll a $\mu\text{g/L}$ and negative correlation with salinity, temperature and DO, pH at both sites. Most important species are ichthyotoxic, hepatotoxic, okadaic acid producers and saxitoxin producers which can cause the fish/ shellfish mortality in this coastal region. Pakistan has limited shellfish harvest and export, but it is imperative to monitor any possible impact of these HAB species on fisheries products and thereby affecting economy of Pakistan.

A NOTE ON EPIZOIC SPECIES OF STARFISH: ANTHENEA RUDIS KOEHLER, 1910 (ASTEROIDEAN: OREASTERIDAE) FROM PAKISTANI WATERS

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During a routine survey of the coast on 20th Dec 2011 , a large specimen of starfish *Anthenea rudis* Koehlar , 1910 belonging to family Oreasteridae was collected from Buleji (L 24° 50' 12N 66° 49' 12E) . A species of a marine insect and a copepod which were starfish epizoic,

were also identified and studied. This type of association is reported first time from Pakistan. A brief account of each species with a note on their association is given in the present paper.

BIOECOLOGY AND POPULATION DYNAMICS OF DEEP SEA COMMUNITIES OF THE NORTHERN ARABIAN SEA

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There was a heating of surface layers during January to June (22-29°C) and gradual cooling of sea water (12-13°C) upto 300-500 m depth. The general pattern of D.O. distribution was from 4.0-6.0 ml/L., highest values were recorded at shallow coastal waters which lowers down to 0.5-0.1 ml/L at 300-500 m depth. In the subsurface waters there was a significant fluctuation in the salinities (35.9-36.8‰), due to evaporation and precipitation. In mid 100-300 m depth it ranged around 36-36.4‰ and decreased to 36.0-35.8‰ at 500 m depth. The higher salinities are due to influence of gulf water which is receding into the Arabian Sea twice daily due to high and low tide. Four species of euphausiids, *Euphausia distinguenda* (67.431%), *E. diomedae* (25.025%); *E. gibboides* (6.093%) and *Pseudophausia latifrons* (1.45%) are found significantly distributed in the northern Arabian Sea. These have one and half to two year of life span, with an average growth rate 0.7 to 1 mm per month, mature in one year or less than one year at 7 to 12 mm T.L., spawn and continue to spawn throughout the year in this tropical and sub tropical climate which also resulted in continuous recruitment. Peak breeding season of *E. distinguenda* and *E. gibboides* is during 13 February to 6 March, *P. latifrons* from 8 March to 15 May, while *E. diomedae* more or less breed evenly from February to June. *E. diomedae* and *E. gibboides* live upto 2000-3000 m depth and ascend to surface layers during night. Three species of *Stylocheron* and one of *Sergestus* also live in deeper waters but do not ascend even to mid depths. *E. distinguenda* and *P. latifrons* live in shallower 100-200 m depth and ascend to surface layers during night.

POPULATION BIOLOGY OF THE *PENAEUS INDICUS*, FROM THE SONMIANI BAY, PAKISTAN

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The extant work was designed to study the population biology of *Penaeus indicus* (Juvenile: 914; Female: 529; Male: 281) were collected from the Sonmiani Bay lagoon, Balochistan. The sex ratio showed that females were found more abundantly than males during the throughout study period. The size of *P. indicus* females (13.10±1.24) was significantly larger than males (12.01±1.10). The relationship of total length (TL) to wet weight (Wt.) and carapace length (CL) of juveniles showed the negative allometry while females and males showed the positive allometry. The values of the exponent b in the length-weight relationships (LWRs) $W = aL^b$ showed significant difference in the Females (2.972) and Males (2.948). This study will be beneficial as baseline information source regarding improvement in Pakistan shellfish industry.

**A SIPUNCULAN *THEMISTE* (*THEMISTE*) *HENNAHI* GRAY, 1828
(GOLFINGIIFORMES:THEMISTIDAE) FIRST REPORT FROM PAKISTAN**

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Sipunculans are exclusively marine unsegmented worms. Single specimens of Sipunculan *Themiste* (*themiste*) *hennahi* Gray, 1828 is collected from Sandspit (Lat. 24° 50' 24" N Long. 66° 49' 12" E). This is the first report of the species collected along the northern Arabian Sea (Pakistan). The species at hand is briefly describe and illustrated with a note on its distribution, ecology and habitat.

**DIVERSITY OF MACROFAUNAL COMMUNITIES INHABITING SANDY BEACH ON
THE WEST BAY OF ORMARA BALOCHISTAN, PAKISTAN**

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Marine invertebrates are widely distributed in intertidal and submerged areas. Intertidal areas have rich biodiversity and serve as a food resource for many species, such as, birds, fish, etc. Pakistan borders the Arabian Sea with a sizeable coastline of about 1120 km, including 348 km of Sindh coast and 772 km in the Balochistan coast. Diverse habitat along the coast provides niches for a wide variety of fauna and flora. For the analysis of diversity of macrofauna on the west bay of Ormara, samples were collected using quadrat (1m²) at low tide during June and December, 2014. Sediment sample taken from each quadrat were sieved to recover macrofauna, Animals were preserved in 10% formalin and stored for further analysis. A total of 24 species of various taxa (gastropods, bivalvia, polychaetes and echinoderms) were found of which gastropods were highly abundant as compare to other macrofauna.

**ALLOMETRIC RELATIONSHIPS OF BLOOD COCKLE, *ANADARA ANTIQUATA*
(FAMILY: ARCIDAE) COLLECTED, (SONMIANI BALOCHISTAN) FROM THE COAST
OF PAKISTAN (NORTHERN ARABIAN SEA).**

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Handpicked 1078 individuals of *Anadara antiquata* were collected from the muddy swamps of Sonmiani Bay from May 2010 to April 2011. The shell length (SL) was ranging 18-99mm and weighing 2-52g recorded. The data was used to estimate allometric relationships of various morphological parameters. The derived equations for various morphological relationships indicated negative allometry between shell length versus width, height, weight, width versus height and wet tissue versus shell weight. The coefficient of determination R^2 indicated strong linearity (>0.78) in length versus width and height whereas rest of the parameters indicated weak linearity (<0.001). The environmental stress during the tidal fluctuations was calculated with the condition

factor (K) 0.01 \square 0.5 and relative condition factor (K_n) 0.2-2.6. The outputs of the study would help in planning and management of an important bivalve species.

Parameters	N	a	b	CI of b	R2
Length/Width	1079	-0.74	1.07	1.03	0.78
Length/Height		1.98	0.46	0.44	0.86
Length/shell wight		2.58	-0.01	-0.08	0.0001
width / height		2.57	0.33	0.32	0.66
Wet tissue/Shell weigh	2.45	0.13	0.07	0.02	0.19

SPOILAGE AND SHELF LIFE OF PENAEID SHRIMPS STORED UNDER TWO DIFFERENT COOLING CONDITIONS (-18°C and 0°C) FROM THE COAST OF PAKISTAN.

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In the seafood's, shrimps are the most important item along with fish, crabs, lobsters and crayfish on the coast of Pakistan. Shrimps are considered as a highly perishable food item and having a very short shelf life and spoil very quickly, their shelf life depends on the initial quality as well as the storage conditions under which it's kept. Microbial growth or activity is responsible to spoilage of fresh shrimp species during the improper handling and inadequate processing. The spoilage rate of shrimp also depends on several factors, the most important of these are temperature, processing and atmospheric conditions during storage. When shrimps are stored at low temperatures, consequently, the shelf life of shrimps is markedly extended because microbial activities are greatly influenced by temperature. The present study has been designed to describe and enumerate the type and number of microorganisms (mainly bacteria and fungi) that are involved in spoilage of the penaeid shrimps at different levels of handling; boat or trawler, mole holders or middle men, Karachi fish harbor, local retail markets and stored at two different temperatures of -18°C and 0°C. This study will be beneficial as baseline information source on some problems being faced regarding to improvement of shelf life of penaeid shrimps in Pakistan shellfish industry.

PLANKTONIC TINTINNIDS COMMUNITY IN THE EAST INDIAN OCEAN

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Planktonic tintinnids community structure was investigated during the spring inter-monsoon in the eastern India Ocean. There was stratification in the vertical characteristic of

temperature, salinity and potential density and deep Chl *a* concentration maximum during the depth of 50-100 m. A total of 131 species belonging to 34 genera were encountered, of which cosmopolitan genera and warm water genera were dominant. Tintinnids ranged from 193 ind./m³ (I206) to 2983 ind./m³ (I106A) in abundance, from 0.99 µg C/m³ (I206) to 14.75 µg C/m³ (I712) in biomass. Notable relationship was not found between species richness and integrated Chl *a* concentration. Tintinnids diversity matrices including taxonomic and morphological diversity were negatively and significantly correlated with the depth of maximum Chl *a* concentration. There was positive relationship between taxonomic and morphological diversity.

ASSESSMENT OF BEACH-CAST REMAINS OF CETACEANS FROM THE COAST OF PAKISTAN

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Marine mammals occurring in Pakistani waters have been largely ignored except for a few reports based on information obtained from fishers and sporadic stranding reports despite that the importance of marine mammals in the marine environment is well understood and documented. Study of cetacean remains and stranded animals provide useful information on the distribution of cetaceans in the area and possible threats that these animals face in the local waters. Extensive beach surveys were conducted along coastal beaches of Pakistan during 2005 to 2013. Cetaceans belonging to five families of order Cetacea, were recorded, including Delphinidae, Ziphiidae, Physeteridae, Balaenopteridae and Phocoenidae. The skeletal materials are archived at the Center of Excellence in Marine Biology (CEMB), University of Karachi. A total of 59 stranded animals including 32 cetaceans were recorded as dead along Sindh coast and 27 dead strandings from Balochistan; including 17 strandings of five whale species and 22 stranding of three dolphin species, 11 unidentified delphinids and 9 strandings of finless porpoises. The data on strandings and skeletal remains confirm a total of 16 cetacean species (7 whales, 8 dolphins and one porpoise) from Pakistani waters. Assessment of unidentified beach cast remains or spotted as dead strandings may possibly add to the list of cetacean species from Pakistani waters.

SEASONAL VARIATION IN OCCURRENCE OF HEAVY METALS IN *PERNA VIRIDIS* FROM MANORA CHANNEL OF KARACHI, ARABIAN SEA

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Seasonal sampling of the edible green mussel *Perna viridis* of two different sizes 6-8cm (n=100) and 4-6cm (n=100) from *Manora channel of Karachi*, were analyzed for their total Hg, Pb, Cu, Ni, Zn, Co, Fe and Mn concentrations. There are large seasonal variation in the metal concentration were found in green mussel *Perna viridis*. The high accumulations of metals were found mostly in small size 4-6 cm mussels as compare to large. The present study has confirmed that green mussels have greater capacity for accumulation of metals (Pb, Cu, Zn, Co, Fe Mn, Ni and Hg). The concentrations of Fe was highest compared to other heavy metals. The results of

heavy metals are in the following descending order of concentration: Fe > Mn > Zn > Pb > Cu > Ni > Co > Hg. It is supposed that in Manora channel water high input of metals and other inorganic and organic substances are coming in the form of industrial and domestic wastes need to monitor on a regular basis.

DISTRIBUTION AND ABUNDANCE OF *OITHONA PLUMIFERA* (COPEPODA: CYCLOPOIDA) IN THE COASTAL WATERS NEAR KARACHI, PAKISTAN

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The genus *Oithona* is a small sized copepod and distributed throughout the world. Their ecological habitat comprises of estuarine, pelagic and Coastal environment. *Oithona* plays a vital role as a food source for other copepods, chaetognaths, fish larvae etc. *Oithona plumifera* has been reported earlier from the coastal waters of Pakistan but the distribution and abundance was not reported. The aim of this study is to evaluate the distribution of *Oithona plumifera* in the coastal waters of Pakistan. The samples for the study of *O. plumifera* were collected from the three selected stations. *Oithona plumifera* was present in all the samples collected from station S2 and was least abundant at station S3. The highest density of *O. plumifera* was also recorded at station S2.

4. PALAEOLOGY

SOME NEW REMAINS OF *MIOTRAGOCERUS GLUTEN* FROM THE MIDDLE MIOCENE OF THE SIWALIKS OF PAKISTAN

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New material attributed to *Miotragocerus gluten* from various localities of the Chinji Formation of the Siwaliks of Pakistan is described and discussed. The Chinji Formation is Middle Miocene in age and has chronological age from 14.2-11.2 Ma. The identification of the newly discovered dental material is based on the morphometric features of the species. The described material comprises both upper and lower dentition. The new material will provide better understanding of the systematics and morphology of the dentition of the described species.

REVIEW OF MIDDLE MIOCENE RHINOCEROTIDAE AND DESCRIPTION OF NEW MATERIAL FROM SIWALIKS OF PAKISTAN

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New and unpublished rhinocerotid remains from different localities of Chinji Formation have been described and discussed in this paper. The identified species include *Gaiotherium browni*, '*Procoelodonta*' (*Pasalarhinus*) *tekkayai*, *Hispanotherium matritense*, *Caenotodon oettingenae*, *Alicornops laogouense*, *Brachypotherium perimense*, *Brachypotherium brachypus*, *Brachypotherium fatehjangense* and *Chilotherium intermedium*. The discovery of '*Procoelodonta*' (*Pasalarhinus*) *tekkayai* and *Brachypotherium brachypus* from the Lower Siwaliks of Pakistan has increased the geographic and stratigraphic distribution of these species and has created the geographic associations between the Siwaliks of Potwar Plateau in Pakistan and other Asian and European regions. The increasing trend of hypsodonty in the recovered dentition of the middle Miocene rhinoceros species indicates the warm and humid climate.

HIPPOTAMUS (MAMMALIA, HIPPOPOTAMIDAE) FROM THE PINJOR FORMATION OF BHIMBER, AZAD KASHMIR, PAKISTAN

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The Siwalik Plio-Pleistocene faunas are characterized by the sporadic presence of hippopotamid taxon, referred to the genus *Hippopotamus* and the species *H. sivalensis*. Due to

rarity its morphological characters are incompletely known. Recently excavated hippopotamid material in the Lower Pleistocene locality of Bhimber (Azad Kashmir, Pakistan) includes almost complete dentary that provides a better knowledge of the taxon's morphology. *Hexaprotodon sivalensis* appears for the first time in the Plio-Pleistocene of the Siwaliks and the species is established wide geographic range extending from the Siwaliks to Eurasia in a very short interval. The best record of *Hexaprotodon sivalensis* is found in the Siwaliks of Pakistan and India. *Hexaprotodon sivalensis* is apparently restricted to Pliocene of the Siwaliks. The rare occurrence of the species in the Siwaliks is noted at Mio-Pliocene boundary, at 5.3 Ma. The studied material extends the biostratigraphic age of the species from the Tatrot Formation Pliocene to the Pinjor Formation Bhimber Pleistocene of the Siwaliks.

DORCATHERIUM (MAMMALIA: TRAGULIDAE) FROM SIWALIK LATE MIOCENE OF PAKISTAN

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Dorcatherium is represented by four species in the Siwaliks of Pakistan: *D. majus*, *D. minus*, *D. nagrii* and *D. minimus*. Out of four, the three species *D. minus* (dominant), *D. majus* and *D. nagrii* have been described in this study. *Dorcatherium* is abundant in the Lower and Middle Siwaliks and almost absent in the Upper Siwaliks. The new tragulid fossils have been reported from the Late Miocene of the Dhok Pathan and Nagri formations of the Middle Siwaliks. The Dhok Pathan Formation is characterized by cyclic alluvium deposition and the Nagri Formation is dominated by sandstone. The scope of the study to add new anatomical features of the genus. The humid environment can be inferred for the Siwalik Late Miocene.

COMPARATIVE STUDY OF THE BOVID (MAMMALIA: BOVIDAE) UPPER PREMOLARS FROM THE SIWALIK OF PAKISTAN

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New dental elements containing upper premolars of the bovids have been recovered from the four sites of the Siwaliks namely Chinji, Dhok Pathan, Hasnot and Padri, situated in the Punjab province, Pakistan. These fossils belong to the Lower and Middle Siwaliks ranging from Middle Miocene to Middle Pliocene (14.2 – 3.4 million years ago). On the basis of the comparative morphology and measurements, the material assigns to *Gazella* sp., *Gazella lydekkeri*, *Elachistoceras khauristanensis*, *Tragoportax salmontanus*, *Tragoportax punjabicus*, *Selenoportax vexillarius*, *Selenoportax lydekkeri* and *Pachyportax latidens*. The fossils provide evidence for the existence of diverse Middle Miocene to Middle Pliocene bovid faunas in Southern Asia. The comparative morphological and metric study of the different bovid species gives interesting information about the size variation within genera and intra-specific level. The Middle Miocene to

Middle Pliocene of the Siwaliks was considerably presented mosaic palaeoenvironment.

**REDISCOVERY OF *PROTANANCUS CHINJIENSIS* (MAMMALIA: PROBOSCIDEA)
FROM CHINJI FORMATION OF PAKISTAN**

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New *Protanancus* remains from the Middle Miocene Chinji Formation of Pakistan are described and discussed in this study. The Middle Miocene proboscideans especially *Protanancus* are very rare and every new finding contributes to their better knowledge. The new material assigns to *Protanancus chinjiensis* and it is reported first time from the locality as well as after the ascription of this species, which was erected by Tassy in 1983. The material originates from Kanati (near Kanhati Garden), situated in the district Khushab, Punjab, Pakistan. The outcrops belong to the Middle Miocene in age. The Chinji Formation can be identified easily on the basis of lithology by having bright red clay. The locality is well known because the presence of the Middle Miocene rhinocerotids and artiodactyls. The mammalian fauna of the locality share many common species with the other Middle Miocene localities of the Siwaliks.

