CONGRESS ORGANIZING COMMITTEE

Prof. Dr. A.R. Shakoori
Prof. Dr. Muzaffar Ahmad
Prof. Dr. M. Afzal Kazmi
Dr. Muhammad Afzal
Dr. Shahzad A. Mufti
Mr. Abdul Aziz Khan
Prof. Dr. S.I.H. Jafri
Dr. Kh. Abdul Mujeeb
Dr. Iftikhar Hussain
Mr. Basharat Ahmad Ch.
Mr. Muhammad Ishaq
Dr. Shahid Mahboob Rana
Dr. Syed Shahid Ali
Dr. Inayat Ali Shahjahan
Prof. Dr. A.M. Cheema
Dr. S. Khan Khattak
Dr. Juma Khan Kakar
Prof. Dr. Ata-ur- Rehman
Prof. Dr. M.D. Shamsi
Prof. Dr. M.H. Qazi
Prof. Dr. A.Q. Ansari
Prof. Dr. S. Altaf Hussain
Dr. Anwar Nasim
Prof.Dr. Khalid M. Khan
Dr. Kasuar Abdullah Malik
Dr. M. Anwar Waqar
Prof. Dr. M.A. Hafeez
Dr. Badruddin Soomro
Prof. Dr. Najma Najam
Dr. Farid Malik
Dr. S.T.K. Naeem
Dr. Afsari S. Qureshi
Prof. Dr. M. Anwar Malik
Prof. Dr. Afsar Mian
Prof. Dr. G.S. Stein, USA
Prof. Dr. A.Sokoloff, USA.
Dr. R.M. Wilkins, UK
Dr. P. Wynn, Australia
Dr. Fereidoon Owfi, Iran
Dr. Gao Xingyi, China
Dr. R. Fotedar, Australia
Prof. Dr. H.H. Gilani, USA
Prof. Dr. G. Nies, Germany
Dr. Louis Evan, Australia
Dr. Mario Carenza, Italy
Dr. M. Mergeay, Belgium
Dr. C. Cervantes, Mexico
Dr. H. Eccles, UK
Prof. Dr. H. K. Poland
Prof. Dr. Nasiruddin

LOCAL ORGANIZING COMMITTEE

Prof. Dr. S. Altaf Hussain
Vice Chancellor (Chief Organizer)

Prof. Dr. Muhammad Rashid
Prof. Najeeb A. Khan
Prof. Dr. Nazir Ahmad Sangi
Prof. Dr. Ali Asghar Chisti
Dr. M. Irfan Khan
Prof. Dr. Afsar Mian
Dr. Azra Khanum
Dr. Yasmin Mussdiq
Mr. Muhammad Afzal
Mr. Abdul Aziz Khan
Dr. S. Azhar Hassan
Dr. Nasreen Muzaffar

PROCEEDINGS OF THE CONGRESS

Editors

Dr. Muzaffer Ahmad

Dr. A.R. Shakoori

Computerized Composed by:

Amjad Ali

PROCEEDINGS

OF

PAKISTAN CONGRESS OF ZOOLOGY

Volume 24, 2004

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

TWENTY FOURTH PAKISTAN CONGRESS OF ZOOLOGY

held under auspices of

THE ZOOLOGICAL SOCIETY OF PAKISTAN

at

Allama Iqbal Open University, Islamabad

March 30 to April 1, 2004

CONTENTS

ACKNOWLEDGMENTS

Allama Iqbal Open University, Islamabad hosted the 24th Pakistan Congress of Zoology (International).

The Zoological Society of Pakistan expresses its deep gratitude to the Vice Chancellor, Allama Iqbal Open University, Islamabad and Dean, Faculty of Sciences, Incharge Division of Environmental Sciences, faculty members and students of the University for extending warm hospitality.

Grants were received from Pakistan Science Foundation, Islamabad, Higher Education Commission, Islamabad and Allama Iqbal Open University, Islamabad.

TWENTY FOURTH PAKISTAN CONGRESS OF ZOOLOGY (INTERNATIONAL)

ALLAMA IQBAL OPEN UNIVERSITY, ISLAMABAD

MARCH 30 – APRIL 1, 2004

PROGRAMME

TUESDAY, MARCH 30, 2004

09:00 AM	Registration	
09:30 AM	Guests to be seated	
09:45 AM	Arrival of the Chief Guest	Prof. Dr. Ata ur Rehman
09:55 AM	Inaugural Session Start	
10:00 AM	Recitation	
10:05 AM	Welcome Address:	Vice Chancellor, AIOU
10:20 AM	Report	Secretary, Zoological Society
10:35 AM	Key Note Address	President, Zoological Society
10:50 AM	Distribution of Medals and Awards	
11:10 AM	Address by the Chief Guest	
11:25 AM	Vote of Thanks	Dean, Faculty of Sciences
11:30 AM	Refreshment	

JOINT SESSION I: (Plenary Lectures)

Chairperson: National Distinguished Prof. M. Akhtar FRS

12:00 AM Prof. Dr. Nasiruddin
Evolving New Concepts in Pursuit of Expanding Vision: Combinatorial Metabolism in *Plasmodium falciparum* Infected Erythrocytes

01:00 PM Lunch and Prayer

LIAQUAT HALL

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

SESSION I
Dedicated to Dr. Din Muhammad Shaikh

	Chairperson:	Prof. Dr. S.N.H. Naqvi
	Co-chairperson:	Dr. Azra Khanum
02:00 AM	Paper reading	
04:30 PM	Tea Time	

SESSION II
Dedicated to Dr. Hamid Khan Bhatti (Late)

	Chairperson:	Dr. Samina Jilali
	Co-chairperson:	Dr. Tasawar H. Khan
05:00 PM	Paper reading	
06:30 PM	Prayer	

SESSION III
Dedicated to Prof. Dr. Ahmed Mohiuddin (Late)

	Chairperson:	Prof. Dr. Shamsuddin Shaikh
	Co-chairperson:	Dr. Naeem Ahmad
06:45 AM	Paper reading	
08:00 PM	Dinner	

JINNAH HALL

SECTION II: PEST AND PEST CONTROL

SESSION I
Dedicated to Prof. Naseeruddin Ahmed (Late)

	Chairperson:	Dr. Sana Ullah Khan Khattak
	Co-chairperson:	Dr. A.G. Arijo
02:00 PM	Paper reading	
04:30 PM	Tea Time	

SESSION II

Dedicated to Dr. M.A. Ghani (Late)

	Chairperson:	Prof. Dr. M. Suleman
	Co-chairperson:	Dr. Mushtaq A. Saleem
05:00 PM	Paper reading	
06:30 PM	Prayer	

SECTION III: ENTOMOLOGY

SESSION I

Dedicated to Dr. S. Nazir Ahmed (Late)

	Chairperson:	Prof. Dr. M.S. Wagan
	Co-chairperson:	Prof. Dr. Syed Kamaluddin
06:45 PM	Paper reading	
08:00 PM	Dinner	

WEDNESDAY, MARCH 31, 2004

JOINT SESSION II: (Plenary Lectures)

Chairman: Prof. Dr. M.H. Qazi

09:00 AM Meritorious Prof. Dr. A.R. Shakoori, School of Biological Sciences, University of the Punjab, Lahore.
Possible Functional Role of Selectins: Prediction Based on Post-Translational Modification Sites.

09:30 AM Dr. Muhammad Irfan Khan, Allama Iqbal Open University, Islamabad.
TRIPs Agreement of WTO and Genetic Resources: Implications and Options for Developing Countries.

LIAQUAT HALL

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

SESSION IV

Dedicated to Prof. Dr. M.H. Qazi

	Chairperson:	Prof. Dr. Afsari Qureshi
	Co-chairperson:	Dr. Muhammad Afzal
10:00 AM	Paper reading	
11:00 PM	Tea Break	

SESSION V

Dedicated to Prof. Dr. Muhammad Arslan

	Chairperson:	Prof. Dr. M. Kaeem Tahir
	Co-chairperson:	Dr. A.R. Abbasi
11:30 AM	Paper reading	
01:00 PM	Lunch and Prayer	

SESSION VI

Dedicated to Prof. Dr. Ashfaq Ahmad (Late)

	Chairperson:	Prof. Dr. Anwar Malik
	Co-chairperson:	Dr. Nematullah
02:00 PM	Paper reading	
04:30 PM	Tea Break	

SESSION VII

Dedicated to Dr. Muhammad Sharif Mughal

	Chairperson:	Prof. Dr. M. Sharif Mughal
	Co-chairperson:	Dr. Wasim Ahmad
05:00 PM	Paper reading	
06:30 PM	Prayer	

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

SESSION I

Dedicated to Dr. Manzoor Ahmad (Late)

	Chairperson:	Prof. Dr. Afsar Mian
	Co-chairperson:	Dr. Iftikhar Hussain
06:45 PM	Paper reading	
07:00 PM	Poster Session	
08:00 PM	Dinner	

JINNAH HALL

SECTION III: ENTOMOLOGY

SESSION II
Dedicated to Dr. Mian Afzal Hussain (Late)

	Chairperson:	Prof. Dr. Imtiaz Ahmad
	Co-chairperson:	Dr. Zahoor Salihah
10:00 AM	Paper reading	
11:30 PM	Tea Break	

SESSION III

Dedicated to Prof. Muhammad Afzal Hussain Qadri (Late)

	Chairperson:	Prof. Dr. Nazir Ahmad Sangi
	Co-chairperson:	Dr. Inayat Ali Shahjehan
11:30 AM	Paper reading	
01:00 PM	Lunch and Prayer	

SESSION IV

Dedicated to Dr. Muhammad Sharif (Late)

Chairperson:	Dr. Azhar Hasan
Co-chairperson:	Dr. Naheed Ali

02:00 PM Paper reading
04:30 PM Tea Break

SECTION IV: PARASITOLOGY

SESSION I

Dedicated to Prof. Dr. Daler Khan (Late)

Chairperson: Prof. Dr. Fatima Mujeeb Bilquees
Co-chairperson: Dr. Aly Khan
05:00 PM Paper reading
06:30 PM Prayer

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

SESSION II

Dedicated to Dr. Ahsan ul Islam (Late)

Chairperson: Prof. Dr. M. Naeem Khan
Co-chairperson: Mr. Javed Iqbal
06:45 PM Paper reading
07:00 PM Poster Session and SZP Executive Council Meeting
08:00 PM Dinner

THURSDAY, APRIL 1, 2004

JOINT SESSION III: (Plenary Lectures)

09:00 AM Dr. Muhammad Naeem Khan, Punjab Fisheries, Department, Lahore.
Impact of WTO Agreements on Fisheries Biology

LIAQUAT HALL

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

SESSION III
Dedicated to Dr. Abu Baker (Late)

	Chairperson:	Dr. Abdul Aleem Chowdhary
	Co-chairperson:	Mr. Abdul Aziz Khan
09:00 AM	Paper reading	
11:00 AM	Tea Break	

SESSION V
Dedicated to Qazi Zakir Hussain

	Chairperson:	Prof. Dr. Qudusi B. Kazmi
	Co-chairperson:	Dr. Itrat Zehra
11:30 AM	Paper reading	
01:00 PM	Lunch and Prayer.	

JINNAH HALL

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

SESSION IV
Dedicated to Nazir Ahmad (Late)

	Chairperson:	Dr. Muhammad Ayub
	Co-chairperson:	Dr. Muhammad Javed
09:00 AM	Paper reading	
11:00 AM	Paper reading	

SESSION VI
Dedicated to Dr. Muhammad Saleem Mahmoon (Late)

Chairperson:	Prof. Dr. S.I.H. Jafri
Co-chairperson:	Dr. Muhammad Irfan Khan

11:30 AM	Paper reading
01:00 PM	Lunch and Prayer.
02:00 PM	General Body Meeting
03:30 PM	Concluding Session
	Recitation
	Congress Report by President SZP
	Award Ceremony
	Concluding Remarks by the Chief Guest
	Vote of Thanks
04:30 PM	Refreshments

MEMBERS OF THE CONGRESS

BAHAWALPUR

Shafiq, M. (Dr.)
Sial, N.

FAISALABAD

Arshad, R.
Ashraf, A.
Ashraf, A.
Chaudhry, T.M. (Dr.)
Khalid, S. (Dr.)
Khaliq, S.
Muzammil, H.M.
Nadeem, S. (Dr.)
Rana, S.M. (Dr.)
Shah, M.S. (Dr.)
Yaqoob, N.
Zubair, H.

GILGIT

Ahmed, K.

HYDERABAD

Shaikh, R.

GUJRANWALA

Sandhu, A.A.

ISLAMABAD

Ahmad, T. (Prof.)
Ahmad, Y. (Dr.)
Akhtar, N.
Ali, M.H.
Ashfaq, G. (Dr.)
Baig, K.J. (Dr.)
Basit, A.
Bukhsh, E.
Chaudhry, A.A. (Dr.)
Fakhar-I-Abbas
Feroze, A.
Hussain, R.
Iqbal, J.
Khan, A.A.
Khan, Z. (Dr.)
Leghari, M.K. (Dr.)
Muzaffar, N. (Dr.)
Naeem, R.
Parveen, K.
Rafique, M.
Shah, N.A.

JAMSHORO

Abbasi, A.R. (Dr.
Anjum, A. (Dr.)
Baloch, W.A. (Dr.)
Bhatti, R.
Bughio, B.A.
Channa, S.A.
Dharejo, A.M. (Prof.)
Gill, N.
Jafri, S.I.H. (Dr.)
Khan, M.M. (Dr.)
Leghari, S.M. (Dr.)
Memon, N. (Dr.)
Memon, Z.
Narejo, N.T. (Dr.)
Shah, S.A.A.
Shahneela
Shaikh, B.B. (Dr.)
Shaikh, G.S.

Shaikh, S.A. (Dr.)
Soomro, A.L.
Soomro, A.N.
Soomro, S.
Sultana, R.
Ursani, T.J.
Wagan, M.S. (Dr.)

KARACHI

Ahmad, I. (Prof.)
Ahmed, S.M.
Akhtar, K. (Dr.)
Ameer, F.
Anwar, N.
Attaullah
Azmi, M.A. (Dr.)
Begum, S.
Ghazi, R.R. (Dr.)
Ghory, F.S.
Hameed, N. (Prof.)
Hameed, S.
Haseeb, M.F. (Dr.)
Hasnain, S.A.
Hassan, H. (Dr.)
Hussain, B.
Hussain, S.M. (Dr.)
Irfan, S.
Kamaluddin, S. (Dr.)
Kazmi, M.A. (Dr.)
Kazmi, Q.B. (Dr.)
Khan, A. (Dr.)
Khatoon, N. (Dr.)
Moizuddin, M. (Dr.)
Mughal, I.
Mughal, S.
Mujib, B.F. (Dr.)
Mushtaq, S.
Mustaquim, J. (Dr.)
Naqvi, S.M.H.M.
Naqvi, S.N.H. (Dr.)
Nisa, N. (Dr.)
Perveen, F.
Qadri, S.S.
Rizvi, S.A. (Dr.)
Saeed, S.A. (Dr.)
Saqib, T.A. (Dr.)
Shamim, S.
Siddiqui, F.A.
Tahera, Q.
Tariq, R.M.Waryani, B.
Tariq, S.
Yasmeen, R. (Dr.)
Yousuf, F.
Zahid, M.
Zehra, I. (Dr.)

KHANEWAL

Saeed, K.I.

LAHORE

Abbas, D.A.
Abbass, N. (Dr.)
Ahmad, M. (Prof.)
Ahsan, M.
Ali, M.A. (Prof.)
Ashiq, N.
Baig, D.N.
Bashir, Q.
Cheema, A.M. (Dr.)
Dar, N.
Farooq, H.
Gardner, Q.A.
Haider, M.S. (Dr.)
Iftikhar, S.
Jalal, A.

Khan, A.U.A.
Khan, M.A.
Khurshed, N. (Dr.)
Mahmood, N.
Mughal, M.S.
Muneer, B.
Munir, M.
Nadeem, M.S.
Nasir-ud-Din (Dr.)
Omer, T.
Qadoos, R.A.
Qamar, S.
Qureshi, M.S.
Rasool, A.G.
Rasool, N.
Rehman, A.
Saleem, F.
Saqib, Z.A.
Shah, A. (Dr.)
Shakoori, A.R. (Prof.)
Shakoori, F. (Dr.)
Sohail, H.M.
Younas, H.
Zafar, T.M.

LARKANA

Shaikh, A.H. (Prof.)

MULTAN

Ahmad, D.
Ahmed, S.
Akram, S.
Anjum, F.
Arif, A.
Asad, S.
Attique, M.
Haleemi, S.
Hameed, A.
Hameed, R.
Iqbal, J.
Jamshed, N.
Kanwal, S.
Khan, A.
Kosar, S.
Khan, T.H. (Dr.)
Nawaz, A.
Naz, R.
Rana, S.I.
Rehman, T.
Saleem, M.A. (Dr.)
Sarwar, H.
Tahir, S.M.
Tahira, N.
Yousaf, S.
Zafar, M.

MUZAFFARABAD, A.K.

Abbasi, A.Z.
Akhtar, M.
Ali, U.
Ashraf, S.
Aslam, T.
Awan, S. (Prof.)
Awan, M.S.
Ayub, J.
Azim, Ch.
Bano, I.
Baseer ud Din
Bashir, M.
Fouzia
Ishaq, Z.
Iwan, S.
Khanum, T.
Kirn, S.
Latif, A.

Musarat, S.
Mushtaq, N.
Naqeena
Naseem, S.
Qureshi, S.
Rafique, M.
Raja, Rizwanullah
Raja, A.
Raja, N.
Rashid, S.
Shafi, N.
Shah, I.
Shakoor, A.
Shaqufat
Shaukat, T.
Siraj, M.
Uzzaman, S.
Yousaf, N.

NOWSHERA

Ahmad, S.

PESHAWAR

Ahmad, M.
Alamzeb
Ali, N. (Dr.)
Arshad, M. (Dr.)
Badshah, Y.
Farid, A. (Dr.)
Khan, A.
Khan, I.
Khan, M.I.N.
Khattak, S.U.K. (Dr.)
Muhammad, A.
Salihah, Z. (Dr.)
Sattar, A. (Dr.)
Suleman, M. (Dr.)

QUETTA

Kakar, J.K. (Dr.)

RAWALAKOT

Mahmood, K. (Dr.)
Shah, W.H.

RAWALPINDI

Akhtar, N.
Farhan, K.
Hussain, R.
Irshad, M.
Jabeen, S.
Khanum, A. (Dr.)
Qayyum, M. (Dr.)
Raja, S.A.
Siddique, J.
Zaidi, M.

SUJAWAL

Jakhar, W.A.

TANDOJAM

Ahmad, N.
Arijo, A.G. (Prof.)
Ashraf, M. (Dr.)
Fatima, B. (Dr.)
Khan, G.Z.
Rashdi, S.M.M.S.
Siddiqui, Q.H.
Soomro, A.H.

BANGLADESH

Chowdhury, A. (Dr.)

EGYPT

Ismail, A.E.S. (Dr.)

IRAN

Esmaeili, H.R. (Dr.)

PROCEEDINGS OF PAKISTAN CONGRESS OF ZOOLOGY
(Proc. Pakistan Congr. Zool.)

Volume 24 **2004**

CONTENTS

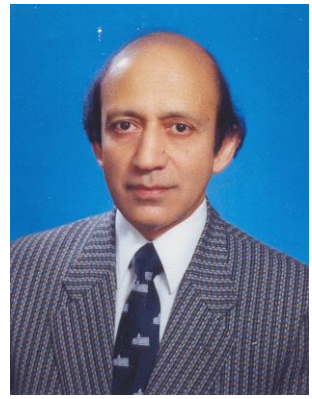
[Abstracted and indexed in Biological Abstracts, Chemical Abstracts, Zoological Records, Informational Retrieval Limited, London, and Service Central De Documentation De L'ORSTOM, Paris. Also listed in Index to Scientific and Technical Proceedings and ISI/ISTP & B Online Data Base of Institute for Scientific Information, Philadelphia, Pennsylvania, USA].

Website: www.zspk.com

Review Article

CITATIONS

RECIPIENT OF ZOOLOGIST OF THE YEAR AWARD 2004*



Dr. Syed Azhar Hassan
Director, Zoological Sciences Division of Pakistan Museum of Natural History, Islamabad

Dr. Syed Azhar Hasan obtained his Ph.D. degree in Zoology (Entomology) from the University of Oxford, U.K. in 1988. He has got 25 years of experience in research and education of biodiversity and conservation. Presently as Director, he is leading Zoological Sciences Division of Pakistan Museum of Natural History, Islamabad.

Dr. Hasan started his career in 1978 as Junior Entomoloogist at Commonwealth Institute of Biological Control and discovered several parasites for natural control of insect pests and weeds. In May 1981, he joined Pakistan Museum of Natural History as Research Associate and subsequently in 1982 he was promoted as Associate Curator. In 1985, he obtained his Ph.D. degree from Oxford University, U.K.

Dr. Hasan has published more than 50 research papers in journals of repute, have written one book "Butterflies of Islamabad and Murree Hills"

*Other nominees of this award were Dr. Sanaullah Khan Khattak, Peshawar and Dr. Muhammad Ayub, Lahore.

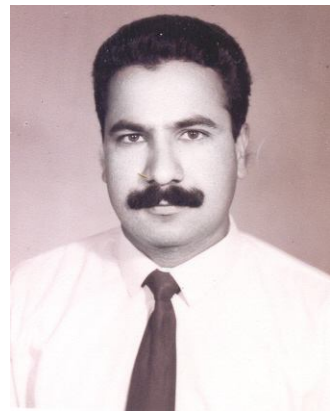
and co-edited another one “Biodiversity of Pakistan”. He has made more than 40 presentations at various national and international conferences including those in U.K., USA and Canada. He has represented Pakistan at the UNESCO’s and UNSCAP’s Meetings of experts of Biodiversity and Biosphere Reserves held in, Germnay, India, Sri Lanka and Thailand. He is an active researcher and have worked on a number of research projects including four in collaboration with international organizations such as University of Oxford, Natural History Museum, London and Florida Museum of Natural History, USA. His research achievements include fully sponsored International Biodiversity Expendition “Millenium Hunza”, organized in collaboration with the Oxford University Natural History Museum, U.K. and the Florida Museum of Natural History, USA to document high altitude biodiversity and ecology of very poorly documented regions of Northern Areas. The Expedition represented the most comprehensive biological survey ever undertaken in the high altitudes of Northern Areas of Pakistan. In context of monitoring environmental changes, Dr. Hasan is also involved for the last seven years on a joint butterfly research project with the University of Oxford, U.K. and has collected valuable data which include several new species to the science.

Dr. Azhar Hasan has rendered advisory services to various governmental and non-governmental organizations on biodiversity and environment related issues including preparation of “Pakistan Biodiversity Action Plan”.

Dr. Hasan has also significantly contributed in formal and informal education and creating awareness among the masses about our nature and natural resources through symposia, training workshops, lectures, setting up of natural history exhibitions/displays, preparation of science posters, pamphlets, popular articles, organizing WALKS for Science promotion etc. He has also supervised Ph.D. and M.Sc. student in their research work.

Dr. Azhar Hasan is fellow of various scientific societies including the Royal Entomological Society, U.K., the Entomological Society, Oxford, U.K., Heteropteran Society, USA and Zoological Society of Pakistan.

RECIPIENT OF
PROF. A.R. SHAKOORI GOLD MEDAL 2004*



Dr. Zafar Iqbal
Associate Professor
Department of Veterinary Parasitology, University of Agriculture, Faisalabad

Dr. Zafar Iqbal was born on 15th June, 1959 in a village in District Toba Tek Singh (Punjab). After primary education from the same village, he completed secondary and higher secondary education from Karachi. He qualified Doctor of Veterinary Medicine degree programe in 1982, M.Sc. in 1985 and Ph.D. in 1991 from Faculty of Veterinary Science, University of Agriculture, Faisalabad. After Ph.D. Dr. Iqbal completed one year post-doctorate fellowship in The Ohio State University, Columbus, Ohio, USA in 1993. He has specialized in the field of Parasitology. Dr. Iqbal earned first class throughout his academic carrier. He was ranked as an excellent researcher by The President, Ohio State University during his post-doc fellowship. He has published 70 research papers in national and international journals of repute.

Dr. Iqbal has completed four research projects and two are in progress. His salient achievements include (i) identification of role of jackal as a final

*Other applicants of this award were Dr. Dileep Kumar Rohra, Karachi, Mr. Arshed Makhdoom Sabir, Murree, Dr. Rubina Abid, Karachi, Dr. Muhammad Asim Beg, Karachi, Dr. Muhammad Farooq Durrani, Peshawar, Dr. Imran Ali Siddiqui, Karachi, Ms. Kherun Nisa, Karachi, and Mr. Abdullah Khatri, Tandojam.

host and wild boar as intermediate host for *Echinococcus granulosus* in Pakistan (ii) development of a research based hydatid control package in Pakistan (iii) development of a bio-climatograph for the incidence of helminths and deworming schedule for the farmers, and (iv) identification of epidemiological factors of major parasitic diseases of livestock in Pakistan.

In addition to above, Dr. Iqbal has edited a monograph on *Parasitic Research in Pakistan* published by University Grants Commission and Proceedings of Regional Seminar on *The Prevalent and Newly Emerging Poultry Diseases in Pakistan*. He has also authored five books on Parasitology.

RECIPIENT OF
PROF. DR. MIRZA AZHAR BEG GOLD MEDAL 2004*

Dr. Muhammad Ali
Assistant Professor of Zoology
Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan

Dr. Muhammad Ali was born in a small village of Jhang District. He did his M.Sc. in Zoology from Punjab University, Lahore with distinction. He started his career from Lawrence College, Murree in 1988. Later he joined Bahauddin Zakariya University, Multan as Lecturer in 1989. He won the "Central Overseas Training Scholarship" in 1995 and went to University of Wales, Aberystwyth, U.K. for his Ph.D. degree, which he obtained in 1999. During his stay in U.K. he won "Best Young Scientist of Europe Award" in Trieste, Italy in 1997.

He has won Prof. A.R. Shakoori Gold Medal 2002, Best Teacher Award 2002 of Higher Education Commission and Agha Hasan Ali Abidi Gold Medal 2001 of Pakistan Academy of Sciences.

He has published more than 50 papers in peer reviewed Proceedings and Journals. He has successfully completed the projects funded by The Royal Society of London; Chinese Academy of Sciences; University Grants Commission and BZU, Multan.

---

*Other applicants of this award were Dr. Rubina Abid, Karachi, Dr. Naeem Tariq Narejo, Jamshoro, Dr. Anas Sarwar, Faisalabad, and Dr. Zafar Iqbal, Lahore.

He is Member Board of Governors and Selection Board of School of Biological Sciences, Punjab University, Lahore. He is elected Member of Senate and Syndicate of Bahauddin Zakariya University, Multan.

RECIPIENT OF
PROF. DR. NASIMA TIRMIZI GOLD MEDAL 2004*

Quddusi B. Kazmi
Professor, Zoology Department, Karachi University, and
Director, Marine Reference Collection & Resource Centre, University of Karachi.

Prof. Dr. Quddusi Bashir Kazmi was born on 18th September, 1946. She obtained her Ph.D. degree in Zoology from Karachi University. She Worked for Ph.D. in British Museum (N.H.) for short duration. She has worked in the Karachi University (Zoology & MRC&RC) in various positions since 1965.

Prof. Kazmi is Author and Editor of 1000 Research publications in journals of national and international repute including books, illustrated keys, proceedings and monographs.

She is Founder Member Pakistan Fisheries Society, Fellow Scientific Scientist of Pakistan; Life-Fellow Zoological Society of Pakistan, Member Pakistan Association of Scientist and Scientific professions; Ex-Member Crustacean Society of India, Member International Association of meiobenthologists; Member International Associate of Copepodologists;

*Other applicants of this award were Dr. Sajjad-ur-Rehman, Faisalabad, and Dr. Razia Sultana, Karachi.

Member Asian Fisheries Society, Philippines; Founder member Asian Fisheries Society, Philippines, Member The Scientific and Cultural Society of Pakistan, Secretary of All Pakistan Association of Oceaners.

She has also won Zoologist of the Year award 2003 by Zoological Society of Pakistan.

RECIPIENTS OF GOLD MEDALS AWARDED BY THE ZOOLOGICAL SOCIETY OF PAKISTAN

1. **Mujib Memorial Gold Medal 2004**
   This Gold Medal is awarded to a student of Karachi University standing first in the recent M.Sc. Zoology examination with specialization in Parasitology. Nine Medals have already been given. This year's Mujib Memorial Gold Medal will go to Ms. Rakshanda Ahmed.

2. **Mohd Afzal Hussain Qadri Memorial Gold Medal 2004**
   This Gold Medal is awarded to a student of Karachi University, standing first in the recent M.Sc. Zoology examination. Seven medals have already been awarded. The eighth medal will be given to Ms Rakshanda Ahmed.

3. **Muzaffar Ahmad Gold Medal 2004**
   This Gold Medal is awarded to a student of the Punjab University, standing first in the recent M.Sc. Zoology examination. Eight Medals have already been given. Muzaffer Ahmad Gold Medal 2004 will be given to Mr. Muhammad Faisal Rehmatullah.

4. **Ahmad Mohiuddin Memorial Gold Medal 2004**
   This Gold Medal is awarded to M.Sc. Zoology student of University of Sindh, Jamshoro standing first in the recent M.Sc. Zoology examination. Three Gold Medals have already been given. This year Ahmed Mohiuddin Memorial Gold Medal 2004 will go to Ms. Fakhra Soomro.

Miss Fakhra Soomro

3. Prof. Imtiaz Ahmad Gold Medal 2004

This Gold Medal is awarded to a student of Karachi University, standing first in the recent M.Sc. Zoology examination with specialization in Entomology. Two medals will given this year. Prof. Imtiaz Ahmed Gold medal 2003 will go to Ms. Sobia Shabbir and Prof. Imtiaz Ahmed Gold Medal 2004 will go to Ms. Ruqqaya Shahzad.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 1-18, 2004.

EFFECT OF ANABOLIC STEROID, DIMETHAZINE, ON THE HEPATIC ENZYMES OF REGENERATING LIVER IN THE PARTIALLY HEPATECTOMIZED RABBITS

A.R. SHAKOORI, SHARIFAN BIBI, SYED SHAHID ALI AND KHALID P. LONE

Department of Zoology, University of the Punjab, New Campus, Lahore

Abstract.- Effect of an anabolic steroid, dimethazine (DM), has been described on the various biochemical components of regenerating liver after partial hepatectomy (PH). The biochemical analysis of liver after PH showed 52, 50 and 70%, increase respectively in ICDH, LDH and aldolase activities, 24 hours after PH. The hepatic ICDH and LDH activities were normalized, while the aldolase activity was drastically reduced (78%) during the next 24 hours. The soluble protein content of control liver decreased 30%, 48 hours after PH. The RNA content increased 62%, 24 hours after PH but at the end of next 24 hours it showed 38% reduction. The DNA content did not exhibit any significant change after PH. After DM treatment the hepatic ICDH and LDH activities showed 99 and 24% increase, respectively, whereas aldolase did not show any appreciable change. The hepatic RNA and DNA contents increased 74% and 27%, respectively, 48 hours after DM treatment. The ICDH activity of control liver is decreased 84% after ethanol treatment of PH rabbits. DM treatment did not help in the recovery of enzyme, instead it showed 81% decrease, 48 hours after treatment. LDH activity decreased 30% and 50%, respectively, by treatment of PH rabbit with ethanol and dimethazine. Both ethanol and DM caused 30% and 17% decrease in aldolase activity when administered to PH rabbits. RNA content decreased by 45% after ethanol treatment, while it reached normal level after the DM treatment of PH rabbit. The DNA content, on the other hand, is not affected by both treatments. The concentration of hepatic enzymes have also been followed in the nuclear as well as in the cytoplasmic fractions of PH rabbit with and without ethanol and or DM treatment. DM seems to accelerate the process of normalization in which biochemical adjustments are involved after PH.

Key words: Hepatic resection, hepatic regeneration, hepatic cellular fractions, isocitrate dehydrogenase, lactate dehydrogenase, aldolase, molecular readjustment.

INTRODUCTION

Partial hepatectomy (PH) is a surgical need in a number of cases and is also very common practice for understanding the mechanism of the liver. A series of morphological and biochemical changes occur in the remaining liver after partial hepatectomy, that results in the reconstruction of the original tissue mass. Shakoori *et al*. (1984) have studied the effect of PH on the liver function

tests of rabbit and have also studied regeneration of liver over a period of 6-10 days (Shakoori *et al*., 1985). Most of the molecular adjustment had been accomplished during the period under study. Faridi (1984) and Shakoori *et al.* (2003) later followed these changes in the blood serum and liver of rabbit during the first 36 hours after PH. The present study has been undertaken to follow changes in rabbit liver during early hours after hepatic resection. The changes have also been planned to be followed in different fractions of the cells. The liver is a centre of the detoxification and drug metabolism. The ability of the liver to metabolize drug after live resection and the effect of number of chemicals on resected liver has been tested a number of times. The effect of thioacitamide (hepatocarcinogene); on the various biochemical components of liver and blood under stress of partial hepatectomy has been assessed in rabbits by Shakoori *et al*. (1985).

Liver function tests are usually recognized as the reliable indicators of liver metabolism (Mann and Bollman, 1926; Leong *et al*., 1950). After PH the sequence of biochemical changes during hepatic regeneration and their relation to intracellular control has been reviewed extensively by Bucher and other (Bucher, 1963, 1967; Bucher *et al*., 1969). Extensive studies on intracellular changes occurring in the liver after PH are available (Ludwig *et al*., 1939; Vars and Gurd, 1947a,b; Gurd *et al*., 1948; Sulkin and Gardner, 1948; Harkness, 1952; Klinman and Erslev, 1963; Bucher, 1963, 1967; Gentile *et al*., 1970; Bucher and Malt, 1971; Levi and Zeppa, 1971; Murray *et al*., 1980, 1981).

Oppenheimer and Flock (1947) have reported elevation of alkaline phosphatase levels in the plasma and liver after 70% PH. Sekas and Cook (1979) have reported the changes in gammaglutamyl transpeptidase, lactate dehydrogenase, glutamic oxaloacetate transaminase and the alkaline phosphatase activities, which increase during very early hours of post PH in rats. The peak of DNA synthesis in the liver of rat after 68% resection has occurred 21 to 23 hours after resection, while in 85% resections, it was delayed ten to fifteen hours. In the dog this curve is not well defined, but the maximum DNA synthesis appears to be three to four days after 70% resection (Sigel *et al*., 1965). The quantitative study of regeneration and the factors which are confined to either the weighing of resected lobes at intervals after PH (Fisher *et al*., 1967; Grindlay and Bollman, 1952; Mann, 1940, 1944) or the counting of the cells of mitosis in tissue sections (Brues *et al*., 1936; Christensen and Jacobson, 1949; Harkness, 1957). Tissue from regenerating liver was found to have striking microscopic evidence of rapid cell division, suprassing that usually seen in neoplasia, with a mitotic index (300

times) that of a normal liver in which the majority of cells remain mitotically inactive for years (Brues *et al*., 1936).

Administration of anabolic steroid may enhance the process of regeneration. In the present series of experiments molecular readjustment in the liver was studied within early hours of PH (0, 24, 48 hours). Steroid (dimethazine) was administered to rabbits after 40% hepatic resection. as a single dose (10 mg/kg body wt.). The response of various biochemical components of different cellular fractions of liver under the stress of PH in the presence of single dose of dimethazine has been assessed in rabbits.

MATERIALS AND METHODS

Animals

Fifty two healthy male domesticated rabbits, *Oryctolagus cuniculus*, weighing 550-800 gms were divided into five groups: (1) Twelve rabbits were partially hepatectomized and were designated as PH group. Six of these were slaughtered 24 hours after hepatic surgery, while the rest of six were slaughtered 48 hours after surgery; (2) Another group of 12 rabbits was partially hepatectomized. Six of these (PH + DM) were administered with dimethazine (DM) immediately after PH and were slaughtered 48 hours after PH. The rest of the six (PH + Eth) were administered with ethanol immediately after PH and were slaughtered 48 hours after PH. Both the dimethazine and ethanol were administered intraperitoneally; (3) Another group of 6 rabbits was administered intraperitoneally with DM and were slaughtered 48 hours after treatment; (4) Three rabbits were administered alcohol and were slaughtered 48 hours after treatment. This group acted as control for group in (3) above as DM was prepared in ethanol.

At the end of each stipulated period the livers were taken out, weighed and processed for isolation of nuclei. The nuclear fractions and various cytoplasmic fractions thus obtained, were analyzed biochemically for the activities of lactate dehydrogenase (LDH), isocitrate dehydrogenase (ICHD) and aldolase, and some other biochemical components like DNA, RNA and protein. The total liver was analyzed in the same way. The methodology adopted has been described in Shakoori *et al*. (2003).

The animals were weighed before hepatectomy and the food was withdrawn. The total body weight was also recorded regularly during post-hepatectomy period.

Isolation of nuclei and cytoplasmic fractions

The method of isolation was described by Hogeboom and Schneider (1952).

Ice cold liver in 0.145M NaCl was immersed in 0.025M Sucrose – 0.0018 $CaCl_2$. The liver slices were blotted and minced in small pieces of about 0.5 cm in thickness. They were weighed and transferred to 9 ml of ice-cold 0.25 M sucrose – 0.0018 M $CaCl_2$. The liver was homogenized for 2 minutes at 6000 rpm by glass homogenizer.

The homogenate was filtered in order to remove the connective tissue and clumps of liver cells through one-fold nylon cloth. Total homogenate was 9 ml, half of this homogenate was processed further for isolation of nuclei, while rest of the 4.5 ml was desiccated for the estimation of nucleic acid contents.

Filtered homogenate (4.5 ml) was layered carefully over 10 ml of 0.34 M sucrose – 0.0018 M $CaCl_2$. The test tubes were centrifuged at 2000 rpm for 10 minutes at 4°C. The supernatant was separated. Total volume of this supernatant was 14.5 ml. It was designated as cytoplasmic fraction I.

The pellet was homogenized for 15 seconds in 5 ml of the 0.25 M sucrose –0.0018 M $CaCl_2$. Introduced beneath the suspension by means of the pasteur pipette with long capillary tube. The tubes were centrifuged for 10 minutes at 2000 rpm at 4°C. The entire supernatant fraction was removed. Total volume of this was 15 ml. It was designated as cytoplasmic fraction II.

The procedure for homogenization of pellet, layering and centrifuge was repeated twice. Entire supernatant fraction was removed. Total volume of this was also 15 ml. It was cytoplasmic fraction III. The nuclei were isolated in the form of a pellet.

The nuclei were suspended in 10 ml of saline (0.145 M NaCl) and sonicated for 1 minute. Centrifugation of sonicated material was done. Clear supernatant was obtained which was used for estimation of enzymes and protein contents.

RESULTS

Effect of PH

Partial hepatectomy (PH) leads to extensive molecular re-adjustment in the liver. Table I shows changes in various biochemical components in liver and its various nuclear and cytoplasmic fractions during post-hepatectomy period.

TABLE I.- EFFECT OF PARTIAL HEPATECTOMY ON SOME ENZYMES AND OTHER BIOCHEMICAL COMPONENTS OF RABBIT LIVER AND ITS ISOLATED NUCLEAR AND CYTOPLASMIC FRACTIONS.

Parameters	**Control**	**Partial hepatectomy**	
	0 hours (n=9)	**24 hours (n=6)**	**48 hours (n=3)**
A) Rabbit liver			
ICDH activity ($x10^3$ SU/g)	19.27±5.62[a]	29.35±1.85 (t=1.75)	14.23±3.57 (t=0.75)
LDH activity ($x10^4$ IU/g)	28.74±1.96	43.4±2.10[d] (t=4.97)	28.61±2.56 (t=0.04)
Aldolase activity (IU/g)	2.60±0.43	4.52±0.29c (t=3.68)	0.57±0.10[d] (t=4.55)
Soluble protein (mg/g)	251.71±10.63	254.34±2.23 (t=0.24)	176.77±7.99[d] (t=11.36)
RNA (mg/g)	4.74±0.36	7.67±0.63[c] (t=4.03)	2.90±0.54[b] (t=2.85)
DNA (mg/g)	3.70±0.49	3.19±0.66 (t=0.61)	5.19±0.60 (t=0.63)
B) Isolated nuclear fraction			
ICDH activity ($x10^3$ SU/g)	1.72±0.32	1.89±0.30 (t=0.38)	2.70±0.34 (t=2.09)
LDH activity ($x10^4$ IU/g)	0.69±0.07	0.79±0.14 (t=0.64)	0.56±0.008 (t=1.87)
Aldolase activity (IU/g)	0.026±0.007	0.04±0.003b (t=2.56)	--
Soluble protein (mg/g)	11.50±1.40	10.20±0.96 (t=0.24)	8.18±0.29 (t=11.36)
RNA (mg/g)	1.97±0.05	0.32±0.03d (t=32.05)	3.73±0.03d (t=33.54)
DNA (mg/g)	2.44±0.10	0.06±0.05d (t=42.84)	0.17±2.75 (t=0.82)
C) Isolated cytoplasmic fraction I			
ICDH activity ($x10^3$ SU/g)	3.33±0.41	3.67±0.19 (t=0.75)	2.90±0.66 (t=0.56)
LDH activity ($x10^4$ IU/g)	5.32±0.49	5.91±0.97 (t=0.313)	5.29±0.63 (t=0.04)
Aldolase activity (IU/g)	0.24±0.02	0.41±0.02d (t=4.44)	0.04±0.003d (t=9.44)
Soluble protein (mg/g)	49.07±3.86	54.34±4.25 (t=0.92)	46.36±0.29 (t=1.88)
RNA (mg/g)	2.24±0.09	0.42±0.17d (t=9.43)	2.00±0.29 (t=0.78)

Continued

Parameters	Control	Partial hepatectomy	
	0 hours (n=9)	24 hours (n=6)	48 hours (n=3)
D) Isolated cytoplasmic fraction II			
ICDH activity ($x10^3$ SU/g)	4.79±0.41	5.63±0.49 (t=1.32)	3.97±0.11 (t=0.20)
LDH activity ($x10^4$ IU/g)	0.76±0.37	-	0.81±0.04 (t=0.13)
Aldolase activity (IU/g)	0.07±0.01	0.04±0.07 (t=0.54)	--
Soluble protein (mg/g)	6.90±0.50	5.08±0.56b (t=2.41)	11.80±0.62d (t=6.14)
RNA (mg/g)	1.64±0.06	2.11±0.15b (t=2.90)	1.25±0.02d (t=5.71)
E) Isolated cytoplasmic fraction III			
ICDH activity ($x10^3$ SU/g)	2.18±0.69	1.53±0.17 (t=0.90)	1.94±0.58 (t=0.26)
LDH activity ($x10^4$ IU/g)	0.77±0.18	--	0.67±0.06 (t=0.14)
Aldolase activity (IU/g)	0.03±0.08	0.04±0.008 (t=0.18)	--
Soluble protein (mg/g)	8.92±2.34	--	7.59±0.43 (t=0.562)
RNA (mg/g)	1.66±0.47	0.17±0.07 (t=1.02)	1.23±0.03 (t=0.90)

[a]Mean±SEM: Student's 't' test; [b]P<0.05; [c]P<0.01; [d]P<0.001.
-, The enzyme did not show any detectable activity under the given experimental conditions.

Normal rabbit liver showed ICDH and LDH activity, which increased 52% and 50%, respectively, 24 hours after PH but appeared to have normalized during the next 24 hours. ICDH and LDH activities in hepatic nuclear fraction remained unchanged 24 hours after PH, but showed a significant increase of 57% in ICDH activity, 48 hours after PH. The hepatic cytoplasmic fractions showed no significant deviation after PH.

A control rabbit liver contained 2.60±0.43 IU/gm aldolase activity, which increases 74% after PH, but was reduced 78% during the next 24 hours. In hepatic nuclear fraction the aldolase activity is 0.026±0.007 IU/gm which increased 74%, 24 hours after PH. In cytoplasmic fraction I, the aldolase showed a 69% increase whereas it is decreased 83% during the next 24 hours. In cytoplasmic fraction II the aldolase activity decreased non-significantly during the first 24 hours. No activity was observed 48 hours after PH. In cytoplasmic fraction III the aldolase activity increased 49%, during first 24 hours.

The soluble protein in liver, its nuclear fraction and cytoplasmic fractions decreased 30%, 29% and 83%, respectively in nuclear and cytoplasmic fractions after 48 hours. The isolated cytoplasmic fraction of normal rabbit liver contained 49.07±3.86 mg/g soluble protein content which showed 70% increase 24 hours after PH. During the subsequent 48 hours, however, these content decreased 83% (Table I).

The isolated cytoplasmic fraction II showed about 26% decrease in the soluble protein content during the initial 24 hours of PH. During the subsequent 24 hours, however, these content increased by 71%. The cytoplasmic fraction III of normal rabbit liver contained soluble protein content of 8.92±2.34 mg/g. This decreased 15%, 48 hours after PH.

The hepatic RNA content increased 62%, 24 hours after PH but at the end of subsequent 24 hours they showed 38 decrease (Table I). In nuclear fraction, the RNA content decreased 84%, 24 hours after PH, but increased 9%, 48 hours after PH, whereas in the isolated cytoplasmic fraction I the RNA content decreased 81% ($t = 9.43$, $P<.001$), 24 hours after PH and attained its normal level within the next 24 hours. In the cytoplasmic fraction II the RNA content increased 28%, 24 hours after PH but reduced significantly (24%) during the next 24 hours after PH. In the cytoplasmic fraction III the RNA content was reduced 90% and 26%, 24 hours and 48 hours after PH, respectively (Table I).

The hepatic DNA content do not exhibit any significant change after PH. In the hepatic nuclear fraction the DNA decreased 102 and 93% 24 and 48 hours after PH, respectively.

Effect of dimethazine

Table II sows effect of DM administered to normal rabbits on various biochemical components of rabbit liver. The ICDH and LDH activity increased 99% and 24% after DM, while ethanol caused 55%, decrease and no change, respectively, 48 hours after treatment. In the isolated nuclear fraction the alcohol treatment caused 89% decrease, while DM treatment resulted in 128% increase.

Alcohol treatment did not cause any significant alteration in ICDH in the cytoplasmic fraction I. The DM treatment caused 54% increase. The ICDH activity of the cytoplasmic fraction II increased by 33%, 48 hours after ethanol treatment, while it decreased 31% after DM treatment. In the cytoplasmic fraction III this activity was reduced by 49% after DM treatment.

TABLE II.- EFFECT OF DIMETHAZINE (ADMINISTERED ONLY ONCE *i.e.* AS 10 mg/kg BODY WEIGHT) ON SOME ENZYMES AND BIOCHEMICAL BIOCHEMICAL COMPONENTS OF RABBIT LIVER AND ISOLATED NUCLEAR AND CYTOPLASMIC FRACTIONS.

Parameters	**Control**	**Dimethazine treatment**	
	0 hours (n=9)	**24 hours (n=6)**	**48 hours (n=3)**
A) Rabbit liver			
ICDH activity ($x10^3$ SU/g)	19.24±5.62a	8.64±2.24 (t=1.56)	28.73±2.29 (t=1.56)
LDH activity ($x10^4$ IU/g)	28.74±1.96	27.10±6.11 (t=0.25)	35.67±3.08c (t=1.2)
Aldolase activity (IU/g)	2.60±0.43	13.69±2.98b (t=3.68)	2.36±0.21 (t=0.48)
Soluble protein (mg/g)	251.71±10.63	142.03±60.67 (t=1.77)	364.48±51.30b (t=2.15)
RNA (mg/g)	4.74±0.36	4.09±1.30 (t=0.49)	40.99±6.08d (t=5.95)
DNA (mg/g)	3.70±0.49	1.53±0.09c (t=3.82)	4.70±0.27 (t=1.78)
B) Nuclear fraction			
ICDH activity ($x10^3$ SU/g)	1.72±0.32	0.19±0.0001c (t=4.76)	3.93±0.73b (t=2.76)
LDH activity ($x10^4$ IU/g)	0.69±0.07	--	0.29±0.04 (t=0.82)
Aldolase activity (IU/g)	0.02±0.007	0.15±0.02c (t=4.89)	0.035±0.002 (t=0.91)
Soluble protein (mg/g)	11.50±1.40	8.82±2.57 (t=0.91)	28.40±3.04d (t=5.04)
RNA (mg/g)	1.97±0.05	0.47±0.11 (t=1.36)	0.46±0.007d (t=33.34)
DNA (mg/g)	2.44±0.10	0.18±1.06 (t=2.12)	0.24±0.85d (t=17.0)
C) Cytoplasmic fraction I			
ICDH activity ($x10^3$ SU/g)	3.33±0.41	3.08±0.54 (t=0.36)	5.13±0.21c (t=3.88)
LDH activity ($x10^4$ IU/g)	5.32±0.49	0.11±0.01d (t=1.53)	4.18±0.83 (t=1.18)
Aldolase activity (IU/g)	0.24±0.02	0.14±0.07 (t=1.30)	0.22±0.06 (t=0.45)
Soluble protein (mg/g)	49.06±3.86	3.19±0.17d (t=11.87)	65.18±3.37b (t=3.14)
RNA (mg/g)	2.24±0.09	2.57±0.10b (t=2.45)	3.22±0.13d (t=187.77)

Continued

Parameters	Control	Dimethazine treatment	
	0 hours (n=9)	24 hours (n=6)	48 hours (n=3)
D) Cytoplasmic fraction II			
ICDH activity ($x10^3$ SU/g)	4.79±0.41	6.38±0.29c (t=4.25)	3.29±0.24d (t=4.54)
LDH activity ($x10^4$ IU/g)	0.76±0.37	--	--
Aldolase activity (IU/g)	0.07±0.01	0.09±0.05 (t=0.047)	0.002±0.002d (t=5.41)
Soluble protein (mg/g)	6.90±0.50	0.69±0.02d (t=12.35)	8.16±1.66 (t=0.73)
RNA (mg/g)	1.64±0.06	3.81±0.11d (t=16.59)	1.99±0.04 (t=4.55)
E) Cytoplasmic fraction III			
ICDH activity ($x10^3$ SU/g)	2.18±0.69a	--	1.12±0.32 (t=1.27)
LDH activity ($x10^4$ IU/g)	0.77±0.18	--	--
Aldolase activity (IU/g)	0.03±0.08	--	0.03±0.001 (t=0.059)
Soluble protein (mg/g)	8.92±2.34	0.61±0.09c (t=3.54)	2.16±0.16b (t=2.87)
RNA (mg/g)	1.66±0.47	--	1.98±0.04 (t=0.66)

[a]Mean±SEM: Student's 't' test; [b]P<0.05; [c]P<0.01; [d]P<0.001.
-, The enzyme did not show any detectable activity under the given experimental conditions.

In the hepatic nuclear fraction the LDH activity remained unaffected after DM treatment. The LDH activity after alcohol treatment in the cytoplasmic fraction I was reduced 98%, after alcohol treatment but attained the normal level after DM treatment. In the cytoplasmic fractions II and III the LDH activity was reduced to an undetectable level after ethanol and DM treatment.

Aldolase activity increased 46%, 48 hours after alcohol treatment, while the DM treatment did not show any appreciable change. The aldolase activity increased 47% after ethanol administration nuclear fraction. The DM treatment did not cause any significant change. The aldolase activity in cytoplasmic fraction I decrease 40% after ethanol treatment, whereas DM treatment did not cause any significant deviation from the control. It remained unaltered in cytoplasmic fraction II after ethanol treatment though reduced 99% after DM treatment. The aldolase activity in cytoplasmic fraction III remained unaltered in all the experimental groups.

The soluble protein content of normal rabbit liver increased by 45% after DM treatment, while alcohol decreased it by 44%. In nuclear fraction, the soluble protein content decreased 23.26% after ethanol treatment, whereas it increased 147% after DM treatment ($P<.001$). The soluble protein content decreased 93% after ethanol treatment, while DM caused 33% increase 48 hours after its treatment in cytoplasmic fraction I. The soluble proteins after alcohol treatment decreased 90 and 75%, respectively, in cytoplasmic fractions II and III. After DM treatment, however, the protein increased 21.45% in cytoplasmic fraction II and decreased 76% in cytoplasmic fraction III, 48 hours after DM treatment.

The total RNA and DNA contents of liver decreased 13% and 58% after ethanol treatment, whereas they showed 74% and 27% increase after DM treatment. The RNA content decreased 76% in the hepatic nuclear fraction after ethanol treatment. In cytoplasmic fractions I, II and III, the RNA content showed 15%, 132% and 19% increase, respectively, after ethanol treatment. These contents, however, increased 43%, 21.45% and 19%, respectively, in cytoplasmic fraction I, II and III after DM treatment. The DNA contents also decreased drastically after ethanol as well as DM treatment.

Effect of dimethazine on PH rabbit liver

Table III shows effects of DM on PH rabbit liver. Ethanol treatment caused a drastic effect on ICDH and LDH activity of PH rabbit liver. The ICDH activity in control rabbit was $20.57\pm3.39\times10^3$ U/g and LDH activity $64.53\pm3.42\times10^4$ U/g, which decreased 84%, and 30% respectively after ethanol treatment. DM treatment did not help in recovery of these activities. Forty eight hours after treating the PH group with DM the ICDH and LDH activities were 81% and 50% less than the control (Table III). The isolated hepatic nuclear fraction had ICDH activity reduced 80%, 48 hours after alcohol group. The DM treatment caused 85% increase. The ethanol treatment of PH rabbits reduced the LDH activity to such a low level that it could not be detected, but when DM was administered to PH rabbits the LDH activity of liver showed 103% increase.

In the hepatic cytoplasmic fractions III, ICDH and LDH activities were drastically decreased after ethanol as well as DM treatment of PH rabbits.

This decrease in cytoplasmic fraction I and II was, respectively 13%, while no activity was detected in the third cytoplasmic fraction. DM treatment caused 16% and 23% decrease in ICDH activity in the cytoplasmic fraction I and II. The LDH activity was reduced 85%, but this activity was reduced to negligible level

TABLE III.- EFFECT OF DIMETHAZINE (ADMINISTERED ONLY ONCE AFTER PH AS 10 mg/kg BODY WEIGHT) ON SOME ENZYMES AND BIOCHEMICAL COMPONENTS OF PARTIALLY HEPATECTOMIZED RABBIT LIVER, ITS NUCLEAR AND CYTOPLASMIC FRACTIONS.

Parameters	**Control**	**PH + Ethanol**	**PH + Dimethazine**
	0 hours (n=9)	**48 hours (n=6)**	**48 hours (n=3)**
A) Rabbit liver			
ICDH activity ($x10^3$ SU/g)	20.57±3.39a	3.20±0.02 (t=0.06)	3.82±1.52 (t=1.45)
LDH activity ($x10^4$ IU/g)	64.53±3.42	40.59±0.66d (t=6.9)	32.49±0.09d (t=9.36)
Aldolase activity (IU/g)	2.26±0.31	1.58±0.12 (t=2.08)	1.88±0.04d (t=54.21)
Soluble protein (mg/g)	245.91±37.43	87.42±0.61c (t=4.23)	164.62±6.47 (t=2.14)
RNA (mg/g)	2.77±0.29	1.53±0.04c (t=4.29)	2.62±0.18 (t=0.44)
DNA (mg/g)	3.75±0.22	3.76±0.06 (t=.028)	4.37±0.26 (t=1.91)
B) Hepatic nuclear fraction			
ICDH activity ($x10^3$ SU/g)	1.02±0.14	0.14±0.02d (t=5.99)	1.88±0.20b (t=3.44)
LDH activity ($x10^4$ IU/g)	0.29±0.068	--	0.60±0.02c (t=4.45)
Aldolase activity (IU/g)	0.02±0.004	0.10±0.006 (t=1.09)	0.02±0.01 (t=0.49)
Soluble protein (mg/g)	5.83±1.47	12.15±2.02c (t=2.53)	7.5±0.44 (t=1.09)
RNA (mg/g)	0.40±0.02	0.44±0.02 (t=1.09)	0.46±0.86 (t=0.06)
DNA (mg/g)	0.62±0.06	0.19±0.67b (t=0.64)	1.43±0.13d (t=5.57)
C) Cytoplasmic fraction I			
ICDH activity ($x10^3$ SU/g)	3.04±0.15	2.64±0.10 (t=2.19)	2.54±0.32 (t=1.39)
LDH activity ($x10^4$ IU/g)	3.20±0.10	4.47±0.01d (t=28.20)	5.55±1.06 (t=2.20)
Aldolase activity (IU/g)	3.38±0.54	5.47±0.07c (t=3.82)	0.22±0.07d (t=5.77)
Soluble protein (mg/g)	19.40±2.27	0.08±0.03	39.56±3.00c (t=5.36)
RNA (mg/g)	2.56±0.50	3.10±0.17 (t=1.03)	1.87±0.09 (t=1.35)

Continued

Parameters	Control	PH + Ethanol	PH + Dimethazine
	0 hours (n=9)	48 hours (n=6)	48 hours (n=3)
D) Cytoplasmic fraction II			
ICDH activity (x10^3 SU/g)	2.88±0.30	1.39±0.07c (t=4.83)	2.21±0.40 (t=1.32)
LDH activity (x10^4 IU/g)	0.69±0.06	--	0.86±0.19 (t=0.82)
Aldolase activity (IU/g)	0.04±0.01	0.36±0.06d (t=5.10)	0.004±0.002b (t=2.68)
Soluble protein (mg/g)	2.62±0.29	0.33±0.006d (t=7.88)	4.68±0.48c (t=3.70)
RNA (mg/g)	2.14±0.50	3.10±0.18 (t=1.77)	1.28±0.87 (t=0.86)
E) Cytoplasmic fraction III			
ICDH activity (x10^3 SU/g)	0.58±0.23a	--	1.52±0.22b (t=2.93)
LDH activity (x10^4 IU/g)	0.79±0.49	--	0.90±0.12d (t=6.47)
Aldolase activity (IU/g)	0.009±0.007	--	2.83±0.001 (t=1.21)
Soluble protein (mg/g)	1.52±0.39	0.35±0.008b (t=3.38)	2.83±0.66 (t=1.71)
RNA (mg/g)	1.75±0.03	1.83±0.02 (t=0.99)	1.24±0.03d (t=10.23)

[a]Mean±SEM: Student's 't' test; [b]P<0.05; [c]P<0.01; [d]P<0.001.
-, The enzyme did not show any detectable activity under the given experimental conditions.

in the second and third cytoplasmic fractions. DM treatment caused an increase in the LDH activity in all the cytoplasmic fractions. This increase was 73.59, 23 and 15%, respectively, in the three fractions.

The aldolase activity likewise decreased after ethanol (30%) and DM treatment (17%) of PH rabbits. In the isolated nuclear fraction the aldolase activity increased 36.47% after ethanol treatment, whereas it showed 17% increase over the control after DM treatment of PH rabbits. This activity increased 62% in the cytoplasmic fraction I after ethanol treatment of PH group, and so it did in cytoplasmic fraction II. In cytoplasmic fraction III the aldolase activity reduced to zero. The DM treatment had just the opposite effect in cytoplasmic fraction I. In the cytoplasmic fraction II and III the aldolase activity reduced 91% and 84%, respectively.

The soluble protein content of control rabbit liver was 245.91±37.43 mg/g. This was reduced to 33% after ethanol treatment of PH rabbits. The soluble protein content decreased 64% in DM treated group. The soluble protein content increased 108% after ethanol treatment of PH rabbits in the nuclear fraction, whereas it increased 29% after DM treatment to PH rabbits. In the cytoplasmic fraction I, it is reduced 99.54%, while in fractions II and III they also showed significant decrease after ethanol treatment. After DM treatment the soluble protein content of cytoplasmic fraction I increased by 103%. These content increased 78% and 86%, respectively in cytoplasmic fractions II and III, 48 hours after DM treatment to PH rabbits.

The control rabbit liver contained 2.77±0.29 mg RNA/g tissue and 3.75±0.22 mg DNA/g tissue. The RNA content decreased 45% when treated with ethanol. Treatment with ethanol as well as DM to PH rabbits did not affect the RNA as well as DNA content. The RNA content of various cytoplasmic fractions deviated significantly from control values after ethanol or DM treatment. The DNA content of hepatic nuclear fraction decreased 69%, after ethanol treatment, though DM treatment caused 130% increase, 48 hours after treatment.

DISCUSSION

Effect of partial hepatectomy on liver regeneration

Partial hepatectomy (PH) is a process in which a part of liver is surgically removed to observe the ability of remaining organ in molecular readjustment and to understand the mechanism of liver regeneration. The time taken for molecular readjustment depends upon the amount of liver taken out. Less than 25% removal may not cause any appreciable disturbance in molecular architecture and hence the function of liver, whereas 90% removal of liver will be more stressful for animals. Sekas and Cook (1979) suggested that most of the molecular readjustment sets in and is completed to a large extent, within a period of one week after the surgical removal of liver in rats. Shakoori *et al.* (1985) also concluded that PH brings about molecular adjustment in the liver of rabbits which is actively accomplished during first 10 days. In the presently reported experiments about 40% liver was removed and then changes in its various enzymes and biochemical components have been assessed, 24 and 48 hours after PH.

Shakoori *et al.* (2003) described changes in various enzymatic activities and other biochemical components within 6 days after PH. The present studies

were restricted to 2 days only. Moreover, the parameters used in the present study are of different nature. The changes in biochemical parameters were followed in various cellular fractions, mainly nuclear fraction and cytoplasmic fraction.

The ICDH, LDH and aldolase activities are increased after PH, but mostly are brought to the normal level after 48 hours. The aldolase activity on the other hand is drastically decreased after an initial increase. This decrease is reflected in low soluble protein content. The RNA content behave just like enzymes, while the DNA content remain unchanged. In the hepatic isolated nuclear fraction the various biochemical components generally behave the same way as the total liver does, whereas these contents remain unchanged in the cytoplasmic fraction. The changes in aldolase activity in the intact hepatic tissue are reflected in the cytoplasmic fraction.

Effect of dimethazine on rabbit liver

The treatment of DM does not result in decrease in the enzymatic activities. All the enzymatic activities increase, so do the soluble protein. The RNA and DNA contents are also increased after DM treatment. In the isolated nuclear fraction, all the enzymatic activities show increased levels. The protein content, however, decreased. On the other hand the RNA and DNA content of isolated nuclei decreased after DM treatment to animals. The DM caused an increase in the nucleic acid content of cytoplasmic fraction. The ethanol treatment, however, results in decreased enzymatic activity.

Effect of dimethazine on hepatectomized rabbit liver

ICDH activity of control rabbit liver is decreased 84% after ethanol treatment of PH rabbits. DM treatment does not help in the recovery of enzyme. It still shows 81% decrease, 48 hours after treatment. LDH activity is decreased by 30% and 50%, respectively, by treatment of PH rabbit liver with ethanol or dimethazine. Both ethanol and DM caused 30% and 17% decrease when administered to PH rabbit. Soluble protein content are also decreased by ethanol or DM treatments. RNA content decreased by 45% after ethanol treatment, while it reached normal level after the DM treatment of PH rabbit liver. The DNA content are not affected by both treatments.

In the isolated hepatic nuclear fraction ICDH activity was reduced 80%, 48 hours after alcohol treatment to PH group, while it increased 85% after DM

treatment. LDH activity in hepatic nuclear fraction was reduced to such a low level that it could not be detected with ethanol treatment. But when DM was administered to PH rabbits, the LDH activity showed 103% increase. Aldolase activity showed 17% increase after DM over the ethanol. The soluble protein content increased by 108% and 29%, respectively, after ethanol and DM treatment to PH rabbits. Nuclear RNA content remained unaffected with PH rabbits after ethanol or DM treatment. DNA content of isolated nuclei decrease by 69% after ethanol treatment, while DM caused increase by 130%, 48 hours after treatment to PH rabbits.

Ethanol treatment caused 13% and DM caused 16% decrease in ICDH activity of cytoplasmic fraction. The LDH activity (74%) decreased after ethanol treatment whereas DM caused an increase in the LDH activity. Aldolase activity is increased after ethanol treatment, but is decreased after DM treatment in cytoplasmic fraction. Soluble protein content decreased by about 100% after ethanol treatment but increased by 103% after DM treatment. RNA content did not show any significant deviation. DM, therefore, appears to accelerate the process of normalization in which biochemical adjustments are involved, after PH.

The enzymatic activities may be decreased as a result of (i) enzyme release from the cells due to hepatic tissue necrosis or (ii) may be due to leeching of enzyme into blood with increased cell membrane permeability without cell necrosis (Bartsokas, 1974; Schmidt *et al*., 1974; Cerda'n *et al*., 1978). The cytoplasmic enzyme are released into circulation within a few hours of injury, which is reflection of cell membrane injury or permeability change before the onset of frank necrosis. Possibility of enzyme activities are increased as a result of the absorption of enzymes release by necrosis of the small amount of hepatic tissue remaining distal to the site of ligation, (iii) it could also be attributed to the increased synthesis of enzymes after PH.

The regeneration does not involve the growth of ramnant stumps, instead it consist of hyperplasia and hypertrophy of all major cellular elements of the remaining unresected lobes of liver. Although a comparable regenerative response can be produced by extirpation and toxic destruction.

Thus PH leads to regenerating process which is definitely included within 48 hours of hepatic surgery. This is a period molecular readjustment is being settled, 5 to 6 days will be required before the rabbits attain their normal molecular balance. Three enzymes have been metabolic indicators in present

study in which TCA cycle enzyme ICDH and glycolytic enzymes like LDH indicate the enzymatic behaviour during readjustment. Aldolase which is an important enzyme leading to conversion of hexose sugar to two trioses appears to be very sensitive enzyme. It is considerably effected and is far below normal level even after 48 hours. The low soluble protein contents are indicators of low enzymatic activity after hepatectomy.

DM, which is an established anabolic steroid, when administered to normal rabbits leads to significant increase in the activity of these enzymes and nucleic acid contents. This steroid, however, helps to understand conditions of stress when it is administered to rabbits after PH. Most of the enzymatic activities seem to recover as generally expected of anabolic steroid. This is true specially during first 48 hours of observation period after hepatic surgery. Entire data should be considered in relation to a fact that single dose of DM was administered (10 mg/kg body weight) after PH. In order to get fast recovery and efficient molecular readjustment, probably higher and repeated doses would be required for this purpose.

REFERENCES

BARTSOKAS, S.K., 1974. Protection of liver function in thioacetmide poisoned rats by heparinoids. *Res. exp. Med.*, **162**: 105-108.

BRUES, A.M., DRURY, D.R. AND BRUE, S.M.C., 1936. Quantitative study of cell growth in the regenerating liver. *Arch. Path.*, **22**: 658-673.

BUCHER, N.L.R., 1963. Regeneration of mammalian liver. *Int. Rev. Clytol.*, **15**: 245-300.

BUCHER, N.L.R., 1967. Experimental aspects of hepatic generation. *New England J. Med.*, **277**: 738-746, 686-696.

BUCHER, N.L.R., SHROCK, T.R. AND MOOLTEEN, F.E., 1969. An experimental view of Hepatic regeneration. *Johns Hopkins Med. J.*, **125**: 250.

BUCHER, N.L.R., AND MALT, R.A., 1971. Regeneration of liver and kidney. In: *New England Journal of Medicines*, Medical Progress series, pp. 1-76. Little and Brown, Bostan.

BUCHER, N.L.R., 1967. Insulin, glucagon and the liver. *Adv. Enzyme Regul*., **15**: 221.

CERDAN, S., CASCALES, M., CHARCON, P., CASCALES, F. AND SANTOS-RUIZ, A., 1978. Hepatotoxic effects of thioacetamide (TAM) on glutamate dehydrogenase. *Arch. Toxicol. Suppl*., **1:** 221-224.

CHRISTENSEN, B.G. AND JACOBSON, E., 1949. Study on liver regeneration. *Acta med. Scand.*, **234**: (Suppl.): 103.

FARIDI, T.A., 1984. *Effect of steroid on the blood serum after partial hepatectomy*. M.Sc. thesis, Zoology Department, Punjab University, Lahore.

FISHER, B., FISHER, E.R. AND LEE, S., 1967. Experimental evaluation of liver atrophy and portcavel shunt. *Surg. Gynecol. Obstet.*, **125**: 1253.

GARUANA, J.A. AND GAGE, A.A., 1980. Increased uptake of insulin and glucagon by the liver as a signal for regeneration. *Surg. Gynecol. Obstet.*, **150**: 390-394.

GENTILE, J.M., AVILA, L. AND GRACE Jr., J.T., 1970. Liver regeneration -old and new concepts. *Am. J. Surg.*, 1**20**: 2-6.

GRINDLAY, J.H., AND BOLLAMAN, J.L., 1952. Regeneration of the liver in the dog after partial hepatectomy. *Surg Gynecol. Obstet*., **94**: 491.

GURD, F.N. VARS, H.M. AND RAVDIN, I.S., 1948. Composition of the regenerating liver after partial hepatectomy in normal protein depleted rats. *Am. J. Physiol*., **152**: 1148.

HARKNESS, R.D., 1952. The spatial distribution of dividing cells in the liver of rat after partial hepatectomy. *J. Physiol*., **116**: 373-379.

HARKNESS, R.D., 1957. Regeneration of liver. *Br. Med. Bull*., **13**: 87.

HOGEBOOM, G.H? SCHNEIDER, W.C., 1952. Isolation of liver cell nuclei in aqueous medium. *J. biol. Chem*., **196**: 111.

KLINMAN, N.R. AND ERSLEV, A.J., 1963. Cellular response to partial hepatectomy. *Proc. Soc. exp. Biol. Med*., **112**: 338-340.

LEONG, G.F., PESSOTTI, R.L. AND BRAVER, R.W., 1959. liver function in regenerating rat liver. CTP~ colloid uptake and bile flow. *Am. J. Physiol*., **197**: 880-886.

LEVI, J.U. AND ZEPPA, R., 1971. Source of hormonal factor that initiates hepatic regeneration. *Ann. Surg*., **174**: 363-370.

LUDWIG, S., MINOR, G.R. AND HORTENSTINE, J.C., 1939. Lipid distribution in rat liver after partial hepatectomy. *Proc. Soc. exp. Biol. Med*., **42**: 158.

MANN, F.C. ,1940. The postal circulation and restoration of the liver after partial removal. *Surgery*, **8**: 25.

MANN, F.C., 1944. Restoration and pathologic reactions of liver. *Mt. Siani J. Med., N.Y*., **11**: 65.

MANN, F.C. AND BOLLMAN, J. L., 1926. Liver function tests. *Arch. Path. Lab. Med*., **1**: 681-710.

MURRAY, A.B., STRECKER, W. AND SILZ, S., 1980. Changes in hepatocyte volume and liver weight in the early phase after partial hepatectomy. *Hepato-Gastroenterology*, **27**: 4-8.

MURRAY, A.B., STRECKER, W. AND SILZ, S., 1981. Ultrastructural changes in rat hepatocyte after partial hepatectomy and comparison with biochemical result. *J. Cell Sci*., **50**: 433-448.

OPPENHEIMER, M.J. AND FLOCK, E.V., 1947. AlKaline phosphatase levels in plasma and liver following partial hepatectomy. *Am. J. Physiol*., **149**: 418-421.

SCHMIDT, E., SCHMIDT, F.W., MOHR, J., OTTO, P., VIDO, I., WROGEMANN, K. AND HERFARTH, C., 1974. Liver morphology and enzyme release: Further studies in the isolated perfused rat liver. In: *Parthenogenesis and mechanisms of liver cell necrosis* (ed. D. Kepplero), University Park Press, Baltimore.

SEKAS, G. AND COOK, R.T., 1979. The evaluation of liver function after partial hepatectomy in rat: Serum changes. *Br. J. exp. Path*., **60**: 447-452.

SHAKOORI, A.R., CHEEMA, A.I.A., RANI, A. AND ALl, S.S., 1984. Evaluation of liver function after thiocetamide treatment of partially hepatectomized rabbits. *Acta Physiol. Pharmacol. Contamin.*, **34**: 304-312.

SHAKOORI, A.R., KAUSAR, N. AND LONE, K.P., 2003. Effect of a single dose of dimethazine

on regeneration of partially hepatectomized rabbit liver. *Proc. Paksitan Congr. Zool*., **23**: 243-270.

SHAKOORI, A.R., RANI. A., CHEEMA, A.I.A. AND ALl, S.S., 1985. Some biochemical and histological changes in regenerating liver after thiocetamide treatmentof partially hepatectomized rabbits. *Pakistan J. Zool*., **17**: 271-288.

SIGEL, B., PECHET, G., QUE, M.Y. AND MACDONALD, R.A., 1965. Tritiated thymidine autoradiography in the regenerating liver of dog. *J. Surge Res*., **5**: 72.

SULKIN, N.M. AND GARDNER, J.H., 1948. The acid and alkaline phosphatase activity in the normal and recovering liver in the rat. *Anat. Rec*., **100**: 143.

VARS, H.M., AND GURD, F.N., 1947a. Role of dietary protein in experimental liver regeneration. A nitrogen balance study. *Am. J. Physiol*., **151**: 391-398.

VARS, H.M. AND GURD, F.N., 1947b. Effect of dietary protein upon the regeneration of liver protein in rat. *Am. J. Physiol*., **151**: 399-404.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 19-23, 2004.

