



Bioaccumulation of Metals in the Organs of Fish inhabiting Ravi River: Serious Threat to Fish and Consumer's Health

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ABSTRACT

Heavy metal contamination of river water adversely affects the growth and productivity of fish and contributes to the human health disasters. River is a trans-country river originating in Himachal Pradesh, India and joins Indus and its tributary rivers in the Punjab province of Pakistan. The present study describes the concentration of heavy metals in the water and different tissues of native fish collected from three different areas. The highest concentration of Cr ($1.451 \mu\text{g L}^{-1}$) and Co ($0.325 \mu\text{g L}^{-1}$) was measured in the river water at Balloki. However, Cd ($0.981 \mu\text{g L}^{-1}$) and Zn ($1.239 \mu\text{g L}^{-1}$) were contributing maximum contamination at confluence of Deg Nallah and river Ravi. We found maximum accumulation of Cr (21.1 ppm) in *Rita rita*, Co (13.1 ppm) in *Channa marulius*, Cd (20.1 ppm) in *Cirrhinus mrigala* and Zn (32.9 ppm) in *Cyprinus carpio*. Maximum accumulation of heavy metals was found in the gills of fish followed by liver, kidneys, muscle and fat cells. Overall, seventeen fish species were recorded in the study areas indicating a decrease in the number of species in Ravi. The results have shown that the water of Ravi is unsafe for aquatic life and irrigation. Metal concentration in the fish meat is higher than the recommended dietary allowance (RDA) parameters indicating a serious health threat to both, the fish and human population.

Article Information

Received 05 May 2018

Revised 30 July 2018

Accepted 10 April 2019

Available online 13 August 2020

Authors' Contribution

SBS and GJ designed the study plan, ZY, SBS and GJ collected the field samples and performed the lab experiments. BNM composed and revised the manuscript and analyzed the data.

Key words

Water pollution, fish, heavy metals, human health, Ravi

INTRODUCTION

Heavy metals are non-biodegradable elements contributing very long-lasting type of pollutants (Ukoha *et al.*, 2014; Sthanadar *et al.*, 2015). Industrial and municipal wastes are the major anthropogenic sources for heavy metal accumulation in the fresh water ecosystems. The subsequent accumulation of heavy metals in aquatic organisms can badly adulterate the food chains. These can also affect the growth and reproduction rate of living organisms (Vosylien and Jankaite, 2006; Hayat *et al.*, 2007). Fish provide an indicative factor for the assessment of water systems, especially for the estimation of quality and suitability of water for the living organisms (Mahboob *et al.*, 2016). Fresh water fish and shellfish accumulates the higher concentrations of metals than the corresponding water and sediments (Al-Weher, 2008; Mahboob *et al.*, 2014). The bioaccumulation of metals depends upon the nature of metals and fish species (Twari-Fufeyin and Ekaye, 2007). Metal ions enter the fish body through exposed epidermal surface or skin, gills and the food tract. Metal accumulation is higher

in the kidneys and liver as compared to the fish muscle (Al-Ghanim *et al.*, 2016; Milanov *et al.*, 2016). Fish remains a key component of human diet comprising all the essential amino acids and fatty acids which reduce the risk of heart diseases (Kuz'mina, 2011). However, the metal toxicity in the fish meat can have serious impact on human health because of their cytotoxic, mutagenic and carcinogenic properties (Rauf *et al.*, 2009; Ahmed *et al.*, 2016). In addition to that the heavy metal stress reduces feed intake and productivity of fish (Sfakianakis *et al.*, 2015).

Indus and its tributary rivers provide the basis for the largest canal system in Pakistan, vastly supporting the agricultural and livestock practices in the region. Ravi River is approximately 720 km long, 320 km of the river's length and most of water is used within India after the Indus Water Treaty (Hayder *et al.*, 2015). The fluctuation of flow rate contribute to pollution and many other management problems. The main sources of pollution in the river Ravi stretching from Lahore Siphon to Balloki headworks are urban, agricultural and industrial wastewaters discharged from various industries like electroplating workshops, steel factories, paper and pulp industries, medicine and scientific laboratories, as well as surface runoff and municipal sewage. Many pumping stations discharging municipal sewage of Lahore city into the Ravi adding to the accumulation of toxic substance and metals in water.

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0030-9923/2020/0006-2027 \$ 9.00/0
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Present study deals with the estimation of heavy metal concentration in the water and in the tissues of fish species of Ravi River.

MATERIALS AND METHODS

Study area

The study area included three points i.e. Balloki area, Chenab near Balloki and at the confluence point of Ravi and Chenab. The sites were Ravi Siphon and Balloki Headwork reservoirs at the River Ravi, it's almost 60 Km Southwest for the collection of naturally occurring freshwater fish species (Fig. 1).

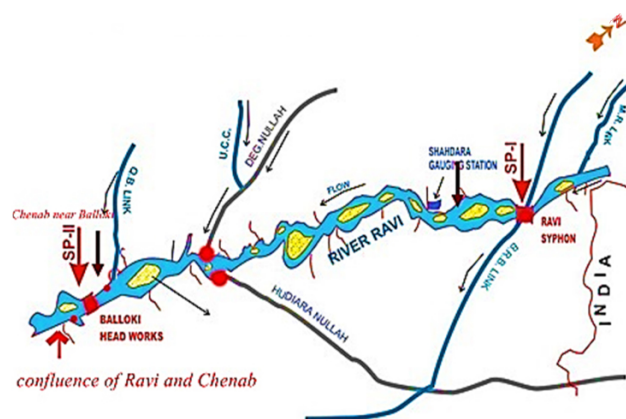


Fig. 1. Study areas, sample collection sites on river Ravi.