**NEW MAMMALIAN FOSSILS FROM THE DHOK BUN AMIR KHATOON OF THE
LOWER SIWALIKS, PUNJAB, PAKISTAN**

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The research work includes the collection, description, comparison and discussion of the mammalian remains from the outcrops of the Dhok Bun Amir Khatoon, district Chakwal, Punjab, Pakistan. The outcrops belong to the Lower Siwaliks having age of the latest Middle Miocene. The specimens include isolated dentitions, mandible fragments and horn cores. The five mammalian genera *Gazella*, *Dorcatherium*, *Miotragocerus*, *Listriodon* and *Conohyus* are identified. This study strengthens the idea that many species of the mammals are found in the Lower Siwaliks during the latest Middle Miocene. The new specimens contribute knowledge about the Siwalik mammals.

**NEW COLLECTION OF *CONOHYUS SINDIENSIS*, A MIDDLE MIOCENE SUID, FROM
CHAKWAL, PAKISTAN**

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New fossils have been collected from Middle Miocene localities of Lawa, Kallar Kahar and Dhok Bun Ameer Khatoon. *Conohyus sindiensis* is very common suid in the Chinji type area of

Pakistan. Its biostratigraphic range varies between Kamlial type area to the base of Nagri Formation. The probable time range of *C. sindiensis* is from 15 to 11 million years. The sample comprises isolated molars. The morphometric description of these specimens will add new information regarding molar morphology as well as the biogeographic distribution of *Conohyus sindiensis*.

TRAGULIDS (RUMINANTIA: TRAGULIDAE) FROM THE MIDDLE SIWALIKS OF PAKISTAN

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New remains of tragulids are identified, described and discussed in this study. The specimens are collected from the Late Miocene sites of the districts Chakwal and Jhelum in the Punjab province, Pakistan. The studied sites include Dhok Pathan and Nagri of the Chakwal district and Padhri of the Jhelum district; these localities are well known for the Late Miocene mammalian fauna. The material represents two tragulid species *Dorcatherium majus* and *Dorcatherium minus*. The recovered tragulid fossils contribute knowledge about the Late Miocene - Early Pliocene tragulids of the Siwalik continental deposits. The Middle Siwalik deposits were formed under swampy and littoral palaeo-environmental conditions.

ANALYSIS OF DENTAL ENAMEL HYPOPASIA AS A STRESS MARKER IN MIDDLE MIOCENE SIWALIK ECOSYSTEMS OF PAKISTAN

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Studies on dental enamel hypoplasia have been used by different palaeontologists as an indicator of stress in evolution of palaeoenvironments. The present study involves the analysis of two giraffid species that were present in the Middle Miocene Chinji Formation of the Siwaliks of Pakistan. The Chinji Formation is Middle Miocene in age and has chronological age from 14.2-11.2 Ma. The results of present study indicates that two giraffid species namely, *Giraffa Priscilla* and *Giraffokeryx punjabiensis* that first appeared and persisted during Middle Miocene Chinji Formation, have dental enamel hypoplasia on lingual as well as buccal side of their fossilized premolars and molars. Enamel hypoplasia is a tooth malady which is mainly caused by the deficiency of food/nutrients. The feeding deficiency is directly linked to the physiological or environmental stress. Enamel is the hardest tissue of body and marks of enamel hypoplasia remain unaltered even during fossilization so presence of enamel hypoplasia in these species indicate significantly the presence of an environmental stress during Middle Miocene that had a key role in evolution and speciation of Siwalik fauna.

5. WILDLIFE, DIVERSITY AND CONSERVATION

BIODIVERSITY LOSS DUE TO ROAD ACCIDENTS

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With the massive increase in population the risk for wildlife to become extinct has increased many folds. Population explosion has resulted in destruction of habitats, reduction of food sources and increase in traffic and roads. This ultimately caused the road accidents resulting in loss of wildlife at considerable rate. A large number of animals die due to road accidents every year. In order to minimize road kills it was very important to know the causes of road accidents. I took a road of 30 KMs and divided it into divisions on the basis of road side which would influence the rate of road accidents of wildlife as agricultural area, residential area, bus stops etc. I recorded the number of road kills on each division. It has been found that road accidents can be reduced by minor efforts like public awareness, reduction in deforestation, fencing of roads and crossings for wildlife.

INVESTIGATION OF POTENTIAL SYNERGIES BETWEEN BIODIVERSITY AND FARM PRODUCTION SUSTAINABILITY

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Agriculture is by far the significant important sector for Pakistan's economy and 99% of exports are directly or indirectly based on agricultural commodities. It not only provides employment opportunities to more than 60% of the country's population but also food to more than 162 million people and supports earning of foreign exchange. Land resources relative to population are limited and have declined over current decades. The hypothesis that if agro-ecosystem is modeled after natural ecosystem, the agro-ecosystem should exhibit many functional attributes and processes that stabilizes the system. A detailed study was conducted to determine floral and invertebrate faunal diversity of the cropland. The relationship of the various synchronous and synergistic taxa of crop fields were estimated along with system cost effectiveness. Five different crop-weed complexes, including Wheat, sugarcane, cotton, Rice and fodder and 39 weed species present in these crops were sampled throughout study duration. A total of 83,543 specimens representing 736 species were captured from cropland of different zones e.g., Mixed-crop zone (MCZ), Cotton-wheat zone (CWZ), Rice-wheat zone (RWZ). All of these species belonging to nine different orders namely, Microcoryphila, Opisthophora, Haplotaxida, Aranae, Chilopoda, Odonata, Orthoptera, Mantodea, Dermaptera, Blattaria, Hemiptera, Coleoptera, Diptera, Lepidoptera, Hymenoptera, Eupulmonata, Neuroptera, Isopoda, Collembola and Pulmonata. Whole fauna was classified into groups on the basis of their trophic status in the field like Prey/Pest, Predators, Omnivores, Detritivores or Scavengers. Prey/pest having herbivore feeding nich were 57.60% and was most dominant group. Predators were second highest group in total cropland fauna with its

total percentage 31.8 with maximum contribution of fodder crops as 8.78% followed by sugarcane 6.97%. The comparison of these systems revealed MCZ (Mixed-crop zone) wheat farms as best with respect to crop production and net biomass production, whereas, recyclable biomass (weeds+ invertebrates) was highest in RWZ. These investigations of these synergies may effectively be utilized for sustainable agro-ecosystem.

DISTRIBUTION AND HABITAT USE OF AVIAN FAUNA OF RAWALAKOT CITY AND ITS SURROUNDING, AZAD KASHMIR, PAKISTAN

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Distribution and habitat use of avian fauna of Rawalakot city and its surrounding was studied from March 2006 to February 2007. The study area was situated in the center of district Poonch. For the convenience of data collection the study area was divided into six study site and systematic surveys were conducted at each site. Direct sighting and indirect evidences were collected from all these sites. A total of 78 bird species was recorded belonging to 32 families and 11 orders. The percentage observation of all the species represent that Passeriformes (83.73%) was dominant order in study area followed by Columbiformes (3.40%), Cuculiformes (2.1%), Apodiformes (1.79%), Psittaciformes (1.7%), Coraciiformes (1.32%), Piciformes (0.76%), Upupiformes (0.31%), Ciconiiformes (0.24%), Strigiformes (0.12%) and Galliformes (0.096%). The habitat destruction due to deforestation, agricultural activities, infrastructure and land sliding and disturbances by the increasing human population were major threats to the avian fauna of Rawalakot.

STUDIES ON GROWTH AND BIOMETRIC TRAITS IN WILD TURKEYS (*MELEAGRIS GALLOPAVO*) FED ON DIFFERENT DIETARY REGIMENS

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Present study on growth performance of wild turkeys (*Meleagris gallopavo*) fed on different dietary regimens was conducted at Captive Breeding Facilities for Birds, Department of Wildlife and Ecology, Ravi Campus, University of Veterinary and Animal Sciences, Lahore. Day-old chicks of *M. gallopavo* were purchased from the market and were arranged into four different groups. Each group was considered a separate treatment and was fed with diets having varying levels of crude protein (CP). The birds in treatment 1 was fed with diets having CP level 16%, treatment 2 birds were fed with diets having CP level of 18%, treatment 3 birds were fed with CP level of 20% and treatment 4 birds were fed with diets having 22% CP. Each chick was weighed and its external body measurements viz. body length, wing length, wingspan, beak length, tarsus length, thigh length, body girth and shank length were measured at the initiation of experiment and thereafter increase in these parameters were recorded on weekly basis. Maximum growth was observed in treatment 4 birds while significantly lower weight gain was observed in birds fed with diets with CP level of 16%.

**BASELINE INFORMATION ON SPERMATOGENESIS OF *PIPISTRELLUS KUHLII*
CAPTURED FROM FAIASLABAD**

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Bats play a very important role in cleaning our ecosystem, performing a vital ecological role of pollinating flowers and dispersing fruit seeds. The kuhl's pipistrellus (*Pipistrellus kuhlii*) one of the commonest and widely distributed bat species in Pakistan. Little is known about the reproductive biology of this bat. The breeding season is however not well defined. Authentic work for studying bats has not been conducted in Pakistan. The present study was designed to investigate the actual period when male bats were reproductively active, to see it's high and low spermatogenic activity. For this purpose, a total of 18 bat species of *P.kuhlii* were captured from different areas of Faisalabad at different season from August 2012-March 2013. Morphometric measurements confirmed the collected samples were exactly the male *P. kuhlii* Histology of the male reproductive organ testes was studied to explore various stages of spermatogenesis using different histological techniques, Fixation, Dehydration, Sectioning, Staining, and preservation. Histometric measurements were also made by image J analysis. All values were taken as Mean±SE. By using one way ANOVA and Fischer LSD the obtained data was analyzed, which showed highly significant values of all parameters, $P<0.005$. In this study, morphological parameters (testis weight, body weight and position of testis) and histological parameters (length and width of testis, length and width of seminiferous tubules, lumen area of seminiferous tubules) were observed, which showed lowest values of all parameters except lumen area, from December to February, which was non spermatogenic period. In August, parameters showed low spermatogenic activity. In October all parameters were at their maximum value, which showed the peak spermatogenic period

**COMPARATIVE STUDIES ON BEHAVIORAL ASPECTS AND MILK COMPOSITION
OF WILD BORN AND CAPTIVE BORN CHINKARA, *GAZELLA BENNETTII* IN
CAPTIVITY**

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Present study on captive management and milk composition of Chinkara (*Gazella bennettii*) was conducted at Captive Breeding Facilities for Ungulates, Ravi Campus, University of Veterinary and Animal Sciences, Lahore. The animals were captured from His Highness Sheikh Zayad Wildlife Park, Rahim Yar Khan, Punjab, Pakistan and kept in captivity in two animal enclosures. These captive facilities covered 200 × 100 feet (length × width) free fenced area, each provided with 20 ft × 20 ft well ventilated room for shelter and separate facilities for drinking water and fodder. The behavioral parameters observed during present study include resting, standing alert, locomotion, feeding, drinking, ruminating, tail pasting, urinating, environmental sniffing, ano-genital sniffing, affiliative interaction and agnostic interaction. Non-significant differences were observed in captive born and the individuals born in semi-wild environment. This may be due to the enclosure design and the time, the wild born populations spent in the animal enclosures prior to this study. The milk composition of Chinkara showed that its milk contains 12.15% fats, 10.62%

solids not fats (SNF), 6.68% protein, 3.58% lactose and 1.03% solids. Average freezing point of milk is 56.88°C and density is 31.55. Three species of endoparasites identified from fecal matter of Chinkara include *Fasciola hepatica*, *Haemoncus conotortus* and *Nematodirus spathigers*.

INHIBITING ROSE-RINGED PARAKEET (*PSITTACULA KRAMERI*) USING THE WIND POWERED HAWK EYE ROTATOR (WPPER) AT THE MATURE AND POST HARVEST STAGE OF WHEAT AND MAIZE IN AN AGRO-ECOLOGICAL SYSTEM IN FAISALABAD, PAKISTAN

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The Rose-ringed parakeet (*Psittacula krameri*) undoubtedly, a serious kind of pest among the cultivations in Pakistan, causes a substantial damage to both the cropped and horticultural interests. The depredation annually brings about economic losses to the farmers and the country, in particular in the guarded situations. The present research is, therefore, aimed at devising a sustainable strategy at the mature and post harvest stages of wheat and maize through the use of wind powered hawk eye rotator which is a bird scaring device. Study was conducted during evening hours, the time when the infestations are far more by the birds on the cropped areas. Present information on the foraging profiles of the rose-ringed parakeet in the unguarded situations. The unguarded situation is the one in which there is no protection to the field crops. The observations were made for five continuous days, while for the next five days, the protection was provided in the form of the hawk eye rotator. It was evident that in the earlier stages, very few parakeets visited the field crop. However, with the passage of time they kept on increasing. Same situation was with regard to leaving the parakeet from crop. The guarded situation using the hawk eye rotator, placed in the center of the field, for inhibiting the rose-ringed parakeet movements, sufficiently, reduced the visitation in the field. It meant that, the installation of a mechanical device, primarily considered as a bird repellent, proved to be advantageous to the field crop. The movements were there but fairly reduced as compared to the one in the unguarded stage. The damage intensity also reduced substantially. It is also worth pointing out that the number of visits of parakeet to the field following the application of the wind powered hawk eye, also reduced. There is an utmost need to reduce its damage by using the non-chemical devices, so, that efficient and environment friendly management can be achieved.

DISTRIBUTION AND POPULATION STATUS OF HIMALAYAN MONAL PHEASANT (*LOPHOPHORUS IMPEJANUS*) IN SALKHALA GAME RESERVE, NEELUM VALLEY AZAD JAMMU AND KASHMIR (PAKISTAN)

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The Himalayan monal pheasant (*Lophophorus impejanus*) is a relatively large size bird within the family Phasianidae. Being a mountain pheasant, it is distributed in different mountainous

areas of Kashmir. Present study was designed to determine distribution and population status of this pheasant in Salkhala Game Reserve, district Neelum, which is situated along ceasefire line between Pakistan and India, and consequently cross border conflicts prevented the completion of any field study during the past many years. The Game Reserve is considered as Endemic Bird Area in the Neelum valley within Western Himalaya. For data collection on distribution and population of this pheasant, study surveys were conducted from March 2014 to September 2014. Study area was divided into four localities with eleven sub-localities and systematic surveys were conducted in each sub-locality. To check the distribution of Himalayan monal in the study area, thorough surveys of the study area were conducted for collecting the evidences of presence of this bird. Direct and indirect evidences noted during the surveys included direct sighting, fresh ground scratching, feathers, fecal material, foot prints, information collected from hunters, locals and wildlife staff. Population of the bird was estimated by line transect method. Site selection was made on the basis of information collected from local people, hunters, shepherds, and wildlife staff. Total sixty six transects were walked in the habitat of Himalayan monal. Transects were surveyed early in the morning (5:00-9:00am) and half an hour before evening, when this bird is more active. During the survey, sighting time, group composition of the detected birds, geographic coordinates, elevation, aspect, slope, weather condition, presence of water bodies, and general habitat conditions were also recorded. The results indicate that this pheasant is distributed in all localities of the study area, preferring high altitude mountains with different aspect between 2300 and 2600 m above sea level (asl). The bird is distributed in the area where third stratum of the vegetation (grasses, herbs, ferns) dominates (63% of total vegetation). Based on transect length and width, and total bird encountered, population density of different localities were calculated. Overall population density of Himalayan monal in the study area was recorded as 2.52 birds/km². The highest density (3.67 birds/km²) was recorded in locality Dohre, followed by locality Chitta Pani (2.56 birds/km²), Tehar Chhamman (2.4 birds/km²) and Khikhri (2.17 birds/km²). The sub-locality Dohre had highest population density (3.67 birds/km²) among eleven sub-localities.

PRELIMINARY INFORMATION ABOUT SPERMATOGENESIS OF GREATER ASIATIC YELLOW HOUSE BAT *SCOTOPHILUS HEATHII* (CHIROPTERA: VESPERTILIONIDAE) CAPTURED FROM FAISALABAD

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The greater Asiatic yellow house bat (*Scotophilus heathii*) is one of the commonest and widely distributed species in Pakistan. Little information is available about its reproductive biology and both sexes roost separately except during January to March, but its breeding season is not well defined yet. This study was designed to investigate the actual breeding period when male bats were reproductively active, to record the high and low spermatogenic activity. For this purpose, a group of 9 specimens were captured from different areas of Faisalabad at different seasons. Morphometric measurements of each specimen were recorded by digital Vernier caliper. Histology of male reproductive organ testes was studied to explore the various stages of spermatogenesis by using different histological techniques e.g. Fixation, Dehydration, Sectioning, Staining, and preservation. Histometric measurements were also made by using image J analysis. It was concluded from the whole study that during November, male were reproductively active and showed peak spermatogenic period. However, during September and October, excitation was low, and it turns

down during February and March. Results of one way ANOVA and Fischer LSD test were significant ($P < 0.001$) for all the parameters.