EFFECT OF INJECTABLE ERYTHROPOIETIN ON BLOOD HEMOGLOBIN LEVELS IN CHRONIC RENAL FAILURE PATIENTS ON HEMODIALYSIS

SHAZIA KHALIQ, KHALIL-UR-REHMAN, TARIQ M. CHAUDHRY, NADEEM YAQOOB, ASMA ASHRAF, M.S. SHAH, NAZIA GHULAM NABI AND TAYYBA HUMMA

Nuclear Institute for Agriculture and Biology, P.O. Box 128, Jhang Road, Faisalabad

Abstract.- Major problem to patients of chronic renal failure (CRF) is the disturbed excretory function of the kidney and low production of erythropoietin (EPO). Low level of EPO causes anemia due to lower levels of hemoglobin (Hb). This results in high levels of blood urea and serum creatinine. The administration of recombinant human erythropoietin (r-HuEPO) is to stimulate the process of erythropoises and hence to cover the state of anemia. In this study two months treatment of EPO was administered subcutaneously to CRF patients on hemodialysis and found it very effective in raising blood hemoglobin levels and to cure anemia. After two months treatment the blood Hb levels increased from 7.20±0.43 g/dl to 9.60±0.47 g/dl. EPO therapy with hemodialysis significantly lowered the levels of blood urea and serum creatinine.

Key words: Erythropoietin, blood urea, serum creatinine, EPO therapy.

INTRODUCTION

Major problem to patients of chronic renal failure (CRF) is the disturbed excretory function of kidneys, less production of erythropoietin (EPO) that decreased the erythropoiesis resulting in low levels of blood hemoglobin (Hb), high levels of blood urea and serum creatinine (Vanella *et al.*, 1983). CRF requiring dialysis or transplantation is known as end-stage renal disease. EPO is a glycoprotein and it behaves like a hormone (Bain, 1995). It is produced primarily in the kidneys in adults and to a lesser extent, in the liver (Koury *et al.*, 1991). Hypoxia is the sole physiological stimulus for EPO production (Piroso *et al.*, 1989). In the kidneys, EPO production of the hormone occurs on an all-or-none basis in each cell, normally there is constitutive production of the hormone that is commensurate with its role as a survival factor (Spivak *et al.*, 1991). EPO acts in several ways to increase the number of circulating red cells. The primary action of EPO is to increase the number of developing erythroid precursors within the marrow by including terminal differentiation of EPO responsive stem cells

(Giblett *et al.,* 1956) The high efficacy of recombinant human erythropoietin (r-HuEPO) in therapy of anemia was first demonstrated in uremic patients on hemodialysis. In the mean time it became clear that all renal failure patients including predialysis and renal transplanted ones, regardless the way of dialysis and etiology of renal disease, benefit of r-HuEPO therapy. EPO proved to be effective in reversing the anemia of CRF (Stojimirovic and Vera, 2000). Optimal use of r-HuEPO should achieve the greatest benefits at the lowest cost. The challenge now is to optimize the r-HuEPO treatment and to allow a wider spectrum of patients to use it.

This study was planned to see the effect of injectable EPO and elevation of blood Hb levels in CRF patients on hemodialysis.

MATERIAL AND METHODS

Twenty anemic CRF patients on hemodialysis, 16 males and 4 females, mean age 41±10.22 years were registered in this study. Quantitative variables blood hemoglobin (Hb), blood urea and serum creatinine were determined before starting treatment. Erythropoietin was administered subcutaneously at a dose of 2000 U/Kg body weight thrice a weak for two months. After completion of treatment biochemical parameters blood Hb, urea and serum creatinine were determined again, according to Kampen and Zijlstra (1965), Hallette (1971) and Bartels *et al*. (1972), respectively.

Statistical analysis

All the variables were described by mean±standard deviations. The t-test was used to compare these variables in paired samples (before and after treatment). Significant differences were defined by $P<0.001$.

RESULTS AND DISCUSSION

Blood Hb ranged between 6.1- 7.9 g/dl before treatment and 8.2 -10.2 g/dl after treatment. Normal blood Hb values are 12-16 g/dl for females and 14-18 g/dl for males (Kampen and Zijlstra, 1965). The values of blood urea and serum creatinine ranged between 159-263 mg/dl and 7.95 - 13.15 mg/dl, respectively before treatment. Two month treatment of r-HuEPO + hemodialysis decreased

the levels of blood urea and serum creatinine to 87- 234 mg/dl and 4.35-12.0 mg/dl, respectively. Normal blood urea values are 10-55 mg/dl (Bartels *et al.*, 1972). Normal serum creatinine values are 0.5 - 1.0 mg/dl for females and 0.7-1.2 mg/dl for males (Hallette, 1971).

The results for whole group of patients before and after treatment are illustrated in the Table I.

TABLE I.- EFFECT OF r-HuEPO ON BLOOD HAEMOGLOBIN, BLOOD UREA AND SERUM CREATININE LEVELS OF CHRONIC RENAL FAILURE PATIENTS ON HEMODIALYSIS.

	Before treatment (Mean±SD)	**After treatment (Mean±SD)**	**Difference of two means**	**Standard error of difference**	**P-value**
Blood Hb (g/dl)	7.20±0.43	9.60±0.47	-2.40	0.144	<0.001***
Blood urea (mg/dl)	202.0±29.9	147.4±4.48	55.4	12.03	<0.001***
Serum creatinine (mg/d)	10.06±1.44	7.37±2.24	55.4	0.596	<0.001***

***Highly significant.

In this study statistically significant rise in mean Hb levels (7.20±0.43 - 9.60±0.47 g/dl) after two-month treatment of r-HuEPO were observed. The value of Hb should be the level at which normal quality of life is possible. Most patients with the anemia of chronic renal failure remained moderately anemic and have not achieved the target Hb (11 to 12 g/dl) (Eschbach, 2000). In this study exact normal levels of Hb is not achieved but it was quit better as reported earlier 7.10±1.4 g/dl - 8.4±1.8 g/dl (Okura, 1996) and 7.22±1.26 g/dl - 8.60±1.66 g/dl (Singh *et al*, 1999). It is revealed that 2000 IU/kg/week increase Hb concentration to 10-12 g/dl in 90% of haemodialysis patients and best marker of benefit of the introduction of rHUEPO is the reduction in need for regular blood transfusion (Winearls, 1998). It is suggested that in CRF patients on hemodialysis r-HuEPO therapy is more effective in rising blood Hb levels. Adequate dialysis is of paramount importance in correcting anemia.

Blood urea and serum creatinine levels were high before treatment (202.9±29.9 mg/dl and 10.06±1.44 mg / dl). After treatment the above levels were significantly decreased to 147.4±4.48 mg/dl and 7.37±2.24 mg/dl

respectively. High flux haemodialysis (HD) had recently been vigorously promoted as a noval standard, and in can indeed efficiently reduces the occurrence of most uremic symptoms (Okada *et al.*, 2000).

Statistical analysis did not reveal any relationship between EPO and creatinine concentrations. A low negative correlation was found between creatinine and Hb values (Zadrazil *et al.*, 1997). It clearly indicates that EPO therapy does not have any effect on blood urea and serum creatinine and this decrease is only due to haemodialysis but it is reported that the direct effect of dialysis adequacy on EPO therapy response is still not completely understood.

This suggests that dialysis adequacy can influence anemia independently and can reduce the r-HuEPO requirement but further intervention studies .are needed to fully confirm this.

REFERENCES

BAIN, B.J., 1995. *Blood cells. A practical guide*. 4th Ed. Blackwell Science Publishing Company, Oxford, pp. 350.

BARTELS, H., BOHMER, M. AND HEIERLIC., 1972. Determination of creatinine in serum plasma and urine. *Clin. Chem. Acta*, **37**: 193.

ESCHBACH, J.W., 2000. Current concepts of anemia management in chronic renal failure. Impact of NKF-DOQI *Semin. Nephrol.*, **20**: 320-329.

GIBLETT, E.R., COLEMAN, D.H., PIRZIO, A.B., DONOHOUE, D.M., MOTULSKY, A.G. AND FINCH., 1956. Erythrokinetics. Quantitative measurements of red cell production and destruction in normal subjects and patients with anemia. *Blood,* **11**: 291-298.

HALLETTE, C., 1971. Determination of urea in serum plasma and urine. *Clin. Chem. Acta,* **35**: 33.

KAMPEN, E.J. AND ZIJLSTRA, W.G., 1965. Calorimetric determination of haemoglobin in whole blood. *Adv. Clin. Chem.*, **8**: 141.

KOURY, S.T., BONDURANT, M.C. AND KOURY, M.J., 1991. Localization cells in murine kidneys by in situ hybridization. *Blood*, **77**: 2497-2503.

OKADA, H., MORIW AKI, K., KANNO, Y., SUGAHARA, S., NAKAMOTO, H., YOSHIZA W.A.M. AND SUZUKI, H., 2000. Vitamin supplementation can improve peripheral polyneuropathy in patients with chronic renal failure on high-flux haemodialysis and human recombinant erythropoietin. *Nephrol. Dial. Transplant.*, 15 : 141O-14

OKURA, Y., OSHIMA, T., Y ASU . U, Y., AMANO, K., MORI, M., SHINOZAKI, K. AND KAJIXAM, G., 1996. Effect of erythropoietin on blood pressure and intracellular concentrations in maintenance hemodialysis patients. *Hypertans. Res*., **19**: 91-95.

PIROSO, E., ERSLEV ANT, A.J. AND CARO, J., 1989. Inappropriate increase in erythropoietin titers during chemotherapy. *Am. J. Hematol.*, **32**: 248-254.

SINGH, L.X., HUANG, J.Y. AND LEE, C.T., 1999. Insulin-like growth factor plays a role in regulating erythropoiesis in patients with end-stage renal disease and erythrocytosis. *J. Am. Soc. Nephrol.*, **10**: 315-322

SPIVAK, J.L., BARNES, D.C. AND FUCHS, E., 1991. Serum immuno reactive erythropoietin in HIV infected patients. *J. Am. med. Assoc*., **261**: 3104-3107.

STOJIMIROVIC, B. AND VERA, K.P., 2000. Optimizing the erythropoietin use in chronic renal failure patients. *Med. Biol*., **7**: 1-6.

VANELLA, A., GERMIA, E. AND PINTURO, R., 1983. Superoxide dismutase activity and reduced glutathione contents in erythrocytes of uremic patients on chronic dialysis. *Acta Haematol*., **70**: 312.

WINEARLES, C.G., 1998. Recombinant human erythropoietin. Ten years clinical experience. *Nephrol. Dial. Tranplant. B. Suppl*., **2**: 3-8.

ZADRAZIL, J., BACHLEDA, P. AND ZANALKOV, J.A., 1997. Endogenous erythropoietin in patients on regular dialysis therapy. *Vnitr. Lek.*, **43**: 649-654.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 25-29, 2004.

PATHOLOGICAL CHANGES IN YOLK SAC WITH *E. COLI* INFECTION IN BROILER CHICKENS

M.S. SHAH, TARIQ M. CHAUDHRY, NADEEM YAQOOB, ASMA ASHRAF AND SHAZIA KHALIQ
Nuclear Institute for Agriculture and Biology,
P.0.128, Jhang Road, Faisalabad

Abstract.- Infection of *E. coli* causes decreased chick quality and reduced hatchability of eggs. This study was conducted to evaluate the pathological changes in yolk sac with *E. coli* infection. For this purpose one hundred, day old broiler chicks were taken and experimental infection with *E. coli* was given via intra-yolk to half of the chicken on day-1 of experiment, while others were kept as control. Parameters studied were the examination of yolk sac and yolk sac to body weight ratio. Yolks were collected from both groups at 48 hours interval. Results of this experiment showed that yolk sac infection with *E. coli* led to gross pathological changes of yolk sac (enlarged, discolored, changed consistency and congested blood vessels). On the other hand, yolk sac to body weight ratio was increased in treated group as compared to control ones. It was concluded that intra yolk infection with *E. coli* resulted in pathological changes of yolk sac and increased yolk sac to body weight ratio.

Key words: Egg hatchability, yolk sac, *E. coli* infection, broiler chicken.

INTRODUCTION

The first two weeks of age are very important in the life of a broiler chick. As in many cases 30-50% mortality may occur in this period. Omphalitis, avian encephalomyelitis, brooder pneumonia, spiking mortality, dehydration, ammonia burns and pullorum disease are major problems of the early life of chickens (Charlton, 1996). Amongst these problems, omphalitis is the major cause of early chick mortality in Pakistan (Anjum, 1997). Major cause of omphalitis is *E. coli* but other bacteria may also be found in single or mixed infections (Deeming, 1995, Rehman *et al.*, 1996, Anjum, 1997, Anonymous, 2000).

The present study was undertaken to evaluate the effect of experimental yolk sac infection with *E. coli* in broilers with the objective to help understand the pathogenesis of yolk retention and thus devise measures for controlling early chick mortality in broiler flocks.

MATERIALS AND METHODS

Preparation of inoculum

Pathogenic strain of *E. coli* was isolated from the birds suspected for colibacillosis. Identification of the organism was done by studying their morphological, cultural and staining characteristics, sugar fermentation and biochemical reactions as described by Jalil and Das (2001) and Khan *et al.* (2002). Serotyping was not done but the as pathogenicity was determined by inoculating overnight cultured broth of *E. coli* to day-old broiler chicks, chicks died within 1-2 days post-inoculation which confirmed pathogenicity of the isolate (Lee and Arp, 1998). Total viable count was done by plate count method as described by Collins *et al.* (1995).

Experimental birds

One hundred day-old chicks were obtained from local hatchery and were reared under optimal managemental conditions in experimental room, feed and water were provided *ad-libitum.*

Experimental design

Chicks were distributed into two groups, A and B, each containing 50 birds on day-1. In Group A yolk sacs were infected with *E. coli* by injecting the bacteria on day-1 of the age. The inoculum of pathogenic isolate of *E. coli* (10^4 c.f. u.f.u/0.1 ml) was injected into the yolk sac of each chick using sterilized insulin syringe as described by Kloryga (1986). The Group B chicks in this group acted as control. Sterile nutrient broth (0.1 ml/chick) was injected into yolk sac on day-l of the age. Chicks were observed for any sign and symptom during experiment to be sure that these chicks were free from any previous infection.

Collection of samples and observations

Ten chicks were slaughtered from each of the above groups at an interval of 48 hours, *i.e.*, on 3^{rd}, 5^{th}, 7^{th} and 9^{th} day post inoculation (PI). Body weights were determined before slaughtering, while the yolks were weighed and collected to record any gross pathological lesions after slaughtering, and to determine yolk sac to body weight ratio in percent.

Statistical analysis

Data thus collected were statistically analyzed by applying unpaired t-test (Steel and Torrie, 1982).

RESULTS AND DISCUSSION

Ten chicks were slaughtered at 3rd, 5th, 7th and 9th day of age and yolks were examined for gross pathological changes. At 3rd day of age yolks of infected chicks were found to be discolored having offensive odor and engorged blood vessels, whereas in control group yolks had normal color and normal blood vessels had no odor. On 5th day of age, yolks of infected chicks were more discolored having more offensive odor and with more engorged blood vessels. At 7th and 9th day of age discoloration of yolks increased whereas offensive odor and engorged blood vessels decreased, while consistency of yolks became hard. In control group at 7th and 9th day of age the yolks were completely absorbed (Table I). Examination of yolk sac revealed that the yolks of infected chicks were discoloured, having abnormal consistency (watery in initial stage and hard in latter stage) and congested yolk sac blood vessels. Similar findings were also reported by Bhatia *et al.* (1970), William (1975), Jordan (1990), Sainsbury (1992), Anjum (1997) and Khan *et al.* (2002).

TABLE I.- GROSS PATHOLOGICAL CHANGES OF YOLK SAC IN CONTROL AND INFECTED GROUP.

Day	Group	Discoloration	Offensive dour	Engorged blood vessels	Consistency		
					Watery	Gaseous	Hard
3	A	+	+++	+++	+++	-	-
	B	-	-	+	+	-	-
5	A	++	++++	++++	++	++	-
	B	-	-	-	-	-	-
7	A	+++	++	++	-	+	++
	B	Absorbed	-	-	-	-	-
9	A	++++	++	++	-	+	++
	B	Absorbed	-	-	-	-	-

-, Negative characteristic; + Positive characteristic; ++ or +++, Degree of severity.

It was evident from the results that body weight of infected chicks was slightly lower than that of control chicks. Reduced weight gain in yolk sac infection was also observed by Gross (1964) and Khan *et al.* (2002). This might be due to refusal of feed by chicks. Yolk sacs of infected chicks were heavier than the yolk sacs in control group Deeming (1995) reported that yolk sacs of infected chicks were bigger than the uninfected yolk sacs from poults of same age. Sander *et al.* (1998) and Khan *et al.* (2002) also reported similar findings.

Reduced weight gain and high yolk sac weight resulted in higher yolk sac to body weight ratio in *E. coli* infected group as compared with control group. At 3rd and 5th day of age the difference of yolk sac to body weight ratio was non significant where as at 7th and 9th day of age the difference of yolk sac to body weight ratio was significant (Table II). Similar observations in *E. coli* infection were also reported by Khan *et al.* (2002).

TABLE-II.- MEAN YOLK SAC TO BODY WEIGHT RATIO IN CONTROL AND INFECTED GROUP.

Groups	Sampling Days (Post inoculation)			
	Day 3	Day 5	Day 7	Day 9
Treatment	4.86	1.792	2.0668*	2.4443*
Control	3.45	1.373	0.2773	0.20331

*Significant difference ($P<0.05$).

It is concluded that intra yolk infection with *E. coli* resulted in pathological changes of yolk sac and increased yolk sac to body weight ratio.

REFERENCES

ANJUM, A.D., 1997. *Poultry diseases*, 2nd Ed., VetAg Publication, Faisalabad, Pakistan. pp. l78-180.

ANONYMOUS, 2000. Controlling yolk sac infection. *Int. Hatch. Pract*., **14**: 27-28.

BHATIA, K.C., SHARMA, U.K. AND CHANDRA, S., 1970. Studies on persistent yolk sac condition in chicks. Pathogenic lesions in experimentally infected baby chicks with Salmonella species. *Bihar J. Vet. Sci. Anim. Husb.*, **1**: 65-68.

CHARLTON, B.R., 1996. *Avian disease manual*. American Association of Avian Pathologists, Pennsylvania, U.S.A., pp 220.

COLLINS, C. H., LYNE, P. M. AND GRANGE, J. M., 1995. *Collin's and Lyne's Microbiological methods*, 7th Ed., Butterworth Heinemann, Oxford, U.K., pp 93, 129-131.

DEEMING, D.C., 1995. Possible effect of microbial infection on yolk utilization in ostrich chicks. *Vet. Rec.*, **136**: 270-271.

GROSS, W. B., 1964. Retained careous yolk sac caused by *Escherichia coli*. *Avian Dis*., **8**: 438-441.

JALIL, H. AND DAS, P., 2001. Biochemical and serological characterization of *Escherichia coli* from commercial poultry. *Pakistan J. scient. Res.*, **53**: 10-14.

JORDAN, F. T. W., 1990. *Poultry diseases*, 3rd Ed., ELBS and Bailliere Tindall, London, U.K., pp. 40.

KHAN, K. A., KHAN, S.A., HAMID, S., ASLAM, A. AND RABBANI, M., 2002. A study on the pathogenesis of yolk sac retention in broiler chicks. *Pakistan Vet. J*., **22**: 175-180.

KLORYGA, M.A., 1986. Pathogenicity of five rough mutants of *Escherichia coli* isolated from broilers. *Avian Pathol*., **15**: 749-759.

LEE, M.D. AND ARP, L.H., 1998. Colibacillosis. In: *A laboratory manual for the isolation and identification of avian pathogens*, 4th Ed., American Association of Avian Pathologists, Pennsylvania, U.S.A., pp 14-16.

REHMAN, R., RABBANI, M., KHAN, S. A. AND SALEEM, C.M., 1996. Pathological aspects of early chick mortality due to bacterial infections. Part-1. *Sci. Res.*, **48**: 10 1-1 07.

SAINSBURY, D., 1992. *Poultry health and management*, 3rd Ed., Blackwell Scientific Publisher, London, U. K., pp 116.

SANDER, I.E., WILLINGHAN, E.M., WILSON, J.I. AND THAYER, S. G., 1998. The effect of inoculating *Enterococcus faecalis* into the yolk sac on chick quality and maternal antibody absorption. *Avian Dis*, **42**: 359-363.

STEEL, R.G.D. AND TORRIE, I.H., 1982. *Principles and procedures of statistics*. 2nd Ed., McGraw Hill, New York. pp. 137-171.

WILLIAM, F. K., 1 975. Omphalitis in poults. *Poult. Digest*, **12**: 490.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 31-36, 2004.

COMPARATIVE STUDY ON THE EFFECT OF EIMERIAZOLE, KALORIVAC AND DARVISUL TO CONTROL COCCIDIOSIS

ASMA ASHRAF, TARIQ M. CHAUDHRY, NADEEM YAQOOB AND M. SALAHUDDIN SHAH
Nuclear Institute for Agriculture and Biology, P.O. Box 128, Jhang Road, Faisalabad

Abstract.- Coccidiosis is one of the most common diseases in poultry causing drastic economic losses to the poultry farmers. It is characterized by droopiness, paleness of the comb and occasionally blood in the droppings. To combat this problem, Eimeriazole (a herbal extract) was used and its efficacy was compared with two commercially available anticoccidial drugs *i.e.* Kalorivac and Darvisul. Oocysts were introduced in to three groups of 23 day old birds, each of 30 chicks, while the four group of 30 chicks was maintained as control . After the completion of the oocyst life cycle, examination of the faeces of the birds was carried out. Eimeriazole, Darvisul and Kalorivac were given to the three diseased groups. After treatment, the faeces of birds were examined. Kalorivac controlled the coccidiosis effectively. However it was found that Darvisul was more effective than Eimeriazole and Kalorivac. Weekly feed intake and weight gain of each chick of all the 4 groups were also studied.

Key words: Coccidiosis, birds, anticoccidial drugs, herbal medicines.

INTRODUCTION

Poultry industry is the second largest industry in Pakistan after the textile industry and about one million people are dependent on poultry (Akram, 2000). According to a survey, poultry meal consumption in Pakistan was 14% in 1986 that showed a remarkable increase upto 59% in 2000 (Anonymous, 2000).

Coccidiosis is one of the most cornmon diseases in poultry. Coccidiosis can occur at any stage of life and in any season, when conditions are favorable. However, it is most prevalent in the summer season (Boado *et al.* 1991). Overall morbidity due to coccidiosis in broilers in district Peshawar was 14.14±0.51% and mortality was 5.71±0.31%. Samad and Chakraborty (1993) reported higher mortality (6.7%) and morbidity (41.4%), whereas, Mahajan *et al.* (1994) reported lower morbidity (8.2-10.8%) and mortality (4.8-7.6%) Cocciidia are protozoan parasites that can grow and multiply in the intestine of chicken. There are seven strains of coccidian, which may infect chickens. These are *Eimeria acervulil1a,*

Eimeria brunetti, Eimeria mitis, Eimeria necatrix, Eimeria praecox, Eimeria renella and *Eimeria maxima. Eimeria tenella* causes ceacal coccidiosis, whereas the other strains cause more damage in the small intestine. Depending on the level of infection, coccidia may cause very severe damage to the intestine leading to the death of bird. The infection stage of the coccidial parasite is called the "oocyst" or egg. It is passed out in the faeces of the infected birds. Williams (1995) reported that deterioration of oocysts started soon after 24 hours when the conditions were not favourable for sporulation, however, viable oocysts up to 23 days of age were also found in the bedding material. Stayer *et al.* (1995) reported a higher number of oocysts (401.91/g of litter) in a house poorly managed than the one managed in a better way (30.63 number). To reduce the sporulation of oocyst, it is important to keep the house dry, clean and follow the appropriate immunization program (Williams, 1994).

At present, many medicines are available in market for the control and treatment of coccidiosis. Comparative studies reported here were conducted for evaluation of three anticoccidial drugs namely Eimeriazole, Kalorivac and Darvisul.

MATERIALS AND METHODS

Isolation and identification of oocyst

Oocysts were isolated from the gut material of the diseased chicks. The gut contents were preserved in the 2.5 % potassium dichromate and centrifuged with saturated sodium chloride or $ZnSO_4$ solution. The supernatants were discarded and 5 times water was added and kept undisturbed overnight. Then sediment was centrifuged at 1500 rpm for 10 min and processed for sporulation at 32-37°C for 48-72hrs. After sporulation counting of the oocyst was done by McMaster technique.

Counting procedure

Feacal sample (2g) was suspended in 30ml of water. One ml of faecal suspension was thoroughly mixed with 1 ml sugar solution. Two chambers of the counting slide were filled with the fecal mixture. The counting of eggs was done after allowing the counting chamber settle for 20 min.

Eggs per gram (EPG) of faeces = x 200, Where X was the number of eggs contained in one counting cell.

$$x = \frac{\text{Total number of eggs}}{\text{Total number of counting chamber}}$$

Experimental design

A total of 120 chicks were equally grouped into four groups A, B, C and D. All groups of birds were given 10,000 oocysts per bird via oral route. When the oocyst completed their life cycle, examination of the faeces of the birds and counting of the oocyst by McMaster technique was done.

Administration of the medicine

Group A was given Eimeriazol prepared by A.B. Pharma, Sahiwal. It has three packets. Before the medication, chicks were kept thirsty for one hour. Then 1st packet was opened and given to the chicks in two liter of drinking water. After two hours the opened medicine was mixed in five liter water for 48 hours. After this second packet was opened. The same procedure was repeated for three days. Group B was treated with Darvisul for three days according to the. prescribed information. Group C was given Kalorivac for three days according to the prescribed information. Group D was not given any treatment - it acted as control group. The first faecal sample was taken before inoculation of oocyst. After inoculation of oocyst three faecal samples were taken after seven-day interval.

RESULTS

Table I shows effect of three different anti-coccidials *i.e.* Eimeriazole, Kalorivac and Darvisul on broiler chicks.

TABLE I.- OOCYSTS PER GRAM OF FAECES BEFORE AND AFTER TREATMENT.

Weeks	**Group A (Eimeriazole)**	**Group B (Darvisul)**	**Group C (Kalorivac)**	**Group D (Control)**
At the time of treatment	14	09	16	12
1st week (after treatment)	247	307	322	376
2nd week	300	245	345	348
3rd weeks	73	22	37	375

There was a non-significant difference in number of oocyts per gram of faeces among all groups prior to drug administration, whereas, there was significant difference between treatment groups (A, B, C) and control group (D) after the medication.

Table II shows the weekly weight gain of chicks after treatment with drugs. In the 1st week of experiment Group A gained 0.115 kg, group B gained 0.091kg, Group C gained 0.111 kg and control group gained 0.124 kg which is comparable with all other groups. Similar increase in weight gain was observed through out the experiment.

TABLE II.- WEEKLY WEIGHT GAIN (Kg) OF THE CHICKENS IN TREATED AND CONTROL GROUPS.

Weeks	**Group A (Eimeriazole)**	**Group B (Darvisul)**	**Group C (Kalorivac)**	**Group D (Control)**
1st week	0.115	0.091	0.111	0.124
2nd weeks	0.297	0.329	0.265	0.323
3rd weeks	0.725	0.735	0.726	0.377

As the birds suffering from coccidiosis showed reluctance in feed intake so the a of feed intake was also recorded to compare this character in all the groups. It is clear from Table III that the feed intake the first week of experiment in group A was 2868 g, group B 2960 g, Group C 2480g and Group D 2961g.

TABLE III.- WEEKLY FEED INTAKE (g) OF THE CHICKENS IN TREATED AND CONTROL GROUPS.

Weeks	**Group A (Eimeriazole)**	**Group B (Darvisul)**	**Group C (Kalorivac)**	**Group D (Control)**
1st week	2868	2960	2480	2961
2nd weeks	4405	4250	3594	2355
3rd weeks	5570	5724	5446	2863

DISCUSSION

As it is clear that Kalorivac controlled the coccidiosis effectively, however, it was found that Darvisul is more effective than Emirezole and Kalorivac. Mathis *et al.* (2003) stated that Toltrazuril completely eliminated all

coccidial lesions and dramatically reduced oocyst shedding. The performance data, lesion scores, and oocyst counts showed that a 2-day treatment with Toltrazuril successfully controlled the coccidiosis with no relapse of infection. Toltrazuril can thus be used for supplemental control with in-feed anti-coccidials or as a primary anti-coccidial with non medicated feed.

The number of oocysts per gram of faeces decreased in all groups where as there was an increased number of oocysts in control group after the medication. Similar results were shown by Das (1993) whose study confirmed that the Zycox (a herbal anti-coccidial drug) is considered to be safe with excenent growth promoting and anti-coccidial activity for broiler production.

The feed intake level in all groups was low due to coccidiosis but after two weeks feed intake improved in treated groups while remain low in control group. Similar trend was observed in weight gain of the chickens in treated and control groups. Similarly Williams and Gobbi (2002) found that vaccinated birds consistently performed at least as well as those treated with the anti-coccidial drug shuttle. The final mean weights of vaccinated birds were significantly greater ($P<0.001$) than those of birds treated with anti-coccidial drugs, both for females at 36/37 days and males at 56 days. Feed conversion ratios, total mortality including culls, the proportion of rejects at the processing plant, and the moisture content of the litter were not significantly different between the two control methods.

REFERENCES

AKRAM, S.H., 2000. *Poultry industry in Pakistan (an overview}.* Poultry Gazette, Pakistan.

ANONYMOUS, 2000. *Executive guide*. WATT Publishing Company. pp. 8-50,

BOADO, E., LAURENL. E., HERRERA, C., QUINTERO, D. AND CANOY A.S.A., 1991. Prevalence of the- major diseases in different categories of poultry during the different seasons of the year. *Poult. Abst*., 019-00164.

DAS, S.N., 1993. Comparative evaluation of zycox a herbal anticoccidial and growth promoter with chemical anticoccidial Jot alone and in combination with growth promoters in broiler production. *Ind. J. Indig. Med*., **10**: 41-6.

MAHAJAN, N. K., JINDAL, N. AND KULSHRESHTHA, R.C., 1994. Major broiler disease in some parts of Harvana. *Ind. J. Anim. Sci*., **64**: 1118-1122.

MATHIS, G.F., FROYMAN, R., IRION, T. AND KENNEDY, T., 2003. Coccidiosis control with toltrazuril in conjunction with anticoccidial medicated or non-medicated feed. *Avian Dis*., **47**: 463-469.

SAMAD, M. A. AND CHAKRABORTY, S. R., 1993. Chemotherapeutic management of acute out breaks of caecal coccidiosis in broiler birds in Bangladesh. *Bangladesh J. Protozool.*, **3**: 140-143.

STAYER, P.A., POTE, L.M. AND KEIRS, R. W., 1995. A comparison of Eimeria oocysts isolated from litter and fecal samples broilers houses at two fanns with different management schemes during on grow out. *Am J. Poult. Sci*., **74**: 26-32.

WILLIAMS, R. B., 1994. Safety .of the attenuated anticoccidial vaccine 'Paracox' in broiler chickens isolated from extraneous coccidial infection. *Poult. Abst*., 020-03111.

WILLIAMS, R. B., 1995. Epidemiological studies of Coccidiosis in the domesticated fowl (*Galus gallus*). Physical condition and survival of *Eimeria acervulina* oocysts in poultry house litter. *J. appl. Parasitol.*, **36**: 90-96.

WILLIAMS, R.B. AND GOBBI, L., 2002. Comparison of an attenuated anticoccidial vaccine and an anticoccidial drug programme in commercial broiler chickens in Italy. *Avian Path*., **31**: 253-265.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 37-50, 2004.

BIOLOGICAL AND CHEMICAL INVESTIGATIONS OF NATURAL SPRINGS OF RANNIKOT, DISTRICT DADU, SINDH, PAKISTAN

S.M. LEGHARI, T.M. JAHANGIR, M.Y. KHUHAWAR AND ABDULLAH LEGHARI

Department of Fresh Water Biology and Fisheries, University of Sindh, Jamshoro (SML), Institute of Chemistry, University of Sindh, Jamshoro (MYK, TMJ) and Institute of Environmental Engineering and Management, Mehran University of Engineering and Technology, Jamshoro (AL)

Abstract: One major and two minor perennial water resources are observed within Rannikot along with Ranni Nai about 120 km north-west of Hyderabad within Kirthar mountainous range. The water samples were collected from the springs and examined for biological and chemical analysis. pH and conductivity were observed in the range 7.48 – 8 and 977 – 3140 µs/cm. The water flow in main spring, before collection in a water pool and was calculated to be 2.5 cusecs and was within the limits of water quality for drinking, agricultural and fisheries purposes. The water resources are surrounded by 7 species of higher aquatic plants and sufficient amount of algal flora consisting *Compsopogan coeruleus Montag* of *Rhodophyta*, *Cyanophyta* (24 species), *Chlorophyta* (27 species) and *Pyrrhophyta* (2 species) to support *Tor putitora*, *Monopterus* sp., *Puntius ticto*, *Puntius sophore* and *Salmostoma bacaila* fish species.

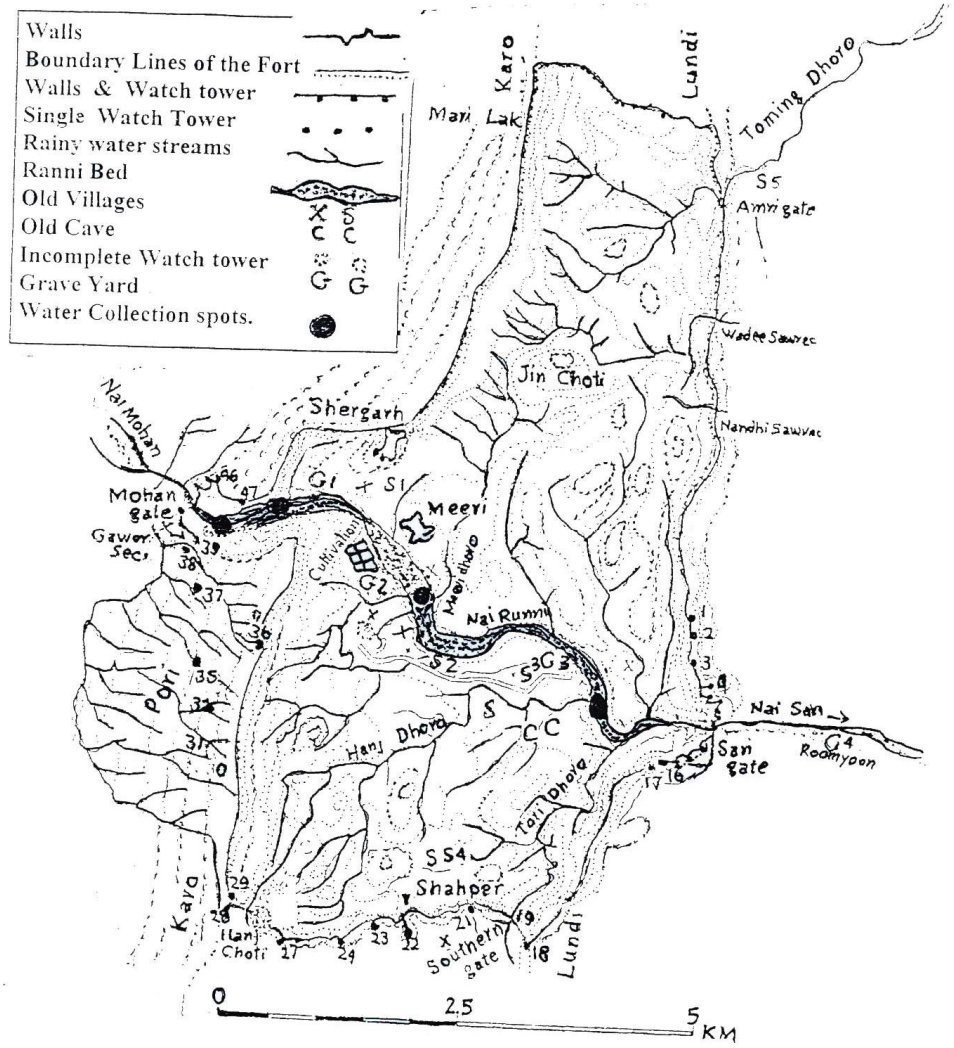
Key words: Flora, fauna and physico – chemical investigation of Rannikot spring.

INTRODUCTION

Rannikot is one of the largest fort in the lower Indus Basin in the Lakki mountainous of Khirthar range, it is situated about 120 km north-west of Hyderabad, 30km in the west from Indus high way from the town of Sunn, District Dadu. It lies at about 35km on the right bank of river Indus and is reported to be 2000 feet above sea level (Sindh Guzetter, 1993; Panhwar, 1984). It is spread over 27 sq.km. The main features of the fort are adequately discussed. (Pithawala, 1959; Rashid, 1960, 1962) The age and reasons for the construction of such huge fort are not well established (Rashid, 1962). One of the reason s for the construction of the fort at the place may due to the presence of perennial natural water resources in the region away from river Indus.

Water resources are based on (1) Ranni Nai and (2) Perennial Ranni springs (Rashid, 1960; Sindh Guzetter, 1993). Ranni Nai enters the Rannikot

through the Mohan gate (Map) and collects the water during rainy season from the mountains in the region and travels about 11 km insides the Rannikot and leaves toward east with a changed name as Sunn Nai. It travels about 35 km in the plains and eventually drains down the water in river Indus.



Map of Rannikot.

The perennial resources of water within Rannikot consist of a main and two smaller springs along the bed of Ranni Nai. The main spring water oozes at Mohan gate from the bed and moves along Ranni Nai. The water collects in a pool of about 25 × 50 m after traveling of approximate 300 m. The water pool works as an artificial water reservoir and is connected with a stream, which is eventually used for the cultivation. Two other water pools were also observed along the bed of Ranni Nai (1) in the south - west of Merrikot and (2) about 1 km from Sunn gate. The natural flow of both the resources is not sufficient to be used for economical purposes. However the water from the latter is being pumped to supply at Sunn gate for human and other consumption. The present work examines the water quality and natural vegetation developed along with the flora and fauna of springs.

MATERIALS AND METHODS

Three samples were collected during 1999 at (1) artificial reservoir; (2) near Merrikot and (3) near Sunn gate. However six samples were collected during 2000. (1) Mohn gate; (2) artificial water reservoir; (3) stream near village; (4) beneath Merrikot; (5) Merrikot lower and (6) near Sunn gate (Map). The water samples were collected in clean pre washed 1.5 L plastic bottles after rinseing several times with sample. Time of collection of samples, temperature air 1 m above the surface of water and water, conductivity, salinity, total dissolved solids (TDS), and dissolved oxygen (DO) were measured on the site, pH, chloride, alkalinity, hardness, silica, nitrate, ammonia nitrogen, Kjeldahl nitrogen, phosphate phosphorous, sodium, potassium, calcium and magnesium were determined in the laboratory (APHA, 1976).

Conductivity, salinity and TDS were determined with WTW 320 conductivity meter; pH was measured with Orion 420A pH meter. Chloride, alkalinity and hardness were estimated by titration with standard silver nitrate, hydrochloric acid and EDTA solution. Dissolved oxygen was measured by Wrinkler method. Ammonia nitrogen and Kjeldahl nitrogen were determined by titrimetery (Alen, 1989; APHA, 1976), silica, nitrate, orthophosphate and total phosphate were determined by spectrophotometry using Hitachi 220 spectophotometer following standard procedures. Orthophosphate was estimated by the reduction of the phosphomolibdate to molybdenum blue with ascorbic acid. Total acid hydrolysable phosphate phosphorous was determined by persulphate oxidation method, followed by phosphate determination as orthophosphate. Nitrate was determined using brucine sulphate as doxinatizing

derivatizing reagent. Sodium, potassium, calcium and magnesium were determined by the flame atomic absorption using Varian AA-20 Spectr atomic absorption spectrometer at the conditions recommended by the manufacturer. Sodium, potassium, calcium and magnesium were determined at 589.0, 766.5, 472.7 and 285.2 nm, respectively. The analysis was carried out in triplicate; with integration time 3 sec. and delay time 3 sec.

Quantity of water discharge of main spring was measured about 50m above, before its discharge in the pool. Quantity (Q) was calculated using the relation,

$$Q = [(B \times D) \times (S / T)]$$

Where B = breadth (feet), D=depth, S=specified distance (feet) and T=time taken by specified distance (sec). Time of the flow over the specified distance, the depth and the width of the flow channel were measured at the site.

All the biological samples were collected by plankton net # 25 µm, hand nets and by hand picking methods and preserved in 3 – 10% commercial formaldehyde and identified with help of taxonomic keys of Prescott (1962), Desikachery (1959) for the algae, higher aquatic plant (Angiospermic) (Cook, 1996) and fishes (Mirza, 1990).

RESULTS AND DISCUSSION

The results of chemical analysis of water samples collected are summarized in Tables I and II. The pH was observed in the range 7.48 – 8.06, conductivity 977 – 3160 µS/cm total dissolved solids 625 – 2022 mg/L, chloride 122 – 800 mg/L, alkalinity 150 – 260 mg/L as $CaCO_3$, hardness 300 – 700 mg/L as $CaCO_3$. The metal ions sodium, potassium, calcium and magnesium were observed in the range of 70 – 328 mg/L, 20 – 56mg/L, 82 – 210 mg/L, 32 – 95 mg/L respectively. The metal ion was observed in decreasing order Na > Ca > Mg > K.

The nutrients nitrogen, phosphorous and silica contents were determined. Ammonia is indicator of pollution was observed below the detection limits and the Kjeldahl nitrogen was present in the range of 0.28 – 1.6 mg/L. Nitrate nitrogen and silica was observed in the range of 150 – 730 µg/L and 11 – 16 mg/L, respectively. Orthophosphate phosphorus and total acid hydrolysable

phosphate phosphorus were observed in the range of 57 – 73 µg/L and 88 – 200 µg/L respectively. The water discharge of the main water source at Rannikot was calculated in January 2000 as 2.5 cusecs and contained dissolved oxygen in the range of 4.5 – 8.2 mg/L to support biological life inhibiting the water body.

TABLE I.- WATER ANALYSIS OF RANNIKOT SPRINGS, TALUKA SEHWEN, DISTRICT DADU *Date of collection 8 – 4 – 1999)

Parameters	Sampling stations		
	1	**2**	**3**
Time	12.30	10.00	9.15
Temperature of air (ºC)	42	38	36
Temperature of water (ºC)	30	29	27
pH	7.92	8.06	7.80
Conductivity (µS/cm)	1380	2800	3160
Salinity (mg/L)	500	1000	1500
TDS (mg/L)	884	1795	2022
Hardness as $CaCO_3$ (mg/L)	300	500	700
M. Alkalinity as $CaCO_3$ (mg/L)	150	230	260
Chloride (mg/L)	308	580	800
Orthophosphate (µg/L)	72	73	57
Total acid hydrolysable phosphate (µg/L)	140	200	88
Nitrate (µg/L)	400	150	730
Silica (mg/L)	11	13	16
Sulphate (mg/L)	120	134	155
Na (mg/L)	144	220	328
K (mg/L)	30	32	55
Ca (mg/L)	102	160	185
Mg (mg/L)	42	78	92

Sampling stations

1. Water escape near Niyamgar mountains from back side gate walls of fort.
2. Torrents and water pool, Merrikot lower. I
3. Water pool near Sunn gate, fort entrance. II

The results of chemical analysis indicate that the water quality at Mohan gate is acceptable as a source of drinking water with TDS of 625 mg/L and pH 7.48, but as the water travels along the bed, it dissolves salts with increase in TDS, chloride, hardness and alkalinity. Two smaller water resources observed along the Ranni Nai south-west of Merrikot and near Sunn gate contain dissolved salts with increasing concentration and indicate higher concentration of TDS, chloride and hardness as compared to main source near Mohan gate.

TABLE II.- WATER SAMPLES FROM RANNIKOT FORT, TALUKA SEHWEN, DISTRICT DADU (Date of collection 16-01-2000).

Parameters	Sampling stations					
	1	2	3	4	5	6
Time	11.00	12.15	13.00	15.40	16.00	16.10
Temperature of air (°C)	29	29	29	29	29	31
Temperature of water (°C)	25.5	25.5	26	26	26.5	26.5
pH	7.48	7.62	8.0	7.5	7.87	7.5
Conductivity (μS/cm)	977	1055	1132	1557	2200	3140
Salinity (mg/L)	200	500	500	700	1200	1000
TDS (mg/L)	625	675	724	996	1408	2009
Chloride (mg/L)	122	300	300	380	500	765
M- Alkalinity as $CaCO_3$ (mg/L)	225	260	250	230	250	227
Hardness as $CaCO_3$ (mg/L)	350	380	400	425	430	450
Silica (mg/L)	17	15	14	17	18	22
Nitrate (μg/L)	2000	1100	1250	600	1700	2200
Kjeldahl nitrogen (μg/L)	1600	300	1000	340	1400	280
Dissolved oxygen (mg/L)	8.2	7.8	8.0	6.5	7.2	4.5
Sodium (mg/L)	70	106	115	158	216	308
Potassium (mg/L)	24	25	25	26	20	56
Calcium (mg/L)	82	135	140	188	190	210
Magnesium (mg/L)	32	46	45	60	75	95

Sampling stations

1. Mohan gate.
2. Water escape near Niyamgar mountains from back side gate walls of fort.
3. Water passes through in front of Hurkan mountains (near village).
4. Torrents and water pool beneath Merrikot.
5. Merrikot lower.
6. Water pool near Sunn gate, fort entrance.

Rannikot springs are calcareous spring water, which supports the biological life. *Typha domingensis, Phragmites vallatoria, Cyperus nutans, Scirpus* species are the emergent microphytes become source of food in the food chain of fish, water fowl (Ducks, wild geese and other animals) which intern form the food for the human beings (Pandit, 1992) and also provide suitable breading and sheltering places for varied aquatic fauna (Pandit *et al.* 1986) and *Potamogeton pectinatus, P. crispus, Najas* major, *Najas* minor are submerged vagetation along with *Adiantum capitulus* growing on moist and shady places under the shadow of the large stones (Table III).

The spring water supports the 59 species of algae belonging to Cyanophyta (24), Chlorophyta (27), Pyrrhophyta (2), Bacillariophyta (5) and (1) species of

TABLE III.- BIOLOGICAL STUDIES OF RANNI KOT SPRING, TALUKA SEHWEN, DISTRICT DADU, SINDH, PAKISTAN

Species	Station I	Station II	Station III
A. Higher aquatic plants (Spermatophytes)			
1. *Cyperus nutans*	++	+	++
2. *Najas minor*	++	+	+
3. *Najas major*	+	+	+
4. *Potamogeton crispus*	+	+	+
5. *Potamogeton pectinatus*	++	++	+++
6. *Phragmites vallatoria*	++	+	++
7. *Typha domingensis*	++	+	++
B. Pteridophyta			
8. *Adiantum capitulus*	++	-	-
C. Algal flora			
i. Rhodophyta			
9. *Compsopogan coeruleus* (Bab) Mont.	+	-	-
ii. Cyanophyta			
10. *Anabaena* sp.	+	+	+
10a. *A. circinalis*	++	++	++
11. *Aphanothece clathrata* W. & W.	++	++	++
12. *Calothrix fusca* (Kutz.)	+	+	++
13. *Chroococcus minor* (Kutz.) Nag.	+	+	++
14. *C. minimus* Lemm.	+	+	++
15. *C. tenax* (Kirch) Hieron.	++	+	+
16. *C. turgidus* (Kutz.) Nag.	+	+	+
17.*Cylindrospermum muscicola* Kutz.	++	++	++
18. *Gloeocapsa alpina* Nag.	++	++	++
19. *G. lacustris* Chodat.	++	+	+
20. *Gloeocapsa compacta* Kutz.	+	+	+
21. *G. magma* (Breb.) Kutz.	++	++	++
22. *G. punctata* Nag.	+	+	++
23. *Gomphosphaeria aponina* Kutz.	++	+	++
24. *Homoeothrix hansgirgi* Lemm.	++	+	++
25. *Lyngbya birgei* Smith.	+	+	++
26. *L. hieronymusii* Lemm.	++	+	++
27. *L. martensiana* Menegh.	+	+	+++
28. *Lyngbya limnetica* Lemm.	+	+	+
29. *Nostoc* sp.	++	+	+
30. *Oscillatoria princeps* Vauch.	+	++	++
31. *Oscillatoria okeni* Ag.	++	+	+
32. *Phormidium calcicola* Gardner.			
33. *Spirulina gigantea* Sch.	+	+	++
34. *Tolypothrix* sp.	+	-	-

Continued

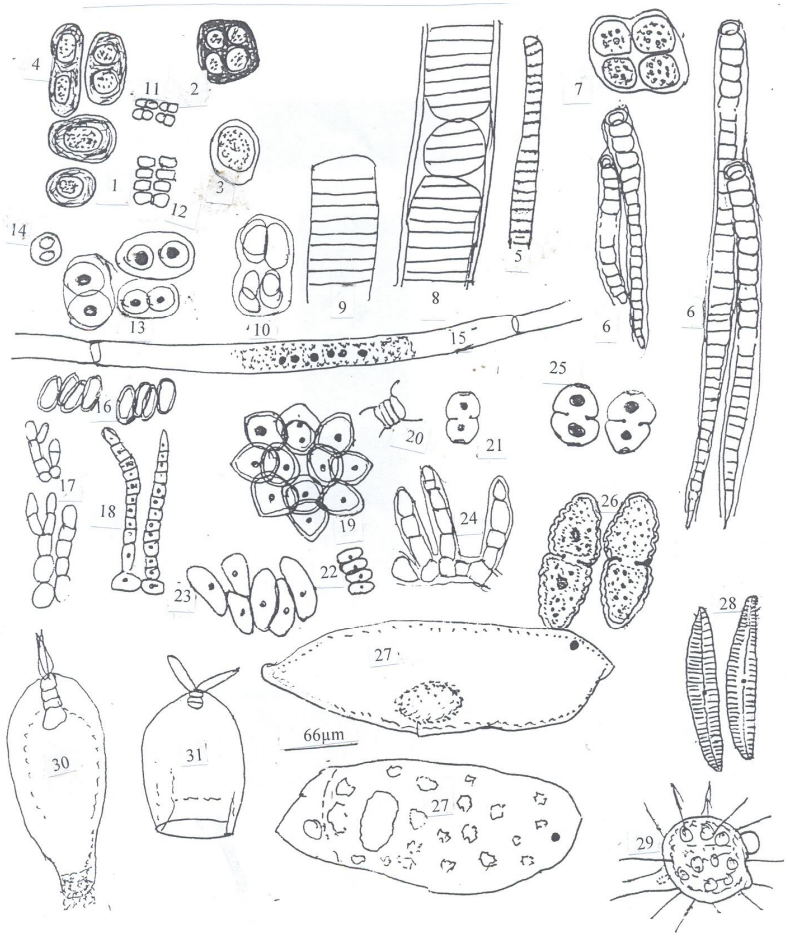
Species	Station I	Station II	Station III
iii. Chlorophyta			
35. *Chara contraria* Kutz.	++	+	+
36. *Chara zeylanica* Wilbron	++	++	++
37. *Cladophora crispata* Kutz.	+++	-	-
38. *C. glomerata* Kutz.	+	+	+
39. *Cosmarium ralfsii*	+	+	+
40. *C. granatum*	+	++	+++
41. *C. regnellii*	+	+	+
42. *C. subtumidum*	+	+	+
43. *Cylindrocystis crassa*	+	+	+
44. *Euglena ehrenbergii* Klebs.	+	+	++
45. *E. splen des* Dang.	+	++	++
46. *Euglena* sp.	+	+	+
47. *Gloeotaenium loitelsbergerianum*	+	+	+
48. *Genochloris pyrenoidosa* Kors.	+	+	+
49. *Mougeotia* sp.	+++	++	++
50. *Microspora tumidula* Hazen.	+	+	+
51. *Nitella dictyosperma* Groves.	+	-	-
52. *Oedogonium* sp.	+	+	+
53. *Oocystis elliptica* W. & W.	+	+	+
54. *Oocystis* Borgei Snow.	+	+	+
55. *Protoderma viride* Kutz.	++	-	-
56. *Phacus minutus* (Plaff.) Pech.	+	+	+
57. *Phacus* sp.	+	+	+
58. *Rhizoclonium fontanum* Kutz.	++	+	+
59. *R. hieroglyphicum* Kutz.	++	+	+
60. *R. crassipelitum* W. & W.	+	+	+
61. *Scenedesmus bijuga* (Turp.) Lag.	++	++	+++
62. *S. acuminatus* (Lag.) Chodat.	++	++	+++
63. *S. quadricauda* (Turp.) Berb.	+	+	+
64. *Spirogyra fluviatilis* Hils.	++	+	+
65. *Spirogyra* sp .	++	+	+
66. *Stigeoclonium nanum* Kutz.	++	+	+
iv. Pyrrhophyta			
67.*Glenodinium Borgei* (Lemm.) Sch.	+	+	-
68. *G. quadridens* (Stein.) Sch.	+	-	-
D. Fishes			
69. *Monopterus* sp. (Goj.)	+	++	++
70. *Puntius sophore* (Ham.) Bidda.	-	++	++
71. *Puntius ticto* (Ham.) Daro.	-	++	++
72. *Salmostoma bacaila* Ham.	-	++	++
73. *Tor putitora* (Maha Sher)	+++	+	+

- absent, + present, ++ abundances, +++ dominant.

Rhodophyta were recorded from the Rannikot springs water. Among these *Calothrix fusca, Compsopogan coeruleus* (Figs. 61 and 32), *Cladophora glomerata, C. crispata* (Figs. 35 and 36), *Gloeotrichia echinulata* (Smith.) Richter, *G. pisum* (Ag.) Thret, *Lyngbya epiphytica, L. nordgaardii* Wille., *Nostoc pruniforme, Nostoc sphaericum, Oedogonium* sp. *Stigeoclonium nanum, Stigeoclonium* sp. *Ulothrix zonata, Uronema* sp. and *Protoderma viride* are epiphytic on the filamentous algae and on the submerged parts of aquatic plants and support the invertibrate fish and gastropodes (Pandit, 1992) and Cyperus sp. and Scripus sp. are directly used cattle feed (Pandit, 1992).

Anabaena circinalis, A. iyengarii, Calothrix fusca, Gloeotrichia echinulata, Nostoc pruniforme, Nostoc sphaericum, Nostoc sp. *Gloeocapsa aeruginosa, Gomphosphaeria aponina, Spirulina major, Oscillatoria limosa, O. princeps, Lyngbya aestuarii, L. limnetica, L. martensiana, Cylindrospermum humicola, C. muscicola* are some important nitrogen fixing blue green algae in the spring water (Singh, 1961), *Anabaena* sp. *Aphanothece clathrata, Chroococcus compacta, C. minor, C. minimus, C. turgidus, C. tenax, Gloeocapsa alpina, G. magma, G. punctata, Gomphosphaeria aponina, Lyngbya hieronymusii, L. martensiana, Oscillatoria tenuis, O. nigra, O. princeps* (Figs. 1 – 16) of *Cyanophyta, Euglena ehrenbergii, E. splendes, Euglena* sp. (Figs. 51 and 52) *Phacus minutus* of *Euglenophyta, Cosmarium granatum, C. leave, C. praecsum, C. regnellii, C. ralfsii, C. subtumidium, Gloeotaenium loitels bergerianum, Genochloris pyrenoidosa, Glenodinium* Borgei, *G. quadridens, Mougeotia viridis, Oocystis* Borgei, *O. eliptica, Spirogyra fluviatilis, S. rhizobrachialis, S. subsalsa, Scenedesmus bijuga and S. quadricauda* are Planktonic. *Homoeothrix fusca, Nostoc pruniforme, Rhizoclonium crassipelitum, R. fontanum, Cladophora glomerata* (Figs. 17 – 29 and 57 – 68) are found growing attached (Epilithic) on the crust and stone of the water channel. *Chara zeylanica* (Fig. 47), *Chara contraria, Cladophorn crispata, C. fracta, Nitella dictyosperma* and *Nitella hyalina* are Rhizobenthos found through out water channel provides shelter and food for the finger lings and water fowl (Vashishta, 1964).

These primary producers support a small group of fishes throughout water channel and pools. There are the *Tor putitora* (Ham.), which can be developed as marketable fish., *Puntius ticto* Ham. (Daro) *Monopterus* sp. (Goj) and *Salmostoma bacaila,* are of small in size and can be developed for recreational and ornamental purposes. Further suggested that *Labeo rohita, Cirrhinus mrigala,* C*tenopharyngodon idella* Vah. (Grass carp) is a voracious eater of several water



Figs. 1-31. Flora and fauna of Rannikot spring. 1. *Gloeocapsa tenax* (Kirch.) Hollb; 2, *G. mgna* Kutz.; 3, *Gloeocapsa* sp.; 4, Fungal spore; 5, *Oscillatoria formosa* Bory.; 6, *Calothrix fusca* Kutz.; 7, *Gloeocapsa alpina* Nag.; 8, *Lyngbya birgei* G. M. Smith.; 9, *Oscillatoria princeps* Vaucher.; 10, *Chroococcus limneticus* Lemm.; 11, *Merismopedia* elegan A.Br.; 12, *Merismopedia punctata* Meyen.; 13, *Gloeothece confluens* Nag.; 14, *Gloeocapsa* Kützingiana Nag.; 15, *Mougeotia* sp.; 16, *Scenedesmus bijugatus* (Turp.) Kuetzing.; 17, *Stigeo clonium* sp.; 18, *Uronema* sp.; 19, *Coelastrum microporum* Naeg.; 20, *Scenedesmus quadricauda* (Turp.) Breb.; 21, *Cosmarium praecisum* Borge.; 22, *Scenedesmus bijuga* Küetz.; 23, *Scenedesmus bijugat* var. *irregularis* Wille.; 24, *Stigeoclonium nanum* Küetz; 25, *Cosmarium laeave* Rab; 26, *Cosmarium* sp.; 27, *Euglena splendens* Dang.; 28, *Cymbella Ehrenbergii* Kutz.; 29, *Rhizochrysis limnetica* G.M. Smith.; 30, *Lacane depressa*; 31, *Lepadell amphitropis* Harring.

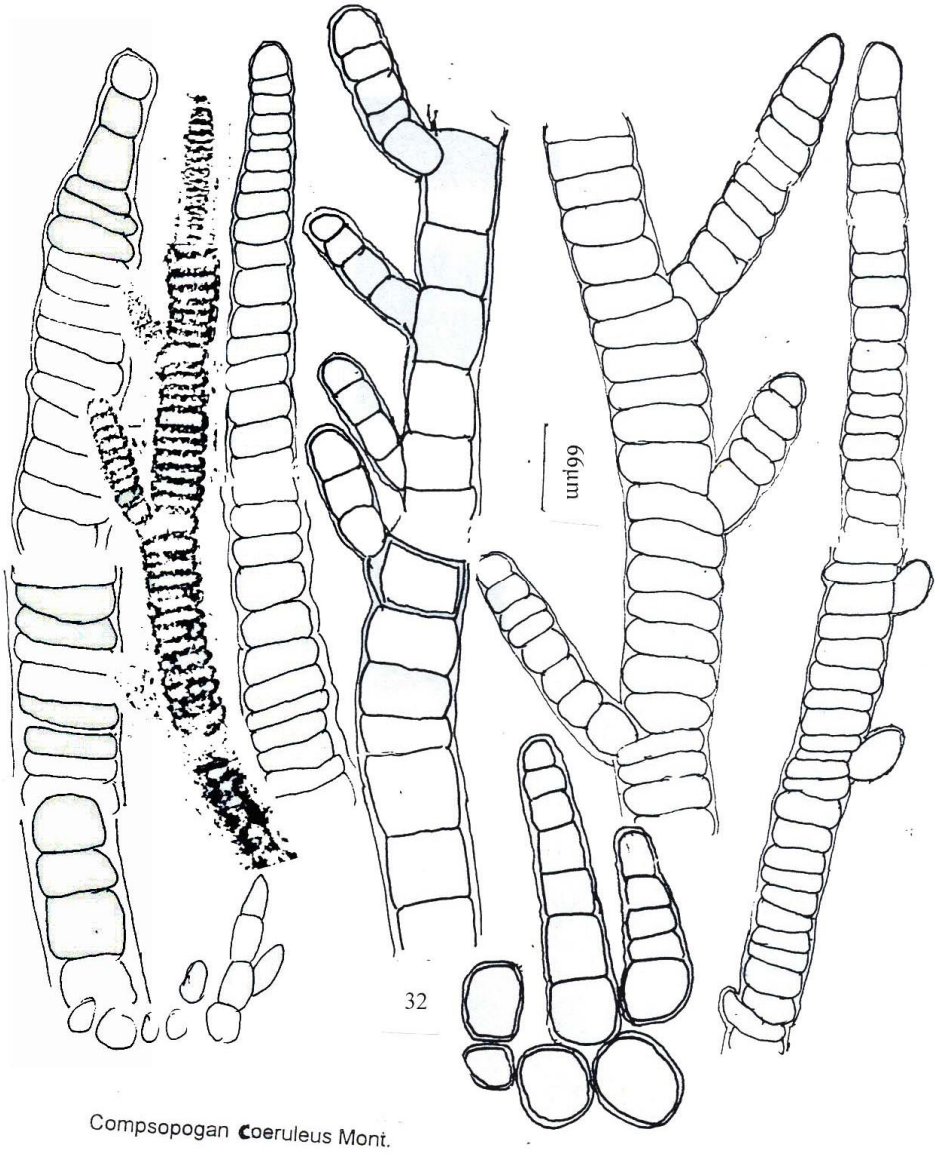
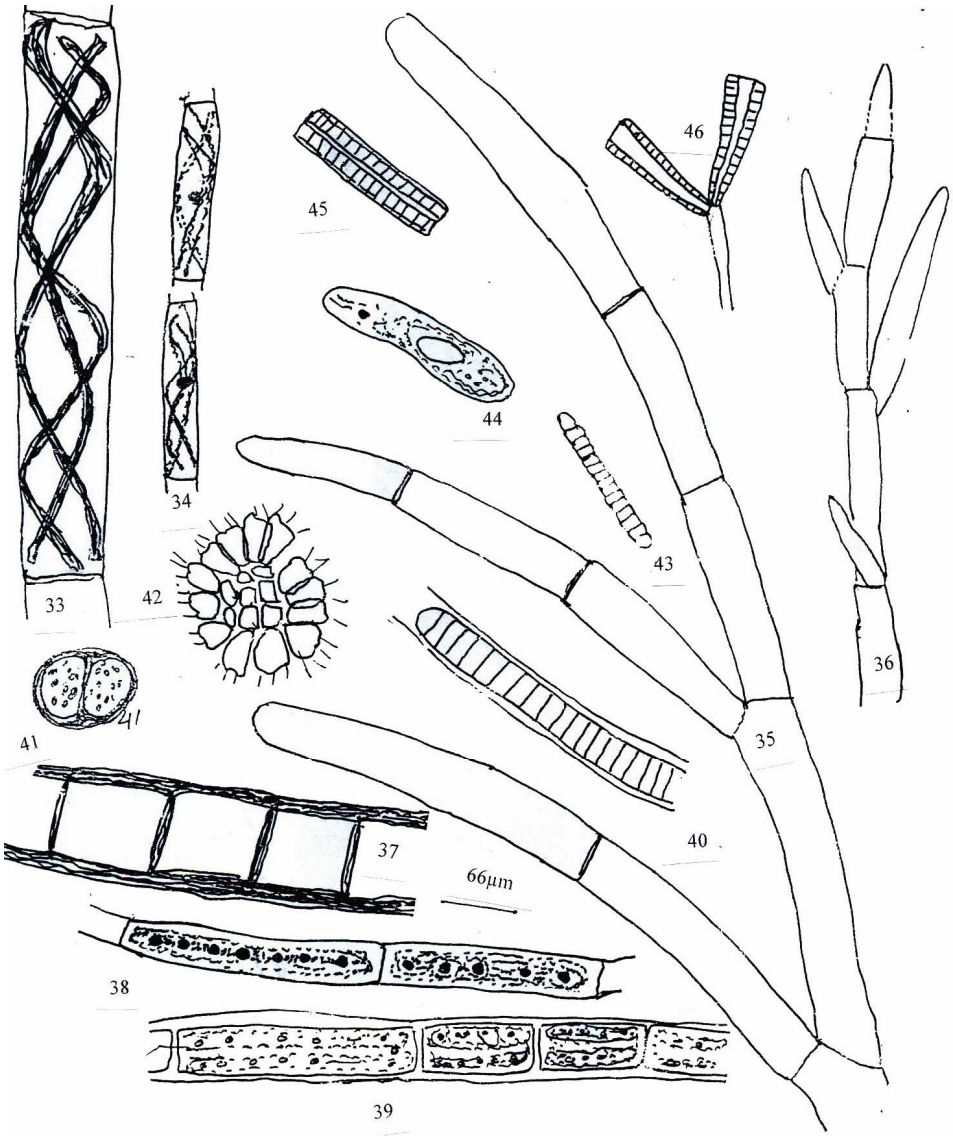
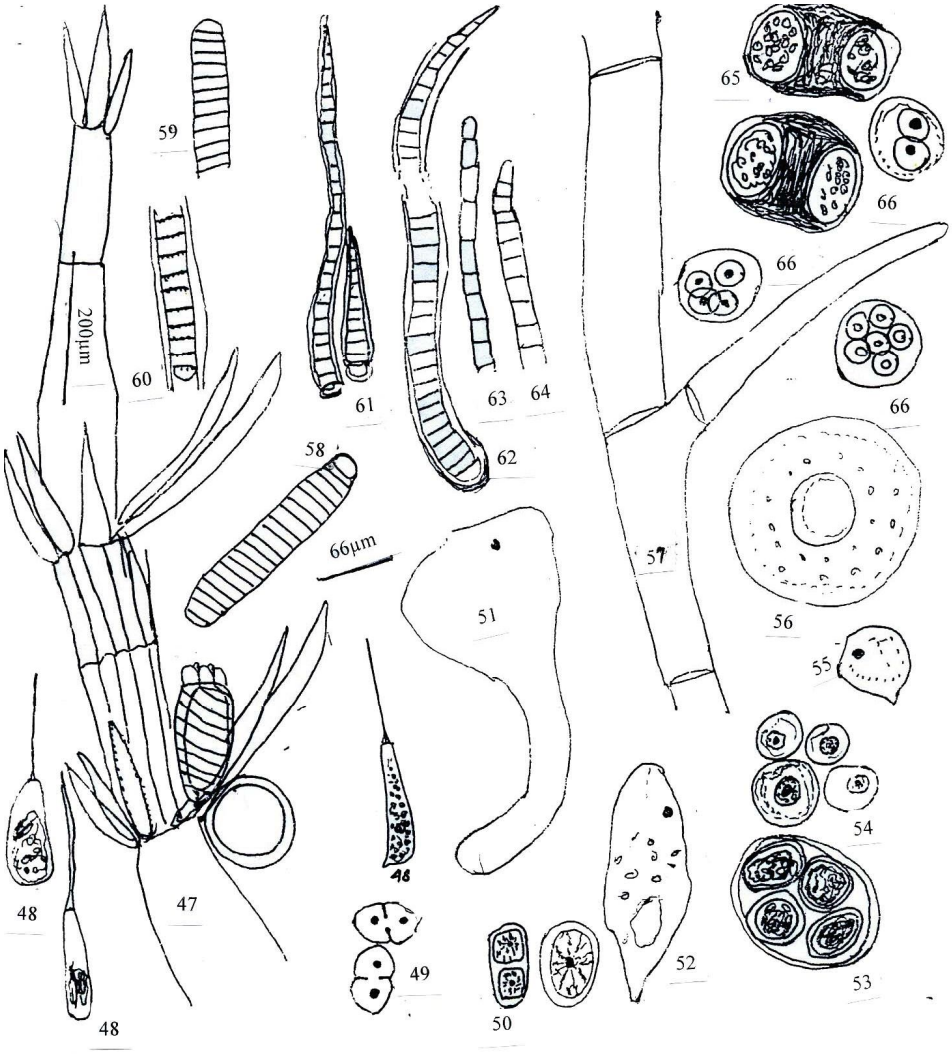


Fig. 32. Flora and fauna of Rannikot spring. 32 *Compsopogon coeruleus* Mont.



Figs. 33-46. Flora and fauna of Rannikot spring. 33-34, *Spirogyra* sp.; 35, *Cladophora crispata* Kutz.; 36, *Cladophora glomerata* (L.) Kutz.; 37, *Microspora tumidula* Hazen.; 38, *Mougeotia* sp.; 39, *Rhizoclonium crassipelitum* W.&W.; 40, *Phormidium calcicola* Gardner.; 41, *Gloeocapsa turgida;* 42, *Gomphosphaeria aponina* Kutz.; 43, *Phormidium* sp.; 44, *Euglena* sp.; 45, *Synedra uluna*; 46, *Gomphonema constrictum.*



Figs. 47-66. Flora and fauna of Rannikot spring. 47, *Chara zeylanica* Willdenow.; 48, *Paranema* sp.; 49, *Cosmarium granatum* Breb.; 50, *Cylindrocystis crassa* De. Bory.; 51, *Euglena obtusa* ; 52, *Euglena* sp.; 53, *Oocystis pusilla* Hansg.; 54, *Chlorococcum* humicola (Naeg.) Rab.; 55, *Euglena* sp.; 56, *Arcella cf. megastoma* Penard.; 57, *Rhizoclonium fontanum* Kutz.; 58, *Oscillatoria vizagapatensis* Rao.; 59, *Oscillatoria limosa* Ag.; 60, *Lyngbya martensiana* Menegh.; 61, *Calothrix fusca* F. Parva.; 62, *Homoeothrix fusca* Starum.; 63, *Oscillatoria limnetica* Lemm.; 64, *Oscillatoria okeni* Ag.; 65, *Gloeotaenium loitelsbergerianum* Hansg.; 66, *Genochloris pyrenoidosa* Kors.

plants as a bioagent of aquatic weeds (Gupta, 1987) can be introduced for fish production and recreational purpose.

ACKNOWLEDGEMENT

Financial support for this project in the form of a grant by the Higher Education Commission Islamabad is acknowledged.

REFERENCES

A.P.H.A., 1976. *Standard methods for the examination of water and wastewater*. 14th Ed, American Public Health Association, Washington D.C, pp. 143 – 628.

ALEN, S.E., 1989. *Chemical analysis of ecological materials.* Backwell Scientific Publications, Second Ed. Oxford.

COOK, C.D., 1996. *Aquatic and wetland plants of India.* William Dawson and Sons, London, pp. 778.

DESIKACHARY, T.V., 1959. *Cyanophyta* I.C.A.R. New Delhi. 686pp.

GUPTA, O.P., 1987. *Aquatic weed management*. Today & Tomorrow's Printers & Publishers, New Delhi, India, pp. 244.

MIRZA, M.R., 1990. *Pakistan Ke Taza Pani Ke Machlian* (Fresh water fishes of Pakistan). Urdu Science Board, Lahore, pp. 128.

PITHAWALA, M.B., 1959. *Historical geography of Sindh*, published by Sind Biology, pp. 176.

PANHWAR, M.H., 1984. Old fort rannikot. *Mehran Quart. Sindh Adbi Bd.,* **33**: 40–59.

PRESCOTT G.W., 1962. *Algae of the Western gate lakes area* . W.M.C. Brown company, Publishers, Dubuque, Iowa, pp. 997.

PANDIT, A.K. and QADRI, M.Y., 1986. *Nurtritive value of some aquatic life forms in Kashmir*. pp. 22 – 30.

PANDIT, A.K., 1992. Macrophytes as a component of Dal lake ecosystem. In: *Aquatic ecology* (eds. S.R. Mishra and D.N. Saksena), pp. 45-67.

RASHID, K.A., 1962. *Rannikot, Military construction in Pakistan.* Karachi, *Mahran Q. Mag.*, **33**: 9-28.

RASHID, K.A., 1960. *Ranni Kot, largest fort in Pakistan Iqbal Rev.* (April), pp. 40-45.

SINDH GUZETTER., 1993. *Dist. Dadu,* published by Board of Revenue, Sindh, pp. 205.

SINGH, R.N., 1961. *Role of blue green algae in nitrogen economy of Indian agriculture.* I.C.A.R. New Delhi.

VASHISHTA, B.R., 1964. *Algae,* Chand Company, Ram Nagar, New Delhi, India.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 51-56, 2004.

OIL ADJUVANTED NEWCASTLE DISEASE VACCINE PRODUCTION USING LOCAL VIRAL ISOLATES

NADEEM YAQOOB, TARIQ M. CHAUDHRY, M. SALAHUDDIN SHAH AND ASMA ASHRAF

Nuclear Institute for Agriculture and Biology, P.O. 128, Jhang Road, Faisalabad

Abstract.- Newcastle disease is havoc for poultry industry responsible for 50% mortalities ill birds in Pakistan. Vaccination is the only solution to overcome this problem. Already available, locally prepared vaccines, in the market against this disease provide short term immunity (3-4) weeks and the vaccine with long term immunity has to be imported at a very high cost. Considering these problems, a project was started to develop an oil adjuvant ND. Vaccine with long immunity duration using local viral strain. For this purpose, local pathogenic strain of virus was isolated and cultivated in 9-10 day old embryonated eggs allantoic route. Then allantoic fluid was collected and inactivated by three different concentrations of formaline. Then three different vaccines were prepared using Paraffin oil as an adjuvant and tween-80 and span-80 as emulsifiers in a specified quantity. Physical properties like color, type of emulsion, stability and viscosity were recorded. Antibodies Geometric Mean Titer (GMT) in the vaccinated birds gave satisfactory results.