Sample collection and initial analysis

Samples were collected on 14th November 2016 and 31st March 2017 from Ravi siphon and the pond areas which was an offshoot of flood at Balloki headworks. Samples were also obtained from the river Ravi at Balloki headworks as well as from the confluence of Ravi and Chenab Rivers. Water samples were taken from two major drains on river Ravi i.e. Deghnallah and Hudiaranallah. Fish samples were stored in zip lock plastic bags and were kept in ice box for transportation. Samples were dried in filter paper, packed in polyethylene bags and kept in the freezer at -20°C until evaluation. Temperature and pH of water was determined for each water sample and the water of fish sampling site. The coordinates of the study area were taken by using the GPS available in the smart phones. Standard identification key by (Mirza and Sandhu, 2007) was used to identify the fish species. The total length of each fish was measured from the tip of snout to longest ray in fish caudal fin by measuring scale. Number of annuli (rings) was counted which provides the fish age, all fish

were weighed.

Digestion method for water samples

Nitric acid (10 ml) was added in 100 ml of water sample and boiled slowly till the sample volume decreased to 20 ml. The solution was cooled, 5 ml of nitric acid was added and heated again to leave 5 ml solution followed by the addition of hydrochloric acid. Heated the solution and 5ml of 5M Sodium hydroxide was added, then filtered. Filtrate was transferred to 100 ml volumetric flask and distilled water was added up to 100 ml. The final sample was subjected to metal detection process.

Preparation of tissue sample

Stock solution of 1000 ppm was prepared for all selective heavy metals. Fish organs such as gills, liver, kidney, muscle and fat cells were dissected and homogenized at -20°C for heavy metal detection. A standard protocol for heavy metal analysis was adopted (Yousufzai *et al.*, 2010).

Estimation of heavy metals

The concentrations of metals (Cd, Co, Zn and Cr) were determined in the fish organs/tissues and water samples using Atomic Absorption Spectrometer (Analyst 800, Perkin Elmer, Massachusetts, USA) as described in the literature (Hashim *et al.*, 2014). The concentration of chromium (Cr), cadmium (Cd), zinc (Zn), and cobalt (Co) in the fish tissues and water. Analytical grade chemicals were used.

Statistical analysis

Data obtained were expressed as mean \pm SE. The results were analyzed using one-way analysis of variances (ANOVA). Statistical version 8.1 was used. P values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

There was no marked difference between the temperatures of all study areas at Ravi Siphon, average temperature was 26°C. The pH of water at Hudiaranallah was 8.3, with an annual discharge of 180 cusecs approximately, this drain is one of the major causes of bio-chemical contamination in Ravi (Table I). Zinc concentration in water was dependent positively and significantly on water temperature. Temperature change may decrease or increase toxicity, which depends on the toxicant plus species. According to the pH of Balloki and DegNala was slightly acidic.

Table I.- Temperature, pH and heavy metal concentration in the river water ($\mu\text{g L}^{-1}$) as measured from different areas in the passage of Ravi river.

| Sr. No. | Study areas | Cr (ppm) | Co (ppm) | Cd (ppm) | Zn (ppm) | Tesmperature | pH |
|---------|--|----------|----------|----------|----------|--------------|-----|
| 1 | Ravi Siphon | 0.144 | 0.015 | 0.115 | 0.135 | 26°C | 7.0 |
| 2 | Balloki headworks | 1.451 | 0.255 | 0.325 | 0.355 | 25°C | 6.5 |
| 3 | Chenab near Balloki | 0.169 | ND | 0.112 | 0.212 | 27°C | 6.0 |
| 4 | Confluence of Ravi and Chenab | 1.266 | ND | 0.122 | 0.132 | 27° C | 6.5 |
| 5 | DegNallah before entering into river Ravi | 1.115 | 0.081 | 0.981 | 1.239 | 26°C | 5.7 |
| 6 | Confluence of DegNallah and River Ravi | 1.015 | 0.012 | 0.123 | 1.038 | 25°C | 6.5 |
| 7 | Hudiara-Nallah before entering into River Ravi | 1.092 | 0.061 | 0.111 | 1.044 | 27° C | 7.0 |
| 8 | Confluence of Hudiara-Nallah and River Ravi | 1.029 | 0.013 | 0.017 | 1.021 | 26°C | 6.0 |

However, that of Ravi Siphon was slightly basic and rest of the areas had neutral pH. Pakistan's soil is almost dry and has a higher concentration of calcium carbonate but low organic matter content, this higher level of lime stone makes Pakistani soil more alkaline with pH near 8 or above. Concentration of Cr in water was highest in Ravi at Balloki ($1.451 \mu\text{g L}^{-1}$) followed by the Confluence point of Ravi and Chenab near Balloki, it was however lowest at Ravi Siphon. This is because of the industrial effluents poured into the river water from Hudiara and Degnallah. Although the water of Chenab and Ravi Siphon is comparatively cleaner and has a diluting effect, the concentration of Cr in Balloki is so high that there is very little change in its concentration at the Confluence of Ravi and Chenab. Concentration of Chromium in all study sites was high as compared to the standards of EPA. The metal concentration was hazarously high at Balloki and in Confluence of Ravi and Chenab. The concentration of Co in water remained in the safe range at all the study sites except at Balloki. The concentration of Cd in the water was above the standard acceptable values of EPA and FAO as measured at all the study sites. However, the maximum concentration was measured in water samples obtained at Balloki. The concentration of Zinc in all the study areas was within the standard acceptable values set by both FAO and EPA. It was however, like Cd, Co, and Cr highest in Ravi at Balloki headworks and lowest at the confluence of Ravi and Chenab. Among all the sites studied, the highest concentration of metal in water was that of Chromium in Balloki headworks (1.451ppm) (Table I). Concentration ($\mu\text{g L}^{-1}$) of heavy metals in Ravi Siphon water was Cr (0.144), Co (0.015), Cd (0.115) and Zn (0.135). Order of the metal concentration from higher to low in water of Ravi siphon was $\text{Cr} > \text{Zn} > \text{Cd} > \text{Co}$. The concentrations of Cd were higher compared to the FAO standard limit i.e. < 0.1 but Zn concentration is less as compared to the FAO standard limit i.e. $7.8 \mu\text{g ml}^{-1}$ (Table II). The concentrations ($\mu\text{g L}^{-1}$) of selective heavy metals were Cr