**MORPHOMETRIC DIFFERENTIATION BETWEEN TWO SNAKE HEAD SPECIES,
CHANNA MARULIUS AND *CHANNA STRIATUS***

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The aim of present research work was to evaluate the difference between morphometric and meristic characters of two snakehead species *Channa marulius* and *Channa striatus*. For this purpose, fish specimens of both species were purchased from a local fish market of Faisalabad. To check phenotypic variation between two experimental species, 12 morphometric characters (Total length, Standard length, Snout length/Pre orbital length, Head length, Eye diameter, Dorsal fin length, Pectoral fin length, Pelvic fin length, Anal fin length, Body weight, Body depth and head width) were measured and 6 meristic characters (Dorsal fin rays, Caudal fin rays, Pectoral fin rays, Pelvic fin rays, Anal fin rays and lateral line scales) were counted. The inferences of present research work showed that four morphometric characters; total length, standard length, dorsal fin length and anal fin length showed highly significant differences ($P < 0.01$) while total weight, pectoral fin length and snout length showed significant differences ($P < 0.05$) between two fish species. Six morphometric characters; head length, eye diameter, pelvic fin length, body depth and head width did not show significant difference ($P > 0.05$) between both species. In case of meristic characters, dorsal fin rays, pectoral fin rays, anal fin rays and lateral line scales were significantly different ($P < 0.01$) while pelvic fin rays and caudal fin rays were equal in both species.

**APPARENT DIGESTIBILITY OF ARTIFICIAL DIETS FOR CHINKARA GAZELLA
*BENNETTII***

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Apparent nutrient (moisture content, crude protein, crude fat, crude fiber and ash) digestibility of artificial diets in combination with three types of fodders as barseem, lucern and sorghum was determined for *Gazella bennettii* (chinkara). All the animals were kept in three groups and each group consisted of three animals. Three combination of diets were used (diet 1: Barseem, black beans along with formulated diet) (diet 2: Lucerne, black beans along with formulated diet) (diet 3: Sorghum, Black beans along with formulated diet). Chromic oxide was added as an indigestible marker. The apparent dry matter digestibility was higher (81.13 ± 6.03) for diet 1. The crude protein digestibility was higher (29.23 ± 4.02) for diet 1 followed by diet 2 (23.08 ± 3.97) and diet 3 (19.42 ± 4.48). Crude fat digestibility was higher (9.58 ± 2.04) for diet 3 as compared to diet 1 (5 ± 9.06) and diet 2 (2.62 ± 0.70). Crude fiber digestibility was higher (18.5 ± 4.4) for diet 1 as compared to diet 2 (14 ± 4.08) and diet 3 (11 ± 4.54). The apparent ash digestibility was higher (49.5 ± 6.11) for diet 1 followed by diet 3 (37.83 ± 7.19) and diet 2 (33.25 ± 9.13).

COMPARISON OF DIVERSIFY INDICES OF AVIAN FAUNA AT MANGLA DAM, AJK**Bushra Nisar Khan¹ and Zulfiqar Ali^{2*}**¹*Centre for the Undergraduate Studies University of the Punjab, Lahore*²*Department of the Zoology University of the Punjab, Lahore**Corresponding Author, dralizulfiqar@gmail.com

To measure the avian diversity at Mangla Dam AJK a study from 2011 to 2014 was conducted. The study was planned to determine the seasonal occurrence, number of birds and threats to their conservation. Direct census method was used for data collection. During the course of study a total of 57896 birds of 187 species belonging to 60 families and 15 orders were recorded. The Maximum values of diversity indices were recorded in 2011 as Shannon-Weiner Diversity Index (H') 3.42 was recorded in 2011; Census Index (CI) was 218/Km²; Simpson's Diversity Index (D) was 0.95 and evenness and of birds was 0.65. According to ranking of occurrence status for the Mangla Dam 24 species were very abundant, 29 were abundant, very common were 67, fairly common were 22 and fairly common were 45. The season wise distribution of bird's species show that 21 percent were year around resident, summer breeder's were 19 percent, winter migrants were 40 percent and 20 percent were passage migrants. The most dominant species during study period recorded at study area were Common Pochard *Aythya ferina* (n=5011), Common Coots *Fulica atra* (n=4782), Little Cormorant *Phalacrocorax niger* (n=4764), Garganey *Anas querquedula* (n= 3863) Mallard *Anas platyrhynchos* (n=3841), Northern Shoveler *Anas clypeata* (n=3743) Tufted Duck *Aythya fuligula* (n=3393) Northern Pintail *Anas acuta* (n=3261) and Gadwall *Anas strepera* (n=2971). The major threats observed included illegal hunting, trapping, unsustainable fisheries practices, deforestation in dam catchment area and water pollution.

HABITAT USE OF MONAL PHEASANT (*LOPHOPHORUS IMPEJANUS*) IN SALKHALA GAME RESERVE, NEELUM VALLEY AZAD JAMMU AND KASHMIR (PAKISTAN)**Fatima Noor, Khawaja Basharat Ahmed*, Muhammad Saddique Awan, Riaz Aziz Minhas and Binish Ghulam Hussain***Department of Zoology, University of Azad Jammu and Kashmir Muzaffarabad.**Corresponding author's e-mail: kbamaknoo@yahoo.com

Monal Pheasant (Himalayan Monal, *Lophophorus impejanus*), a large, dimorphic mountain pheasant, belongs to genus *Lophophorus*, family Phasianidae in the order Galliformes. A study was carried out on the habitat use of Monal Pheasant in Salkhala Game Reserve, Neelum valley, Azad Jammu and Kashmir. Salkhala Game Reserve falls under Important Bird Area category, an Endemic Bird Area of Western Himalaya. Sum total of 15 surveys from March 2014 to September 2014 were conducted to collect Phytosociological data for habitat analysis of Monal pheasant. For the convenience, study area was divided into four main localities, which were further sub-divided into eleven sub-localities. Quadrature method was used to calculate important values of dominant and co-dominant vegetation of Monal habitat. Quadrates were laid down accordingly along each transect as per suitability and natural contours of the habitat. Trees were analyzed by using quadrature of 10×10m, while shrubs were sampled by 5×5m Quadrature and herbs were sampled by 1×1m quadrature and Phytosociological attributes (density, frequency, relative density, relative frequency) were measured in each case. The data were used to determine relationship of Monal pheasant population with various variables of habitat. Phytosociological habitat comparison of dominant and

co-dominant vegetation of eleven sub-localities of four localities showed that following plant species were common characteristic of each habitat. Kayal *Pinus wallichiana* (IVI=126.03) Deodar *Cedrus deodara* (IVI=125.84) Parath *Prunus percisa* (IVI=70.07) Loonrhe *Cotoneaster bacilaris* (IVI= 64.65) Barnh kantha *Indigofera gerardiana* (IVI=68.83) Peanthrean *Withania thaina sumenifera* (IVI= 61.22) Hola *Rumax hustatus* (IVI= 75.81) Gogun (IVI=77.45). A total of 59 species were recorded in study area including trees (n=12) shrubs (n=10) and herbs, ferns and grasses (n=37). The overall flora of the habitat was dominated by third strata of vegetation consisting of herbs, grasses and ferns (62.71%) followed by trees (20.33%) and shrubs (16.94%).

COMPARATIVE STUDY OF PARTICULATE MATTER FRACTION IN THE TRANSPORT MICROENVIRONMENT (BUSES) OF PAKISTAN AND UK

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Transport microenvironments can contain higher levels of particulate matter due to infiltration from the roads, vehicular exhaust and commuter's activities. Continuous exposure to elevated PM concentrations can lead to a variety of respiratory and cardiopulmonary disorders and people who travel frequently are therefore more at risk. The present study monitored PM, CO₂, CO, Temperature and relative humidity levels in Diesel-powered buses in Pakistan and United Kingdom. Two routes of almost same travelling distance were selected in Pakistan and UK and indoor air quality of the buses monitored to determine the exposure level faced by the commuters. The average levels of PM_{Total} during travel from Lahore to Islamabad was observed to be 249.96µg/m³ while PM_{Total} value was considerably lower i.e. 57.33µg/m³ in bus while travelling from London to Birmingham. While the observed levels in both countries were not in compliance with the WHO guidelines of 25µg/m³, level of particulate matter was much higher in Pakistan than the concentrations in UK.

HABITAT AND FOOD PREFERENCES OF LADAKH URIAL (*OVIS VIGNEI VIGNEI* BLYTH, 1841) IN GILGIT BALTISTAN, PAKISTAN

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Ladakh urial (*Ovis vignei vignei*) is a threatened wild sheep distributed in northern Pakistan. Gilgit Baltistan supports largest population of urial in Pakistan. The present study was conducted to determine habitat use and feeding preference of these urial in Gilgit Baltistan. The habitat use of urial was determined on the basis urial direct or indirect evidences (e.g. animal sightings, fecal pellets and hairs) in different habitats. Information on food consumption was

collected by using scan sampling technique and information collected from local peoples, hunters and shepherds (n=78). Feeding animals were scanned with the help of telescope and spotting scope. Ladakh urial preferred montane dry sub-tropical scrub zone habitat with 41.87% evidences of its presence followed by alpine meadows/alpine scrub zone (21.14%), sub-alpine scrub zone (13.41%), and dry temperate coniferous forests (8.54%). On the other hand, agricultural lands (3.24%) and dry alpine zone/permanent snowfield (3.66%) were the least preferred habitats of urials in Gilgit Baltistan. Although, the evidences of these urials were found in different habitats, but they generally preferred rocky terrain (48%) between 2600-3000 m elevations (above sea level) in montane dry sub-tropical scrub (41.87%), and alpine meadows and scrub zone (21.14%) with intermediate slope (51°-70°, 56%) along the southern aspects (40%). A total of 36 plant species were recorded to be consumed by the Ladakh urial in Gilgit Baltistan. Ladakh urial used *Artemisia maritima* (n=53) with 18.34% of observations followed by *Olea ferruginea* (n=28, 9.69%) *Ephedra intermedia* (n=25, 8.65), *Pistacia khinjuk* and *Ephedra gerardiana* (n=23, 7.69%). Out of total 36 plant species, 15 were consumed during summer (June to August), 10 in spring (April-May) and 6 in autumn (September-October) and 5 in winter (February-March). This vulnerable species has a significant role in the local economy by generating revenue through trophy hunting programs. The conservation of this species could be achieved by protecting potential habitat and preferred food plant species of urial.

STATUS OF LADAKH URIAL (*OVIS VIGNEI VIGNEI* BLYTH, 1841) IN GILGIT BALTISTAN, PAKISTAN

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Ladakh urial (*Ovis vignei vignei*) is an Endangered wild sheep present in small pockets of northern Pakistan including Gilgit Baltistan (GB). Field surveys were conducted in Ladakh urial habitat in GB during January to September, 2013. Data was collected through direct observations, using trail walks and vantage points, as well as by collecting information from local residents, shepherds, and hunters using a questionnaire. A total of 172 individuals were encountered from four main localities (Bunji, Nanga Parbat, Nagar, Skardu) comprising twenty four herds (mean size=7.17±3.19) with 1.91 animals/km² overall population density. Of these, 128 individuals could be classified as females (n=66; 38.37%) and males (n=62; 36.05%), while remaining 44 (25.58%) individuals including young (n=29) could not be sexually identified. Besides direct sighting minimum population (172 individuals), an overall estimated population comprising ca. 432 individuals (4.79 urials/km²) was also figured out based on local information as collected through questionnaires. Majority of the local peoples were well aware off about the urial, having very crucial information about its status in the area. The population of Ladakh urial, as reported by local community as well as observed during the study, has been declined during the last few decades due to illegal hunting (70 killings were reported during 2000-2013) and habitat degradation by increasing livestock population and extraction of wood, fodder and medicinal plants. Furthermore, its distribution range has also been shrunken and they have been extirpated from at least six previously known localities during the last decade. To ensure the continuous survival of existing

urial population, a comprehensive research based integrated strategy is highly required.

**ORNITHOLOGICAL MONITORING FOR A 50 MW WIND POWER PROJECT AT
THATTA, PAKISTAN**

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Ornithological monitoring of a 50 MW Wind Power Project at Jhimpir, Thatta, Pakistan was conducted during May and November, 2011 with the objectives to investigate any possible and potential direct and indirect threats to the resident as well as the migratory birds during construction and production phase of the proposed wind energy project. All the potential habitats around the project site were studied, an inventory of existing avian fauna was prepared and threats to the birds from proposed wind farm were investigated by interviewing different local residents. Ninety one avian species were recorded including 49 (53.8%) resident, 33 (36%) winter visitors, 8 (8.8%) irregular year-round visitors and one (1%) summer breeder. Two threatened species were also recorded including Pallas's Fish-eagle (*Haliaeetus leucoryphus*) and Egyptian vulture (*Neophron percnopterus*). Rests of the 89 species have Least Concern status according to IUCN Red list of Threatened Species. Twenty five species were representing agricultural habitats, 32 terrestrial/desert habitats and 34 aquatic habitats. None of the recorded bird species was found endemic to Pakistan. Since the proposed wind farm is located on main migratory route (Indus Flyway) of birds therefore, three types of possible threats to birds from the proposed wind farm were identified including; direct habitat loss due to development activities under the proposed project, collision risks of birds with rotor blades and displacement of birds from the area around wind turbines. However, it was concluded that the establishment of wind farm will have little impact on the resident as well as migratory birds and will not act as a barrier to the movement of birds. Secondly, learning process among birds is very fast and any change in the existing habitat is well noted by the birds and soon they adapt themselves according to the changing environment, habitat and climatic conditions. In practice, birds avoid flying through rotating blades and generally, are able to avoid collisions and do not simply blindly fly into the electric poles, wires and wind turbines.

**A COMPARATIVE STUDY OF SOME BREEDING BEHAVIOR PARAMETERS IN
CAPTIVE AND WILD CHINKARA (*GAZELLA BENNETTII*)**

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Wildlife plays momentous and important biological role to maintain the balance of different ecosystem and make the planet earth habitable. Pakistan is rich in endemic and exotic wildlife. Chinkara (*Gazella bennettii*) is specie of *Gazella* which belong to South Asia.

Unfortunately, the distribution of chinkara in Pakistan has been greatly reduced by overhunting and although still widespread, populations are scattered in Cholistan desert. The present study was conducted to determine the baseline difference of breeding behaviour (vigilance, resting and grooming) between the wild and captive male and female chinkara population. Both the populations were studied at different locations; the captive population was studied at University of Veterinary and Animal Sciences (UVAS), while the wild population was studied at His Highness Sheikh Zayed Wildlife Park, Rahim Yar Khan. The vigilance of wild and captive females was significantly different, the wild female population was too vigilant than the captive females. Resting parameter was significantly high in captive females than wild ($P < 0.05$) while grooming showed non-significant results for captive and wild population. The same parameters for wild and captive males were observed. It was noticed that wild males are more vigilant ($P < 0.05$) than the captive males, while the captive males spent significantly high ($P < 0.05$) time in resting than wild males. Grooming was high ($P < 0.05$) in captive males than wild ones. Thus the findings of the study showed that female behaviour is more variable in both the locations than males. The male tend to be less effected than the environmental factors than females thus much more focus is needed for the conservation and comfort of females in captivity.

BREEDING AND SPATIO-TEMPORAL VARIATIONS IN ABUNDANCE OF RED-WATTLED LAPWING (*VANELLUS IIDICUS*) IN NORTH PUNJAB, PAKISTAN

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Present study was conducted to determine the association of Red-wattled Lapwing (*Vanellus Iidicus*) with various habitats such as croplands, wetlands, urban areas and scrublands and seasonal variation in habitat association, in Rawalpindi and Islamabad area from December, 2013 to June, 2014. The spatial extent of the study area was categorized as urban area, wetland area, cropland area and open scrubland area. We used distance sampling using line transect method to collect data on abundance of Red-wattled lapwing and record variables such as substrate, surrounding cover objects and vegetation. Of all the models tested, uniform/ cosine yielded best fit for urban areas, scrubland and the study area (Chakwal, Islamabad and Rawalpindi) while hazard-rate/ simple polynomial adjustments and half-normal/ cosine yielded best fit our data for wetland and cropland areas, respectively. Overall population density of 0.52 individuals ha⁻¹ of Red-wattled Lapwing was recorded from the study area with highest population density of 9.27 individuals ha⁻¹ in wetland areas and the lowest population density of 0.38 individuals ha⁻¹ in cropland areas. We recorded a population density of 0.40 and 0.22 individuals ha⁻¹ during winter and summer, respectively. It revealed that number of Red-wattled Lapwing did not differed ($P= 0.89$) significantly between the seasons but differed significantly ($P < 0.05$) among the habitats. The univariate generalized linear model revealed that the interaction between the effects of habitats and seasons on abundance of Red-wattled Lapwing was non-significant ($F_{(3, 47)} = 0.41, p = 0.98$). Four nests and ten chicks of Red-wattled Lapwing were observed along the transects during study which provided evidence of its breeding during summer season (May-June). The mean nest diameter was 30.75cm (± 0.75) while clutch size comprised of 3-4 eggs. The nests were made on ground in open

fields with stones, pebbles and dried twigs in urban, scrubland and cropland areas at a distance of less than 200 meters from water bodies in most instances.