Key words: Newcastle Disease, vaccine, immunity.

INTRODUCTION

Poultry is one of the fast expanding industries in Pakistan. A number of diseases, both infectious and non-infectious, affect the economy of the poultry farmers. Among these, Newcastle Disease (ND) is a highly contagious viral disease, which infects birds of all ages, particularly chicken (Alexander. 1991). During earlier days of commercial poultry farming, the mortality losses were so great that it was not considered viable to venture into this business. Absolute success was not achieved due to ineffective vaccines, antigenic variation and other fallacious administration. The present research project was designed to develop a potent vaccine with longer immunity period than the locally available ones using the local viral isolates.

MATERIALS AND METHODS

Isolation of virus

Spleen samples were collected for the isolation of ND virus from clinically infected birds. These were stored at -20°C.

Isolation and confirmation of ND virus was conducted using the method described by OIE Terrestrial Manual (2004). The spleen was grinded in phosphate buffer saline (pH 7.0-7.4) having antibiotics *e.g.* pencilline (2000 units/ml), streptomycin (2 mg/ml) and gentamycin (50 µg/ml). Incubate it at 37°C for 1-2 hrs then it was centrifuged at 1000g for 10 minutes at 4°C temperature, supernatant was collected for inoculation into embryonated eggs.

Candling and drilling

Two hundred fertile hen eggs were incubated at 37°C at 60-70% humidity for a period of 10 days. After incubation. the eggs were candled and only those eggs were selected which showed the active movement of embryo head. The boundary of air-space and position of the head were marked with a lead pencil. A site for the inoculation was selected a few millimeters below the area of air space on the side of the egg where the chorioallantoic membrane was well developed but was free of the large blood vessels. The area of the shell covering the air space was swabbed with methylated spirit. A hole was made through the shell with the help of a sterilized sharp end of the nail.

Inoculation in embryonated eggs

A 28 gauge needle, fitted to a 1 ml sterile insulin syringe containing the treated organ suspension inoculum was inserted into the allantoic cavity by passing it through the' hole in the shell, parallel to the long axis of the eggs. Inoculum of 0.2 ml was injected in allantoic cavity through the hole. The hole. was sealed with sterilized melted paraffin wax. The inoculated eggs were replaced in the incubator at 37°C at 60-70 percent relative humidity.

Each inoculated egg was re-candled after 24 hours post inoculation. The eggs having dead embryos were discarded. Eggs having live embryos were incubated further for a period of 24-48 hours.

Harvesting of allantoic fluid

The eggs were placed vertically and swabbed with 70 percent ethyl alcohol and allowed to dry. The egg-shells were decapitated around the air sac with a sterilized pair of scissors and forceps. The embryo was displaced to one side with the help of a sterile spatula. The allantoic fluid was aspirated with .the help of a peristaltic pump and collected in a sterilized flask. It was stored at -20°C until further use.

Confirmation and quantification of virus

The Newcastle Disease virus in the allentoic fluid was identified by applying Haemagglutination and Haemagglutination inhibition tests (Allan *et al.*, 1978; Mahboob *et al.,* 1996). Micro-haemagglutination assay (HA) test was conducted to get the quantitative determination. A two fold serial dilution of 50 µg allantoic fluid was made in 50 µg phosphate buffer saline in one row of the wells of round bottom micro-titration plates. A 50 µl of washed 1% chicken erythrocytes was added in each well and placed at room temperature for 20 minutes. The highest dilution of virus showing haemagglutination pattern with granular mat was considered the reciprocal of its titre and four haemagglutination units were calculated. The antigen is diluted up to 4HA dilution.

Preparation of vaccine

For inactivation of virus, three batches (1-3) of virus were prepared by adding 0.12%, 0.2% and 0.3% formalin in the antigen and incubating for 36 hours at 37°C.

Oil adjuvant vaccine was prepared by using emulsifier tween-80 and span-80 and paraffin oil. These components were homogenized with the inactivated virus in the specific ratio and for specific time interval, The vaccine prepared was checked for the emulsion type stability, injectibilty and sterility (Table I).

TABLE I.- PROPERTIES OF PREPARED VACCINES.

Batch No.	**Stability (upto 1 year)**	**Emulsion type**	**Viscosity (Time taken to flow due to gravity through 1 ml. glass pipette held vertically) (Second)**	**Bacterial/Fungal contamination (Growth in nutrient agar plate incubated overnight at 37°C)**
1.	No separation (Homogeneous)	Water in oil	7	Nil
2.	No separation (Homogenous)	Water in oil	9	Nil
3	No separation (Homogenous	Water in oil	6	Nil

Safety test of the vaccine

To check the safety of the vaccine, 0.1ml of inactivated antigen was injected in to ten 9 days old embryonated eggs and incubated at 37°C for 168 hrs. Then these eggs were transferred to 4°C in refrigerator. Stunting effects of embryos were determined, collected the allantoic fluid from these eggs and inoculated in to second set of 10 embryonated eggs. Incubated these eggs at 37°C for 168 hrs and determined the stunting effects. Lack of stunting effects of the fluid was considered as complete inactivation of the virus (OIE Manual, 2000; Council of Europe, 1997).

Potency test of the vaccine

For potency testing of the vaccine, four groups (A, B, C and D) of layers, each of 10 chicken were reared in the NIAB Animal House. Group D was taken as control while the A, B, and C groups were injected intramuscularly 0.2 ml of prepared vaccines of batch 1, batch 2 and batch 3, respectively at day 7 and then at day 18. Antibody titre was determined using Haemagglutination Inhibition Assay (HI) as described by Alexander and Chettle (1977). Allan *et al*. (1978). The blood was collected at different time interval for three months. The appearance of button formation in the wells provided the quantitative estimation of the antibody titre present in the vaccinated birds (Table II).

Challenge protection test of the vaccine

At the age of 50 days, fifty percent birds of each group were exposed to challenge by injecting intramuscular velogenic Newcastle disease virus (local isolate) in a single dose (0.1 ml). The birds were observed up to 7 days post challenge for any mortality and morbidity.

RESULTS AND DISCUSSION

The results showed that the vaccine is water-in-oil type, very stable at room temperature, easily injectable and contamination free (Table I). The test on bird flock showed that it provided the immunity upto three months and the antibody titre is quite high which is satisfactory for the vaccination (Table II). In challenge protection test no mortality was found in vaccinated birds, while 70% mortality was observed in control group. These results demonstrated the effectiveness of all the three vaccines. Batch 1 and batch 3 vaccines were however more immunogenic than vaccine of batch 2.

TABLE II.- ANTIBODY TITRE DETERMINATION OF PREPARED VACCINE.

Sampling day	Average antibody titre (Dilution factor) A (Vaccinated with batch 1)	B (Vaccinated with batch-2)	C (Vaccinated with batch-3)	D (non-vaccinated)
10th	2	Nil	2	0
20th	16	8	8	0
30th	64	16	32	0
40th	128	64	128	0
50th	256	128	256	0
60th	256	64	128	0
70th	256	128	256	0
80th	64	32	32	0
90th	8	8	32	0

The efficacy of ND oil emulsion vaccine has been tested in the past also with favorable results (Aitken and Survashe, 1974; Stone and Xie, 1990; Folitse *et al*., 1998; Youn *et al*., 1993), while the virus inactivation by formalin is considered as the most common, time saving and cheap method with reliable results (Wesslen *et al*., 1957).

This project was initiated to develop oil adjuvanted ND vaccine with long term immunity and the results suggest that NIAB ND Vaccine has the potential to induce higher level of protection against Newcastle Disease for longer period of time against the infection that is currently realized than the already available vaccines prepared locally.

REFERENCES

AITKEN, I.D. AND SURVASHE, B.D., 1974. Observations on the serological and dermal response of turkes to a single subcutaneous inoculation of inactivated Newcastle Disease vaccine in mineral oil adjuvant. *Avian Pathol*., **3**: 211-222.

ALEXANDER, D.J., 1991. Newcastle disease and other paramyxovirus infection. In: *Diseases of poultry* (eds. B.W. Calnek, H.J. Barnes, C.W. Beard, W.M. Reidand and H.W. Yoder Jr), 9th ed. pp. 496-519. Wolf Publishing Ltd.

ALEXANDER, D.J. AND CHETTLE, N.J., 1977. Procedures for the haemagglutination haemagglutination inhibition test. *Avian Path.*, **6**: 9-17.

ALLAN, W.H., LANCASTER, J.E. AND TOTH, B., 1978. *A report on the production of ND vaccine*. Food and Agricultural Organization of UN, Rome, pp. 57-62.

COUNCIL, OF EUROPE, 1997. *European Pharmacopia*, 3rd ed. Editions of the Europe, Strasbough, France, pp. 1745-1796.

FOLITSE, R., HAVORSON, D.A. AND SIVANANDAN, V., 1998. Efficiency of combined killed-in-oil emulsion and live Newcastle disease vaccine in chickens. *Avian Dis.*, **42**: 173-178.

MAHBOOB, T., ARSHAD, M. AND AFZAL, H., 1996. Preparation and evaluation of disease oil emulsion vaccine at hydrophile-lipophile balance 7.0. *Pakistan J. biol. Sci*., 489.

OIE MANUAL, 2000. http://www.oie.int/

OIE TERRESTRIAL MANUAL, 2004. *Newcastle disease*, Chapter 2.1.15, pp. 271-272.

STONE, H.D. AND XIE, Z., 1990. Efficacy of experimental Newcastle Water in oil emulsion vaccine formulated from squalane and squalene. *Avian Dis.*, **34**: 979-983.

WESSLEN, T., LYCKE, E., GARD, S. AND OLIN, G., 1957. Inactivation of poliomyelitis virus by formaldehyde. *Arch. Ges. Virusf*., **7**: 125-135.

YOUN, H.J., NAMGOONG, S., KIM, J.H. AND NA, M.C., 1993. The quality control of inactivated oil-emulsion vaccine of Newcastle disease, the change of potency according to the change of quality. *J. agric. Sci*., **35**: 733-739.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 57-66, 2004.

LIMNOLOGICAL STUDY OF KHADEJI SPRING, KARACHI, SINDH, PAKISTAN

S.M. LEGHARI, M.Y. KHUHAWAR, T.M. JAHANGIR AND ABDULLAH LEGHARI

Department of Fresh Water Biology & Fisheries University of Sindh, Jamshoro (SML), Institute of Chemistry, University of Sindh, Jamshoro (MYK, TMJ), Institute of Environmental Engineering and Management, Mehran University of Engineering and Technology Jamshoro (AL)

Abstract.- Khadeji Nai originates from Sari mountains of Thana Bola Khan area. Water seeps out in the form of stream and flow was calculated 4.5 – 8.5 cusecs which travel about 4 – 5 km and water then drops down as a water fall. The water collected in a pool. Four samples of spring water were collected and analyzed for physico – chemical and biological life inhabiting in the water. The conductivity and total dissolved solid were observed 970 – 1468 µS/cm and 620 – 926 mg/L with pH in the range 7.6 – 8.8. Spring water contained 9 species of aquatic plants in which *Typha domingensis*, *Phragmite australis*, *Scripus debilis*, *Scripus lineatus* in shallow water and water logged area. *Chara zeylanica, Hydrilla verticillata*, *Potamogeton pectinatus*, *Najas* minor, *Najas* major, and *Nitella hyalina* were found submerged plants. In the spring water the growth of 41 species of Cyanophyta, 39 species of Chlorophyta, 4 species of Bacillarophyta, 2 species of Zooplankton and 6 species of fishes were recorded. In which *Aphanius dispar* (Ruppel), *Puntius ticto* (Ham.), *P. conchonius* (Ham.) and *Tilapia mossambica* are dominant.

Key words: Khadeji spring water quality, flora and fauna.

INTRODUCTION

Natural water resources in mountainous area with a low rain fall in a dry and arid region have a special significance. The source could be used by humans, animals, fishes development and agriculture. Khadeji spring is located at a distance of about 100 km from Hyderabad and 65 km from Karachi and 1.5 km from Super Highway near Sui gas pumping station. The water seeps out in the Nai bed and collects in the form of small stream, which travels as water channel forming water pools. The channel travel for about 4 – 5 km and drops down the hills as water fall. The water is collected as water pool. The water is used for the agricultural purpose and as a source of drinking water for humans, animals and chicken forms.

A number of studies are reported on the natural water springs of Karachi

and Dadu district Leghari *et al.* (1995, 2001, 2002), Jehangir *et al.* (2001). Beg *et al* (1984) have described Khadeji as seasonal dependent spring with water discharge upto 9 cusecs. Nazneen and Saeed (1987) described Khadeji as a perennial river and identified six species of fishes. Panhwar (1988) described the location of Khadeji as spring with water discharge of 2 to 4 cusecs. The present work examines the water quality and biological life inhabiting in the water during 2000 and 2001.

MATERIALS AND METHODS

The perennial spring is located in Khadeji area of Karachi and is indicated in sheet No. 33 – 0/8 with latitude 25.1′ – 25.3′ and longitude 67.25′ - 67.30′. It is 220 – 230 feet above sea level. Four samples were collected during the years 1999 – 2001. (1) Khadeji fall near Chakwal chicken form, seepage of spring 300 ft long and 10 ft wide; (2) small pool of water from where Khadeji fall originates; (3) Khadeji pool near military camp; and (4) beneath from valley and 2 km far from Army camp. Water samples were collected from the depth of about 3 to 9 inches. The water was collected in 1.5 L plastic bottle, which was rinsed several times with sampling water before collection of samples. The temperature of water and air (1meter above the surface of water) were noted. The conductivity, salinity and total dissolved solids were recorded with WTW 320 conductivity meter at the sampling site. The pH was measured with Orion 420 A pH meter. Total phosphate, silica, nitrite and sulphate were determined by spectrophotometer. Nitrite was determined using brucine sulphate as a derivatizing reagent. Total phosphate was determined by persulphate digestion method, followed by reducing phosphomolybdic acid formed with ascorbic acid to molybdenium blue, slica was determined as molybdosilicic acid. Sulphate was determined by turbidiometric method as barium sulphate (APHA, 1976).

Chloride, alkalinity and hardness were determined by titration with standard silver nitrate, hydrochloric acid and E.D.T.A. respectively. Dissolved oxygen was evaluated by Wrinkler method. Sodium, potassium, calcium and magnesium were determined by Varian Spectr AA – 20 atomic absorption spectrophotometer with air – acetylene flame using standard burner at the conditions recommended by the manufacturer. The sodium, potassium, calcium and magnesium were determined at 589.0nm, 766.5nm, 422.7nm and 285.2 nm respectively with integration time 3 sec and delay time 3 sec.

All the biological samples were collected by using plankton net # 25/55 µm, hand nets and hand picking methods. The samples were preserved in 3 to

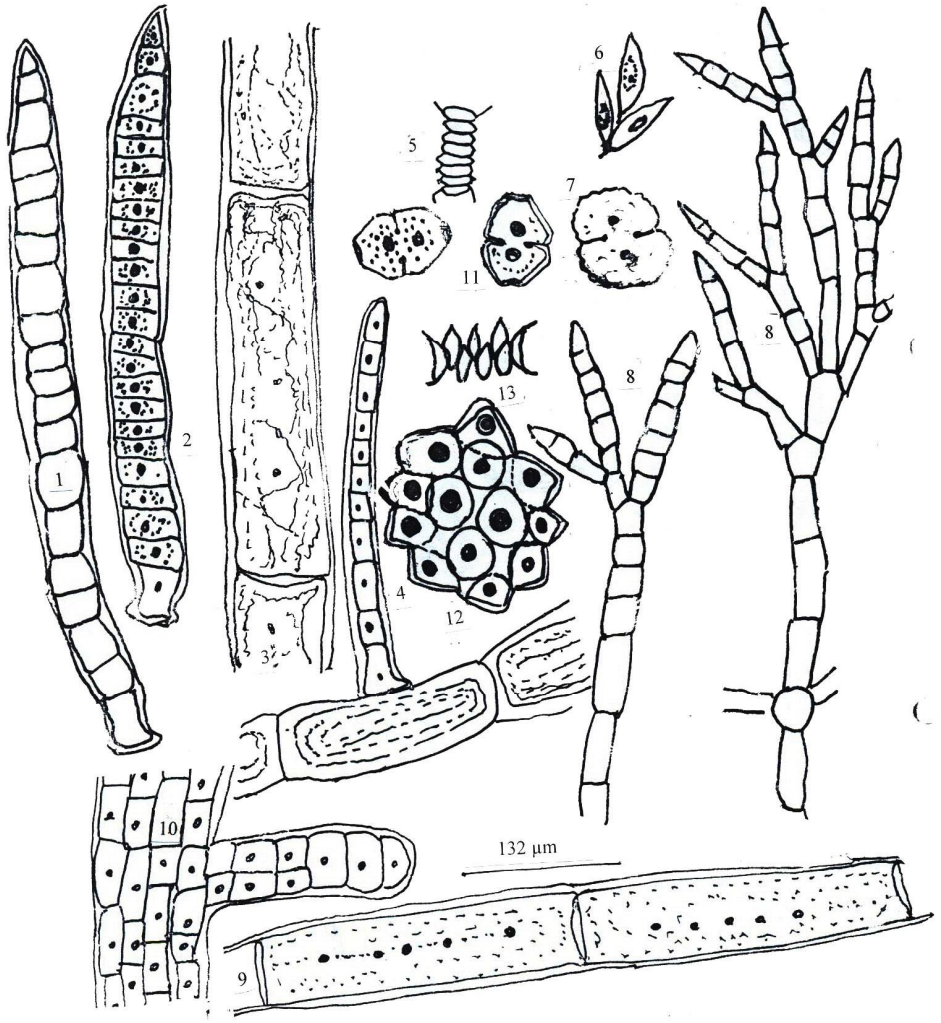
10% formaldehyde and identified with the help of taxonomic keys of Desikachary (1959) and Prescott (1962) for algae, Cook (1996) for higher aquatic plants (Spermatophyta) and Ward and Whipple (1959) for zooplankton.

RESULTS AND DISCUSSION

The water temperature of transparent water was observed from where water seeps and along the flowing channel varied with atmospheric temperature. The flow of water was observed as 4.5 – 8.5 cusecs and indicated acceptable water quality with conductivity and total dissolved solids in the range of 970 to 1860 µS/cm and 620 to 1190 mg/L. The pH was within the limits of 7.60 to 8.85 at the time of collection of the samples. The water contained sufficient amount of dissolved oxygen (DO) for the biological life inhabiting in the water in the range of 8 to 8.4 mg/L. Chloride, alkalinity and hardness were observed in the ranges of 142 – 365 mg/L, 60 – 95 mg/L and 100 – 130 mg/L respectively. The water of the spring were also collected in the form of pool which remain partially connected with the main flow of the channel. Two such pools were also collected which indicated slightly higher conductivity and TDS with 1448 – 1860 µS/cm and 926 – 1190 mg/L (Table I).

The metal contents in the water were in the following decreasing order Na > Ca > Mg > K.

Khadeji spring water has a flow in the form of the small channel. *Typha domingensis, Phragmite australis, Scripus debilis, Scripus lineatus* are growing on the side of channel and inside of the water channel. *Najas minor, Hydrilla verticillata, Potamogeton pectinatus* are present in the shallow ponds and pools. *Chroococcus minor, Chroococcus tenax, Chroococcus turgidus, Gomphosphaeria aponina, Merismopedia tenuissima, M. punctata, M. elegans, Lyngbya martensiana, Oscillatoria princeps, O. curviceps, O. formosa* are planktonic belong to Cyanophyta were observed in the ponds (Figs. 14-19). In the channels *Ankistrodesmus falcatus, A. convolutus, Coelastrum microporium, Cosmarium leave* are free floating green plankton (Figs. 4-7, 11, 13, 20-28). While *Spirogyra* sp. *S. rhizobrachalis, Mougeotia viridis, Cladophora glomerata* and *Zygnema* sp are found free floating and attached to stone and grasses in the ponds and channel. The submerged stones were covered with the Periphytic algae, *Cladophora glomerata, Chaetophora attenuata, C. elegans, Stigeoclonium lubricum, S. stagnatile, Spirogyra rhizobrachalis* (Figs. 1-3, 8-12). Some small fishes were also identified namely *Aphanius dispar* (Ruppel.), *Puntius ticto, P.*



Figs. 1-13: Flora and fauna of Khadeji Springs, Karachi, Sindh Pakistan. 1&2.*Uronema confervicolum* Lager; 3, *Rhizoclonium hieroglyphicum* Kuetz.; 4, *Uronema* sp.; 5, *Scenedesmus opoliensis* Richter.; 6, *Characium ambiguum* Herman.; 7, *Cosmarium undulatum* Corda.; 8, *Stigeoclonium subsecundum* Küetz.; 9, *Mougeotia sphaerocarpa* Wolle.; 10, *Enteromorpha intestinalis* (L.) Nees.; 11, *Cosmarium granatum* Breb.; 12, *Coelastrum microporum* Naegeli.; 13, *Scenedesmus incrassatulus* Bohln.

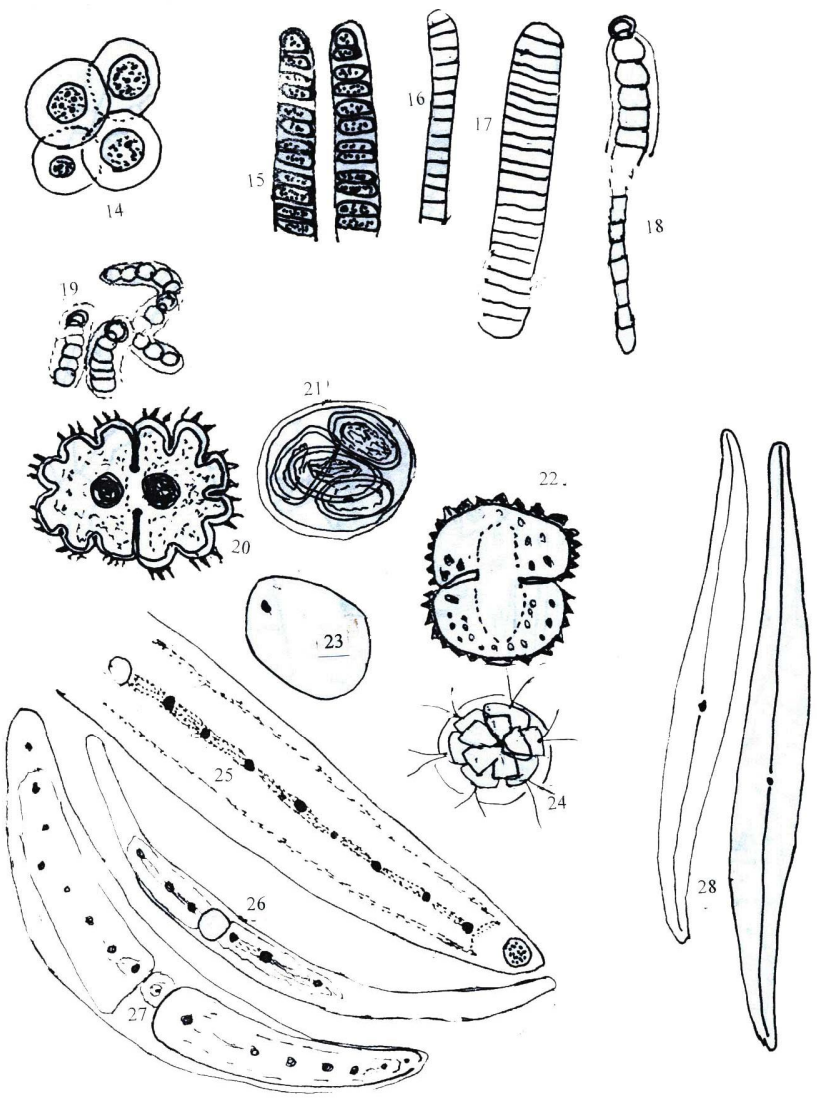


Fig. 14-28. (Continued) 14, *Gloeocapsa chroococcoides* Novacek.; 15, *Johannesbaptistia pellucida* (Dickie) Taylor.; 16, *Oscillatoria nigra* Vaucher.; 17, *Oscillatoria limosa* (Roth) Ag.; 18, *Calothrix epiphytica* W.&W.; 19, *Nostoc microscopicum* Carmichael.; 20, *Euastrum spinulosum* Delp.; 21, *Oocystis elliptica* W.&W.; 22, *Cosmarium reniformae* (Ralf.) Arch.; 23, *Lepocinclis playfairiana* Deflandre.; 24, *Pandorina morum* (Muell.) Bory.; 25, *Closterium parvulum* Naege.; 26, *Closterium dianae var. dianae.* Ehernb.; 27, *Closterium* sp.; 28, *Gyrosigma attenuatum* (Kutz.) Rabli.

TABLE I.- PHYSICO – CHEMICAL ANALYSIS OF WATER QUALITY OF KHADEJI SPRING NEAR GAS FIELD, DISTRICT KARACHI, SINDH, PAKISTAN. (n = 4).

Parameters	**Sampling stations**			
	1	**2**	**3**	**4**
Time	9.35	10.50	12.30	14.50
Temperature of air (°C)	29	36	38	38
Temperature of water (°C)	28	31	32	32
Color	Trans.	Trans.	Trans.	Trans.
pH	8.10	8.85	7.60	8.30
Conductivity (µS/cm)	970	1446	1448	1860
Salinity (g/L)	0.2	0.5	0.5	0.7
TDS (mg/L)	620	925	926	1190
Chloride (mg/L)	142	301	237	365
P- Alkalinity as $CaCO_3$ (mg/L)	BD	50	BD	BD
M-Alkalinity as $CaCO_3$ (mg/L)	80	60	95	90
Hardness as $CaCO_3$ (mg/L)	100	120	130	120
DO (mg/L)	8.4	8.2	8.0	8.4
Nitrate (mg/L)	0.6	BD	0.7	BD
Total acid hydrolysable phosphate (µg/L)	300	150	100	250
Silica (mg/L)	2	2	7	5
Sulphate (mg/L)	80	120	125	154
Sodium (mg/L)	77	137	120	194
Potassium (mg/L)	5	6	8	5
Calcium (mg/L)	38	30	60	56
Magnesium (mg/L)	26	18	28	25

BD= Below detection limit; Trans.= Transparent

Sampling stations
1 Khadeji fall near Chakwal Chicken form, Seepage of spring 300 ft long & 10 ft wide.
2 Small pool of water from where Khadeji fall originates.
3 Khadeji pool near military camp.
4 Beneath from valley and 2 km far from Army camp.

conchonius, *Tor putitora* (Hem.), and *Tilapia mossambica* (Peters) as reported Nazneen and Saeed (1987). All the species of fish growth with thick population only *Tor putitora* (Mahaseer) is commercial, but all the other fishes are small size with no commercial value (Table II). Therefore it is suggested that *Cyprinus carpio* (Gulfam), *Labeo rohita* (Rohou), *Catla catla* (Thaila), *Cirrhinus mrigala* (Mori), *Ctenopharyngodon idella* (Grass carp), *Hypophthalmichthys molitrix* (Silver carp) may be cultured with proper ratio. The grasses are used as food for the domestic animals. The main source of water in this valley is used for the cultivation of various type of vegetables and can considered as suitable source of drinking water for humans and animals. The easy availability of the source of water in the region has led to develop chicken forming within the area.

TABLE II.- FLORA AND FAUNA OF KHADEJI SPRINGS, KARACHI, SINDH PAKISTAN

Species	Up Stream	Down Stream
Aquatic Plants		
Spermatophyta		
Hydrilla verticillata (L.) Royle.	++	++
Najas minor Allioni.	++	+
Najas graminea Raff.	+	++
Phragmite australis (Cava) Trinius.	+	++
Potamogeton pectinatus Linn.	+	+
P. nodosus Poiret.	++	+
Typha domingensis Persoon.	++	++
Vallisneria spiralis Lin.	++	++
Cyanophyta		
Anabaena variabilis Kutz.	+	+
Aphanocapsa biformis A.Br.	++	+
Aphanocapsa sp.	+	+
A. littoralis Hansg.	++	+
Aphanothece stagnina (Spreng.) A. Br.	+	++
Aphanothece saxicola Nag.	+	+
Calothrix epiphytica W&W.	+	+
Calothrix fusca (Kutz.). B&F.	+	+
Chroococcus limneticus Lemm.	++	++
Chroococcus pallidus Nag.	++	++
Chroococcus turgidus Kutz.	++	+
Coelosphaeium kuetzingianum Nag.	+	++
Cylindrospermum majus Kutz.	+	+
Cylindrospermum muscicola Kutz.	+	+
Gloeocapsa rupestris Kutz.	+	+
G. chroococcoides Nova.	+	++
Gloeotheca rupestris (Lyngb.) Born.	+	+
Gomphosphaeria aponina Kutz.	+	++
Gomphosphaeria aponina f.cordiformis Wolle.	++	+
Johannesbaptistia pellucida (Dickie) Taylor.	+	++
Lyngbya epiphytica Wille.	+	+
L. hieronymusii Lemm.	+	+
L. limnetica Lemm.	+	++
L. martensiana Menegh.	+	+
Merismopedia elegans A. Br.	+	++
Merismopedia glauca (Eh) Nag.	+	+
Merismopedia tenuissima Lemm.	++	++
Microcystis aeruginosa Küetz.	++	+++
Microcoleus paludosus (Kutz.) Gom.	+	+
Nostoc muscorum (on soil) Ag.	+	+

Continued

Species	Up Stream	Down Stream
N. microscopicum Car.		
Oscillatoria amphibia Agardh.	+	+
O. amoena Gom.	+	+
O. chalybea Mertenss.	+	+
O. formosa Bory.	+	+
O. granulosa Martens.	+	+
O. limosa Ag.	+	+
O. limnetica Lemm.	++	+
O. nigra Vauch.	+	+
O. princeps Vauch.	++	++
O. sancta (Küetz) Gom.	+	++
Spirulina laxissima Wert.	+	++
Synechocystis salina Wislouch.	+	+
Chlorophyta		
Characium ambiguum Herman.	++	++
Chara zeylanica Willdnon.	++	++
Chaetophora elegana (Roth.) Ag.	+	--
Cheatophora attenuata Hazen.	+	--
Chlamydomonas globosa Snow.	+	+
Cladophora glomerata (L) Küetz.	++	+
Closteriopsis longissima Lemm.	+	++
Coelastrum microporum Naegli.	+	+
Coelastrum sphaericum Naegli.	+	++
Closterium dianae Eher.	+	+
C. purvulum Naeg.	+	+
Cosmarium granatum Berb.	+	++
Cosmarium leave Berb.	+	+
C. undulatum Corda.	+	+
C. reniformae Arch.	+	+
Crucigenia quadrata Morren.	--	+
Enteromorpha intestinalis (L.) Nees.	+	+++
Ermosphaera viridis (Snow.) Printz.	+	--
Euglena sp.	+	+
Euastrum spinulosum Delp.	+	+
Glenodinium quadridens (Stein.) Schiller.	--	++
Kirchneriella contorta (sch) Boh.	+	++
Lepocinclis ovum (Ehernb.) lemm.	--	++
Mougeotia robusta (De. Bary.) Wittrock.	--	+
Mougeotia scalaris Hassall.	--	+
M. sphaerocarpa Wolle.	+	+
Nitella hyalina (DC.) Ag.	++	++
Oedogonium epiphyticum. Transeau & Tiffany	++	+

Continued

Species	Up Stream	Down Stream
Oocystis pusilla Hansgirg	+	+
O. elliptica W. & W.	+	++
Pediastrum duplex Meyen.	+	+
P. duplex var clathratum (A. Br) Lagarhe	+	+
P. tetras (Ehernb.) Ralfs.	+	+
Phacus orbicularis Huebner.	--	+
Pandorina morum Bory.	+	++
Rhizoclonium fontanum Kutz.	+	+
R. hieroglyphicum Kutz.	+	++
Rhizochrysis limnetica GM Smith.	+	++
Scenedesmus bijuga (Trup.) Lagerhein.	--	+
S. bijuga var alternans (Rein.) Hans.	--	+
S. quadricauda var. longispina (Chod.) Smith.	--	+
S. quadricauda var. quadrispina Smith.	+	++
S. incrassatulus Bohlin.	++	++
Spirogyra crassa Küetz.	++	+
Spirogyra sub salsa Küetz.	+	+
Spirogyra rhizobrachialis Jao.	++	+
Stigeoclonium attenuatum (Hazen.) Coll.	++	--
Stigeoclonium lubricum (Dillow.) Kutz.	+	--
Stigeoclonium stagnatile (Hazen.) Collins	++	--
Stigeoclonium subsecundum Kutz.	++	--
Uronema confervicolum Lager.	+	+
Ulothrix aequalis Kutz.	+	--
Zooplankton		
Lacane depressa	++	++
Lacane sp.	++	+
Lepadella amephitrops	+	+
Fishes		
Aphanius dispar (Ruppel)*.	+++	+++
Cyprinion watsoni (Day)*.	+	+
Puntius conchonius (Ham.)*.	+	+
Puntius ticto (Ham.)*.	+++	+++
Tilapia mossambica (Peters.)*.	+	+
Tor putitora (Ham.)*.	+	++

(-) absent, (+) present, (++) abundance, (+++) dominance and
(*) commercial and ornamental.

ACKNOWLEDGEMENT

Financial support in the form of a grant for this project by the Higher Education Commission Islamabad is acknowledged.

REFERENCES

APHA., 1976. *Standard methods for the examination of water and wastewater*. 14th Ed, American Pub. Health Assoc., Washington D.C. pp: 143 – 628.

BEG, M.A.A, MAHMOOD, S.N. AND NAEEM, S., 1984. Characterization of waters. Part-I. Spring waters in and around Karachi. *Pakistan J. scient. indust. Res.*, **27**: 131-136.

COOK, C.D., 1996. *Aquatic and wet land plants of India*. William Dawson and Sons, London, pp: 778

DESIKACHARY, T.V., 1959. *Cyanophyta*. ICAR. New Delhi, 686 pp.

JAHANGIR, T.M., KHUHAWAR, M.Y., LEGHARI, S. M. AND LEGHARI, A., 2001. Physico – chemical and biological study of Mangho Pir Euthermal spring, Karachi, Sindh, Pakistan. *OnLine J. biol. Sci.*, **1**: 636–639.

LEGHARI, S.M; SAHITO, G.N. AND KHUHAWAR, M.Y., 1995. Physico – chemical and floral studies of Pokhan and Zai springs of Hilly areas near Thano Bola Khan Distt. Dadu, Sindh, Pakistan. *Scient. Khyber*, **8**: 37 – 41.

LEGHARI, S.M., JAHANGIR, T.M., KHUHAWAR, M.Y. and LEGHARI, A., 2001. Physico – chemical study of Dhabeji springs, Malir, Karachi, Sindh, Pakistan. *OnLine J. Biol. Sci*., **1**: 623–627.

LEGHARI, S.M., JAHANGIR, T.M., KHUHAWAR, M.Y. and LEGHARI, A., 2002. Study on the natural springs at Clifton, Karachi, Sindh, Pakistan. *Proc. Pakistan Congr. Zool*., **22**: 125–131.

NAZNEEN, S. AND SAEED, B., 1987. Note on the fishes of Khadeji River (Sindh, Pakistan). *J. Pure. appl. Sci.*, **6**: 55 pp

PANHWAR, M.H., 1988. Some important spring of Mahal Kohistan. *Mining Engg. J. Pakistan*, **7**: 9-13.

PRESCOTT, G.W., 1962. *Algae of the Western Great Lakes area*. W.M.C. Brown Company Publishers, Dubuque, Iowa, USA. 977pp.

WARD, H.B. AND WHIPPLE, G.C., 1959. *Freshwater biology*. John Wiley & Sons. New York, pp. 1248.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 67-75, 2004.

STUDIES ON THE OPTIMIZATION OF PROTEASE PRODUCTION BY *BACILLUS SUBTILIS* IH-16

IKRAM-UL-HAQ AND HAMID MUKHTAR
Biotechnology Research Centre, Department of Botany, Government College University, Lahore, Pakistan

Abstract.- Present study describes the optimization of submerged fermentation parameters for the production of neutral protease by *Bacillus subtilis* IH-16 isolated from the garden soil. Different cultural conditions such as fermentation medium, incubation temperature, medium pH and fermentation period were optimised. M2 medium consisting of (w/v %) Sunflower meal 1.0; casein, 0.5; Glucose, 1.0; Peptone, 1.0 and KH_2PO_4, 0.1 (pH 7.0) was found to be the best medium for biosynthesis of neutral protease by the organism. It was found that a temperature of 35°C, pH 7.0 and fermentation period of 48 h were the optimum conditions for *Bacillus subtilis* IH-16 to produce high titers of neutral protease in submerged fermentation. The maximum production of neutral protease during the course of present studies was 8 U ml^1.

Key words: Proteolytic enzyme, fermentation, pH, incubation temperature.

INTRODUCTION

Proteases are the hydrolytic enzymes which occupy a pivotal position as far as their application in physiological and commercial fields is concerned. Today, microbial proteases account for approximately 40% of the total enzyme sales in various industrial market sectors (Gupta *et al.,* 2002). These are degradative enzymes which catalyse the total hydrolysis of proteins. Microbial proteases are classified into various groups *i.e.,* acidic proteases, neutral proteases and alkaline proteases depending on whether they are active under acidic, neutral or alkaline conditions and on the basis of characteristics of the active site group of the enzyme *i.e.*, metallo-, aspartic-, cysteine-, sulphydral or serine type (Kalisz, 1988).

Bacterial neutral proteases are active in a narrow pH range *i.e.,* pH 5.0-8.0. These are characterized by low thermotolerance which is mainly due to their autolysis at higher temperatures (Eijsink *et al.,* 1991; Hardy *et al.,* 1993). Neutral proteases also have an intermediate rate of reaction which generates less bitterness in the hydrolysed food than do the animal proteases and hence are valuable for use in the food industry (Rao *et al.,* 1998). Their low thermo-

tolerance is useful for controlling their reactivity during the production of food hydrolysate having low degree of hydrolysis. Neutral protease which is insensitive to the natural plant proteinase inhibitors is useful in the brewing industry (Felix and Villettaz, 1983). Neutral protease may be the metalloprotease type and thus requiring divalent metal ions for their activity or they may be serine protease type thus are not affected by chelating agents (Rao *et al.*, 1998).

Although a variety of proteolytic fungi and bacteria are known which produce neutral proteases, but only few provide high activities with commercial success. *Bacillus* species are specifically and extensively used for the production of neutral proteases (Priest, 1977; Markland and Smith, 1971). *Bacillus* species have the capability to grow in a wide range of pH from 7.0 to 11.0 and produce neutral to alkaline proteases (Adinaryana and Ellaiah, 2002). Among *Bacillus* species, the common proteolytic species include *Bacillus subtilis* (Uehara *et al.*, 1974; Qadeer *et al.*, 1990), *B. thermoproteotticus* (Jiang and Bond, 1992), *B. cereus* (Pauptit *et al.*, 1988; Stark *et al.*, 1992) and *B. stearothermophilus* (Kubo *et al.*, 1988; Nakamura *et al.*, 1997).

Production of extracellular proteases by microorganisms is strongly influenced by the composition of media such as variation in C/N ratio, presence of some easily metabolizable sugars (Beg *et al.*, 2002) and divalent metal ions in the fermentation medium (Varela *et al.*, 1996). In addition to these, different environmental and fermentation parameters such as pH, aeration, temperature, agitation, inoculum density and incubation period also affect the amount of protease produced (Hameed *et al.*, 1999; Puri *et al.*, 2002). So the research efforts have been directed towards the optimization of above said factors.

The present investigation is aimed at optimization of some culture parameters such as fermentation medium, incubation temperature, pH of the medium and fermentation period for the biosynthesis of neutral protease by *Bacillus subtilis* IH-16.

MATERIALS AND METHODS

Microorganisms

A new strain of *Bacillus subtilis* IH-16, isolated from the garden soil, was used as producer of neutral protease. The strain was grown at 37°C and was maintained on nutrient agar slants by weekly transfers on to new slants. For storage, the 24 h old culture was kept at 4°C in a cold room.

Inoculum preparation

The inoculum was prepared by transferring a loopful of bacteria from a 24 h old slant culture, into 250 ml cotton wool plugged Erlenmeyer flask containing 50 ml of sterilized inoculum medium. The inoculum medium was composed of 0.8 % nutrient broth (Peptone and yeast extract) and was sterilized in an autoclave at 15 Ibs/ $inch^2$ pressure. The flask was kept in a rotary shaker at 160 rpm at 37°C. One ml of this culture containing $1.2x10^7$ cells/ml was used as inoculum.

Fermentation experiments

The shake flask experiments for the production of neutral protease were carried out in 250ml Erlenmeyer flasks containing 50 ml of culture medium. The culture medium was consisted of (w/v %) Sunflower meal, 1.0; Casein, 0.5; Glucose, 1.0; Polypeptone, 1.0 and KH_2PO_4, 0.1. The pH value of the production medium was adjusted to 7.0 before sterilization by adding IN NaOH or IN HCl. The flasks were cotton plugged and sterilized in an autoclave at 15Ibs / $inch^2$ pressure (121°C) for 15 minutes. After the medium was cooled at room temperature, it was inoculated with 1ml of the inoculum containing $1.2x10^7$ cells/ml, as prepared earlier. The flasks were placed in an incubator shaker at 160 rpm and 30°C.

After an incubation period of 24 h, the flasks were taken out of the shaker and their contents were centrifuged at 5000rpm for 10 minutes. The clear supernatant obtained after centrifugation was used to determine the enzyme production. All the experiments were carried out in triplicate and average values were reported. During all the experiments, the chemicals of analytical grade and calibrated lab ware were used.

Fermentation media

Different culture media were tested for the production of neutral protease by *Bacillus subtilis* IH-16. The pH of all the media was adjusted to 7.0 by adding IN NaOH or IN HCl. The media include:

M1: (w/v %) Dextrin, 3.0; $(NH_4)SO_4$, 1.0; peptone, 1.0; KH_2PO_4, 1.0 and $MgSO_4$, 1.0 (Mohamed *et al.*, 1998).

M2: (w/v %) Sunflower meal 1.0; casein, 0.5; glucose, 1.0; peptone, 1.0 and KH_2PO_4, 0.1.

M3: (w/v %) Milk powder, 1.0; yeast autolyzate, 0.3; cornstarch and malt, 0.5 (20ml per 100ml of medium) (Michalik *et al.,* 1995).
M4: (w/v %) Soyabean meal, 1.0; glucose, 2.0; polypeptone, 1.0; KH_2PO_4, 0.1 (Fujiwara & Yamamoto 1987).
M5: (w/v %) Casein-hydrolyzate, 0.48; gelatin, .6.0 and 3ml of glycerol (separately sterilized in oven at 121°C for 30 min.) (Hameed *et al.,* 1996).

Assay of proteases

The method of McDonald and Chen (1965) was used for the assay of proteases. Casein (1%) was incubated with one ml of enzyme sample at 30°C for one hour. The reaction was arrested by the addition of five ml of 5% trichloroacetic acid solution. The mixture was centrifuged at 5000 rpm for 10 min and one ml of supernatant was mixed with five ml of alkaline reagent. To this mixture one ml of 1N NaOH was added to make the contents of the tube alkaline. After 10 min, 1.0 ml of Folin-Ciocalteau reagent (diluted with distilled water at the ratio 1:1) was added to the test tubes and mixed. The blue colour produced was measured (CECIL, CE 7200, Cambridge, England) at 700 nm after 30 min. One unit of protease activity is defined as the amount of enzyme required to produce an increase of 0.1 in optical density at 700 nm under defined conditions.

RESULTS AND DISCUSSION

Optimum medium

The fermentation experiments were carried out in different culture media to find out the best medium for maximum production of neutral proteases by *Bacillus subtilis* IH-16. Five different culture media were examined and M2 was found to be the best for maximum production of protease (Fig. 1). M2 consisted of (w/v %) sunflower meal, 1.0; casein, 0.5; glucose, 1.0; peptone, 1.0 and KH_2PO_4, 0.1 (pH adjusted to 7.0).

It seems that M2 contained all the essential nutrients and a fairly good C/N ratio for the growth and subsequent production of neutral protease by *Bacillus subtilis* IH -16. Glucose and peptone present in the medium were simple and easily utilizable carbon and nitrogen sources for the growth of organism. Many other workers also reported the maximum production of proteases using glucose as carbon source (Qadeer *et al.,* 1990).

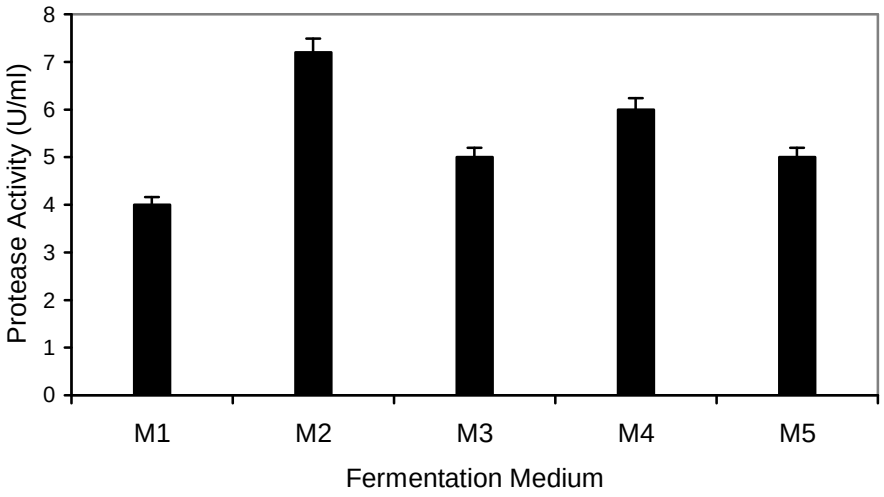


Fig. 1. Selection of medium for the production of neutral protease by *Bacillus subtilis* IH-16.

Optimum temperature

The effect of different incubation temperatures on the production of neutral protease by *Bacillus subtilis* IH-16 was investigated. For that purpose, fermentation experiments were carried out at different temperatures such as 25, 30, 35, 40 and 45°C. The results of the Fig. 2 show that the maximum production *of* neutral protease (7.8 U ml^{-1}) was achieved when fermentation was carried out at 35°C. Below and above this temperature, enzyme biosynthesis was decreased.

The incubation temperature is one of the important parameters in the growth of organism and production of enzymes. Temperature affects the growth of the organism as well as activity of the enzyme produced. Higher temperature may damage the structure of enzyme, hence lowers the rate of growth of the organism. Some other workers have also reported the production of protease at the same temperature (Kassem and Lasztity, 1995; Qadeer *et al.*, 1990).

Optimum pH

For the selection of optimum pH for maximum enzyme production by *Bacillus subtilis* IH-16, the fermentation was carried out at varying levels of pH

such as 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0. It was found that the best initial pH for the growth *of Bacillus subtilis* IH-16 and maximum production of neutral protease (8.0 Uml^{-1}) was 7.0 (Fig.3).

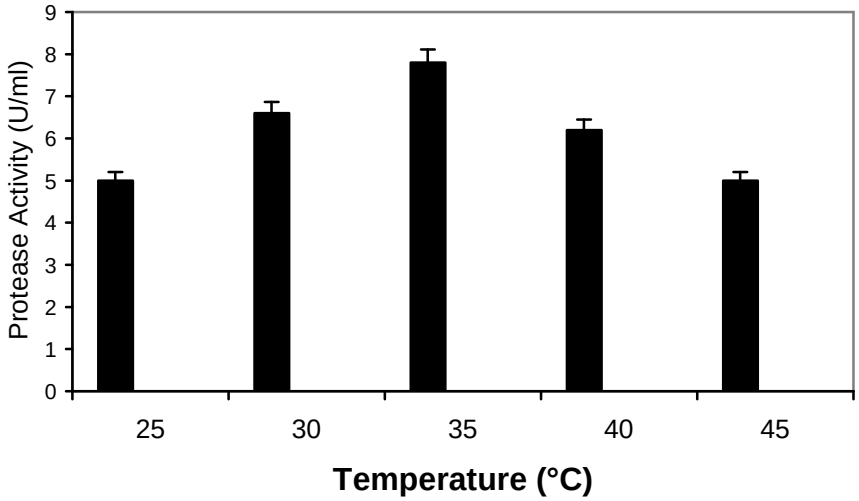


Fig. 2. Selection of optimum temperature for the production of neutral protease by *Bacillus subtilis* IH-16.

The maximum growth of the organism at pH 7.0 shows that the organism was a mesophile and the protease produced by *B. subtilis* IH -16 was a neutral protease. Change in pH of the medium results in the decreased growth of the organism and also affects the structure of the enzyme and substrate. It causes the ionization of substrate components that becomes unavailable for the organism. It may also causes the ionization of the active site of enzymes, which then show reduced activity (Prescott *et al.,* 1999). Many other *Bacillus* species have also been reported to produce proteases at pH 7.0 (Uehara *et al.,* 1974; Nakamura *et al.,* 1997).

Optimum period of fermentation

The period of fermentation for the production of neutral protease by *Bacillus subtilis* IH-16 was also optimised in the present studies. For that purpose, fermentation flasks were incubated for different time periods such as 12, 24, 36, 48 and 6.0 hours. The investigations revealed that the maximum production of neutral proteases (8.0 Uml^{-1}) was reached after 48 h of fermentation (Fig. 4).

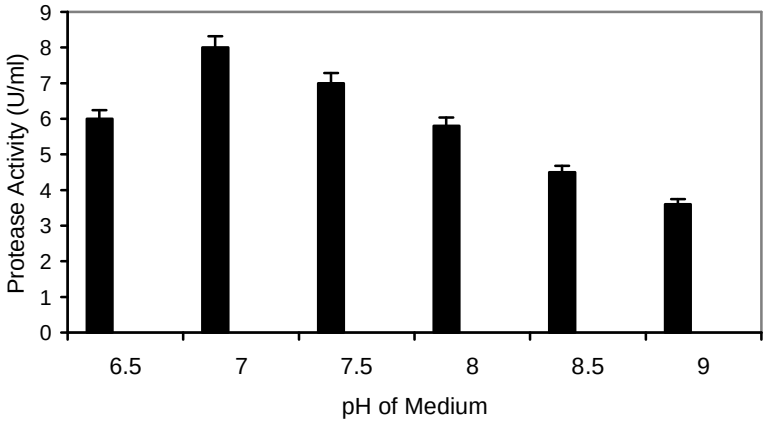


Fig. 3. Selection of optimum pH for the production of neutral protease by *Bacillus subtilis* IH-16.

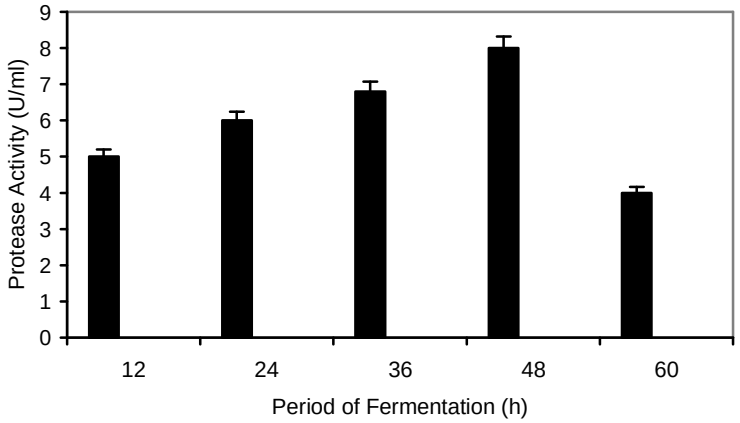


Fig. 4. Selection of period of fermentation for the production of neutral protease by *Bacillus subtilis* IH-16.

Protease production usually occurs at the late logarithmic to stationery phase of growth of the bacterium (Atalo and Gashe, 1993). Maximum production of protease occurred after 48 h of incubation when the organism was at late exponential and early stationery phase of growth. Beyond this period, the enzyme production was decreased which may be attributed to the autolysis of cells due to

decreased availability of nutrients. Our results are similar to many other workers who have also reported maximum production of proteases after 48 h of incubation (Haque *et al.,* 1990; Qadeer *et al.,* 1990).

REFERENCES

ADINARAYANA, K. AND ELLAIAH, P.J., 2002. Response surface optimization of the critical medium components for the production of alkaline protease by a newly isolated *Bacillus* sp. *J. Pharm. Pharmaceut. Sci.,* **5**: 272-278.

ATALO, K. AND GASHE, B.A., 1993. Protease production by a thermophilic *Bacillus* species (P-001A) which degrades various kinds of fibrous proteins. *Biotech. Lett.,* **15**: 1151-1156.

BEG, Q.K., SAXENA, R.K. AND GUPTA, R., 2002. De-repression and subsequent induction of protease synthesis by *Bacillus mojavensis* under fed-batch operations. *Process Biochem.,* 37: 1103-1156.

EIJSINK, V.G.H., VAN DENBURG, B., VRIEND, G. BARENDSEEN, H.J.C. AND VENEMA, G., 1991. Improving the thermostability of neutral protease of *Bacillus stearothermophilus* by replacing a burried aspargine by leucine. *Biochem. Int.,* **24**: 517 –525.

FELIX, R. AND VILLETAZ, J.C., 1983. Wine. In: *Industrial enzymology* (eds. T. Godfrey and J. Reichelt), pp. 410-421. MacMillan, London.

GUPTA, R., BEG, Q.K. AND LORENZ, P., 2002. Bacterial alkaline proteases: molecular approaches and industrial applications. *Appl. Microbiol. Biotechnol.,* **59**: 15-32.

HAMEED, A., KESHAVARZ, T. AND EVANS, C.S., 1999. Effect of oxygen tension and pH on the production of extracellular protease from a new isolate of *Bacillus subtilis* K2, for use in leather processing. *J. Chem. Technol. Biotechnol.,* **74**: 5-8.

HAQUE, H., NADEEM, B.A. AND QADEER, M.A., 1990. Biosynthesis of enzyme by solid state fermentation III. Production of protease by *Bacillus subtilis. Sci. Int.,* **2**: 31-34.

HARDY, F., VRIEND, G., VELTMAN, O.R., VAN DER VINNE, B., VENEMA, G. AND EIJSINK, V.G.H., I993. Stabilization of *Bacillus stearothermophilus* neutral protease by introduction of prolines. *FEBS Lett.,* **317**: 89-92.

JIANG, W. AND BOND, J.S., 1992. Families of metalloendopeptidases and their relationship. *FEBS Lett.,* **312**: 110-114.

KALISZ, M.H., 1988. MicrobiaL proteinases. *Adv. Biochem. Eng. Biotechnol.,* **36**: 17-55.

KASSEM, AE. AND LASZTITY, R., 1995. Production of neutral protease by *Serratia marscens* using rice bran. *Per. Pol. Chem. Eng.,* **39**: 43-54.

KUBO, M., MURAYAMA, K., SETO, K. AND IMANAKA, T., 1988. Highly thermostable neutral protease from *Bacillus stearothermophilus. Ferment.Technol.,* **66**: 646-649.

MARKLAND, J.E. AND SMITH, E.L., 1971. *Subtilisins: primary structure, chemical and physical properties* (ed. P.D. Boyer). The Enzymes, 3rd, New York: Academic press, pp. 561-608.

McDONALD, C.E. AND CHEN, L.L., 1965. Lowry modification of the Folin reagent for determination of proteinase activity. *Annls. Biochem.,* **10**: 175.

MOHAMED, A. N., ISMAIL, A-M.S., AHMED, S.A. AND AHMED F., ABDEL FATTAH, 1998. Production and Immobilization of alkaline protease from *Bacillus Mycoides. Biores.*

Technol., **64**: 205-210.

NAKAMURA, S., TANAKA, T., YADA, RY. AND NAKAI, S., 1997. Improving the thermostability of *Bacillus stearothermophilus* neutral protease by introducing proline into the active site helix. *Protein Engin.*, **10**: 1263-1269.

PAUPTIT, R.A., KARLSSON, R, PICOT, D., JENKINS, J.A., NIKLAUS, J.A., REIMER, AS. AND JANSONIUS, J.N., 1988. Crystal structure of neutral protease from *Bacillus cereus* refined at 3.0A° resolution and comparison with the homologus but more thermostable enzyme thermolysine. *J. mol. BioI.*, **199**: 525-537.

PRESCOTT, L.M., HARLEY, J.P. AND KLEIN, D.A., 1999. *Microbiology*, Fourth Edition, The McGraw-Hill Companies, Inc. USA, P: 125-128.

PRIEST, F.G., 1977. Extracellular enzyme synthesis in the genus *Bacillus. Bact. Rev.*, **41**: 711-753.

PURI, S., BEG, Q.R. AND GUPTA, R., 2002. Optimization of alkaline protease production from *Bacillus* species using response 'surface methodology. *Curr. Microbiol.*, **44**: 286-290.

QADEER, M.A., RAHMAN, M., IQBAL, J. AND AHMAD, A., 1990. Studies on the production of extracellular proteolytic enzymes by *Bacillus subtilis. J. Pure Appl. Sci.*, **1**: 11-17.

RAO, M.B., TANKSALE A.M., GHATGE, M.S. AND DESHPANDE, V.V., 1998. Molecular anc biotechnological aspects of microbial proteases. *Microbiol. Mol. BioI. Rev.*, **62**: 597-635.

STARK, W., PAUPTIT, R.A., WILSON, K.S. AND JANSONIUS, J.N., 1992. Properties of bacterial neutral proteases. *Eur. J Biochem.*, **207**: 781-791.

UEHARA, H., YONEDA, Y, YAMANE, K. AND MAMO, B., 1974. Regulation of neutral protease productivity in *Bacillus subtilis:* Transformation of high protease productivity. *J. Bact.*, **19**: 82-91.

VARELA, H., FERRARI, M.D., BELOBRADJIC, L., WEYRAUCH, RAND LOPERANA, M.L., 1996. Effecl of medium composition on the production by a new *Bacillus subtilis* isolate oj protease with promising unhairing activity. *World J. Microbiol. Biotechnol.*, **12**: 643-645.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 77-84, 2004.

PROGRESSIVE CHANGES IN THE BLOOD CELLS OF *CHANNA PUNCTATUS* INFECTED WITH EPIZOOTIC ULCERATIVE SYNDROME

AKHTARUNNESSA CHOWDHURY AND ASMA HUSSAIN
Department of Zoology, University of Dhaka, Bangladesh

Abstract.- This study was aimed at studying haematological changes in a population of *Channa punctatus* living under wild conditions with an object to elucidate the role of different blood cell types in EUS infected fish and to identify critical point of epidemic state from morphological change and deviation from the normogram . Dermatitis in 25% infected fish indicated early stages of lesions whereas smaller lesions with haemorrehgic broader and deep patch with fungal hyphae in 9% indicated the late stage. Large lymphocytes showed lymphocytosis– a leukemic condition in diseased fish. All parameters of diseased fish except lymphocytes, Mean Corpuscular Volume (52.83%) and Mean Corpuscular Haemoglobin (59.63%) showed higher fluctuation rates. Lower Mean Corpuscular Volume and Mean Corpuscular Haemoglobin with normal Mean Corpuscular Haemoglobin concentration results in normochromic increase in erythrocyte size showed anisocytosis which is the first indication of anaemic stage of the cells. This further amplified by poikilocytosis resulting in macrocytic state of the cell.

Key words: EUS epidemic, anisocytosis, macrocytic, lymphocytosis.

INTRODUCTION

Ulcerative disease syndrome (UDC) is a disease characterized by large cutaneous lesions. These lesions of wild and cultured fresh water fishes periodically result in death of fish populations. The Epizootic Ulcerative Syndrome (EUS) is the most important disease in the Indo-Pacific region. Use of the term syndrome highlights the complexity of the condition, involving the interaction of a specific monoclonal fungus, a wide variety of environmental factors and a range of secondary invading pathogens, which differ with each outbreak. It is now recognized to be synonymous with the condition Mycotic Granuloma (MG) first described from Japan 1971 and Red Spot Disease (RSD) described from Australia in 1972 (Chinabut and Roberts, 1999).

First incident of Ulcerative Syndrome was first reported from Chandpur area of Bangladesh in February in 1988 and continued to make it epidemic appearance till 1999, destroying as many as 31 fresh water fish species of

Bangladesh (Barua, 1989). Worse affected group among these was the Channaede fishes, which have five species. Of this *Channa punctatus* was found surviving much more severe, chronic lesions. Disease and parasites of fishes constitute one of the most important problems confronting the modern fish culturists. Intensive culture system with high stocking densities, poor water qualities encounter much of disease problems. The disease outbreak may occur from a variety of sources. Whatever the sources may be these factors provide a congenial atmosphere for rapid multiplication of these pathogens.

Blood is an important diagnostic indicator of disease in fish (McCarthy *et al.*, 1973). This study was aimed at evaluating haematological changes in *Channa punctatus* infected with EUS living under normal wild conditions.

MATERIALS AND METHODS

A total of 135 *Channa punctatus,* 11.14 - 16.25cm long and 46.65 - 63.5gm, were collected form five markets of Dhaka city and acclimatized them for 48 hours before experimentation. Forty six fishes were found to have ulceration.

Blood samples were collected by veno-puncture following Blaxhall and Daisly (1973). EDTA was used as anticoagulant. Erythrocytic and leucocytic counts were done by a standard clinical method (Improved Neubaur rulling haemocytometer) using Daice's fluid with brilliant cresyl blue, a modification suggested by Hesser (1960). For differential count of the leucocytes, blood smears were stained (Klontz, 1972) with Giemsa and Leishmen stain and the cells were counted with the help of a leucocytometer (Digital 9 key Erma Inc.). Identifications of blood cells were done following Mahajan and Dheer (1979) and Ellis (1977). Cyanmethaemoglobin method was used for the haemoglobin estimation (Rahman and Begum, 1992). Erythrocyte sedimentation rates were determined by Wintrobe method using microhaematocrit tube (1.1-1.2mm, internal diameter and 75mm length) and Paul Hellar reagent as suggested by Blaxhall and Daislay (1973). MCV, MCH and MCHC values were calculated from the erythrocyte count, haemoglobin, ESR and haematocrite values (Khalaque, 1987).

RESULTS

Morphological, behavioral and haematological changes in *Channa punctatus* caused by epizootic ulcerative syndrome infection showed

morphological changes including partial necrosis of fin tissue, scale loss and loss of pigmentation, together with haematological changes

Early stages of the lesions with dermatitis were observed in 25% of the fish (34±2.5) with a 2.6-5.3mm diameter patch (Fig. 1). A decreased patch of 2.4mm diameter with 2-4mm depth was the characteristic of 9% of the fish (13±1.02) in the late stage of lesions (Fig. 2). Although some fin erosion was observed, but there was no fish with total caudal fin erosion.

Fig. 1. Showing primary dermatitis of the head region and deep patch of lesion in *C. punctatus.*

Fig. 2 Showing late haemorrhagic lesion of the caudal peduncle of *C. punctatus.*

The total erythrocyte count in diseased fish showed a wider range (3.35-4.30 $x10^6mm^{-3}$) than the healthy fishes (3.03-3.55 $x10^6mm^{-3)}$, but the mean values were closely similar. Morphology of the erythrocytes was distinct by thin cell wall and peripheral shifting of nucleus. Other cellular parameters like total leucocytes (68.88±6.4 $x10^3mm^{-3}$) and differential count of W.B.C like thrombocytes (5.96±0.24 $x10^3mm^{-3}$), monocytes (6.65±0.12 $x10^3mm^{-3}$), neutrophils (14.61±1.02 $x10^3mm^{-3}$), eosinophils (2.36±0.15 $x10^3mm^{-3}$) and basophils (2.21±0.50) of healthy *Channa punctatus* showed higher means than the diseased fish. The increases in the number of lymphocytes (37.50±2.69 $x10^3mm^{-3}$) was conspicuous in diseased fish (Table I). Similar morphological changes like erythrocyte were also observed in some varieties of leukocytes.

All of the eosinophils, some neutrophils and one or two thrombocytes retained their morphological structure in diseased fish throughtout the infection (Fig. 3C). The fluctuation rates of all the cellular parameters except the large lymphocytes (8.57%) were higher in diseased fish, highest among those were basophils (161.29%) (Table I).

Erythrocytic characteristic like haemoglobin concentration of both diseased and healthy *Channa punctatusa* have the same means (11.46±0.71 g 100 mm^{-1} and 11.26±1.16 g 100 mm^{-1}) but the haematocrit value (44.61±1.79%) and ESR (6.44±0.27 mm/h) of healthy *Channa punctatus* were higher than the diseased fish. Fluctuation rates of all the three parameters were higher in diseased fish (Table I).

The diseased fish became anaemic through loss of blood as shown by their lethargic movement and decreased feeding activities. Anisocytosis which is increased erythrocyte size (0.05μ-0.06μ) with thinning of the cell wall and shifting of the nucleus towards the periphery of the erythrocyte is observed in early stages of lesions (Fig. 3A). This became more conspicuous with the change in the shape of erythrocytes-the poikilocytosis of the RBC (Fig. 3B), resulted in macrocytic condition.

MCV (12.00±1.72fl.) and MCH (30.77±5.0pg.) values of the healthy *Channa punctatus* showed higher values and higher fluctuation rates in (52.8% and 59.63%) MCHC (52.88±1.50 and 21.67±2.24) were the same for both the healthy and diseased fish with higher fluctuation in diseased fish (Table I).

Table I

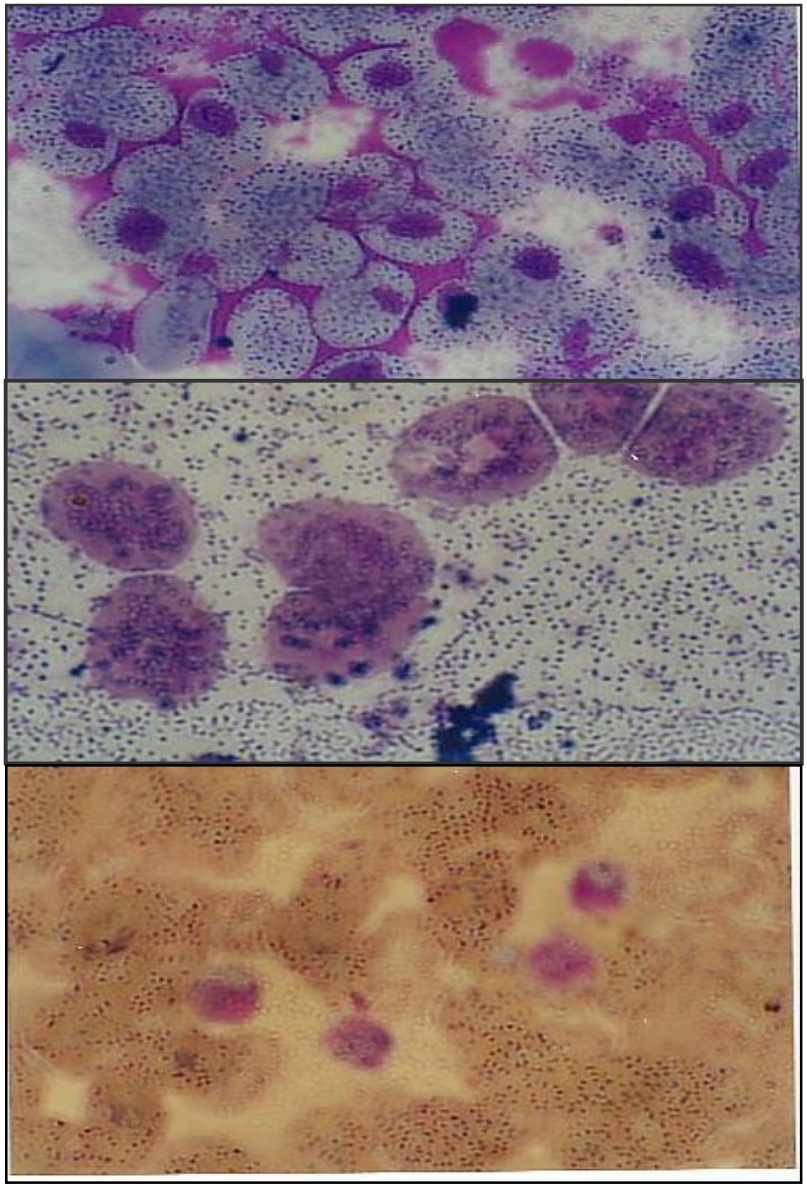


Fig. 3. Blood cells of diseased C*hanna punctatus* showing anisocytosis of the RBC (A), poikilocytosis of the erythrocytes (B), and poikilocytosis of the erythrocytes with unchanged monocytes neutrophils and thrombocytes (C).

DISCUSSION

The RBC, WBC counts and differential count of WBC, haemoglobin concentration, haematocrit values, haematological absolute values comparable to the values reported for other airbreething teleosts *Anabus testudineus* (Banerjee, 1966; Dube and Munshi, 1973) and *Amphipaness cuchia* (Sreevastava *et al*., 1979). The higher fluctuation rates of the parameters indicate the stress susceptibility of the diseased fishes, which may be the cause of their infestation by fungus and bacteria. Morphological changes of 25% infected fish indicating early stages of lesions, while in late stage 9% fish with smaller lesions with hemorrhagic broader and deep patch may have facilitated the invasion of fungal hypae. While complete fin erosions were reported by others, no complete erosion was observed in this investigation.

Of the 15 blood parameters, 10 showed higher mean values, 4 showed the same means and only large lymphocyte showed the increased count (37.58±2.69) in diseased fish resulting in lymphocytosis - a leukemic condition. Other leukocytes did not show any deviation.

Erythrocytic characteristic like haemoglobin, haematocrit and erythrocyte sedimentation rate showed higher mean in healthy fish but higher fluctuation rates were the characteristic features of the diseased fish (Table I).

The means of the haematological absolute values, MCHC, MCV and MCH were also higher in healthy fish but MCV (52.83%) and MCH (59.63%) were with higher fluctuation rate of the diseased fish indicate the normochromic state of the erythrocytic cells (Khalaque, 1987).

Increase in erythrocyte size from .05μ to .07μ indicate the macrocytic condition of the cell, which is the first indication of anaemic stage of the cell (Khalaque, 1987). This is further worsened by poikilocytosis. The macrocytic state when coupled with normocromic condition result in macrocytic normochromic anaemia.

To concluded, macrocytic normochromic anaemia and leukemic condition along with high basophilic fluctuation rate could be the diagnostic tool for epizootic ulcerative syndrome. Anisocytosis in early lesion and poikilocytosis in late stage may be of help to identify the critical point of the disease. Anaemia is often associated with chronic disease or infection and may represent a stage precursor to epidemic.

REFERENCES

BARUA, G., 1989.Epizootic ulcerative syndrome- A fish disease of major concern in Bangladesh. First workshop on ulcerative fish disease and environment 20-24th March,1989, Bangladesh & Thailand, pp.16.

BLAXHALL, P.C. AND DAISLY, K.W.,1973.Routine haematological methods for use with fish blood. *J. Fish Biol*., **5**: 771-781.

BANERHEE, V., 1966. A note on the haematological studies of *Anabus testudeneus* (Bloch). *Sci. Cult*., **32**: 326-327.

CHINABUT, S. AND ROBERTS, J.R., 1999.*Pathology and histopathology of Epizootic Ulcerative Syndrome (EUS)*. AAHRI, DOF, Bangkok, Thailand. 1999. pp.33.

DUBE, S.C. AND MUNSHI, J.S.D., 1973. A quantitative study of the erythrocyte and haemoglobin in the blood of air breathing fish *Anabus testudeneus* (Bloch) in relation to its body size. *Folia Hematol*., **100**: 436-446.

ELLIS, A.E., 1977. The leucocytes of fishes. A review. *J. Fish Biol*., **11**: 435-490.

HESSER, E.F., 1960. Methods for routine fish haematology. *Prog. Fish Cult*., **.22**: 164-170.

KHALAQUE, K.A., 1987. *Practical haematology*. 7th ed. Polwell Printing Press. Dhaka, Bangladesh.

KLONTZ, G.W., 1972. Haematological techniques and immune response in rainbow trout. *Sym. Zool. Soc. London*, **30**: 89-99

MAHAJAN, C.L. AND DHEER, J.S., 1979. Cell types in the peripheral blood of an air breething fish *Channa punctatus. J. Fish Biol*., **14**: 481-487.

MCCARTHY, D. H., ROBERTSON, J. P. AND ROBERTS, M.S., 1973. Some blood parameters of the rainbow trout. 1. The Kamploops variety. *J. Fish Biol*., **5**: 1-8.

RAHMAN, T. AND BEGUM, H.A., 1988. *Baboharic Rogatutta (Practical pathoilogy.)* Palton Press, Dhaka, Bangladesh.

SRIVASTAVA, A.K., 1968. Studies on the hematology of certain freshwater teleost. I, Erythrocytes. *Anat. Anz.*, **124**: 233-249.