(1.451), Co (0.255), Cd (0.325) and Zn (0.355) in water of Balloki headworks. Order of the metal concentration in water of Balloki headworks was $\text{Co} > \text{Cd} > \text{Zn} > \text{Cr}$. The concentrations ($\mu\text{g L}^{-1}$) of Cd and Cr was higher than the FAO permissible limit i.e. < 0.1 and 0.29 , respectively and concentrations $\mu\text{g L}^{-1}$ of Zn and Co are less according to FAO permissible limits i.e. 7.8 and $1-5$. Concentrations ($\mu\text{g L}^{-1}$) of selective heavy metals were Cr (0.690), Co (0.000), Cd (0.112) and Zn (0.212) in water of Chenab near Balloki. Order of the metal concentration in water of Chenab near Balloki was $\text{Cr} > \text{Zn} > \text{Cd} > \text{Co}$. The concentration ($\mu\text{g L}^{-1}$) of Cd was higher than the FAO standard limit i.e. < 0.1 . Selective heavy metal concentrations ($\mu\text{g L}^{-1}$) were Cr (1.266), Co (ND), Cd (0.122) and Zn (0.132) in water from the confluence of Ravi and Chenab. metal concentration in water from the area confluence of Ravi and Chenab was $\text{Cr} > \text{Zn} > \text{Cd} > \text{Co}$. The concentration of Cr and Cd are higher as compare to the FAO standard limit i.e. $0.29 \mu\text{g L}^{-1}$ and $< 0.1 \mu\text{g L}^{-1}$ showed the comparison of water concentration of study areas with heavy metal FAO permissible limits in fresh water, it clearly showed that the concentration of Chromium was high in Ballokiheadworks and in confluence of Ravi and Chenab thus, it showed that Cr concentration also exceeded the permissible limits set by EPA and FAO standard limits of heavy metals concentrations. The main sources of pollution in River Ravi are municipal sewage, scientific laboratories, paper and pulp industries, steel factories and electroplating workshops (Khan, 2011) the highest and lowest concentration of heavy metals in major drains of river Ravi, Zn concentration was higher before entering into the river Ravi because of the excessive pollution load in Nallah Deg. All the factories drain their waste in it and it becomes more polluted 105 reported factories have been observed near Nallah Deg on Sheikhpura road Lahore Punjab Pakistan these factories mostly discharge effluents containing heavy metals such as Zn, Cd and Cr into Deg.

Table II.- Fish species found in the study areas.

| Sr. No. | Fish species of Siphon | Fish species of Chenab near Balloki | Fish species of confluence of Ravi and Chenab |
|---------|------------------------------------|-------------------------------------|---|
| 1 | <i>Chanda nama</i> | <i>Chanda nama</i> | <i>Chanda nama</i> |
| 2 | <i>Rita rita</i> | <i>Rita rita</i> | <i>Pseudeutropius atherinoides</i> |
| 3 | <i>Channa marulius</i> | <i>Channa marulius</i> | <i>Channa marulius</i> |
| 4 | <i>Hypophthalmichthys molitrix</i> | <i>Cirrhinus mrigala</i> | <i>Hypophthalmichthys molitrix</i> |
| 5 | <i>Labeo rohita</i> | <i>Gudusia chapra</i> | - |
| 6 | <i>Sperata sarwari</i> | <i>Pseudeutropius atherinoides</i> | - |
| 7 | <i>Catla catla</i> | <i>Labeo nigripinnis</i> | - |
| 8 | <i>Wallago attu</i> | - | - |
| 9 | <i>Cyprinus carpio</i> | - | - |
| 10 | <i>Cirrhinus mrigala</i> | - | - |

Overall, 15 species were found in the investigated area. The number of species was maximum at Siphon.