MORPHOMETRIC AND GRAVIMETRIC MEASUREMENTS OF COMMON SKITTERING FROG (*EUPHLYCTIS CYANOPHLYCTIS*) WITH FIRST REPORT ON DEFORMED ANURANS FROM PAKISTAN

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Present study was conducted to record morphometric and gravimetric measurements of Common Skittering Frog (*Euphlyctis cyanophlyctis*) inhabiting cropland (rice crop, where pesticides are used, experiment site) areas in district Jugranwala and control cropland area in Rawalpindi/Islamabad where no pesticides were used (control site). We recorded mean values of snout-vent length, inter-orbital width, distance from front of eyes to the tip of snout, inter-nasal space, distance from nostrils to the tip of snout, distance from tympanum to the back of eyes, forelimb length, body weight and renosomatic index of females and inter-orbital width, inter-nasal space, distance from nostrils to the tip of snout, forelimb length and hepatosomatic index of males collected from experiment and reference cropland sites which differed significantly among both sites ($P < 0.05$). Statistically significant difference ($F_{(27, 122)} = 3.90$, $p < .0005$; Wilk's $\Lambda = 0.537$, partial $\eta^2 = 0.46$) was found in the measurements of male and female frogs. We also recorded statistically significant difference ($F_{(27, 122)} = 7.42$, $p < .0005$; Wilk's $\Lambda = 0.378$, partial $\eta^2 = 0.62$) in the measurements of both male and female frogs collected from reference and experiment sites. We found strong significant ($R^2 > 0.90$; $P < 0.05$) relationship between snout-vent length and body weight of female frogs collected from reference and cropland site and of male frogs ($R^2 = 0.84$; $P < 0.05$) collected from cropland site. However, relationship between snout-vent length and body weight of male frogs collected from reference was weak but significant ($R^2 = 0.40$; $P < 0.05$). We recorded one deformed (micromelic) and one abnormal (gas bubble disease) specimen from a eutrophic pond of our reference site. We assumed that high levels of agricultural nutrients might have caused the deformities. We suggest further detailed studies on impacts of eutrophication on populations of Common Skittering Frog.

A BASELINE STUDY ON ABUNDANCE AND HABITAT OF WHITE-BREASTED WATERHEN (*AMAURORNIS PHOENICURUS*) IN RAWALPINDI-ISLAMABAD AREA, PAKISTAN

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Information on ecology, abundance and habitat of wildlife species is essential to devise management strategies. The present study was conducted to gather information regarding

population density and habitat of White-breasted Waterhen (*Amaurornis phoenicurus*) in Rawalpindi-Islamabad area from September, 2013 to July, 2014. Line transect distance sampling method was used to gather data on population and line intercept method to sample vegetation. Data on substrate, surrounding land cover, percentage refugia and sympatric water bird species was recorded along the transect line. Overall population density of 5.18 individual ha⁻¹ of White-breasted Waterhen was estimated in the study area. Highest population density of the bird i.e. 6.76 birds ha⁻¹ and 6.64 birds ha⁻¹ was recorded from Khasala Nullah, Rawalpindi and Tumair Nullah, Islamabad, respectively. These areas were dominated by marshy habitat, lotic water, with vegetation, logs as surrounding cover. The marsh habitat was dominated by herb species such as *Parthenium hysterophorus*, *Centaurea calcitrapa*, *Carthamus oxyacantha*, *Conzya canadensis* and grasses including hydrophytes such as *Cynodon dactylon*, *Typha angustata* and *Phragmites karka*. Common Moorhen (*Gallinula chloropus*), Pond Heron (*Ardeola grayii*), Little Egret (*Egretta garzetta*) and White-breasted Kingfisher (*Halcyon smyrnensis*) were recorded as sympatric water bird species. The logistic regression model explained variables recorded from the study area had contributed 73.0% in the abundance of White-breasted Waterhen. The Wald test revealed that variables such as surrounding land cover (%), refugia (%) and percentage cover of herbs, shrubs, grasses and trees influenced the abundance and distribution of the species.

IDENTIFICATION OF THREATS TO GREY PARTRIDGE (*FRANCOLINUS PONDICERIANUS*) IN SALT RANGE, PUNJAB PAKISTAN

Sangam Khalil, Maqsood Anwar, Iftikhar Hussain, Tariq Mahmood, Muhammad Rais and Bushra Allah Rakha.

Grey partridge (*Francolinus pondicerianus*) is a medium size game bird, also serving as biological control agent. Population of grey partridge has declined over the time mainly due to excessive hunting and habitat destruction. Research studies on biological and ecological aspects Grey partridge area lacking especially in the Salt Range, Punjab. The current study was conducted in two protected areas i.e. Chumbi Surla Wildlife Sanctuary (CSWS) and Diljabba Domeli Game Resrve (DDGR) to generate information about major threats affecting Grey partridge population in the Salt Range. Data was collected through questionnaire survey (n=100) from hunters, local people and wildlife staffs. Threats in both study sites were identified as hunting, trade, habitat destruction, predation, livestock pressure, agriculture activities, fuel wood collection, land clearing and stone crushing. Threat to Grey partridge population and habitat reported in CSWS included; 38% by illegal hunting, 18% by agriculture activities, 18% by land clearing, 06% by trade, 06% by habitat destruction, 04% by predation, 04% by live stock pressure, 04% by fuel wood collection, and 02% by stone crushing. Similarly threat to this game bird reported by respondents in DDGR were; 46% by illegal hunting, 18% by agriculture, 16% by fuel wood collection, 04% by trade, 04% by predation, 04% by live stock pressure, 04% land clearing, 02% by habitat destruction, and 2% by stone crushing. Among all the threats, illegal hunting was found to be major threat in both study sites, followed by agriculture activities and fuel wood collection. These activities adversely affect and causes decline in Grey partridge population in the study area. Strict protection measures are required by the wildlife department to protect population and habitat of Grey partridge in Salt Range. Results would help in conservation of Grey partridge, ultimately helping in sustaining the population of this important game bird in the Salt Range.

DIET ANALYSIS OF STRAIGHT-HORNED MARKHOR (*CAPRA FALCONERI JERDONI*) IN TORGHAR MOUNTAINS, BALOCHISTAN

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Markhor (*Capra falconeri jerdoni*) is a gregarious ungulate restricted to Torghar mountain range of Balochistan, Pakistan. Since the existing literature lacks any information on food habits of this endangered sub-species the present study therefore was conducted to investigate its diet composition. In total 33 composite fecal samples along with potential reference plant species contributing in the diet of the Markhor were collected during November 2013 through August 2014 for analysis by standard micro-histological technique, used to study herbivore food habits. Absolute and relative quantification of identifiable food remains found in the fecal matters were determined. Analysis of spring fecal samples showed occurrence of 51% tree leaves, 12% shrub parts and 39% grasses whereas the samples collected in summer were composed of 41% tree leaves, 29% shrubs and 30% grasses while the winter samples were consisted on 59% tree leaves, 29% shrubs and 12% grasses. The results indicated dominance of tree leaves during all the seasonal samples designating this ungulate dominantly a browser. However, the proportion of three groups of vegetation changed from season to season indicating dominance of grasses over the shrubs during spring and summer. In total, remains of ten plant species were identified including four trees (*Juniperus excelsa*, *Pistacia khinjuk*, *Pistacia atlantica*, *Fraxinus xanthoxyloides*), three shrubs (*Ephedra gerardiana*, *Prunus eburnae*, *Prunus begernae*) and three grasses (*Mentha longifolia*, *Stipa pinnata*, *Chrysopogon serrulatus*). Relative frequency of occurrence of plant remains showed that the leaves of *J. excelsa* and *E. gerardiana* were the most preferred trees and shrubs respectively, recovered from all the three seasonal samples. *M. longifolia* was the most preferred grass in winter and spring while *S. pinnata* was the most preferred grass in summer season. The Markhor consumed significantly higher ($P < 0.05$) amount of *J. excelsa* and *E. gerardiana* during the winter compared to same plant species in spring and summer whereas the consumption of these plant species did not vary significantly ($P > 0.05$) between spring and summer seasons. The proportion of grass parts (all three spp.) were significantly higher ($P < 0.05$) in spring and summer seasons as compared to winter while there was no difference ($P > 0.05$) in their occurrence between spring and summer seasons. The study suggest to conclude that the most favorite plant species i.e. *J. excelsa* (tree), *E. gerardiana* (shrub) and, *M. longifolia* and *S. pinnata* (grasses) shall be protected and propagated in the distribution range of Markhor to conserve/maintain its population.

POPULATION ESTIMATION, HABITAT ANALYSIS AND CONFLICT OF INDIAN GREY MONGOOSE (*HERPESTES EDWARDSII*) IN MIRPUR DISTRICT, AZAD JAMMU & KASHMIR

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To study distribution, population status, habitat utilization and conflict of Indian grey mongoose (*Herpestes edwardsii*) 18 localities were sampled from 3 zones created in Mirpur district

(Azad Jammu and Kashmir). Random transects of fixed length were laid and number of active burrows counted and habitat analysed using quadrat method. Mongoose conflict was evaluated using questionnaire method. Mongoose was distributed with average population density of 9.8 animal/km², with highest (17.8 animal/km²) density at Kalyal and lowest (3.5 animal/km²) at Panyam. Most favored altitudinal range was 450 m to 550 m (asl). Population density was the highest (12 animals/km²) in October and lowest (7.44 animals/km²) in May. Dominant vegetation included *Acacia nilotica* (IV= 108.9), *Acacia modesta* (IV= 94.23), *Brossonnetia papyrifera* (IV= 94.19), *Calotropis procera* (IV= 145.46), *Dodonea viscosa* (IV=120.17), *Cynodon dactylon* (IV= 174.94), *Chrysopogon serrulatus* (IV=152.96) and *Artemisia scoparia* (IV= 105.69), *Parthenium hysterophorus* (IV= 113.82), *Saccharum spontanicum* (IV= 199.88) *Ziziphus mauritiana* (IV= 68.54), *Lantana indica* (IV= 92.86) and *Morus alba* (IV= 31.38). A total of 490 poultry and pets were depredated by mongoose during April 2013 to March 2014, inflicting a loss of 126 thousand PKR. Most (66%) of the attacks were observed in summer and in the morning hours. Three retaliatory killings were reported.

**HUMAN-GREY WOLF (*CANIS LUPUS*) CONFLICT IN SHOUNTHER VALLEY,
DISTRICT NEELUM, AZAD JAMMU AND KASHMIR, PAKISTAN**

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Human-wolf conflict is a major issue in various parts of the world due to predation on livestock. Shounther valley (a sub valley of Neelum valley, AJK) harbors pasture-based economy in summer season, thereby livestock predation by grey wolf (*Canis lupus*) creating a serious conflict between formers and wildlife. Present study is first attempt in this area that aimed to assess the intensity of the conflict in term of livestock depredation. Study area was divided in to 8 study sites and data were collected by five months (May to September, 2011) field surveys at each study site. Result revealed that a total of 76 livestock heads were depredated by grey wolf during study period, out of that highest depredation was recorded at Bhedian study site (22%) followed by Mali (185) and Dukk (13%). Sheep (67%) were more vulnerable to depredation as compared to goats (26%) and horses (4%). Most depredations (24%) were noted at the age groups of > 2 years and preferred time of depredation was recorded at 11 pm to 5 am (65%) whereas open pens were the preferred sites for depredation (61%) as compared to fenced pens (22%) and pastures (17%). Overall depredation during study period resulted a loss of PKR 4,33,000 to formers that raise rage against wolf and consequent a retaliatory killing of three wolves; one was gunned down, second was poisoned and third was killed by guard dogs. Watch and ward and herding practices are very poor as most of the depredations (61%) were occurred in absence of protection. No compensation claim was filed to the compensation agencies, showed miserable cooperation between formers and these bodies. Conflict is rising day by day and is a serious question mark for the conservation of

wolf in the study area. A comprehensive awareness program should be started with the consultation of local popular person to improve herding practices, better watch and ward conditions and to change people attitude toward the wolf presence in the study area. This base-line study would provide a step toward the conservation efforts of this species in Shouther valley.

**BREEDING BIOLOGY OF BARN SWALLOW (*HIRUNDO RUSTICA*) AT TEHSIL
BALAKOT, PAKISTAN**

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The study was conducted at four sites (Shohal Najaf, Shohal Mazullah, Bissian and Tranna) in Tehsil Balakot of District Mansehra during the breeding season February to June 2014. Details about nests and eggs characteristics are given. Different materials and methods used including nest height and other nest parameters (i.e. nest diameter and nest depth) were measured by using common measuring tape. Egg length and breadth was measured by using Vernier Calliper with Least Count 0.1 mm. Egg volume was calculated by using Hoyt (1979) formula i.e. $V = 0.51 \times L \times B^2 / 1000$ and egg shape index was simply calculated by formula $ESI = L/B$. Egg weight were taken on Digital Weighing Bar. Statistical analyses were performed by using One Way Anova. Result of this study shows that nests were positioned on vertical walls, roofs of buildings and situated at average height of 2.8 ± 0.43 m above ground, while nest diameter was measured 14.78 ± 3.13 cm, nest depth 3.97 ± 0.90 cm, nest cup diameter 10.91 ± 2.46 cm and nest cup depth 3.27 ± 0.80 cm. Nests attached to cemented walls were (46.3%), plastic surfaces (20.4%), wooden materials (16.7%), soil walls (11.1%) and to mirrors (5.6%). The average clutch size recorded was 3.7, egg ranged 2-5. Mean egg length was measured 18.5 ± 1.6 mm, breadth 13.6 ± 1.2 mm, egg volume 1.80 ± 0.5 cm³ and egg shape index was calculated to be 1.36 ± 0.03 . Mean egg weight was recorded to be 1.81 ± 0.1 g. Egg and nest success was 76% and 85%. Hatchling and fledgling produced per nest was 2.84 and 2.44. Main causes for reproductive failures were unhatched and broken eggs, predation and observer's disruption.

**BREEDING PERFORMANCE OF RED-VENTED BULBUL (*PYCNONOTUS CAFER*) IN
DISTRICT MANSEHRA, PAKISTAN**

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Breeding performance of Red-vented *Bulbul Pycnonotus cafer* was studied at the District of Mansehra, KP Province, Pakistan during the breeding season from May to August 2013. Pair formation in Red-vented Bulbul occurred in March and April, followed by nest construction. Different materials and methods used i.e. plant and nest height was measured by a common measuring tape. Egg length and breadth was measured by using Vernier Calliper with precision of 0.1 mm. Egg volume was calculated by using Hoyt (1979) formula i.e. $V = 0.51 \times L \times B^2 / 1000$ and egg shape index was calculated by L/B . Murray (2000) was followed to calculate egg success and

nest success as measure of reproductive success. Statistical analyses were performed by student *t*-test. All the mean values are given with Standard Deviation (Mean±SD). Result showed that Red-vented Bulbul preferred leafy dense shrubs and smaller trees for nest construction. Average plant and nest 2.2±0.2m and 1.9±0.3m, respectively. Mean clutch size was 2.3 ranged 1-3. Egg length and breadth was 20.2±0.6 and 16.0±0.6mm. Mean egg volume was 2.6±0.2cm³ and egg shape index was 1.24±0.02. There was significant correlation found between egg length and breadth in relation to clutch size ($P < 0.05$). Egg success was 55.6% and nest success was 59.4%. Main reasons for breeding failures were unhatched eggs, weather conditions and observer's disturbance. Average fledgling produced per nest was 2.2.

MORPHOLOGICAL AND MOLECULAR CONFIRMATION OF NEW WOLF SPECIES (*CANIS HIMALAYENSIS*) IN PAKISTAN

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New Wolf Species population was located in Himalayan foothill region of Pakistan, it was observed different from the Indian wolf (*Canis lupus pallipes*) found in Planes and plateau of Pakistan and Tibetan wolf (*Canis lupus chanco*) found in northern areas of Pakistan. However, the morphological characteristics were visualised close to Himalayan wolf (*Canis himalayensis*) was reported to occur in Eastern Himalayan region like Nepal Bhutan. International experts were consulted and they suggested the same. Himalayan wolf is declared critically endangered and not sighted since last ten years in the wild. Current results of mitochondrial DNA analysis suggest that the Himalayan wolf is phylogenetically distinct from the Tibetan wolf *Canis lupus chanco* and Indian wolf (*Canis lupus pallipes*). Furthermore, molecular taxonomy was conducted by amplifying the folmer region of the CO1 gene of the mitochondrial genome. This 700bp region was then sequenced and aligned with sequence of other wolf species and confirmed as Himalayan wolf "*Canis himalayensis*"

COMPARATIVE STUDY OF WILDLIFE DIVERSITY OF TAUNSA BARRAGE WILDLIFE SANCTUARY AND CHOLISTAN GAME RESERVE

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Wildlife diversity performs variety of ecological services including the production of food, recycling of nutrients, regulations of micro-climate and local hydrological process, inhibition of undesirable micro-organism and detoxification of harmful nutrient chemicals. Pakistan due to its unique geographical and climatic conditions, however, hosts a variety of ecosystems and associated biological diversity. Protecting biodiversity and nature conservation have been major concerns for the human society for a long time, even though reasons and strategies to do so have varied greatly. The aim of the study was to enlist all observed and identified wildlife diversity of Cholistan Game Reserve and Taunsa Barrage Wildlife Sanctuary. During field surveys, the wildlife diversity data has been collected by direct census method and point count method. During the surveys, among birds 150 species, 12 mammals' species, 03 amphibian species, 09 reptiles' species and 17 fish

species were recorded at TBWS. Similarly, at Cholistan Game Reserve, among birds 63 species, 09 mammals' species, 01 amphibian's species, and 07 reptiles' species were identified. Both study sites were very famous due to the presence of one of the world's rarest and second most endangered species Indus dolphin (*Platinista minor*) and Chinkara (*Gazella bennettii*) respectively. Hunting and habitat exploitation in protected areas were found to be the major threat for wildlife. It was concluded that protected areas are cornerstone of the conservation of wildlife diversity. It is suggested, therefore, to take immediate steps for the protection of the protected areas to conserve the wildlife.