TABLE I.- BLOOD CELLS COUNTS HAEMOGLOBIN CONCENTRATION, HAEMATOCRIT, ESR AND VARIOUS HAEMATOLOGICAL INDICES OF IN HEALTHY AND DISEASED *CHANNA PUNCTATUS.*

Parameters	**Healthy *Channa punctatus***		**Diseased *Channa punctatus***	
	Range	**Mean**	**Range**	**Mean**
Blood cells counts				
Eythrocytes (x 10^3mm^{-6})	3.03-3.55	3.35±0.17	3.35-4.30	3.56±0.24
Leucocytes (x 10^3mm^{-3})	60.50-79.90	68.88±6.4	55.78-73.59	63.32±6.74
Thrombocytes (x 10^3mm^{-3})	6.08-6.66	5.96±0.24	4.0-5.0	4.40±0.24
Large Lymphocytes (x 10^3mm^{-3})	32.6-39.66	33.68±2.10	36.26-46.0	37.58±2.69
Small Lymphocytes (x 10^3mm^{-3})	28.37-30.1	28.83±0.49	26.91-33.50	28.17±1.69
Monocytes (x 10^3mm^{-3})	6.43-7.0	6.65±0.12	3.15-3.56	3.39±0.19
Neutrophils (x 10^3mm^{-3})	14.21-18.0	14.61±1.02	8.37-15.5	9.74±1.88
Eosinophils (x 10^3mm^{-3})	3.2-2.6	2.36±0.15	1.71-2.5	1.93±0.28
Basophils (x 10^3mm^{-3})	1.65-3.33	2.12±0.50	0.0-3.0	1.83±0.43
Haemoglobin (g $100mm^{-1}$)	10.97-13.66	11.46±0.71	10.69-15.00	11.26±1.16
Haematocrit (%)	43.79-51.00	44.61±1.79	40.46-51.50	42.01±2.90
ESR (mm/h)	6.27-7.33	6.44±0.27	5.84-7.00	6.2±0.31
Haematological indices				
MCHC (%)	21.01-26.6	21.88±1.51	20.58-28.85	21.69±2.24
MCV (fl.)	10.69-17.03	12.00±1.71	8.91-11.95	9.71±0.79
MCH (pg.)	27.08-45.43	30.77±5.0	23.59-34.60	25.80±2.96

Proc. Pakistan Congr. Zool., Vol. 24, pp. 85-98, 2004.

SOME OBSERVATIONS ON THE ANIMALS AT CLIFTON BEACH, KARACHI AFTER THE OIL SPILL FROM TASMAN SPIRIT

Q.B. KAZMI AND R. NAUSHABA
Marine Reference Collection & Resource Centre, University of Karachi.
Karachi-75270, Pakistan
E-mail: qbkazmi@mrcrc.ku.ed.pk razianaushaba@yahoo.com

Abstract.- The Clifton beach at Karachi was monitored regularly for 37 days after an oil spill from the ship Tasman Spirit in 2003. The monitoring continued until April 2004. Live and dead specimens were collected from an area of approximately 200 meters in length. A large number of animal groups were collected from the area during the study, but *Dotilla blanfordi*, a scopomerine ocypodid crab, was specially noted for its abundance, habit and survival. The effects of oil on marine life, with particular emphasis on *Dotilla blanfordi*, are described in this paper.

Key words: Tasman spirit, oil spill, Clifton beach, *Dotilla*, Ocypodid crab, leucosid crab.

INTRODUCTION

It cannot be overemphasized that oil in the sea from anthropogenic sources has been always a major environmental problem, particularly from major oil spills like the recent Tasman Spirit. This spill received considerable public attention, because of the obvious attendant environmental damage, including the oil-coated shorelines of Pakistan at Clifton (Fig. 1A) and dead or moribund marine life including the propagules of mangroves plants.

This paper focuses on the results of a preliminary investigation made after the spill on the Clifton beach with the more emphasis on two ocypodid crab species during the course of the study of the oil-affected animals. Of the macrofauna, which included several groups of invertebrates and vertebrates, both living and dead, the crabs and fish were singled out for special attention because of their visibility in oil and sand. Among the recovered animal life, the two ocypodid crabs were observed alive after six days of exposure to oil spill. The first was a solitary specimen of a large ghost crab of the genus *Ocypode*, the other species was a population of small soldier crabs of *Dotilla blanfordi*. The oiled and sheened sediments were colonized by natural populations of *Dotilla* crabs which are characteristics of sand flats. A note on other animals is included.

Fig. 1. A, Location of stranded oil tanker Tasman spirit (Courtesy WWF-Pakistan) B, Oil slick on the surface of water and sand as “Chocolate mouse”; C, study site at Clifton beach.

The Marine Reference Collection & Resource Centre (MRC), University of Karachi since its inception in 1968, has been surveying the Clifton Beach and its marine fauna along with the other coastal areas. The beach is 8 km long, has a gentle slope (1-6°), low wave action, and the sediment characteristics are: sand largely reworked sand and silt of the Indus, rich in mica flakes with carbonate content of 7% and 8.2% heavy minerals (Ibrahim *et al.*, 1992). The primary productivity is 1.5-1.8gC/m^2 day. The food web can be summarized as having a number of pathways of energy flow generated primarily from a detrital base. The animals depend on phytoplankton and organic debris brought in by waves and on predation for food.

Brachyuran crustaceans in general, and particularly of two groups, the ocypodids and leucosid crabs of genus *Philyra*, are important elements of the intertidal sand flats at Clifton. Quantitative data on the intertidal crab fauna of Pakistani coasts are not available.

The beach like other intertidal areas worldwide is subjected to considerable anthropogenic influences that range from direct physical disturbance to indirect biological and chemical disturbances. This has led to a loss of biodiversity, adverse effect in the standing stocks of resources and alternation of ecological processes. This was noted while comparing the unpublished old and new entries of catalogued collection at MRC depository.

Soldier crab fact file

The populations of *Dotilla blanfordi* (Fig. 2A) are characteristic of the middle levels of tidal sand flats. The crabs are allied to fiddler crabs *(Uca)* and are called soldier crabs or dhobi crabs. These small crabs measuring four mm in carapace length occur in enormous numbers on sand, which is literally riddled by their burrows. They seem to emerge spontaneously from their burrows, their emergence seems to be triggered by the falling tide. Like other ocypodid crabs, *Dotilla* shows a range of morphological and behavioural adaptations to intertidal life (Ansell, 1989; Pereira and Goncalves, 2002). The crabs are air-breathers and need areas that regularly are not submerged to provide time for feeding (Gherardi *et al.*, 1999). These crabs are surface deposit-feeders - an adaptation similar to that of other ocypodid crabs. Water, originally stored in the gill chambers, is used to separate suspended organic material from sand. As surface-deposit feeders they need areas that are regularly covered by the tide to renew the surface food supply. These crabs make temporary burrows in sand when tide rises too high. From mouthfuls of sand the crabs extract the organic particles (mostly diatom

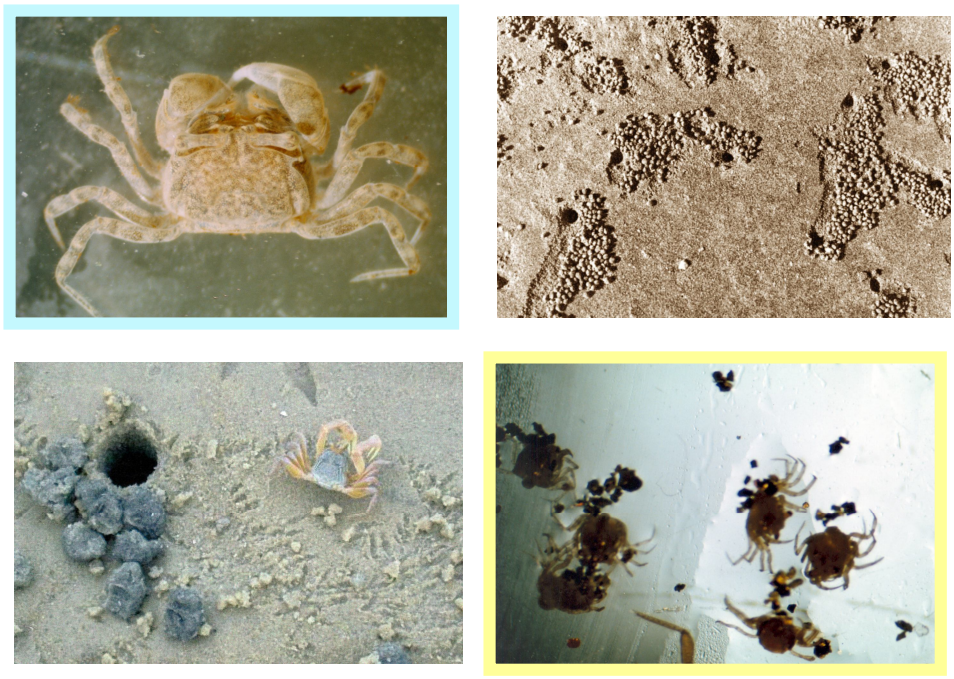


Fig. 2. A, Adult *Dotilla blanfordi*; B, Pseudofeacal pellets in radiating manner; C, A closer view of entrance of burrow and pseudofeacal pellets; D, Megalopae and juveniles of *Dotilla blanfordi*.

cells, but also bacteria, protozoans, blue green algae, nematodes and detritus) by flushing them out with a stream of water and discard the clean sand in star-like radiating patterns as compact, spherical "pseudofaecal" pellets (Fig. 2B,C) around the entrances to their burrows, the patterns differing among species of *Dotilla.* The burrows may be 3.9 cm in diameter (Rajabai, 1960) and 5-10 cm in depth (Kaestner, 1970); the digging is made obliquely from 6-8" (Kemp, 1915) with a corkscrew motion. Burrowing is more pronounced in larger individuals. Juveniles do not excavate burrows. The burrows provide refuges from disturbance, predation and thermal extremes. There are two breeding seasons. The first is in the spring to summer (Tirmizi *et al.*, 1993; Tirmizi and Ghani, 1996) and the second in winter (Tirmizi and Siddiqui, 1990) in the Arabian Sea. Zoeal stages have been reported from open sea samples by Tirmizi *et al.* (1993) during Dr. Fridtjof Nansen Cruise, 1977 (St. 32, 15, 16 and 45) as well as from inshore waters (Ghory, 2001). Megalopae have been reported from the Manora

Channel by Tirmizi and Siddiqui (1993). The present study provided an opportunity to observe the megalopae and crab instars (Fig. 2D) in August 2003 and April 2004 at Clifton. There is evidence that recruitment follows a lunar periodicity (Paula and Dray, 1995) and present study.

Dotilla crabs, because of their abundance and burrowing habits, play a major role in the ecology of sandy shores, as they are responsible for rapid sediment turnovers. Additionally, they constitute an important prey item for the local bird communities. Seventy species of water birds have been reported from the area (Hasnain and Ghalib, 1998). Migratory water birds also come here in August.

MATERIALS AND METHODS

Under the directives of Federal Ministry of Environment, the Sindh Environmental Protection Agency conducted an assessment study on the impact of oil spill from Tasman spirit on Clifton beach. A committee was formulated where Marine Reference Collection & Resource Centre also gave input.

In all, nine trips in 37 days (19 Aug. - 27 Sep. 03) and five trips from January to April 2004 were made to collect and study samples. The collection site (Fig. 1C) was an area about 200 meters long at Clifton beach where a 4-5 cm thick deposit of oil on sand was noticed, after the oil spilled from the Tasman Spirit on 13 Aug. 2003 [near the Sea View residential area next to Marine Point building on the beach of Clifton. The dead macrofauna was picked with the large forceps, placed in polythene bags, and brought to laboratory where the animals were placed in a tub and washed thoroughly to remove the oil. For some samples detergent was required to remove the oil. Because oil sedimentation was seen 45 cm deep in the sand (Fig. 3A). Sand samples were collected for interstitial organisms using a metallic core (5cm diameter) and the samples were stored in containers. The animals were sieved from the sand in the laboratory.

The burrows of soldier crabs in a one square meter quadrate near the low water mark were counted at the point where they were abundant. Each burrow was considered one individual. But the actual number of individuals was higher since juveniles were also associated with adult burrows. The burrowing crabs were picked out using a shovel, stored in a bag and brought to the laboratory to determine the developmental stages and sex.

Fig. 3. A, Pit dug to show oil sedimentation; B, Assemblage of dead invertebrate fauna; C, Dead eel; D, Dead ray.

RESULTS AND DISCUSSION

The known impact of oil on marine organisms is either direct killing or the fitness is reduced through sublethal affects, which may include impairment of feeding mechanisms, growth rate, differential growth of body parts (deformity), occasional anomalies in development of organs, energetic, reproductive output, recruitment rates, increased susceptibility to disease and histopathological disorder (Capuzzo, 1987). The spill from Tasman Spirit at Clifton has not virtually extinguished life but disturbed structure and function of communities and ecosystem can be expected.

Sublethal effects on Dotilla blanfordi

It cannot be said with certainty that the malformations, genetic damage, mortality, decrease in size at hatching and impaired swimming occurred or not since we stopped the survey work after this preliminary investigation. No baseline data were available except the megalopae (CL. 1.36 mm) of *Dotilla* of pre-oil spill period (1994) were available for comparison. When compared from the samples of 2004, few malformations in the mouths parts were observed. These are in the form of rasps on various segments of the mandibles, maxilla and maxilliped, which are not seen in earlier samples of 1994 (Fig. 4).

Recovery of Dotilla blanfordi

Some 128 specimens were counted in one square meter in the year 2003 in one sample. In addition to adult male crabs, berried females, both megalopae and juveniles were present. All specimens were oil soaked. The settlement of larvae of *Dotilla blanfordi* at Clifton beach after the oil spill, their survival and abundance seemed to be dependent on or could be explained by:

Weathering and emulsification of oil

Weathering seems to affect the surface oil where 25% is lost due to evaporation, the rest looses buoyancy and eventually sinks in water and sand. The crude oil itself does not readily penetrate the sand but emulsified oil, following the use of dispersal has been reported. This process changes the oil as "chocolate mouse" in viscous pancakes like patches of various sizes. We expect that these processes would have altered the toxicity of oil spill like they did as reported for previous oil spills (Krebs and Burns, 1977; Ho *et al.*, 1990; Apel, 1994; Jones *et al.*, 1996).

Planktonic larvae

Though early development stages of animals are especially vulnerable to hydrocarbon exposure more sensitive than their adults (Fingas, 2000), the larvae of most of the crustaceans are adaptively significant in that they occupy distinctly different niches from their parents. Some crabs have tendencies for high larval tolerance of physico-chemical stress. The planktonic larval stages of *Dotilla*, which move with tides into the open seawater that is oil free, are in better position for survival. Dense colonization of *Dotilla* after spill showed that the influx of planktonic larvae was not low and the environmental conditions were favourable for larval settlement.

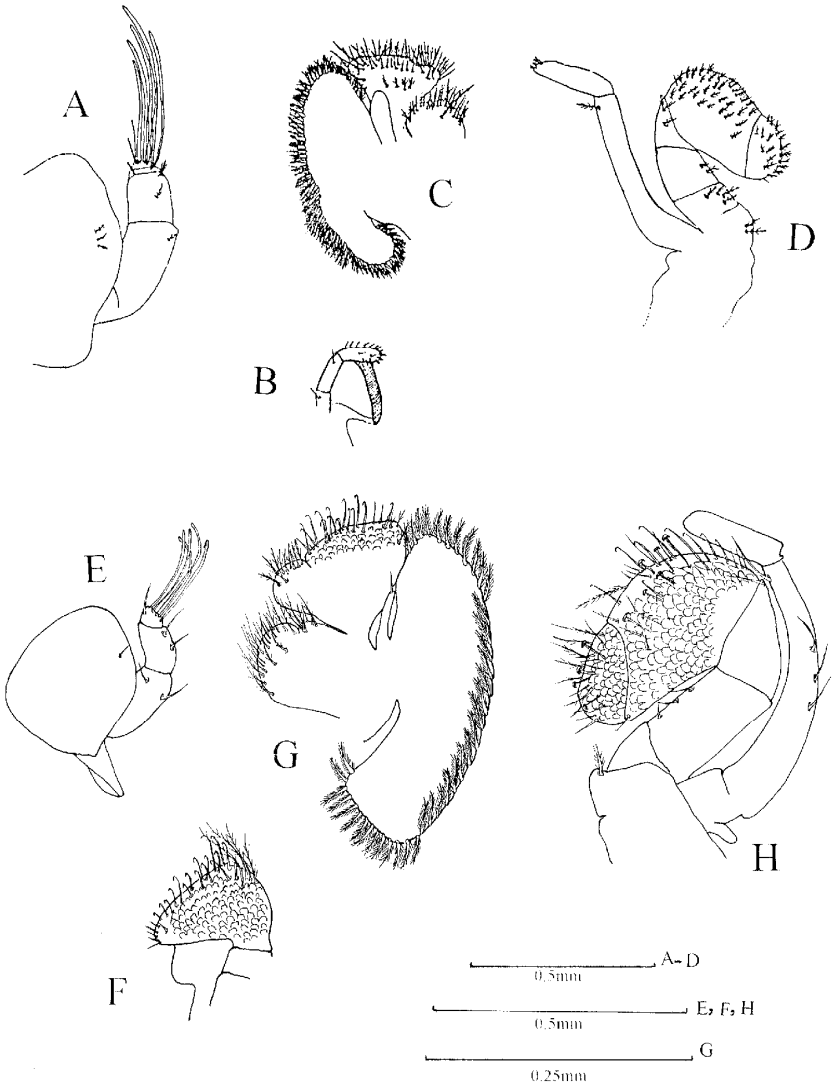


Fig. 4. *Dotilla blanfordi*, Malformations in mouth parts: A-D, normal appendages from megalopal stage (carapace length 1.36 mm) collected from Manora Channel (dated 22-10-1994); E-H, appendages with rasp developed from megalopa stage (carapace length 1.56 mm) collected from Clifton (dated 30-8-2003). A, E antennutes; B, F mandibles; C, G maxillae; D, H maxillipeds II.

Migration and burrowing habit

The genus has an ability to 'switch' modes of activity that allow responses to both the predictable and the present unpredictable elements of the intertidal environment .The well-reported migrating behaviour of *Dotilla* (Macnae and Kalk, 1962; Hartnoll, 1973; Fishelson, 1983; Branch *et al.,* 1995), following tidal movements would have helped in avoiding oil on the surface of water.

The intertidal area of Clifton is already affected by pollution of all sorts as expected for one of the beaches of a mega city like Karachi. The burrowing activities of the soldier crabs turnover the sediments and may transport all pollutants in elements in to the overlying water. (AhsanuIIah and Ying, 1993). The ability of *Dotilla blanfordi* to switch modes of activity can be suggestive as its mode of survival in chronic pollution further intensified by the oil spill.

Highly nutritive sand and feeding habits

The presence of mica in the Clifton sand and organic material seems to be helping in survival. The structure of setae on second maxiIIipeds in the genus *Dotilla* indicates this value, they are developed in *D. blanfordi* in a manner which can retain organic and silt particles in a better way as compared to other ocypodid (Vogel, 1984).

High wave action

This may be an effective phenomenon for cleansing of the beach according to Fatima and Moazzam (2004). We did not observe this ourselves.

Effective cleanup measures

A quick recovery of certain populations living in upper layers of sand could be due to the reason that the coastal authorities did not use very intrusive techniques.

Lack of predation by coastal birds

The birds avoided the area due to oil which might be another aspect that could have contributed to the density of crabs. No baseline studies are available to compare. However, there were kites on the ground in large number, probably to feed on dead fish.

Biodegradation

Indigenous bacteria could have biodegraded 40% - 80% of oil that led to recovery.

Observations on Ocypode *sp.*

The other live species of crab, the ghost crab (*Ocypode*), was spotted on one of the visits, coming from the sea to the land. The bioavailability of petroleum was noticed in the form of surface coating of oil, but the extent of bioaccumulation by the crab was not studied further. It is already known that an ocypodid crab can survive for as long as six weeks without renewal of cleaner water, which it stores in its gill cavity and it can metabolize hydrocarbons that enter its body through water and food by converting them into soluble derivatives. In severe oiling, depuration can take months (Lee *et al*., 1976) and in clean seawater a rapid decline in the naphthalene may occur in tissues.

Note on dead macrofauna

Invertebrates (Fig. 3B)

The major chunk of soiled sand piled up at the effected site consisted of rotting and thick smothered majid spider crabs, *Doclea*, which are benthic, slow moving and mud dwellers, thus indicating that oil settled on the bottom in the subtidal region. There were gastropods, hermit crabs, pistol shrimps, and penaeid shrimps (in dying conditions due to narcosis) and a few live polychaetes, starfish, mole crabs, mud crabs, porpitas, seapens, insects and barnacles. The gastropods both empty and dead were abundant. Since the oxygen deficient water is commonly observed in summer and may cause mortality and effect on population structure of benthic organisms and form death sphere of organisms. Perhaps it has not been documented for the Arabian Sea but some insight may be derived from Shimoyama and Hamano (l988) who have given data for Hakata Bay, Japan. The appearance of oxygen deficient water result in the production of large amount of empty shells.

Vertebrates (Fig. 3C, D)

A number of shallow water fish as such as skates, rays, eels, mugils, ladyfish, catfish, flatfish, tripods were also among the deads. A loss of 500-600kg was estimated. Restoration of this loss is expected after the toxic material biodegrades. One dead turtle and porpoise were also observed (WWF-P, 2003).

Interstitial fauna

The oil that sedimented was expected to be effecting the benthic meiofauna. These tiny invertebrates are an important food source for fish, which thereby pass contamination up through food chain. The effect of oil contamination on the food chain required a thorough study. However during preliminary investigations in the laboratory few nematodes, ostracodes, archiannelids and copepods were found from the samples taken at near surface depth. The number of meiobenthic groups in intertidal sand is generally high in pre-monsoon pre-spill period (unpublished data, 1998). Therefore, beach erosion due to summer monsoon could also be one of the reasons of low biodiversity. It can be pointed out here that the presence of *Dotilla*, which are efficient bioturbers of the uppermost few cm of the sand, can significantly affect the abundance of interstitial fauna like nematodes and harpacticoids. lt was interesting to note that the sand of pseudofeacal pellets of *Dotilla* had more harpacticoids than in the surface sand.

We can say that gaps still exist in our understanding of the effects of petroleum hydrocarbons in the oil on populations of marine organisms and ecosystems since the marine ecosystem is a complex multi-scale, spatial and temporal environment. We are challenged to detect change caused by oil in the sea and to assess the damage at the level of individuals, population communities and ecosystem. Assessing recovery after pollution is perhaps even more challenging than assessing initial damage (Report NRC, USA, 2003). The lack of baseline information on the intertidal crab communities made it more difficult to assess the effect of the Tasman Spirit oil spill at Clifton and to evaluate the recovery process.

Much research efforts like discoveries of the anoxic layer and water table depth determinations in these areas before environmental hazards as a result of contaminant input could be addressed adequately. There is much work to be done on the subject, especially if one takes into consideration the chronic and persistence spills in the area because transfer to marine biota and the human consumer, and toxicological effects on the ecosystem are dependant on the availability and persistence of these contaminants within benthic environment.

Suggestions for conservation

Soldier crabs as deposit feeders play a considerable role in the transfer of energy in food webs. Their burrowing behaviour can strongly affect the dynamics

of the sediment. Therefore, understanding the ecology of this group and its habits are fundamental for the protection and management of intertidal flats. For their spatial variability, heterogeneity of the surface of the beach sand is important. Therefore, reduction of this heterogeneity by human trampling, coastal development or by driving vehicles over a sand flat could effect their distribution, although they survived the perturbance due to oil. The oil spill might have been a blessing in disguise, as it added a new dimension to the task of establishing another marine sanctuary in our area, and that requires international action.

Recommendation

The use of a powder developed in 1994 by a private entrepreneur Reliable Business System (personal communications) in Karachi with the support of World Bank can be tried on crude oil as test in laboratory. The company claims that it can capture/gelatinize waste oil at room temperature after which it can be recycled or destroyed.

ACKNOWLEDGMENTS

The Sindh Environmental Protection Agency is acknowledged for financial support. Dr. Patsy McLaughlin of Shannon Point, Washington, USA has very kindly improved the text.

REFERENCES

AHSANULLAH, M. AND YING, Y., 1993. Tidal rhythm and accumulation of cadmium from water and sediment by soldier crabs. *Mar. Pollu. Bull.*, **26**: 20-23.

ANSELL, D.A., 1998. Migration or shelter? Behavioural options for deposit feeding crabs on tropical sandy shores. In: *Behavioral adoptions to intertidal life* (eds. G. Chelazzi and M. Vannini), pp. 15-26. Plenum Press, New York and London. [NATO ASI series).

APEL, M., 1994. Effect of 1991 Gulf war oil spill on the crab fauna of intertidal mudflats in the Western Arabian Gulf. *Courier Forsch-Inst. Senckenbrg*, **66**:40-46.

BRANCH, G.M., GRIFFITH, C.L., BRANCH, M.L. AND BECKLEY, L.E., 1995. *Two oceans - A guide to the marine life of Southern Africa*, David Philip, Cape Town, pp. 259.

CAPUZZO, J.M.,]987. Biological effect of petroleum hydrocarbons: As assessments from experimental results. In: *Long-term environmental effects of offshore oil and gas development* (eds. D.F. Boesch and N.N. Rabalais), pp. 343-410. Elseiver Appled Science, London.

FATIMA, M. AND MOAZZAM, N., 2004. A preliminary report on the effect of "Tasman Spirit" oil spill on intertidal and subtidal animals. In: *Marine environment, resource, pollution and*

management, 2004. CEMB, University of Karachi, Abstract.- 29.

FINGAS, M., 2000. Basics of oil spill cleanup. (2nd Ed.). In: *Environment Canada* (ed. J. Charles), pp. 182.

FISHELSON, L., 1983. Population ecology and biology of *Dotilla sulcata* (Crustacea, Ocypodidae) typical for sandy beaches of the Red Sea. In: *Sandy beaches as ecosystem* (eds. McLachlan and T. Erasmus), pp. 643-654. Dr. W. Junk Publishers, The Hague.

GHERARDI, F., RUSSO, S. AND ANYONA, D., 1999. Burrow-orientated activity in the ocypodid crab, *Dotilla fenestrata*, living in a mangrove swamp. *J. mar. bioI. Ass. UK.*, **79**: :281-293.

GHORY, F.S., 2001. *Occurrence and abundance of brachyuran larvae in the Manora channel* (Karachi, Pakistan). M.Phil. thesis, Marine Reference Collection and Resource Centre, University of Karachi.

HARTNOLL, R.G., 1973. Factors affecting the distribution and behaviour ofthe crab *Dotilla fenestrata* on East African shores. *Estuar. coast. mar. Sci*., **1**: 137-152.

HASNAIN, S.A. AND GHALIB, S.A., 1995. Waders of the Karachi coast. In: *The Arabian Sea, Living marine resources and the environment* (eds. M.F. Thompson and N.M. Tirmizi), pp. 107-110. Vanguard Books, Lahore.

HO, K., PATTON, L., LATIMER, J.S., PRUELL, R.J., PELLETIER, M., MCKINNY, R. AND JAYARAMAN, S., 1990. The chemistry and toxicity sediment affected by oil from the North Cape spilled into Rhode Island sound. *Mar. Pollut. Bull.*, **38**: 314-323.

IBRAHIM, N., MUHAMMAD, M.J. AND ZAIDI, S.M.S., 1992. Textural and compositional variation in beach sand along Karachi coast. *Pakistan J. mar. Sci*., **1**: 87-93.

JONES, D.A., WATT, I., WOODHOUSE, T.D. AND RICHMOND, M.D., 1996. Intertidal recovery in the Dawhat ad-Dafi and Dawhat al-Musallamiya region (Saudi Arabia) after Gulf war oil spill. *Courier Forsch-Inst. Senckenberg*, **166**: 27-33.

KAESTENER, A., 1970. *Invertebrate Zoology Crustacea 3*. Translated and adopted from German by W. Levi and Lorna R. Levi. Interscience Publishers, New York. pp. 523.

KEMP, S., 1915. Crustacea Decapoda. Fauna of Chilka Lake. *Mem. Indian Mus*., **5**: 199-325.

KREBS, C.T. AND BURNS, K.A., 1977. Long-term effects of an oil spill on populations of the salt marsh crab, *Uca pugnax. Science*, **197**: 484-487.

LEE, R.F., RYAN, C. AND NEUHAUSER, M.L., 1976. Fate of petroleum hydrocarbons taken up from food and water by the blue crab, *Callinectes aspidux. Mar. Biol*., **37**: 363-370.

MACNAE, W. AND KALK, M., 1962. The fauna and flora of sand flats at Inchaca Island, Mozambique. *J. Anim. Ecol*., **31**: 93-128.

NATIONAL RESEARCH COUNCIL, (U.S.), 2003. *Oil in the sea III: input, fates, and effects* (Committee on oil in the sea: Inputs, Fates and Effects). The National Academies Press, pp. 265.

PAULA, J. AND DRAY, T., 1995. Biological and ecological aspect of the population of the crab, *Dotilla fenestrata* (Hilgendrof, 1869) (Brachyura, Ocypodidae) in the tidal flats of Inhaca Island. In: *Interlinkages between Eastern African coastal ecosystem* (ed. M.A. Hemminga), pp. 254-263. EEC Contract N°ts3*-CT92-0114. Final Report.

PEREIRA, M.A.M. AND GONCALVES, P.M.B., 2000. A preliminary investigation of the effect of human physical disturbance on the ecology of the solider crab *Dotilla fenestrata*

(Crustacea, Ocypodidae) at Paria da Costa do Sol, Maputo. In: *Proceedings of the 11 National Conference on Coastal Zone Research* (ed. M.A.M. Pereira), Maputo, pp. 58-64.

RAJA BAI, K.G., 1960. Studies on the larval development of Brachyua. I. The early and post larval development of *Dotilla blanfordi* Alcock. *Ann. Mag. nat. Hist.*, **13**: 129-135.

SHIMOYAMA, S. AND HAMANO, T., 1988. The effect of oxygen-deficient water on the molluscan Thanatocoenosis in Hakata Bay. *Atti. Quart. Simpos. Ecol. Palecol. Comun. Bent.*, pp. 753-772.

TIRMIZI, N.M., SIDDIQUI, F.A. AND AMIR, N., 1993. Distribution of brachyuran larvae collected by R.V. Dr. Fridtjof Nansen, 1977 (Cruise 1,2) from coastal waters of Pakistan. In: *Proc. national seminar on the study and management in coastal zone in Pakistan* (eds. N.M. Tirmizi and Q.B. Kazmi), pp. 181-188. MRC and UNESCO, Karachi.

TIRMIZI, N.M. AND GHANI, N., 1996. Marine fauna of Pakistan: 5 Crustacea: Brachyura, Brachyrhyncha Part-I (Xanthidae, Goneplacidae, Pinnotheridae, Ocypodidae, Grapsidae). CEMB Publication, pp. 188.

TIRMIZI, N.M. AND SIDDIQUI, F.A., 1993. Developmental stages of *Dotilla blanfordi* Alcock (Crustacea, Decapoda: Ocypodidae). *Proc. Pakistan Congr. Zool*., **13**: 130.

VOGEL, F., 1984. Comparative and functional morphology of the spoon-tipped setae on the Ocypodidae). *Crustaceana*, **47**: 225-234.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 99-104, 2004.

REDESCRIPTION OF *CHRYSORABDIA VIRIDATA* WALKER (LEPIDOPTERA: ARCTIIDAE: LITHOSINAE) FROM PAKISTAN AND ITS CLADISTIC RELATIONSHIP

SYED KAMALUDDIN AND SAEEDA NARGIS VIQAR
Department of Zoology, Federal Urdu University of Arts, Sciences and Technology, Gulshan-e-Iqbal Campus (SK), and Government Degree College for Women, Block-M, North Nazimabad (SNV)

Abstract.- *Chrysorabdia viridata* Walker is described first time from Pakistan with special reference to its head appendages, venations of fore and hind wings and male and female genitalia, it also compared with its closset ally *C. bivitta* and its cladistic relationship is also briefly discussed.

Key words: *Chrysorabdia viridata* Walker, Arctiidae, Pakistan, cladistic relationship.

INTRODUCTION

Cotes and Swinhoe (1887) listed three species of the genus *Chrysorabdia* Butler *viz. bivitta* Walker, *strigata* (Moschler) and v*iridata* Walker in their catalogue on moths of India. Hampson (1894) described only two species *viridata* and *bivitta* based only on the superficial characters. Watson *et al.* (1980) listed *Chrysorabdia* in their generic list. Hashmi and Tashfeen (1992) listed only two species *viridata* and *bivitta* in their checklist on lepidopterous fauna of Pakistan.

MATERIALS AND METHODS

The adult Tiger moths, *Chrysorabdia viridata* Walker were collected with the help of light trap from Donga Gali, Ayubia, Pakistan and were identified with the help of Hampson (1894). For the study of genital complex the abdomen was excised at the base and boiled in 10% KOH solution for about 5 minutes and then washed with tap water. The genitalia were removed from the abdomen for detailed examination. The individual elements of the genitalia and the associated structures were removed, examined, and sketched using ocular grid under Leitz Weitzler dissection microscope.

RESULTS

Genus: *CHRYSORABDIA* Butler

Chrysorabdia Butler, 1877, *Trans. Ent. Soc*.:357; Cotes and Swinhoe, 1877, *Cat. moths Ind. Bombyces* **1**: 102; Hampson. 1894, *Faun. Brit. Ind.* **2:** 73; Watson *et al.* 1980, *Brit. Mus. (Nat. Hist.)* **2:** 3a; Hashmi and Tashfeen, 1992, *Proc. Pakistan Congr. Zool.* **12**: 172

Diagnostic features

Body generally yellowish with blue black patches on pronotum and two bands on fore wings, palpi short and porect, antennae of males with short cilia and bristles, fore wings very long and narrow, veins R_3 and R_4 originate from cell, vein R_5 absent, vein M_1 originates from upper angle of cell, hind wing with vein M_3 absent, cell open, in males parameres highly developed, broad and curved, uncus and gnathos well developed, aedeagus with prominent thecal appendage, in females both apophysesses moderate, ductus bursac long.

Comparative note

This genus is most closely related to *Eilema* Moore in having general appearance, fore wings narrowed with prominent anal lobe but it can easily be separated from the same in having frons broadly rounded, fore wings with veins R_3 and R_4 largely stalked and directly originate from cell, hind wings without cell, in males the parameres long, inwardly highly curved in contrast frons narrowly rounded, forewing with veins R_3 and R_4 either stalked or separated but not directly originate from cell, in males the parameres not curved inwardly in *Eilema* and by the other characters as noted in the description.

Types species

Lithosia viridata Walker.

Distribution

Oriental region (Assam, Burma, India and Pakistan).

Chrysorabdia viridata Walker
(Figs. 1-3)

Chrysorabdia viridata Walker 1860, *Cat. 21*:225; Moore 1878, *P.Z.S.*: 19; Cotes and Swinhoe 1887, *Cat. moths. Ind. Sphinges. 1*:103; Hampson 1894, *Faun. Brit. Ind.* **2** :74; Hashmi and

Tashfeen, 1992, *Proc. Pakistan Congr. Zool. 12:172. Gnophria stringata,* Moschler, 1872, *Stelt. Ent. Zeit.,* 353.

Fig. 1. *Chrysorabdia viridata* (Walker), entire, dorsal view.

Colouration

Body generally yellow except antennae, a median path on pronotum, a costal and sub-medial bands on fore wings in female, a narrow costal band, a sub-apical and a triangular sub-median band in male bluish black.

Head (Fig. 2A)

Eyes large, fascated with blocks, frons broadly convex, palpi anteriorly porect with small hairs, 2nd segment largest, more than 3X the length of 3rd, proboscis very large and coiled.

Fore wing (Fig. 2B)

Fore wing very large with apex sub-acute, veins Sc and R_1 parallel to each other, veins R_3 and R_4 largely stalked originated from aeriole, M_1 originates from upper angle of cell, vein M_2 originates from lower angle of cell, only one anal vein 1A is present.

Hind wing (Fig. 2C)

Hind wing broad, some what triangular shape with apex sub-acute, cell is open, veins R_5 and M_1 stalked, M_2 and M_3 stalked, three anal veins (1A, 2A, 3A) are present.

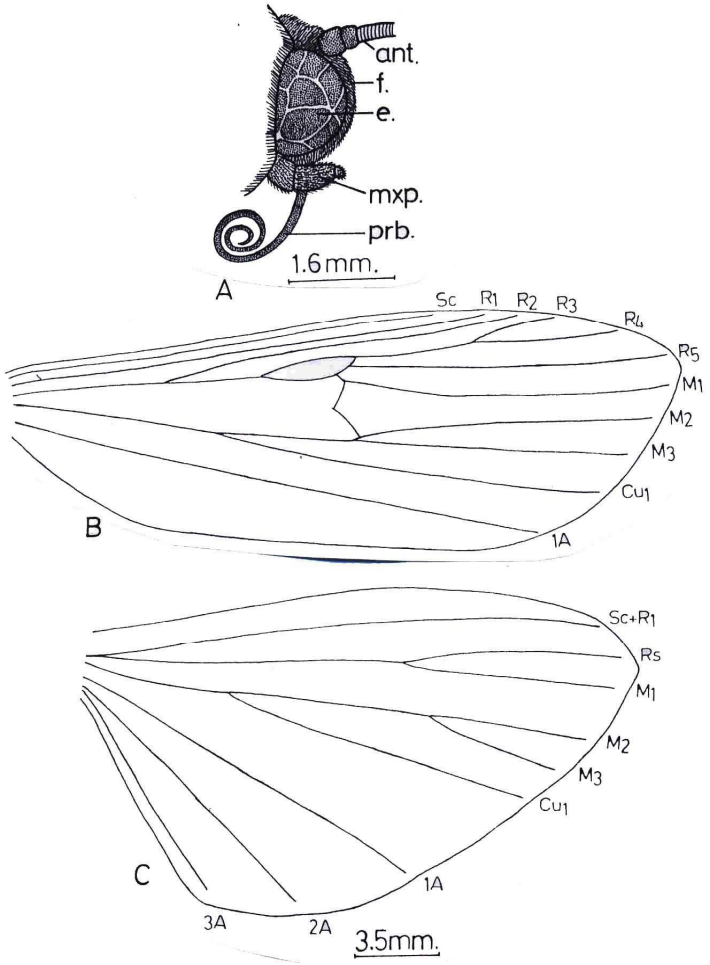


Fig. 2. *C. viridata* (Walker); A, head, lateral view; B, fore wing, dorsal view; C, hind wing, dorsal view.
ant., antenna; e, eye; f., frons; mx. p., maxillary palp; prb., proboscis; 1A-3A. Anal vein 1 to 3; Cu_1, Cubital vein 1; M_1-M_3, Median vein 1 to 3; R_1-R_5, Radius vein 1 to 5; sc., Subcastal vein.

Male genitalia (Figs. 3A-3C)

Tegumen large somewhat spherical shaped, uncus short, highly sclerotized inwardly directed, apically bifurcated, gnathos membranous, longer than uncus, paramere large, bilobed, inner lobe short, membranous beset with hairs, outer

lobe highly sclerotized, very large, inwardly curved, beak-shaped with apex narrowly rounded, lateral margin sinuated beset with a group of small setae and a group of large setae, juxta very large dorsally directed, theca (Fig. 3C) broad with bifurcated thecal appendage, membranous conjunctival lobe large, curved apical half semisclerotized.

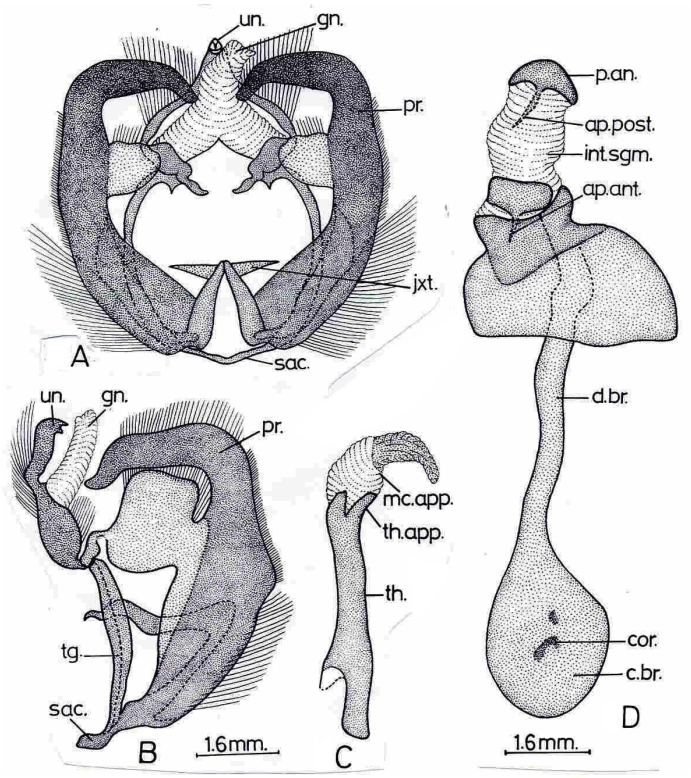


Fig. 3. *C.viridata* Walker; A, tegumen, ventral view; B, same, lateral view; C, aedeagus, lateral view; D, female genitalia, lateral view.
ap. ant., apophyses anteriors; ap. post., apophyses posteriors; c.br., corpus bursae; cor., cornuti; d. br., ductus bursae; gn., gnathos; int. sgm., inter segmental membrane; jxt., juxta; mc. app., membranous conjunctival appendage; p. an., papillae anales; pr., paramere; sac., saccus; tg., tegument; th., theca; th. app., thecal appendage; un., uncus.

Female genitalia (Fig. 3D)

Papillae anales lunar-shaped, apophyses posteriors large, much longer than

apophyses anteriors, lobus vaginalis reduced, ductus bursae very large, tubular, corpus bursae balloon-like with two cornuti.

Material examined

Four males, 4 females, Pakistan: Donga Gali, Ayubia, 20-7-2002, on light, leg. Syed Viqar Ali, Nargis Viqar, Raja Rizwan, loged at Ali Museum of Insecta research center, Karachi.

Comparative note

This species is most closely related to *bivitta* Walker in having the general appearance and colour patterns and hind wing with the cell open but it can easily be separated from the same in having body smaller sized, thorax with black spot in contrast body large sized more than 50cm, thorax without black spot as in *bivitta* and by the other characters as noted in the description.

DISCUSSION

The genus *Chrysorabdia* Butler of the sub-family Lithosinae included only three species distributed in Assam, Burma, Sikkim, Sylhet, Himalays, India and Pakistan mostly on high altitude. The present species is recorded from Donga Gali, Ayubia and Ghora gali about 5,000 feets.

The present species *Chrysorabdia vridata* Walker isolated from other species *bivitta* Walker *strigata* (Moschler) by its autapomorphies body moderate sized, hind wings pale yellow, 3rd palpus segment very short, in males paramere large bilobed with outer lobe highly sclerotized inwardly curved, thumb-like, uncus vertically bifurcated, juxta well marked, aedeagus with two thecal appendages, in female papillae anales lunar-shaped, apophyses posteriors much longer than apophyses anteriors, ductus bursae very long and tubuler.

REFERENCES

COTES, E.C. AND SWINHOE, C.C., 1887. A catalogue of the moths of India. *Cat. Moths. Ind. Sphinges*, **1:** 103.

HAMPSON, G.F., 1894. Fauna of British India including Ceylon and Burma. *Faun. Brit. Ind.* **2:** 74.

HASHMI, A.A. AND TASHFEEN, A., 1992. Lepidoptera of Pakistan. *Proc. Pakistan Congr. Zool.*, **12**: 172.

WATSON, A., FLETCHER, D.S. AND NYE, I.W.B., 1980. The generic name of moths of the world. *Brit. Mus. (Nat. Hist.)*, **2**: 39.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 105-113, 2004.

EFFECTS OF ACIDIC WATER AND MERCURY ON EGGS AND NEWLY HATCHED LARVAE OF HERRING, *CLUPEA HARENGUS* L.

H.A. JASTANIA AND A.R. ABBASI
King Abdul Aziz University, Jeddah, Saudi Arabia (HAJ) and Department of Fresh Water Biology and Fisheries, University of Sindh, Jamshoro, Pakistan (ARA)

Abstract.-Atlantic herring, *Clupea harengus* L. is an estuarine spawner. Adult fish, which were about to spawn were collected from Castle Beach, Milford Haven, South Wales, U.K. Eggs were stripped from these fish and artificially fertilized. Fertilized eggs were exposed to different pH and 1.0mgl^{-1} mercury added in acidic water. The effects on eggs and larvae hatched from previously exposed eggs were observed. These were (1) Premature hatching 1 to 2 days earlier than control (2) Larvae were shorter in length about 2.8mm as compared to control (3) Jaw and vertebral column abnormalities were 35% and 40% respectively in acidic waters, while 68% and 100%, respectively, in mercury added solutions (4) The ratio of eye and otic capsule diameters to body length are significantly affected in acidic solution with and without mercury treatment.

Key words: Herring, pH, mercury.

INTRODUCTION

Water acidification has become the most important environmental factor affecting fish life at various stages. Acidic water adversely affects egg development and inhibits spermatogenesis (Ruby *et al.*, 1978; Lee and Gerking, 1980). High mortality of fertilized eggs and anomalies in embryonic development were reported at low pH in *Catostomus commersoni, Cyprinodon nevadensis,* trout, perch and roach (Trojnar, 1977a,b; Johansson and Milbrink, 1976; Daye, 1980; Lee and Greking, 1980). High mortalities of fish larvae (Trojnar, 1977a; Daye and Garside, 1980; Peterson and Martin-Robichaud, 1980), inhibition of growth (Leivestad and Muniz, 1976; Menendez, 1976; Nelson, 1982) and pathological affects on liver and gut epithelial lining, muscle layers and connective tissues (Shrivastava Dwivedi, 1979) have been reported in low acidic water. Mohammad-Nagib(1987) reported premature hatching and structural abnormalities at low pH in *Clupea harengus.* A decreased frequency of trunk movement and pectoral fin movements were also noted at low pH in the larvae of *Salmo salar* (Peterson and Martin-Robichaud, 1983). Recently, the

involvement of heavy metals in enhancing the toxicity of low pH has been documented (Campbell and Stokes, 1985; McDonald *et al.,* 1988). At low pH, inorganic heavy metals are leached from the soil and bed rock, thus increasing aquatic heavy metals concentration and the toxicity of aluminum increased at low pH (Goss and Wood, 1988). Daye and Garside (1975) found lowest lethal limit of mercury at pH 3.2 to 3.6 in the fingerling, *Salvelinus fontinalis.*.

Little is, however, known on the toxicity of high or low pH to marine fish. Ocean dumping and discharge from mine tailings can ultimately lead to a change in pH in the marine environment. Apart from the direct effect of the pH, the chemistry of the saline waters can change as natural pH of sea water changes.

The present study was undertaken to assess the effects of water acidification plus mercury toxicity on the survival of eggs and larval development of Atlantic herring, *Clupea harengus* L. which spawn in estuarine waters where pollution may occur.

MATERIALS AND METHODS

During the spawning season in March 1989, adult herring, *C. harengus* were collected from Castle Beach, Milford Haven, South Wales, U.K. by gill netting. Eggs were stripped from females on to clean glass slides and fertilized with sperms stripped from males (Alderdice *et al*., 1979). All fertilized eggs were placed in plastic containers with artificial sea water (Ocean Synthetic Sea Salts) diluted to 30 $^{o}/_{oo}$ salinity and aerated. Sea water with pH of 4.5-5.0, 7.5-8.0 or 9.0, was prepared by adding dilute hydrochloric acid or sodium hydroxide. pH was measured directly by a pH meter, using a single glass electrode. Other solutions were prepared with pH 4.5-5.0, 7.5-8.0 or 9.0 with 0.1 µg/l mercury. Solutions were changed at 24h intervals to avoid any significant change in the concentration of mercury or pH. The jars were washed in 10% HNO_3 and then rinsed in double distilled water before use. All experiments were carried out at constant temperature of 9°C. After hatching, larvae were reared under similar conditions until yolk absorption was complete. At intervals of 16, 18, 21, 23, 24, or 27 days after fertilization, samples of larvae were examined. The frequencies of jaw and vertebral abnormalities were determined from random samples of 20-35 larvae. The incubation period (time from fertilization to 50% hatching), body length and eye and otic capsule diameters at hatching were also measured.

RESULTS

Table I shows the incubation period (time from fertilization to 50% hatching) was 16 days at pH 7.5-8.0, 9.0, whilst at pH 4.5-5.0, this was 15 days. Table I shows that the incubation period was 12 days in 0.1 µg/l mercury solution, at pH 4.5-5.0, but at pH 7.5-8.0 and 9.0 this was 13 days. The mean body-length of larvae decreased on exposure to pH 4.5-5.0 but it increased on exposure to pH 9.0 compared to that of the controls (pH 7.5-8.0). In the presence of 0.1 µg/l mercury the mean body length decreased in pH 4.5-5.0 but in pH 9.0 the mean body length remained the same as controls (pH 7.5-8.0). Jaw and vertebral column abnormalities were high in pH 4.5-5.0 and pH 9.0 when compared with the controls (pH 7.5-8.0). Similar results were obtained in the presence of 1.0 µg/l mercury. However, 100% abnormalities were observed in the vertebral column when fish were exposed to different pH's (low, 4.5-5.0 and high, 9.0) together with 0.1 µg/1 mercury (Table I). Thus mercury had a marked effect on the incidence of vertebral column abnormalities.

TABLE I.- INCUBATION PERIOD (TIME FROM FERTILIZATION TO 50% HATCHING) OF EGGS, MEAN BODY LENGTH OF THE LARVAE HATCHED FROM THE EGGS, AND JAW AND VERTEBRAL COLUMN ABNORMALITIES OF *C. HARENGUS* L. EXPOSED TO ACIDIC WATER AND ACIDIC MERCURY SOLUTIONS.

	pH	Acidic water	Acidic water + Mercury
Incubation period (day)	4.5-5.0	14.5	12
	7.5-8.0	16	13
	9.0-9.5	16	13
Body length of larvae (mm)	4.5-5.0	5.84±0.566 (n=28)	5.04±0.764 (n=23)
	7.5-8.0	7.96±0.743* (n=26)	7.02±0.851 (n=38)
	9.0-9.5	8.55±0.717**** (n=24)	6.173±1.09 (n=24)
Jaw abnormalities (%)	4.5-5.0	35	35
	7.5-8.0	13	13
	9.0-9.5	37	37
Vertebral column abnormalities (%)	4.5-5.0	40	40
	7.5-8.0	20	20
	9.0-9.5	38	38

Mean±SD; Student's `t' test.
Significantly different (* P<0.001; **** P<0.0001) from the control
N= number of larvae m= mean length of larvae s.d.= standard deviation.

The eye diameter to body-length ratios of the larvae treated with different pH are shown in Figure 2. The relative eye diameter increased significantly at pH 4.5-5.0 and 9.0 as compared with control pH 7.5-8.0. The otic capsule diameter to the body length ratios at hatching are shown in Figure 1. There is a significant decrease in the relative diameter of the otic capsules of larvae hatched in pH 4.5-5.0 ($P<0.05$) from the controls, but at pH 9.0 there was significant decrease compared with control.

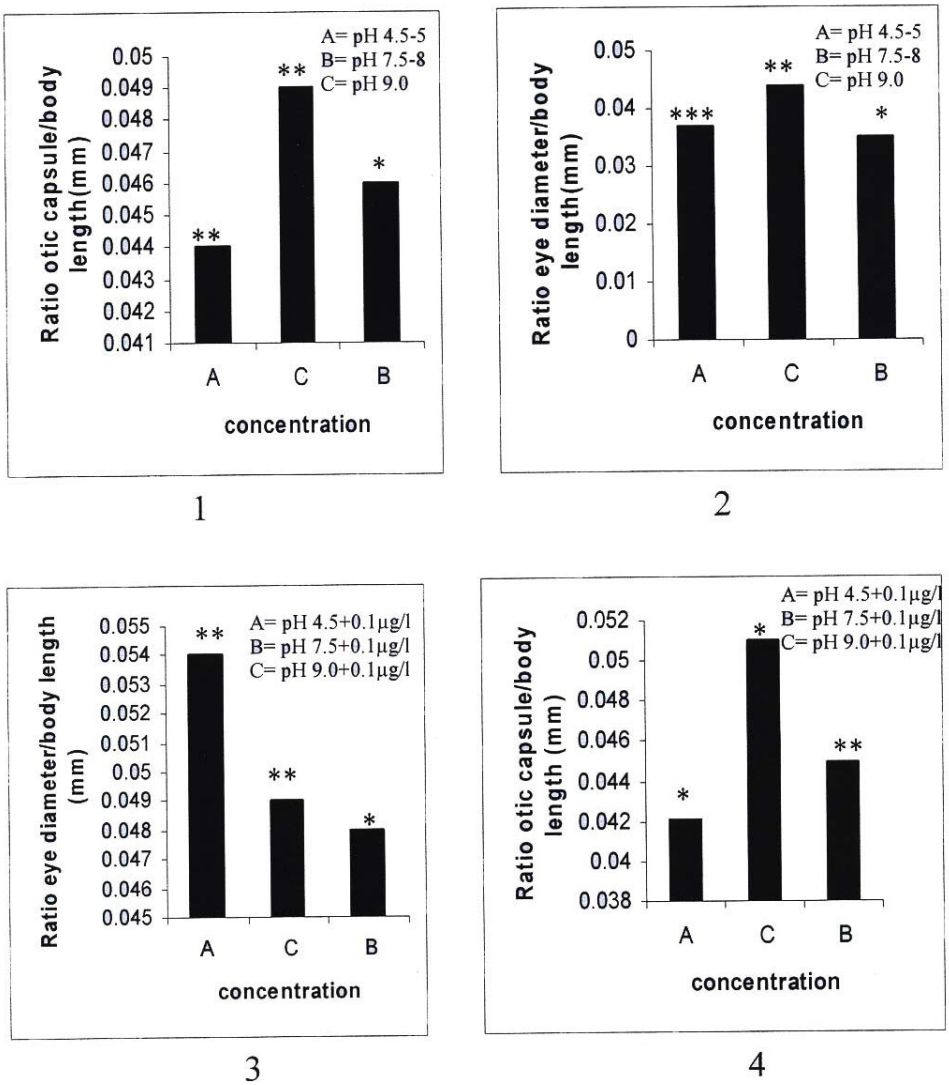
Figure 3 show effect of 0.1 µg/l mercury at pH 4.5-5.0. The ratio of eye diameter to body length showed a significant increase. The otic capsule diameter to body length ratios at hatching is also significantly decreased at pH 4.5-5.0 and increased at pH 9.0 ($P<0.001$) compared with controls.

DISCUSSION

Mason (1981) reported that pH plays an important role in interactions between heavy metals and other chemical parameters such as hardness and dissolved organic compounds. Wright (1975) stated that in many Norwegian lakes, the pH decreased to <pH 4 as a result of acid precipitation, and that low pH inhibits the decomposition of organic matter and decrease the amount of phytoplankton and other invertebrates present in these lakes. In southern Norway, mass mortality in the trout population has been recorded as a result of pH changes during thawing of snow (Schofield, 1976). Harvey (1982) found that the size of the fish populations in acidified fresh waters is reduced and fish growth was decreased probably due to the combined effects of metabolic stress population density and food supply. Harriman and Morrison (1982) showed that, in an area of central Scotland, trout were generally absent from streams in long established forests and that these streams were acidified.

The present study shows that the incubation period of *C. harengus* eggs in acidified sea water (pH 4.5-5.0) is shorter by one day. A reduction in the incubation period has also been observed in Baltic herring eggs exposed to a low pH (Kinne and Rosenthal, 1967; Mohammed-Nagib, 1987).

The premature hatching was observed in the present study in the presence of the mercury and acidified sea water. Carrick (1979) reported the highest mortality in pH 6.0 to 7.0, and observed no obvious difference in the percentage hatching in the pH range 4 to 5, although pH 3.5 was lethal within 10 days to all eggs of trout, *S. salar* and brown trout, *Salmo trutta.* Trajonar (1977) reported that the eggs of brook trout, *S. fontinalis* have been found to hatch earlier at low



Figs. 1-4. Effect of acidic water (1,2) and mercury (0.1 µg/l) administered in acidic water (3,4) on the relationship of otic capsule and body length (1, 4) and eye diameter and body length (2, 3).
Significantly different (*, P<0.001; **P<0.05; ***P<0.002) from control.

pH. Hall (1925) suggested that high hydrogen ion concentrations which are still acceptable for fertilization are too high for later embryo development. When pH interacts with water hardness, moderately soft water causes lowering in plasma

ion concentration and high mortalities in rainbow trout *S. gairdneri* (McDonald *et at.,* 1980).

McDonald and Wood (1981) found that low pH impaired gill ion regulation in *S. gairdneri,* as indicated by continuous bronchial (gill) losses of Na^+, Cl^- and K^+ and decline in plasma Na^+ and Cl^- levels, because there was significant contribution by the intracellular compartment both to the total body ion losses and to buffering the body acid load. Fish dying from acid stress have the lowest plasma Na^+ and Cl^- levels (McDonald, 1983).

In the present study, fertilized eggs incubated at low pH, hatched into smaller larvae with large yolk sacs and appeared to hatch prematurely under these stressful conditions. Menendez (1976) and Trojnar (1977) reported similar findings. Nelson (1982) related slowing down of yolk sac resorption to deficient protein synthesis in acidic medium. Johansson and Kihlstron (1975) developed pike larvae in water acidified to pH 4.2 which exhibited structural changes and slower resorption of yolk sac. Korwin-Kossakowski and Jezierska (1988) reported prolongation of yolk sac resorption and inhibition of development when *Cyprinus carpio* larvae were reared in low pH (5.0-5.2).

. The present study concludes that the deformities among prematurely hatched larvae could affect their activity and survival potential. The higher percentage deformity occurring in the present study was in the vertebral column at low pH (4.5). Mohammed-Nagib (1987) found a high deformity in jaw and vertebral column of *C. harengus* when fish were exposed to low pH of 5.2. Runn *et at.* (1977) observed cartilaginous elements of perch, *Perca fluviatilis* when exposed to acid media. Nelson (1982) suggested that inability of acid stressed larvae to ossify skeletal parts would be contributory factor to recruitment failure. In addition, the inability of larvae to ossify structures such as teeth and fin rays could have implications at swimming when predatory competence is essential for survival. These abnormalities in vertebrae and jaws have also been seen as responses to environmental factor such as exposure to extremes of temperature, salinity, radiation and pollutants (Rosenthal and Aderdice, 1976). Peterson and Martin-Robichaud (1982) suggested that the physiological stress imposed by low levels of pH 4.5, may create an ionic imbalance which interferes with the requirement for normal maintenance and feeding.

Enhanced mobilization of heavy metal occurs in acid water (Wright and Gjessing, 1976) and acidity can affect the uptake of heavy metals in aquatic organisms by its influence on physiological processes and ecological levels. In

the present study eggs of *C. harengus* exposed to pH 4.5-5.0, 7.5-8.0 and 9.0 with 0.1 ppm mercury added, produced 100% abnormalities in jaws and vertebral column. It may be that the chemistry of the heavy metals is modified by ambient pH, such as the formation of monomethyl mercury which is accelerated by low pH (Jemelov, 1980), dimethyl mercury becomes progressively more unstable below pH 9.0. Eggs of *C. harengus* exposed to pH 4.5 and 9.0 produced larvae with 80% deformities but no mortality occurred. Trojnar (1977a) reported that the eggs of the white sucker, *Catostomu commersont* when subjected to pH 4.5 with high water conductivity (tap water), produced few abnormal prolarvea which soon died, but in soft water at pH 4.5 or below, no egg survived to hatch.

CONCLUSIONS

It is concluded that; (1) pH less than 4.5 is toxic to eggs and larvae of estuarine-spawner teleosts. (2) pH more than 5.0 is safe to eggs and stages of embryo and larval development. (3) Toxicity of mercury increases in the acidic water. (4) Premature hatching occurs 1 to 3 days earlier in acidic and acidic/ mercury waters. (5) Inhibition of growth, abnormalities of jaws and vertebral column in newly hatched larvae result-in both acidic and acidic/mercury waters. (6) pH higher than normal 7.5-8.0 show no any obvious effects on eggs and larvae.

REFERENCES

ALDERDICE, D.F., ROSENTHAL, H. AND VELSEN, F.P.J., 1979. Influence of salinity and cadmium on capsule strength in Pacific herring eggs. *Helgoland wiss. Meeresunters*, **32:** 163-178.

CAMPBELL, P.G. AND STOKES., P.M., 1985. Acidification and toxicity of metals to aquatIc biota. *Can. J. Fish. aquat. Sci.,* **42:** 2034-2049.

CARRICK, T.R., 1979. The effect of acid water on the hatching of salmonid eggs. *J. Fish Biol.*, **14:** 165-172.

DAYE, P.G. AND GARSIDE, E.T., I975. Lethal levels of pH for brook trout, *Salvelinua fontinalis* M. *Can. J. Zool.*, **53:** 639-641.

DAYE, P.G. AND GARSIDE, E.T., 1980. Structural alterations in embryos and alvins of the Atlantic salmon, *Salmo salar* L. induced by continuous or short term exposure to acidic levels of pH. *Can. J. Zool.,* **58:** 27-43.

GOSS, G.G. AND WOOD, C.M., 1988. The effects of acid and acid/aluminum exposure on circulating plasma cortisol levels and other blood parameters in the rainbow trout, *Salmo gairdneri. J. Fish. Biol.,* **32**: 63-76.

HALL, A.R., 1925. Effects of oxygen and carbon dioxide on the development of the white fish *Ecology,* **6:** 104-116.

HARRIMAN, R. AND MORRISON, B.RS., 1982. Ecology of streams draining forest and non-forest catchments in an area of central Scotland subject to acid precipitation. *Hydrobiologia,* **88**: 251-263.

HARVEY, H.H., 1982. Population responses of fishes in acidified waters. In: *Acid rain fisheries.* Proceeding of an international symposium on acid rain and fisheries. Impacts on north eastern North America (ed. R.E. Johnson), pp. 227-242. American Fisheries Society, Bethesda, MD (USA).

JERNELOV, A., 1980. The effects of acidity on the uptake of mercury in fish. In: *Polluted rain.* Plenum publishing Co.

JOHANSSON, N.G. AND KIHLSTROM, J.K., 1975. Pikes, *Esox lucius* L. showing to be affected by low pH value during first week after hatching. *Environ. Res.,* **9:** 12-17.

JOHANSSON, N.G. AND MILBRINK, G., 1976. Some effects of acidified water on early development of roach, *Rutilus rutilus* and perch, *Perca jluviatilis. Water Res. Bull.,* **12:** 39-48.

KINNE, O. AND ROSENTHAL, H., 1967. Effects of sulfuric water pollutants on fertilization, embryonic development and larvae of the herring, *Clupea harengus. Mar. Biol.,* **1**: 67-83.

KORWIN-KOSSAKOWSKI, M. AND JEZIERSKA, B., 1985. The influence of temperature on carp fry survival in acidic water. *J. Fish. BioI.,* **26:** 43-47.

LEE, R.M. AND GERKING, S.D., 1980. Survival and reproductive performance of the desert pupfish, *Cyprinodon n. nevadensis* E&E, in acid waters. *J. Fish. BioI.,* **17:** 507-515.

LEIVESTAD, H. AND MUNIZ, I.P., 1976. Fish kill at low pH in Norwegian river. *Nature* (Lond.), **259:** 391-392.

MASON, C,F., 1981. *Biology of fresh water pollution*. Longman, New York.

McDONALD, D.G., 1983. The effects of pH upon gills of fresh water fish. *Can.. J. Zool.,* **61:** 691-703.

McDONALD, D.G., HOBE, H. AND WOOD, C.M., 1980. The influence of calcium on the physiological responses of the rainbow trout, *Salmo gairdneri* to low environmental pH. *J. exp. Biol.,* **88:** 109-131.

McDONALD, D.G., READER, J.P. AND DALZIEL, T.K.R., 1988. The combined effect of pH and trace metals on fish ionoregulation. In: *Acid toxicity and aquatic animals* (eds. R. Morris, D.J.A. Brown, E.W. and TaylorI J.A. Brown). Society of Experimental Biology Seminar Series, Cambridge University Press.

McDONALD, D.G. AND WOOD, C.M. 1981. Branchail and renal acid and ion fluxes in the rainbow trout, *Salmo gairdneri,* at low environmental pH. *J. exp. Biol.,* **93:** 101-118.

MENENDEZ, R.,1976. Chronic effects of reduced pH on brook trout, *Salvelinus fontinalis. J. Fish. Res. Bd. Can.,* **33:** 118-123.

MOHAMMED-NAGIB, F.J.B., 1987. *Effects of heavy metals on the biological activities of marine fishes, with special reference to their reproduction and development.* Ph.D. thesis, University of Wales, UK.

NELSON, J.A., 1982. Physiological observations on developing rainbow trout, *Salmo gairdneri* (Richardson), exposed to low pH and varied calcium ion concentrations. *J. Fish Biol.,* **20:** 359-372.

PETERSON, R.H. AND MARTIN-ROBICHAUD, D.J., 1982. Water uptake by Atlantic salmon

ova affected by low pH. *Trans. Am.. Fish. Soc.,* **111:** 722-774.

PETERSON, R.H. AND MARTIN-ROBICHAUD, D.J., 1983. Embryo movements of Atlantic salmon, *Salmo salar,* as influence by pH, temperature and state of development. *Can. J Fish. aqua. Sci.,* **40**: 777-782.

ROSENTHAL, H. AND ALDERDICE, D.F., 1976. Sublethal effects of environmental stressors, natural and pollutional on marine fish eggs and larvae. *J. Fish. Res. Bd. Can.,* **33**: 2047-2065.

RUBY, S.M., ACZEL, J. AND CRAIG, G.R., 1978. The effects of depressed pH on spermatogenesis in flagfish, *Jordanella floridae. Water Res.,* **12**: 621-626.

RUNN, P., JOHANSSON, N. AND MILBRING, G., 1977. Some effects of low pH on the hatchability of eggs of perch, *Perca fluviatilis* L. *J. Zool.,* **5**: 115-125

SCHOFIELD, C.L., 1976. Acid precipitation: effects on fish. *Ambio,* **5**: 228-230.

SHRIVASTAVA, R.S. AND DWIVEDI, P., 1979. Studies on the effects of acidic medium on the fry of *Cyprinus carpio. Acta Hydrochem. Hydrobiology,* **7:** 421-430.

TROJNAR, J.R., 1977a. Egg and larval survival of white suckers, *Catostomus commersoni* at low pH. *J. Fish. Res. Bd. Can.,* **34**: 262-266.

TROJNAR, J.R., 1977b. Egg hatchability and tolerance of brook trout, *Salvelinus fontmalis* fry at low pH. *J. Fish. Res. Bd. Can.,* **34**: 574-579.

WRIGHT, R.F., 1975. *Acid precipitation and its effects on fresh water ecosystem in Norway*. The first international symposium on acid precipitation and the forest ecosystem, pp. 35-36.

WRIGHT, R.F. AND GJESSING, E.T., 1976. Acid precipitation changes in the chemical composition of lake. *Ambio,* **5**: 219-223.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 115-123, 2004.

CHEWING LICE (INSECTA: MALLOPHAGA) OF DOMESTIC CHICKENS AT JAND (DISTRICT ATTOCK)

NAHEED ALI* AND ADNA BIBI
Department of Zoology, University of Peshawar, Peshawar
*E-mail: naheedsherpao@yahoo.com

Abstract.- One hundred and six chickens, from 47 houses were examined from November 2002 to February 2003 for chewing or biting lice infestation at Jand (a Tehsil of district Attock, Punjab). Four species of lice were found: *Menopon gallinae, Cuclotogaster heterographus, Goniodes gigas* and *Goniocotes gallinae.* The infestation was found to be 51.89%. Chickens were observed to be generally infested with a single species but in some cases infestation by more than one species was also found. The most common species was happen to be *Menopon gallinae* recorded from 33 chickens. The species *Cuclotogaster heterographus* was confined to head and neck area while other three species, *Menopon gallinae, Goniocotes gallinae* and *Goniodes gigas* were found in rest of host's body.

Kew words: Biting lice infestation, *Menopon gallinae*, ectoparasite.

INTRODUCTION

Poultry has been confronted with various infectious, contagious and parasitic diseases. The chewing or biting lice are small wingless (apterous), flat bodied insects belonging to order Mallophaga (Mallos-hair, phagein-to eat) of class Insecta and are adapted for an ectoparasitic life. The lice of this order are also referred as bird lice, as 85% are parasitic on birds (Kettle, 1995). They are characterized by possession of chewing type of mouthparts, feed mostly by chewing feathers and hair of hosts. Different species of the lice attack different types of poultry and domestic mammals and each species usually infest a particular part of the host body (Borror and Delong, 1970).

The most important factor about lice is their ability to multiply rapidly (Pfadt, 1985). Biting lice usually do not spread pathogens but heavy infestation in poultry can cause severe skin irritation, weight loss and reduced egg production. Lice are not highly pathogenic to mature birds, but louse infested chicks may die (Hofstad *et al*., 1978). Clinical evidence indicates that lice may irritate nerve endings thus interfering with the rest and sleep so necessary to immature animals. They further stated that lousiness frequently accompanies manifestation of poor health such as internal parasitism, infectious disease and malnutrition as well as

poor sanitation. Lice can cause direct losses by adverse effects on the body development and weight and low egg yield (Buriro, 1982).

This paper is about the types of Mallophaga infesting the domestic chickens at Jand. It also includes the study on prevalence of infestation in chicken specificity of various Mallophaga species on different parts of host body. Abundance of various lice species on chicken at Jand is also included.

MATERIALS AND METHODS

The survey to check the chicken lice infestation was carried out from November 2002 to February 2003 at Jand (a Tehsil of Attock district). For the collection of lice 47 houses were visited and their domestic chickens were thoroughly examined.

The search for lice was carried out manually. The feathers on different parts were examined carefully. Lice were picked from the host with a brush moistened with alcohol and were preserved in 70% alcohol. Date of collection and other information about the hosts were recorded. Specimens collected from different parts of the same host were preserved separately.

The permanent slides of preserved sample were prepared according to Borror and Delong (1970). The preserved specimens was first cleared in 10-15% KOH for about 24 hours in case of family Philopteridae (hard bodied) and 1 hour in the case of family Menoponidae (soft bodied), then dehydrated in various grades of alcohol 5-10 minutes for each grade, and finally mounted in Hoyer's media. Identification was done mostly with the help of a key provided by Furman (1967).

RESULTS

Prevalence of infestation

Table I shows the prevalence of infestation with different species of Mallophaga. A total of 106 Chickens were examined. Among these 55 were found to be infested with different species of Mallophaga. These chickens were infested either by a single species or more than one species at the same time.

Hundred percent infestation was found once in the month of February while no infestation (0%) was found three times during the study, (one time in each of the three months). The overall incidence of infestation was 51.89%.

TABLE I.- PREVALENCE OF INFESTATION WITH DIFFERENT SPECIES OF MALLOPHAGA IN CHICKENS AT JAND, ATTOCK.

Date	No. Examined	No. infested	infestation%
29.11.002	4	2	50
2.12.002	6	3	50
3.12.002	5	2	40
11.12.002	3	2	66.67
14.12.002	9	4	44.44
15.12.002	6	2	33.33
22.12.002	4	2	50
25.12.002	4	0	0
29.12.002	5	4	80
1.1.003	4	2	50
8.1.003	7	3	42.85
9.1.003	3	0	0
11.1.003	5	2	40
18.1.003	6	3	50
26.1.003	9	6	66.67
27.1.003	8	6	75
9.2.003	6	6	100
13.2.003	4	0	0
19.2.003	8	6	75
	106	55	51.89

Infestation and relative abundance of different species of Mallophaga

Four species *viz*. *Menopon gallinae* (Linnaeus, 1758), *Cuclotogaster heterographus* (Nitzsch, 1866), *Goniodes gigas* (Taschenberg, 1879), *Goniocotes gallinae* (de Geer, 1778), were identified. *M. gallinae* belonged to family Menoponidae, while the rest of the three species belonged to family Philopteridae.

The most common species of lice was *M. gallinae*, which was collected from 33 chickens showing the highest infestation. The next abundant species was *C. heterographus* which was collected from 21 chickens. The other two species *G. gallinae* and *G. gigas* were relatively rare species found only in 8 and 7 chickens, respectively, showing high difference with the occurrence of first two species. Out of a total of 588 lice collected, the highest number collected was that of *M. gallinae* (223) followed by *C. heterographus* (159), *G. gallinae* (109) and *G. gigas* (97), thereby showing 37.93%, 27.04%, 18.54% and 16.5% relative abundance, respectively.

Location of different species of lice on different parts of hosts body

C. heterographus was found to be confined to the head and neck region of host, while the other three species were collected from almost all parts of the body except head and neck regions. The wings were not infested by all the three species, but specimens belonging to *G. gigas* were found in this region. *M. gallinae* and *G. gallinae* were found on the shafts and fluffs, while *G. gigas* was found in quills of the host body.

Comparison of the number of male and female lice of all four species

The Chi square analysis for *M. gallinae* showed no significant difference in the number of male and female, while the other three species showed significant difference (Table II).

TABLE II.- COMPARISON OF THE NUMBER OF MALE AND FEMALE LICE OF ALL FOUR SPECIES AT JAND, ATTOCK BASED ON POOLED DATA:

Species	Total	Male		Female		Comparison
		No.	%age	No.	%age	χ^2
M.gallinae	223	102	45.74	121	54.26	1.61 ns
C.heterographus	159	56	35.22	103	64.78	13.89 *
G.gallinae	109	44	40.37	65	59.60	4.04 *
G.gigas	97	32	32.99	65	67.01	11.26 *
Total	588	234	39.79	354	60.20	24.48 *

Test to check the randomness of all four species on chickens

Table III shows the species complex in chickens. Among 106 chickens, 51 had no louse, 43 were infested by only one species of lice, while two and three species were found on 10 and 2 chickens, respectively. The data were subjected to Poisson distribution to check for randomness of the prevalence of all four species on chickens.