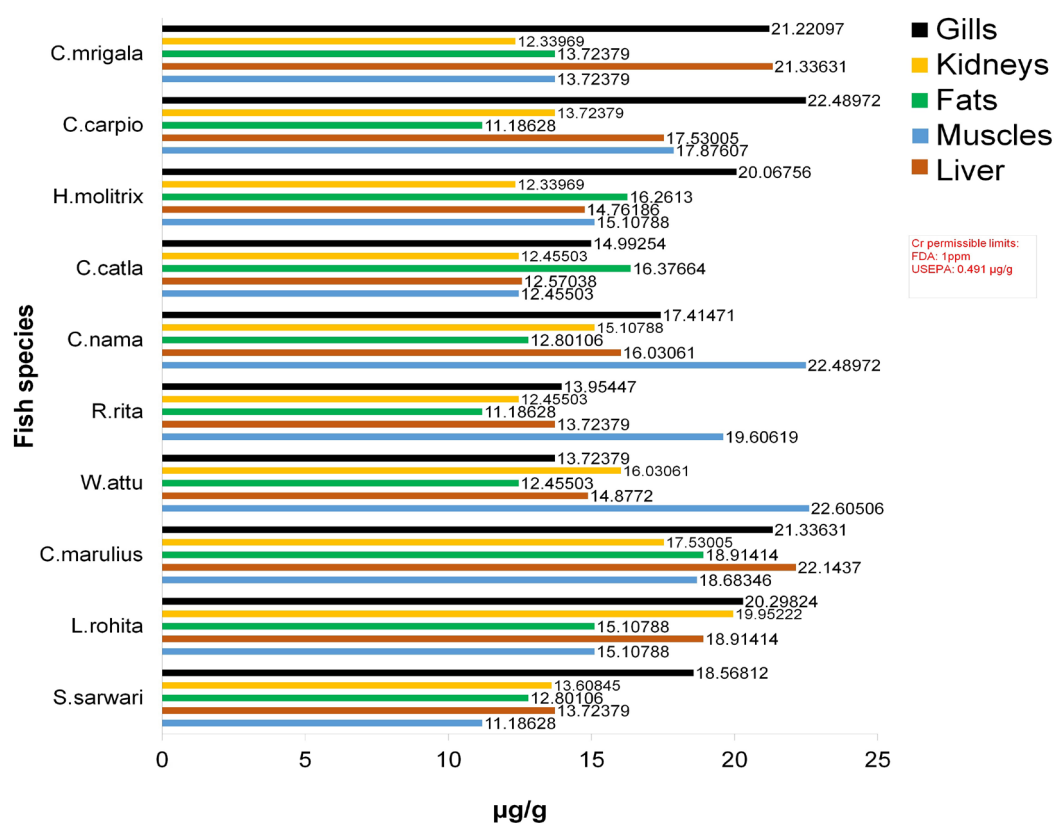


Fig. 2. Cr ($\mu\text{g g}^{-1}$) in different organs of different fish species from Ravi Siphon Lahore.

Zn is used in can food products for preservation of food in many countries because it is a non rusting compound (Rauf and Javed, 2007). Results showed that the concentrations $\mu\text{g/l}$ of Zn 1.021-1.239 And Cr 1.015-1.115 are higher than Co: 0.012-0.081 and Cd .981-.017. Hudiara Nallah

showed less concentration of heavy metals as compared to NallahDeg because it is flowing from river Ravi and carries pollution load of Ravi only.

Knowledge of heavy metal concentrations in fish is important with respect to nature of management and

human consumption of fish. Bioaccumulation of metals in fish can be considered as an index of metal pollution in the aquatic bodies (Emmaneul *et al.*, 2012). The fish samples collected from the River Ravi at Balloki showed a considerable drop in the number of fish collected from Ravi Siphon (Table II). Present results showed maximum accumulation in fish muscles and that is the part which is more eaten by humans whereas the rest is thrown away, other fish body tissues are used in production of chicken feed. In Pakistan 2kg per year capita consumption and world's average per capita consumption is 17kg per year. (Papagiannis *et al.*, 2004). Fish diversity is higher in river Ravi Siphon because fish farming practices are less here because of the Pak army control and water conditions are also suitable for aquatic life pollution load is less in river Ravi Siphon. A study conducted by Emmaneul *et al.* (2012) found more medium tolerant specimens of macro-invertebrates as compared to tolerant ones. Balloki area showed more medium tolerant specimens as compared

to tolerant ones but the presence of sensitive species then indicated much cleaner water as compared to the present study (Karadede and Unlu, 2007). Same study was conducted by Ather (Arther, 2015). *Chlamydomonas*, *Chlorella* and *Volvox* are the sensitive species whose number was quite low because of the polluted water Among the medium tolerant species are *Cosmarium*, *Microsporia* and *Synedra*. The diatoms species obtain from selective sampling sites were *Pinnularia*, *Nitzschia* and *Navicula*, all these diatoms species can survive in high polluted water environment (Javed, 2006). *Euglena* and *Cosmarium* are the species which can survive in high heavy metal deposited environment these all macro-invertebrates are the diet of Fish and this showed that due to polluted water and less availability of food for Fish can lead to shortage of Fish in river. Fish fry age was 1 month. Fish fry from river Ravi Siphon showed high level of Zn concentration in fish *C. carpio* that is 32.987µg/g (Table III). In fish *C. marulius* high levels of Zn and Cr were identified,

Table III.- Metal accumulation in the tissues of fish species collected from Ravi River at different study areas.