**HABITAT PREFERENCE OF INDIAN PANGOLIN (*MANIS CRASSICAUDATA*)
INHABITING MARHALLA HILLS NATIONAL PARK, ISLAMABAD, PAKISTAN**

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Indian pangolin (*Manis crassicaudata*), the only member of order Pholidota that occurs in Pakistan, has been recently declared as "Endangered" by IUCN, so it faces a high risk of extinction due to illegal hunting pressure for obtaining its scales. In the current study, we investigated its habitat preference in the Margallah Hills National Park Islamabad, from September 2011 to August 2012. Three different habitat types viz, Human vicinity area, wild area and agricultural lands were searched for recording the signs of the species. Data were collected by recording both direct and indirect signs of Indian Pangolin using 85 line transects established in 17 different sampling sites. Five line transects were established at each study site (each with 500m length and 200m width). A total of 323 signs of Indian pangolin were recorded; 299 were burrows, 10 were live individuals and 14 were scats. Maximum number of signs were found in wild area (n=178) 55.1%, followed by agricultural area (n=80) 24.76% and least signs were found in Human vicinity (n=65) 20.12% area. Maximum signs (38.70%) were recorded in Malpur (n=125) and least (0.92%) were found at Trail3 (n=3). The results indicate that the Indian pangolin prefers wild or natural area in its habitat over human vicinity and agricultural land.

**SPECIES DIVERSITY, SEASONAL VARIATION AND ABUNDANCE OF RESIDENT
AND MIGRATORY BIRD FAUNA OF PERI-URBAN AREA AT KARACHI**

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Peri-urbanization involves the forms of land which are altered for use resulting in complete restructuring of vegetation and composition of species. The aims to describe the species richness and species diversity through the identification, assessment of abundance of resident species as well as of migratory birds in relation to seasonal variation in a peri-urban area at Gulshan-e-Maymar. The study was conducted at three sectors including eight subsectors and three parks, and adjacent raw areas. The status of each bird species was recorded. The study was based on a time frame of eleven months from February to December. Diversity indices like Shannon's Diversity Index,

Simpson's Index and Margalef's Index were employed, identification of different species was completed by the use of field guides as well different plant habitats were identified and methods for determining abundance, specie richness were conducted. The study provided a great deal of information about surveillance of bird species capable of existing in close proximity with humans in an area of fragmented urban and rural characteristics.

**EFFECT OF LOCAL BIRDS TRADE IN DIMINISHING WILD BIRD'S POPULATIONS;
A SURVEY REPORT FROM RAWALPINDI CITY, PAKISTAN**

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Bird trade at local and commercial level has been witnessed profitable in commerce because of widespread and large population sizes, diversity and trade worth of many species. It is presumed that bird trade at local level in addition to commercial scale business is affecting the population dynamics of many species. Present study was designed to investigate the number and proportion of different birds species used for trading at local level. The data was collected from the local hunters, traders and birds markets through surveys and interviews. During the one year survey a total of 50 different species were enlisted which were used for local business. Out of 50 only 13 species were identified with greater proportional representation in local trade. These species include Bank myna (*Acridotheres ginginianus*) 15%, Red Munia (*Amandava amandava*) 11.89%, House sparrow (*Passer domesticus*) 9.17%, Common myna (*Acridotheres tristis*) 9%, Spotted munia (*Lonchura punctulata*) 7.86%, Common starling (*Sturnus vulgaris*) 5.2%, Baya weaver (*Ploceus philippinus*) 5%, Rose-ringed parakeet (*Psittacula krameri*) 4.87%, Rock pigeon (*Columba livia*) 4.8%, Streaked weaver (*Ploceus manyar*) 3.3%, Collared dove (*Streptopelia decaocto*) 2.81%, Citrine wagtail (*Motacilla citreola*) 2.54% and Pied myna (*Gracupica contra*) 2.24%. These birds has greater market value because of their usage as a cage bird mostly however they were also used in diet for their exceptional medicinal and energy values. It is concluded that the current trade ratio of different bird's species in local markets is one of the most overlooked prospect which may seriously affect the wild population of different species.

**THE STUDY ON HABITATS AND POPULATION OF BLACK FRANCOLIN
(FRANCOLINUS FRANCOLINUS) AND GREY FRANCOLIN (FRANCOLINUS
PENDICERIANUS) IN TEHSIL NARA, DISTRICT KHAIRPUR MIR'S, SINDH,
PAKISTAN**

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The present study was designed to estimate the habitats and population of Black Francolin (*Francolinus francolinus*) and Grey Francolin (*Francolinus pendicerianus*) species in Tehsil Nara, District Khairpur Mir's Sindh, Pakistan. The Black and Grey Francolins are the prime game birds

of Pakistan occupying a variety of habitats. They prefer to live in grassland and agriculture ecosystem. Vegetation exploited as trees are cut to be used as timber and looped for firewood. Shrubs, herbs and grasses are heavily grazed by livestock, destroying breeding sites of ground nesting birds. Such factors have adversely affected population of Black and Grey Francolin along with other wild life species. The population of Black and Grey Francolin were estimated using direct (*line transect method*) and indirect methods ("calls" of two species). Data were collected weekly from October 2014 to December 2014. The result showed as average population densities of $0.47 \pm 0.09/\text{ha}$ and $0.06 \pm 0.01/\text{ha}$ for Grey and Black Francolin respectively. For an assessment of the habitat 50 species of plants were identified from the francolins habitats in Tehsil Nara. The Black Francolin appeared in high densities in the areas having high vegetation cover, but the grey francolin prefer to the areas having low vegetation cover. Overall results indicated that the population of both these francolin species was not encouraging. Excessive hunting and habitat degradation were identified as important factors affecting francolins. Strict watch and ward and habitat improvement with the co-operation of forest Department will be helpful to conserve these species

MORPHOMETRICS AND URBAN ADAPTATIONS OF ASIAN PIED MYNA (*STURNUS CONTRA*) IN RAWALPINDI CITY, PAKISTAN

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Asian Pied Myna (*Sturnus contra*) was first reported in Pakistan at Changa Managa forest plantation in Kasur District in April 1982. Recently, a shift in the populations of pied myna (*S. contra*) from rural to sub-urban and urban habitat has been noticed. Present study was designed to investigate the extent of expansion of population of pied myna in urban areas of Rawalpindi. For this purpose we conducted rekey surveys to find the potential habitat of pied myna (*S. contra*) and identify its seasonal population trends in the city. A total of five different sampling sites were identified for survey and periodic monitoring. Total these sites mainly consists of human settlement areas where marshes formed by the sanitation water. Point count method was used to estimate the population at selected site and observation was made through binocular (250x) for a period of a whole year. A high concentration of *S. contra* was observed during the month of November (12.08 ± 1.01), December (12.08 ± 1.01), January (12.08 ± 1.01) and February (12.4 ± 1.16) suggesting some migratory influx during winter months. The average observed population of pied myna was (9.46 ± 0.86) throughout the city. During the whole study period, a stable population of 3-4 pairs at each study site was observed at sites which prefer to developed nest on electric and telephonic poles, feed upon dumped house garbage and animal dungs. Average morphometrics of ten captured individual was as: total length (23.83 ± 0.23 cm), tail length (7.13 ± 0.13 cm), beak length ($2.63 \text{cm} \pm 0.04$), and average weight (82 ± 1.73 g). It is predicted that alongwith feeding and nesting opportunities, marshy vegetation in urban open spaces and hiding places developed in urban construction which provide cover attracts *S. contra* to adopt in urban environment and it may extends with urban expansion in the coming years.

**AVIFAUNA OF BARA GALI SUMMER CAMPUS, UNIVERSITY OF PESHAWAR,
KHYBER PAKHTUNKHWA**

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Survey of avian fauna of Bara Gali Summer Campus, University of Peshawar situated in Abbottabad was conducted from April to October, 2013. A total of 21 species belonging to 5 orders and 15 families were recorded. Out of these, 6 were resident, 12 summer visitor and 3 rare. Order Passeriformes was represented by 16 species which are *Certhia himalayana*, *Megalaima virens*, *Phylloscopus trochiloides*, *Garrulax lineatus*, *Passer rutilans*, *Corvus macrorhynchos*, *Hypsipetes leucocephalus*, *Acridotheres tristis*, *Delichon dasypus cashmeriensis*, *Hirundo rustica*, *Muscicapa thalassina*, *Saxicola ferrea*, *Myiophoneus caeruleus*, *Parus melonolophus*, *Parus rufonuchalis*, *Parus monticolus*, belonging to 11 families. Two species *Dendrocopos himalayensis* and *Picus squamatus* belongs to only one family Picidae of order Piciformes. Among rest of the three orders each is represented by only a single species; Accipitriformes by *Accipiter virgatus*, Coraciformes by *Upupa epops* while order Psittaciformes has been represented by *Psittacula himalayana*. The distribution and abundance varied with season and maximum number of species were found during the monsoon season when most of the birds migrate for breeding. Some habits and behaviors like nesting, feeding, breeding and vocalizations were also studied which are very unique from other birds found on lower elevations. Among bird species adapted to diverse habitat in the field, Himalayan Jungle Crow, Common Mynas, Bulbuls, Barn Swallows, barbets were prominent. Interesting feature of the avian fauna is its familiarity with flora, was also observed during the present studies that some birds are very quick and active in their movement on a tree surface i.e. *Certhia himalayana*.

**POPULATION DISTRIBUTION AND HABITAT USE OF *LISSEMYS PUNCTATA* IN
DISTRICT MIRPUR, AJK, PAKISTAN**

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Present study aimed to investigate the species distribution, population density, habitat characteristics and threats to freshwater turtle in Mirpur, Azad Jammu and Kashmir. Study area was divided in 3 zones such as Mirpur, Dadhyal and Chakswari, which were further sub-divided in to 18 localities (each zone consisted upon 6 localities). Species were identified with the help of keys and photographs used by earlier researchers. Quadrature method was used to assess population density and Phytosociological attributes of the study area. Questionnaire method was used to evaluate threats to freshwater turtle. Analysis of data revealed that 4 freshwater turtle species including *Lissemys punctata*, *Aspideretes hurum*, *Aspideretes gangeticus* and *Hardella thurjii* were identified and distributed in all study zones. *L. punctata* was recorded as the most common (73.72%) direct sighted while according to respondent's opinion 62% observed this turtle species. Maximum population density (21.5turtle/km²) recorded in Afzalpur locality and minimum population density was recorded at Darharii (2 turtle/km²). Most favorite altitudinal level was below 300 m with highest population density of 13.30 turtle/km² whereas lowest population

density of 3.50 turtle/km² was recorded in Class III (above 400 m). Maximum population density (13 turtle/km²) was recorded in the month of June and January-2013 while minimum population density (5 turtle/km²) was noted in the month of March and April-2013. Seasons during *L. punctata* observed was 44% in summer, 39% in rainy season, 12% in winter and 5% in spring. Dominant plant species of the in the study zone included *Medicago spp*, *Mentha*, *Parthenium*, *Equisetum spp*, *Rumex spp.*, *Albizia lebbeck* and *Ficus carica*. Habitat sites where *L. punctata* mostly observed were 53% near river water, 29% near grasses, 16% sandy area near water and 2% on elevated rocks inside water. Freshwater turtles faced various threats including killing (45%), pollution (32%), habitat destruction (10%), destruction of eggs and nest (6%) and killing of hatchlings. According to respondents causes of killing were for trade (73%), for medicinal purpose (13%), conflict among local and turtles was (8%) and killing without any reason (6%).

FIRST RECORD OF LAEMOBOTHRION NITZCH, 1818 (LAEMOBOTHRIDAE: AMBLYCERA: PHTHIRAPTERA) AND DEGEERIELLA NEUMANN, 1906 ON STEPPE EAGLE AQUILA NIPALENSIS (HODGSON) (ACCIPITRIDAE: FALCONIFORMES)

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Five birds of Steppe Eagle, *Aquila nipalensis* (Hodgson) (Accipitridae: Falconiformes: Aves) were examined for their chewing lice (Phthiraptera). All the birds were infested by two species of Amblyceran chewing lice including *Laemobothrion* Nitzsch, 1818 (Laemobothriidae) and *Colpocephalum* sp. (Menoponidae), and three species of Ischnoceran chewing lice of family Philopteridae, are *Degeeriella fusca* (Denny, 1842), *Degeeriella* sp. and *Falcolipeurus suturalis* (Rudow, 1869). All the chewing lice species have new records for locality as well as for the host from Sindh region, Pakistan. However *F. suturalis* was first reported from Pakistan on *Milvus migrans govinda* Sykes-Black Kite (Cheel). The genus *Laemobothrion* and genus *Degeeriella* are first reported from steppe eagles in the world.

EFFECT OF EGG ADAPTED GAMETOCYTIC VACCINE (LOCAL ISOLATES) ON LESION SCORE IN COCCIDIOSIS IN POULTRY

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Egg adapted gametocytes (local isolates) *E. tenella*, vaccine(s) were used against coccidiosis in chickens. A total of two hundred day old chicks were utilized in this study. On day fifth of their age, they were divided into four groups, viz Group-I, group-II, Group-III and Group-IV having 50 chicks in each groups. These groups were distributed as; Group-I, Group-II, Group-III and Group-IV and were administered vaccine(s) for immunization orally viz; Vaccine I (gametocytes), to chicks of Group-I, Vaccine II (gametocytes inactivated) to Group-II, Vaccine III (gametocytes sonicated inactivated) to Group-III and Group-IV served as control given normal saline. On day 15th post- immunization, chicks were challenged with 60,000–70,000 sporulated

oocysts of mixed species of *Eimeria*. On day 21st post vaccination, birds were subjected to postmortem and their lesions score were recorded. A maximum of 46 (92%) birds having lesions in intestine and caeca of Group-IV were observed while a minimum of 17 (34%) birds having slight lesions in intestine and caeca were observed in Group-III. There was non-significant difference ($P>0.05$) in lesions score of Group-I, II and IV. Lesions scores in Group-III were significantly different ($P>0.05$) from Group-I, II and IV. It is concluded that on the basis of lesions score the egg-adapted vaccine saved the chicks against coccidiosis on challenge.

EFFECTS OF DIFFERENT LEVELS OF PROTEIN ON THE PERFORMANCE OF BROILER

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A six-week trial was conducted to study the effect low and high crude protein (CP) level on the performance of broilers, at Poultry Farm near Bahawalpur during March – April, 2012. One hundred and twenty day-old chicks were randomly distributed into 12 experimental units, each having 10 chicks and fed on four CP levels 16% (T1), 19% (T2), 22% (T3) and 25% (T4), respectively. Each Treatment were randomly allotted to experimental units such that each treatment received three replicates. The experimental diets were fed to birds from day 1st to 42nd. Performance of birds was monitored in terms of weight gain, feed consumption and feed conversion ratio (FCR). The results revealed that broiler growth were affected significantly by increasing the protein level. Weight gain at treatments T1, T2, T3 and T4 was 2164.00 g, 1447.33 g, 2271.33 g and 2192.00 g/bird, respectively. Results of the trials suggested that weight gain was significantly ($P<0.01$) increased in birds on diets with 22% crude protein.

BIODIVERSITY DETERIORATION IN THAL ZONE IN SCENARIO OF CLIMATE CHANGE

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Thal zone comprised of six districts; Mianwali, Bhakkar Layyah, Muzaffarabad, Dera Ghazi Khan and Rajanpur. It is characterized by sand dunes, wind cyclones and common drought prevalence. Under ground water is usually brackish, unsuitable for drinking and irrigation purpose. Climate change have brought harsh weather extremes. Rise in temperature and reduction in annual precipitation have questioned the survival of several fauna and flora species of this domain. There is rampant occurrence of torrential, unpredictable and erratic rainfalls in monsoon leaving no chance of infiltration of water for storage for chickpea, moong bean and wheat cultivation. Temperature fluctuation of day and night adversely affect the pollination process in these field crops. In addition to this, long drought spells due to climate change have extended the chances of blight and pod borer infestation in gram in this area. Deforestation due to climate change have

resulted in drastic changes in the agro ecological conditions of Thal tract. Even the common forest species of Frash (*Tamarax aphylla* L.), Beri (*Zizyphus* sp.) and the most drought resistant species of kikar (*Acacia* sp.) have become extinct from this sector. Honey production in this region has almost gone to nil. Wildlife birds like partridges (*Ammoperdix heyi* L.), quail (*Coturnix coturnix* L.), chukar (*Alectoris chukar* L.) and pheasant (*Pucrasia macrolopha* L.) which were very common in this pocket are now rare. Similarly, wildlife herbal weeds like wild cucurbit (*Mukia maderasputana* L.) and Puncture Clover (*Tribulus terrestris* L.) are not seen in the nomadic life of Thal zone. Edible mushrooms previously extensively found in Thal territory are exceptional among public under the umbrella of Federal Ministry of climate change integrating all the departments of agri. Extension, wildlife, forestry and fisheries from provincial set up to mitigate the adverse effects of climate change.