The null hypothesis (Ho) is accepted. The observed frequency fitted the poison distribution. The distribution of different species of lice on chickens was random. Hence All chickens had equal chance of being infested by any species of lice.

TABLE III.- POISSON DISTRIBUTION TO CHECK THE RANDOMNESS OF LICE SPECIES ON CHICKENS AT JAND, ATTOCK (N = 106; infested = 55)

No. of lice species received per host (x)	No. of hosts (observed frequency) (Oi)	Expected poison frequency (e_i)	O_i-e_i	$(O_i-e_i)^2$	$X^2 = \frac{(o_i - e_i)^2}{e_i}$
0	51	55.4	-4.4	19.36	0.35
1	43	35.9	7.1	5041	1.40
2	10	11.7			
3	2	2.5	-2.8	7.84	0.53
4	0	0.6			

($\chi^2 = 2.28$; df = 1, P> 0.05)

Analysis of species complex on chickens

The species complex in infested chickens is analyzed in Table IV. The analysis shows that all the four species live solely on their host as well as in association with an other species. *Cuclotogaster heterographus* was found to be in association with all other three species. *Menopon gallinae* and *Goniodes gigas* were found in association with two other species while *Goniocotes gallinas* was found in association with only one species.

TABLE IV.- INFESTATION OF CHICKENS BY A SINGLE, TWO AND THREE SPECIES OF LICE AT JAND, ATTOCK. THE SPECIES WERE SHOWN BY ALPHABETIC LETTERS, WHEREAS THE HOST NUMBERS ARE SHOWN IN PARENTHESIS.

	A	B	C	D
A	A (23)	AB (5)	AC (0)	AD (3)
B		B (13)	BC (1)	BD (1)
C			C (6)	CD (0)
D				D (1)
AB			ABC (0)	
AD		ABD (1)	ACD (1)	

A, *Menopon gallinae;* B, *Cuclotogaster heterographus;* C, *Goniocotes gallinae;* D, *Goniodes gigas.*

In majority of the cases all the three species were found solely on their hosts. Just in few cases more than one species was found in association with

other species, however, AB (*M. gallinae* and *C. heterographus*) combination appeared to be relatively the highest.

The occurrence of two types of groups (ABD and ACD) was found, while the other possible combination (ABC) was not found. Although association of C with D and A with C were not observed but association ACD was found. Again chicken infestation by group of lice species simultaneously was rare and only one each case could be reported for both the combinations.

Lice burden in infested chickens

Table V shows number of lice on infested chickens. The total number of lice collected from different chickens was divided into seven categories. This Table shows that the most prevalent category is the first one *i.e.*, 27 chickens were being infested by 1-5 lice. The second common category is the third one having 11-15 lice and was found on 11 chickens. However, the rest of the categories are least common.

TABLE V.- LICE BURDEN IRRESPECTIVE OF VARIOUS SPECIES IN 55 INFESTED CHICKENS AT JAND, ATTOCK.

S. No.	No. of lice	No. of host
1	1 – 5	27
2	6 – 10	6
3	11 – 15	11
4	16 – 20	2
5	21 – 25	4
6	26 – 30	2
7	above 30	3

DISCUSSION

This study revealed the existence of four species viz. *Menopon gallinae, Cuclotogaster heterographus, Goniocotes gallinae* and *Goniodes gigas.* The study had some limitations: (1) The host study was restricted to domestic chickens, (2) The study of parasites included only the biting lice (Mallophaga), (3) The study area was confined to Jand, and (4) The available literature was extremely limited. On the basis of available records it appears that *M.gallinae* and *G. gigas* have also been reported from Sindh by Buriro and Akbar (1978)

and Buriro (1982) and from N.W.F.P. by Iqbal (1987), while *C. heterographus* has been reported only from N.W.F.P. by Iqbal (1987). The survey conducted by Iqbal (1987) reported five species of Mallophaga in chickens *i.e. Menopon gallinae, Menacanthus stramineus, Cuclotogaster heterographus, Goniodes gigas* and *Goniodes dissimilis* from Peshawar and Mansehra. The occurrence of *M.gallinae, C.heterographus* and *G.gigas* indicate their adoptability irrespective of abiotic and biotic factors as these are found both in previous as well as present study.

All the four species of lice found in the present survey seemed to feed only on barbs, fluffs and dander of hosts. The other studies which confirmed such situation in these species is by Cheng (1973) in the species of *M. gallinae* as quoted by Iqbal (1987) and Kettle (1995) in *C. heterographus.* These species feed on barbs and scales of feathers and are not generally blood ingester. He also reported that fluff louse *G. gallinae*, is a small louse occur in down feathers anywhere on body and generally cause little irritation.

No severe infestation was observed as the work was carried out in winter, when the lice were not so active and reproductive as in summer. There were only 3 chickens in which more than 30 parasites were found. According to Metcalf and Flint (1962) poultry lice generally breed faster and become more abundant in summer than in cold weather. Hofstad *et al*. (1978) reported that lice tend to increase during autumn and winter.

Prevalence of infestation of lice species does not give any clear picture in this study. Both, 100% as well as 0% infestation was found during the study. These variations among results may be due to certain additional factors such as, hygienic condition of chickens. The seasonal variation of Mallophaga as well as factors affecting the prevalence of infestation of Mallophaga need further investigations.

G. gigas was found on all parts of the host body including quills and *C. heterographus* was found on shaft and skin of head and neck. These species have strongly chitinized bodies and of dark colour. The other two species, *M. gallinae* and *G. gallinae* were found on body fluff and have less chitinized bodies and light colour. The probable reason of these different types of morphological characters may be due to differences in their habitat. The former two species live in relatively hard parts of the body, while the latter two species live in soft parts of the feathers and are therefore adapted accordingly.

M. gallinae was observed to be the most prevalent species. It was not only found on a large number of chickens but also in comparatively greater number on their hosts. This louse is extremely active and quick in movement therefore, it is assumed to be easily transmitted to the other hosts.

C. heterographus was collected from the head and neck region and not from the body, while other three species were found in all parts of the body but not in the head and neck region. *C. heterographus* was observed to be present not only solely but also with other species. The probable explanation could be, that because these species were infesting different parts of the host, which make possible the presence of different species on the same host, due to lack of direct inter-species competition.

No chicken was found to be infested by all the four species at the same time. Iqbal (1987) also reported the absence of all five species on the same host. The reason may be that the inter species competition increases as species number increase on a host. So they prefer to live solely or with minimum possible number of species.

The comparison in the number of male and female individuals shows significant differences in sex ratio. Female individuals were found more in number as compared to male. Iqbal (1987) reported the same situation in her study. The possible reason for these differences may be that either the males are short lived or their birth rate is low. The literature is silent about this problem and needs further investigation.

ACKNOWLEDGEMENTS

We are grateful to Professor Dr. Mohammad Suleman of Department of Zoology, University of Peshawar for his help in statistical analysis.

REFERENCES

BORROR, D.J. AND DELONG, D.M., 1970. *An introduction to the study of insects*. Holt. Rinehart and Winston, Columbus, Ohio, pp. 812.

BURIRO, S.N. AND AKBAR, S.S., 1978. Incidence and occurrence of ecto-parasites of poultry in Pakistan. *Z. angew, Ent.*, **89**: 22-25.

BURIRO, S.N., 1982. *Incidence and epizootology of ecto and endoparasites of poultry in urban and rural areas of Sindh.* Final annual technical report, Deptt. of Parasitology Sindh Agriculture University (quoted in Iqbal,1987).

CHENG, T.C., 1973. *General parasitology.* Academic Press, New York, pp. 803-808.

HOFSTAD, M.S., CALNEK, B.W., HEMBOLDT, C.F., REID, W.M AND YODER, H.W. Jr., 1978. *Diseases of poultry*, 7th ed. Iowa State University Press, Ames, Iowa, USA, pp. 949.

IQBAL, S., 1987. *Biting lice (Mallophaga) of chickens of Peshawar and Mansehra*. M.Sc. thesis, Department of Zoology, University of Peshawar. pp. 58.

KETTLE, D.S., 1995. *Medical and veterinary entomology*, 2nd ed. CAB International UK, pp. 725.

METCALF, C.L. AND FLINT, W.P., 1962. *Destructive and useful insects, their habits and control*. 4th ed. McGraw Hill International Book Co. Singapore, 1087 pp.

PFADT, R.E., 1985. *Fundamentals of applied entomology.* 4th ed. Macmillan Publishing Company, New York, 742 pp.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 125-130, 2004.

A REVISION OF *TACHENGIA* CHINA (HEMIPTERA: PENTATOMIDAE: PENTATOMINAE: HALYINI) AND ITS CLADISTIC RELATIONSHIPS

IMTIAZ AHMAD
Department of Zoology, University of Karachi, Karachi-75270, Pakistan.

Abstract.- The halyine stinkbug genus *Tachengia* China is redescribed with a key to all its species including the type species *T. ascra* China with reference to its male genitalia trom various localities of China. *Tachengia* is compared with its closest allies within Halyini and in this light considering their zoogeography the cladistic relationships of the included taxa are also briefly discussed.

Keywords: Stink bug, *Tachengia,* Pentatomidae, Haylini.

INTRODUCTION

China (1925) described a new halyine stink bug genus *Tachengia* to accommodate a new species *ascra* from an altitude of 7300 ft. in Tacheng Yunnan, China. Wu (1935) described *Tachenia* also trom China but Stichel (1960-62) synonymised it with *Tachengia* China. Hsiao and Cheng (1977) described and illustrated *T. viridula* and *T. yunnana* with their distributions from Szechuan and Yunnan in China respectively. Rider *et al.* (2002) listed the above three species with their distributions in China. China (1925) showed his genus closely related to the new world genus *Brochymena* Amyot and Serville but he also considered metasternal orifice auriculate as in *Dalpada.*

The present author not only studied the new world halyine genera *Brochymena* and Parabrochymena Lariviere 1992 (Ahmad and Mc-Pherson, 1998) but also *Dalpada* (Ahmad and Afzal, 1984) and related groups (Ahmad and Ahmad, 1993). Presently, therefore not only ihe forgotten genus *Tachengia* is redescribed with reference to male genitalia with a key to all its three known species trom China in the eastern Palaearctic region, but in this light the cladistic relationships of the included taxa are also briefly discussed.

MATERIALS AND METHODS

The holotype of *T. ascra* was part of Prof. Gregory's Collection, collected from Tacheng, Yunnan, 7300 ft., in the Open Valley on 1st August 1922 and

deposited at Natural History Museum, London (BMNH) and was examined by the courtesy of Mr Mick Webb, Incharge Hemiptera section of that Museum. The data of other two species *i.e. T. viridula* and *T. yunnana* was scanned from Hsiao and Cheng (1977), Hsiao *et al.* (1978) and Rider *et al.* (2002). For measurements and illustrations the conventional procedures especially those used by Ahmad *et al.* (2004) were used.

RESULTS

Genus Tachengia *China*

Tachengia China 1925: 450, figs. C and D; Hsiao *et al.* 1978: 298, figs. 265, 266; Rider *et al.* 2002: 145.

Head narrowing slightly towards the apex, as long as pronotum and about as long as width across eyes, sides with a small tooth infront of the eyes and a large rounded tooth towards the apex; paraclypei not contiguous apically, much longer than clypeus; latter depressed and narrowed apically, and scarcely passing beyond the anterior lateral tooth of the paraclypei; eyes small, diameter only one third width of head, very prominent, almost stalked; ocelli placed with their anterior margins on a level. with the posterior margin of the eyes; antenniferous tubercles visible from above, first antennal segment short, scarcely reaching the apex of the paraclypei, second nearly 2x the length of the first, third subequal with the second, fourth a third longer than the second, fifth a little shorter than the fourth; labium with basal segment extending slightly beyond the bucculae to the middle of the eyes, second reaching the fore caxae, third reaching the middle coxae and the fourth extending beyond hind caxae. Pronotum 2X as wide as long, anterior margin slightly wider than head including eyes, sides strongly concave, slightly serrate anteriorly and smoothly carinate posteriorly, humeral angles dentiform, rather prominent and some what elevated, base broadly emarginate, anterior half of disc more or less depressed; scutellum with apical third narrowed with the sides more or less parallel, frena extending to base of narrowed apical third; hemelytra with membrane extending beyond the apex of the abdomen; femora umarmed, tibiae simple, not dilated, merosternum carinate, metathoracic ostiolar peritreme auriculate, abdomen with basal median furrow indistinct, pygophore in the male with a large central emargination and two smaller lateral ones, lateroinner tooth on each side prominent (Fig. IB).

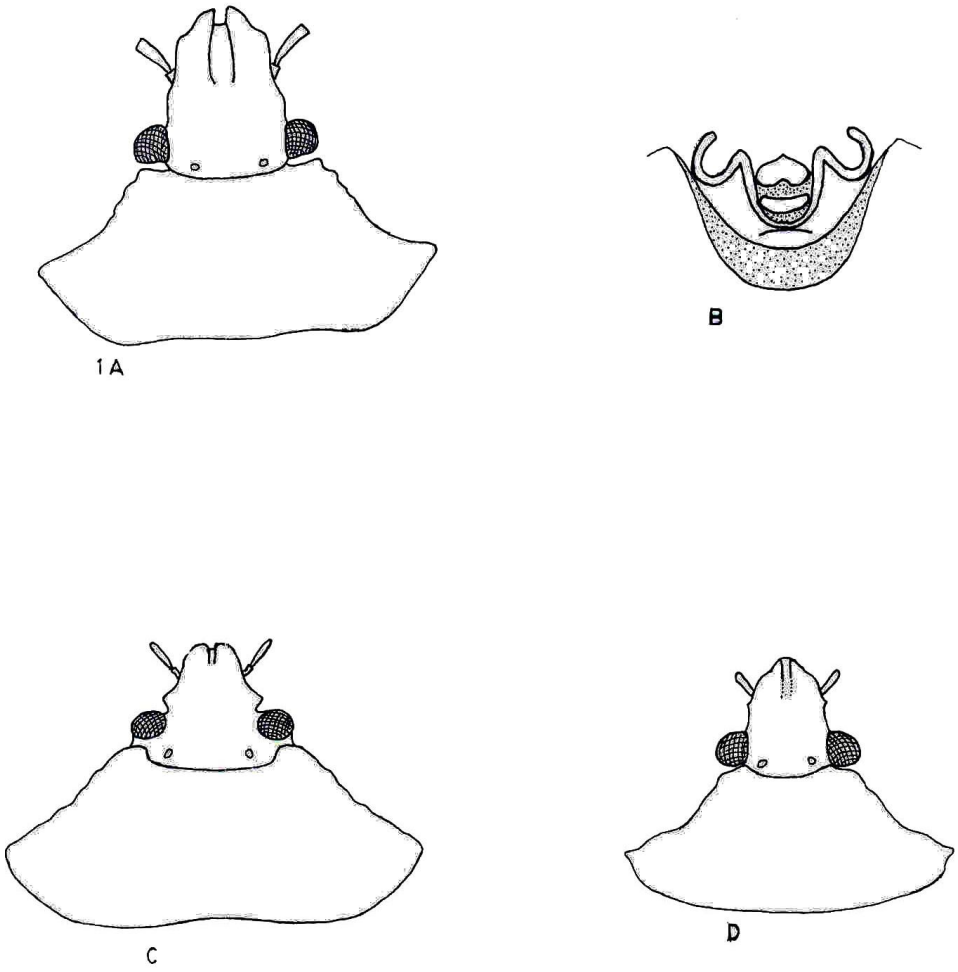


Fig. 1. Head dorsal view: A, *T. ascra*; C, *T. viridula*; D, *T. yunnana.* B, Pygophore, dorsal view: *T. ascra.*

Type species

Tachengia ascra China.

Comparative note

Tachengia is most closely related to the genus *Dalpada* not only in the

auriculate well developed with elongate and curved ostiolar peritreme as noted by the original author (China 1925) but in the structure of head especially in the presence of two teeth at sides, fIrst in front of the eyes and second larger, rounded tooth towards the apex (Fig. lA, lC-D) in the prominent almost substylate eyes, labium passing beyond hind coxae, anteriolateral margins of pronotum crenulated, humeral angles prominently spinose or nodulose and slightly elevated and in male pygophore with medially deep ventral excavation and two smaller lateral ones. The species of *Dalpada,* however, can easily be separated from the species of *Tachengia* in having prominently dilated anterior tibiae. The male genitalia, especially the paramere, as shown by Ahmad and Afzal (1984) of *Dalpada* species and as shown by Hsiao and Cheng (1977) of *T. achengia* species also clearly separate these very closely related generic sister groups.

Key to the species of *Tachengia* China

1. Body much longer, measuring 18 mm in length, head pronotum and scutellum metallic green in colour ... *T. viridula* Hsiao and Cheng (Guizhou, Sichuan, Xizang, Yunnan, China)
- Body much shorter, measuring upto 13.0 mm in length, head pronotum and scutellum not as above. 2
2. Paraclypei equal to in length, not at all longer than clypeus (Fig. ID), head, pronotum and scutellum brown to purplish brown in colour, pronotum with 4–6 metallic green stripes .... *T. yunnana* Hsiao and Cheng (Xizang, Yunnan, China)
- Paraclypei not contiguous apically, much longer than clypeus, latter depressed and narrowed apically and scarcely passing beyond the anterior lateral teeth of paraclypei, head black with a faint purplish lusture, pronotum and scutellum blackish, flecked with ochraceus .....*T. ascra* China (Tacheng 7,300 ft, Open Valley, Guizhou, Yunnan, China).

DISCUSSION

China's description of *Tachengia* was based on Prof. Gregory's (1922) collection about which he suggested that the bulk of the species taken were typical of Blanford's Transgangetic subregion which includes the forest area of the Himalayas, Assam, N. Burma, Siam (Malaya) and Tonkin (China). Although China (*op. cit.*) himself regarded that there was little evidence of the connection between S.W. Asia and tropical America but he considered that *Tachengia* was more closely related to the American genus *Brochymena* Amyot and Serville than to the oriental forms of the Halyini. China (*op.cit.*) did not mention in which character or characters the two genera closely resembled but probably the resemblances he found in the shape of paraclypei with two teeth i.e. basal in front of eyes and apical towards the apex, in the similar shape of prominent substylate eyes and in the similar form and placement of ocelli. These characters are indeed

specific as noted by Lariviere (1992) in Brochymena. China (1925) differentiated his genus from *Brochymena* in the much less dentate lateral margins of the pronotum and in the greater length of the paraclypei (than clypeus). The latter character also appears to be specific as is evident from the present key and figs. (lA, C, D).

The more dentate lateral margins of pronotum in *Broclymena* certainly represent an apomorphic trait as the large remarkably deep central emargination and the two smaller lateral ones also appear clearly apomorphic characters which not only separate *Tachengia* from *Brochymena* but also link *Tachengia* species with those of *Dalpada.* Although China (*op. cit.*) claimed, as cited above, that *Tachengia* is more closely related to the american genus *Brochymena* than to the oriental forms, he himself in the same paper stressed that metathoracic ostiolar peritreme is auriculate as in *Dalpada.* Presently under comparative note of *Tachengia* it is shown beyond all doubt that the closest ally of *Tachengia* is *Dalpada* for sharing many apomorphic traits. The latter is also IndoMalayan in distribution. Certainly *Dalpada* appears more advanced than *Tachengia* in having dialated anterior tibiae.

Within the genus *Tachengia, T. viridula* and *T. yunnana* probably appear more primitive with paraclypei more or less as long as clypeus in length. Purplish brown colour of head, pronotum and scutellum of *T. yunnana* and 4-6 metallic green stripes on its pronotum shows its autapomorphic traits. *T. ascra* in having reduced size appears probably its sister group. Its head with a faint purplish lusture also probably supports this view.

T. ascra however, appears more advanced than *T. yunnana* with its apomorphies of remarkably reduced clypeus than paraclypei and for its black head, pronotum and scutellum. On the other hand *T. viridula* appears most primitive in this complex with longest body size (18.0 mm in length). However, its slightly reduced clypeus in length than paraclypei and its metallic green head, pronotum and scutellum probably show its autapomorphies.

REFERENCES

AHMAD, I. AND AFZAL, M., 1984. Revision of the Indo-Malayan genus *Dalpada* Amyot et Serville (Halyini, Pentatomidae, Heteroptera). *Zool. Anz.*, **213**: 170-176.

AHMAD, I. AND AHMAD, S. 1993. Revision of the palaearctic genus *Apodiphus* Spinola (Heteroptera: Pentatomidae) harmful to fniits and trees in Pakistan. *Mitt. schweiz. Ges.*, **66**: 219-232.

AHMAD, I. AND MCPHERSON, J.E., 1998. Additional information on male and female genitalia of *Parabrochymena* Lariviere and *Brochymena* Amyot and Serville (Hemiptera: Pentotomidae). *Ann. ent. Soc. Am.,* **91**: 800-807.

AHMAD, I., MEMON, N. AND KAMALUDDIN, S. 2004. A revision of Hayline stinkbug genus *Erthesina* Spinola (Hemiptera: Pentatomidae) from Indo Pakistan subcontinent and their cladistics. *Pakistan J. Zool.,* **36**: 285-293.

CHINA, W.E., 1925. Expedition to Yunnan, with synonymic notes on allied species. *Ann. Mag. nat. Hist. Ser.,* 9. **16**: 449-452.

HSIAO, T.Y. AND CHENG, L.Y., 1977. In Rider *et al.,* 2002 (as cited below).

HSIAO, T.Y., CHENG, L.Y., REN, S.Z. AND JEN, S., 1978. In: Rider *et al.,* 2002 (as cited below).

LARIVIERE, M.C., 1992. Description of *Parobrochymena*, new genus, and redefinition and review of *Brochymena* Amyot and Audinet-Serville (Hemiptera: Pentatomidae), with considerations on natural history, chorological affinities, and evolutionary relationships. *Mem. ent. Soc. Can.,* **163**: 1-75.

RIDER, D.A., ZHENG, L.Y. and KERZHNER, I.M., 2002. Checklist and nomenclatural notes on the Chinese Pentatomidae (Heteroptera). II. Pentatominae. *Zoosysts. Ross.,* **11**: 135-153.

STICHEL, W. 1960-62. Illustrierte Bestimmungstabellan der wanzen. II. *Europa* Hemiptera. *Heteroptera Europae* Berlin, **15-25**: 449-800.

WU, M. 1935. In Stichel, W. (1960-62), as cited above (original not seen).

Proc. Pakistan Congr. Zool., Vol. 24, pp. 131-139, 2004.

NATATOLANA INSIGNIS HOBBINS & JONES 1993 A NEW RECORD OF THE SPECIES OF CIROLANIDAE (ISOPODA: FLABELLIFERA) FROM THE PAKISTAN COAST

REHANA YASMEEN
Department of Zoology, University of Karachi, Karachi-75270, Pakistan

Abstract.- The species *Natatolana insignis* is reported for the first time from the Pakistan coast. *Natatolana insignis* is described and illustrated in detail. A list of the known species of the genus *Natatolana* from Indian Ocean is provided. Synonymy and diagnosis for the species are given.

Key words: *Natatolana insignis*, Cirolanidae, Pakistan coast

INTRODUCTION

The genus *Natatolana* was established by Bruce (1981), for *Ciralana hiritipes* Milne Edwards 1840. The *Natatolana* species has been recorded from temperate and cold temperate waters. The present species was obtained from the gut of a fish belonging to genus *Plectarhynchus,* collected from the fish harbour, Karachi.

Systematic Account

Genus *Natatolana* Bruce, 1981

Natatolana Bruce, 1981b: 957; 1986: 52; Brusca & Iverson 1985:37; Botosaneanu *et al.*, 1986: 412; Wetzer *et al.,* 1987: 2; Brandt, 1988: 102; Kens1ey & Schotte, 1989:139; Brusca *et al.,* 1995: 74; Bruce & Olesen, 1995: 213; Keable & Bruce, 1997:657. Yasmeen, 2003: 16.

Diagnosis

Body 2.5-3.0 times longer than broad, smooth without ornamentation, cephalon with or without minute rostral point. Frontal lamina narrow, 3-4 times longer than wide. C1ypeus flat, labrum narrower than c1ypeus. Pleon of 5 free pleonites, pleonite 5 encompassed by pleonite 4. Pereopods 1-3 mediodistal angles of ischium and merus produced bearing long simple setae, basis of pereopods 4-7 markedly flattened and provided with long setae. Pleopods rami bearing marginal plumose setae except endopod of pleopod 5, Pleopod peduncles

broader than long, proximomedial angle of pleopod 5 endopod weakly lobed. Appendix masculina inserted basally or subbasally.

Following 11 species in the Indian Ocean represents *Natatolana* (after Brian Kensley: 2001).

1. *Natatolana albicaudata* stebbing, 1900. Papua New Guinea. Bombay India (Barnard 1936).
2. *Natatolana anopthalma* (Kussakin & Vasina, 1982a). Kerguelen Is. St. Paul & Amsterdam Is., 1430-1600m (Kensley 1989).
3. *Natatolana hiritipes* (H.Milne Edwards, 1840). South Africa, India) Burma, SE Africa (Barnard 1936).
4. *Natatolana insignis* Hobbins & Jones, 1993. Red Sea, 73-1825m.
5. *Natatolana lurur* Bruce, 1986a. western Australia, 150m.
6. *Natatolana natalensis* (Barnard, 1940). Natal, South Africa; shallow infratidal. Madagascar (Roman 1970).
7. *Natatolana nitida* (Hale, 1952). Kerguelen Is. 75-290m. Crozet Is. (Kensley 1980b).
8. *Natatolana pallidocula* (Kussakin & Vasina, 1982a). Kerguelen Is., 310m.
9. *Natatolana pi/ula* (Barnard, 1955). South Africa, 18-66m.
10. *Natatolana vieta* (Hale, 1925). South Australia. Victoria; Western Australia, New South Wales, Australia 6-156m (Bruce 1986a).
11. *Natatolana viri/is* (Barnard, 1940). Algoa Bay, South Africa) 66-80m.

***Natatolana insignis* Hobbins & Jones, 1993**

(Figs. 1-3)

Cirolana albicaudata, Thielemann, 1910: 8; Richardson, 1910: 5; Nierstrasz, 1931: 152; Barnard, 1936: 152; lwasa., 1956: 14;
Natatolana albicaudata, Bruce, 1981b: 958; 1986:71;
Natato/ana insignisi Hobbins & Jones, 1993:11.

Material examined

Dissected male, 10.0mm from the gut content of the fish *Plectorhynchus* 100males, 8.0-10.5mm, 12 females, 8.0-9.0mm, same data as of dissected male.

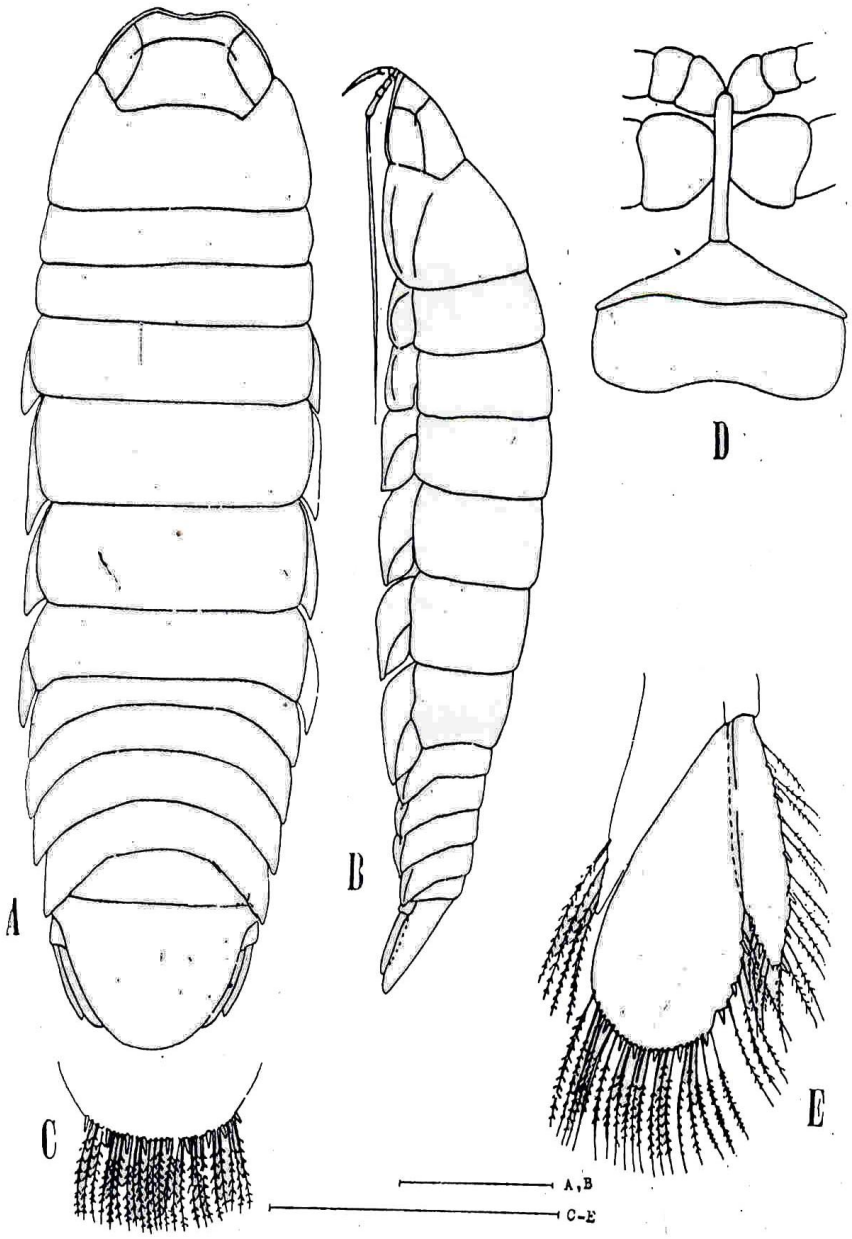


Fig. 1. *Natatolana insignis* male, 10.0 mm. A, dorsal view; B, lateral view; C, pleotelson apex; D, clypeal region; E, uropod. Scale 1.0 mm.

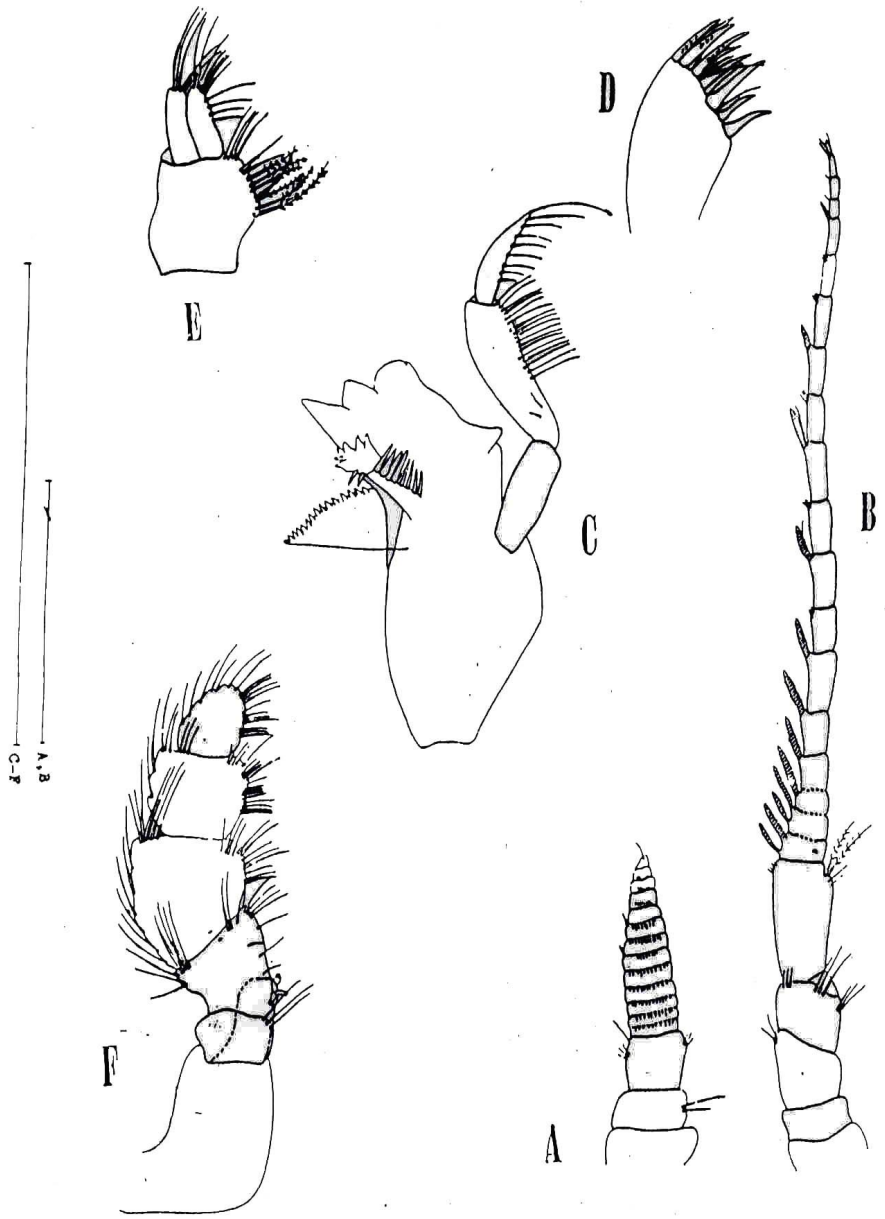


Fig. 2. *Natatolana insignis* male, 10.0 mm. A, antenna 1; B, antenna 2; C, mandible; D, maxilla 1; E, maxilla 2; F, maxilliped. Scale 1.0 mm.

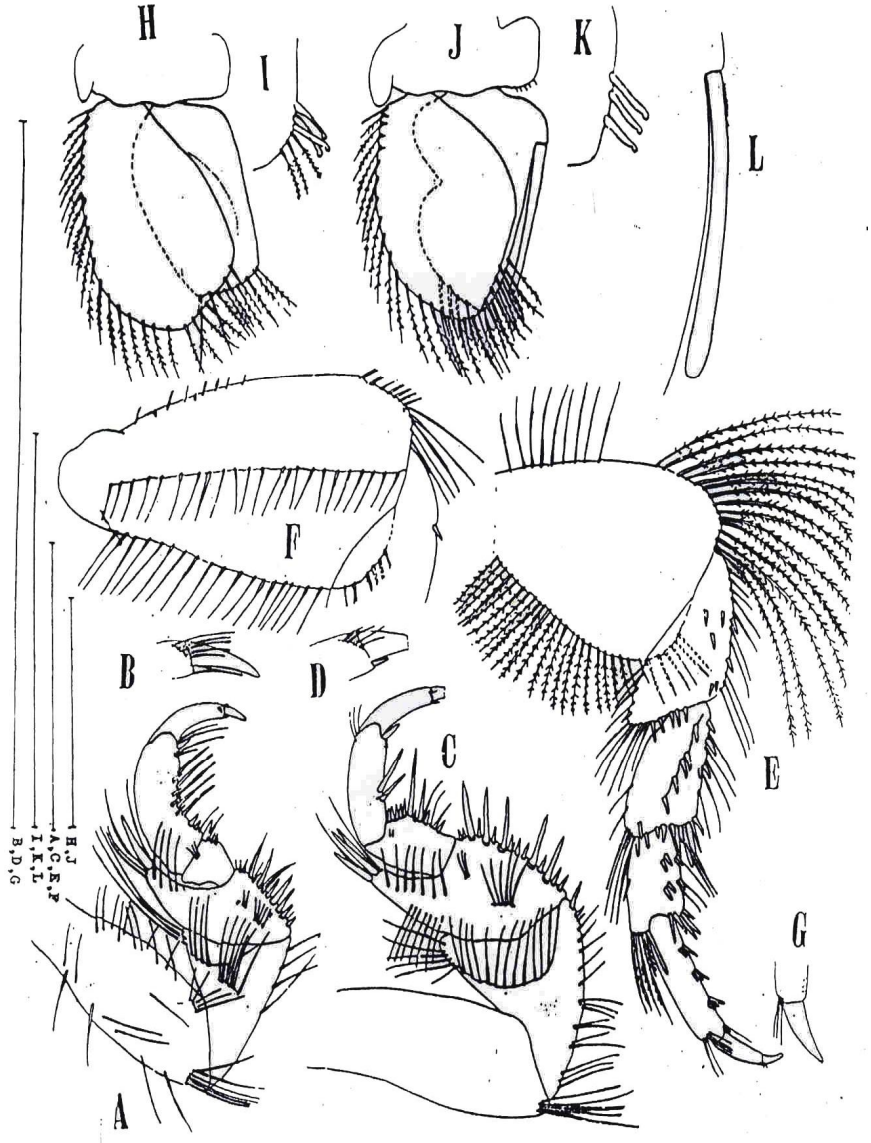


Fig. 3. *Natatolana insignis* male, 10.0 mm. A, pereopod 1; B, pereopod dactylus; C, pereopod 2; D, pereopod 2 dactylus; E, pereopod 7; F, pereopod 7 basis; G, pereopod 7 dactylus; H, pleopod 1; I, pleopod 1 peduncle; J, pleopod 2; K, pleopod 2 peduncle; L, appendix masculina. Scale 1.0 mm.

Diagnosis

Cephalon with minute rostral process, pleonites 1-4 posterolateral margin produced and acute. Posterior margin of pleotelson truncate, armed with 9 spines, bearing 3 plumose setae in between every 2 spines, appendix masculina arising subbasally from the medial margin of pleopod 2, distal margin not extending to apex of endopod. Uropod shorter than notch pleotelson, exopod lateral margin with 6 spines and long plunmose setae, distolateral margin of endopod with notch bearing a spine.

Adult male

Body about 3 times as long as wide, surface smooth, without tubercles and setae. Cephalon (Fig. 1A) with minute rostral process. All coxae (Figs. 1A,B) with entire carinae, 4-7 visible dorsally, posterolateral angles of coxae 6 and 7 acute, pleonites 1-4 each with a lateral longitudinal carina, pleotelson shorter than pleon, lateral margin convex, postererior margin (Fig. 1C) truncate, armed with 9 spines bearing 3 plumose setae between 2 spines.

Frontal lamina (Fig. 1D) narrow, elongated, anterior margin rounded lateral margin straight, parallel, clypeus triangular and flat ventrally.

Antenna (Fig. 1B) short, not extending to postererior margin of eye, peduncle articles (Fig. 2A) I and 2 subequal in length, article 3 longest, flagellum I3-articled. Antenna 2 (Fig. 1B) long, extending beyond posterior margin of pereonite 3, peduncle articles (Fig. 2B) I and 2 short, articles 3 and 4 longer than I and 2, bearing simple setae on distal portions, article 5 longest distolateral margin bearing 2 long plumose and 2 short simple setae, I8-articled, basal 4 articles more or less fused, bearing 6 digitiform process arising from medial margin, proximal 1 and 2 articles each with 2 digitiform process and articles 3, 4, 5, 6, 8, 10, 12 and 15 each bearing single digitform process. Right mandible (Fig. 2C) with broad tridentate incisor, palp article 2 longest, lateral margin fringed with simple setae, article 3 with setae. Maxilla 1 (Fig. 2D), lateral lobe with 10 apical spines and 3 setae. Maxilla 2 (Fig. 2E) medial lobe with 6 plumose and 7 simple and long setae, central lobe bearing many marginal simple setae, lateral lobe with 5 long simple setae. Maxilliped (Fig. 2F) palp article 1 with 3 simple setae on distomedial margin, articles 2-5 with both margins setose endite with 3 coupling spines only.

Pereopods (Figs. 3A- 3D) 1-2 biunguiculate, pereopods 3-7 (Fig. 3E - 3G) without accessory unguis.

Penes absent.

Pleopod 1 (Fig. 3H) endopod truncate and narrower distally than exopod, peduncles of pleopod 1 and 2 (Fig. 3J) broader than long, lateral margin produced into lobes, medial margin of pleopod I peduncle (Fig. 3I) with 3 coupling spines, and 3 plumose setae, that of pleopod 2 (Fig. 3K) bearing only 3 coupling spines, appendix masculina (Fig. 3L) arising subbasally from the medial margin of pleopod 2 endopod not extending to the distal margin of endopod.

Uropod (Figs. 1A,E) not extending to apex of pleotelson, peduncle medial angle strongly produced, and acute, distomedial margin of peduncle with 4 long plumose setae, exopod shorter than endopod, distal margin pointed bearing a spine, lateral margin with 6 spines and long plumose setae, distomedial margin with 2 spines among long plumose setae, more than half proximolateral margin of endopod straight and naked, distolateral margin with 3 spines, long plumose setae, and a small notch distally, distal margin rounded bearing 6 spines among long plumose setae, medial margin without spines and setae.

Remarks

The present material agrees closely with the description and Illustrations given by Hobbins and Jones (1993) especially in one of the most interesting characters of *N. insigins,* the unusual form of antennal flagellar segments, which are bundles of elongate aesthetascs.

Distribution

N. insignis is centred on the West Indian Ocean which records from the Red Sea and Gulf of Oman (John Murray Expedition), (Hobbins and Jones) 1993, Barnard, 1936). Present materials extends the known range northwards in the Indian Ocean to Pakistan, northern Arabian Sea.

ACKNOWLEDGEMENTS

The author wish to express her hearty thanks to Dr. Niel L. Bruce Marine Biodiversity and systematic, National Institute of Water & Atmosphere Research,

New Zealand for identification of species and providing helpful comments. Specials thanks are due to Prof. Dr. Waqar Javed, Ph.D. Supervisor, who always. ready to help me in research.

REFERENCES

BARNARD, K.H., 1936. Isopods collected by R.I.M.S. "Investigator". *Rec. India Mus.*, **38**: 147-191. Calcutta.

BOTOSANEANU, L.N.L., BRUCE AND NOTENBOOM, J., 1986. Isopoda: Cirolanidae:. In: *Stygofauna Mundi* (ed. L. Botosaneanu), pp. 412-422. E.J. Brill, Leiden, Netherlands.

BRANDT, A., 1988. Antarctic Serolidae and Cirolanidae (Crustacea: Isopoda) new genera, new species and redescription. Keoltz Scientific Books, Koenigstein (Theses Zoologicae. Vol. 10).

BRUCE, N.L., 1986. Cirolanidae (Crustacea: Isopoda) of Australia: Diagnosis of *Cirolana* Leach, *Metacirolana* Nierstrasz, *Neocirolana* Hale, *Anopsilana* Paullina Debouteville and three new genera. *Natatolana, Politolana* and *Cartetolana. Aust. J. Mar. Freshw. Res*., **32**: 945-966.

BRUCE, N.L., 1986. Cirolanidae (Crustacea: Isopoda) of Australia. *Rec. Austral. Mus. Suppl.* **6**: 1-239. Sydney.

BRUCE, N.L. AND OLESEN, J., 1995. *Natatolana mukumbutho*, a new species (Crustacea: Isopoda: Cirolanidae) from deep water of Suva, Fiji. *Proc. Biol. Soc. Washington*, **108**: 212-219.

BRUSCA, R.C. AND IVERSON, E.W., 1985. A guide to the marine Isopoda Crustacean of Pacific Costa Rice. *Rev. Biol. Trop*., **33**: Supplement 7: 1-77.

BRUSCA, R.C., REGINA, W. AND FRANCE, S.C., 1995. Cirolanidae (Crustacea: Isopoda: Flabellifera) of the tropical eastern Pacific. *Proc. San Diego Soc. Nat. Hist*., pp. 1-96.

HOBBINS, C.ST.C. AND JONES, D.A., 1993. New species of Dead Sea Isopods from the Red sea and North West Indian Ocean: families Cirolanidae and Corallanidae. *Senek. Mari*., **23**: 115-134.

IWASA, M., 1965. Isopod Crustaceans collected by the surveying ship Soyo maru on the continental shelf bordering Japan during the years 1926-1930. *Bull. seikei Univ*., **3**:13-19.

KEABLE, S.J. AND BRUCE, N.L., 1997. Redescription of the Northern Atlantic and Mediterranean species of *Natalolana* (Crustacea: Isopoda: Cirolanidae). *J. Mar. Biol. Assoc. U.K.*, **77**: 658-705.

KENSLEY, B. AND SCHOTTE, M., 1989. *Guide to the marine isopod crustaceans of the Caribbean*. Smithsonian Institution Press, Washington, DC.

KENSLEY, B., 2001. Biogeography of the marine isopoda of the Indian Ocean with a checklist of species and records. In: *Isopod systematics and evolution. Crustacean Issues*, vol. 13, pp. 205-264.

NIERSTRASZ, H.F., 1931. Die Isopodan der Sibogo-Expedition 3. Isopoda Genuina 2. Flabellifera. *Sibogo-Exp. Monogr*., **32C**: 123-233.

RICHARDSON, H., 1910. Marine isopods collected in the Philippines by the United States Fisheries steamer "Albatross" in 1907-1908. *Dept. Comm. Lab. Bur. Fish. Doc*., **736**: 1-44.

THIELEMANN, M., 1910. Beitrage zur kenntnis der isopodan fauna ostasiens. Biitrage zur Naturgeschichte Ostasiens. *Abh. Math. Physik. Klasse Koenig. Bayer. Akad. Wissensch.*, **3**(Suppl. 2): 1-110.

WETZER, R., DELANEY, P.M. AND BRUSCA, R.C., 1987. *Polito/ana Wickstenae* new species, a new cirolanid isopod from the Gulf of Mexico and the review of the "Cinolera genus group" of Bruce (1986). *Natural History Museum of Los Angeles Country, Contribution in Science*, **392**: 1-10.

YASMEEN, R., 2003. New record of cirolanid genera *Anopsilana* Paulian & Deboutteville, 1956 and *Natato/ana* Bruce, 1981 from Karachi coast, Northern Arabian Sea. *J. nat. hist. Wildl.*, **2**: 11-19.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 141-154, 2004.

A REVISION OF ETHIOPIAN GENUS *MYROCHEA* AMYOT AND SERVILLE (HEMIPTERA: PENTATOMIDAE: PENTATOMINAE: MYROCHEINI) WITH A NOTE ON ITS TAXONOMIC POSITION AND PHYLOGENETIC RELATIONSIPS

IMTIAZ AHMAD AND MUHAMMAD ZAHID
Department of Zoology, University of Karachi (IA) and Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal, Karachi (MZ)

Abstract.- *Pentatoma oculeata* Westwood the type species of the genus *Myrochea* Amyot and Serville as well as type species of sub genus *Myrochea sensu stricto, sensu* Linavouri 1982, *Sciocoris cribrosa* KIug the type species of *Dorpius* Distant and *Dymantis subvittatus* Stål the type species of the genus *Dymantis* Stål as well as the type species of the subgenus *Eomyrochea* Linnavuori 1982 and *Neodius aethiopicus* Distant the type species of *Stysicoris* Ahmad and Kamaluddin 1985, all the four species placed under the genus *Myrochea* and under its two subgenera *viz*., *Myrochea sensu stricto* and *Eomyrochea* by Linnavuori 1982 are redescribed and keyed with their respective genera and tribes Aeliini Stål which is Dymantini Distant and Aeptini Stål with special reference to their genitalia and in this light their cladistic relationships are also briefly discussed. *Myrochea* appears to be a monotypic genus and the type genus of the tribe Myrocheini Stål including the genera *Dorpius* and *Stysicoris* but *Dymantis* with its type species *D. subvittatus* is clearly excluded from this tribe and *Eomyrochea* is synonymised with *Myrochea* as a superfluous and unnecessary subgeneric name.

Key words: *Myrochea*, Heteroptera, Pentatomidae.

INTRODUCTION

After Linnavuori's (1982) treatment of the genus *Myrochea* Amyot and Serville, it contains two subgenera *Myrochea sensu stricto* with *Myrochea oculeata* (Westwood) the type of the genus and of the subgenus, described by Ahmad and Kamaluddin (1985), *M. cribrosus* (Klug) the type species of *Dorpius* Distant described by Ahmad and Afzal (1989) and *M. aethiopicus (*Distant) described as type species of their new genus *Stysicoris* by Ahmad and Kamaluddin (1985), *M. subvittatus (*Stål) the type species of *Dymantis* and also the designated type species of his new subgenus *Eomyrochaea* by Linnavuori (*op. cit.*). In his recent draft catalogue of Pentatomidae (July, 2004, unpublished and also personal communication) Rider in his Myrocheini not only treated *Dorpius* as junior synonym of *Myrochea* but also included *Dymantis* Stål within this division.

This action of Rider following Linnavuori necessitated the present work which includes a key to separate Aeliini (correct name of Dymantini/Aeptini) alongwith and brief descriptions of their type genera *Myrochea* and *Dymantis (D. subvittatus* is the type species of *Dymantis* and if it is transferred to *Myrochea* the genus *Dymantis* would be a junior synonym of that genus and for the included species of this genus by Linnavuouri (1982) a new generic name would be needed) and *Dorpius* and *Stysicoris* with complete synonymy and with a brief discussion on the cladistic relationships of the included taxa.

Linnavuori (1982) revised *Ethiopean* genus *Myrochea* Amyot and Serville and included the following four species divided into two subgenera as under:

Myrochea sensu stricto including; *Myrochea oculeata* (Westwood), type species of *Myrochea* and of subgenus *Myrochea sensu stricto; Dorpius cribrosus* (Klug), type species of *Dorpius*, thus synonyrnising *Dorpius* with *Myrochea; Neodius aethiopicus* Distant, type species of Ahmad and Kamaluddin's new genus *Stysicoris* placed under Myrocheini but treated by Rider (*op. cit*.) as a synonym of *Myrochea* following Linnavuori (*op. cit*.) who transferred *N. aethiopicus* under *Myrochea* and *Dymantis subvittatus* Stål, type species of *Dymantis* and type species of Linnavuori's new subgenus *Eomyrochea* (thus inadvertently synonyrnising the entire genus *Dymantis* Stål with *Myrochea,* which he did not do because he also transferred *Dymantis* (excluding *subvittatus)* into his Myrocheini.

Rider (*op.cit.*) also recognized *Stysicoris aethiopicus* separate from *Myrochea* in opposition to Linnavuori 1982 but following Linnavuori (*op. cit.*) considered *Dorpius* as a synonym of *Myrochea* and included *Dymantis* under his Myrocheini in agreement with Linnavuori (1982). Therefore to (1) resurrect *Dorpius* as an independent genus separate from *Myrochea* (2) to resurrect *Stysicoris* as an independent genus separate from *Myrochea* and (3) to remove *Dymantis* including its type species *subvittatus* from Myrocheini and transferring it to its original tribe Aeliini the present work was under taken.

MATERIALS AND METHODS

We examined the holotypes of the following species (in contrast to Linnavuori who did not examine any of these holotypes).

1. *Pentatoma oculeata* Westwood after Ahmad and Kamaluddin (1985).
2. *Neodius aethiopicus* Distant after Ahmad and Kamaluddin (1985).

3. *Sciocoris cribrosus* Klug after Ahmad and Afza1 (1989).

We consulted Ståls (1861) original description and that of Linnavuori (1982), based our results on the examination of holotypes and paratypes and examined holotype and paratypes of *Dymantis subvittatus* Stål. We followed the techniques of Ahmad and Kamaluddin (1985) and Ahmad and Afzal (1979, 1989) for measurements, illustrations and description and for examination of female terrninalia and spermatheca and for inflation of aedeagus and examination and illustration of male genitalia that of Ahmad (1986) and Ahmad and McPherson (1990, 1998).

RESULTS

DYMANTIS Stål
Dymantis Stål (Figs. lA-B, 2A, 3A-E, 4A-C, 5A) and also the figs. 85a, 86f, 87d, 88b in Linnavuori 1982

Dymantis Stål 1861: 199; 1865: 79, 110, 1876: 51: Linnavuori 1982: 68 (excluding *D. subvittatus* Stål).

Type species

D. subvittatus Stål (subsequent designation as type species by Kirkaldy (1909).

Head with anteocular region much longer than remainder of head; eyes touching anteriolateral margin of pronotum; ocelli minute and placed farther apart, nearer to the eyes than to each other; labium with 2nd segment longer than posterior two segments together, third segment tumid (very small). Pronotum with anterior margin medially smooth but much shallowly concave upto the eyes, anterior angles subrounded and never pointing anteriad but usually laterad, humeral angles round, posterior margin subround sublaterally and concave medially. Fore femora unarmed. Metathoracic ostiolar peritreme minute. In males, pygophore with prominent or subprominent lateral lobes and without medio-inner process; paramere with large and basally much broader hypophysis in continuation with distal portion of stem, with very little distance between curved bow-shaped blade and inner median process (apophysis). In female terminalia, outer margin of ninth paratergites markedly convex; first gonocoxae with posterior margin sinuate but not uniformly markedly concave.

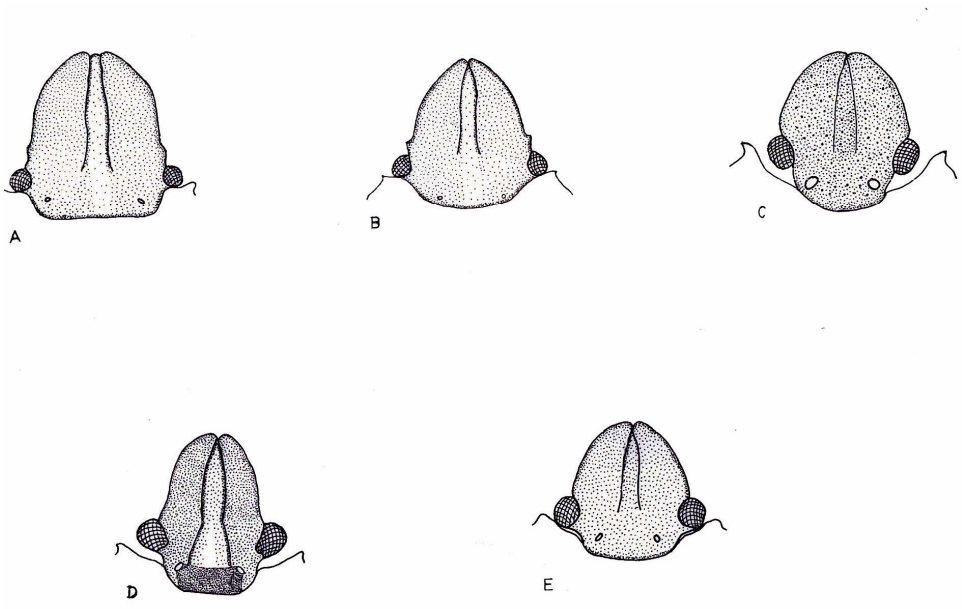


Fig. 1. Head, dorsal view: A, *Dymantis aias*; B, *Dymantis subvittatus*; C, *Dorpius cribrosus*; D, *Myrochea oculeata*; E, *Stysicoris aethiopicus.*

Materials examined

Holotype male and paratypes 2 females with labels "South Africa", "Caffraria" and J. Wahlberg in Stockholm Museum, Sweden.

Myrochea oculeata (Westwood)
(Figs. lD, 2B, 3G, 4E, 5C) and also the figs. 5-9, 13 of
Ahmad and Kamaluddin 1985

Pentatoma oculeata Westwood 1837: 44; *Myrochea oculeata,* Stål, 1865: 177; Kirkaldy 1909: 207; *Myrochea vittata* Amyot and Serville, 1843: 136.

Type species

Myrochea oculeata (Westwood) subsequent type designation by Stål 1865.

Head with anteocular region almost equal to remainder of head; eyes not touching anteriolateral margin of pronotum; ocelli well developed and placed

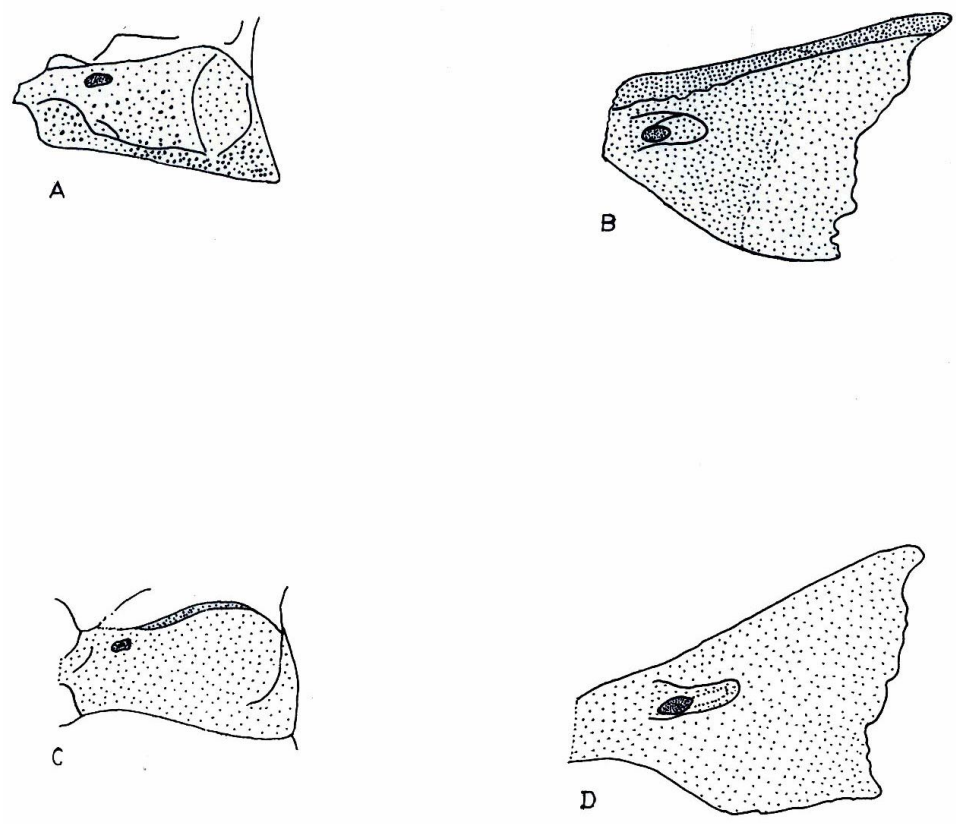


Fig. 2. Metathoracic scent ostiole and ostiolar peritreme, ventral view: A, *Dymantis grisea*; B, *Myrochea oculeata*; C, *Dorpius cribrosus*; D, *Stysicoris aethiopicus.*

nearer to each other and farther apart from the eyes; labium with 2nd labial segment shorter than posterior two segments together, third segment not tumid. Pronotum with anterior margin medially deeply concave, sublaterally shallowly concave, anterior angles tooth-like directing anteriolaterad, humeral angles acutely produced laterad, posterior margin remarkably sinuate sublaterally and deeply concave medially. Fore femora armed with distal spines. Metathoracic ostiolar peritreme short but visible. In males, pygophore with prominent lateral lobes and with medio-inner processes; paramere with basally narrower apophysis in continuation with basal portion of blade, inner median processes (apophyses) not in close association with curved blade. In female terminalia, outer margin of ninth paratergites convex; first gonocoxae with posterior margin straight.

Material examined

Holotype male, with labels *"Pentatoma oculeata* Hope 1837", "Cat. Hope 1: 44" in Hope Museum, Oxford, 1 male Nuer Dist. B. Cl. 6th V.H. Fergussen, 7.9.23 "Ent. Coll. 1937", "Pres. By Imp. Bur. of Ent.", *"Myrochea oculeata* (West.) var. det. B. Uvarov, 1927-209", in British Museum (Natural History) London; 1 a Senegal, "Coil. Star', serial Nos. 313-316", in Riksmuseum, Stockhom, 2 a, 2 ~ Senegal with labels *"Myrochea oculeata* (Westwood)", det., G. Schmitz" in Musee Roay del. Afrique Centrale, Tervuren, Belgium.

Dorpius cribrosus (Klug)
(Figs. lC, 2C, 3F, 4D, 5B) and also the figs. 1-3,6-7 of Ahmad and Afzal 1989

Sciocoris cribrosus Klug, 1845: 44; *Dorpius cribrosus,* Kirkaldy 1909: 182; Linnavuori, 1975: 29; *Myrochea cribrosus,* Linnavuori 1982: 70. *Dorpius cribrosus:* Ahmad and Afza11989: 249. *Dorpius typicus* Distant 1900: 165.

Type species

Dorpius cribrosus (Klug) original designation by Distant 1900 of *D. typicus* synonymised with *cribrosus* Klug by S. Schouteden (in Rider 151 July 2004, unpublished).

Head with anteocular region slightly longer that remainder of head; eyes not touching and not nearer to anteriolateral margin of pronotum; ocelli well developed and placed more or less at equidistance from each other and to the eyes; labium with 2nd segment usually not longer than posterior two segments together, third segment not tumid. Pronotum with anterior margin medially remarkably concave and sublaterally slightly concave, anterior angles toothed directing anteriad, humeral angles subround, posterior margin substraight sublaterally and medially. Fore femora armed distally. Metathoracic ostiolar peritreme very minute with only ostiole visible. In males, pygophore with prominent lateral lobes and with medio-inner processes; paramere with basally narrower apophysis in continuation with basal portion of blade, former not in close association with curved distal portion of the blade. In female, terminalia with outer margin of 9th paratergites sinuate, first gonocoxae with posterior margin sublaterally deeply concave, medially convex.

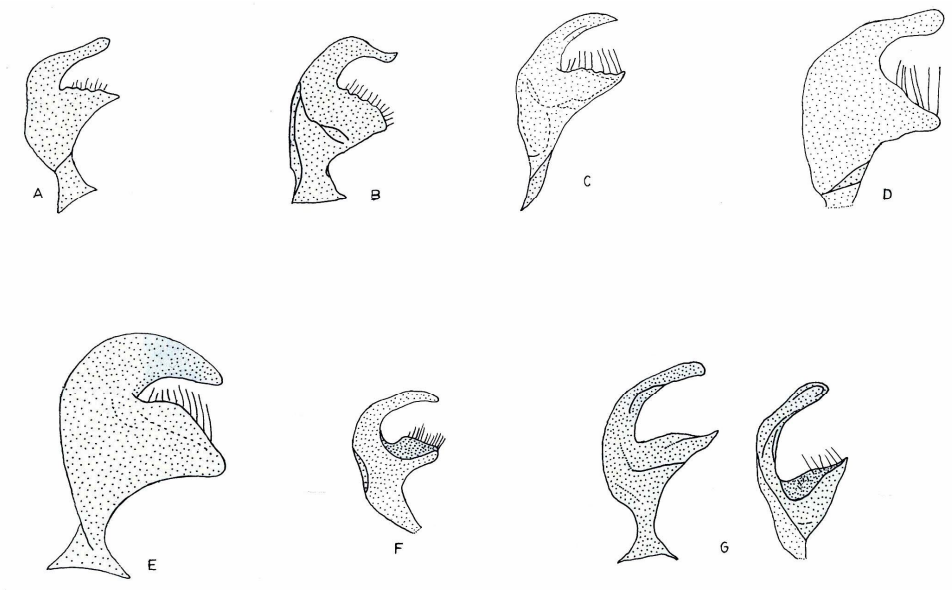


Fig. 3. Paramere, inner view: A, *Dymantis aias;* B, *Dymantis grisea*; C, *Dymantis plana*; D, *Dymantis relata*; E, *Dymantis subvittatus*; F, *Dorpius cribrosus*; G, *Myrochea oculeata.*

Material examined

Holotype Congo "Dist. Coll." at BMNH l male, Arabia: Shuqaiq, "7.11.1937" II. st. J.B. Philly, "1937-228 in BMNH; 2 females, Arabia: Asir. nr. Sabya, "1-1945," "A.R. Waterstone," "1947-349," "at light, in BMNH.

Stysicoris aethiopicus (Distant)
(Figs. 1E, 2D, 4F) and also the figs. 1-3 of Ahmad and Kamaluddin 1985

Neodius aethiopicus Distant 1903: 469; *Caystrus aethiopicus*, Bergroth (in Rider *op. cit.*) *Stysicoris aethiopicus* (Distant) Comb. n. Ahmad and Kamaluddin 1985: 2.

Type species

Stysicoris aethiopicus (Distant) by monotypy.

Head with anteocular region almost equal to remainder of head; eyes not

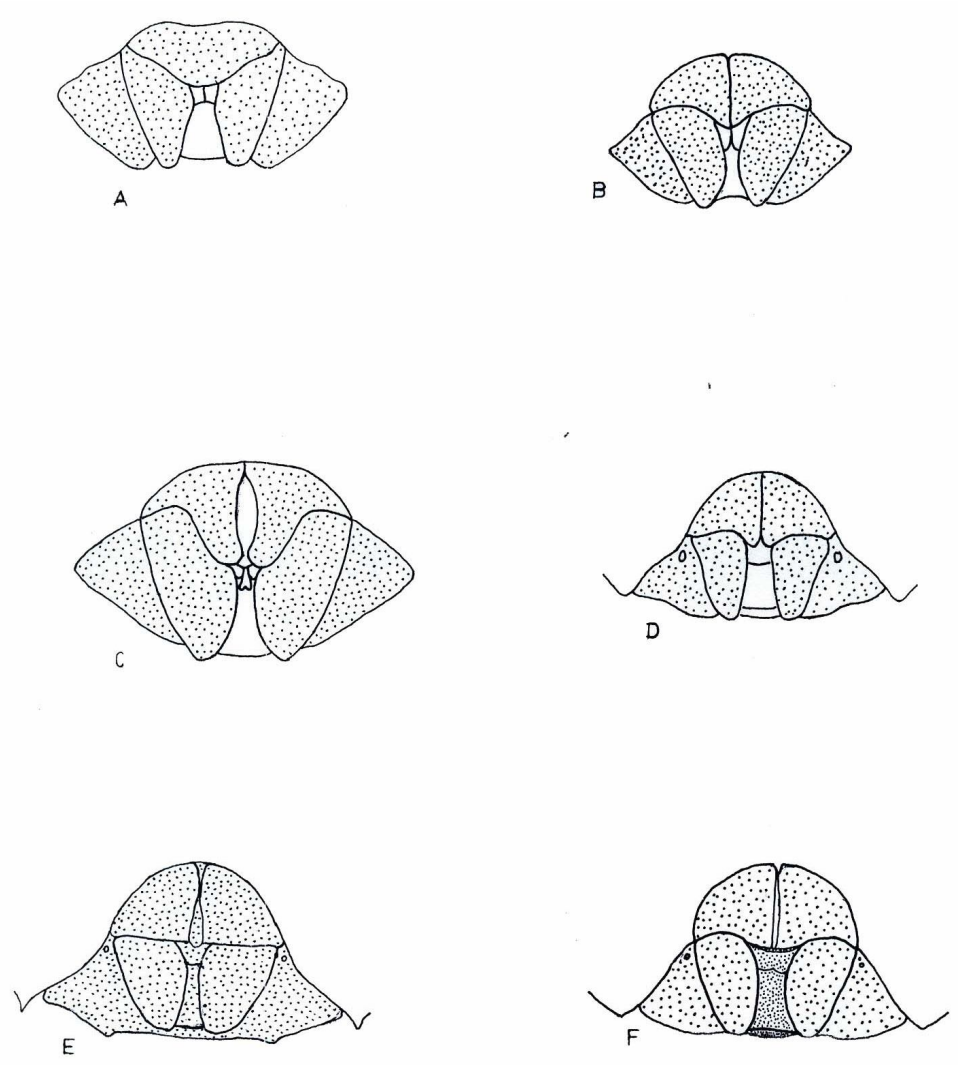


Fig. 4. Female terminalia, ventral view: A, *Dymantis aias*; B, *Dymantis grisea*; C, *Dymantis plana*; D, *Dorpius cribrosus*; E, *Myrochea oculeata*; F, *Stysicoris aethiopicus.*

touching but very nearer to anteriolateral margin of pronotum; ocelli more developed and placed nearer to each other and farther apart from eyes; labium with 2nd segment shorter than posterior two segments together, third segment not tumid. Pronotum with anterior margin medially deeply concave, sublaterally

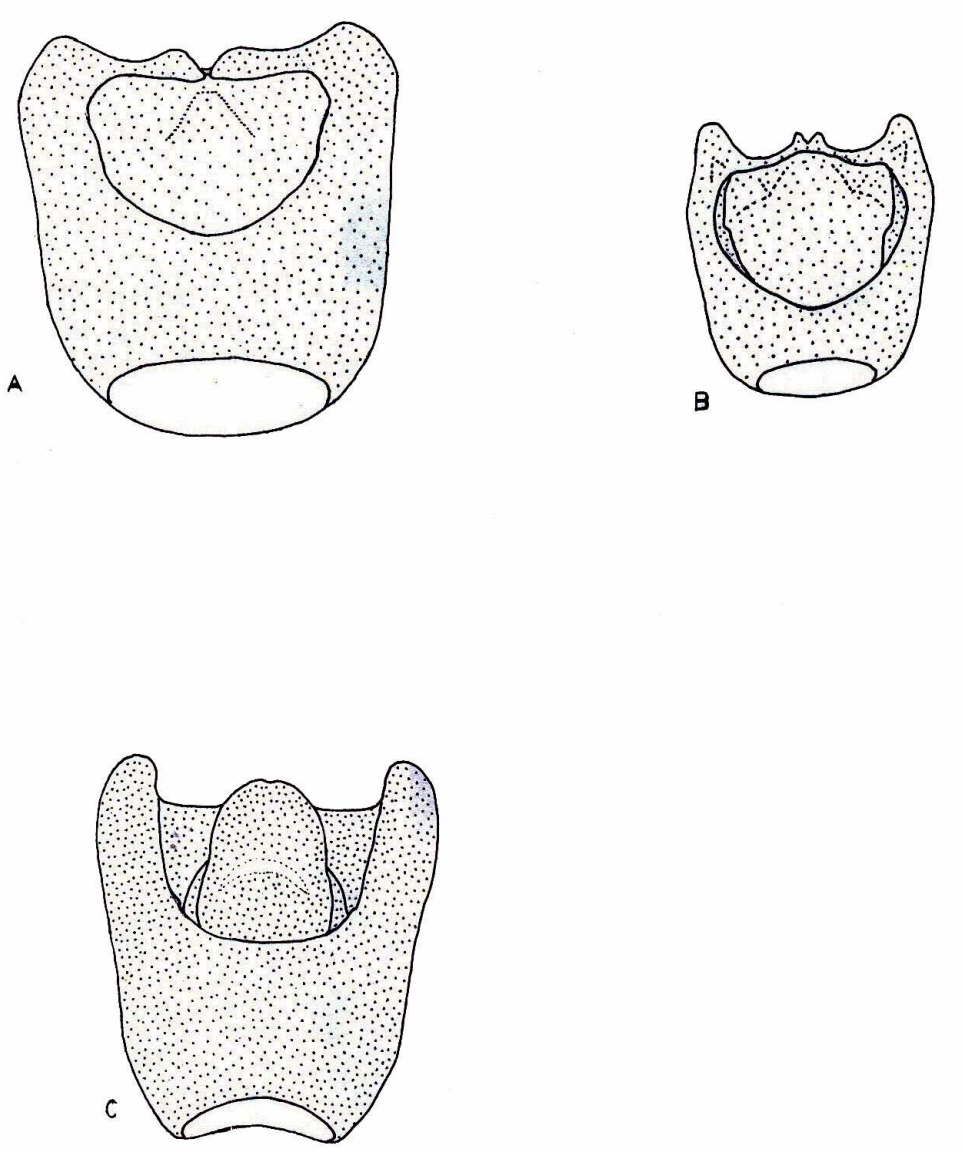


Fig. 5. Pygophore, dorsal view: A, *Dymantis subvittatus*; B, *Dorpius cribrosus*; C, *Myrochea oculeata.*

shallowly concave, anterior angles subround, directing anteriolaterad, humeral angles subround, posterior margin sinuate sublaterally and deeply convace

medially. Fore femora armed distally. Metathoracic ostiolar peritreme invisible, ostiole distinct. In female terminalia outer margin of ninth paratergites *substraight* or *sinuate,* first gonocoxae with posterior margin sublaterally deeply concave and medially convex.

Material examined

Holotype female, with labels *"Neodius aethiopicus* Distant", Brit. Mus.", "Dist. CoIl." In British Museum (Natural History), London; 1 female, with labels *"Neodius aethiopicus* Distant", "det. and compared by (Late) M.R.J. Izzard 1965", "P. Vanderijst" in Koninklijk Museum V oor Midden Afrika, Tervuren, Belgium.

KEY TO THE INCLUDED SPECIES

1. Second labial segment longer than apical two segments together, third tumid, ocelli reduced, placed farther apart from each other than from the eyes, larter touching anterior margin of pronotum, metathoracic ostiolar peritreme remarkably reduced and dorsolateral lobes of pygophore ill-developed ..... ……. Tribe Aeliini (Aeptini/ Dymantini) ..... *Dymantis subvittatus* Stål (South Africa: Caffraria; Caffrarian range)
- Second labial segment distinctly shorter than apical two segments together, third not tumid, ocelli and other characters not as above..... "Tribe Myrocheini 2
2. Anterior angles of pronotum markedly projected anteriorly ..... *Dorpius* Distant……...*Dorpius cribrosus* (Klug) …….. .....(Sohelian and Eremian range (the Sudan, Somalia, Arabia), Indo-Pakistani areas)
- Anterior angles of pronotum not as above, slightly and laterally or anteriolaterally projected 3
3. Membrane of hemelytra distinctly exposing posterior most portion of ninth abdominal tergum, humeral angles of pronotum smoothly rounded ..... Stysicoris Ahmad and Kamaluddin …….*Stysicoris aethiopicus* Distant ..... (Nigeria: Abutshi River, Guinea: Comkry, Camayen Ivory Coast, Foro-Foro West Sudanese region)
- Hemelytra distinctly concealing posterior portion of ninth abdominal tergum, humeral angles of pronotum atleast angulate, spinously projected in holotype ..... …….*Myrochea* Amyot and Serville ..... *Myrochea oculeata* (Westwood) (Nigeria: W. St: He-Ife, Ibadan, Ivory Coast: Foro-Foro)

DISCUSSION

Linnavuori (1982) primarily synonymised *Dorpius* Distant basing his conclusion only on the Ethiopian type species *S. cribrosus* Klug with *Myrochea.* He also transferred *N. aethiopicus* Distant under *Myrochea* primarily because he found variation in the shape of humeral angles of pronotum in *Myrochea oculeata* (Linnavuori, 1982: Figs. 91, 92). His obvious .misinterpretation that

lateral margins curved and humeral angles rounded in the species of *Dorpius*, straight or insinuated with small apical tooth and sharp humeral angles in *Myrochea oculeata* was not sufficient for keeping the genera apart, owing to great individual variability in the shape of pronotum in *Myrochea oculeata* especially since the structure was otherwise similar.