| Sr. No. | Fish species found at Siphon | Body Weight (g) | Body Length (cm) | Age of Fish (years) | Cr ppm | Zn ppm | Cd ppm | Co ppm |
|--|------------------------------------|-----------------|------------------|---------------------|--------|--------|--------|--------|
| Fish species and associated parameters investigated in the present study at Ravi Siphon area | | | | | | | | |
| 1 | <i>Sperata sarwari</i> | 545.8 | 36.5 | 3 | 20.8 | 32.9 | 13.7 | 10.9 |
| 2 | <i>Labeo rohita</i> | 487.9 | 34.5 | 3 | 16.5 | 21.1 | 20.1 | 12.9 |
| 3 | <i>Channa marulius</i> | 456.3 | 36.0 | 4 | 21.1 | 20.1 | 12.8 | 10.9 |
| 4 | <i>Wallago attu</i> | 567.9 | 40.5 | 3 | 18.6 | 21.9 | 10.9 | 13.1 |
| 5 | <i>Rita rita</i> | 676.1 | 44.5 | 3 | 19.8 | 30.9 | 14.2 | 10.1 |
| 6 | <i>Chanda nama</i> | 845.2 | 55.0 | 4 | 17.5 | 19.1 | 18.1 | 13.4 |
| 7 | <i>Catla catla</i> | 662.8 | 49.7 | 4 | 20.1 | 18.3 | 15.7 | 14.9 |
| 8 | <i>Hypophthalmichthys molitrix</i> | 560.8 | 52.9 | 3 | 18.1 | 20.2 | 11.9 | 15.2 |
| 9 | <i>Cyprinus carpio</i> | 498.0 | 44.5 | 4 | 19.6 | 17.2 | 14.8 | 11.9 |
| 10 | <i>Cirrhinus mrigala</i> | 575.9 | 53.2 | 4 | 16.6 | 19.7 | 12.9 | 13.8 |
| Fish species and associated parameters investigated in the present study at Ravi near Balloki | | | | | | | | |
| 1 | <i>Chanda nama</i> | 558.9 | 44.3 | 3 | 32.8 | 30.9 | 29.7 | 11.9 |
| 2 | <i>Rita rita</i> | 653.2 | 49.8 | 3 | 35.5 | 32.1 | 32.1 | 10.9 |
| 3 | <i>Channa marulius</i> | 667.9 | 49.5 | 3 | 30.1 | 34.1 | 14.8 | 11.9 |
| 4 | <i>Cirrhinus mrigala</i> | 587.1 | 42.0 | 3 | 18.1 | 20.2 | 11.9 | 15.2 |
| 5 | <i>Gudusia chapra</i> | 782.5 | 60.5 | 5 | 19.6 | 17.2 | 14.8 | 11.9 |
| 6 | <i>Pseudeutropius atherinoides</i> | 795.0 | 61.8 | 5 | 16.6 | 19.7 | 12.9 | 13.8 |
| 7 | <i>Labeo nigripinnis</i> | 678.2 | 50.5 | 4 | 16.6 | 19.7 | 12.9 | 13.8 |
| Fish species and associated parameters investigated from the confluence of Ravi and Chenab | | | | | | | | |
| 1 | <i>Chanda nama</i> | 458.3 | 34.0 | 3 | 21.2 | 20.3 | 13.8 | 11.9 |
| 2 | <i>Pseudeutropius atherinoides</i> | 557.2 | 41.5 | 4 | 15.6 | 20.9 | 11.9 | 12.1 |
| 3 | <i>Channa marulius</i> | 666.4 | 43.5 | 3 | 18.8 | 26.9 | 13.2 | 12.3 |
| 4 | <i>Hypophthalmichthys molitrix</i> | 834.5 | 54.0 | 4 | 16.5 | 19.1 | 16.1 | 13.1 |

its body wt (g) was 456.3, length (cm) of 36.0 and age 3 years Fish *H. molitrix* showed high 104.6µg/g Cd concentration with its body wt (g) 560.8, length (cm) 52.9 and age 4 years (Table III). Cr concentration in muscles, gills and in liver of fish species from Ravi siphon was low between 11.1µg/g-22.6µg/g (Fig. 2) as compare to other sampling sites like Balloki. In Siphon water order of Cr concentration in fish organs was this liver > gills > muscles > kidneys > fats that was 22.6µg/g > 22.4µg/g > 22.1µg/g > 19.9µg/g > 18.9µg/g. Cr was high 22.6µg/g in liver of fish *W. attu* and in liver of *C. namai*.e.22.4µg/g (Fig. 3) and its carnivorous fish which feed on small other fish that is why metal accumulated more in *W. attu* due to magnification. Order of Cr concentration in fats of different fish in water of river Siphon was *C. carpio*>*C. marulius*>*C. mrigala*>*L. rohita*>*H. molitrix* Gills also showed high 22.4µg/g concentrations of Cr in fish *C. carpio*. Heavy metal analysis of the whole fish specimens showed concentration for Chromium which showed an increase from fish fry stage to

age 3 and age 4. However, as muscles and fats are the only things most commonly and directly consumed by man. Concentration of Cr increased in the fish samples when compared with fish fry the complete sample fish fry of *C. marulius* showed a Cr concentration of 18.6ppm whereas only the muscles of this fish had Cr. Concentration of 22.1437ppm and 18.914ppm was detected in fats of the same fish. Similarly, in all four species an increase was observed from fry stage to age 3 and 4 years. Results of Ravi Siphon (Fig. 3) showed that the concentration of Zn was higher between 45.5µg/g-36.6µg/g in fish muscles especially in fish *C. carpio* and *S. sarwari*. Increasing order of Zn concentration in different tissues of different fish species was this muscles > liver > kidneys > fats > gills ranged as 45.5µg/g > 32.6µg/g > 28.8µg/g > 22.4µg/g > 20.0µg/g, respectively. Fish *C. marulius* showed maximum 276.5µg/g Zn concentration as compare to the other fish species of same area. Zn concentration in the whole fish fry was analyzed and compared with the