AVIAN DIVERSITY OF LAHORE ZOO SAFARI IN WINTER SEASON LAHORE, PAKISTAN

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The study was carried out to observe the avian diversity during winter season at Lahore Zoo safari. Lahore zoo safari is located in (31° 22' 57" N, 74° 12' 47" E and elevation is 208m) district Lahore, Punjab, Pakistan. The ecosystem is a composite of wetland, terrestrial and irrigated plantation. The survey was carried out from October 2014 to December 2014 to record the bird diversity. Total no of birds individuals were 2085, belonging to 52 species covering 30 families and 12 orders. The season wise distribution was resident were 37 (71.1%), winter migratory 11 (21.1%), and summer breeder 4 (7.6%). Shannon-Weiner diversity index 1.93 shown a low profile of avian diversity along with, Simpson's diversity index 0.64, species evenness 0.49, species richness 52 and census index was 1021.7 birds/km². Among most dominant species were house crow *Corvus splendens* (n=600), house sparrow *Passer domesticus* (n=85), common myna *Acridotheres tristis* (n=63), jungle babbler *Turdoides striatus* (n=50) and black crowned night heron *Nycticorax nycticorax* (n=30). A reduction of 17 species was noted relative to former survey conducted in 2004. The speedy urbanization may be the cause of reduction in species number around the study area.

AMPHIBIANS AND REPTILES OF DISTRICT KASUR, PUNJAB, PAKISTAN

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This six months survey extending from April through September, 2014 was conducted in district Kasur in Punjab province, Pakistan. A total of 60 amphibian specimens representing 3

species, 3 genera and 2 families while 71 reptilian specimens representing 15 species, 14 genera and 10 families were captured from the study area. Amphibian species captured of the study area included *Bufo stomaticus*, *Hoplobatrachus tigerinus* and *Euphlyctis cyanophlyctis* while reptiles included *Varanus bengalensis*, *Amphiesma stolatum*, *Ptyas mucosus*, *Echis carinatus*, *Calotes versicolor*, *Bungarus caeruleus*, *Lycodon aulicus*, *Hemidactylus flaviviridis*, *Ablepharus grayanus*, *Typhlops ductuliformes*, *Eryx Johnii*, *Lissemys punctata Andersoni*, *Ablepharus grayanus* and *Eutropis macularia*.

PREVALENCE OF MASTITIS IN DOMESTIC ANIMALS IN DISTRICT BHAWALPUR

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The present study was carried out to determine the prevalence of mastitis in lactating domestic animals on the clinic of University College of Veterinary and Animal Sciences, Islamia University of Bahawalpur during the period of October 2013 to October 2014. Mastitis was diagnosed by the physical examination of the udder and various tests were applied for the detection of mastitis which was white side test, surf field mastitis test and California mastitis test. A total of 1177, lactating local buffaloes (n= 54), local cow (n=171) goats (n=748) and sheep (n=203) were included in this study. An overall prevalence of 25% (29/1177) of mastitis was reported. Prevalence of mastitis in buffalo level was 7.40% in which 4 buffaloes were affected from 54, in cow 5.84% in which 10 cows were affected with mastitis, in goats 1.73% in which 13 goats showed the symptoms of mastitis and in sheep only 2 sheep were affected with this disease. In conclusion, this study revealed the prevalence of mastitis in buffalo was more than the prevalence of mastitis in cow. In case small ruminants the prevalence of mastitis in goats was more than the prevalence of mastitis in sheep.

GENETIC HISTORY AND DIVERSITY OF COCKFIGHTING CHICKENS OF PAKISTAN

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The Asian subcontinent cockfighting chickens are famous for their vigour, alertness, fighting behaviour and disease resistances. The origin of the 'breed' is unknown; it might have been associated to the domestication of the species for recreational purpose. Seals depicting cockfighting, perhaps as old as to 2500 BC have been found at Mohenjo-Daro (Sindh Province).

The number of cockfighting population breeds in Pakistan is unclear with 4 to 7 varieties recognized. This study aims to understand the origin and diversity of Pakistani cockfighting chicken and to relate this diversity to other indigenous population and commercial birds. Here, we report our results on the mtDNA diversity of 185 birds from all Pakistani cockfighting varieties and 10 captive red junglefowl (*Gallus gallus murghi*). A 397 bp fragment of the mtDNA D-loop was sequenced. Within and between population haplotypes diversity and relationships (neighbor-joining tree and median-joining network) are presented. We address the expected origin and phylogeographic history of the birds with reference to wild red junglefowl and non - Pakistani chicken populations. Cockfighting chickens have had a substantial influence on the dispersal of the species throughout the world providing a proxy for the understanding of past trading relationships between human communities and civilizations.

STUDY ON THE TREND AND PATTERNS OF VIRAL BROILER DISEASES IN DISTRICT HYDERABAD, PAKISTAN

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The present study was conducted to investigate the trend and patterns of two viral broiler diseases of poultry i.e., Hydropericardium syndrome and New Castle, diseases of broiler poultry birds in district Hyderabad and its adjacent areas 2014. The data was collected randomly by personal survey of five cities such as Hyderabad, Hala, Tando Muhammad Khan, Tando Allahyar and Matyari. The samples were brought at poultry disease diagnosed laboratory Hyderabad for confirmation of diseases. The results according the regression line of Hydro pericardium syndrome, the number of birds, affected, died and survived showed declining trend and trend of disease in all cities, in which Hyderabad city showed highest over all mean of died birds 22.08±2.82 followed by Tando Muhammad khan 20.75±3.45, Tando Allahyar 19.66±1.21, Matyari 10.33±1.25, Hala 10.90±0.67. While regression line for New Castle disease showed declining trend in Hyderabad city showed highest mortality 60.00±9.96 and lowest at Taluka Matyari 24.33±4.42. It was concluded from the present study that the incidence of viral disease such as Hydropericardium Syndrome (HPS) and New castle disease (NDV) had high mortality in district Hyderabad and its adjacent areas.

THE INVASION OF GOLDEN APPLE SNAIL (GAS) IN HALEJI LAKE: ITS OCCURRENCE AND REPRODUCTION

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Pomacea canaliculata (Golden Apple Snail) was introduced in Pakistan through the aquarium trade in 2009. First wild populations were observed in early 2012 in Haleji Lake, Sindh. Haleji Lake is located about 75 kilometers away from Karachi on the left hand side of the National Highway with a surface area of 16.9 square kilometer (174 hectares). *P. canaliculata* range of distribution has been highly expanding. Presently, it has dispersed into many locations of Thatta district including Haleji Lake, Gujjo River, Makli River, Ali Mohammad Samo village,

Rahmattullah Jokhio village, Haji Gulu Palijo village, RBOD and Keenjhar Lake. The GAS is also found in highly polluted freshwater canals. It is reported from the paddy fields of the nearby areas of Haleji Lake. However, any significant damage to rice fields in the area is not yet documented. Monthly samplings for water quality while fortnight samples for snail population and clutch size were taken from September 2013 to August 2014. There were nine stations set at shallow bank side areas of the lake. Work on morphometric, seasonal variation in population, clutch size and water quality was undertaken. Water chemistry included temperature, pH, TDS, electric conductivity, salinity and transparency. Apple Snails appeared from September to November showed highest population (18snails/m²) early October 2013 while no snails were seen from late December 2013 to early March 2014. Mean clutch size was 6.8 cm and weight 4.7 g. Mean number of eggs in per gram weight was 385. Higher mean temperature was recorded (31°C) in mid-September while lowest (19°C) in January. Higher TDS was (28 mg/l) in January while low (22 mg/l) in November 2013. Pakistan is having diverse aquatic habitats, which are on threat of highly invasive snail and thus requires considerable attention.

**BIODIVERSITY IN THE BIVALVE FAMILY CARDIIDAE THE LARGER THE ISLAND
OF KNOWLEDGE, THE LONGER THE SHORELINE WONDER**

Jan Johan ter Poorten,
The Netherlands

The bivalve family Cardiidae is one of the principal heteroconch families. Ranging from cockles to giant clams, the family is conchologically and anatomically highly heterogenous. Occupying a remarkable variety of infaunal, semi-infaunal and epifaunal habitats worldwide and forming important components of various marine ecosystems and of fisheries and aquaculture production. Compared to other bivalve groups, cardiiids have received fairly extensive attention in the past decades, resulting in reviews at subfamily and genus level; regional faunal monographs and various phylogenetic studies. In this session, a closer look at the time scale and intensity of taxonomic research of the past 250 years is given. Periods with intense research alternate with intervals where progress was slow. In some periods mainly professionals were involved whereas in other decades amateurs took the lead. One would expect that after such a long time of research a high saturation level would have been reached. This is not the case: 25% of the currently accepted valid species have been described after 1980. Results from the past few decades make clear that diversity is much more focussed in the spatially complex tropical Indo-Pacific than ever thought before. Until 1975, 45% of the described valid species focussed in the Indo-Pacific, whereas 75% of the cockles described after 1975 come from this fauna province. Moreover, the number of described cockles has increased enormously since 1980 and the alpha-taxonomic work is now almost exclusively being carried out by amateurs. Taking as an example the cardiid fauna of Western Australia, a supposedly well documented region, with a well-known regional monograph published in 1977, the increase in the known diversity is from 29 species (1977) to 60 (2015). Being aware of these numbers, how will the situation be in less intensively explored regions like the northern and western Indian Ocean? The importance of local biodiversity studies cannot be underestimated and will lead to a better understanding of species boundaries, allopatric speciation events and biogeographical + evolutionary patterns. Extensive morphologic and molecular phylogenetic research shows that our taxonomic picture needs to be firmly adjusted at all levels. At subfamily level, it shows that the current classification is unsatisfactory with most of the subfamilies being polyphyletic. At species level, several hidden species are revealed thanks to

DNA research. Therefore, molecular research forms an important tool in order to further sharpen our views with regards to regional diversity. It especially forms a great aid for documenting the complex Indo-Pacific fauna province: centre of hidden molluscan diversity.

A COMPARATIVE STUDY OF AMPHIBIAN POPULATION IN DISTRICT JAMSHORO AND LARKANA, SINDH-PAKISTAN

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Present study was proposed to record the population status of amphibians comparatively in District Jamshoro (11,517 Km²) and Larkana (7423 Km²) to assess which environment sustain them more abundantly. Amphibian diversity in both areas i.e. *Bufo stomaticus*, *Hoplobatrachus tigerinus*, *Euphlyctis cyanophlyctis* and *Allopa hazarensis* was already confirmed. The population status was investigated by monthly surveys from March to October during the three years (2011-2013) at fixed habitations using the Global Positioning System. Pitfall trap was used to capture all the adult specimens from each pond and collected into aquarium till calculated at the spot. Subsequently each studied specimen was released into the same pond where it was captured. The comparative study indicated *B. stomaticus* population more abundant (56.4%) in District Larkana than in District Jamshoro. Meanwhile only 19.0% population of *A. hazarensis* was found merely from District Jamshoro, however in District Larkana lowest population was presented by *E. cyanophlyctis*. The study indicated *B. stomaticus* densely inhabited with 37.8% population in both Districts. *H. tigerinus* and *E. cyanophlyctis* existed with remarkably same status of 28.5% population in both study zones. *A. hazarensis* occurred in only 5.2% population. The study also displayed higher amphibian population (55.0%) in District Larkana during the year 2011, while in 2012 and 2013 population rate remained persistently higher (54.1% and 51.7%) in District Jamshoro. Entire study of three years in both Districts showed approximately same population (50.3% and 49.7%) of amphibians respectively in District Jamshoro and Larkana. Present investigation indicated District Jamshoro containing higher diversity may because of its variety of habitats. Whereas amphibian population was relatively same in both districts due to their similar environmental conditions.

A STUDY OF AMPHIBIAN ENVIRONMENT IN DISTRICT HYDERABAD, SINDH PAKISTAN

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Amphibians are highly sensitive to water impurities because of their respiratory skin. Their eggs and larvae are more vulnerable as they confine aquatic place till completion of their development hence if water quality is not favorable, they may not be able to flourish successfully and encounter with abnormalities or mortality. Due to great impact of water pollution on amphibians, present study was conducted in District Hyderabad (3,198 Km²) to evaluate whether amphibian environment is protected from pollution or not. Present investigation discovered 12 permanent amphibian dwellings in agricultural ponds which were surveyed from January to December during the year 2014. Sampling was conducted during day time between 10 am to 05 pm

and water was collected in Van Dorn plastic bottles and kept in stopper polyethylene plastic bottles prior to evaluation. All the water samples were delivered to the laboratory where analysis of certain parameters *i.e.* pH, electric conductivity (EC), total dissolved solids (TDS), total hardness (T-Hard), total alkalinity (T-Alk) and carbon dioxide (CO₂) was carried out. pH meter (Orion 420) was used to record the hydrogen ion concentration, whereas as conductivity meter (WTW 320) was used to measure the volume of EC and TDS. However T-Hard, T-Alk and CO₂ were analyzed through titration procedures. The results revealed highly polluted status of whole aquatic environment mainly due to EC, TDS, T-Hard and T-Alk. The value of pH and CO₂ remained within favorable limit throughout the study period. All the parameters exhibited seasonal variation in their volume. The maximum level of all the parameters was recorded in July, however their level decreased to minimum level in November. However CO₂ displayed variation in its value antagonistic to other parameters. This destructed environment requires urgent management for the conservation of amphibian fauna in District Hyderabad.

MORPHOMETRIC AND BURROWS CHARACTERISTICS OF THE TWO MONGOOSE SPECIES (*HERPESTES JAVANICUS* AND *H. EDWARDSII*) FROM POTHWAR PLATEAU, PAKISTAN

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Mongoose are small carnivore mammals belonging to one of the oldest known genus "Herpestes" that dates back 30 million years. Out of 10 mongoose species world over, only two are native to Pakistan viz. small Indian mongoose (*Herpestes javanicus*) and the grey mongoose (*H. edwardsii*). The two species are predicted to be sympatric. Here we investigated the burrows (diameter and depth) characteristics and the external body measurements of the two mongoose species from Pothwar Plateau. Morphometric measurements were recorded by trapping the two species using especially designed live traps from the selected sampling sites during 12 trapping events from November 2011 to June 2013. Average burrow diameter of the small Indian mongoose was found to be 10.57 as against 19.44 cm that of the grey mongoose, while the burrows depth was 184.06 and 212.33 cm respectively, for the two species. Statistical analysis by ANOVA model in "R" showed a significance difference in burrow diameters of the two species at 0.001 level of significance ($F = 1240.16$, $df = 1$, $p < 2e-16$) and burrow depth at 0.05 level of significance ($F = 9.092$, $df = 1$, $p = 0.0028$). Average body weight was 436.65 and 927.24 grams body length 30.43 and 40.18 cm, tail length 25.07 30.05 cm, total body length 55.83 and 78.23 cm and ear length 3.33 and 4.19 cm, respectively, for small and the grey species. and a significant difference at 0.001 level of significance was found in weight ($F = 675.1$, $df = 1$, $p = 2e-16$), body length ($F = 606.5$, $df = 1$, $p = 2e-16$), tail length ($F = 1303.0$, $df = 1$, $p = 2e-16$), total length ($F = 1036.0$, $df = 1$, $p = 2e-16$) and ear length ($F = 158.4$, $df = 1$, $p = 2e-16$) of two the mongoose species in the study area.

SECTION - VI**POSTER SESSION****DIABETES MELLITUS AND ASSOCIATED SKIN INFECTIONS IN LOCALITIES OF SINDH, PAKISTAN****Younis Jogi, Shamsuddin Shaikh, Javed AhmedUjjan* and Tanveer Ahmed Soomro***Shah Abdul Latif University Khairpur, Sindh, Pakistan*

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Diabetes Mellitus is one of the most common disease and the infections due to diabetes are also very know now a days. Diabetes increases susceptibility to various types of infections. The most common sites of infection in diabetic patients are the skin and urinary tract. An attempt was made to study the skin manifestations in the diabetic patients of Non-Insulin Dependent Diabetes Mellitus NIDDM, type-2, in localities of Sindh. The patients were examined by taking history about the skin disorders and the bacterial types. Both male and female patients were equally infected. It was also found that the patients of 45 age and above were mostly suffered the skin infections. Type-2 disorders from 1-7 of the table 1 were infections and possess different types of bacteria 1, nail infection (paronchia): *Staphylococcus lugdunensis*. 2, keralosis: *Staphylococcus aureus*. 3, *Erythrasma*: *Escherchiacoli* type-1. 4, *Acanthosis nigricans*: *Morgnellamorgannii*. 5, *Pityriasisrosea*: *Streptococcus species*, *E.coli* type-2. 6, Guttate plaque : *Acinetobacterlwoffii*. 7, Lichen planus: *Pseudomons species*. Few disorders were non-infecteious 8, Pruritus(itch). 9, Vitiligo (depigmentation). 10, Skin tags. 11, Xerosis no any bacterial species was found in these disorders, probably cause of these manifestations is the skin water shortage and allergy.