Ahmad and Kamaluddin (1985) justified their action of erecting a new genus *Stysicoris* for *aethiopicus* noting that it strikingly differs from all the taxa described under *Dorpius* (and also under *Myrochea)* in having pronotum only obsoletely produced anteriolaterad and labium much longer, reaching the 3rd abdominal sternum in addition to several genital characters and metathoracic scent apparatus with a small but distinctly visible ostiole (see also present description and key). Distant (1902) and Ahmad and Afzal (1989) have illustrated these characters in all the species of *Dorpius* from Indo-Pakistan area and it is remarkably different not only from *S. aethiopicus* but also from *Myrochea oculeata* and *Dymantis subvittatus* Stål. In none of his Illustration showing great individual variability, Linnavuori (1982, Figs. 91, 92) has shown similar shape of anterior angles of pronotum as shown in all the species of *Dorpius* by Ahmad and Afzal (*op. cit*.), Ahmad and Kamaluddin (1985) or even by Linnavuori (1982, Fig. 92) in his *N. aethiopicus.*

On the other hand, Linnavuori (1982) obviously has excluded *subvittatus* Stål from *Dymantis* Stål and has designated it as type species of his subgenus *Eomyrochea* thus not only placing *subvittatus* under *Myrochea* Stål but also transferred it to the tribe *Myrocheini* Stål from Aeliini Stål (the correct name for Aeptini Stål/Dymantini Distant) Ahmad *et al.* (1974), Ahmad (1979) and Ahmad *et al.* (1979) and Stål (1867, 1871, 1876) clearly separated his Aeptini from closely related *Myrocheini* (which according to him included *Aeptus* Dallas, *Dymantis* Stål, *Menestheus* Stål and *Eribotus* Stål) by the more elongate second labial segment longer than apical two together and the femora usually being unarmed. Surprisingly, Linnavuori (1982) not only separated his *Dymantis* (excluding *subvittata)* from *Myrochea* on this very character (*i.e.* second labial segment much longer than apical two segments together and third segment tumid in *Dymantis* and second labial (he inadvertently wrote antennal instead of labial) segment generally shorter (42:50) than third and fourth segments together; if shorter, then third segment not tumid.

Linnavuori (*op. cit*.) also separated his subgenus *Eomyrochea* from his subgenus *Myrochea sensu stricto* using the same above character which he used to separate his *Dymantis* (excluding *subvittatus)* from *Myrochea i.e.*, second

labial segment longer than third and fourth segments together and third segment tumid in monotypic *Eomyrochea* with only *subvittatus* as its type species but second labial segment shorter than third and fourth segments together and third not tumid in *Myrochea sensu stricto.* This situation appeared actually because it is type species of *Dymantis* and was placed in *Dymantis* by Stål (*op.cit.*) and therefore also has the same labial character as in other species of *Dymantis* and different from *Myrochea oculeata* as noted by L innavuori (*op.cit.*) and earlier by Stål (1871, 1876). Also its fore femora is unarmed as in other species of *Dymantis* and different from that of *Myrochea* (not noted by Linnavuori (*op. cit*.) but noted by Stål (*op. cit*.). Many other characters of *subvittatus* in head, pronotum, metathoracic scent auricle and male and female genitalia as per present description and key also support these facts.

No doubt *subvittatus* shares the similar shape of paraclypei and clypeus with *oculeata* and different from that in *D. aias* (L.) (Fig. lA-B, D) *i.e.*, apical part of clypeus concealed by paraclypei (not by geneae as noted by Linnavuori (*op. cit*.) but this character does also vary from species to species in *Dorpius* and *Laprius* and on this basis these two genera alongwith *Myrochea* should not be synomised with *Sciocoris* (because its species also share this character) the type genus of a closely related tribe Sciocorini (Ahmad *et al.,* 1996).

Linnavuori (*op. cit*.) should also have noticed the shape of head in his own illustrations that ocelli in all the species of *Dymantis* (including *subvittatus)* and in fact in those of the tribe Aeliini (Aeptini/Dymantini) are minute (reduced as Linnavuori described them) and farther apart from each other than to the compound eyes in comparison to all the species belonging to Myrocheini (*i.e. Myrochea* (excluding *subvittatus), Dorpius* and *Stysicoris).* The characters of metathoracic ostiolar peritreme much reduced in those of *Dymantis* including *subvittatus* and in other species of Aeliini in comparison to atleast slightly developed in other species of Myrocheini (see as above) (Figs. lA-D) and other characters of genitalia as presently noted in the description and key (Figs. 4A-F, 5A-C).

The characters of reduced ocelli, reduced ostiolar peritreme, tumid third labial segment, reduced dorsolateral lobes of pygophore all indicate that *Dymantis* (including *subvittatus)* is more advanced than *Myrochea* and its allies (i.e. *Dorpius* and *Stysicoris).* The smaller size of aelliines (including that in *Dymantis* spp.) and fore femora with spines probably lost, all support this conclusion.

Within Myrocheini, *Dorpius* spp. appear most advanced with anterior angles of pronotum markedly projected anteriad (Fig. 1C) remarkably reduced metathoracic ostiolar peritreme (Fig. 2C) and quite reduced dorsolateral lobes of pygophore (Fig. 5B). The smaller size of *Dorpius* spp., (Ahmad and Afzal 1989, Fig. 1) and reduced size of labium never quite reaching hind coxae also seem to point out in this direction. *Stysicoris* appears more advanced than *Myrochea* in having remarkably reduced hemelytra exposing most of connexiva and posterior portion of ninth abdominal tergum (Ahmad and Kamaluddin, 1985, Fig. 1) and labium much longer reaching third abdominal venter. Its comparatively smaller size (12.4 mm) and its endemic distribution confined to western sudanese area also probably support this conclusion. On the other hand, in *Myrochea* more developed hemelytra passing beyond abdomen (Ahmad and Kamaluddin 1985, Fig. 5) and its comparatively larger size (13.8 mm) all show it to be more primitive in its tribe Myrocheini. Its wider distributional range in Holosudanese region also support this stand. Its mostly spinose or nodulose humeral angles probably is its autapomorphy.

REFERENCES

AHMAD, I., 1979. A revision of the check-list of Coreidae and Pentatomidae of the superfamilies Coreoidea and Pentatomoidea (Heteroptera: Pentatomomorpha) from Pakistan with phylogenetic considerations. *Karachi ent. Soc. Suppl.,* **4**: 1-113.

AHMAD, I., 1986. A fool-proof technique for inflation of male genitalia in Hemiptera. *Insecta. Pakistan J. Ent. Kar.,* **1**: 111-112.

AHMAD, I. AND AFZAL, M., 1979. Resurrection of the tribe *Caystrini* Ståls (Heteroptera, Pentatomidae, Pentatominae) with description of two new genera from Oriental region. *Annotnes zool. bot. Bratislava,* **133**: 1-14.

AHMAD, I. AND KAMALUDDIN, S., 1985. A new genus for *Caystrus aethiopicus* (Distant) (Pentatomidae: Pentatominde: Myrocheini) with redescription of *Myrochea oculeata* (Westwood) and their relationships. *Annotnes zool. bot. Bratislava,* **170**: 1-10.

AHMAD, I. AND AFZAL, M., 1989. A revision of Myrocheini (Pentatomidae: Pentatominae) from Indo-Pakistan Area. *Oriental Insect,* **23**: 243-268.

AHMAD, I. AND McPHERSON, J.E., 1990. Male genitalia of the type species of *Corimelaena* White, *Galgupha* Amyot and Serville, and *Cydnoides* Malloch (Hemiptera: Cydnidae: Corimelaeninae) and their bearing on classification. *Ann. ent. Soc. Am.,* **83**: 162-170.

AHMAD, I. AND McPHERSON, J.E., 1998. Additional information on male and female genitalia of *Parabrochymena* Lariviere and *Brochymena* Amyot and Serville (Hemiptera: Penatotomidae). *Ann. ent. Soc. Am.,* **91**: 800-807.

AHMAD, I., ABBASI, Q.A. AND KHAN, A.A., 1974. Generic and supergeneric keys with reference to a check-list of pentatomid fauna of Pakistan (Hemiptera: Pentatomidae) with notes on their distribution and food plants. *Karachi ent. Soc. Suppl.,* **1**: 1-103.

AHMAD, I., AFZAL, M. AND KAMALUDDIN, S., 1979. Anatomy of three Myrocheines (Heteroptera: Pentatomidae: Pentatominae) from Pakistan. *Acta ent.. Bohemoslovaca,* **76**: 219-222.

AHMAD, I., SCHAEFER, C.W. AND ONDER, F., 1996. The first species of Myrocheini (Hemiptera: Pentatomidae: Pentatominae) from the Palaearctic region. *Eur. J. Ent.*, **93**: 255-262.

AMYOT, C.lB. AND SERVILE, J.G., 1843. *Histoire naturelle des insectes. Hemipteres* . 681 pp. 12 plates. Paris: Fain et Thunot.

DISTANT, W.L., 1900. Underscribed genera and species belonging to the Rhynchotal family Pentatomidae. *Trans. ent. Soc.. Lond.*, **1900**: 163-174.

DISTANT, W.L., 1902. *The fauna of British India including Ceylon and Burma,* Rhynchota, 1: 1-438, Taylor and Francis, London.

DISTANT, W.L., 1903. Rhynchotal notes-Heteroptera: Pentatomidae. *Ann. Mag. nat. Hist. Ser.*, **7-12**: 469.

KIRKALDY, G.W. 1909. Catalogue of the Hemiptera (Heteroptera). I. Cimicidae. *Berlin,* 244-246.

KLUG, C.J.F., 1845. In Ehrenberg: *Symbolae physicae seuicones and description insectorum.* V. Berlin: ex officina academica Impresis G. Reimeri: 44.

LINNAVOURI, R.E., 1975.. Hemiptera of Sudan, with remarks on some species of the adjacent countries 5. Pentatomidae. *Bol. Soc. Portuguesa Cienc. Nat.*, **15:** 5-128.

LINNAVUOR!, R.E. 1982. Pentatomidae and Acanthosomidae of Nigeria and the Ivory coast, with remarks on species of the adjacent countries in West and Central Africa. *Acta. zool. fenn.*, **163**: 1-77.

STÅL, C., 1861. Nya Hemiptera, -Ofv. Svenska Vet. Akad. Forhandl. 1861: 199-200.

STÅL, C., 1865. Hemiptera africana I. - Holmiae. 266 pp.

STÅL, C., 1867. Bidragtill Hemipteremas Systematik Ofvers *K. Svenska. Vetensk Akad. Forh.*, **24**: 491-560.

STÅL, C. 1 871. *Enumeratio Hemipterorum* Part I . *K. svenska. VetenskAkad. Handl*., **10**: 1-159.

STÅL, C., 1876. Enumeratio Hemipterorum. *K. Svensk. VetenskAkad. Handl.*, **14**: 1-162.

WESTWOOD, J.O. 1837-42. *Catalogue of Hemiptera in the collection of Rev.* F.W. Hope Part 1 and 2 London.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 155-161, 2004.

SUBLETHAL TOXICITY OF TANNERY EFFLUENTS TO COMMON CULTURABLE FISHES OF PAKISTAN

MUHAMMAD IRFAN KHAN, ABDUL REHMAN, MUHAMMAD RASHID, MUHAMMAD AMJAD SALEEM, NAZIR AHMAD AND ISRAR HUSSAIN SHAH

Department of Environmental Science, Allama Iqbal Open University, Islamabad (MIK), Environmental Research Laboratory, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan (MR, MAS & NA) and Post Graduate Centre of Zoology, Government College, Multan (AR &IHA).

Abstract.- Fingerling stage of four species of common culturable fishes, the grass carp, *Ctenopharyngodon idella* (Valenciennes), the Mori, *Cirrhinus mrigala* (Hamilton), the Rahu, *Labeo rohita* (Hamilton) and the Thaila, *Gibelion catla* (Hamilton) were exposed to a range of sub-lethal concentrations (0, 5, 10 and 20 mlL^{-1}) of leather tannery effluent for 90 days under laboratory conditions in four separate experiments for investigating their effects. The results indicated sublethal toxicity, determined by measuring the growth in terms of increase in mean body length and weight of the fish. The response of each species was variable with respect to sensitivity to different treatment levels but was similar qualitatively. The degree of susceptibility of these fishes to leather tannery effluent were graded as *L. rohita* > *C. idella* > *C. mrigala* > *G. catla*.

Key Words: Sublethal toxicity of chromium, tannery, industrial effluent, culturable fishes, hexavalent chromium.

INTRODUCTION

The effluents from leather tanneries pose a serious threat to human health and to the ecology and biodiversity of freshwater and land. Chromium is one of the heavy metals present in tannery effluent, which is the most hazardous component.

Studies have been carried out on acute as well as chronic chromium toxicity to fish and a variety of responses like effect on enzyme activity, cytogenetic damages, hematological and pathological abnormalities, growth retardation and behavioural abnormalities have been reported (Barron and Adelman, 1984; Babich *et al.,* 1986; Gill and Pant, 1987; McCuloch and Rue, 1989; Bog *et al.,* 1992; Khangarot and Tripathi, 1992; Venugopal and Reddy,

1992, 1993; Roche and Boget, 1993; Sarkar and Konar, 1993, 1995; Al-Sabti *et al.*, 1994; Hutchinson, 1994; Sornaraj *et al.*, 1995; Ali *et al.*, 1996; Srivastava *et al.*, 1996a,b, 1998). The studies describing effects of other chemical component of tannery effluent on fish are also available but none has reported the toxicity of tannery effluent as a whole except Khan and Rashid (2001).

Keeping in view current trends in ecotoxicology the present study was conducted in which four culturable species *i.e.* the grass carp, *Ctenopharyngodon idella* (Valenciennes), the mori, *Cirrhinus mrigala* (Hamilton), the rohu, *Labeo rohita* (Hamilton), and the thaila, *Gibelion catla* (Hamilton) were used.

MATERIALS AND METHODS

Procurement of material

Fingerlings from the same cohort of each fish, that is *Ctenopharyngodon idella, Cirrhinus mrigala, Labeo rohita* and *Gibelion catla* were procured from hatchery of Punjab Fisheries Department at Muzzafargrah and acclimatized to the laboratory conditions for 15 days prior to experimentation. The effluent used in the experiments was taken from outlet drain of Mahar Ghulam Dastgir Leather Tanneries, Nawabpur Road, Multan.

Experimental design

Four separate experiments, one for each species were conducted simultaneously in the same laboratory conditions. Standard 90-day toxicity tests were conducted as recommended by ASTM (1990). For each experiment 16 plastic tubs of 50-litre capacity each were set up in 4 groups, each of 4 replicates. Ten fish were released in each tub, hence a total of 160 healthy and active fishes were exposed to four levels of treatments in each experiment.

Experimental protocol

Fishes were treated with 0, 5, 10 and 20 mlL^{-1} of raw effluent for 90 days and fed regularly *ad libitum* with commercial fish food, about 3% of the body weight twice daily as described by Lanno (1989). Body 1ength and the weight of each fish after 90 days were recorded and used as growth parameter.

Data analysis

The difference in the mean body weight and length of fish of each species after 90 days exposure in different treatments was determined by performing the Analysis of variance (ANOVA) and student's `t' test using SPSS version.

RESULTS AND DISCUSSION

The 90-day treatment of four species of common freshwater fishes with leather tannery effluent in the laboratory conditions showed sublethal toxicity to fishes in terms of mean body weight and length.

Weight

The treatment with tannery effluent affected the weight gain of the fish with the increasing treatment levels. The mean body weights of four fish species treated with different concentrations of tannery effluent are shown in Table I. The mean body weight of *Ctenopharyngodon idella* exposed to 10 mlL^{-1} and 20 mlL^{-1} treatments was significantly low *(F* = 3.391; P<0.05) than the fish in control but not in 5 mlL-1 treatment (*t* = 0.32, P>0.05). In case *of Cirrhinus mrigala* the mean body weights in all three treatments were significantly low *(F* = 3.75; P<0.05) than the control. The response *of Labeo rohita* (*F* = 100.719, P<0.05) and *Gibleon catla* (*F* = 3.73, P<0.05) were also similar and increased with increasing concentration level.

TABLE I.- MEAN BODY WEIGHTS WITH STANDARD ERRORS OF FISH EXPOSED TO TANNERY EFFLUENTS FOR 90 DAYS AND.

Fishes	**Mean Body Weight with SE (gm)**			
	Control	**5 mlL^{-1}**	**10 mlL^{-1}**	**20 mlL^{-1}**
Ctenopharyngodon idella	0.65±0.04	0.60±0.03	0.53±0.027	0.53±0.04
Cirrhinus mirgala	1.22±0.04	1.15±0.03	1.06±0.01	1.0±0.06
Laboe rohita	4.00±.042	3.43±.035	3.31±042	3.04±.042
Gibelion catla	1.18±0.07	1.08±0.08	0.94±0.03	0.84±0.09

Length

The treatment with tannery effluent affected the ability of the fish to grow in length of all species except in *G. catla.* The response in general was also variable and it did not increase with increasing treatment levels. The mean body

length of four fish species treated with different concentrations of leather tannery effluent is shown in Table II.

TABLE II.- MEAN BODY LENGTH WITH STANDARD ERRORS OF FISH EXPOSED TO TANNERY EFFLUENTS FOR 90 DAYS.

Fishes	**Mean Body Weight with SE (cm)**			
	Control	**5 mlL^{-1}**	**10 mlL^{-1}**	**20 mlL^{-1}**
Ctenopharyngodon idella	3.95±0.08	3.84±0.05	3.70±0.05	3.62±0.09
Cirrhinus mrigala	5.29±0.061	5.07±0.06	5.15±0.04	5.07±0.08
Laboe rohita	7.50±0.058	07.24±0.03	07.18±0.05	06.88±0.08
Gibelion catla	5.10±0.10	5.13±0.11	3.71±0.05	4.67±0.18

The response of *Ctenopharyngodon idella* was same as for *G. catla i.e.*, the mean body length of fish treated with leather tannery effluent was significantly short *(*$F = 4.06$; $P < 0.05$) in 10 mlL^{-1} and *20* mlL^{-1} treatments when compared with control but not in 5 mlL^{-1} treatment ($t = 0.40$, $P>0.05$). In case of *Cirrhinus mrigala (*$F = 0.61$, $P<0.05$) and *Labeo rohita* ($F = 16.91$, $P<0.05$), the mean body length was significantly affected than the fish in control. The response increased with the increasing concentration of leather tannery effluent. The mean body length of *Gibleon catla* was short only in 20 mlL^{-1} treatment if compared with control *(*$t = 5.5$, $P<0.05$) but in lower treatment levels, the difference was not significant *(*$F = 2.23$, $P>0.05$).

The results of this study indicate that, if compared with the control, the growth of fishes is significantly affected. The response of four species was variable to the same treatment level. This may be either due to variation in susceptibility of each species or adapted tolerance of some of the species as described by Ali *et al.* (1996) that living system has the ability to defend itself by induction of various mechanisms.

Chromium is an essential element required for biochemical processes as trace element, it affects the metabolism and alter the histological architecture of liver, kidney and testes (Hamilton and Mehler, 1986). The hampering of growth and reproduction of a common fish *Oreochromus mossambieus* has been reported when exposed to sub-lethal concentration of chromium and a detergent Ekalin F, (Sarkar and Konar, 1995). Tannery effluents contain a reasonable quantity of detergents, which also have growth retarding effects in combination with

chromium. Moreover Chromium forms different chemical combinations with the intestinal enzymes thus retarding their activity as reported by the Bog *et al.* (1992) in rainbow trout *(Oncorhychus mykiss)*. Gastric juices work in acidic medium in general and the effluent keeps high alkalinity due to which proteinic part of the food is not likely to be utilized for body growth. Bile of the fish is greatly affected by the phenolic compounds, which are present in tannery effluent. According to Brumley *et al.* (1995) concentration of certain conjugated phenolic compounds in fish bile can be very much greater than their concentration in the surrounding water. In general, as described by Little and Finger (1990) that changes in swimming behaviour caused by sub-lethal exposure to contaminants may impair the ability of fish to feed.

Similar factors as described above are involved in retardation of body length increase. For the growth of bones an efficient supply of oxygen is necessary which essentially the hemoglobin carries out. Moreover hemoglobin contains iron, an essential part of the bones. Different studies have shown that different pollutants especially Chromium greatly affect the respiratory system as studied by Khangarot and Tripathi (1992) in a catfish, *Sacerobranchus fossili* when exposed to chromium. Putte (1982) studied"the histological and hematological changes in *Salmo gairdneri* due to toxicity of Cr^{+6}. Similar cytogenetic damages were observed by AI-Sabti *et al.* (1994) in Prussian carp *(Carassius auratus gibelio)* where there was an increase in the number of micronuclei in erythrocytes, and this was due to tannery effluent coming into the river. Due to such respiratory failures there may be a hypoxic condition in the body tissue, which not only retarded the growth but also decreased the hemoglobin contents as chromium has been reported to induce this response (Gill and Pant, 1987) in a fresh water fish. In general, pollutants especially heavy metals like Cr^{+6} affect at micromolecular level such as DNA, RNA and protein synthesis which result in decreased rate of mitosis, reduced protein synthesis and ultimately reduced growth (Barron and Adelman, 1984).

ACKNOWLEDGMENTS

The authors are grateful for the cooperation of Mahar Ghulam Dastgir Tanneries, Nawabpur Road, Multan for providing the effluent and allowed us to visit their tan yard many times. The Punjab Fisheries Department's Muzzaffargrah Hatchery's staff is also gratefully acknowledged for providing the fish stock and help for this project. Cooperation by the Transport wing of B.Z. University, Multan is also acknowledged.

REFERENCES

ALI, S.S., HUSSAIN, A. AND SHAKOORI, A.R. 1996. Effect of hexavalent chromium on the liver biochemistry of fish *Oreoehromis mossambieus. Biologia,* **42 (1&2)**: 101-110.

AL-SABTI, K., FRANKO, M., ANDRIJENIC, B., KENZ, S. AND STEGNER, P., 1994. Chromium induced micronuclei in fish. *J. appl. Toxicol*., **14**: 333-336.

ASTM, 1990. Standard guide for conducting acute toxicity tests with fishes, macro invertebrates and amphibians. *Ann. Book ASTM Stand*., **3**: 360-379, ASTM, Philadelphia, P A.

BABICH, H., PUERNER, J.A. AND RENFREUND, E., 1986. *In vitro* tytotoxicology of metals to bluegill (BF-2) CELLS. *Arch. environ. Contam. Toxicol*., **15**: 31.

BARRON, M.S. AND ADELMAN, J. R., 1984. Nucleic acid, protelt.-content and growth of larval fish sub-lethally exposed to various taxicants. *Can. J. Fish. aquat. Sci,* **41**: 141-150.

BOG, G., LEYDET, M. AND HONVET, D., 1992. The effect of hexavalent chromium on the activity of alkaline phosphatase in the intestine of rainbow trout *(Oneorynehus mykiss). Aquat. Toxicol*., **23**: 247-260.

BRUMLEY, C. M., HARITOS, U.S., AHOKAS, J. T. AND HOLDWAY, D.A., 1995. Validation of sand flathead as a bioindicator species of marine pollution exposure. *Aquat. Toxicol*., **31**: 249-262.

GILL, P. S. AND PANT, J., 1987. Hematological and pathological ef~s of chromium toxicopis in the fresh water fish *Barhus eonehonius* (Ham). *Water Air Soil Pollut*., **35**: 241-250.

HAMILTON, I.E. AND MEHRLE, D.F., 1986. Effect of hexavalent chromium on fish metabolism. *Trans. Am. Fish. Soc.*, **11:** 596-609.

HUTCHINSON, T.H., 1994. The toxicity of cadmium, hexavalent chromium and copper to marine fish larvae *(Cyprinodon variegate). Mar. environ. Res.,* **38**: 275-290.

KHAN, M.I. and RASHID, M. (2001) Toxicity of leather tannery effluent to common fish *Labeo rohita* (Hamilton), *Pakistan J. Zool.,* **33**: 255-256.

KHANGAROT, B. S. AND TRIPATHI, D. M., 1992. The stereoscan observations of the skin of catfish, S. *fossilis,* following chromium exposure. *J. environ. Sci. Hlth.,* Part-A, **27**: 1141-1148.

LANNO, R.P., HICKlE, B.E. AND DIXON, D.G., 1989. Feeding and nutritional considerations in aquatic toxicity. *Hydrobiologia,* **188/189**: 525-531.

LITTLE, E.E. AND FINGER, R., 1990. Behavioural toxicology: stimulating challenges for a growing discipline. *Environ. Toxicol. Chem.,* **9**: 1-2.

McCULLOCH, W.L. AND RUE, W.J., 1989. Evaluation of a seven day chronic toxicity estimation test using *Cyprindon variegates*. In: *Aquatic toxicology and hazard assessment*. 12th volume.

PUTTE, K. A. 1982. Effects of hexavalent chromium in rainbow trout *(Salmo gairdneri)* after prolonged exposure at two different pH levels. *Ecotoxicol. environ. Saf.,* **6**: 246-257.

ROCHE, H. AND BOGET, G., 1993. Effects of Cu, Zn, and Cr on activities *in vitro* of red blood cells of a marine fish, *Dicentrarchus tabrax. Toxicology,* **7**: 223-229.

SARKAR, V. K. AND KONAR, S. K., 1993. Sublethal effects of pesticide, heavy metal, detergent and petroleum product in three combinations on fish. *Environ. Ecol.,* **11**: 609-615.

SARKAR, V.K. AND KONAR, S.K., 1995. The combined effects of pesticide thiodon, heavy

metal chromium and detergent ekaline FI on fish and fish food organisms. *J. environ. Biol*., **16**: 19-26.

SORNARAJ, R., BASKARAN, P. AND THANALAKSHMI, S., 1995. Effects of heavy metals on some physiological responses of air breathing fish *Channa punctata* (Bloch.). *Environ. Ecol*., **13**: 202-207.

SRIVASTAVA, S. J., SINGH, N.D., SINHA, R. AND SRIVASTAVA, A. K. 1996a. Acute toxicity of fuchsin basic (Magenta) and its effects on blood parameters of a freshwater catfish, *Heteropneustes fossils* (Bloch). *J. Adv. Zool*., **17**: 38-43.

SRIVASTAVA, A. K., ROY, D., SINHA, R., SINGH, N.D. AND SRIVASTAVA, S. J., 1996b. Dyes induced changes in the hematological parameters of a freshwater catfish *Heteropneustes fossilis. Ecol. environ. Cons.*, **2:** 155-158.

SRIVASTAVA, S. J., SINGH, N. D., SINHA, R. AND SRIV AST A V A, A. K., 1998. Ma1chite green induced histopathologicall lesions in the liver of a freshwater catfish, *Heteropneustes fossilsis* (Bloch). *J. Adv. Zool*., **19**: 46-49.

VENUGOPAL, N. B. R. AND REDDY, S. L. N., 1992. Nephrotoxic and hepatotoxic effect of trivalent and hexavalent chromium in a teleost fish *Anabas scandens. Ecotoxicol. environ. Saf.,* **24:** 287-293.

VENUGOPAL, N. B.R. K. AND REDDY, S. L. N., 1993. In vivo effects of trivalent and hexavalent chromium in renal and hepatic ATPases of a freshwater teleost *Anabas scandens*. *Monit. Assess.*, **28**: 131.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 163-169, 2004.

STUDIES ON HABITATS AND LIFE CYCLE OF *OEDALEUS SENEGALENSIS* (KRAUSS) (ORTHOPTERA: ACRIDOIDEA) IN THE DESERT AREA OF LASBELLA BALOCHISTAN

MOHAMMAD MOIZUDDIN
Coastal Agricultural Research Station, Southern Zone Agricultural Research Centre, Pakistan Agricultural Research Council, Karachi University Campus, Karachi

Abstract.- The studies on habitats and life cycle of *Oedaleus senegalensis* (Krauss) were conducted in the desert area of Lasbella, Balochistan in the field conditions. Both hoppers and adults cause damage to agricultural crops and pasture. Number of eggs varies from 8 to 25. The hoppers emerge after summer rainfall in July and become adult in 3-4 weeks. Adults are in abundance in August. Eggs laid during September to November remain in diapause for 8-12 months and hatch in the next summer season after rainfall. It completes one generation in a year.

Key words: Grasshopper, *Oedaleus senegalensis*, life cycle, eggs diapause.

INTRODUCTION

The grasshopper *Oedaleus senegalensis* (Krauss) is a serious pest of agricultural crops such as barley, burlish millet, cowpea, groundnut, guinea corn, lucerne, maize, water melon, rice, wheat and various grasses in Cape Verde Island, Morroco, Mauritania, Senegal, Mali, Moussa, Niger, Nigeria, Sudan, Saudi Arabia, Oman, Iran, Afghanistan, Pakistan and India (Batten, 1969; Bhatia and Ahluwalia, 1963, 1967; Cheke *et al.*, 1980; Gentry, 1965; Joyce, 1952; McAleer, 1977a, 1977b; Moizuddin, 1991; Popov, 1974; Saraiva, 1962). Its attack on cereal crops at seedling stage and at milky stage of grain formation causes complete loss (Bhatia and Ahluwalia, 1963; Cheke *et al*., 1980; McAleer, 1977a, 1977b; Popov, 1974). Severe damage to millet, sorghum, rice, *Cenchrus biflurus*, *Tribulus* sp. and fronds of young Hyphaene plants has been reported from Niger, Mali and Benin by Cheke *et al*. (1980). Joyce (1952) from Sudan reported its attack and damage to millet and young cotton plants.

It has been recorded that in W. Africa it migrates northwards at the start of rainy season and southwards at the end of rainy season (Launois 1978a, 1978b). Batten (1969) and Joyce (1952) reported that the hoppers form bands and Batten (*op.cit*.) and Popov (1974) reported that the adults form swarms and fly by day time in W. Africa.

Heavy outbreak of this species was recorded in 1974 in W. Africa by Popov (*op.cit*) in which the hoppers form bands and the adults form swarms causing severe damage to millet and pasture. Bhatia and Ahluwalia (1963) reported its swarming and in 1967 reported its damage to millet causing plague in Rajasthan, India. Similarly, Cheke *et al*. (1980) reported its outbreak in the South Eastern Nigeria which caused tremendous loss to millet.

The life cycle, habitats, diapause of eggs and hopper development period vary greatly from place to place. Taking into consideration its economic importance and gap in our knowledge on its life cycle in field conditions, the present work was undertaken.

MATERIALS AND METHODS

Regular survey was conducted in the desert area at Chachai, district Lasbella. Balochistan for five years. The collection of solitary adult was made at least twice in a month. The average number of adults collected per man, per hour, per month was calculated from the collection of adults made by different collectors. The collection was made between 10:00 hours to 13:00 hours.

RESULTS

Location

The studies on grasshopper *Oedaleus senegalensis* (Krauss) were conducted in the breeding place in the desert area at Chachai, district Lasbella, Balochistan. It is situated between 25 15°N and 66 45°E. It is about 5 kilometres away from the coastal belt of Gadani. It is considered as arid/semi arid zone.

Climatic conditions

During summer season from April to October the maximum temperature ranges from 26°C to 46°C and the minimum temperature ranges from 12°C to 29°C and during winter season from November to March the maximum temperature ranges from 16°C to 35°C and the minimum temperature ranges from 1°C to 12°C. The rains occur in summer season during July to September and in winter season during December to March. The average rainfall is 150 mm in summer season and 32 mm in winter season. The average annual rainfall is 183 mm. Sometimes there is no rain in one season or both the seasons.

Life cycle

The life cycle comprises egg, hopper and adult stages and vary greatly in each stage in number of days. The temperature and the seasonal rain appear to be the most important factors in each development stage.

Habitats

The grasshopper *Oedaleus senegalensis* lives and feeds on various agricultural crops and variety of grasses which are used as pasture. They lay egg pods in the sandy soil. The hoppers and the adults both attack and damage millet *Penisetum americana*, sorghum *Sorghum bicolor* and maize *Zea mays*, They also damage pastures particularly to *Gynandropis gynandra*, *Cleon viscosa*, *Cenchrus setigerus* and *Dactyloctinium agyptiu*m which grow after summer rainfall.

Egg laying and egg diapause

The egg pods are laid during the summer season from August or September to November in the light sandy soil at the depth about one inch. The number of the eggs per pod varies from 8 to 25 with an average of 15 eggs. The eggs remain in diapause till next summer season and hatch in July or August after summer rainfall. The eggs remain in diapause for 8-12 months.

Hopper development

The hoppers which hatch in July or August after summer rainfall become adult in about a month. The hopper development period usually lasts for 3-4 weeks.

Maturation

The fledglings or immature adults become adult within a week and start copulation. It completes one generation in a year.

Emergence of adults

The adults usually appear in August or September depending on early or late rains of summer. The population rapidly increases and become abundance in next month in September or October. The population gradually decreases in the following months from September or October to December. The adults are not

available in the field during winter season from December till next summer season *i.e.* August or September.

TABLE I.- AVERAGE NUMBER OF ADULTS COLLECTED, PER MAN, PER HOUR, PER MONTH

Months	Number of adults
July	0
August	10
September	25
October	15
November	8
December	0

Seasonal variation

Table I shows the average number of adults collected per man, per hour, per month from July to December. During the period of study sufficient rains occurred in early July. The hoppers emerged and the adults appeared in the first week of the August, The population was in peak in September. The population of adults gradually decreased in the following months and disappeared in December with the advent of winter season. Figure-1 shows the line of graph of peak formation of the adults in September when rains occurred in July.

DISCUSSION

The life cycle of this species is not fully understood. Batten (1969) noted that hatching occurs in June or July, the adults appear by the end of July and the second generation starts before November when eggs are laid which diapause until the next year. Venkatesh *et al*. (1971) observed that hatching occurs usually within two weeks of rainfall in India. However, Bhatia and Ahluwalia (1967) noted that in India eggs laid in June hatched in 13 days and the five hopper instars lasted 24 days. Similarly, Joyce (1952) from Sudan reported that the hatching occurs in June and July. It is most abundant in October. McAleer (1977a, 1977b) from West Africa reported that the minimum development period from hatching to oviposition is 35 days. In the present study it has been noted that the hatching occurs during July or August depending on summer rains. The hoppers become adult in 3-4 weeks. The population is in abundance in August or September depending on early or late summer rains. The hatching of this species in Pakistan occurs one month late due to late summer rains in Pakistan as compared to India.

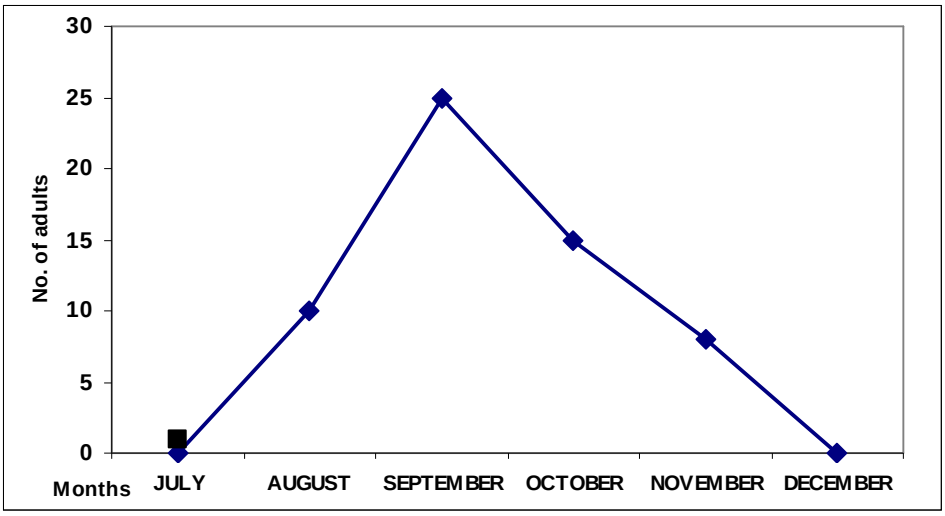


Fig. 1. Seasonal variation in *Oedaleus senegalensis* (Krauss), when rain occurred in July.

Venkatesh *et al.* (1972) from India noted that the eggs spend a dry period of six to thirteen months. Similarly, Jago (1979) from Ethopia noted that this species spend the dry season which usually runs from November until June as eggs. On the other hand Cheke *et al.* (1980) from West Africa recorded that the eggs diapause for twenty two months in laboratory conditions and Saraiva (1962) from East Africa noted that the eggs remain in diapause for even five years. In the present study it has been noted that the eggs laid from August or September to November hatch in next summer season after rainfall in July or August. Thus the eggs remain in diapause for 8 to 12 months. The results correlate with the findings of Venkatesh *et al.* (*op.cit*) and Jago (*op.cit*).

Cheke *et al.* (1980) noted 8 to 37 eggs per pod. In the present study 8 to 25 eggs per pod with an average of 15 eggs have been recorded which is close to the findings of above authors.

Darvey *et al.* (1959) and Batten (1969) reported one to three generations in a year according to latitude in Africa and Joyce (1952) reported one generation in a year in Sudan. In the present study it has been observed that it completes one generation in a year which is similar to the findings of Joyce (*op.cit*) but contrary to Davey *et al.* (*op.cit*) and Batten (*op.cit*).

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Dr. Ulfatun Nabi Khan, Director General, STARC, Pakistan Agricultural Research Council, Karachi, for providing research facilities. Thanks are also due to Prof. Dr. Imtiaz Ahmed, Department of Zoology, University of Karachi, for providing valuable literature.

REFERENCES

BATTEN, A., 1969. The Senegalese grasshoppers *Oedaleus senegalensis* (Kraus). *J. appl. Ecol.*, **6**: 27-45.

BHATIA, D.R. AND AHLUWALIA, P.J.S., 1963. Swarming of *Oedaleus senegalensis* Krauss (Orthoptera: Acrididae) in Rajasthan, India. *Indian J. Ent.*, **24**:222.

BHATIA, D. R. AND AHLUWALIA, P.J.S., 1967. *Oedaleus senegalensis* (Krauss) (Orthoptera: Acrididae-subfamily Oedipodinae) plague in Rajashthan. *Pl. Prot. Bull*. New Delhi, **18**: 8-12.

CHEKE, R.A., FISHPOOL, L.D.C. SND FORREST, G.A., 1980. *Oedaleus senegalensis* (Krauss): an account of the 1977 outbreak in West Africa and notes on eclosion under laboratory conditions. *Acrida,* **9**:107-132.

DAVEY, J.T., DESCAMPS, M. AND DEMANGE, R., 1959. Notes on the Acrididae of the French Sudan, with special reference to the Central Niger delta. *Bull. Inst. Fr. Afr. Noire (A)* **21**: 60-112.

GENTRY, J.W., 1965. Crop insects of northeast Africa-Southwest Asia. *Handb. Agric. Res. Serv*. U.S., **273**: 1-210.

JAGO, N.D., 1979. Light trap sampling of the grasshopper. *Oedaleus senegalensis* (Krauss) (Acrididae: Oedipodinae) and other species in West Africa: A critique. *Proc. Symp. P.A.A.S. Montana U.S.A.,* **1979:** 1-20.

JOYCE, R.J.V., 1952. The ecology of grasshoppers in East Central Sudan. *Anti Locust Bull*., **11**:1-96.

LAUNOIS, M., 1978a. *Modelisation ecologique et simulation operationnelle an Acridologie: application a Oedaleus senegalensis (Krauss).* Ministere de la cooperation de la Republique Francaise: Paris, pp. 214.

LAUNOIS, M., 1978 b. *Manuel pratique d' identification des principacex acridiens du Sahel.* Ministere de la Cooperation de la Republique Francaise, Paris, pp. 297.

McALEER, I.P.B., 1977a. *Oedaleus senegalensis.* Centre for Overseas Pest Research. London, pp. 20 pp.

McALEER, I.P.B., 1977b. *OSRO Plant Protection Project: on grasshopper pests of the Sahel.* Centre for Overseas Pest Research. London, pp. 27.

MOIZUDDIN, M., 1991. Studies on *Oedaleus senegalensis* (Krauss) (Orthoptera: Acridoidea) with special reference to its habitat and genitalia. *Proc. Pakistan Congr. Zool.*, **11**:107-117.

POPOV, G.B., 1974. *The 1974 outbreak of grasshoppers in Western Africa*. Organisation Commune de Lutte Antiacridienne et de Lutte Antiaviare, Dakkar (Unpublished). Report in COPR Archives.

SARAIVA, A.C., 1962. Plague locusts – *Oedaleus senegalensis* (Krauss) and *Schistocera gregeria* (Forskal) – in the Cape Verde Islands. (In Portuguese with English summary). *Estudos Agron.*, **3**:61-89.

VENKATESH, M.V., AHLUWALLIA, P.J.S. AND HARJAI, S.C., 1972. Influence of rainfall on the diapause of *Oedaleus senegalensis* (Krauss) (Orthoptera: Acrididae). *Pl. Prot. Bull.* New Delhi, **23**: 20-27.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 171-178, 2004.

HISTOPHYSIOLOGICAL CHANGES IN PITUITARY GONADOTROPHIC HORMONE CELLS IN RELATION TO SEASONAL CHANGES IN TESTES OF THE FISH, *OREOCHROMIS MOSSAMBICUS*

T.J. URSANI AND S.A. SHAIKH
Department of Zoology, University of Sindh, Jamshoro, Pakistan

Abstract.- Seasonal changes in the testes and its correlation with the pituitary gonadotrophs of tilapia fish, *Oreochromis mossambicus,* were studied throughout the year. The reproductive cycle of this fish is divided into three main periods *i.e.* recrudescent (Nov.-April), spawning (May-August) and quiescent (Sept.-Oct.). The results are based on the mean values of the percentage of gonadosomatic indices and histological studies.Testes attained maximum mean GSI values (1.36 ± 0.19) in the breeding season. During this period the testes containing large lobules filled with sperms and minimum mean GSI (0.44 ± 0.08) was observed during quiescent period. The pituitary GTH-cells during recrudescent were small and granulated. Degranulation was noticed at the time when seminiferous lobules were fully filled with spermatozoa. Chromophobic condition was achieved at the time of spawing and continued till the testes reached in quiescent phase.

Key words: Pituitary gland, GTH cells, seasonal changes.

INTRODUCTION

Tilapia, *Oreochromis mossambicus* is an exotic fish which belongs to family Cichlidae. It was introduced in Pakistan from Malaya in 1951(Naik, 1973).These fish are also widely distributed in fresh waters of Africa and Middle East. The present paper deals with the data on the structure of testes on the basis of during seasonal variation of reproductive cycle.

Considerable information is available on the breeding activity of various fishes throughout the year (Chubb and Potter, 1984; Cyrus and Blaber, 1984; Jalali and Haider, 1985; Shaikh and Jalali, 1991).

This is well established that the pituitary gland has pivoted role in maturation of the gonads in all vertebrates. The physiology of reproduction in fishes have been done by several investigators (Dodd, 1955; Hoar, 1955; Pickford and Aatz, 1962). The teleosts generally have lobular type testes which develop cyst during the recrudescent period (Cyrus and Blaber, 1984; Jalali and Haider, 1985).Most teleostean species manifest annual rhythm of breeding which

in many cases is synchronized or controlled by environmental factors. In large number of species the gonadotrops exhibit hypertrophy vacuolization, granulations, numerical increase and other signs of intense activity in correlation with maturation of the gonads (Sundaraj, 1959; Sathyanesan, 1963; Rai, 1965; Oztan, 1966). Attempt has been made to correlate the pituitary gonadotrophs to annual reproductive cycle in the male *Oreochromis mossambicus.*

MATERIALS AND METHODS

Specimens of male fish, *Oreochromis mossambicus*, were collected with the help of cast net from the waters around Hyderabad city. The fish was transported to the laboratory in live condition, twice a month for a period of one year. The fish were kept outside the water in order to allow them to die. The dead fish were cleaned and dried with filter paper and weighed.

Histological examination

Testes were removed from the fish and weighed. A single middle piece from both the testes were taken and fixed in the Bouin's fluid for histological studies. At the same time pituitary gland of each fish was isolated carefully and was fixed in Bouin's fluid. The testes and pituitary glands were processed for section cutting. The sections (6-8 μm) of the testes were stained in haemotoxylin and eosin while those of pituitary glands were stained in periodic acid Schiff's reagent (PAS).

RESULTS

Gonadosomatic index (GSI)

The change in the gonadosomatic index (GSI) in the seasonal reproductive cycle is shown in Figure 1. The highest value of GSI was recorded during breeding period. The testes were full of large lobules filled with sperms. However, minimum GSI values (0.44 ± 0.08) were observed during recrudescent period. The GSI gradually decreased as the breeding was over and remained the same till the recrudescent period started again.

Histological observation of testes

Recrudescent period

The histological structure of testes shows thick testicular wall with

leydig's cells in the interstitial space and empty lobule during the recrudescent period (Fig.2) The spermatogenic cells were clearly visible inside the lobule and few lobules among them contained smallest eosinophilic cells in the lumen of the testes (Fig.3). Histologically it was observed that the lobules were present at the periphery containing spermatogonia, restricted to the testicular wall and spermatocytes and spermatids located inside during the recrudescent period (Fig.4). These lobules were empty and increased with the increase in the number of spermatocytes with darkly stained chromatin material. Darkly stained spermatids were also observed in large lobules.

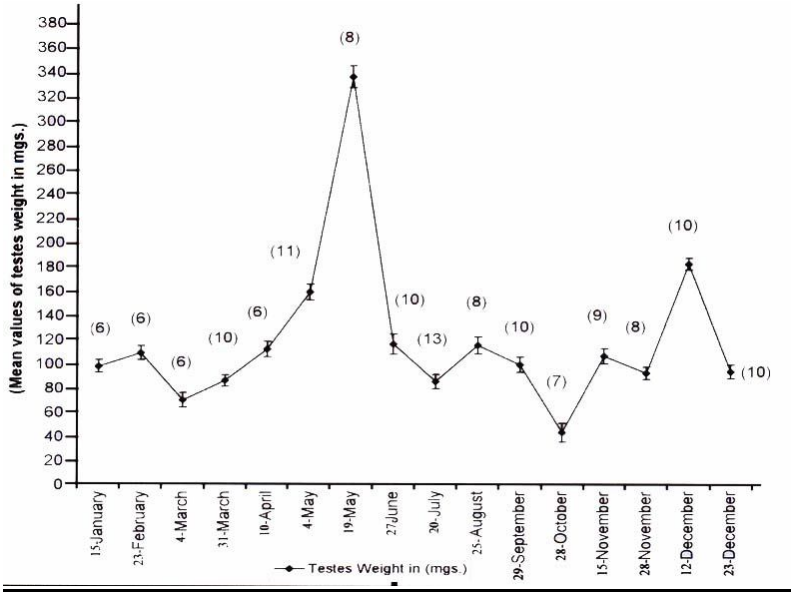


Fig. 1. Annual changes in testicular weight in the male *Oreochromis mossambicus*. The number within parenthesis represent the number of animals.

Breeding period

During this period lobules at the centre of the testes were very large and filled with sperms. Histologically the results revealed that most of the lobules present at the lumen were half filled with spermatozoa (Fig.5).The lobules also possessed few cysts which were filled with spermatocytes and spermatids (Fig.6).The lobules at the lumen were even larger than those of previous months containing many cysts (Fig.7).

Figs. 2-5. Histological structure of testis of *Oreochromic mossambicus* during December (2), March (3), April (4) and June (5), showing tunica albuginea (ta), interstitial tissue (IT) and leydig's cells (1c); cysts filled with spermatids (st); lobules filled with spermatozoa (sz) and showing lobules at the lumen half filled with spermatozoa (sz). Magnification 1, 1000x, all other 400x.

Figs. 6-11. Histological structure of testes of *Oreochromis mossambicus* during July (6), August (7), October (8), December (9), April (10), and May (11), showing elongated lobules containing cysts (cy) of different cells in Fig. 6, two types of lobules: lobules at lumen are large with many cysts (cy) and cysts at periphery containing single type of cells in Fig. 7, regressed cysts (cy) with pycnotic cells in Figs. 8, 9-11 shows, histological structure of pituitary of early recrusdescent period (December) showing small sized GTH-cells, pituitary of recrudescent period (April) showing granulated (g) GTH-cells and pituitary of breeding season (Late May) showing degranulated (dg) GTH-cells x 1000. Stain: 9-11, AB-PAS-Orange G. Magnification: 6, 260x; 7, 9, 400x; 8, 250x; 10, 11, 1000x.

Quiescent period

In this period testes regressed (Fig.8), and lobules of the testes were compressed or empty. This indicated that the testes were in the resting state.

Pituitary gonadotrophs

The pituitary GTH-cells during recrudescent period were granulated and small in size (Fig.9). During breeding season the GTH-cells became large but were less granulated (Fig.10). Complete degranulation was noted at the end of the breeding season (Fig.11). When spawning was over, the GTH-cells were reduced in the number.

DISCUSSION

The study of seasonal changes in the testes and their relationship with the gonadotrophs in the fish, *Oreochromis mossambicus* revealed that the gonadal development was group synchronous. Reproductive cycle showed three main periods, recrudescent period, breeding period and quiescent period.

At least two population or clutches can be distinguished in the testes of the *Oreochromis mossambicus* (Stacey *et al.,* 1979). Maximum gonadal weight was recorded during the main breeding season (May). Other studies show that, the rise in the gonadal weight was also recorded in August. This rise in testicular weight reveals that the temperature has a role to synchronize the gonadal development. This type of temperature dependent development already reported by many workers (de Vlaming, 1972, 1975; Hanyu *et al.*, 1983; Henderson, 1963; Jafri, 1989; Shaikh and Hafeez, 1993). In the present study, the spermatogenic activity was recorded during the months of May to August. In many clupids and *Gerres* species breeding period has not been restricted to the particular periods (Ellis, 1971; Cyrus and Blabber, 1984). Whereas, in *Barilius vagra* Ham. (Jalali and Haider, 1985), the breeding period extends from March to May and *Illisha africana* (Marcus and Kusemiju, 1984) breed during May to December.

The gonadotrophs exhibited a gradual increase in size and number. These were very active and more in number when the testes were in prespawning and spawning phase (April to May). During the resting phase (September to October) of the testes, the gonadotrophs were numerically reduced. These findings are in

agreement with those reported from other laboratories (Kerr, 1948; Beach, 1959, Sundararaj, 1960; Sathyanesan, 1963; Sathyanesan and Singh, 1963; Barr and Hobsan, 1964; Lagios, 1965). The role of gonadotrops in the testicular activity was assessed by the changes in the GSI which seems to be more reliable criterion (Pickford and Atz, 1957). The GSI in *Oreochromis mossambicus* was minimum during the resting phase of the testes. It progressively increased during the preparatory and prespawning phase and reached to its peak at the spawning phase. The maximal GSI in *Oreochromis mossambicus* coincided with the hightened activity of the gonadotrophs. This was followed by sudden decrease (Ahsan, 1966).

It is concluded that changes in gonadotrops in relation to testicular cycle in the fish *Oreochromis mossambicus* show almost the other fishes reported elsewhere.

REFERENCES

AHSAN, S.N., 1966. Effects of gonadotropic hormones on male hypophysectomized lake chub, *Couesius plombeus*. *Canadian J.Zool.*, **44**: 703-717.

BARR, W.A. AND HOBSON., B.M., 1964. Endocrine control of the sexual cycle in the plaice, *Pleurenectes platessa* (L) iv. Gonadotropic activity of the pituitary gland. *Gen. Comp. Endocr*., **4**:608-613.

BEACH, A.W., 1959. Seasonal changes in the cytology of the ovary and the pituitary gland of the gold fish. *Canadian J. Zool*., **37**:615-625.

CYRUS, D.P. AND BLABER, S.J.M., 1984. The reproductive biology of *Gerres* in natural estuaries. *J. Fish Biol.*, **24**:491-504.

CHUBB, C.F AND POTTER, C.I., 1984. The reproductive biology and estuarine movements of the gizzard shad, *Nematolosa vlaminghi* (Munro).*J. Fish Biol*., **25**: 527-543.

DeVLAMING, V.L., 1972**.** Environmental control of teleost reproductive cycles: A brief review. *J. Fish Biol*., **4**:131-140**.**

DeVLAMING, V.L., 1975. Effects of photoperiod and temperature on gonadal activity in the cyprinid teleost, *Notemigonus crysoleucas*. *Biol. Bull. Woods Hole, Mass*., **148**: 402-415.

DODD, J.M., 1955. The hormones of sex and reproduction and their effects in fish and lower chordates. *Mem. Soc. Endocr*., **4**:166-187.

ELLIS, C.K.A., 1971.The size of maturity and breeding seasons of sardines in southern lake Tanganyika. *Afr. J. Trop. Hydrobiol. Fish.*, **1**: 59-66.

HANYU, I., ASAHINA, A.S., RAZANI, H. AND KANEKO, T., 1983. Environmental regulation of reproductive cycle in teleost. *Proc. 2nd N. Pac. Aquaculture Symp*., September 1983, Tokyo, pp. 173-188.

HENDERSON, N.E., 1963.Influence of light and temperature on the reproductive cycle of the eastern book trout, *salvelinus fontinalis* (Mitchell). *J. Fish. Res. Bd. Can.*, **20**:859-897.

HOAR, W.S., 1955.Reproduction in the teleost fishes. *Mem. Soc. Endocr*., **4**:5-22.

JALALI, S. AND HAIDER, G., 1985.Seasonal variation in the testicular androgen and histology of fish *Barilius vagra*. *Hum. Biol.*, **13**: 129-146.

JAFRI, S.I.H., 1989. The effects of photoperiod and temperature manipulation on reproduction in the roach, *Rutilus rutilus* (1) (Teleostei). *Pakistan J. Zool.*, **21**: 289-299.

KERR, T., 1948.The pituitary in normal and parasitized roach (*leucisus rutilus* Flem). *Q. J. microsc. Sci.*, **89**: 129-137.

LAGIOS, M.D., 1965. Seasonal Changes in the cytology of the adenohypophysis, testes and ovaries of the black surf perch, *Embiotoca jacksoni*, a viviparous percomorph fish . *Gen. Comp. Endocr*., **5**: 207-221.

MARCUS, O. AND KUSEMIJU, K., 1984. Some aspects of the of the clupeid reproductive biology**,** *Illisha africana* (Bloch) off the Lagos Coast. *Nigeria J. Fish Biol*., **25**: 679-689.

NAIK, I.U., 1973. Studies on *Tilapia mossambica* (Peters) in Pakistan. *Agric. Pakistan*, **24**: 47-76.

PICKFORD, G.E. AND ATZ, J.W., 1957. *The physiology of the pituitary gland of fishes*. Network Zoological Society, New York. pp. 613.

OZTAN, M., 1966. The fine structure of the adenohypophysis of *Zoarces vivparus* L. *Z. Zellforsch*., **69**: 699-718.

RAI, B.P., 1965.Histophysiology of the pituitary gland in correlation with the ovarian cycle in tor (*Barbus tor* Ham). *Z. Zellforsch.*, **72**:574-582.

SATHYANESAN, A.G., 1963. Histological changes in the pituitary and their correlation with gonadal cycle in some teleosts. *Cellule, Louvain*, **63**: 281-290.

SATHYANESAN, A.G. AND T.P. SINGH., 1963. Some observation on the seasonal changes in the "Periodic acid-schiff" positive cells in the pituitary of a catfish, *Mystus vittatus* Bloch. *J. zool. Soc. India*, **14**: 217-243.

SHAIKH, S.A. AND HAFEEZ, M.A., 1993. Effects of photoperiod and temperature on gonadal response in the cyprinid fish, *Cyprinion watsoni*. *Pakistan J. Zool*., **25**: 233-241.

SHAIKH, S.A. AND JALALI, S., 1991. Seasonal changes in the ovary of the cyprinid fish, *Cyprinion watsoni. Pakistan J. Zool.*, **23**: 119-125.

SHAIKH, S.A. AND JALALI, S., 1989. Seasonal variation in the testes of the fish, *Cyprinion watsoni*. *Proc. Pakistan Congr. Zool*., **9**: 47-59.

STACEY, N.E., COOK, A.F. AND PETER, R.E., 1979. Ovulatory surge of gonadotropin in the gold fish. *Gen. Comp. Endocr*., **37**: 246-249.

SUNDARARAJ, B.I., 1959. A study on the correlation between the structure of the pituitary gland of the Indian catfish *Heteropneustes* and the seasonal changes in the ovary. *Actanat.*, **37**: 17-80.

SUNDARARAJ, B.I., 1960. A study on the correlation between the structure of the pituitary gland and the changes in testes of the Indian catfish, *Heteropneustes fossilis*. *J. zool. Soc. India*, **40**: 305-322.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 179-184, 2004.

SOME MORPHOMETRIC CHARACTERS AND THEIR RELATIONSHIP IN CARP, *CIRRHINUS REBA* (HAMILTON), FROM FISH POND, DISTRICT JACOBABAD, SINDH

P. K. LASHARI, N.T. NAREJO, A. M. MASTOI AND M. A. MAHAR

Department of Fresh Water Biology and Fisheries, University of Sindh, Jamshoro, Sindh (PKL, NTN, MAH) and Directorate of Fisheries Research and Development, Sindh, Karachi (AMM)

Abstract.- Some morphometric characters and their relationship in carp, *Cirrhinus reba* (Hamilton) collected from district Jacobabad, Sindh were recorded between October to December 2004. The results revealed that the standard length, pelvic fin length, pectoral fin length, dorsal fin length, anal fin length and the head length (dependent variables) are highly correlated with the total length (independent variables), while the eye diameter (dependable variable) is highly correlated with head length (independent variable).

Key words: Morphometric characters, carp, *Cirrhinus reba.*

INTRODUCTION

Information on morphometric measurements of fishes and the study of statistical relationship among them are essential for taxonomic work (McConnel, 1978). Moreover, to know the origin of stock, separation of stocks or identification of commercially important species of fishes, morphometric characters are frequently used (Godsil, 1948; Schaffer, 1948; Pillay, 1957; Royce, 1963; Kramholz and Cavanah, 1963).

Now days, small indigenous fishes like *Gudusia chapra, Cirrhinus reba, Puntius sophore, Chela cachius* and *Amblypharyngodon mola* are being cultured in many countries of the world as an important source of quality food for the ever increasing human population (Kohinoor, 2000). *Cirrhinus reba* (Hamilton) locally known as Sunhee, belongs to the family Cyprinidae of the order Cypriniformes. It is commercially important, small indigenous food fish commonly occurs in India, Bangladesh and Pakistan (Rahman, 1989). There is no published report on the biological aspects of this species in Pakistan. Body measurements and their proportions are extensively used in identification of this species. Significance study and statistical relationship between the measurements

of different body parts of fishes has also been recognised in all taxonomic and systematic studies. Hence the present study was undertaken to reckon the relationship between the various morphological body parts of C. *reba* and to establish mathematical equations relating to the various morphometric relationship which could be utilized for the conversion of one measurement into another.

MATERIALS AND METHODS

A total number of 164 specimens of *Cirrhinus reba* with total length ranging in size from 101 to 220 mm were collected from fishpond of district Jacobabad, Sindh, during the period of October to December 2004. All the measurements were taken on a millimeter scale. The eye diameter was measured with the help of vernier calliper. The following measurements were used for each specimen: Total length (TL) from the tip of the snout to the tip of the tail; Standard length (SL) from tip of the snout to the hind margin of hyporal bone, Fork length (FL) from tip of the snout to the point where caudal fin is forked, Pelvic fin length (Pel. FL), base length, greatest distance measured in a . straight line between the anterior most and posterior most point of junction with the body; Pectoral fin length (Pec. FL) base length, greatest distance measured in a straight line between the anterior most and posterior most point of junction with the body; Dorsal fin length (DFL), base length, greatest distance measured in a straight, Anal fin length (AFL), base length, greatest distance measured in a straight line between the anterior most and posterior most point of junction with the body; Head length (HL), the distance from the most anterior part on snout to the posterior edge of opercular bones; Eye diameter (ED), the greatest distance across the cornea, that is between the margin of the eye ball; Girth (G), circumference of the body at dorsal fin.

Experimental fish were divided into 20 mm length groups. In describing the relationship between different morphometric measurements, the equation used for the regression lines was $Y = a + bx$, where x stands for the total length, and Y for the variables, viz. SL, FL, AFL. FL, Pec. FL, DFL, AFL, HL, ED and G. To show the rate of growth of different variables on a percentage basis the following equation was used (Lagler, 1956).

$$\text{Growth rate} = \frac{.\ Y}{X} \times 100$$

where Y is the observed of the variables and X is the total length.

RESULTS AND DISCUSSION

The comparative data relating to various body measurements and their percentage ratios are presented in Table I, in order to compare the growth rate of various morphometric relationships in relations to total length (Table I) have been calculated from the data presented in Table I. The following equations were obtained in regression analysis between:

I. Total length and standard length - SL = -5.62 + 0.84 TL (r = 0.99).

II. Total length and fork length - FL = - 3.44 + 0.41 TL (r = 0.98).

III. Total length and head length - HL = 0.904 + 0.285 TL (r = 0.97).

IV. Total length and dorsal fin length - DF = 19.0 + 0.36 TL (r = 0.96).

V. Total length and pectoral fin length - FL = 1.55 + 0.05 TL (r = 0.97).

VI. Total length and pelvic fin length - FL = -2.67 + 0.68 TL (r = 0.96).

VII. Total length and anal fin length - AFL = -0.15 + 0.16 TL (r = 0.99).

VIII. Head length and eye diameter - ED = 2.19 + 0.17 HL (r = 0.98).

It was evident that the SL, FL, DFL, Pect FL, Pel FL, AFL and HL were highly. correlated with TL while ED was correlated with HL. Relationship between TL and SL, FL, DFL. Pect FL, Pel FL and AFL were found to be linear (Table I). The higher values of r (correlation coefficient) showed that the variables were highly correlated. Hoque (1984) stated that FL, DFL, Pect FL, Pel FL, AFL and HL were highly correlated with TL. While ED was highly correlated with HL in *Harpodon nehercus*. Similar findings also been made by Ganguly *et al.*, (1959), Chonder (1977) and Prakash and Verma (1982) in the fishes studied by them.

Growths of various variables in relation to total length are presented in Table I. Literature available regarding the growth rates of the various variables in relation to total length indicates that the growth of various morphological body parts varies from species to species in fishes. In the case of *Ophicephalus gachua,* Mehta and Bapat (1977) stated that the growth of the standard length and height of the body increased with the increase of the total length. In *Harpodon*

Table I

nehercus, Hoque (1984) reported that the growth rates of the variables like length of anal fin and eye diameter shows no definite change, while the growth rates of variables like the length of pectoral fin, pelvic fin and dorsal fin decreases with the increase in total length. From Table 2 it is evident that in C. *reba*, the growth rate of variables, like standard length, fork length, pelvic fin length, pectoral fin length, anal fin length and eye diameter show no definite change. On the other hand the head length decreases with the increase in total length up to 200 mm and than gradually increases with the increase in total length.

From the above observations and discussions it is concluded that in *C. reba*, the standard length, fork length, pelvic fin length, pectoral fin length, dorsal fin length, anal fin length and head length showed strong correlation with the total length, while eye diameter is highly correlated with head length.

REFERENCES

CHONDER, S.L., 1977. Morphometric characters and their relationship in *Gudusia chapra* (Ham.). *Proc. Ind. Acad. Sci. B.,* **80**: 57-67.

GANGULY, D. N., MITRA, B. AND BHATTACHARYA, A., 1959. On the interrelationship between the total length, standard length, depth and weight of *Lates calcarifer. Proc. Nat. Inst. Sci. India,* **25**: 175-158.

GODSIL, H. C., 1948. A preliminary population study of the yellow fin tuna and the albacore. *Calif. Fish Game,* 70, 90 pp.

HOQUE, B., 1984. Morphometric characters and their relationship in Bombay duck, *Harpodon nehercus* (Hamilton). *Bangladesh J. Zool.,* **2**: 105-108.

KOHINOOR, A.H.M., 2000. *Development of culture technology of three small indigenous fish, mola* (Amblypharyngodon mola), *punti* (Puntius sophore) *and chela* (Chela cachius) *with notes on some aspects of their biology*. Ph.D. thesis, Department of Fisheries Management, BAU, Mymensingh 365 pp.

KRAMHOLZ, L. A. AND CAVANAH, S. H., 1963. Comparative Morphometry of freshwater drum from two midwestem localities. *Trans. Am. Fish. Soc.,* **97**: 429-441.

LAGLER, K. F., 1956. *Freshwater fishery biology.* 2nd ed. W. C. Brown Co. Dubuque, Lowa.

McCONNEL, R.H.L., 1978. Identification of freshwater fishes. In: *Methods for assessment of fish production in freshwater* (ed. T. Bagenal). Blackwell Scientific Publications, London, pp. 48-83.

MEHTA, D. P. AND BAPAT, S. S., 1977. Statistical relationship in between body measurements of *Ophicephalus gachua* (Hamilton-Buchanan). *Marthwada Univ. J. Sci.,* **16**: 74-77.

PILLAY, T.V.R., 1957. A morphometric study of the population of *Hilsa ilisha* (Ham.) of the river Hooghly and of the Chilka lake. *Indian J. Fish.,* 4: 344-386.

PRAKASH, M. AND VERMA, B. R., 1982. Morphometric characters and their relationship in *Notopterus notopterus* (Pallas). *Bangladesh J. Zool.,* **10**: 14-21.

RAHMAN, A.K.A., 1989. *Freshwater fishes of Bangladesh*. Zoological Society of Bangladesh, University of Dhaka, 364 pp.

ROYCE, W. F., 1963. A morphometric study of yellow fin tuna, *Thunnus albacore* (Bonnaterre). U. S. Fish & Wildlife Service, *Fish. Bull.*, **63**: 395-443.

SCHAFFER, M. B., 1948. Morphometric characters and relative growth of yellow fin tuna *(Neothunnus macropterus)* from Central America. *Pacif. Sci.*, **2**: 114-120.

TABLE I.- MORPHOMETRIC MEASUREMENTS OF CARP, *CIRRHINUS REBA* (HAMILTON) FROM FISH POND OF DISTRICT JACOBABAD, SINDH, PAKISTAN. THE RATE OF GROWTH OF DIFFERENT BODY PARTS IN RELATION TO THE TOTAL LENGTH ARE SHOWN IN PERCENT IN PARENTHESIS.

Length group (20 mm)	**No. of fish**	**Mean TL (mm)**	**Mean SL (mm)**	**Mean FL (mm)**	**Mean HL (mm)**	**Mean ED (mm)**	**Mean DFL (mm)**	**Mean PFL (mm)**	**Mean Pel. FL (mm)**	**Mean AFL (mm)**	**Mean Girth (mm)**
101-120	11	115	92 (80)	101(88)	20(17)	4 (20)	14 (12)	4 (3)	4 (3)	7 (6)	59 (51)
121-140	33	130	105(81)	112(86)	22(17)	5(22)	15 (12)	5 (4)	5 (4)	8 (6)	70 (54)
141-160	45	155	125(81)	134(86)	24(15)	5(21)	16 (12)	5 (3)	5 (3)	9 (6)	80 (53)
161-180	29	176	144(82)	154(88)	26(15)	6(21)	18 (11)	6 (3)	6 (3)	10 (6)	95 (54)
181-200	18	189	152(80)	161(86)	28(15)	6(22)	20 (12)	7 (4)	7 (4)	11 (6)	103(55)
201-220	28	207	168(81)	180(87)	30(16)	7(22)	22 (12)	8 (4)	8 (4)	12 (6)	112(55)
Mean	164	162	131(81)	140(87)	25.5(16)	5.5(21)	18.8(12)	5.8 (4)	5.8 (4)	9.5 (6)	86.5(54)
SD ±	-	4.80	3.90	4.0	0.75	0.22	0.62	0.15	0.15	0.25	2.85

Proc. Pakistan Congr. Zool., Vol. 24, pp. 185-198, 2004.

ISOLATION AND CHARACTERIZATION OF PENTACHLOROPHENOL DEGRADING BACTERIA

JAVED IQBAL QAZI, RAMLA SHAHID AND SYED SHAHID ALI
Microbiology Laboratory, Department of Zoology, University of the Punjab, Lahore 54590, Pakistan
E-mail: qazi@scientist.com & ssalipuniv@hotmail.com

Abstract.- Four water samples collected from Nala Dage, a streamlet receiving effluents of Kala Shah Kakoo industrial site, Lahore, were screened using M9 selective media containing various concentrations of pentachlorophenol (PCP). Six PCP resistant strains were isolated, characterized and optimized for growth conditions. The bacterial isolates PCP-1, PCP-2, PCP-4 and PCP-6 were Gram negative. All these strains revealed bacilli cells except the PCP-4, which was of cocci morphology. The isolates PCP-3 and PCP-5 were Gram positive, while cell morphology ranged from cocci and bacillus to streptobacilli. The long chain streptobacilli revealed a sheathed nature of the bacteria. All the bacterial strains showed optimum growth under aerobic condition at 37°C. Optimum inocula sizes ranged from 1 to 5% in LB and 10% in M9 medium .The isolates showed variable responses to pH indicating acidophilic, alkaliphilic and neutrophilic nature of bacteria. All the isolates exhibited high potential for PCP degradation (upto10µg/ml). Invariably all the strains at concentrations above 30µg/ml PCP could not complete the job of PCP degradation up to the end of experimental period of 120 hours. The results of this study are promising with respect to the bioremediation of PCP polluted soil and water habitats. The information throws light on the importance of maximum limit of the toxic substance within a bioreactor for its economically feasible biotransformation. Augmentation of these strains by optimum growth conditions for proper period of time may lead to rehabilitation of PCP contaminated soil or habitats improving the quality of the environment.

Key words: Pesticide biodegradation; metal resistant bacteria; environmental rehabilitation.

INTRODUCTION

To get better crop yields, the use of pesticides and herbicides has increased manifold over the last couple of decades resulting in soil contamination. Pentachlorophenol (PCP) is a chlorinated aromatic molecule that has extensively been used as a fungicide and wood preservative (Beck *et al.,* 2000; Li and Sengupta, 2000; Sharma *et al.,* 2000; Wilconox, 2000). PCP is of quite persistent nature having half life of 15hrs in rats, 78hrs in monkey, and 30-50hrs in man and 45days in ground (Augustijn-beckers *et al.,* 1994). It is moderately toxic

through oral route but is readily absorbed through skin which is most dangerous route of exposure (Gasiewicz, 1991). But most common way of persistent pesticides to enter in human body is through food chain. Pentachlorophenol and its intermediates have been detected from cow's milk and eggs of chicken which forage in PCP contaminated area. (Pfender *et al.,* 1996; Fries *et al.,* 1999). Its toxicological effects include gynecological and endocrine abnormalities and ovarian and adrenal dysfunctions (Gerhard *et al.,* 1999) and carcinogenicity in rats and man (Sharma *et al*., 2000). It could react with ozone to form oxalic acid so could contribute in the ozone depletion (Kim and Moon, 2000; Weavers *et al*., 2000). Bypassing its official status, the chemical is being widely used in many developing countries. Considering management of post application environmental hazards of the pesticides, recently bioremediation has been considered an important method of detoxification. Some algae have been reported that grow in PCP contaminated soils. (Tikoo *et al.,* 1997). Similarly, fungi also degrade PCP and are used in bioreactors for biotransformation of PCP (Pall *et al*., 1997; Engwell *et al*., 1999).

Owing to the fact that PCP has been and is being used in our country, the pesticide would have polluted the soil and water habitats. However, studies describing PCP degradation have not been reported. The present investigation was undertaken to isolate bacteria from a streamlet, Nala Dage receiving agriculture runoff as well as industrial effluents. The isolates reported here afford results for designing PCP biodegradation and detoxification strategies.

MATERIALS AND METHODS

Sample collection

Four water samples were collected from Nala Dage streamlet in the vicinity of Kala Shah Kako industrial area. It receives agricultural runoff of upstream area and various industrial effluents. Running water samples from the stream were collected in sterile bottles from the same spot at 5 minutes interval. The samples were soon transported to the laboratory and kept in a refrigerator. On the next day they were processed for the isolation of bacteria.