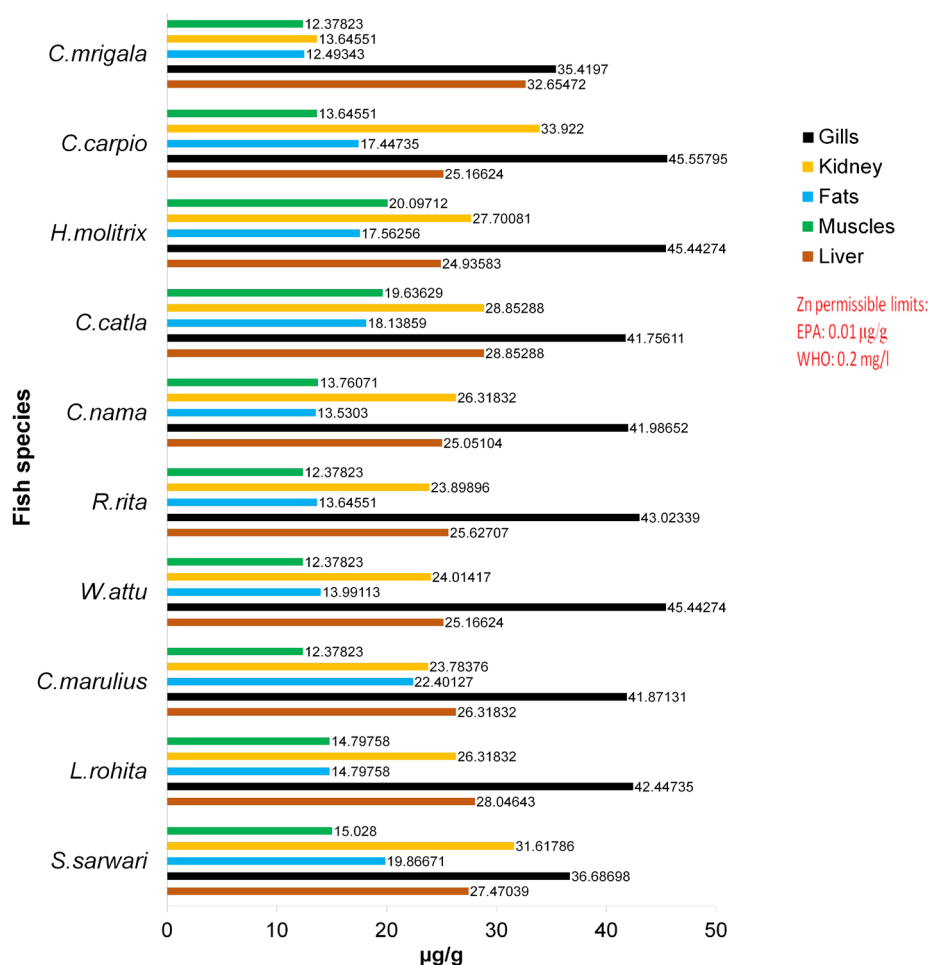


Fig. 3. Zn (µg g⁻¹) in different organs of different fish species from Ravi Siphon, Lahore.

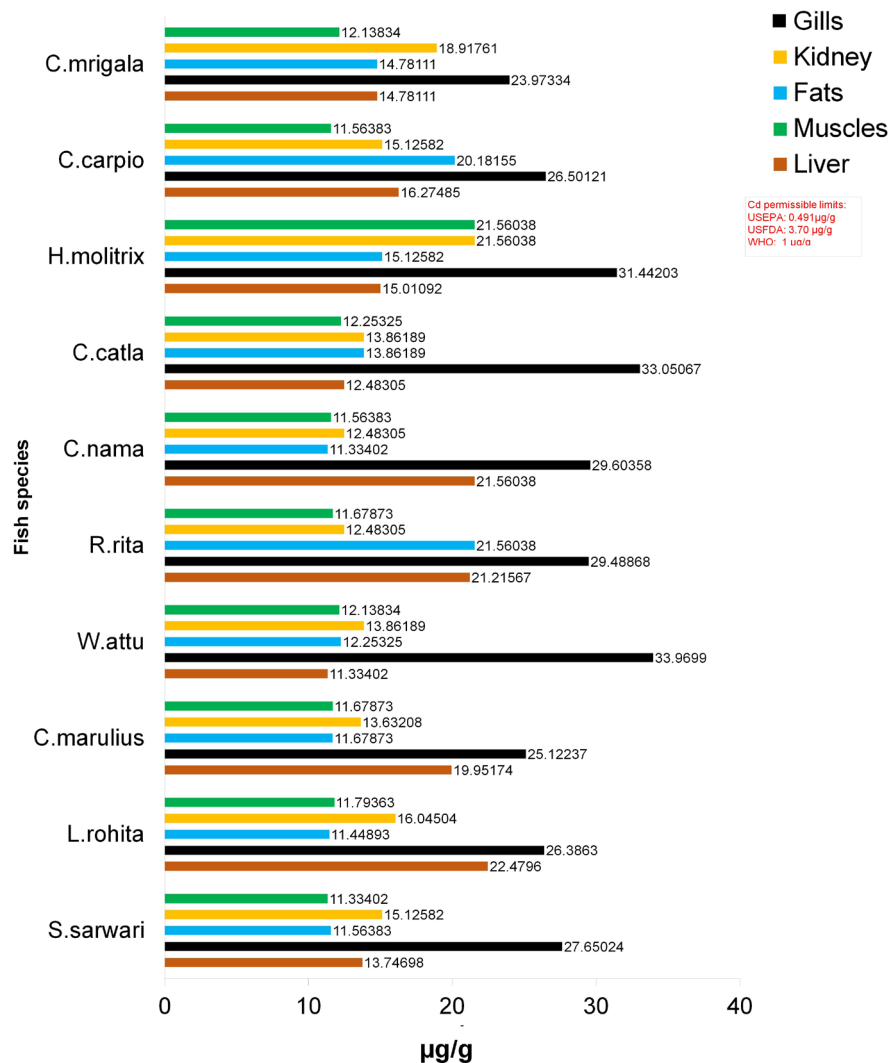


Fig. 4. Cd ($\mu\text{g g}^{-1}$) in different organs of different fish species from Ravi Siphon, Lahore.

concentration of Zn in the adult specimens of the same species at ages 3 and 4 years. All four fish studied showed a marked rise in Zn concentration even if only fish muscles were considered. All these readings were much higher than the acceptable standards for fish. The observed Cd levels in fish muscles collected from Ravi siphon showed high 33.9 $\mu\text{g/g}$ concentration in muscles as compare to the other organs of same fish species were presented in (Fig. 4). *H. molitrix* showed high Cd concentration 104.6 $\mu\text{g/g}$ among other fish species of Ravi Siphon. From all other fish tissues muscles accumulated more Cd in increasing order muscles (33.9 $\mu\text{g/g}$) > liver (21.5 $\mu\text{g/g}$) > fats (20.1 $\mu\text{g/g}$) > kidneys (18.9 $\mu\text{g/g}$) > gills (12.5 $\mu\text{g/g}$). *C. marulius* showed high level of Zn with its body weight (g) 350.2, body length 35.9(cm) and age 3 years. *H. molitrix* showed high