IN VITRO INHIBITION OF DETOXIFYING ENZYMES BY METALS (Cu & Cr) IN SPIDERS (ARANEAE: LYCOSIDAE)**Nida Aziz and Abida Butt***Department of Zoology, University of the Punjab, Lahore*

Heavy metals are natural components of our environment but their excess can have adverse effect on living organisms. Metals pollution can induce biochemical defensive mechanisms in spiders enabling them to survive additional stress. In view of that spiders can be used as bioindicator of heavy metal pollution in terrestrial ecosystem. Present study was conducted to evaluate the role of detoxifying enzymes viz., glutathione s-transferase (GST), acetylcholine esterase (AChE) and carboxylesterase (CarE) in *Pardosa oakleyi* exposed against heavy metals (Cu and Cr). For this purpose, spiders were collected from fallow land of University of the Punjab, Lahore by visual search method and *in vitro* assay was performed with different concentration Cu and Cr. Level of GST and CarE showed positive correlation with the concentration of both heavy metals. The quantity of AChE showed positive correlation with Cr but decreases as concentration of Cu increases. The result of this tudy showed that quantity of detoxifying enzymes in spiders can be used to assess the heavy metal pollution in the environment.

**STUDY ON THE CONSERVATION BIOLOGICAL CONTROL USING
ENTOMOPATHOGENIC FUNGI AGAINST PEST**

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The locusts and grasshoppers are the most feared insect pest of cultivators living around the world major deserts. It caused incredible devastation in the cropping season in many tropical countries. Control of these pest species has traditionally relied on synthetic insecticides and pesticides. On contrary to this, growing awareness of the environmental issues associated with pest population control as well as the high costs of emergency control is expanding the demand for biological control that includes: pathogenic group i-e bacterium, virus, protozoan, nematodes and fungus. At the present some observation were made to investigate the effects of entomopathogenic fungi against many pest species of locusts and grasshoppers. All species of entomopathogenic fungi play major roles in the natural regulation of many insects' species they can develop dramatic epizootics that lead to rapid declines in host population in field as well as in laboratory. It should be possible that the best/frequent use of this fungus against pest at wider range eradicate the chemicals use to a large level in future.

**ASSOCIATION OF SOME SNPS IN eNOS GENE AND SERUM NITRITE LEVELS IN
HEART PATIENTS OF PAKISTANI POPULATION**

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Majority of the cardiovascular diseases (CVDs) are associated with a process known as atherosclerosis, which causes the narrowing and hardening of arteries ultimately leading towards myocardial infarction (MI) commonly known as heart attack. The association of CVDs with single nucleotide polymorphisms (SNPs) in endothelial nitric oxide synthase (eNOS) gene has not been investigated extensively in Pakistani population. Thus, the aim of the present study was to investigate the association of MI with two major eNOS SNPs i.e. G894T and T786C in Pakistani population. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used for genotyping analysis of 100 MI patients and 82 healthy individuals. Moreover nitrite level of MI patients and healthy individuals was also measured by Griess reaction and was found to be correlated with different genotypes resulting from SNPs in eNOS gene. The analysis of eNOS G894T SNP revealed a strong association between mutant TT genotype and MI. It was found to be 23% among MI patients and 12.19% in healthy individuals. Similarly heterozygous GT genotype was also found to be higher (47%) in MI patients as compared with 35.5% in control subjects. Hence, eNOS G894T polymorphism can be used as a genetic marker for MI. On the other hand, eNOS T786C SNP did not revealed a significant difference between the two study groups, it was 9% among patients and 8.5% in healthy individuals for homozygous mutant CC genotype. Hence T786C SNP in eNOS gene was not found to be associated with MI. Furthermore, both types of polymorphisms i.e. G894T and T786C polymorphisms were associated with increased nitrite levels. Serum nitrite level was found to be highest (23 µmol/ml) in patients

with homozygous mutant TT genotypes and heterozygote GT had lower nitrite level i.e. 18 $\mu\text{mol/ml}$, whereas in case of T786C polymorphism individuals with heterozygous TC genotype showed highest nitrite level of 12 $\mu\text{mol/ml}$. Thus presence of a mutated allele, either in homozygous or heterozygous condition also resulted in increased nitrite levels in patients with MI.

**GROWTH PARAMETERS OF THE TWO SPECIES OF FAMILY PORTUNIDAE:
PORTUNUS PELAGICUS AND P. SANGUINOLENTUS IN SONMIANI BAY LAGOON
BALOCHISTAN, PAKISTAN**

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Family Portunidae of brachyuran crabs include economic and commercially important species which are widely distributed across the Indo-West Pacific region. Two species of swimming crabs of family Portunidae, *Portunus pelagicus* and *P. sanguinolentus* are valuable seafood items of great demand both in the domestic market and export industry of Pakistan. Population parameters of the blue swimming crab, *Portunus pelagicus* and three spotted crab *P. sanguinolentus* in Sonmiani bay lagoon Balochistan, Pakistan were estimated. Sampling was carried out through the gill net on the monthly basis from March 2005 to April 2007. Three morphometric relationships were estimated (CW-Wt, CL-CW, CW-Wt) for both species. These relationships demonstrated that growth in both species were allometric ($p < 0.05$). Growth parameters of the Bertalanffy (1938) growth model (L_{∞} and K), total mortality coefficient (Z), natural mortality coefficient (M) and fishing mortality coefficient (F) were estimated. Asymptotic carapace length as (L_{∞} : 168 mm); growth parameter as (K: 1 yr⁻¹); total mortality (Z: 2.299); natural mortality (M: 2.06186) and fishing (F: 0.23714) respectively by applying FAO-ICLARM Fish Stock Assessment Tools (FiSAT II).

**MOVEMENT OF COMMON LEOPARD (*PANTHERA PARDUS*) IN PAKISTAN: SSR
MARKER-BASED STUDY CONFIRMS LONG DISTANCE MIGRATION**

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Simple Sequence Repeat (SSR) markers were used to ascertain the origin of a common leopard (*Panthera pardus*) killed in Sukkar, Pakistan, using skin biopsy samples. We analysed the individual's nuclear DNA, using 3 SSR markers which amplified a total of 6 alleles, suggesting low genetic diversity. This low diversity could be due to small population sizes, and thus the potential for genetic fixation under a population bottleneck (in recent past). The leopard killed in Sukkar had a very high similarity index (1.000) to the Azad Jammu and Kashmir populations, and a lower similarity with Indian (0.33) and Balochistan (0.48) populations. The riverine forest along the river Indus likely provided the opportunity for local movement of leopard.

**HABITAT AND GENDER EFFECT ON METAL ACCUMULATION IN *UCA IRANICA*
FROM COASTAL AREAS OF KARACHI, PAKISTAN**

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Metals in sediment and its accumulation in marine organisms are essential to understand the impacts of metals on their life processes and for monitoring the pollution contribution through the food chain. The current study provides elementary aspect on metal concentration in two major habitats and the assessment of ecological status and health quality of their allied biota. The concentrations of heavy metals (Cu, Cr, Cd, Co, Pb, Fe, Zn and Ni) in *Uca iranica* (fiddler crab) and sediment from mangrove and non-mangrove areas were measured to evaluate the biotope disparity. Analysis of variance reveals that significant variations ($p < 0.05$) were observed for metal concentrations in sediment according to biotope. The bio-concentration factor of all metals except Cd, were observed less than 1.0 in mangrove area, signify no active accumulation while the BCF values for Cu, Cd and Zn were greater than 1.0 in the non-mangrove area. Cd showed a higher bio-concentration factor for both habitats suggesting the active accumulation of this toxic metal. The gender differences were observed in Zn, Cu and Cd concentrations. Strong correlations were identified in both sexes among the most of the metals in mangrove areas. The results were analyzed by multivariate statistical techniques to identify the major factors explaining the variance of metal concentrations in sediment and crabs.

**DETECTION OF MUTATIONS IN RPOB GENE IN LOCAL ISOLATES OF MULTI
DRUG RESISTANT TUBERCULOSIS (MDR-Tb)**

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Tuberculosis is the disease of poverty and Pakistan is at fourth highest in terms of multidrug resistant tuberculosis (MDR-TB) prevalence. MDR-TB is caused by *Mycobacterium tuberculosis* (*M.tb*) that is resistant to at least isoniazid (INH) and rifampicin (RIF), the two influential TB drugs. The progress of drug resistance occurs due to assemblage of changes or mutations in genomic content. A number of genes participate in resistance to isoniazid like katG, ahpC, inhA, and the oxyR-ahpC, rifampin (rpoB gene), streptomycin (rrs, rpsL gene), ethambutol (embB gene), pyrazinamid (pncA gene) resistance and quinolones (gyrA gene) but mostly MDR-TB cases are because of mutation in rpoB gene that encodes the β -subunit of RNA polymerase. MDR-TB flourishes due to poor chemotherapy of TB patients; therefore, the study of molecular basis of MDR-TB is very important to have insight of its mechanism and control. Thus, the present study was conducted to molecularly characterize the rpoB mutations associated with MDR-TB by using PCR-SSCP as screening method and sequencing was carried out to validate the results of PCR-SSCP. It was found that 50 % MDR samples carry mutation (CAC to TAC) at codon 526, 37% carry (GAC to TAC) mutation at codon 516 of rpoB gene. One novel mutation GAA to AAG at codon 543 was also found and 13% MDR samples carry this mutation. In present study the

specificity and sensitivity calculation of PCR-SSCP showed that it has 90.91% specificity with 95% CI of 58.67% to 98.49% and 96.77 sensitivity with 95% confidence interval of 83.24% to 99.46%. Thus PCR-SSCP with sequencing is efficient, cost effective, specific and sensitive technique as diagnostic procedure for those poor countries which have high prevalence of MDR-TB.

NEW RECORD OF CAMPTANDRIID CRAB (CRUSTACEA: DECAPODA: BRACHYURA) FROM INTERTIDAL MUDFLATS OF PAKISTAN

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The brachyuran crabs of family Camptandriidae are characterized by extremely reflexed pleopod (G1), these small sized crabs are inhabitant of intertidal mudflats of tropical and sub tropical shores of the world. So far two species (*Opusia indica* and *Nasima dotilliformes*) were reported. Current study reports one more new species from monotypic genus i.e *Manningis arabicum* which was collected from Sonmiani Bay lagoon, a west coast of Pakistani site of North Arabian Sea. This species was previously considered as endemic from Arabian Gulf and Gulf of Oman. Current study extends the geographical range of this species.

CLONING AND EXPRESSION OF HUMAN HAIR KERATIN K31 ROD DOMAIN FOR HAIR COSMETICS APPLICATION

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K31 is the major protein type of human hair keratin. Loss of K31 decreases the hair tensile strength. The present study based on the recombinant production of human hair type I acidic keratin K31 (Hha 1) Rod domain (Kr) in *E. coli* as a possible straightening and strengthening agent for damaged and curly hair. The K31 Kr domain amplified and cloned in pTZ cloning vector, digesting the recombinant pTZ-Kr vector with *NdeI* and *EcoRI* restriction enzymes was inserted in T7 promoter based pET22b (+) vector to generate Kr-pET22b expression plasmid. The correct integration of Kr in pET22b was confirmed by restriction digestion and colony PCR. The recombinant Kr-pET22b was then used to transform *E. coli* BL21 expression host. The transformants were grown at 37°C up to an OD₆₀₀ = 8 in 100 ml M9NG medium in which 10 mM lactose was used to induce Kr expression in *E. coli*. A prominent band of □□ 33 kDa could be seen on 12% SDS-PAGE. Expression levels achieved amounted to 30% of the total *E. coli* cellular proteins. The higher cell density (OD₆₀₀=10) was achieved in auto-inducing M9NG medium when cells were grown at 28°C. The expressed protein was, however, in the form of inclusion bodies, were solubilized in 1 M urea under alkaline conditions (pH 12) and the denaturant was subsequently removed by step-dialysis while reducing the buffer pH to 8. Keratin rod domain protein along with NaOH treatment, when applied on damaged curly hair, not only resulted in straightening but also restoring their natural beauty.

AN OVER CLAWED (WITH TWO ENLARGE CHELA) MALE CRAB OF *UCA (TUBUCA) URVILLEI* (OCYPODIDAE: UCINAE) ALONG THE COAST OF KARACHI, PAKISTAN

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Uca (Tubuca) urvillei (H. Milne Edwards 1852) is one of the most abundant narrow front Fiddler crab species in the Pakistan mangrove forest, The male of this narrow front species *Uca urvillei* (Milne- Edwards, 1852) has been recorded as super male (with two large chelipede) first time from the natural habitat of Sandspit, Karachi, Pakistan. Specimen posses two enlarged chelipeds rather than only cheliped along with other small one. Before this no any such supermale has been found so far in natural habitat.

IN-SILICO PREDICTION OF PROTEIN KINASE DOMAIN INTERACTION WITH ATP IN AN ATYPICAL KINASE-FIKK 4.2 OF *PLASMODIUM FALCIPARUM*

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During the developmental stage of *Plasmodium falciparum* extensive changes in shape, structure, permeability, deformability and adhesiveness of the membrane occurs. The different structures of red blood cells (RBCs) like the membrane, becomes phosphorylated, where it loses its integrity leading to deformation of the membrane. During this developmental stage in RBCs it gives rise to some clinical manifestations designated as malaria. Recent genomic study of *Plasmodium* has identified a novel family of kinases which has a conserved amino acid motif phenylalanine (F), Isoleucine (I) and lysine (K) hence named FIKK kinases. FIKK 4.2, a member of this kinase family has been proposed to phosphorylate various cytoskeleton proteins such as spectrin and demntin and has a role in the remodeling of the RBC membrane. To understand the interaction between plasmodium and its host cell membrane proteins, FIKK 4.2 and its interaction with ATP was investigated. Sequence homology studies revealed that FIKK 4.2 has a catalytic domain similar to prokaryotes like protein kinase domain of eukaryotic kinases. A 3D structure of FIKK 4.2 protein kinase domain (76 residues) was developed by using the bioinformatic tool PHYRE. It was found that 41% formed α -helix, 16 % formed β -strands and rest of the sequence was predicted to be forming loops. The protein kinase domain interaction with ATP was found possible via five different amino acid clusters, making up the ATP binding pocket of the domain. One cluster showing maximum score for ligand interaction (residues 8-11, 14, 62, 65 and 75-77) was accepted as ATP binding pocket. This information can be helpful to further investigate and predict the models of interaction of FIKK 4.2 with host RBC membrane proteins. It can also be helpful to devise effective antimalarial therapies targeting such kinases to reduce the pathogenesis of *Plasmodium*.

DEGRADATION OF PHENOL BY LOCALLY ISOLATED BACTERIAL STRAINS**Memoona Ramzan and Abdul Rehman***Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore*

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Phenol and its many derivatives are produced at the rate of about 6 million ton/year worldwide as a waste of different industries such as agriculture chemical, textile, plastic, paper and pulp, wood preservation, oil refineries, pharmaceuticals, wine distilleries and coal coking. Usually, this waste is directly dumped into the water without any treatment. On its way to other water bodies, the presence of phenol becomes a major threat to aquatic and terrestrial biota. Therefore, it is necessary to explore and devise certain effective and safe methods for removal of phenol and its derivatives from water. In this study, phenol degrading activity by bacterial isolates collected from industrial effluent showed maximum survival in the presence of phenol up to the concentration of 300µg/ml with an initial concentration of 100µg/ml. Two isolates were selected for further characterization and both were gram negative and aerobic bacteria. Both exhibited maximum growth in the presence of phenol at 37°C and pH of 7. They were identified as *Stenotrophomonas maltophilia* and *Bacillus subtilis* by 16S rRNA analysis. Furthermore, these strains were analyzed for enzyme activity involved in degradation of phenol. Among intracellular and extracellular enzyme activity, extracellular was found comparatively predominant. The extracellular enzyme activity was observed under wide ranges of pH (5-9), temperature (30°C-90°C) and presence of different metal ions (Na⁺, Mg²⁺, Mn²⁺, Zn²⁺ and Cu²⁺). Maximum enzyme activity was observed at pH 5 and temperature as 90°C. Protein assessment by SDS-PAGE and HPLC studies confirmed that both bacterial species possess efficient ability to degrade phenol. Conclusively, bacterial strains can be exploited for removal of phenol from polluted water or land, which will be cost effective and safe as well.