Isolation of bacteria

One ml of each water sample was diluted with 9ml of autoclaved distilled water and 50µl of the dilution was then spread on modified M9 agar media containing 0.001%, 0.002% and 0.005% of pentachlorophenol (Merck) as a

carbon source instead of glucose. These media were designated as M9-PCP-1, M9-PCP-2 and M9-PCP-3, respectively. The other ingredients of M9 media were 0.6g of di sodium hydrogen phosphate, 0.3g of potassium di hydrogen phosphate , 0.05g NaCl , 0.1g of ammonium chloride, 1.5g agar, 200µl of 1M $MgSO_4$ and 10µl of $CaCl_2$ per 100 ml of distilled water. pH of the media was adjusted between 7.2-7.4. Petri plates were incubated at 37°C for one week and observed daily for bacterial growth. However, colonies were enumerated at the end of the incubation period (1-week). For pure culturing, widely separated colonies were selected and streaked onto their respective PCP containing media, followed by streaking on nutrient agar. The pure cultures were preserved in agar slants routinely and the glycerol stocks kept in refrigerator for further use.

Characterization of isolated bacteria

Morphology and color of the bacterial colonies were observed with the help of a colony counter. Biochemical characterization *viz*. Gram staining, oxidase, catalase, motility, growth on MacConkey agar, starch hydrolysis, citrate utilization, nitrate reduction and H_2S production tests were performed according to Benson (1994), while Voges Proskauer, methyl red and oxidation/ fermentation tests were performed after Cheesbrough (1993).

Optimizing growth conditions

Bacterial isolates were optimized for pH, temperature and inoculum size both in M9 and LB broths. All the experiments were performed in triplicates. Media with varying pH were prepared by the addition of 1M HCl or 1M NaOH. Five ml of broth in each test tube was inoculated with 100µl of overnight incubated culture that was prepared by transferring a loop full of bacterial growth from an agar slant into 5ml LB broth. All the test tubes were incubated at 37°C for over night period in shaker. Optical densities of the cultures were then recorded at 600nm. Growth of each strain was also checked in aerated condition (130 rpm) and without shaker for over night period. The bacteria were similarly incubated in media having corresponding optimum pH at 25ºC, 30ºC, 37ºC and 45ºC for over night period. Growth of each strain was then determined at optimum pH and temperature with 1,2,5 and 10% inocula within 5ml of culture volume.

Growth on Pb^{2+}, Cr^{6+} and antibiogram

The bacterial isolates were also grown on LB agar plates containing

$Pb^{2+}$and Cr^{6+} and antibiogram. Different concentrations of Pb^{2+} and Cr^{6+} (.0.0001, 0.001, 0.01, 0.1, 0.2%) were used to check the growth of bacteria by addition of $Pb(CH_3COOH)$ and $K_2Cr_2O_7$, respectively. Bacterial growth on these media was checked after 24 hours.

For antibiotics susceptibility test, LB agar plates were prepared and 50µl of fresh culture of each bacterial isolate was spread on them. Sensitivity discs (Oxiod) of erythromycin 15µg, doxycyclin 30µg, oxytetracyclin 30µg, co-trimaxazol 25µg, amoxicillin 25µg and clarithromycin 15µg were placed on the surface of the inoculated plates, which were subsequently incubated at 37°C. On the next day the plates were observed for the appearance of inhibition zones around the antibiotic discs.

Pesticide degradation / removal ability of the bacterial isolates

Method for estimation of monohydric phenols described by Thomas and Chamberlain (1975) was adopted for quantifying the pentachlorophenol.

For standard curve preparation various dilutions of PCP were prepared in M9 broth. Three ml of each of dilution was taken in a test tube and 0.5ml of phosphate buffer was added followed by the addition of 0.05ml of 2% 4-aminoantipyrin and 0.05ml of 8% potassium ferricyanide aqueous solutions. The contents in each test tube were shaken and absorbance was taken on spectophotometer at 500nm after 10 minutes to make standard curve.

The bacteria were grown in M9 broth containing different concentrations of PCP under optimum growth conditions. After every 24hr (up to 120 hrs), 3ml of broth was taken from each culture and centrifuged at 3000 rpm for 3-5 minutes. The supernatant was processed for estimation of PCP as mentioned above.

RESULTS

Cultural and physio-biochemical characterization of the bacterial isolates

Six types of bacterial colonies were observed following spreading of the samples on the selective media containing varying amounts of pentachlorophenol. The colonies differed in colors, margins and shapes. Two bacterial isolates expressed large and round colonies and one of these had wavy, while the other smooth margin. Two other strains had small colonies, one having convex and the

other flat elevation. Another pair of the colonies was medium and round, again one with convex and the other with flat elevation. All the colonies appeared white except one strain, designated as PCP-3, which expressed yellow color (Table I). Regarding the effects of concentration of pentachlorophenol on the total number of bacterial colonies appearing /plate from the sample spread, it was observed that the number decreased retrogressively with increase in concentration of the pesticide.

Only two of the isolates, PCP-3 and PCP-5 were found Gram positive. The strains PCP-1, PCP-2, PCP-3 and PCP-6 depicted rod shaped, while, PCP-4 and PCP-5 as coccus morphology. Regarding the cellular arrangements, strains PCP-1was found Gram negative diplobacilli, while the terminal portion of the cells and some times some spots at the lateral edge of the cell gave Gram-positive reaction (Table II).Gram positive streptobacilli of PCP-3 comprised of few cells to 24 cells chains and appear to be sheathed as revealed microscopically by very prominent gaps between the cells of each chain comprising of three cell stage onwards.

All the isolates showed positive and negative tests for catalase and oxidase, respectively. All the bacteria were found motile .The isolates PCP-1, PCP-2 and PCP-4 showed growth on MacConkey agar plates changing red color of the media to pale yellow. The isolates, PCP-1, PCP-4 and PCP-6 hydrolyzed starch in agar medium. The bacterial strains PCP-3 and PCP-5 indicated positive Voges Proskauer test. PCP-1 and PCP-4 showed positive results for citrate utilization. Bacteria differed for nitrate reduction and H_2S production. (Table II).

Optimization of growth conditions

The isolates, PCP-3 and PCP-5 grew optimally at initial pH 8, while the PCP-1 and PCP-6 at pH 6. Only the strain number PCP-4 had neutral optimum pH in M9 broth. When these bacteria were grown in LB broth the strains PCP-2, PCP-3 and PCP-5 indicated optimum population densities at pH 6.The isolates PCP-3, PCP-6 and PCP-1 showed best growth at 7 and 9 pH values, respectively. Optimum incubation temperature was found as 37°C for all the isolates (Fig.1). Similarly, all the bacteria grew best in aerated condition (Fig.2). For all the isolates 10% inoculum as found optimum in M9 media. However, the inoculum size varied in LB broth and it was 2% for PCP-1, PCP-2 and PCP-4, while 1% for PCP-3 and 5% for PCP-4 and PCP-6 (Fig.3).

TABLE II-. MORPHOLOGICAL AND PHYSIOBIOCHEMICAL CHARACTERISTICS OF THE BACTERIAL ISOLATES

Characteristics	Bacterial isolations					
	PCP-1	PCP-2	PCP-3	PCP-4	PCP-5	PCP-6
Gram's reaction	-ve	-ve	+ve	-ve	+ve	-ve
Cell morphology	baccili	baccili	strepto-bacilli	cocci	cocci	baccili
Oxidase activity	-ve	-ve	-ve	-ve	-ve	-ve
Catalase activity	+ve	+ve	+ve	+ve	+ve	+ve
Motility	+ve	+ve	+ve	+ve	+ve	+ve
Growth on MacConkey agar	+ve	+ve	-ve	+ve	-ve	-ve
Starch hydrolysis	+ve	-ve	-ve	+ve	-ve	+ve
Voges-Proskauer test	-ve	-ve	+ve	-ve	+ve	-ve
Methyl red test	-ve	-ve	-ve	-ve	-ve	+ve
Citrate utilization	+ve	-ve	-ve	+ve	-ve	-ve
Nitrate reduction	-ve	-ve	-ve	-ve	-ve	-ve
Oxidation / fermentation	-ve	-ve	-ve	-ve	-ve	-ve
H_2S production	+ve	+ve	+ve	+ve	+ve	+ve

Bacterial isolates PCP-5 and PCP-6 grew in the presence of 0.1%, while other four strains were inhibited at 0.2% of Pb^{2+}. Strains PCP -1, PCP-2, PCP-3, PCP-5 and PCP-6 could not grow at concentration of 0.1% of Cr^{6+}, while PCP-4 was found resistant up to 0.2% of the metal. All the six strains were found resistant to erythromycin and sensitive to doxycyclin. All the strains except, PCP-3 were sensitive to oxytetrocyclin. Only PCP-2 was sensitive to co-trimoxazole. Except PCP-2 all the isolates appeared resistant to amoxycilin. The isolates PCP-3, PCP-4 and PCP-6 were found resistant to clarithromycin.

Pentachlorophenol degradation /removal by the bacterial isolates

After 24hrs, the bacterial strain PCP-1 degraded the compound up to 74% and 28% in the media having 10 and 20µg of PCP /ml, respectively. The process for these two concentrations was completed up to 100% by the end of 4^{th} day under optimum growth conditions. However, for the higher doses the microbes utilized the PCP scarcely so that at the end of the experimental period about 66, 30 and 24% of the substrate was degraded in the media containing 30, 40 and 50 µg of the pesticide/ml, respectively. (Fig.4A). Other strains depicted more or less same patterns as explained for the isolate PCP-1, that is in case of the media having low concentrations of the pesticide (10 and 20µg/ml) 100% and earlier

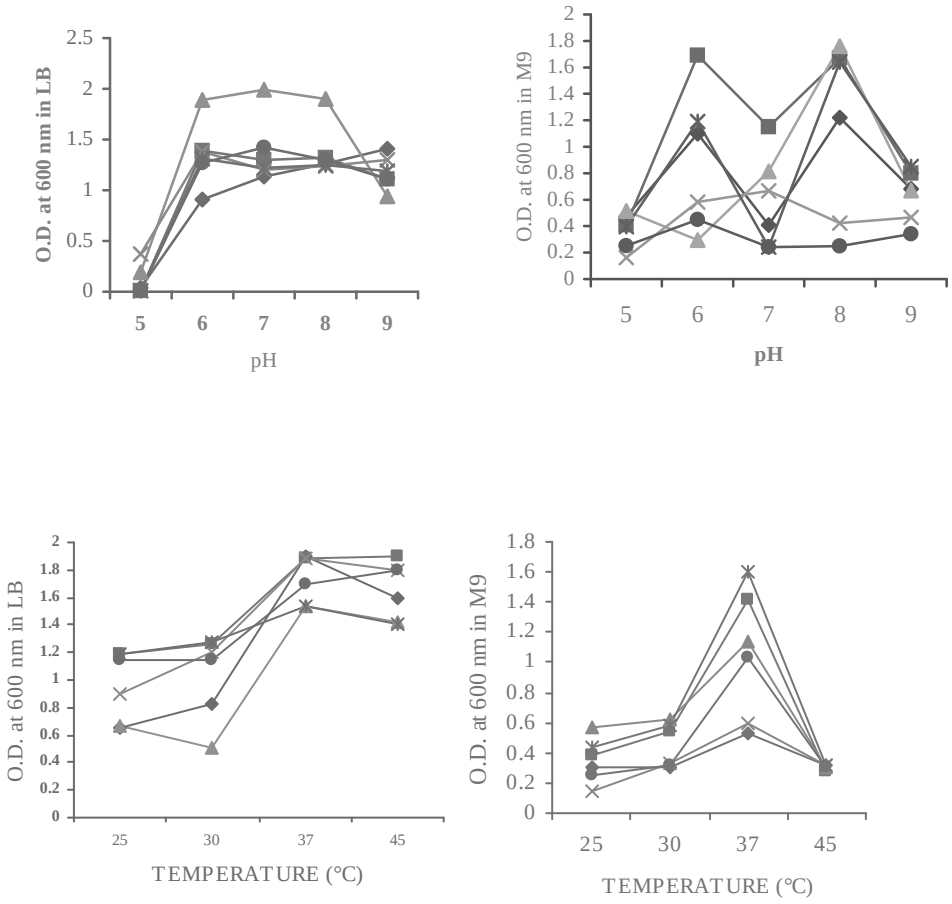


Fig 1.Growth of the bacterial isolate at different pH and temperatures in LB and M9 broths.

degradation/disappearance of PCP was achieved, except for the isolate PCP-4 that degraded 100% of PCP only in the lowest concentration (Figs. 4D). An accessory conclusion can also be pointed out here that presence of these PCP degrading bacteria in Nala Dage, which appeared resistant to other pollutants such as heavy metals and antibiotics, throws light on the diversity of the pollutants and their load in the upstream agricultural area.

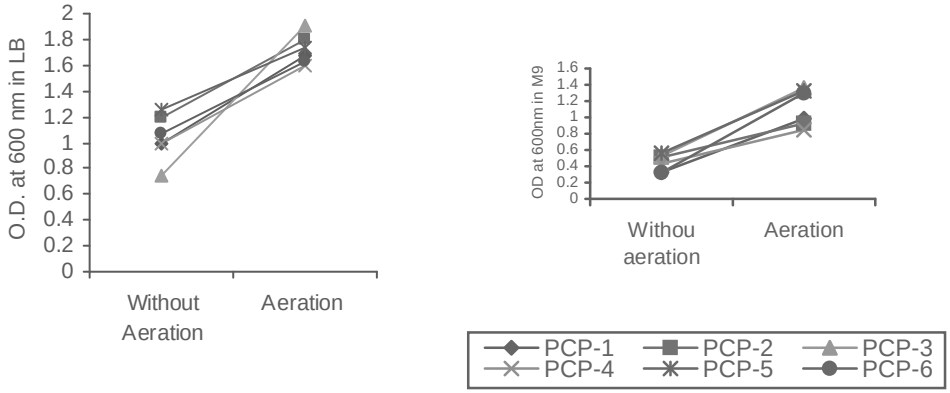


Fig 2. Growth of the bacterial isolates in aeration and without aeration in LB and M9 broths

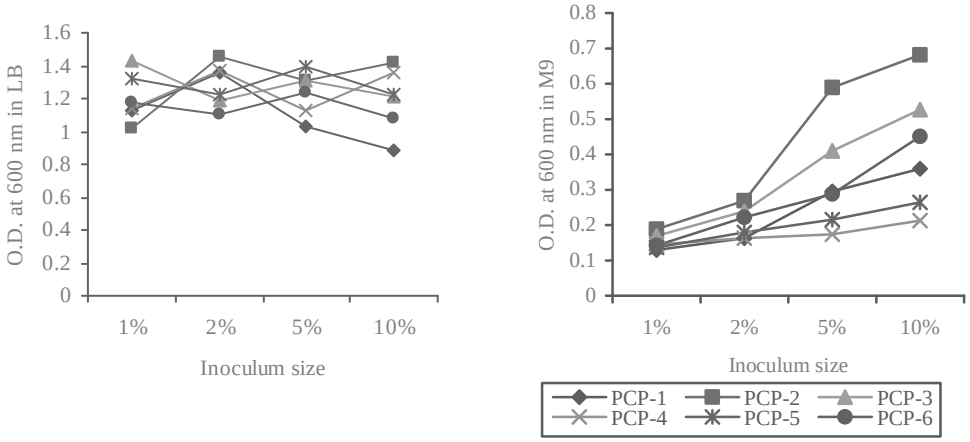


Fig 3.Growth of bacterial isolates started with different inocula sizes in LB and M9 broths.

DISCUSSION

The bacterial isolates reported here showed remarkable ability to consume/ degrade PCP upto 20µg/ml in the M9 media, when it was supplied as sole carbon source. The bacteria in general, were able to make the 100% disappearance of the

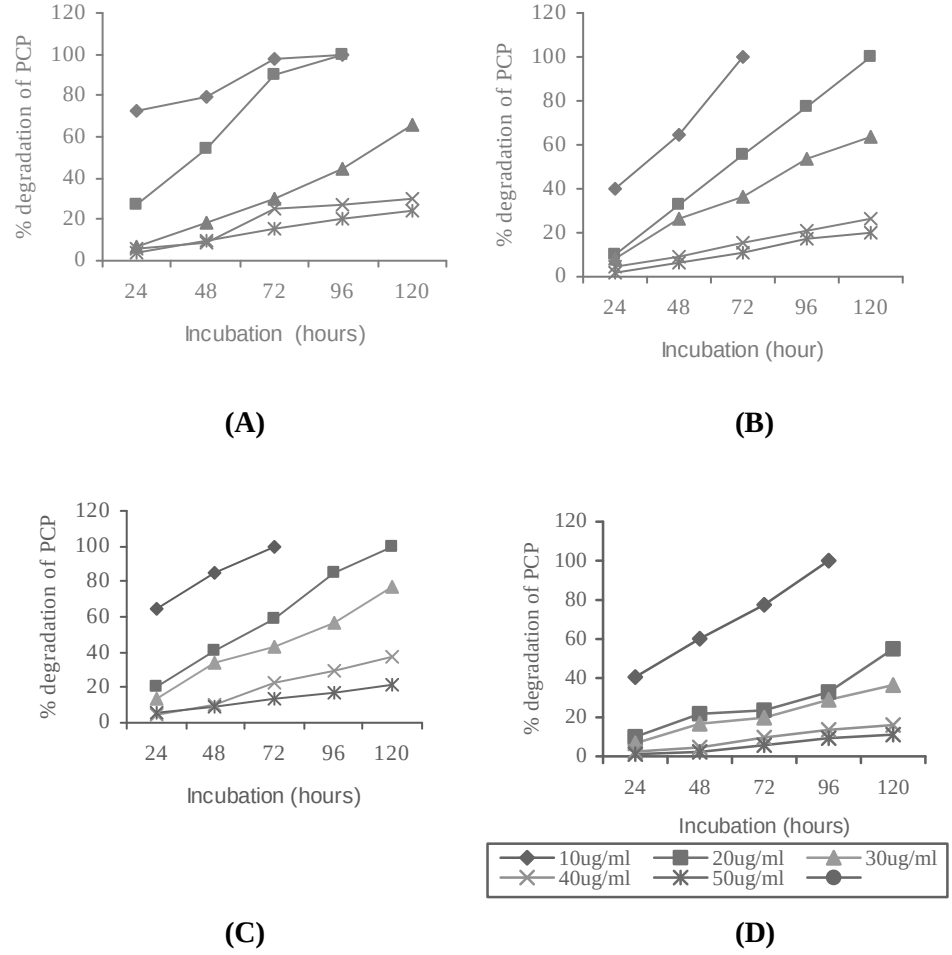


Fig. 4. Percent degradation of PCP by the bacterial isolates, PCP-1 (A); PCP-2 (B); PCP-3 (C) and PCP-4 (D) in M9 media having different concentrations of the pesticide.

pesticide in the media within few days of incubation. However, the higher doses of the insecticide that is 30 to 50 µg/ml did not allow the bacteria to grow and degrade the substance comparable to the situations for the first two doses. From these findings it can safely be concluded that the bacterial strains are fully capable for the degradation of the pesticide but the higher doses do exert toxic effect on these PCP resistant microbes. Edghil (1996) worked on laboratory reactor and used feed that contains PCP as primary carbon source. He found that the system was unable to competently respond to step increase in PCP feed concentration within four months because of the ammonia in the feed and by

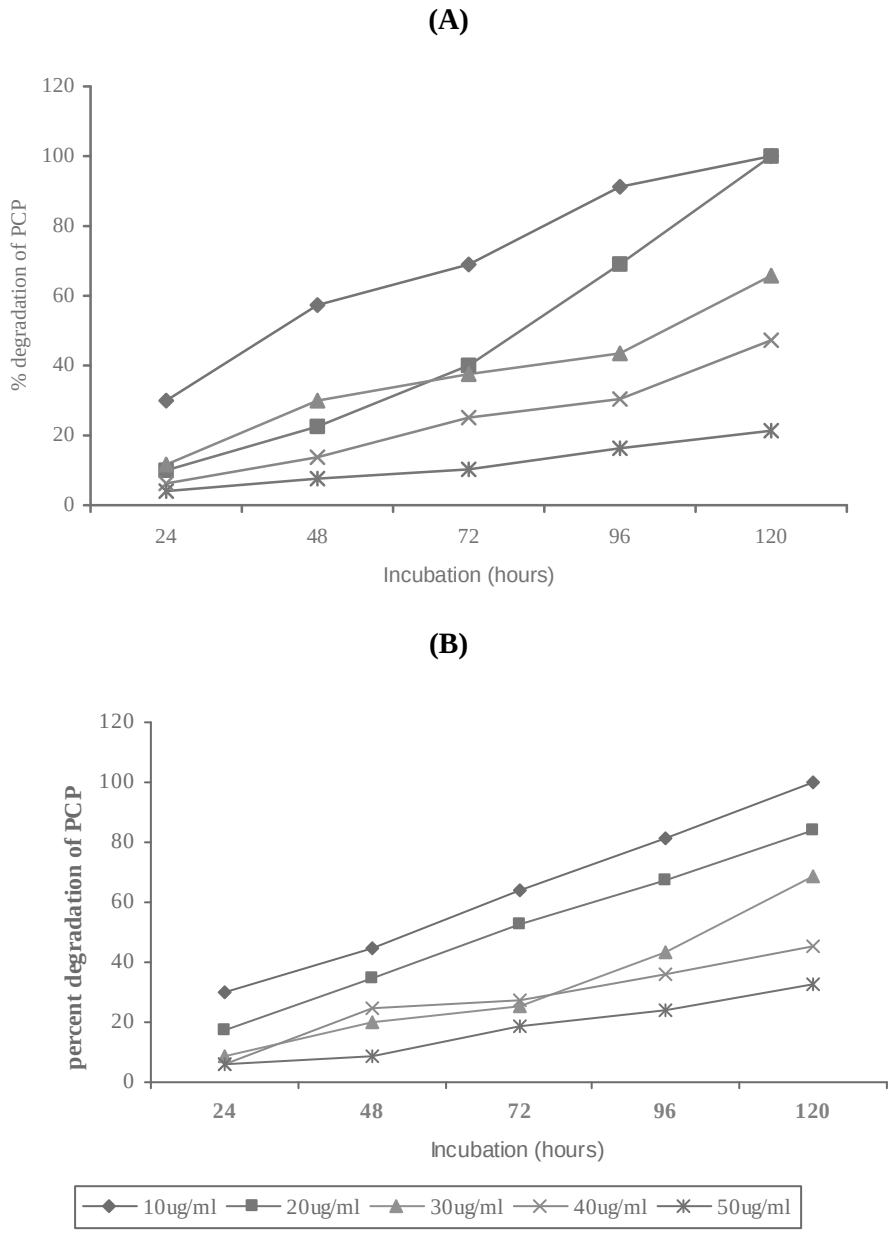


Fig. 5. Percent degradation of PCP by the bacterial isolates PCP-5 (A) and PCP-6 (B) in M9 media having different concentrations of the pesticide.

replacing the ammonia with nitrate, PCP removal efficiency reinstated. Other workers have also reported PCP degrading bacteria (Topalova *et al*., 1999; Maniso *et al*., 2001).

Of the six strains, only two gave Gram positive reaction. Nature of bacterial isolates is of course a variable phenomenon at different habitats. However, an earlier study also indicated high prevalence of Gram-negative PCP degrading bacteria. Manisto *et al*. (2001) isolated the pentachlorophenol degrading bacteria and reported that 86% of them were Gram negative. The bacterial isolates indicated tolerance to Pb^{2+} and Cr^{6+}. At higher concentrations of the metals growth appeared after 9 hours .The delayed growth may be the result of effective heavy metal binding with essential functional group that modifies the active conformation of biological molecules (Collins and Stotzky, 1989; Guzzo *et al*., 1991). All the strains showed multi drug resistance to two to three antibiotics. The isolate PCP-1 was found resistant to catrimoxazole, erythromycin, amoxicillin and clarithromycin. This multi drug resistance may be due to wide spread use of antibiotics (Erova *et al*., 1989).

It is known that presence of relatively stable and especially toxic substance will alter the environment so that either the pollutant tolerating/resistant organisms will be selected or the chemical can induce evolutionary process especially in bacteria (Mollah and Allen, 1999). However, bacteria capable of utilizing such pollutants are not totally immune from their toxic effects. Surely, it is the dose that differentiates between the manageable and intolerable limits of a substance. Further work is needed to explore low cost substances that may augment tolerance and degradability of these microbes for PCP. However, the present information suffice to conclude that these bacteria appear suitable for the biodegradation and rehabilitation of the PCP contaminated soils and water when the pollutant is brought in the manageable range *i.e.* 10-20µg/ml. The acidiophilic and alkaliphilic natures of some of the isolates is very interesting .In fact, sampling location also receives highly acidic and alkaline industrial effluents about 300-400 meters upstream. Such low or high pH liking PCP utilizing bacteria can be considered potential candidates for the bioremediation of the PCP contaminated environment accompanied by low or high pH conditions.

REFERENCES

AUGUSTIJN-BECKERS, P.W.M., HORNSBY, A.G. AND WAUCHOPE, R.D., 1994. SCS/ARS/CES Pesticide properties database for environmental decision making II. Additional Compounds. *Rev. environ. Contam. Toxicol.,* **137**: 1-82.

BECK, H. A., BOZOKI, Z. AND NIESSNER, R., 2002. Screening of pentachlorophenol-contaminated wood by thermodesorption sampling and photoacoustic detection. *Anal. Chem*., **72** : 2171-2196.

BENSON, H.J., 1994. *Microbiological applications. Laboratory manual in general microbiology*. Wan. C. Brown Publisher, Duque, Malbourn, Oxford.

CHEESBROUGH, M., 1993. *Medical laboratory practice in tropical countries*, vol. II. *Microbiology*. ELBS University Press, Cambridge.

COLLINS, Y. AND STOTZKY, G., 1989. Factor affecting toxicity of heavy metals to microbe In: *Metals in bacteria* (eds. T.J. Beveridge and R.J. Doyle), John Wiley and Sons Inc, Toronto.

EDGEHILL, R.U., 1996. Degradation of pentachlorophenol (PCP) by *Arthrobacter* strain ATCC 33790 in biofilm culture. *Water Res*., **30**: 357-363.

ENGWALL, M.A., PIGNATELLO, J.J. AND GRASSO, D., 1999. Degradation and detoxification of the wood preservatives creosote and pentachlorophenol in water by the photo fenton reaction. *Water Res*., **33**:1151-1158.

EROVA, T.E., ANISOMAVA, L.A., SMOLYANSKYA, A.Z. AND BORONIN, A.M., 1989. Plasmid and genetics determinants of antibiotic resistance in Gram negative bacteria. *Antibiot. Khimioter*., **34**:365-370.

FRIES, G.F., PAUSTENBACH, D.J. AND MATHER, D.B., 1999. A congener specific evaluation of transfer of chlorinated dibenzo-p-dioxins and dibenzofurans to milk of cows following ingestion of pentachlorophenol-treated wood. *Environ. Sci. Tech*., **33**: 1165-1170.

GASIEWICZ, T.A., 1991. Nitro compounds and related phenolic pesticides. In: *A handbook of pesticide toxicology* (eds. W.J. Hayes Jr. and E.R. Laws Jr.), Academic Press, New York, NY.

GERHARD, I., FRICK, A. AND MONGA, B., 1999. Pentachlorophenol exposure in women with gynecological and endocrine dysfunction. *Environ. Res. Sec*., **80**:383-388.

GUZZO, A., DIORIO, C. AND DUBAW, M.S., 1991. Transcription of *E. coli* fli C gene is regulated by metal ions. *Appl. environ. Microbiol*., **57**: 2255-2259.

KIM, J.Y. AND MOON, S.H., 2000. A kinetic study on oxidation of pentachlorophenol by ozone. *Air Waste Managem. Assoc*., **50**:555-562.

LI, P. AND SENGUPTA, A,K., 2000. Intraparticle diffusion during selective sorption of trace contaminants: the effect of gel versus macroporous morphology. *Environ. Sci. Tech.*, **34** : 5193-5200.

MANNISO, M.K., SALKINOJA, S.M.S. AND PUHAKKA, J.A., 2001. *In situ* polychlorophenol bioremediation potential of the indigenous bacterial community of boreal groundwater. *Water Res*., **35**: 2496-2504

MOLLAH, A.H. AND ALLEN, D.G., 1999. Biodegradation and detoxification of wood leachate from pentachlorophenol-treated poles . *Canadian J. Chem. Engin.*, **77**: 942-947.

PAL, N., KORFIATIS, G.P. AND PATEL, V., 1997. Sonochemical extraction and biological treatment of pentachlorophenol contaminated wood. *Hazard. Mat.*, **53**: 165-182.

PFENDER, W.F., 1996. Bioremediation bacteria to protect plants in pentachlorophenol-contaminated soil . *Environ. Qual*., **25**: 1256-1260.

SHARMA, A.K/. JOSEPHSON, G.B. AND CAMAIONI, D.M., 2000. Destruction of pentachlorophenol using glow discharge plasma process. *Environ. Sci. Tech*., **34**: 2267-2272.

THOMAS, L.C. AND CHAMBRLIN, G.J., 1975. *Colorimetric analytical methods*. 9th Edition, The Tintometer Ltd., Salisbury, England.

TIKOO,V., SCRAGG, A.H. AND SHALES, S.W., 1997. Degradation of pentachlorophenol by microalgae. *Chem. Tech. Biotech*., **68**: 425-431.

TOPALOVA, Y., KOZUHAROV, D. AND DIMKOV, R., 1999. Adaptive response of trivial activated sludge towards toxic effect of ONP, PCP and combination ONP/PCP. *Waste Managem.*, **19**: 247-254

WEAVERS, L.K., MALMSTADT, N. AND HOFFMANN, M.R., 2000. Kinetics and mechanism of pentachlorophenol degradation by sonication, ozonation, and sonolytic ozonation. *Environ. Sci. Tech.*, **34**: 1280-1285.

WILCOXON, J.P., 2000. Catalytic photooxidation of pentachlorophenol using semiconductor nanoclusters . *J. Phys. Chem. B.*, **104**: 7334-7343.

TABLE I.- NUMBER OF DIFFERENT BACTERIAL COLONIES OBTAINED FROM DIFFERENT SAMPLES INOCULATION ON PCP- CONTAINING MEDIA AND COLONIAL CHARACTERISTICS OF THE ISOLATES ON NUTRIENT AGAR.

Strain No.	No. of colonies on PCP containing media									Colony characteristics				
	M9-PCP-1[a]			M9-PCP-2[a]			M9-PCP-3[a]							
	1	2	3	1	2	3	1	2	3	Color	Margins	Elevation	Size	Shape
PCP-1	11	13	11	9	11	13	-b	-	-	White	Smooth	Flat	Small	
PCP-2	17	15	14	-	-	-	-	-	-	White	Smooth	Flat	Large	
PCP-3	13	10	16	-	-	-	-	-	-	Yellow	Smooth	Convex	Small	
PCP-4	12	12	16	12	-	-	-	-	-	White	Smooth	Flat	Medium	
PCP-5	12	28	27	12	17	21	11	13	15	White	Smooth	Convex	Medium	
PCP-6	13	14	11	-	-	13	-	-	04	White	Wavy	Flat	Large	

a, The media M9-PCP-1, M9-PCP-2 and M9-PCP-3 contained 0.001,0.002 and 0.005% of pentachlorophenol, respectively; b, Colony did not appear.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 199-204, 2004.

ASSESSMENT OF TVBN AND K FACTOR WITH SPECIAL REFERENCE TO MARKETING AND HANDLING OF FRESHWATER EDIBLE FISHES OF SINDH

MUHAMMAD IQBAL AND SULTAN AHMED
Department of Zoology, University of Karachi, Karachi
E-mail: iqbalkhi@khi.wol.net.pk

Abstract.- In order to sell the catch at a higher price as food or as ingredients for dried products, freshness becomes the most important factor, which is affirmed by using an index in the ship storage or landing place. The index that is more frequently used is the chemical index, total volatile basic nitrogen (TVB-N). The measurement principle is that as the freshness of the fish product decreases and the process of decay starts, the total amount of ammonia, methylamine, dimethylamine, trimethylamine and other total volatile basic nitrogen's gradually increases. The criterion of TVB-N is that fish meat has a value of 5-10 mg/100g, fish meat starting to decay has the value 30-40 mg/100g and the decayed fish meat has over 50mg/100g. However, in sharks and other cartilaginous fish that contain urea and therefore generate a large amount of ammonia, the TVB- N value exceeds 100mg/100 g, hence this method cannot be utilized for sharks. In present paper initial studies have been done on some commercially important edible fishes of Sindh region.

Key words: TVBN factor, freshwater, edible fishes, Pakistan transport.

INTRODUCTION

Large quantities of fish particularly harvested from inland waters are lost during handling, storage, transport and marketing due to their quick perish ability and lack of preservation facilities. Modern methods of fish preservation, refrigerated transport and cold storage at marketing centers can keep damage caused by the action of harmful bacteria to a very low level. If and when these methods can be more generally applied, substantial supplies of fish which are now wasted will be saved for human consumption. There are, however, quite a few problems involved. Almost 50% of fish catch is wasted as trash fish and converted into fish meal (Table I) and per capita consumption is less than 3 kg per person per year. The dearth of appropriate information on marketing, handling and preservation as well as lack of training of local fisherman to ascertain freshness of catch is one of the main drawbacks for the management and optimum utilization of the fisheries resources of Pakistan. Lot of fishes

(almost 50%) are sold as trash most of them are of small size but some are not properly preserved and are unfit for human consumption. The methodology presented here was developed by Jetro (2002) for Pakistan resources. The present work on TVBN factor and K-values is an attempt towards partial fulfillment of the basic requirements in the above direction. No information is available on any aspect of such work from Pakistan. Naturally it becomes necessary to affirm the freshness of each catch using an index in the ship's storage or landing place. The index that is most frequently used is the chemical index, total volatile basic nitrogen (TVBN). The measurement principle is that as the freshness of the fish products decrease and the process of decay starts, the total amount of ammonia, methylamine, dimethylamine, trimethylamine and other total volatile basic nitrogen gradually increases. An index of freshness is obtained by measuring this value. The criterion of TVBN is that in very fresh fish meat there is only 5-10 mg/l 00 g, in fish meat starting to decay 30-40 mg/l 00g and in decayed fish meat over 50 mg/l 00 g (Commel, 1995).

TABLE I.- FISH UTILIZATION PATTERN IN PAKISTAN.

	Quantity	**% of total**
Fishmeal	224	55
Shrimp for export	34	6
Finfish for export	10	3
Curing for export	29	7
Finfish export fresh	3	1
Domestic consumption	108	26
Total	405	100

MATERIALS AND METHODS

For measuring total volatile basic nitrogen (TVBN), the following procedure was adopted.

Sample (10 g) was ground in 50 ml water in a mortar. The protein was precipitated by adding 10 ml 20% trichloric acetic acid and churn. The mixture was filtered after 10 minutes. The filtrate (1 ml) was placed in the Conway units external room and Boric acid absorbing solution (Dissolve 10 g of boric acid in 200 ml ethanol in 1 litre flask. Add 10 ml of mixed indicator prepared by equally mixing 0.0066% methyl red and 0.0066% bromocresol green in absolute ethanol, make up the volume to 1 litre with distilled water) in the internal room. Potassium carbonate solution (50%, 1 ml, was added to the external room to

make it alkaline and to extricate the total volatile basic nitrogen. Lid was placed on the Conway unit to seal it with grease. The unit was left at 20°C for l20 minutes and then at 30° C for about 100 minutes. The boric acid absorbing solution absorbs the total volatile basic nitrogen during this period. The lid of the Conway unit was opened and boric acid was titrated with 0.02N-sulfuric acid. The color tone will display a change from green to colorless to rose red (at the end point). TVB-N was calculated using the formula

$$\text{TVB-N (mg/100 g)} = 0.28x\ (X-b)\ x\ fx100/0.1$$

where X is Blank test titration value, f is 0.02 N-sulfuric acid factor and b is sample solution. Fishes were identified using Western Indian Ocean Region 51 identification sheets (FAO, 1995).

RESULTS AND DISCUSSION

The results are summed up in Table II. TVB-N has a strong correlation with standard plate count (SPC), but even in the stage of autolysis before the growth of bacteria, a drop in freshness is seen. The degree of autolysis is considered to be very important factor as it has major influence on the taste and texture of fish. Convenient measurement methods have been developed in order to measure the K value. The principle is that after the fish dies, the ATP in the muscles are broken down ezymatically to ADP $\rightarrow$ AMP $\rightarrow$ inosinic acid (IMP) $\rightarrow$ inosine (HxR) $\rightarrow$ hypoxanthine (Hx). However, as freshness drops, nucleotide HxR or purine derivative Hx increases. At the same time, the ATP content is maintained for some time after the fish dies, but when this ATP content decreases, the muscles become rigid. Some fish species accumulate HxR, while others accumulate Hx or both. In each cases, the percentage of HxR and Hx within the ATP related material constituents become an index of freshness.

The fish that has K value 1-10% has just died and the one with K value of 11-20% is suitable for Sashimi*. The fresh fish has K value of 20-30% and is suitable for marketing, whereas values 30-80% represent decay stages. When K value goes above 50%, fish is unfit for human consumption. It could perhaps be used as fish meal manure or poultry feed. At K value of 50, if the fish is stored at 3-5°C, it will take 4 days to decay, whereas at 13-15°C, it will take a few hours to decay. At –20°C, the fish will remained fresh.

*Sashimi is a Japanese fish dish, in which raw fish is eaten.

TABLE II.- K VALUES OF DIFFERENT EDIBLE FISHES.

S.No.	Scientific name	Local Name	Code No.	K-value
1.	*Notopterus chitala*	Moh	NOTNO01	25-30
2.	*Labeo rohita*	Rohu	CYPLA09	38-48
3.	*Labeo calbasu*	Kalbans	CYPLA05	30-35
4.	*Labeo goinus*	Seereba	CYPLA10	35-40
5.	*Cirrhinus mrigala*	Morakha	CYPCI02	40-55
6.	*Tor tor*	Mahasher	CYPT)91	22-28
7.	*Tor pituttora*	Mahasher	CYPTO02	25-30
8.	*Cyprinus carpio*	Gulfam	PODPO06	25-30
9.	*Catla catla*	Theila	CYPCL01	40-45
10.	*Rita rita*	Khagga	BAGRI01	30-40
11.	*Aorichthys aor*	Singharee	BAGAO01	30-35
12.	*Bagarius bagarius*	Knigar	SISBA01	35-40
13.	*Wallago attu*	Mullee	SILWA01	40-50
14.	*Heteropneustes fossilis*	Singhee	HETHE01	15-20
15.	*Channa punctatus*	Soul	CHACH01	15-20
16.	*Channa striatus*	Daulanga	CHACH02	15-20

Packaging

The type of container to be used for transport of fish has a strong bearing upon the condition of fish when it is distributed to the public and as such is a matter that required careful consideration. With the use of a suitable container, the problem of preservation during transport can be tackled more easily.

In Pakistan the fish is transported to near and far places in mat or bamboo baskets, gunny bags and quite rarely in wooden boxes. These baskets and bags have big inter-spaces in them with the result that ice melts quickly. Moreover, the fish, being in direct contact with warm atmospheric air, deteriorates rapidly. The soft material of which these are generally made cannot protect the fish from becoming badly crushed.

Wooden boxes are most suitable for fish packaging. But when they are used a number of times without being properly cleaned and disinfected there is every likelihood of the presence and. growth of spoilage bacteria on fish which stick to the corners and joints of boxes. It is, therefore, most desirable that wooden fish containers should be non-returnable boxes; light and less timber may be used and such boxes may be reinforced by being bound with wire to withstand long transport. These wooden boxes should be neither too large nor too

small but of moderate and convenient size. In large boxes the fish at the bottom of the box is some time badly crushed and spoiled. Very small boxes are not economical.

Transportation

One of the most important functions with respect to fish preservation is concerned with the transport of fish. The situation, as prevailing in Pakistan. demand that there should be proper provision, and better use of transport facilities, especially to bring fish deficit areas within reach of existing fish supplies. In a sub-tropical country like Pakistan it has indeed a very strong bearing upon the condition of fish when they reach the market for sale. In some places the spoilage of fish is as high as 80% of the total catches mainly due to lack of transport facilities.

All available means of transport, road, rail, water and air should therefore, be brought into the highest level of efficiency. Speedy handling and provision of insulated or refrigerated vans bogies chambers of holders to carry the fish are top priority undertakings. In Express trains refrigerated compartments be attached for transport of perishable commodities. It is needed that all fast trains should be provided with refrigerated bogies for transporting fish from Karachi, Kotri, Rohri, Bubak Road, and Sehwan stations to various cities in Pakistan. trucks and lorries with insulated body should be provided between landing and marketing centers, especially at Karachi, Thatta, Hyderabad, Dadu Districts and in other divisions from various lakes, reservoirs and other big water areas to the cities of Lahore, Rawalpindi, Multan Sargodha and Peshawar.

Large quantities of fish particularly harvested from inland waters are lost during handling, storage, transport and marketing due to their quick perishability and lack of preservation facilities. Modern methods of fish preservation, refrigerated transport and cold storage at marketing centers can keep damage caused by the action of harmful bacteria to a very low rate. If and when these methods can be more generally applied, substantial supplies of fish which are now wasted will be saved for human consumption.

In reality, there are many obstacles to implementation, which come from various directions. Pakistan's fishing industry has enormous potential if these hurdles can be overcome (Bykov, 1985; Govindan, 1990).

ACKNOWLEDGEMENT

Thanks are due to JETRO and Export Promotion Bureau for providing training and literature in their workshop. Thanks Due to Dean Faculty of Science and V.C. for providing research funds.

REFERENCES

BYKOV, V.P., 1985. Marine Fishes. In: *Chemical composition and processing properties* (ed. A.A Balkema), (Publishers) Rotterdam.

COMMEL, J.J., 1995. *Control of fish quality*. The University Press, Cambridge.

FOOD AND AGRICULTURE ORGANIZATION, 1995. *Species identification for data sheets for fisheries purpose*. FAO, Rome

GOVINDAN, T.K., 1990. *Fish processing technology*. Oxford and IBH Publishing Company Pvt.Ltd. India.

JETRO, 2000. *Workshop on Fish processing technology and problems facing Pakistan Fish Industry*. Organized by Japan External Trade Org. (JETRO) and EPB, Karachi.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 205-211, 2004.

ENHANCING TRAP-CATCHES OF MELON FRUIT FLIES *BACTROCERA* SPP. (DIPTERA: TCPHRITIDAC) BY INTEGRATION OF VISUAL AND OLFACTORY CUES

ABDUL LATIF, KHALID ABDULLAH AND MUHAMMAD AKRAM
Agricultural Research Institute, Dera Ismail Khan 29020
E-mail: fruitflydik@brain netpk

Abstract.- A series of experiments was conducted to evaluate and optimize the design of an economical trap for masstrapping of fruit flies attacking muskmelon. Yellow triangular cardboard (10 x 18 cm) trap with the appropriate attractant effectively trapped fruit flies in melon fields. A combination of the sex attractants (Methyl eugenol and Culture) in the same trap decreased trap catches However, combination of a sex attractant with food, attractant (Protein hydrolysate) led to increase trap catches.

Key words: Muskmelon, Fruit flies, Visual and olfactory cues, Trap catches

INTRODUCTION

Visual and olfactory attractants are becoming increasingly important in Fruit fly control. Studies with fruit mimicking objects of different shapes and colors have been carried out with a number of species including *Rhagoletis pomonella* (Prokopy and Owens, 1978; Garcia, 2001), *Ceratitis capitata, Dacus tryoni* (Hill and Hooper, 1984) and the onion, fly, *Delia antiqua* (Harris and Miller, 1983). These studies showed that these flies have specific attraction to objects of the same shape, color and size as the host There are several important examples using olfactory attractants in fruit fly control. *Bactrocera dorsalis* was eradicated from the Mariana Islands by the use of male lure methyl eugenol plus naled (Steiner *et al.*, 1970; Abdullah *et al.*, 2002) *B. cucurbitae* populations were reduced by 90% by the use of cuelure in Hawaii (Cunningham and Steiner, 1972; Ball and Meats, 2000). Protein hydrolysate has been used to suppress the population of *Dacus tyroni* in Australia (Prokopy and Roitberg, 1984) and Mediterranean fruit fly, *C. capitata,* oriental fruit fly, *B. dorsalis,* and melon fruit fly, *B. cucurbitae,* in Hawaii (Harris *et al.*, 1971; Broumas *et al.*, 2002)

Traps with more than one type of attractants have been tested by some researchers and reported contrasting results. For example, Delrio and Zuemreoglu (1983) and Vita *et al*. (1986) found increased responses of flies

when food and sex attractants were placed in the same traps, while Hill (1986) reported a reduction in number of flies caught The major objectives of this study was to systemically enhance the visual and olfactory responses of fruit fly attracting melons and obtained an efficient and economical trap for their control.

MATERIALS AND METHODS

Experiments were carried out on farmers' fields in the vicinity of Agricultural Research Institute, D.I. Khan, Pakistan. Systematic evaluation of traps was conducted under four experiments, designed in a randomized complete block. Experiments 1-3 were carried out in one field, while experiment 4 was conducted in a second field.

Experiment 1

Yellow and white triangular cardboard traps, measuring 28 x 18 cm were used. The adhesive stikam, covering the upper surface of a 8 x 28 cm removable card lining the bottom of the trap, was used in order to facilitate the counting and removing of captured flies. Protein hydrolysate (PH) was used as standard food attractant in yellow and white colored card-hoard traps Three milliliters were injected into a cotton-wick (4 x 2 cm), which was wrapped at the middle with cellophane tape (I 5 cm width), and the wick was hung from the ceiling of the trap. The traps were placed in the field with 20 m trap to trap distance and were hung from a stand made of wooden stick at 0.75 m above the ground level. Two treatments, yellow (T1) and white (T2) traps baited with the food lure, were replicated five times for four days.

Flies trapped were counted daily according to species and sex. The position of traps was re-randomized on each day. Data were transformed by square root (X + 0.5) and then subjected to a two-way ANOVA for statistical analysis.

Experiment 2

Yellow and white traps were tested with male sex-attractants, methyl eugenol (ME) and cuelure (CL). The following four treatments were replicated five times for four days with a trap to trap distance of 15 m.

T1, yellow trap + ME; T, white trap + ME; T, yellow trap + CL; T, white trap + CL.

Four milliliters of each chemical attractant were injected into a cotton wick and installed in traps. The number of trapped flies was counted at the end of each day and statistical analysis was conducted according to the procedure as mentioned above Scheffe's test was used for mean separation.

Experiment 3

Evaluation of size of yellow ~raps with olfactory lures. Two sizes of yellow traps, measuring 28 x 15 cm (large size) and 18x10 cm (small size) were evaluated. Each trap was baited with food or sex-attractants. The design of experiment was a randomized complete block, consisting of the following six treatments with live replications and repeated for 3 days. Distance between traps was kept at 10m.

The same procedure for loading of chemicals in traps, recording number of captured flies, and statistical analysis was followed as mentioned above.

Experiment 4

Food and sex-attractants alone and in combination in small size (18 x 10 cm) yellow traps: The following six treatments were replicated five times for four days. The distance between traps were kept at 10m with total plot size of 80 x 70 m.

T1, PH; T2, ME; T3, CL; T4, PH + ME; TS, PH + CL; T6, PH + ME + CL.

The same procedures relating to loading of traps with lures, counting of flies captured, and statistical analysis of the data recorded, were followed as mentioned above.

RESULTS

Two species, *B. zonatus* and *B. cucurbitae* were found in traps during this trial. Yellow and white traps baited with the food attractant showed no significant difference at the 0.05 level (Table I). However, overall performance of yellow traps was better than for white traps in attracting both sexes of flies of each species.

TABLE I.- MEAN NUMBER OF FLIES CAPTURED/TRAP/DAY USING FOOD ATTRACTANTS WITH WHITE OR YELLOW TRAPS.

Coloured Traps	Number of flies / trap / day (n=20)			
	Bactrocera zonata		*B. cucurbitae*	
	Male	Female	Male	Female
Yellow	22.0a	20.0a	04.0a	15.0a
White	14.0a	13.0ab	04.0a	0.0b

Means followed by the same letter do not differ significantly at 0.05, ANOVA on square root transformed data.

Methyl eugenol and cuelure showed specific attraction for males of *B. zonatus* and *B. cucurbitae*, respectively. No significant differences ($p > 0.05$) were found between yellow or white color fraps baited with ME, while CL attracted significantly higher number ($p < 0.05$) of flies in yellow traps (Table II). However, overall captures rates of flies remained at low levels for both species.

TABLE II.- MEAN NUMBER OF FLIES CAPTURED/TRAP/DAY USING SEX ATTRACTANTS WITH WHITE OR YELLOW TRAPS (n=20).

Trap colour + lure	Number of flies / trap / day (n=20)	
	Bactrocera zonata	*B. cucurbitae*
	Male	Male
Yellow + ME	117.5a	0.00
White + ME	67.5b	0.00
Yellow + CL	0.00	60.0a
White + CL	0.00	27.5b

Means followed by the same letter do not differ significantly at 0.05, ANOVA on square root transformed data.

Small and large size yellow traps baited either with food or sex attractants showed no significant difference ($p > 0.05$) regarding size of traps (Table III). Traps with protein hydrolysate attracted males and females of both species, while traps baited with ME and CL attracted male flies, respectively.

A test of small (18 x 10 cm) yellow traps, baited with food and/or sex attractant alone or in combination (Table IV) revealed that more flies were caught when the food attractant (protein-hydrolysate) was combined with a single sex attractant. Significantly more ($p < 0.05$) male *R. zonatus* were attracted to T4 (PH + ME) than to other treatments. The lowest number of flies was caught when

food and both sex attractants were assembled in the same trap in treatment T6 (Table IV).

TABLE III.- EFFECT OF TRAP SIZE + ATTRACTANT ON CAPTURE OF FLIES/TRAP/DAY USING FOOD ATTRACTANTS WITH WHITE OR YELLOW TRAPS.

Bait + Trap size	Number of flies / trap / day (n=20)			
	Bactrocera zonata		*B. cucurbitae*	
	Male	Female	Male	Female
Food + Small*	02.0b	10.0a	03.0a	10.0b
Food + Large	00.6b	17.0a	0.00	27.0a
ME + Small	120a	10.0a	0.00	0.00
ME + Large	77.0a	03.0a	0.00	0.00
CL + Small	0.00	0.00	17.0a	0.00
CL + Large	0.00	0.00	20.0a	0.00

Means followed by the same letter do not differ significantly at 0.05, ANOVA on square root transformed data.
*Small size = 18x10 cm. Large size = 28x15 cm.

TABLE IV.- EFFECT OF COMBINATION OF SEX AND FOOD ATTRACTANTS.

Baited Traps	Number of flies / trap / day (n=20)			
	Bactrocera zonata		*B. cucurbitae*	
	Male	Female	Male	Female
Food	0.00	15.0a	03.0b	40.0a
ME	90.0a	0.00	0.00	0.00
CL	0.00	0.00	35.0b	0.00
Food + ME	120.0a	15.0ba	0.00	30.0b
Food + CL	10.0b	0.00	80.0a	10.0b
Food + ME + CL	40.0b	0.00	05.0	0.00

Means followed by the same letter do not differ significantly at 0.05, ANOVA on square root transformed data.

DISCUSSION

The result of trials comparing capture efficiency of traps indicated that small yellow rectangular (18 x 10 cm) traps, baited with the food attractant and a single male sex-attractant (either methyl eugenol or cuelure) can capture fruit flies efficiently. Although the number of flies captured in yellow and white colour traps were not significantly different, yellow traps generally attracted more flies. Thus yellow traps arc recommended for trap-out studies. Other

studies reporting on the impact of colours of haps for other tephritid flies showed that yellow is effective for Mediterranean fruit flies, *C. capitata* (Villeda *et al.*, 1988), and olive fruit flies, *D. oleae* (Haniotakis, 1986). It is thought that yellow constitutes a "supernormal" foliage-like stimulus, eliciting host and food seeking responses (Prokopy, 1972). Larger traps were not more efficient than smaller traps, even though the larger traps had a greater sticky surface (28 x 8 cm) than the smaller ones (18 x 6 cm). This may be due to the fact that a number of attracted flies could .have escaped through the larger opening of the large traps. Increased capture rates with smaller traps may also be due to some correspondence between size of traps and size of host fruit (see Prokopy, 1968 for an example of this phenomenon in *R. pumonell*. A combination of the food attractant with both sex attractants in the same traps lead to a decrease in the capture rate of flies of each species. It was observed earlier that methyl eugenol and cuelure specifically attract male *B. zonatus* and *B. cucurbitae*, respectively. However, when the two male sex attractants were placed in separate traps already baited with the food attractant, overall capture rate of flies was increased. These results agree with some of the studies carried out on other species of fruit flies. For instance, Tan (1983) reported comparison of means between traps baited with methyl eugenol and cuelure either mixed together or separate. Results show that the number of male *B. dorsalis* and *B. umbrosus* caught was significantly higher when sex attractants were separate than in traps baited with both sex attractants. The highest captures of *C. capitata* were recorded when Jackson traps were baited both with food attractant (protein hydrolysate) and a sex attractant, trimedlure (Hendrichs *et al*., 1989). Result presented here are not in agreement with those reported by Ito *et al*. (1976), in which a fiber block impregnated with a 6 g mixture of cuelure and methyl eugenol was shown equally attractive to *B. dorsalis* and *B. cucurbitae* when compared with methyl eugenol and cuelure separately.

ACKNOWLEDGEMENT

We are thankful to the PARC for providing partial financial support under the ALP project.

REFERENCES

ABDULLAH, K., AKRAM, M. AND ALIZAI, A.A., 2002. Non-traditional control of fruit flies in guava orchards in D.I. Khan. *Pak. J. agric. Res.*, **17**: 195-196.

BALL, G.D. AND MEATS, A., 2000. Influences of the odour of fruit yeast and Culure on the flight activity of the Queensland fruit fly *(Bactrocera tryom* (Froggatt) (Dipetra:

Tephritidae). *Aust. J. Ent.*, **39**: 195-200.

BROUMAS, T., HANIOTAKIS, G., LIAROPOULOS, C., TOMAZAU, T. AND RAGOUSSIS, N., 2002. The efficacy of an improved form of the mass trapping method for the control of olive fruit fly *Bactrocera olivae* (Gmelin) (Diptera: Tephritidae) pilot scale feasibility studies. *J. appl. Ent.*, **126**: 217-222.

GARCIA, J., 2001. A better fruit fly trap. *Agric. Res*., pp. 14-15.

HANIOTAKIS, G.E., 1986. Effect o size, color and height of pheromone baited sticky traps on capture of *Dacus oleae* flies. *Entomol. Hallen*, **4**: 55-61.

HARRIS, M.O. AND MILLER, J.R., 1983. Color stimuli and oviposition behaviour of the onion fly, *Delia antique* (Meigen) (Diptera: Anthomyidae). *Ann. ent. Soc. Am.*, **76**: 766-771.

HENDRICH, J., REYES, J. AND ALUJA, M., 1989. Behaviour of female and male Mediterranean flies, *Ceratitis capitata* in and around Jackson traps placed on fruiting host trees. *Insect Sci. Appl.*, **10**: 285-294.

HILL, A.R., 1986. Reduction in trap captures of female fruit flies (Diptera: Tephriidae) when synthetic male lures are added. *J. Aust. ent. Soc*., **25**: 211-214.

HILL, A.R. AND HOOPER, G.H.S., 1984. Attractiveness of various colours to Australian tephritid fruit flies in the field. *Ent. exp. Appl*., **35**: 119-128.

ITO, Y., TERYU, T. AND SAKIYAMA, M., 1976. Attractiveness of fiberblocks containing mixtures of methyl eugenol and cuelure on *Dacus dorsalis* and *D. cucurbitae*. *Res. Rep. Okinawa agric. exp. Stn*., **2**: 29-43.

PROKOPY, T.J., 1968. Visual responses of apple maggot flies, *Rhagoletis pomonella* (Diptera: Tephriidae) Orchard studies. *Ent. exp. Appl.*, **11**: 403-422.

PROKOPY, R.J., 1972. Responses of apple maggot flies to rectangles of different colors and shades. *Environ. Ent*., **1**: 720-723.

PROKOPY, R.J. AND OWENS, D.E., 1978. Visual generalist-visual specialist phytophygous insects: Host selection behaviour and application management. *Ent.. exp. Appl.*, **24**: 409-420.

PROKOPY, R.J. AND ROITBERG, B.D., 1984. Foraging behavior of true fruit flies. *Am. Sci.*, **72**: 41-49.

STEINER, L.F., HART, W.G., HARRIS, E.J., CUNNINGHAM, R.T., OHINATA, K. AND KAMAKAHI, D.C., 1970. Eradication of the Oriental fruit fly from the Mariana Islands by the methods of male annihilation and sterile insect release. *J. econ. Ent.*, **63**: 131-135.

TANK, K.H., 1983. Response of *Dacus* (Diptera: Tephritidae) on *Ocumum santum* (Linn.) extracts and different synthetic attractants in penang, Malaysia. In: *Fruit flies of economic importance* (ed. R. Cavlloro), pp. 513-520. A.A. Balkema, Rotterdam.

VILLEDA, M.P., HENDRICHS, J., ALUJA, M. AND REYES, J., 1988. Mediterranean fruit flies, *Certitis capitata*: Behaviour in nature in relation to different Jackson traps. *Fla. Entomol*., **71**: 154-162.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 213-236, 2004.

BIOCHEMICAL AND HAEMATOLOGICAL ABNORMALITIES IN HUMAN POPULATION EXPOSED TO HEXAVALENT CHROMIUM IN EFFLUENTS OF TANNERIES IN INDUSTRIAL AREA OF KASUR DISTRICT*

MUHAMMAD MASHHOOD AHSAN AND ABDUL RAUF SHAKOORI**
Department of Zoology, University of the Punjab, New Campus, Lahore

Abstract.- Chromium is one of the toxic heavy metals, which is extensively discharged in the environment through a large number of industrial operations such as metal finishing industries, petroleum refining, leather tanning etc. To evaluate the clinical toxicity of chromium, 600 human blood samples were collected from Niaz Nagar and Din Ghar of District Kasur and were analyzed heamatologicaaly as well as biochemically for various liver function tests. About 100 blood samples were collected from Khanuspur, Ayubia as control samples. Almost all liver function tests were found to be affected in the Kasur blood samples and were significantly different from those of the normal population of Khanaspur. The albumin, total protein and aminotransferases were higher, whereas bilirubin (direct and total) and glucose content are lower in the chromium exposed population. The exposed population had higher values for total chromium (327%) and chromium VI (1618%) in the blood serum of exposed population. There was a general trend of increased red blood cell count, platelets count and packed cell volume in the exposed population, whereas, other parameters such as haemoglobin content, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration decreased in the exposed population. The white blood cell count, mean corpuscular volume and erythrocyte sedimentation rate remained unchanged. No definite pattern has been observed in different haematological and biochemical parameters consequent to chromium exposure. Slight variations in different parameters may be due to multitude of factors in addition to possible effects of chromium toxicity.

Key words: Chromium toxicity, hexavalent chromium, liver function tests.

INTRODUCTION

The intensive development of industries and water disposal without efficient emission control, to protect the ambient environment, may cause the accumulation of high amount of heavy metals in soils, which cannot be degraded by any natural biological process. Consequently these harmful substances enter the food chain of man and accumulate in the tissues (Moore and Ramamoorthy,

---

* Part of Ph.D. thesis of first author.

** Corresponding author: arshak@brain.net.pk, Present address: School of Biological Sciences, University of the Punjab, New Campus, Lahore.

1984) and have varying toxic potentials in the soil (Mukherjee, 1998). At low concentrations heavy metals are essential to living system but are toxic at sufficiently high concentrations. Their toxicity occurs through displacement of essential metals from their natural native binding sites or through ligand interaction. Toxicity results from alterations in conformational structure of nucleic acid and protein interference with oxidative phosphorylation and osmotic balance (Poole and Gadd, 1989). These toxic metals interact with essential cellular components through covalent and ionic bonding. At high levels, both essential and non-essential metals can damage cell membrane, alter enzyme specificity, disrupt cellular function and damage the structure of DNA (Bruins *et al*., 2000; Blasiak *et al.,* 1999).

The fate of chromium in soil is partly controlled by redox potential and soil pH. Chromium normally exists in oxidation states ranging from chromium II to chromium VI. However, the three major forms of chromium that commonly exist in the environment are chromium (O), chromium (III) and chromium (VI) (Amacher and Baker, 1982; Bartlett and James, 1988; Weng *et al*., 1994). Only the trivalent (III) and hexavalent (VI) forms are of biological significance. All forms of chromium can be toxic at high levels but hexavalent compounds are more toxic than its trivalent form. Metallic chromium O is relatively non-toxic. In the natural environment, Cr VI is less stable than Cr III; Cr VI will convert to Cr III in the presence of organic and inorganic reducing agent. It has been estimated that the average half-life for Cr VI in the ambient air is less than 24 hrs (an estimation of 13 hrs was given in 1988; Research Triangle Institute, 1988).

The essentiality of dietary Cr has been demonstrated in numerous species, such as the mouse (Schroeder *et al.,* 1963), rat (Schwartz and Mertz, 1963; Schroeder e*t al.,* 1965; Mertz *et al.,* 1965), guinea pig (Seaborn *et al.,* 1994), turkey (Rosebrough and Steele, 1981), fish (Shiau and Lin, 1993), pig (Lindemann *et al.,* 1995) as well as children (Gurson and Sauer, 1973). For optimal health, as a supplement, women should take between 200-400 µg daily and men should take between 400-600µg daily. On the other hand, elevated amounts of chromium may be hazardous to fauna and flora (Nriagu *et al.,* 1988; Losi *et al.,* 1994).

High incidence of skin ulceration and nasal septum perforation was frequent consequences of occupational exposure to hexavalent chromium compounds in some industrial facilities for tanning, electroplating and chromate production. Tissue damage, irritation lesions of the skin and respiratory tract and cell mediated allergic reactions are also caused by the exposure of hexavalent

chromium (Samitz *et al.,* 1962; Samitz, 1955). The initial toxic signs of ingesting hexavalent chromium compounds by humans are abdominal pain, vomiting, diarrhea and intestinal bleeding (Sanz *et al.,* 1990). These are followed by renal failure resulting from tubular necrosis (Michie *et al.,* 1991). Hepatic failure secondary to primary hepatocellular damage, encephalopathy, methaemoglobinaemia and hemolysis are frequent complications (Pedersen and Morch, 1978).

Liver and kidney enzyme activities and blood glucose and heamoglobin have been reported to increase after 15 or 30 d of dermal exposure to nickel and chromium, besides increasing concentration of these metals in tissues (Mathur and Gupta, 1994; Milkovic-Kraus and Macan, 1996; Roto *et al.,* 1996). Chromium possesses both acute and chronic toxicities mainly associated with hexavalent chromium compounds such as dermatitis, allergic and eczematous skin reaction, skin and mucous ulceration, perforation of the nasal septum, allergic asthmatic, bronchial carcinomas, gastroenteritis, leukemia, Hodgkin's hepatocellular deficiencies and renal oligoanuric deficiencies (Goyer, 1986; Baruthio, 1992; Ptashekas, 1992; Gurjar *et al.,* 1996; Costa, 1997).

Industrial wastes laden with heavy metal are posing serious problems in Pakistan where the environmental awareness is abysmally low. Waste recycling treatments and disposal of effluents is not according to the world standards. In the province of Punjab, there are about 46,000 industrial units of various categories, out of which 4,600 units are considered to be the major contributors of pollution (Khalil *et al*., 1991). In Kasur the effluents from tanneries are discharged in open fields, rendering the agricultural land into waste land and the atmosphere absolutely smelly and the air unbreathable. The water of Bangla Kamboan (Kasur) has been reported to be harmful even for irrigation (personal communication). Hasnain and Sabri (1992) have reported the presence of toxic metals in concentration much higher than permissible limits in the industrial wastewaters of Lahore, including Kasur.

The aim of present project is to assess and evaluate the biochemical and haematological abnormalities in human population exposed to chromium contaminated effluents of tanneries in industrial area of district Kasur.

MATERIALS AND METHODS

Sampling sites

Kasur, a small industrial town, about 50 Km in the east of Lahore was

selected as sampling site for the presence of tanneries, which dispose off their effluents containing toxic materials, principally chromium directly into open fields without pretreatment. The effluents ultimately drain through open channels, which end in big ponds in and around the industrial area. These channels run through the residential areas and leave a distinctive odor, not now noticeable to the local inhabitants. Toxic chemicals have been seeping in the earth's core of these pounds, for over 40 years. This contaminates the potable water accessed by boring turbines. For control sites, Murree Hills, Ayubia and Khanaspur, which have no industry, and hence environmental hazards of the types found in Kasur do not exist, were taken as control sites.

Human subjects

The population of Kasur city is 2.365 million and that of Murree Hills is 2 million and generally belong to low economic group. The industrial area population which is indirectly exposed to chromium through food chain was considered as exposed (treated) population, whereas the population of Murree Hills, Ayubia and Khanspur, which is not exposed to chromium, was taken as control population.

Blood samples

Blood samples of 600 residents from Niaz Nagar and Din Ghar areas including those dealing with supply of dyes and chemicals used for leather processing residents, school children and teachers of Kasur Public High School and Sir Sayed High School, the ages of whom ranged between 5-60 years, both males and females, were taken. People not in proximity of the tanneries and on the out-skirts of Kasur were not included.

Blood (5ml) was drawn from the brachial vein in 5 ml disposable (BD) syringe of which 2.5 ml was dispensed in a 5 ml sterile glass test tube containing 3.75 mg of dipotassium salt of ethylenediamine tetra-acetic acid (EDTA) as an anti-coagulant, for the analysis of different haematological parameters. The remaining 2.5 ml blood was used for the biochemical analysis. For separation of serum, the blood samples were centrifuged at .1000x g for 30 minutes. An aliquot of serum samples (300µl) was stored in a freezer (-20^{o}C) for the estimation of total and hexavalent chromium estimation.

Water samples

Five water samples were collected from vicinity of tanneries, dumping ponds, potable water tanks, local turbines and the control areas. From each area a 50 ml water sample was taken in screw capped autoclaved bottles and used for the qualitative and quantitative analysis. The water samples were also processed for the estimation of total chromium, hexavalent chromium, lead and cadmium.

Haematological analysis of blood

The various haematological parameters such as RBC count, WBC count, packed cell volume (PCV), platelet count, and erythrocyte sedimentation ratre (ESR) were determined according to the procedure given in Dacie and Lewis (1950). Haemoglobin was estimated according to Van Kampen and Zijlstra (1961) and Eilers (1967). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were also calculated according to Dacie and Lewis (1950).

Biochemical analysis of blood serum

The blood serum was used for the estimation of total protein according to Henry *et al.* (1969), albumin according to Doumas *et al.* (1997) and Grant (1987), alkaline phosphatase (AP) according to King and King (1954), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) according to to Reitman and Frankel (1957), direct and total bilirubin according to Lo and Wu (1983), and serum glucose according to Barham and Trinder (1972).

Estimation of total chromium in blood samples

The blood serum (200µl) was digested according to Humphries (1958) by heating it in two 25 ml conical flasks with 0.5 ml of HNO_3 and 0.1 ml of $HClO_4$, on sand bath. The brown fumes of HNO_3 emitted from the flasks in the beginning were converted into dense white fumes. When solution became clear, flasks were removed from sand bath and after cooling the solution was transferred into 10 ml graduated cylinder and volumes were made up to 5 ml with distilled water. The diluted samples were used for chromium estimation (Rand *et al.*, 1979), for which 1 ml of digested sample (1 ml) was taken in 250 ml conical flasks in three replicates. Methyl orange (2-3 drops) was added as an indicator in these flasks. Pink colour appeared which showed acidic nature of the solution. Then NH_4OH was added with the help of burette till the pink colour of solution turned yellow,

which showed that solution had turned basic. Now 2-3 ml distilled water and H_2SO_4 (1:1) was added. The volume was made up to 40 ml with distilled water. Flasks were heated to boiling and then 2-3 drops of $KMnO_4$ were added. The colour of solution changed to purple or dark red. If the red / purple colour persisted then 0.5 ml of sodium azide was added in flask and boiled for two minutes. At the end solution was colourless. After cooling, 25 ml of H_3PO_4 was added, and the final volume was made upto 100 ml by adding distilled water. The mixture was shifted back to conical flask, to which 2 ml of diphenyl carbazide was added and then kept in dark for 30 minutes. Purple colour developed. Optical density was measured at 540 nm on spectrophotometer (200-D) by using water as a blank and the standard curve was used for the estimation of total chromium.

For preparation of standard curve chromium concentration ranging from 5µg to 100µg were processed as above. Potassium dichromate was used as chromium source.

Hexavalent chromium estimation

Blood serum (200 µl) was taken in a 50 ml conical flask in three replicates. 0.1 ml phosphoric acid was added, and final volume was made by adding 15 ml distilled water with the help of graduated cylinder and than 1 ml diphenyl carbazide was added and then kept in dark for 30 minutes. Purple colour developed. Optical density was taken at 540 nm on spectrophotometer (200-D) by using water as a blank. Hexavalent chromium was calculated by using the standard curve.

Estimation of chromium in water samples

Water sample (5 ml) was centrifuged at 1000x g for 15 minutes to separate the supernatant. The supernatants were processed for the estimation chromium by atomic absorption spectrophotometer at 357.9 nm. The concentration of chromium was estimated from the standard curve.

Statistical analysis

For statistical analysis the data was subjected to student's t test in order to determine the significant differences between treated and control samples.

RESULTS

Haematological studies

Table I shows haematological values of population of different age groups residing in industrial area of Kasur and also of those areas in Murree Hills, Ayubia and Khanaspurnt. There is a general trend of increased RBC count, platelets count and PCV value in the exposed population as compared with the control population. Whereas, other parameters such as haemoglobin content, MCH and MCHC are decreased in exposed population. The WBC count, MCV and ESR remained unchanged.

Age group 1 - 20 years

The control population had, on the average, 14.52±0.18 g haemoglobin per 100 ml. In population residing in industrial area the haemoglobin content was 4% less. In control population, the age group 1-20 years had 5.06 x 10^6 RBC /µl, 8.98 x ^{3} WBC /µl, 245.23 x 10^3 platelets /µl and PCV of 42.35%. These parameters increased 12%, 5%, 12%, and 7% respectively in the population of the same age residing in industrial areas (Table I). The other haematological parameters decreased 10% (MCH) and 11% (MCHC), in the exposed population.

Age group 21 – 40 years

The trends in various haematological parameters of this age group are the same as in the age group 1 – 20 years. The RBC count and PCV of blood of exposed population increased 11% each, whereas MCH and MCHC showed 8% decrease each.

Age group 41 – 60 years

The trend of changes in the various haematological parameters is the same as depicted in the first two age groups (Table I). Although WBC and platelet counts showed some increase, but significant increase of 6% in RBC count and 5% in PCV was recorded, whereas MCH and MCHC showed 10% decrease in both the parameters.

Biochemical analysis of blood serum

Table II shows the biochemical analysis of blood sera of treated population residing in industrial area of Kasur, which has been compared with those from the non-industrial areas. Almost all the parameters of liver function have been affected in the exposed population and are significantly different from those of the normal population. The albumin, total protein and aminotransferases are higher, whereas bilirubin (direct and total) and glucose content are lower in exposed population when compared with those of control population.

The population residing in industrial area showed increasing trend of total chromium and chromium VI in the blood serum when compared with that of non-industrial area.

Age group 1 – 20 years

The bilirubin content, both total and direct, were significantly lower *i.e.* 33% and 32%, respectively, in population residing in industrial area as compared with the control population. The other parameters such as ALT, AST, albumin and total protein showed significant increase of 31, 7, 9 and 17%, respectively. AP activity did not show any significant change. The glucose level in blood serum of exposed population was found to be 26% lower than in control population.

Total chromium and chromium VI showed 169% and 349% increase, respectively, when compared with the control population.

Age group 21 – 40 years

The pattern of changes in this age group is the same as in younger age group. The direct and total bilirubin contents were 44% and 48% lower, respectively, in the exposed population than in the control population. The ALT and AST activities, and albumin and total protein contents were 53%, 35%, 6% and 14% respectively more in the population residing in industrial area than those in the control area. The AP showed only 6% increase, which is non-significant. The glucose content, like in the previous age group, was 50% less in the exposed population than that of control population.

The population residing in industrial area showed 187% and 460% increase in the total chromium and chromium VI content of serum, which is significant as compared with the control population.

Age group 41 – 60 years

The direct and total bilirubin content in population residing in industrial area showed 56% and 58% lower values respectively, than those of the control population. The total protein, albumin, AP and ALT showed 18, 11, 15 and 54% higher values, respectively, in population residing in industrial area as compared with the control population. The AST values were not much different. The glucose content in blood serum of this age group was 32% lower in the exposed population.

The total chromium and chromium VI showed 327% and 1618% highly significant increase in population residing in industrial area as compared with the control population

DISCUSSION

The present study showed that residents of industrial area had significantly higher concentration of total and hexavalent chromium. The Kasur's drinking water had 250µg/l hexavalent chromium, compared to the control area drinking water, which had 22µg/l. It is obvious that Kasur water is highly contaminated.

Present study showed the biochemical and haematological defects in human population exposed to chromium in effluents of tanneries in industrial area of district Kasur. There are many other toxic agents/chemicals or heavy metals, which are related to this particular industry and affect the population under study. Arsenic is a common tannery chemical and has long been associated with lung cancer in workers who are exposed to it on a regular basis. Several studies have established links between sinus and lung cancers and the chromium used in tanning. Studies of leather-tannery workers in Sweden and Italy found cancer risks "between 20% and 50% above those expected (Labreche, 1997). The toxic affects of these agents are already established on the human bone marrow. Most important of these agents is benzene, which is related to many haematologic disorders including leukaemias, myelodysplasia and bone marrow depression. With this background the analysis of the haematological results need to be taken with utmost caution to avoid any misinterpretation.