levels of Cd and Cr with its body weight (g) 454.3, body length 32.5(cm) and age 3 years. Results showed that metal accumulate and increase its concentration in fish tissues as the fish increases in body weight and body length (Table III). At confluence of Ravi and Chenab Cr concentration was higher 634.5 $\mu\text{g/g}$ total weight of fish *H. molitrix*. Increasing order of Cr concentration in different organs of different fish species at sampling site of confluence of Ravi and Chenab was this muscles (318.1 $\mu\text{g/g}$) > liver (221.4 $\mu\text{g/g}$) > kidneys (81.0 $\mu\text{g/g}$) > fats (24.2 $\mu\text{g/g}$) > gills (7.1 $\mu\text{g/g}$) Fish *H. molitrix* and *P. atherinoides* showed highest ranged as 634.5 $\mu\text{g/g}$ and 499.0 $\mu\text{g/g}$, respectively Cr concentration among other fish from confluence of Ravi and Chenab (Fig. 5). Zn concentration in different fish tissues of different fish

species from confluence of Ravi and Chenab, it showed that fish *C. marulius* accumulated Zn more i.e. $510.5\mu\text{g/g}$ than other fish species of same area. Increasing order of Zn concentration was this muscles > liver > fats > kidneys > gills ranged as $230.7\mu\text{g/g}$ > $215.7\mu\text{g/g}$ > $90.7\mu\text{g/g}$ > $9.8\mu\text{g/g}$ > $4.42\mu\text{g/g}$, respectively. The muscles of fish *C. marulius* accumulated high level $197.4\mu\text{g/g}$ of Cd concentration. Increasing order of Cd levels in tissues of fish species from area of confluence of Ravi and Chenab was this muscles > liver > fats > gills > kidneys ranged as $197.4\mu\text{g/g}$ > $192.5\mu\text{g/g}$ > $11.5\mu\text{g/g}$ > $11.3\mu\text{g/g}$ > $2.14\mu\text{g/g}$, respectively.

There is no marked difference in cobalt concentration. *C. marulius* showed high levels of Cd and Cr with its body weight of 678.2g, length 50.5cm and age 4 years. Fish *P. atherinoides* showed high level of Zn accumulation with its body weight of 795.0g, length of 61.8cm and age 5 years (Table III). Cr. concentration in fish liver of *C. marulius* and *P. atherinoides* was high ranged as $94.3\mu\text{g/g}$ and $82.8\mu\text{g/g}$, respectively at Chenab near Balloki site more than the fish

of other selective sampling sites but in the muscles of *C. marulius* fish concentration of Cr was as $81.8\mu\text{g/g}$. Heavy metal Cr concentration in fish organs at Chenab near Balloki site was this liver > muscles > kidneys > gills > fats ranged as $94.3\mu\text{g/g}$ > $81.8\mu\text{g/g}$ > $74.9\mu\text{g/g}$ > $57.0\mu\text{g/g}$ > $54.5\mu\text{g/g}$, respectively. Results of Zn concentration at Chenab near Balloki site showed higher concentration in fish *C. nama* muscle $164.4\mu\text{g/g}$. Increasing order for Zn concentration in different fish tissues of different fish species was this muscles > liver > fats > kidneys > gills ranged as $164.4\mu\text{g/g}$ > $151.7\mu\text{g/g}$ > $112.6\mu\text{g/g}$ > $22.4\mu\text{g/g}$ > $13.8\mu\text{g/g}$, respectively. The Chenab near Balloki study showed higher levels of Cd in fish *C. marulius* $209.4\mu\text{g/g}$. Fish *G. chapra* showed higher levels in its tissues such as Muscles > fats > gills > kidneys ranged as $112.4\mu\text{g/g}$ > $27.6\mu\text{g/g}$ > $21.9\mu\text{g/g}$ > $20.1\mu\text{g/g}$, respectively. There is no mark difference in cobalt results. From Balloki *R. rita* showed higher level of Zn concentration that is 34.132ppm. *C. mrigala* and *C. nama* showed high levels of Cd, Zn and Cr with body