ISOLATION OF OLEAGINOUS YEASTS AND DESIGNING A MICROBIAL CONSORTIUM BASED BIOPROCESS FOR LOW-COST CULTIVATION**Hira Javaid and Javed Iqbal Qazi***Microbial Biotechnology Lab. Department of Zoology, University of the Punjab, Lahore (54590)*

Thirty strains of oleaginous yeasts were isolated from organic rich soils sampled from Lahore, Pakistan. Different carbon sources like glucose, xylose, glycerol and lactose were employed in different isolation media. Six different isolation media were prepared using the four different carbon sources. Media A and C contained glycerol and 9 and 3 yeast strains were isolated, respectively, on them. Whereas media B and E contained glucose and yielded 7 and 3 isolates, respectively. The medium D contained xylose as carbon source and 6 strains were isolated on this and media F contained lactose as carbon source and 3 strains were isolated. Maximum oleaginous yeast strains were isolated from glycerol isolation media. The colonies were differentiated ON the basis of colonial morphology. All the strains were checked for oil production utilizing a nitrogen-deficient medium. All the isolates were incubated at 30°C for four days. The lipids were extracted using methanol and chloroform in 1:1 ratio. Quantity of lipid and dry mass were calculated. Microbial consortia based bio-fermenter has been designed to employ low-cost substrates based fermentation process for yeast biolipid production. Biologically saccharified low-cost cellulytic substrates syrup will be fed to the yeast culture for maximum production of lipids. The designed

bioprocess will likely lead to the economic provision of biodiesel and yeast extract while utilizing agri/urban wastes.

MAPPING OF LOCI FOR RARE GENETIC DISORDER IN PAKISTAN

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Rare disorders are those diseases which affect a very small percentage of population. In contrast to research on genetics of more common disorders, work on rare disorders is very limited. Four families with multiple affected individuals were recruited from different cities of Punjab, Pakistan. Three families contained only affected male individuals, with different types of movement disorders. For these linkage to known genetic loci on X chromosome was excluded first. In order to map the responsible locus for disease, linkage analysis was performed. The whole X chromosome was typed by using 41 microsatellite markers spanning the X chromosome. A new locus for movement disorder associated with abnormal hands and feet morphology segregating as an X linked recessive trait, was mapped to chromosome Xq27.3-q28 in family RDHR-08 with a maximum LOD score of 2.6 for *DXS8011* at $\theta=0$. *MTM1*, *BGN*, *EMD*, and *FLNA* were considered as possible candidate genes for the trait in this family. X chromosome for family RDHR-01 and RDHR-04 was excluded which means that the causative gene for the trait of these family is present on an autosome.

ISOLATION AND CHARACTERIZATION OF CADMIUM RESISTANT BACTERIA AND THEIR POTENTIAL USE IN DECONTAMINATION OF METAL POLLUTED WASTEWATER

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Heavy metal contamination due to natural and anthropogenic sources is a global environmental concern. Heavy metals are the most abundant pollutants in the sewage and in wastewater and are one of the main causes of water and soil pollution. Heavy metal pollution is badly affecting human health as it gets entry into human food chain and drinking water. Removal of metal pollutants from the contaminated environment has been a challenge for a long time. It is important to establish an efficient and low cost method for the removal of toxic metal ions. Microorganisms present in industrial effluents carrying toxic chemicals show adaptation and acclimation to their environment. Many of them have achieved the capability of metabolizing toxic metal ions. Microorganisms with the ability to grow in the presence of heavy metals and with a significant metal uptake have a potential use in bioremediation of polluted waters. The present research work aims at the isolation, biochemical and molecular characterization, optimum growth conditions with reference to temperature and pH and tolerance to cadmium toxic ions of microorganism from industrial wastewaters of different industrial areas of Pakistan. Glutathione and non-protein thiols were estimated in Cd-treated samples to know the role of these molecules in cadmium resistance. Cadmium uptake ability of microorganisms will be assessed with a view to

using them to detoxify industrial wastewaters contaminated with cadmium ions. Cadmium resistance mechanism will also be assessed in these bacteria.

MOLECULAR CHARACTERIZATION OF ARSENITE RESISTANT BACTERIA FROM INDUSTRIAL EFFLUENTS AND THEIR POTENTIAL USE IN WASTEWATER TREATMENT

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In the present investigation arsenite oxidizing bacteria, isolated from industrial wastewater of Lahore, Pakistan, were found to have resistance against arsenite (40 mM) and other heavy metals (10 mM Pb; 8 mM Cd; 6 mM Cr; 10 mM Cu and 26.6 mM As⁺⁵). Bacterial isolates characterized on the basis of morphological, biochemical and 16S rRNA as *Bacillus cereus* (1.1S) and *Acinetobacter junii* (1.3S) under accession numbers KF003021 and KF003019, respectively. Both isolates showed maximum growth after 24 h of incubation at pH 7 and temperature 37°C as optimum. The AgNO₃ assay performed to determine arsenite oxidase activity as by brownish precipitation. Safranin assay performed to quantitatively determine arsenite oxidase activity extracellularly up to 73% and 75% for and *A. junii*, respectively. Presence of arsenite increased the concentration of glutathione (GSH) and non-protein thiol in *B. cereus* and *A. junii* (170% and 108%) and (72% and 69%), repetitively at the concentration of 100 mg/l. Oxidation of As⁺³ in original industrial wastewater determined upto 92% and 88% in the presence of *B. cereus* and *A. junii*, respectively. The microbial treated wastewater used for the growth of *Vigna radiata* (mung beans) plant and improved growth of *Vigna radiata* observed in microbial treated wastewater as compared to untreated wastewater. It indicates that these bacterial isolates have fair ability to oxidize As⁺³ into As⁺⁵ and may find some potential applications in wastewater treatment systems to transform arsenite into less toxic form, arsenate.

CHARACTERIZATION OF AZO DYES DEGRADING BACTERIA FROM INDUSTRIAL CONTAMINATED WASTEWATER

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A developing country like Pakistan requires the establishment of new industries in order to develop its economy. Synthetic dyes especially azo dyes which are widely used in many industries such as textiles, cosmetics, paper, leather and pharmaceutical, are the major constituents of the wastewater discharged from such industries. Due to its non-degradability and biomagnifications properties, it persists in the environment and imposes significant effects on human and animal health. However, bacteria are responsible for their degradation under anaerobic and aerobic conditions which is eco-friendly and cost-competitive alternative to chemical decomposition process. The present study deals with isolation, biochemical and physiological characterization, growth curve, temperature, pH, and metal ions effect of azo dye resistant bacteria. Wastewater samples were collected from Kot Lakhpat industrial estate, Lahore and azo dye resistant aerobic and anaerobic bacteria were isolated. Their physiological and molecular characterization is in process now a days and the dye degradation conditions will be optimized on lab scale and further

checked on large scale. Moreover, microbial and phytotoxicity assays will be performed in order to analyze the toxic effects of decolorized dyes wastewater.

LOW SERUM VITAMIN D LEVELS AND SUSCEPTIBILITY OF TUBERCULOSIS

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Vitamin D modulates monocyte-macrophage activity in the body and plays a role in human innate immunity to certain infectious agents. This role may be important in the body's defence against tuberculosis. We conducted a case control study and a longitudinal study to investigate the effect of Vitamin D on tuberculosis. We recruited 260 patients initiating treatment for smear positive pulmonary tuberculosis in Lahore, Pakistan and 100 age match controls. Vitamin D status and genotypes for polymorphism in VDR, DBP and CYP2R1 were determined at baseline in TB patients and controls. Analysis was conducted to determine vitamin D association with active TB, to determine susceptibility of TB and genetic determinants. The clinical and genetic determinants of baseline vitamin D status were also analysed. Profound vitamin D deficiency was observed in TB patients ($p < 0.001$); significant association of rs2060793 in TB patients (p -value 0.04) was found. Vitamin D deficiency was independently associated with female vs. male sex, recruitment in October to March inclusive, bilateral vs. unilateral disease and Bsm polymorphism. Vitamin D deficiency is a risk for active tuberculosis in Pakistan.

OXIDATIVE STRESS AND GLUTATHIONE RESPONSE TO HEAVY METALS IN MULTI-RESISTANT YEAST PATHOGEN, *TRICHOSPORON ASAHII*

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A yeast strain, *Trichosporon asahii*, isolated from industrial effluents was grown well at 37°C (pH 6) in YPD medium. The minimum inhibitory concentration (MIC) observed was 10 mM (Cd), 33 mM (Cu), 30 mM (As) and 35 mM (Pb). Overall MIC was ranged from 10-35 mM and increased in the following order: Pb > Cu > As > Cd > Cr. The total glutathione (GSH and GSSG) and cysteine levels were assayed by growing (24 h) cultures and exposed to 100 mg/l of each heavy metal ion for 2 days. Cadmium showed remarkable increase in GSH level followed by arsenic, chromium, lead and copper. However, stressed cells had much more oxidized GSH than unstressed cells. A decreased reduced/oxidized glutathione ratio was observed in cadmium in contrast to copper, arsenic and lead and no difference was detected in chromium treated cells. Statistical analysis revealed significantly higher levels of cysteine and non-protein thiols in metal treated samples as compared to control. Analysis of relative arsenite and arsenate concentrations in metal contaminated cultures revealed that arsenite disappearance and arsenate appearance was due to enzymatic oxidation by arsenite oxidase. Arsenic and cadmium removal potential was 83 and 81% whereas 91 and 93% removal was determined by copper and lead from the medium. This metal uptake capacity of yeast can be exploited in wastewater treatment strategies. Marked differences in

protein banding pattern were observed in heavy metal exposed and unexposed yeast cells. Both arsenic and lead treatment showed increased intensity of 80kDa and 20 kDa protein bands as compared to control.

MUTATIONAL ANALYSIS OF *flt3* GENE IN DIFFERENT FORMS OF LEUKEMIA

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Leukemia is a disease of uncontrolled proliferation of hematopoietic stem cells. Leukemia is of four basic types, Acute Lymphocytic Leukemia (ALL), Acute Myeloid Leukemia (AML), Chronic Lymphocytic Leukemia (CLL) and Chronic Myeloid Leukemia (CML). The ratio of AML is higher than all other forms of leukemia, while in children ALL is most common. Most frequent genetic aberrations in AML are the mutations in FLT3 gene. Present study is aimed to find mutations in FLT3 in different forms of leukemia. Mutations in exon 14 to exon 15 of FLT3 were screened by PCR-SSCP and further confirmed by sequencing. Peripheral blood of 10 normal samples and 26 leukemia samples was collected. Out of which 9 were of ALL, 3 AML, 9 CML, and 5 of lymphoma patients. SSCP revealed obvious differences in banding pattern of 4 patient samples; P7, P14, P25 and P26, which were selected for sequencing. Sequencing results revealed mutations in 3 of 4 patients. Greatest sequence variations could be found in AML patients. FLT3 mutations produce adverse form of leukemia and are common in AML patients. These mutations have an effect on progression and prognosis of the disease. Patients with FLT3 mutations need modification in treatment with chemotherapy. These patients respond better by targeting FLT3 signaling proteins. Modern methods to cure leukemia use nucleoside analogs, vaccines, immunological targeting, tyrosine kinase inhibitors, and allogenic stem cell transplants.

PRODUCTION OF ARTEMIA CYSTS ON PILOT SCALE

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Artemia (Linnaeus 1758) commonly known as brine shrimp is an essential feed for larval stages of finfish and shellfish. Thus the production of *Artemia* is important for the development of aquaculture industry particularly in developing countries. In the present work *Artemia* cysts were collected through micro-sieving from salt pans near Younusabad. The de-capsulation and storage of the cysts were accomplished during 45 days by following six different steps. The cyst hydration, filter and rinse cysts, transfer of cysts to *Artemia* tanks and de-capsulation processes have been carried out in Hawksbay hatchery with 80-82% hatching rate of nauplii. After that the nauplii were transfer to ponds near Ambra Creek Thatta, where 75-78% survival rate has been observed. Finally as the female *Artemia* starts to produce cysts which were collected and stored. Most of the hatcheries are dependent on imported *Artemia* cysts which makes hatchery operations expensive therefore, this study would contribute towards the development of aquaculture industry in Pakistan.

ISOLATION OF OLEAGINOUS MICROALGAL SPECIES FOR BIODIESEL PRODUCTION FROM FRESHWATER LOCALITIES OF PUNJAB, PAKISTAN

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Oleaginous microalgal species e.g, *Chlorella* sp., *Scenedesmus quadricauda*, *Anabaena* sp. and *Chlamydomonas* sp. were isolated from different freshwater localities of Punjab, Pakistan, during summer of 2014. These were maintained in BG11 and BBM media, with a photoperiod of 18 hours of artificial light/6 hours' dark, at a temperature of 28°C. Nile red (9-diethylamino-5H-benzo[α] phenoxaphenoxazine-5-one) staining was carried out to detect intracellular lipid droplets with the help of fluorescence microscope. These species appear potential candidates for economic biodiesel production while utilizing food industrial effluents and sunlight.

BIODIVERSITY OF FAUNA ASSOCIATED WITH MANGROVE ECOSYSTEM OF PAKISTAN

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Mangroves are angiosperm, halophytic, viviparous shrubs or trees having specialized vascular system. They provide nutrients in the form of organic matter and they are heavily populated by variety of organisms which includes invertebrate fauna such as arthropods, cnidarians, annelids, molluscs and vertebrate fauna like fishes, amphibians, reptiles, birds and mammals. The ecosystem is considered as most productive due to its biodiversity, and provides protection against erosion, storm surge and tsunamis. Mangroves serve as a critical nursery grounds for commercially important fisheries and therefore play an important role in the economic well-being of fishermen. Pakistan has one of the world's largest arid mangroves in the world. It benefits the national economy to earn foreign exchange of 100 million US dollars/annum from exports. Mangrove biodiversity is highly threatened in their existence by several causes, the main source of these **threats** are induced by human activities. Society of Pakistan is learning now, how to protect mangroves and use them in sustainable ways. In order to restore our mangrove areas to its former pristine glory, we must create awareness and implement afforestation along with anti-pollution policies on a large scale.

MOLECULAR EPIDEMIOLOGY OF DIFFERENT CLINICAL ISOLATES OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* OF LAHORE BASED ON SPA TYPING

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Staphylococcus aureus has become the commonest nosocomial infectious agent throughout the world, causing a wide range of hospital infections. Epidemiological studies suggest that

hospitals of all sizes are facing the problem of the resistant form of *Staphylococcus aureus* that is Methicillin Resistant *Staphylococcus aureus* (MRSA). Protein A of *Staphylococcus aureus* is a virulence factor whose encoding gene, *spa*, shows a variation in length among different strains of MRSA. In this study Ninety-six strains were collected from private and public sector hospitals of Lahore. Sixty-seven (70%) strains were actual MRSA after the reconfirmation by DNase test and Kirby Bar disk diffusion test. The remaining (30%) isolates were misdiagnosed as other *Staphylococcus* species. Subsequently, Polymorphic X region of the *spa* gene was amplified using specific primers of *spa* gene by the polymerase chain reaction (PCR). Four different patterns, ranging in length between 300 to 500 bp, showed polymorphism of the *spa* gene. Maximum strains showed 98 % homology with isolate *spa 7 Staphylococcus aureus* IgG protein. Those sequences would be analyzed by the *Spa* Ridom database and will be served as a rapid diagnostic tool for the identification of MRSA during epidemic.

SARDINES AND ANCHOVIES OF PAKISTAN

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Sardines and anchovies, pelagic shoaling fishes are found on the near surface area throughout the fishing grounds of Pakistan but chiefly in a 16 km strip of coastal waters and specially during breeding season in Sonmiani bay lagoon. These species feed upon plankton. They were caught with boat seines, gillnets, shore seines and cast nets. They were also caught in the trawl net owing to the shallowness of the habitat. It reveals that these fishes were also found in near shore shallow water at 10 – 12 meters depth in the Pakistani Sea waters. Among several species (Bianchi, 1985), 25 species of Sardines and anchovies were found commercially importance in the study area catches. Sardines found dominant with 17 species in which *Hilsa kelee*, *Ilisha megaloptera*, and *Escualosa thoracata* found abundant in most of the year whereas anchovies ranked second with 8 species among *Thryssa setirostris*, *Thryssa vitirostris* and *Thryssa malabarica* found abundant. The major fluctuation of species was cause by salinity and temperature of the area, so the species diversity and catch sizes varied with the months and the biomass distribution of catch as well as species showed a seasonal pattern in Pakistani Sea waters.

ESTIMATION OF GENE EXPRESSION OF CELL TO MATRIX AND CELL-CELL ADHESION GENES IN CANCER CELL LINES AT DIFFERENT TIME POINTS IN CELL CYCLE

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Cancer is one of the major causes of death worldwide and most important reason this high mortality rate is the metastasis in which cells break their connection from their primary site of origin and reach the distant parts of body, develop new adhesion junctions their and invade in surrounding tissue. Cell to cell and cell to matrix adhesion has a crucial role in cancer development

and metastasis in glioblastoma (an invasive type of brain tumor). This adhesion is due to different genes such as E-cadherin, N-cadherin, Ep-CAM, N-CAM and integrin which express at different time points. Complete cell to matrix attachment (in vitro) is achieved within 24h and during this time all these genes undergo a unique pattern of expression. In our study the pattern of expression of these genes during 24h at different time points (0h, 1h, 3h, 6h, 12h and 24h) was estimated in SF767 (glioblastoma), MDA-MB231(breast cancer), Hela (Cervical cancer) cell line using both colorimetric quantification and real time PCR techniques and results were compared with the visual observations obtained from photographs of cell culture at 0,1,3,6,12 and 24h. Genes responsible for cell to matrix adhesion were constantly expressed but expression reached to highest point after 6h of initial attachment. Genes responsible for cell to cell adhesion were over expressed in SF767 and comparatively less expression was observed in breast cancer cell line. All these genes have complex cross talk among each other and this complex communication allows cancer cells to detach from primary site of tumor and attach to the secondary site of tumor.