Haematological parameters

The peripheral blood picture was not greatly affected by the short or long term exposure to chromium. Mild to moderate changes were evident, which is again consistent with earlier investigations and documentations (Canli, 1995; Willie, 1996).

White blood cell count

Increasing trend in WBC count was observed in the industrial area population and decreasing trend was observed in tannery workers and non-workers. The age group I and II of the industrial area population Although a number of investigators (Paustenbach *et al.,* 1996; Minoia and Cavalleri, 1988) have examined the uptake and effects of chromium in the red blood cells, little is known about chromium uptake in the white blood cells. Suggestions have been made on the use of WBC as a target for the development of biomarkers for chromium exposure. This may be based on the investigations that WBC accumulates a greater extent of hexavalent chromium, than do the RBC's. WBC accumulates Cr III both *in vitro* and *in vivo.* In addition, white blood cells accumulate chromium to a greater extent than red blood cells (Coogan *et al.,* 1991).

Red blood cell count

Determination of total chromium in serum and the red blood cells has showed a significant increase of chromium levels in erythrocytes of workers exposed to chromium VI (Cavalleri and Minoia, 1985). This increase was more in the younger age groups and seems to get lesser with the advancing age. The industrial area age group I, II and III appeared to have a 12%, 11% and 6% increase in the RBC count, respectively..

Paustenbach *et al.* (1996) reported that chromium concentrations of red blood cell and plasma returned rapidly to background levels within a few days after cessation of dosing since the concentration of chromium in the RBC would not decrease quickly if the chromium had entered the RBC as chromium VI. Concentration of Cr 10mg/l or less in drinking water of exposed humans appears to be completely reduced to Cr III prior to systemic distribution.

Studies on human blood revealed that RBC fraction apparently has the capacity to reduce Cr VI at concentration in blood up to 15,000 µg/l and that the

rate of Cr VI uptake into RBC may not exceed the rate of intracellular reduction at these concentration (Valeri *et al*., 2002). Significant increase of Cr levels in erythrocytes of workers exposed to Cr VI was observed. Cr III was absorbed through respiratory tract and distributed in the body (Cavalleri and Minoia, 1985). Oxidation state of Cr largely influences uptake, mechanism of absorption, transport and organ distribution as well as toxicity of Cr containing compounds. Cr VI hence is more toxic to occupationally exposed subjects, while Cr III has little effects.

Haemoglobin

In this study not much difference was observed in haemoglobin content in all the three categories of population. A 4% decrease was observed in age group I of industrial area population, 2% decrease in age group II and 5% decrease in the age group III, when compared to the control population. Flores-Tena and Martinez-Tabchet (2001) have reported that haemoglobin contents in *Limnodiluson* decreases significantly when Cr concentration increases above 1 µg/g dry weight.

PCV, platelet count, ESR and erythrocytic indices

The PCV in industrial area population was on relatively higher side in most subjects as compared to the control population. The age group I showed 7% higher value, age group II and III showed 5% higher PCV values as compared with the control group. of increase (8.5-9%) in the PCV values, when compared with the control. In the present study Platelet count was raised to 12% in the age group I, only 1% in the age groups II and 8% in the age group III.

Chromium exposure seemed to have no effect on ESR. Chromium concentration values were higher in plasma, erythrocytes and platelets 248%, 61% and 91%, respectively, and lower in the non nuclear leukocytes (35%). These are also age and sex dependent (Rukgauer and Zeyfang *et al.,* 2002).

Little or no change was observed in MCV in the industrial area population. The MCH and MCHC followed the same pattern of changes. The MCH decreased 10% in age group I, 8% in age group II and 10% in the age group III.

Liver function tests

Present study showed no significantly adverse effects on liver function

tests and haematological parameters of occupationally exposed human as well as animal. Present data principally shows insignificant rise or fall in total proteins (mainly albumin), AP and ASAT. Chen *et al.* (2001) reported that dietary chromium supplementation did not significantly influence serum constituents, including insulin, HDL, VLDL, total protein, albumin and gamma globulin. Uyanik (2001) has reported that no significant differences were found in total protein, albumin, ALAT, and ASAT. He also reported that chromium supplementation may affect carbohydrates and lipid metabolism and lipid deposition in lamb.

Albumin, total proteins, AP, ALAT, ASAT, total bilirubin, direct bilirubin and glucose, albumin, total proteins and ALAT are significantly higher in all exposed population of all the three age groups *i.e.* I, II and III age groups, when compared to the control population. ASAT remained unchanged in the I and III age group, but increase was observed in the age group II. Bilirubin (total and direct) and glucose contents are lower in exposed population when compared to control population. Similar decreasing trend in bilirubin (total and direct) and glucose was observed both in the male and female in industrial area population

Albumin

Blood serum albumin showed 9, 6 and 11% increase in the age group I, II and III of industrial area, respectively, when compared with the control group. Apparently resident of industrial areas are not adversely affected. Uyanik (2001) has reported a non-significant decrease in the albumin in sheep because of dietary chromium. Chen *et al.* (2001), on the other hand, have not shown significant effect on the serum constituents, including total protein, albumin, gamma globulin, insulin, HDL, VLDL, HDL-C and VLDL-C, after dietary chromium supplementation.

Some workers have referred to insignificant alteration in albumin levels. In patient who develop long standing or serious liver damage due to chronic chromium exposure as well as from other heavy metals are expected to have a lower albumin level. The higher albumin level in exposed population may actually reflect the increased production of albumin to accommodate increased transport of chromium in them. Ananth *et al.* (2000) have postulated molecular interaction of bovine serum albumin with potassium dichromate to form relatively stable chromium (V) as well as Cr (III) mediated cross-links in the proteins.

Alkaline phosphatase (AP)

Blood AP showed non-significant 1.6, 6 and 15%, increase, respectively, in the three age groups of industrial populations when compared with the controls population. Behari *et al.* (1978) have reported inhibition of acid phosphatase, ATPase and SDH after administration of trivalent and hexavalent chromium. Nehru and Kaushal (1993), on the other hand, reported significant increase in AP activity after lead intoxication.

Alanine and aspartate aminotransferases

The industrial population showed significant increase in the ALT activity in all the three age groups, 31%, 53% and 54%, respectively. AST, on the other hand, showed only 7% rise in the age group I, 35% in the age group II and 3% in age group III in the industrial area population.. The present studies showed that the liver function tests as reflected by the enzyme activities (ALT and AST) have variable patterns, with an overall increase in all age and sex groups when compared with the control population. This increase may be statistically significant, but are of no clinical significance as the obtained values remain well with in the normal range.

The chromium and other heavy metals have been reported to raise the level of aminotransferases. Awadallah and Hanna (1980) have reported that the serum AST was significantly higher in animals injected with chromium than cobalt, zinc and manganese; while serum ALT levels were higher in cobalt than in chromium, zinc and manganese. Bavazzano *et al.* (1981) reported that ALT and AST enzymatic activities are higher in tannery worker as compared to workers in the shoe factory. Kim and Na (1990) reported significant increase in serum lactate and pyruvate after intraperitoneal injection of sodium dichromate in rate (20 or 40 mg/kg). Vaglio and Landriscina (1999) reported increased ALT and AST in the serum after intoxication of cadmium. Wu *et al.* (2000) reported that a 33 years old white woman taking 6-12 time more chromium picolinates compared to recommended dose, presented with weight loss, anemia, thrombocytopenia, hemolysis and raised liver enzymes (15 to 20 times) as compared to the normal values. Guyton and Hall (2001) reported hepatic membrane damage due to continuous arsenic feeding, probably due to reduction of glutathione and anitoxidant enzymes through drinking water. Fatty liver with elevated serum aminotransferases has also been reported. Kumar *et al.* (1985) reported alteration in the distribution of AP acid phosphatase, glucose-6-

phosphatase and cholinesterase after chromium poisoning. Chromium has also been reported to inhibit these enzymes.

Total bilirubin

In the present study a significant decrease in the total bilirubin was observed *i.e.* 33% in the age group I, 48% in the age group II and 58% in the age group III. The total bilirubin did not show any appreciable change in the different age groups of industrial area population. Wu *et al.* (2000) have reported that the 6-12 time increased dose of chromium picolinate resulted weight loss, anemia, thrombocytopenia, hemolysis, liver dysfunction and renal failure. It is obvious that the chromium supplementation may cause serious liver and renal impairment when ingested in excess.

Direct bilirubin

The direct bilirubin content of industrial area population gave lower values as compared with the control population. The age group I showed 32% decrease, age group II 44% decrease and age group III 56% decrease. The total and direct bilirubin was found to be higher in control population as compared with the industrial area population. One of the possible factors may be a higher haemoglobin concentration at high altitudes (control population) resulting in high haemoglobin break down products like bilirubin. This however may be one of the contributing biochemical factors. Frank *et al.* (2000) reported increased concentration of total bilirubin in goats, deficient of Cu^{2+} and Cr^{6+}.

Total protein

An inconsistent pattern of increase was observed in all age groups for the total proteins. In industrial area population the total seral proteins in the age group I showed 17% increase, age group II showed 14% and age group III showed 18% increase. Total protein level increased in the industrial area populations, which most likely reflect the level of albumin. The possible variation may relate to different levels of exposure and liver toxicity (Uyanik, 2001; Chen *et al.*, 2001; Canli, 1995; Shrivastava and Nair, 2000).

Glucose metabolism

By far the most abundant of the absorbed monosaccharides is glucose, usually accounting for more than 80 per cent of carbohydrate calories absorbed.

The remaining 20 per cent of absorbed monosaccharides are composed almost entirely of galactose and fructose (Guyton and Hall, 2001). Causes of hyperglycemia are diabetes mellitus, adrenal cortical hyperactivity (Cushing's syndrome), acromegaly and obesity. Persistent hyperglycemia appear in insulinoma, adrenal cortical insufficiency, hypopituitarism, galactosemia and ectopic insulin production from tumors (Sood, 1999).

The effects of chromium in decreasing blood glucose have been well documented in literature (Awadallah and Hanna, 1980; Vincent , 2000; Kim *et al.,* 2002; Appleton *et al.,* 2002). A similar decrease was evident in this study. The age group I showed 26% decrease, the age group II showed 50% decrease and the age group III showed 32% decrease, when compared with the control population.

Fujimoto (1987) has reported that chromium, which is contained in glucose tolerance factor, showed lower blood concentration in patients with severe complications, such as retinopathy or nephropathy. Therefore, it appears that chromium plays an important role in advancing diabetes mellitus. Vincent (2000) has reported that chromium is an essential trace element for mammals and is required for maintenance of proper carbohydrate and lipid metabolism. Recently it has been revealed that the chromium-binding oligopeptide chromodulin may play a unique role in the autoamplification of insulin signaling. McCarty (2000) has reported that chromium picolinate can also aid muscle insulin sensitivity, and initial reports suggest that it is an effective therapy for type II diabetes. The evident ability of fiber-rich cereal products to decrease diabetes risk, as documented in prospective epidemiological studies. Kim *et al.* (2002) have reported that Cr supplementation can be considered to improve carbohydrates and lipid metabolism in patients receiving corticosteroid treatment and they suggest that Cr supplementation in dexamethasone treated rats can relatively reverse a catabolic state and increase insulin sensitivity. Ghosh *et al.* (2002) have reported that Cr supplementation seems to improve glycaemic control in type 2 diabetic patients, which appears to be due to an increase in insulin action rather than stimulation of insulin secretion. These effects being mostly beneficial towards the diabetic condition. Chromium supplementation has shown to improve blood glucose tolerance and improve insulin efficiency. Exposed population showed significantly lower random blood glucose levels in exposed and both in male and female population when compared with the control population. This observation of lower glucose level in exposed population directly correlates with the already documented affects of chromium supplementation in improving glucose tolerance (Awadallah and Hanna, 1980;

Vincent *et al.,* 2000; Kim *et al.,* 2002; Appleton *et al.,* 2002). The difference of blood glucose level between tannery workers and non-workers or the vicinity of industrial site that affected area was found to be insignificant.

Chromium uptake and toxicity

Total chromium levels showed an increase of 169%, 187% and 327% in age group I, II and III, respectively of population of industrial area. A staggering rise of the hexavalant chromium in serum levels was observed. A 349% rise in age group I, a 460% rise in the age group II and a 1618% rise in the age group III was found. Hexavalent chromium in Kasur water was recorded 165 µg/l in comparison to 12 µg/l, of the controls. This raised level would be the result of long-term direct exposure to chromium, ingestion through the drinking water, and inhalation of air borne chromium particulates.

The intensive development of industries and water disposal without efficient emission control, to protect the ambient environment, may cause the accumulation of high amount of heavy metals in soils, which cannot be degraded by any natural biological process. Consequently these harmful substances have entered the food chain of man. These heavy metals have long half-lives and accumulate in the tissues (Moore and Ramamoorthy, 1984) and have varying toxic potentials in the soil (Mukherjee, 1998).

Chromium is the 3rd largest chemical, which is used in lather tannery. Male workers have more tendencies to accumulate chromium in their body tissues. Male workers have higher concentration of chromium in their sera, because 95% of the workers are males in tanneries and the chromium accumulates with the passage of time (Kornhauser *et al*., 2002).

With regard to toxicity, pure metallic chromium is reported to be nontoxic. Chromium III is poorly absorbed, and much less toxic than chromium VI. The industrial monitoring for toxicity is mainly related to total and hexavalent chromium. The effect of chromium related to different parameters is primarily based on data obtained from experimental animals, *viz.* mice (Bagachi *et al.,* 2002; Wunder *et al.,* 2002), cats (Appleton *et al.,* 2002), sheep (Uyanik, 2001), rats (Jarrar and Mahmoud, 2000, Sutherland *et al.,* 2000), *Limnodrillus hoffmeisteri* (Oligochaeta: Tubificidae) (Flores-Tena and Martinez-Tabchet, 2001) and guinea Pig (Mathur and Gupta, 1994).

CONCLUDING REMARKS

No definite pattern has been observed in different haematological parameters. The differences observed in various parameters are mostly insignificant. Even when the differences are significant the mean values obtained are well within the normal range. Slight variation may be due to multitude of factors in addition to possible effects of chromium toxicity. The factors involved may include the non-exposed population residents of high altitude where the haemaglobin concentration is known to be higher. Similarly a relatively raised red cell count observed in exposed population particularly in age group II may partially be explained on the fact that this age group included a high percentage of heavy smokers resulting in higher red cell count but because of possible underlying nutritional deficiencies, which are considered to be quite common in these population, cells tend to remain hypochromic (low MCH). PCV, which is higher in exposed population, relates to higher RBC count. MCV is raised insignificantly in exposed population but remains within the normal range. A normal MCV with reduced MCH may be an indication of borderline iron deficiency state. WBC shows no significant change in count. Platelets appear to be relatively increased in exposed population but are well within the normal range.

No adequate evidence of carcinogenicity has been found in classical epidemiological studies, in industries using mainly trivalent chromium, such as the tanning industry (IARC, 1990; Langård, 1990). This industry uses trivalent chromium compounds in the processing of animal hides into leather. Although its carcinogenicity has been known for several years, there is still a considerable lack of knowledge of the mechanism(s) of toxic action of hexavalent chromium and the risks associated with various routes of exposure to both hexavalent and trivalent chromium compounds.

The significance of this study lies not on the results, which are by and large consistent with results from other parts of the world, but on the fact that human subjects were the focus of study. Such long term and consistent exposure to toxic elements are rarely found at any level in the western world. The enforcements of checks and balances on environmental pollution and the legalities of proper toxic disposals ensure that the residing populations are not exposed to such toxins. Pakistan, unfortunately lacks such essential checks, thereby the leverage of permissive dumping of chromium out in the open environment. Human beings are subjected to such exposure, but this give us an

opportunity to study the harmful effects. Such a level of study has rarely been documented in Pakistan. Such evident adverse effects would also help stress the need for proper and more stringent procedures towards the free and liberal use of numerous toxins, their safe handling and proper disposal, leaving the environment and the world a cleaner and safer place to live in.

REFERENCES

AMACHER, M. L. AND BAKER, D.E., 1982. *Redox reactions involving chromium, plutonium and manganese in soils. DOE/DP/0*4515-1 University Park, : Pennsylvania State University, PA, USA.

ANANTH, K. M., RAJARAM, R. AND RAMASAMI, T., 2000. Stabilization of chromium (V) species in bovine serum albumin. *Biochem. biophys. Res. Commun.*, **273:** 1138-1143.

APPLETON, D. J., RAND, J. S., SUNVOLD, G. D. AND PRIEST, J., 2002. Dietary chromium tripicolinate supplementation reduces glucose concentrations and improve glucose tolerance in normal-weight cats. *J. Fel. Med. Surg.*, **4:** 13-25.

AWADALLAH, R. AND HANNA, A., 1980. Serum enzyme changes due to trace amounts of some transition metal ions on the induction of experimental diabetes. *Z. Ernahrungswiss.*, **19:** 103-110.

BAGACHI, D., HASSOUN, E. A., BAGCHI, M. AND STOHS, S. J., 1995. Chromium induced excretion of urinary lipid metabolites, DNA damage, nitric oxide production and generation of reactive oxygen species in sprague-Dawley rats. *Comp. Biochem. Physiol.*, **110:** 177-187.

BARHAM, D, AND TRINDER, P., 1972. An improved colour reagent for the determination of glucose. *Analyst*, **97:** 142-145.

BARTLETT, R. J., 1991. Chromium cycling in soils and water: links, gaps and method. *Environ. Hlth. Perspect*., 92: 17-24.

BARTLETT, R. J. AND JAMES, B. R., 1988. Mobility and bioavailability of chromium in soils. In: *Chromium in the natural and human environments* (eds. J.O. Nriagu and E. Nieboer), pp. 267-304. John Wiley, New York,

BARUTHIO, F., 1992. Toxic effects of chromium and its compounds. *Biol. Trace Elem. Res*., **32:** 145-153.

BAVAZZANO, P., BENASSI, S., FORZIERI, R. AND PETRIOLI, G., 1981. Levels of aspartate aminotransferase and alanine aminotransferase in two factories with various hepato-toxic risks. *Quad. Sclavo. Diagn.*, **17:** 407-420.

BEHARI, J., CHANDRA, S. V. AND TANDON, S. K., 1978. Comparative toxicity of trivalent and hexavalent chromium to rabbits. III. Biochemical and histological changes in testicular tissue. *Acta Biol. Med. Ger*., **37:** 463-468.

BLASIAK, J., TRZECIAK, A., MALECKA-PANAS, E., DRZEWOSKI, J. AND IWANIENKO, T., 1999. DNA damage and repair in human lymphocytes and gastric mucosa cells exposed to chromium and curcumin. *Teratog. Carcinog. Mutagen.*, **19:** 19-31.

BRUINS, M. J., SOETERS, P. B. AND DEUTZ, N. E., 2000. Endotoxemia affects organ protein metabolism differently during prolonged feeding in pigs. *J. Nutr.*; **130:** 3003-3013.

CANLI, M., 1995. Effects of mercury, chromium and nickel on some blood parameters in the carp *Cyprinus carpio*. *Turkish J. Zool.*, **19:** 301-311.

CAVALLERI, A. AND MINOIA, C., 1985. Serum and erythrocyte chromium distribution and

urinary elimination in persons occupationally exposed to chromium VI and chromium III. *G. Ital. Med. Lav.*, **7:** 35-38.

CHEN, N. S. C., TSAI, A. AND DYER, I.A., 1973. Effect of chelating agents on chromium absorption in rats. *Nutrition*, **103:** 1182-1186.

CHEN, K. L., LU, J. J., LIEN, T. F. AND CHIOU, P. W., 2001. Effects of chromium nicotinate on performance, carcass characteristics and blood chemistry of growing turkeys. *Br. Poult. Sci.*, **42:** 399-404.

COOGAN, T. P., MOTZ, J., SNYDER, C. A., SQUIBB, K. S. AND COSTA, M., 1991. Differential DNA-protein crosslinking in lymphocytes and liver following chronic drinking water exposure of rats to potassium chromate. *Toxicol. appl. Pharmacol.*, **109:** 60-72.

COOGAN, T. P., SQUIBB, K. S., MOTZ. J., KINEY, P. L. AND COSTA, M., 1995. Distribution of chromium within cells of the blood. *Toxicol. appl. Pharmacol.*, **109:** 60-72.

COSTA, M., 1997. Toxicity and carcionogenicity of Cr (VI) in animal models and humans. *Crit. Rev. Toxicol.*, **27:** 431-432.

DACIE, J.V. AND LEWIS, S. M., 1991. In: *Practical haematology*. Seventh edition, Churchill Livingstone, Edinburgh.

DOUMAS, B. T., WATSON, W. A. AND BIGGS, H. G., 1997. Albumin standards and measurement of serum albumin with bromcresol green. *Clin. Chim. Acta*, **258:** 21-30.

EILERS, R. J., 1967. Notification of final adoption of an international method and standard solution for haemoglobinometry specifications for preparation of standard solution. *Am. J. clin. Path.*, **47:** 212.

FLORES-TENA, F. J. AND MARTINEZ-TABCHET, L., 2001. The effect of chromium on the haemoglobin concentration of *Limnodrilus hoffmeisteri* (Oligochaeta: Tubificidae). *Ecotoxicol. Environ. Saf.*, **50:** 196-202.

FRANK, A., DANIELSSON, R. AND JONES, B., 2000. Experimental copper and chromium deficiency and additional molybdenum supplementation in goats. II. Concentration of trace minor element in liver, kidneys and ribs: haematology and clinical chemistry. *Sci. Total Environ.*, **249:** 143-170.

FUJIMOTO, S., 1987. Studies on the relationship between blood trace metal concentration and the clinical status of patients with cerebrovascular diseases, gastric cancer and diabetes mellitus. *Hokkaido Lgaku. Zasshi*, **62:** 913-32.

GHOSH, D., BHATTACHARYA, B., MUKHERJEE, B., MANNA, B., SINHA, M., CHOWDHURY, J. AND CHOWDHARY, S., 2002. Role of chromium supplementation in Indians with type 2 diabeties mellitus. *J. Nutr. Biochem.*, **13:** 690-697.

GOYER, R. A., 1986. Toxic effects of metals. In: *Casarett and Deull's Toxicology, the basic science of poisons* (eds. C. D. Klaassen, M. D., Amdur and J. Doull). Macmillan Publishing company, New York.

GRANT, G. H., 1987. *Amino acids and proteins; Fundamentals of clinical chemistry* (eds. N.W. Tietz), Third addition, W.B. Saunders Company, Philadelphia, USA, pp. 328-329.

GURJAR, B. R., MOHAN, M. AND SIDHU, K. S., 1996. Potential health risk to carcinogens in the atmosphere environment in India. *Regul. Toxocol. Pharmacol.*, **24:** 141-148.

GURSON, C. T. AND SAUER, G., 1973. Effects of chromium supplementation on growth in marasmic protein-calorie malnutrition. *Am J. Clin. Nutr.*, **26:** 988-991.

GUYTON, A. C. AND HALL, J. E., 2001. *Texbook of medical physiology*. Tenth edition. W. B. Saunders Company, New York.

HASNAIN, S. AND SABRI, A., 1992. Effect of temperature and pH on conjugal transfer of Zn-resistant plasmids residing on gram-negative bacteria isolated from industrial effluents. *Environ. Poll.*, **76:** 245-249.

HENRY, J. B., BEELER, M. F., COPELAND, B. E. AND WERT, E. B., 1969. A format for description of methods in clinical pathology. *Am. J. Clin. Pathol.,* **52:** 296-299.

HUMPHRIES, E. C., 1958. Mineral Component and ash analysis. In: *Modern methods of plant analysis* (eds. K. Paech and M. Y. Traley), Springer Verlag, Berlin.

IARC., 1990. *IARC monographs on the evaluation of carcinogenic risks to humans. Chromium, nickel and welding.* In: IARC, vol. 49. World Health Organization, Lyons, France. pp. 49-256.

JARRAR, B. M. AND MAHMOUD, Z. N., 2000. Histochemical demonstration of changes in the activity of hepatic phosphatases induced by experimental lead poisoning in male white rats (*Rattus norvegicus*). *Toxicol. Ind. Hlth.,* **16:** 7-15.

KHALIL, M., ADEEB, F., JHASSAN, S. AND IQBAL, J., 1991. *Annual progress report of EPA research laboratories (1990-1991).* Environmental Protection Agency, House Physical Environment Planning Department, Government of the Punjab, Pakistan.

KIM, E. AND NA, K. J., 1990. Acute toxic effect of sodium dichromate on metabolism. *Arch. Toxicol.,* **64:** 644-649.

KIM, D. S., KIM, T. W., PARK, I. K., KANG, J. S. AND OM, A. S., 2002. Effects of chromium picolinate supplementation on insulin sensitivity, serum lipids, and body weight in dexamethasone-treated rats. *Metabolism,* **51:** 589-594.

KING, P. R. N. AND KING, E. J., 1954. Estimation of plasma phosphatases by determination of hydrolysed phenol with aminoantipyrine. *J. clin. Pathol.,* **7:** 322-326.

KORNHAUSER, C., WROBEL, W., MALACARA, J.M., VAVA, L.E., GOMEZ, L. AND GONZALEZ, R., 2002. Possible adverse effect of chromium in occupational exposure of tannery workers. *Ind. Hlth.,* **40:** 207-213.

KUMAR, A., RANA, S. V. AND PRAKASH, R., 1985. Dysenzymia induced by hexavalent chromium in rat liver. *Int. J. Tiss. React.,* **7:** 333-338.

LABRÈCHE, F., 1997. Occupations and breast cancer: Evaluation of associations between breast cancer and workplace exposures, McGill University, Montréal, 23 December.

LANGARD, S., 1990. One hundred years of chromium and cancer. A review of epidemiological evidence and selected case reports. *Am. J. ind. Med.,* **17:** 198-215.

LINDEMANN, M. D., WOOD, C. M., HARPER, A. F., KORNEGAY, E. T. AND ANDERSON, R. A., 1995. Dietary chromium picolinate addition improve gain: feed and carcass characteristics in growing-finishing pigs and increase litter size in reproduction sows. *J. Anim. Sci.,* **73:** 457-465.

LO, D.H. AND WU, T.W., 1983. Assessment of the fundamental accuracy of the Jendrassik-Graf total and direct bilirubin assay. *Clin. Chem.,* **29:** 31-36.

LOSI, E., AMREIN, C. AND FRANKENBERGER, W. T., 1994. Environmental biochemistry of chromium. In: *Reviews of environmental contamination and toxicity* (ed. G.W. Ware), vol. 136, Springer-Verlag, Heidelberg, pp. 91-121.

MATHUR, A. K. AND GUPTA, B. N., 1994. Dermal toxicity of nickel and chromium in guinea pigs. *Vet. Hum. Toxicol.,* **36:** 131-132.

McCARTY, M. F., 2000. Toward practical prevention of type 2 diabetes. *Med. Hypoth.,* **54:** 786-93.

MERTZ, W., ROGINSKI, E. E. AND REBA, R. C., 1965. Biological activity and fate of trace quantities of intravenous chromium (III) in the rat. *Am. J. Physiol.,* **209:** 614-618.

MICHIE, C. A., HAYHURST, M., KNOBEL, G. J., STOKOL, J. M. AND HENSLEY, B., 1991. Poisoning with a traditional remedy containing potassium dichromate. *Hum. exp. Toxicol.,* **10:** 129-131.

MILKOVIC-KRAUS, S. AND MACAN, J., 1996. Can pre-employment patch testing help to

prevent occupational contact allergy. *Cont. Dermat*., **35:** 226-228.

MINOIA, C., AND CAVALLERI, A., 1988. Chromium in urine, serum and red blood cells in the biological monitoring of workers exposed to different chromium valency states. *Sci. Total Environ.*, **71:** 323-327.

MOORE, J. AND RAMAMOORTHY, S., 1984. *Heavy metals in natural waters*. Kedar NathRam Nath, Meerut, India, pp. 435-441.

MUKHERJEE, A. B., 1998. Chromium in the environment of Finland. *Sci. Total Environ.*, **217**: 9-19.

NEHRU, B. AND KAUSHAL, S., 1993. Alteration in the hepatic enzymes following experimental lead poisoning. *Biol. Trace Elem. Res*., **38:** 27-34.

NRIAGU, J. O., PACYNA, J. M., MILFORD, J. B. AND DAVIDSON, C. L., 1988. Distribution and characteristic features of chromium in the atmosphere. In: *Chromium in the natural and human environments* (eds. J. O. Nriagu and E. Niebour), pp. 125-172. John Wiley, New York.

PAUSTENBACH, D. J., HAYS, S., BRIEN, B., DODGE, D. G. AND KERGER, B. D., 1996. Observation of steady state in blood and urine following human ingestion of hexavalent chromium in drinking water. *J. Toxicol. environ. Hlth.*, **49:** 453-461.

PEDERSEN, R. S. AND MORCH, P. T., 1978. Chromic acid poisoning treated with hemodialysis. *Nephron*, **22:** 592-595.

POOLE, R. AND GADD, G, M., 1989. *Metal-microbe intraction.* IRL, Press, Oxford.

PTASHEKAS, J. R., 1992. Environmental monitoring in Lithuania. Environmental distress signals: gastrointestinal epithelial barrier after exposure to chemical agents. *J. environ. Pathol. Toxicol. Oncol.*, **11**: 299-302.

RAND, M. C., ARNOLD, E. AND MICHEAL, J., 1979. *Standard methods for the examination of water and waste water*. American Public Health Association.

REITMAN, S. AND FRANKEL, S., 1957. A colorimetric method for the determination of serum glutamate oxaloacetate and glutamate pyruvate transaminase. *Am. J. clin. Pathol*., **28:** 56-63.

RESEARCH TRIANGLE INSTITUTE, 1988. *The fate of hexavalent chromium in the atmosphere,* Research Triangle Park, N. C. RTI/3798/00-01F.

ROSEBROUGH, R. W. AND STEELE, N. C., 1981. Effect of supplemental dietary chromium or nicotinic acid on carbohydrate metabolism during basal, starvation and refeeding periods in poult. *Science*, **60:** 407-417.

ROTO, P., SAINIO, H., REUNALA, T. AND LAIPPALA, P., 1996. Addition of ferrous sulfate to cement and risk of chromium dermatitis among construction workers. *Cont. Dermat.*, **34:** 43-50.

RUKGAUER, M. AND ZEYFANG, A., 2002. Chromium determinations in blood cells: clinical relevance demonstrated in patients with diabetes mellitus type 2. *Biol. Trace Elem. Res*., **86:** 193-202.

SAMITZ, M. H., 1955. Some dermatological aspects of the chromate problem. *Arch. Ind. Hlth.*, **11:** 361-367.

SAMITZ, M. H., SHRAGER, J. D. AND KATZ, S. A., 1962. Studies on the prevention of the injurious effects of chromates in industry. *Ind. Med. Surg*., **31:** 427-432.

SANZ, P., NOGUE, S., MUNNE, P., TORRA, R. AND MARQUES, F., 1990. Acute potassium dichromate poisoning. *Hum. exp. Toxicol.*, **10:** 228-229.

SEABORN, C. D., CHENG, N., ADELEYE, B., OWENS, F. AND STOECKER, B. J., 1994. Chromium and chronic ascorbic acid depletion effects on tissue ascorbate, manganese and C^{14} retention from C^{14}-ascorbate in guinea pigs. *Biol. Trace Elem. Res.*, **41:**279-294.

SCHROEDER, H. A., VINTON JR. W.H. AND BALASSA, J.J., 1963. Effect of chromium, cadmium and other trace metals on the growth and survival of mice. *J. Nutr.*, **80:** 48-54.

SCHROEDER, H. A., BALASSA, J. J. AND VINTON, H. H., 1965. Chromium, cadmium, and lead in rats: effects on life span, tumors, and tissue levels. *J. Nutr.*, **86:** 51-66.

SCHWART, K. AND MERTZ, W., 1963. A glucose tolerance factor and its differentiation from factor 3. *Arch. Biochem. Biophys.*, **85:** 292-295.

SHIAU, S. Y. AND LIN, S. F., 1993. Effect of supplemental dietary chromium and vanadium on the utilization of different carbohydrates in tilapia. *Oreochromis niloticus* X *O. aureus*. *Aquaculture*, **110:** 321-330.

SHRIVASTAVA, H. Y. AND NAIR, B. U., 2000. Protein degradation by peroxide catalyzed by chromium (III): Role of coordinated ligand. *Biochem. biophys. Res. Commum.*, **270:** 749-754.

SOOD, R., 1999. In: *Medical laboratory technology: Methods and interpretations.* Fifth edition. Jaypee Brothers Medical Publishers (P) LTD, New Delhi.

SUTHERLAND, J. E., ZHITKOVICH, A., KLUZ, T. AND COSTA, M., 2000. Rats retain chromium in tissues following chronic ingestion of drinking water containing hexavalent chromium. *Biol. Trace Elem. Res.*, **74:** 41-53.

TROW, L. G., LEWIS, J., GREENWOOD, R. H., SAMPSON, M. J., SELF, K. A, CREWS, H. M. AND FAIRWEATHER-TAIT, S. J., 2000. Lack of effect of dietary chromium supplementation on glucose tolerance, plasma insulin and lipoprotein levels in patients with type 2 diabetes. *Int. J. Vitam. Nutr. Res.*, **70:** 14-18.

UYANIK, F., 2001. The effects of dietary chromium supplementation on some blood parameters in sheep. *Biol. Trace Elem. Res.*, **84:** 93-101.

VAGLIO, A. AND LANDRISCINA, C., 1999. Changes in liver enzyme activity in the teleost *Sparus aurata* in response to cadmium intoxication. *Ecotoxicol. Environ. Saf.*, **43:** 111-116.

VALERI, C. R., PIVACEK, L. E., CASSIDY, G. P. AND RAGNO, G., 2002. Volume of RBCs 24-and 48-hours posttransfusion survivals, and the lifespan of (VI) Cr and biotin-X-N-hydroxysuccinimide (NHS)-labeled autologous baboon RBCs: effect of the anticoagulant and blood pH on (VI) Cr and biotin-X-NHS elution *in vivo*. *Trasfusion*, **42:** 343-348.

VAN KAMPEN, E. J. AND ZIJLASTRA, W.G., 1961. Standardization of haemoglobinometry 11. The haemoglobin cyanide method. *Clin. chim. Acta*, **6:** 538-544.

VINCENT, J. B., 2000. Elucidating a biological role for chromium at a molecular level. *Acc. Chem. Res.*, 33**:** 503-510.

WENG, C. H., HUANG, C. P., ALLEN, H. E., CHENG, A. H.-D. AND SANDERS, P. F., 1994. Chromium leaching behavior in soil derived from chromite ore processing waste. *Sci. Total Environ.*, **154:** 71-86.

WILLIE, G. R., 1996. Trace element. In: *Laboratory test handbook* (eds. D.S., Jacobs, W.R. Demott, H.J. Grady, R.T. Harvat, D.W. Huestis, and B.L. Kasten), 4th Ed. pp 587-596. Lexi-Comp, Inc. Hudson, Cleveland, USA.

WU, F. Y., KUO, H. W., TSAI, C. H., WU, F. Y., WANG, R. Y. AND LAI, J. S., 2000. Cytogenetic study of workers exposed to chromium compounds. *Mutat. Res*., **24**: 289-296.

WUNDER, C., BROCK, R.W., MCCARTER, S.D., BIHARI, A., HARRIS, K., EICHELBRONNER, O. AND POTTER, R.F., 2002. Inhibition of haem oxygenase activity increase leukocyte accumulation in the liver following limb ischaemia-reperfusion in mice. *J. Physiol.*, 540**:** 1013-1021.

TABLE I.- HAEMATOLOGICAL ANALYSIS OF BLOOD OF EXPOSED POPULATION FROM TANNERIES OF KASUR INDUSTRIAL AREA. THE POPULATION OF MURREE HILLS, KHANSPUR AND AYUBIA HAS BEEN TAKEN AS CONTROL POPULATION, AS THERE IS NO TANNERY AND NO OTHER INDUSTRY IN THAT AREA OF POPULATION.

Haematological parameters	**Age group upto 30 years**		**Age group 21-40 years**		**Age group 41-60 years**	
	Control (n=30)	**Treated (n=159)**	**Control (n=50)**	**Treated (n=356)**	**Control (n=20)**	**Treated (n=85)**
WBC [b] ($x10^3/\mu l$)	8.98±0.39 [a]	9.44±0.16	8.44±0.29	8.89±0.11	8.35±0.52	9.16±0.30
RBC ($x10^6/\mu l$)	5.06±0.07	5.68±0.29*	5.12±0.07	5.66±0.03***	5.23±0.10	5.56±0.08 *
Hb (g/dl)	14.52±0.18	13.92±0.13**	15.52±0.23	14.79±0.08	15.28±0.24	14.51±0.19*
PCV (%)	42.35±0.56	45.26±0.39***	42.94±0.62	47.58±0.25***	45.15±0.70	47.21±0.53**
MCV (fl)	83.93±0.97	84.32±0.56	84.09±0.95	84.43±0.35	86.41±1.45	85.50±0.79
MCH (pg)	28.79±0.37	25.90±0.21***	28.42±0.36	26.27±0.14***	29.33±0.44	26.32±0.31***
MCHC (g/dl)	34.34±0.25	30.71±0.16***	33.80±0.21	31.13±0.11***	33.99±0.26	30.74±0.24***
Plt ($x10^3/\mu$)	245.23±10.9	275.11±6.12*	256.16±11.63	259.22±4.38	227.30±12.31	245.81±7.50
ESR (mm/hr)	7.40±0.79	7.14±0.31	6.02±0.57	6.86±0.29	7.10±1.13	7.69±0.57

a. Mean ± SEM, *P<0.05; **P<0.01; ***P<0.001

b. Abbreviations used; ESR, erythrocyte sedimentation rate; Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PCV, pecked cell volume; Plt, platelets count; RBC, red blood cells; WBC, white blood cells.

c. Population living in industrial area of Kasur, which predominately comprises tanneries.

TABLE II.- BIOCHEMICAL ANALYSIS OF BLOOD OF POPULATION RESIDING IN INDUSTRIAL AREA OF TANNERIES OF KASUR. MURREE HILLS, AYUBIA AND KHANASPUR WAS TAKEN AS CONTROL POPULATION, AS THERE IS NO TANNERY AND NO OTHER INDUSTRY IN THAT AREA.

Biochemical parameters	Age group upto 30 years		Age group 21-40 years		Age group 41-60 years	
	Control (n=30)	Treated (n=159)	Control (n=50)	Treated (n=356)	Control (n=20)	Treated (n=85)
Alb [b] (g/dl)	4.71±0.06 [a]	5.15±0.05 ***	4.79±0.04	5.07±0.02 ***	4.59±0.06	5.09±0.04 ***
AP (U/l)	394.47±55.52	400.80±19.85	230.00±10.18	243.31±3.61	228.35±24.99	262.95±8.00
ALT (U/l)	23.00±2.41	30.22±1.66 *	23.54±1.54	35.97±1.39***	23.90±4.48	36.75±2.71*
AST (U/l)	29.30±2.52	31.20±1.12	24.84±1.31	33.41±0.87***	30.10±3.86	30.96±1.78
D.Bili (mg/dl)	0.34±0.02	0.23±0.01 ***	0.44±0.04	0.25±0.01 ***	0.48±0.07	0.21±0.01 ***
T.Bili (mg/dl)	0.83±0.07	0.56±0.02 ***	1.07±0.10	0.56±0.02 ***	1.15±0.18	0.49±0.02 ***
T.Prot (g/dl)	6.77±0.13	7.91±0.06 ***	6.98±0.07	7.97±0.04 ***	6.74±0.15	7.93±0.07 ***
Glu (mg/dl)	112.20±2.35	83.05±5.22***	110.82±1.76	74.05±1.46***	117.05±4.33	79.37±4.67***
T.Cr (µg/l)	26.83±5.04	72.11±2.89***	21.80±3.52	62.64±1.66***	15.25±4.71	65.24±3.68***
Cr. VI (µg/l)	2.67±1.04	12.01±0.99***	1.50±0.29	8.41±0.49 ***	0.5±0.96	8.59±0.96 **

a. Mean ± SEM, *P<0.05; **P<0.01; ***P<0.001

b. Abbreviations used; Alb, albumin; ALT, alanine aminotransferases; AP, alkaline phosphatase; AST, aspartate aminotransferases; Cr. VI, chromium VI; D.Bili, direct bilirubin; Glu, glucose; T.Bili, total bilirubin; T.Cr, total chromium; T.Prot, total protein.

c. Population living in industrial area of Kasur, which predominantely comprises Tanneries.

Proc. Pakistan Congr. Zool., Vol. 24, pp. 237-245, 2004.

TRIPS AGREEMENT OF WTO AND GENETIC RESOURCES: IMPLICATIONS AND OPTIONS FOR DEVELOPING COUNTRIES

MUHAMMAD IRFAN KHAN
Department of Environmental Science, Allama Iqbal Open University, Islamabad
Email: irfankhan_64@yahoo.com

CONTENTS

Abstract.- The WTO has no specific agreement dealing with the environment. However, a number of the WTO agreements include provisions dealing with environmental concerns like agreement on Trade Related Intellectual Property Rights (TRIPS). The clause 27.3 (b) of Section V of TRIPS Agreement extends an exemption that allows WTO members to refuse to grant patents for plants and animals (other than microorganisms and non-biological and microbiological processes). It is argued that TRIPS Agreement will affect the ownership of genetic resources like local seeds, plants and animals and, as a result, will endanger the food security of developing countries like Pakistan. This paper analyzes the implications of this agreement in trade-environment context and proposes options for developing countries to deal with such issues.

Key words: World Trade Organization, GATT, Intellectual Property Rights, Multi-lateral Environmental Agreements.

INTRODUCTION

A multilateral trading system was created in 1948 as the General Agreement on Tariffs and Trade (GATT). In 1995, GATT became the World Trade Organization (WTO) an international organization based in Switzerland. WTO was established through the latest round of the GATT negotiations, the 1986-94 Uruguay Round. WTO is the only international organization dealing with the rules of trade between nations. At its heart are the WTO agreements, negotiated and signed by the bulk of the world's trading nations and ratified in their parliaments. There are 146 members including Pakistan, 30 observers; and others include UN, UNCTAD, IMF, WB, FAO, WIPO, OECD. The difference between GATT and WTO is that General Agreement on Tariffs and Trade

(GATT) allowed the member states to select which agreements they wanted to be signatories to; whereas WTO is based on a single undertaking *i.e.* member countries have to agree to abide by all agreements in their entirety (Cosbey, 2003). The objectives of WTO are to help trade flow smoothly, freely, fairly and predictably. The WTO's rules - the agreements - are the result of negotiations between the members. The major WTO agreements are: (i) Agreements on Goods, (ii) Agreements on Services, (iii) Agreements on Intellectual Property, (iv) Agreement on Dispute Settlement, (v) Agreement on Trade Policy Review.

The WTO Agreement designed to protect intellectual property is called TRIPS- Trade Related Intellectual Property Rights, which requires all member countries to set up systems to protect intellectual property rights within their borders. Creative ideas and expressions that have commercial value is intellectual property. Intellectual property rights are a legally enforceable but a limited monopoly granted by the state to the inventor for a specific time period during which others may not copy the innovator's idea allowing him or her to commercialize it and recoup any investments made on research and development (Hafter and Litowitz, 2003).

Forms of Intellectual Property Protection included in TRIPS are (i) Patents, (ii) Copyright and related rights, (iii) Trademarks, including service marks Geographical indications, (iv) Industrial design, (v) Layout-designs (topographies) of integrated circuits and (vi) Undisclosed information, including trade secrets. A patent is that form of intellectual property protection that gives a monopoly right to the innovator to exploit an invention for a period of 17-20 years. In exchange for the monopoly the inventor discloses information about the invention. To be patentable an invention must be novel, inventive (not a discovery) and capable of industrial application. It is generally argued that TRIPs Agreement will affect the ownership of local seeds, plants and animals and, as a result, will endanger the food security of developing countries.

CRITICAL ANALYSIS

The increased emphasis on environmental concerns in relation to trade policies is relatively recent at international level. There are about 200 international agreements dealing with various environmental issues currently in force. They are called multilateral environmental agreements (MEAs). About 20 of these include provisions that can affect trade but WTO has no agreement specifically dealing with environment.

The first decade of the 21st century is a critical time in the development of agriculture across the developing world. Key international decisions are being made that will determine how to feed ourselves, and what impact this will have on the natural environment. Analysis of the problems facing agriculture, how trade policy interacts with them, and the positions our government is taking at the WTO reveals immense disparities between the design of current trade regimes and the need to develop alternative policies to deliver secure food supplies through sustainable agriculture.

National agriculture policies come within, and will continue to evolve under, the Agreement on Agriculture (AoA)- the global framework for agricultural trade relations. This international instrument, which is overseen by the WTO, promotes the liberalization of agricultural markets between its members. After the re-negotiations, the outcome AoA will determine the extent to which agricultural trade policy contributes to more environmentally sound agriculture, secure and equitable supplies of food, and the survival of viable rural communities. As far as, WTO agreement on trade related aspects of intellectual property rights is concerned, it has a comprehensive set of new rules and standards for Intellectual Property Rights (IPRs), which must be adopted by all WTO member countries from 1996 onwards, depending on the status of development of the individual Member State. Section V of TRIPs obliges Member States to provide patent protection for all inventions, both products and processes. Clause 27.3 (b) of Section V is about how plants and animals be patented.

TRIPS contain an exemption that allows WTO members to refuse to grant patents for plants and animals (other than micro-organisms). But if members wish to opt patents to plants, they must protect them by some "effective *sui generis* regime" a system specially designed for a certain type of intellectual property - or a combination of the two systems.

The draftsmen of the TRIPS Agreement, undoubtedly, had in mind the International Convention for the Protection of New Varieties of Plants (DPOV Convention)-a regime that many countries are using, very much close to patents. But some developing countries are creating their own *sui generis* systems, citing aspects of DPOV on which they want to improve. In the mandated review of the TRIPS Agreement in the WTO (starting in 2000), many developed countries are expected to push for less flexibility to develop such regimes.

The civil society organizations' viewpoint is that all forms of life including micro- organisms and microbiological processes should be excluded from Section V. of TRIPs (Laura, 1999). If the World has yet to go with the present state of agreement, anyway, it is argued that the word effective *sui generis* system had made the provision given in clause 27.3 (b) doubtful. Although, some developing countries are creating their own *sui generis* systems, citing aspects of DPOV on which they want to improve, but it is generally feared that DPOV model will be set as the "effective *sui generis* system" and all member countries will be pressurized to opt DPOV (Lehman, 2003). Developing countries involved in formulating such laws, such as Plant Breeders Right's (PBR) Act in Pakistan, are already facing bilateral pressures to join DPOV. Some argue that three elements of DPOV's 1991 Act may conflict with sustainable development objectives:

1. Duration of protection: Twenty years of protection, which may be too long from a consumer's perspective.

2. Breeders' exemption: Limited scope for breeders' exemption-the traditional free access of breeders to protected material for research purposes. If the new variety is "essentially derived" from the original variety, the intellectual property rights must be shared with the original

3. Farmers' rights vs breeders' rights: Strong protection of breeders' rights-the intellectual property rights of formal innovators-but no protection of farmers' rights- the intellectual property rights of informal (typically poor) innovators.

Based on this, it is generally argued (Correa, 2002) that TRIPS Agreement will affect the ownership of local seeds, plants and animals and, as a result, will be a concern for food security of developing countries like Pakistan. Some of its impacts on farmers' rights, indigenous knowledge, bio-diversity and food security are discussed below.

SOCIO-ECONOMIC AND ENVIRONMENTAL IMPLICATIONS

Impact on farmer's rights

WTO agreement on TRIPS disregards the interests of consumers, farmers and developing countries. Farmers constitute majority population of most of the developing countries and will directly be affected by this agreement. TRIPS

agreement is threatening the real owners of natural resources on demands being made by some of the industrialized countries. TRIPS are a protectionist device promoting corporate monopolies of seeds, genes and medicine. It shifts the balance of control away from public interest to the private gains of patent holders. Who will gain control over crop seeds and foodstuffs? Concern is shown that transnational companies (TNCs), through genetic modification technology, will acquire patents and will, eventually, control everything from genes, seeds, plants, and agricultural harvests to foodstuffs. It shows, in fact, a monopolistic competition among TNCs and farmers. Farmers lack the scientific capability to innovate and patent genetic materials and are not even able to catalogue the natural resources they currently have. On the other hand, bio-tech TNCs are putting increasingly more resources and expertise to patent these. This is also true in developed countries where farmers are notable to contend with companies. There is a famed case of Percy Schmeiser-a Canadian farmer who was accused of illegally using Monsanto genetically engineered seed. In April 2001, the Supreme Court of Canada ordered him to pay eighty thousand dollars to Monsanto for violating its patent rights. Schmeiser's seed had, in fact, been accidentally contaminated by the flight of pollen. Now, the scandal in this is that the court was of the opinion that it was irrelevant whether the farmer's seed had been accidentally contaminated by the flight of pollen or if Monsanto's crop seed had, in fact, been used deliberately. Enforcing the corporation's patent rights had in all events priority.

Similarly, in Pakistan farmers used Monsanto's maize seed in the Swabi District and failed to produce any thing. They complained about it to the seed dealer and they were given another bag of seed on credit basis. It is shocking to report that again they failed to harvest maize crop. Now the point is that the farmer has neither the right nor the power to take legal action against such a big Trans National Company. Eventually, they re- ploughed all their fields and sowed the local maize seed for their subsistence with a certain degree of success.

Impact on indigenous knowledge

It is a recognized fact that poverty has not been alleviated so far in developing countries because the indigenous socio-economic systems have been neglected and excluded from the development and decision-making processes. WTO agreements will further affect the indigenous knowledge of farmers. Under the TRIPS agreement, patents and effective *sui generis* systems, such as PBR Act in Pakistan, will restrict farmers to continue centuries' old traditional system of seed storage, sharing and multiplying.

Impact on bio-diversity

A serious concern is the rapidly shrinking genetic diversity of cultivated species, as farmers switch from traditional varieties to new high-yielding strains developed by professional breeders. During the Green Revolution, farmers turned away from traditional varieties to adopt modem strains that promised better yields and better resistance to pests and disease. By providing incentives to breeders to develop the new improved varieties, strengthened intellectual property rights contribute to this decline in diversity, although they are only one of a host of contributing factors. As a matter of fact, high-yielding varieties (HYVs) have already displaced less profitable crop seeds. Now, with the advent of WTO agreements, corporations will increasingly prevent access to their patented genetic resources through using exclusive rights. The other threat is that under the umbrella of WTO, corporations of the North are pirating biodiversity of the South by declaring the genes that have been "discovered" by them, and the crop seeds belonging to them, as their "property".

TRIPS is also conflicting agreement with the UN Convention on Biological Diversity (CBD). CBD recognizes the sovereign rights of States over their biological and genetic resources. The Convention requires parties to protect and promote the rights of communities, farmers and indigenous people including their customary use of biological resources and knowledge systems. However, TRIPS does not reinforce the provisions of CBD. It does not require the patent holder to either disclose the source of origin, get prior informed consent from the genetic resource/knowledge holder (thus encouraging bio-piracy), or ensure that there is an equitable benefit sharing. Patenting and plant breeders' rights under TRIPS carry with them the aims of politics of control and legalise bio-piracy (Tansey, 1999).

Impacts on food security

In the age of modern agriculture, poor communities face difficulty to produce or purchase food. Food distribution system is already a problem and once the food production and distribution system will be in the hands of corporations, poor communities will not be able to purchase food at company's price. The consumers have to see monopoly of patent owners, since they will have exclusive rights to prevent third parties to make, use, sell or import patented food items. Farmers will not be allowed to multiply seeds without their consent.

The other important thing to note is that food security is not merely a question of enough amounts of foodstuff in quantitative terms as it is often expressed in the official documents. The quality of food available for the masses is also important. Apart from safety of food, the question of food security also means diverse and quality food for healthy lives. Now in a situation when corporation will produce and market food, nobody will be able to question its quality.

CONSEQUENCES, IMPLICATIONS AND OPTIONS FOR DEVELOPING COUNTRIES

Consequences

This allows companies and individuals to patent microorganisms, new medicines, vaccines and even human gene sequences giving them the exclusive right to make, use or sell biological resource and life itself. Article 27.3(b) also raises serious concerns regarding food security in the South. TRIPS ban the exchange or reuse of seeds thus forcing farmers to buy new seed stocks each year. This makes farmers more dependants on transnational seed companies and increases corporate control over our food supply. TRIPS offer no protection to indigenous knowledge and genetic resources resulting in a cost free transfer of precious resources and knowledge from the South to the North.

Implications

TRIPS extends both product and process patents making it illegal for local pharmaceuticals in the South to reproduce drugs at a lower cost and catch-up through "copying". TRIPS limits research and development by allowing firms in the North to restrict access to useful technologies. TRIPS increases the bargaining power of technology owners allowing them to set higher prices and royalty rates. TRIPS may result in patent holders exporting their product rather than manufacturing it in the developing countries. TRIPS allows Northern pharmaceutical MNCs operating in South to patent the knowledge and resources of the South for their profit. More than 7,000 compounds in Western pharmacopoeia are derived from plants found in the South. Profits derived from these compounds are not shared with stakeholders in the South.

Worry for developing countries

An estimated 83% of bio prospecting occurs in developing countries in the

South. This bio prospecting is the basis of tremendous profits in the seed and pharmaceutical industry in developed countries in the North. But communities in the South who are the stewards of this rich biodiversity have no claim over these profits since CBD declares all biodiversity to be common heritage of mankind.

Implications for developing countries

Already MNCs from the North are bidding to patent valuable biological resources, *e.g*., Monsanto's patent for the fungicidal and insecticidal uses of Neem wax and oil, Rice Tec patent on Basmati rice etc. Patents and copyrights will result in a sharp increase in the price of patented materials in developing countries. The infrastructure required to establish and implement the prescribed IPR regime is often too expensive for developing countries.

Options for developing countries

The developing countries have possibly the following options:

(i) Establish the primacy of CBD over TRIPS
(ii) Develop an appropriate *sui generis* system.
(iii) Insist on fair and equitable benefit sharing schemes as part of patent applications.
(iv) Argue for the exclusion of plants and animals from patentability.
(v) Interpret the TRIPS Agreement in a manner that favours their interests.

Initiative taken by developing countries

Brazil, China, Cuba, Dominican Republic, Ecuador, India, Pakistan, Peru, Thailand, Venezuela, Zambia and Zimbabwe had made a submission in the TRIPS Council on the relationship between the TRIPS Agreement and the CBD and the protection of Traditional Knowledge in June 2002. The submission was made under paragraphs 12 and 19 of the Doha Ministerial Declaration. This submission was preceded by several papers and submissions from developing countries to develop an effective and consistent framework so as to enaple the WTO members to meet their obligations under both the TRIPS and the CBD. The key issues raised were that the TRIPS Agreement should be amended in order to provide means that members shall require that an applicant for a patent relating to biological materials or to traditional knowledge shall provide, as a condition to

acquiring patent rights:

1. Disclosure of the source and country of origin of the biological resource and of the traditional knowledge used in the invention.
2. Evidence of prior informed consent through approval of authorities under the relevant national regime; and
3. Evidence of fair and equitable benefit sharing under the relevant national regime.

The Doha Ministerial Declaration provided a mandate to address the outstanding implementation issues on a priority basis by the end of year 2002. The deadline recommended by the Doha Ministerial Declaration has now passed, without any recommendation to the TNC. It is therefore incumbent on the TRIPS Council to treat this matter as one of great urgency, so as to arrive at some practical proposals for the TNC. It is therefore incumbent on the TRIPS Council to treat this matter as one of the great urgency, so as to arrive at some practical proposals for TNC. The Doha Ministerial Declaration reaffirmed the objective of sustainable development. In order to achieve that objective and to fulfill the commitment to the interests of developing countries, it is incumbent upon the Members to arrive at an appropriate decision on this issue.

REFERENCES

CORREA, C.M., 2002. *Intellectual Property Rights, The WTO and Developing Counties. The TRIPS Agreement and Policy Options.* Zed Books, Third World Network.

COSBY, A., 2003. *The sustainable development effects of the WTO TRIPS agreement – A focus on developing countries.* The Gateway Development Information Services. www.eldis.org

HEFTER, R.L. AND LITOWITZ, R., 2003. What is intellectual property? *Vanderb. J. Transnat. Law*, **36**: 961-993.

LAURA, K. (ed.), 1999. *Patients and food security options for research and action*, Action Aid, London.

LEHMAN, B.A., 2003. *Support for economic and political freedom – A developing countries, perspective*, U.S. International Information Program. http://usinfo.state.gov

TANSEY, G., 1999. *Trade, intellectual property, food and biodiversity, key issues and options: Review of article 27.3(b) of the TRIPS agreement.* Discussion paper Agricultural biotechnology support project (ABSP), Institute of International Agriculture, Michigan State University, East Lansing, Zen Internet Limited. www.eldis.org

Proc. Pakistan Congr. Zool., Vol. 24, pp. 237-245, 2004.

TRIPS AGREEMENT OF WTO AND GENETIC RESOURCES: IMPLICATIONS AND OPTIONS FOR DEVELOPING COUNTRIES

MUHAMMAD IRFAN KHAN
Department of Environmental Science, Allama Iqbal Open University, Islamabad
Email: irfankhan_64@yahoo.com

CONTENTS

Abstract.- The WTO has no specific agreement dealing with the environment. However, a number of the WTO agreements include provisions dealing with environmental concerns like agreement on Trade Related Intellectual Property Rights (TRIPS). The clause 27.3 (b) of Section V of TRIPS Agreement extends an exemption that allows WTO members to refuse to grant patents for plants and animals (other than microorganisms and non-biological and microbiological processes). It is argued that TRIPS Agreement will affect the ownership of genetic resources like local seeds, plants and animals and, as a result, will endanger the food security of developing countries like Pakistan. This paper analyzes the implications of this agreement in trade-environment context and proposes options for developing countries to deal with such issues.

Key words: World Trade Organization, GATT, Intellectual Property Rights, Multi-lateral Environmental Agreements.

INTRODUCTION

A multilateral trading system was created in 1948 as the General Agreement on Tariffs and Trade (GATT). In 1995, GATT became the World Trade Organization (WTO) an international organization based in Switzerland. WTO was established through the latest round of the GATT negotiations, the 1986-94 Uruguay Round. WTO is the only international organization dealing with the rules of trade between nations. At its heart are the WTO agreements, negotiated and signed by the bulk of the world's trading nations and ratified in their parliaments. There are 146 members including Pakistan, 30 observers; and others include UN, UNCTAD, IMF, WB, FAO, WIPO, OECD. The difference between GATT and WTO is that General Agreement on Tariffs and Trade

(GATT) allowed the member states to select which agreements they wanted to be signatories to; whereas WTO is based on a single undertaking *i.e.* member countries have to agree to abide by all agreements in their entirety (Cosbey, 2003). The objectives of WTO are to help trade flow smoothly, freely, fairly and predictably. The WTO's rules - the agreements - are the result of negotiations between the members. The major WTO agreements are: (i) Agreements on Goods, (ii) Agreements on Services, (iii) Agreements on Intellectual Property, (iv) Agreement on Dispute Settlement, (v) Agreement on Trade Policy Review.

The WTO Agreement designed to protect intellectual property is called TRIPS- Trade Related Intellectual Property Rights, which requires all member countries to set up systems to protect intellectual property rights within their borders. Creative ideas and expressions that have commercial value is intellectual property. Intellectual property rights are a legally enforceable but a limited monopoly granted by the state to the inventor for a specific time period during which others may not copy the innovator's idea allowing him or her to commercialize it and recoup any investments made on research and development (Hafter and Litowitz, 2003).

Forms of Intellectual Property Protection included in TRIPS are (i) Patents, (ii) Copyright and related rights, (iii) Trademarks, including service marks Geographical indications, (iv) Industrial design, (v) Layout-designs (topographies) of integrated circuits and (vi) Undisclosed information, including trade secrets. A patent is that form of intellectual property protection that gives a monopoly right to the innovator to exploit an invention for a period of 17-20 years. In exchange for the monopoly the inventor discloses information about the invention. To be patentable an invention must be novel, inventive (not a discovery) and capable of industrial application. It is generally argued that TRIPs Agreement will affect the ownership of local seeds, plants and animals and, as a result, will endanger the food security of developing countries.

CRITICAL ANALYSIS

The increased emphasis on environmental concerns in relation to trade policies is relatively recent at international level. There are about 200 international agreements dealing with various environmental issues currently in force. They are called multilateral environmental agreements (MEAs). About 20 of these include provisions that can affect trade but WTO has no agreement specifically dealing with environment.

The first decade of the 21st century is a critical time in the development of agriculture across the developing world. Key international decisions are being made that will determine how to feed ourselves, and what impact this will have on the natural environment. Analysis of the problems facing agriculture, how trade policy interacts with them, and the positions our government is taking at the WTO reveals immense disparities between the design of current trade regimes and the need to develop alternative policies to deliver secure food supplies through sustainable agriculture.

National agriculture policies come within, and will continue to evolve under, the Agreement on Agriculture (AoA)- the global framework for agricultural trade relations. This international instrument, which is overseen by the WTO, promotes the liberalization of agricultural markets between its members. After the re-negotiations, the outcome AoA will determine the extent to which agricultural trade policy contributes to more environmentally sound agriculture, secure and equitable supplies of food, and the survival of viable rural communities. As far as, WTO agreement on trade related aspects of intellectual property rights is concerned, it has a comprehensive set of new rules and standards for Intellectual Property Rights (IPRs), which must be adopted by all WTO member countries from 1996 onwards, depending on the status of development of the individual Member State. Section V of TRIPs obliges Member States to provide patent protection for all inventions, both products and processes. Clause 27.3 (b) of Section V is about how plants and animals be patented.

TRIPS contain an exemption that allows WTO members to refuse to grant patents for plants and animals (other than micro-organisms). But if members wish to opt patents to plants, they must protect them by some "effective *sui generis* regime" a system specially designed for a certain type of intellectual property - or a combination of the two systems.

The draftsmen of the TRIPS Agreement, undoubtedly, had in mind the International Convention for the Protection of New Varieties of Plants (DPOV Convention)-a regime that many countries are using, very much close to patents. But some developing countries are creating their own *sui generis* systems, citing aspects of DPOV on which they want to improve. In the mandated review of the TRIPS Agreement in the WTO (starting in 2000), many developed countries are expected to push for less flexibility to develop such regimes.

The civil society organizations' viewpoint is that all forms of life including micro- organisms and microbiological processes should be excluded from Section V. of TRIPs (Laura, 1999). If the World has yet to go with the present state of agreement, anyway, it is argued that the word effective *sui generis* system had made the provision given in clause 27.3 (b) doubtful. Although, some developing countries are creating their own *sui generis* systems, citing aspects of DPOV on which they want to improve, but it is generally feared that DPOV model will be set as the "effective *sui generis* system" and all member countries will be pressurized to opt DPOV (Lehman, 2003). Developing countries involved in formulating such laws, such as Plant Breeders Right's (PBR) Act in Pakistan, are already facing bilateral pressures to join DPOV. Some argue that three elements of DPOV's 1991 Act may conflict with sustainable development objectives:

1. Duration of protection: Twenty years of protection, which may be too long from a consumer's perspective.

2. Breeders' exemption: Limited scope for breeders' exemption-the traditional free access of breeders to protected material for research purposes. If the new variety is "essentially derived" from the original variety, the intellectual property rights must be shared with the original

3. Farmers' rights vs breeders' rights: Strong protection of breeders' rights-the intellectual property rights of formal innovators-but no protection of farmers' rights- the intellectual property rights of informal (typically poor) innovators.

Based on this, it is generally argued (Correa, 2002) that TRIPS Agreement will affect the ownership of local seeds, plants and animals and, as a result, will be a concern for food security of developing countries like Pakistan. Some of its impacts on farmers' rights, indigenous knowledge, bio-diversity and food security are discussed below.

SOCIO-ECONOMIC AND ENVIRONMENTAL IMPLICATIONS

Impact on farmer's rights

WTO agreement on TRIPS disregards the interests of consumers, farmers and developing countries. Farmers constitute majority population of most of the developing countries and will directly be affected by this agreement. TRIPS

agreement is threatening the real owners of natural resources on demands being made by some of the industrialized countries. TRIPS are a protectionist device promoting corporate monopolies of seeds, genes and medicine. It shifts the balance of control away from public interest to the private gains of patent holders. Who will gain control over crop seeds and foodstuffs? Concern is shown that transnational companies (TNCs), through genetic modification technology, will acquire patents and will, eventually, control everything from genes, seeds, plants, and agricultural harvests to foodstuffs. It shows, in fact, a monopolistic competition among TNCs and farmers. Farmers lack the scientific capability to innovate and patent genetic materials and are not even able to catalogue the natural resources they currently have. On the other hand, bio-tech TNCs are putting increasingly more resources and expertise to patent these. This is also true in developed countries where farmers are notable to contend with companies. There is a famed case of Percy Schmeiser-a Canadian farmer who was accused of illegally using Monsanto genetically engineered seed. In April 2001, the Supreme Court of Canada ordered him to pay eighty thousand dollars to Monsanto for violating its patent rights. Schmeiser's seed had, in fact, been accidentally contaminated by the flight of pollen. Now, the scandal in this is that the court was of the opinion that it was irrelevant whether the farmer's seed had been accidentally contaminated by the flight of pollen or if Monsanto's crop seed had, in fact, been used deliberately. Enforcing the corporation's patent rights had in all events priority.

Similarly, in Pakistan farmers used Monsanto's maize seed in the Swabi District and failed to produce any thing. They complained about it to the seed dealer and they were given another bag of seed on credit basis. It is shocking to report that again they failed to harvest maize crop. Now the point is that the farmer has neither the right nor the power to take legal action against such a big Trans National Company. Eventually, they re- ploughed all their fields and sowed the local maize seed for their subsistence with a certain degree of success.

Impact on indigenous knowledge

It is a recognized fact that poverty has not been alleviated so far in developing countries because the indigenous socio-economic systems have been neglected and excluded from the development and decision-making processes. WTO agreements will further affect the indigenous knowledge of farmers. Under the TRIPS agreement, patents and effective *sui generis* systems, such as PBR Act in Pakistan, will restrict farmers to continue centuries' old traditional system of seed storage, sharing and multiplying.

Impact on bio-diversity

A serious concern is the rapidly shrinking genetic diversity of cultivated species, as farmers switch from traditional varieties to new high-yielding strains developed by professional breeders. During the Green Revolution, farmers turned away from traditional varieties to adopt modem strains that promised better yields and better resistance to pests and disease. By providing incentives to breeders to develop the new improved varieties, strengthened intellectual property rights contribute to this decline in diversity, although they are only one of a host of contributing factors. As a matter of fact, high-yielding varieties (HYVs) have already displaced less profitable crop seeds. Now, with the advent of WTO agreements, corporations will increasingly prevent access to their patented genetic resources through using exclusive rights. The other threat is that under the umbrella of WTO, corporations of the North are pirating biodiversity of the South by declaring the genes that have been "discovered" by them, and the crop seeds belonging to them, as their "property".

TRIPS is also conflicting agreement with the UN Convention on Biological Diversity (CBD). CBD recognizes the sovereign rights of States over their biological and genetic resources. The Convention requires parties to protect and promote the rights of communities, farmers and indigenous people including their customary use of biological resources and knowledge systems. However, TRIPS does not reinforce the provisions of CBD. It does not require the patent holder to either disclose the source of origin, get prior informed consent from the genetic resource/knowledge holder (thus encouraging bio-piracy), or ensure that there is an equitable benefit sharing. Patenting and plant breeders' rights under TRIPS carry with them the aims of politics of control and legalise bio-piracy (Tansey, 1999).

Impacts on food security

In the age of modern agriculture, poor communities face difficulty to produce or purchase food. Food distribution system is already a problem and once the food production and distribution system will be in the hands of corporations, poor communities will not be able to purchase food at company's price. The consumers have to see monopoly of patent owners, since they will have exclusive rights to prevent third parties to make, use, sell or import patented food items. Farmers will not be allowed to multiply seeds without their consent.

The other important thing to note is that food security is not merely a question of enough amounts of foodstuff in quantitative terms as it is often expressed in the official documents. The quality of food available for the masses is also important. Apart from safety of food, the question of food security also means diverse and quality food for healthy lives. Now in a situation when corporation will produce and market food, nobody will be able to question its quality.

CONSEQUENCES, IMPLICATIONS AND OPTIONS FOR DEVELOPING COUNTRIES

Consequences

This allows companies and individuals to patent microorganisms, new medicines, vaccines and even human gene sequences giving them the exclusive right to make, use or sell biological resource and life itself. Article 27.3(b) also raises serious concerns regarding food security in the South. TRIPS ban the exchange or reuse of seeds thus forcing farmers to buy new seed stocks each year. This makes farmers more dependants on transnational seed companies and increases corporate control over our food supply. TRIPS offer no protection to indigenous knowledge and genetic resources resulting in a cost free transfer of precious resources and knowledge from the South to the North.

Implications

TRIPS extends both product and process patents making it illegal for local pharmaceuticals in the South to reproduce drugs at a lower cost and catch-up through "copying". TRIPS limits research and development by allowing firms in the North to restrict access to useful technologies. TRIPS increases the bargaining power of technology owners allowing them to set higher prices and royalty rates. TRIPS may result in patent holders exporting their product rather than manufacturing it in the developing countries. TRIPS allows Northern pharmaceutical MNCs operating in South to patent the knowledge and resources of the South for their profit. More than 7,000 compounds in Western pharmacopoeia are derived from plants found in the South. Profits derived from these compounds are not shared with stakeholders in the South.

Worry for developing countries

An estimated 83% of bio prospecting occurs in developing countries in the

South. This bio prospecting is the basis of tremendous profits in the seed and pharmaceutical industry in developed countries in the North. But communities in the South who are the stewards of this rich biodiversity have no claim over these profits since CBD declares all biodiversity to be common heritage of mankind.

Implications for developing countries

Already MNCs from the North are bidding to patent valuable biological resources, *e.g*., Monsanto's patent for the fungicidal and insecticidal uses of Neem wax and oil, Rice Tec patent on Basmati rice etc. Patents and copyrights will result in a sharp increase in the price of patented materials in developing countries. The infrastructure required to establish and implement the prescribed IPR regime is often too expensive for developing countries.

Options for developing countries

The developing countries have possibly the following options:

(i) Establish the primacy of CBD over TRIPS
(ii) Develop an appropriate *sui generis* system.
(iii) Insist on fair and equitable benefit sharing schemes as part of patent applications.
(iv) Argue for the exclusion of plants and animals from patentability.
(v) Interpret the TRIPS Agreement in a manner that favours their interests.

Initiative taken by developing countries

Brazil, China, Cuba, Dominican Republic, Ecuador, India, Pakistan, Peru, Thailand, Venezuela, Zambia and Zimbabwe had made a submission in the TRIPS Council on the relationship between the TRIPS Agreement and the CBD and the protection of Traditional Knowledge in June 2002. The submission was made under paragraphs 12 and 19 of the Doha Ministerial Declaration. This submission was preceded by several papers and submissions from developing countries to develop an effective and consistent framework so as to enaple the WTO members to meet their obligations under both the TRIPS and the CBD. The key issues raised were that the TRIPS Agreement should be amended in order to provide means that members shall require that an applicant for a patent relating to biological materials or to traditional knowledge shall provide, as a condition to

acquiring patent rights:

1. Disclosure of the source and country of origin of the biological resource and of the traditional knowledge used in the invention.
2. Evidence of prior informed consent through approval of authorities under the relevant national regime; and
3. Evidence of fair and equitable benefit sharing under the relevant national regime.

The Doha Ministerial Declaration provided a mandate to address the outstanding implementation issues on a priority basis by the end of year 2002. The deadline recommended by the Doha Ministerial Declaration has now passed, without any recommendation to the TNC. It is therefore incumbent on the TRIPS Council to treat this matter as one of great urgency, so as to arrive at some practical proposals for the TNC. It is therefore incumbent on the TRIPS Council to treat this matter as one of the great urgency, so as to arrive at some practical proposals for TNC. The Doha Ministerial Declaration reaffirmed the objective of sustainable development. In order to achieve that objective and to fulfill the commitment to the interests of developing countries, it is incumbent upon the Members to arrive at an appropriate decision on this issue.

REFERENCES

CORREA, C.M., 2002. *Intellectual Property Rights, The WTO and Developing Counties. The TRIPS Agreement and Policy Options.* Zed Books, Third World Network.

COSBY, A., 2003. *The sustainable development effects of the WTO TRIPS agreement – A focus on developing countries.* The Gateway Development Information Services. www.eldis.org

HEFTER, R.L. AND LITOWITZ, R., 2003. What is intellectual property? *Vanderb. J. Transnat. Law*, **36**: 961-993.

LAURA, K. (ed.), 1999. *Patients and food security options for research and action*, Action Aid, London.

LEHMAN, B.A., 2003. *Support for economic and political freedom – A developing countries, perspective*, U.S. International Information Program. http://usinfo.state.gov

TANSEY, G., 1999. *Trade, intellectual property, food and biodiversity, key issues and options: Review of article 27.3(b) of the TRIPS agreement.* Discussion paper Agricultural biotechnology support project (ABSP), Institute of International Agriculture, Michigan State University, East Lansing, Zen Internet Limited. www.eldis.org