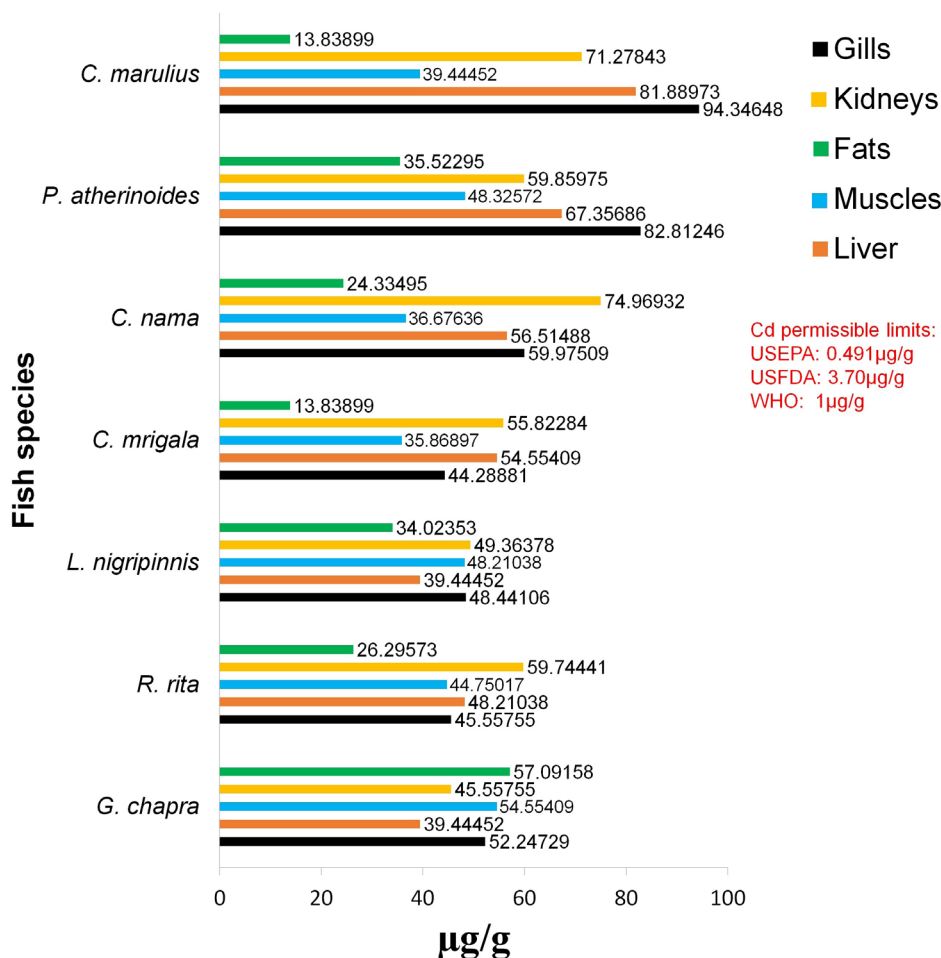


Fig. 5. Cd ($\mu\text{g g}^{-1}$) in different fish organs of different fish species from Balloki.

weight (g) 456.9, 440.2, length (cm) 35.9, 35.9 and age was same in both fish species 3 years, respectively (Table III). Cr concentration was high $76.05\mu\text{g/g}$ in fish *C. nama* muscle at Balloki. Order of concentration was, muscles > liver > gills > kidneys > fats, ranged as $76.05\mu\text{g/g}$ > $75.6\mu\text{g/g}$ > $43.1\mu\text{g/g}$ > $42.6\mu\text{g/g}$ > $33.9\mu\text{g/g}$, respectively. In literature, the concentration mg L^{-1} of Cr in *Cirrhinamrigala* ranged as 5.94-6.56, 6.60-7.14 in winter and summer, respectively. Above all ranges showed higher levels of Cr as compare to the ranges set by, USEPA permissible limit of chromium is $0.491\mu\text{g/g}$, EUROPA chromium limit is $0.100\mu\text{g/g}$, NEPA chromium limit $\mu\text{g/g}$ is 500, FAO Cr limit is $1\mu\text{g/g}$ and 1ppm from international and national standard values FDA. All the findings of this study showed higher range of Cd in fish organs such as in muscles and in liver of different fish species from river Ravi as compare to the permissible limits of Cd in fish. Lowest value $167.8\mu\text{g/g}$ of Cd was showed in different fish organs of different fish species of Ravi Siphon. All statistical analysis showed significant ($p < 0.005$) values of Cr concentration in different fish tissues of different areas. Zn levels in different fish organs of different fish species from Balloki, showed Zn concentration ranged as $96.7\mu\text{g/g}$ > $72.4\mu\text{g/g}$ > $68.4\mu\text{g/g}$ > $48.5\mu\text{g/g}$ > $39.5\mu\text{g/g}$ in different fish organs such as muscles > gills > kidneys > liver > fats, respectively. Fish *C. mrigala* showed high accumulated level $307\mu\text{g/g}$ among other fish of Balloki. Higher ranges of Cd concentration in fish *C. mrigala* $545.3\mu\text{g/g}$ and in its organs such as in muscles > liver > fats > gills > kidneys ranged as $255.9\mu\text{g/g}$ > $145.8\mu\text{g/g}$ > $76.9\mu\text{g/g}$ > $37.5\mu\text{g/g}$ > $37.4\mu\text{g/g}$, respectively. As compared to other tissues of different fish species of Balloki, Cd concentration increasing order was, muscles > liver > fats > kidneys > gills ranged as $225.9\mu\text{g/g}$ > $145.8\mu\text{g/g}$ > $76.9\mu\text{g/g}$ > $42.3\mu\text{g/g}$ > $37.5\mu\text{g/g}$, respectively. Cd has high potential for bioaccumulation in fish organs. Highest Cd ranges were found in different fish species from confluence of Ravi and Chenab, fish organs such as liver and muscles were highly contaminated from Cd but in Balloki pond water fish muscles and fats are highly contaminated (Fig. 3). In our results, the observed concentrations for Cd found to be in high ranges as compared to the permissible limits set by different organizations (FAO/WHO, 1989; USEPA, 1989). Observed values of Co-concentrations in different organs of fish species were below the standard limits although the detectable and published limits of Co. in fish were from 0.04 – 0.26 mg/kg and 0.05 – 0.28 mg/kg (Yilmaz, 2009). Fish fats showed least concentrations than all the body organs. Liver and muscles showed higher concentration of metals than all the body organs. Muscle is the main body organ of fish which is eaten by humans in Pakistan, liver and kidneys are not edible but in world many countries like China eat

other fish organs such as eyes, liver, gills and kidneys etc. Kidney is the main organ for metal detoxification. In kidney, significant amounts of metals were collected (Mustafa *et al.*, 2008). The results clearly demonstrate that the fish of river Ravi faces metal pollution in particular areas for Zn, Cd, Co and Cr. Zn and Cd are high in concentration present in river Ravi water.

CONCLUSION

Herein, fish species including *C. marulius*, *C. nama*, *H. molitrix* and *C. mrigala* showed high levels of metal accumulations in their body tissues, measurement difference in body weight and length was showed in same fish species from selective study areas. Higher metal concentrations have resulted in the decrease of fish species found in Ravi during the recent years. Further studies are required to explore the phenomenon in detail.

ACKNOWLEDGMENTS

Authors would like to thank the staff members of 'Wild Life Balloki' and Department of Environmental Sciences, Kinnaird College, Lahore for their help. Prof. Abdul Rauf Shakoori was consultant to the research project.

Conflict of Interest

The authors declare no conflict of interests.

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