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Estimation of selected heavy metals in muscles of *Cirrhinus mrigala* and *Cyprinus carpio* From Bhakkar District, Punjab – Pakistan

Faiza Zubair[a]*, Naila Riaz[a], Naila Amjad[b], Muhammad Imran[a]

[a]Department of Zoology, University of Sargodha; [b]Department of Zoology, Institute of Molecular Biology and Biotechnology, University of Lahore, Sargodha Campus, Pakistan

ABSTRACT

Background: Heavy metals have long been a cause for concern in our environment due to their toxicity when found in concentrations higher than permitted. These metals are discharged through a number of methods, including industrial processes in air, soil, and aquatic ecosystems. The most common kind of food in aquatic ecosystem is fish. This study aims to estimate the concentration of selected heavy metals with regards to toxicity in fish and health surveillance of human beings. Fish occupies the top position in food pyramid and act as medium of transfer of heavy metals to humans. The basic purpose of this study was to determine concentration of heavy metals (Cu, Pb, Mn and Cr) in the muscles of *Cirrhinus mrigala* and *Cyprinus carpio*. **Methods:** Samples were collected from District Bhakkar (Dera-Bhakar bridge). Defrosted muscle samples were processed through wet acid digestion method following Flame Atomic Absorption Spectrophotometry for estimation of metal concentration. The numerical data was analyzed using ANOVA, SPSS software (version 20.0). **Results:** Results showed the non-significant ($p<0.05$) concentration of heavy metals in muscle samples. Results revealed the highest level of Cu (25.62±3.23 mg/kg) in the examined samples. There was also a significant rise in the level of Pb. The descending order of mean values were present in order of Cu>Pb>Mn>Cr. **Conclusion:** Based on estimated levels of targeted heavy metals, it is concluded that the concentration of heavy metals is found in permissible limits of WHO/FAO thus eating such fish does not present any risks to humans.

Corresponding author:
Faiza Zubair
Department of Zoology, University of Sargodha, Pakistan.
faiza.zubair@uos.edu.pk

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INTRODUCTION

In the last decennary, the evolution of industry and agriculture has raised the exposure of heavy metals causing pollution, which is a momentous environmental risk for invertebrates, fish, and humans (Uluturhan and Kucuksezgin, 2007). Among the other environmental pollutants, heavy metals should be highlighted due to the heir bioaccumulation (Nadmitov *et al.*, 2014). Significant concentration of heavy metals is released into water bodies, where they are concentrated and biomagnified consequently causing lethal or sub-lethal effects to aquatic populations (Protano *et al*., 2014, Xie *et al*., 2014).

The toxic concentration of trace metals has been a serious concern, as they are not removed from aquatic ecosystems by self-purification (Harikumar and Nasir, 2010) and are concentrated in the form of sediments and suspended particulates (De Jonge *et al.*, 2012), consequently, threatening human health and ecosystems through the food web causing a serious health concern for aquatic life and human consuming them (Eslami *et al.*, 2011). This scenario makes it crucial to evaluate the ecological and health risk due to the accumulation of trace elements (Mario *et al.*, 2012).

The contamination of aquatic ecosystem both by natural and anthropogenic activities have affected the health as well as economic status of the populations that inhabit the ecosystem. These activities cause pollution as they are the cause of heavy metals, pesticides and herbicides entry into aquatic ecosystem. Most important of these are, the heavy metals accumulation in water. Thus, the pollution caused by heavy metals is increasing at high rate and have become a major worldwide problem (Malik *et al*., 2010).

Cirrhinus mrigala is benthopelagic species i.e. living near the bottom of river as well as in mid waters and so called as potamodramous. This fish can inhabit freshwater as well as brackish waters because it can tolerate high levels of salinity but requires swift waters for spawning purposes. Food of *Cirrhinus mrigala* includes phytoplankton, zooplankton and insect larvae and also feed on algae. *C. mrigala* is eurythermal species and can tolerate as low temperature as 14 ºC, but usually breeds at 28-30ºC (Iqbal and Kausar, 2009). Gonadal weights of *C. mrigala* are in correlation with photoperiod and temperature of water. This fish can live up to 12 years in natural waters (Lone and Husain, 2009).

Cyprinus carpio is an important fish as human diet and is also important economically for a country. As their habitat *C. carpio* loves to be in an area of abundant vegetation and detritus bottom in natural lakes, so these are found in natural lakes, ponds and slow moving rivers. Deep waters are preferably occupied by the elder *C. carpio* while in younger age these fish occupied the shallow waters. Normally these fish species like warm waters for their habitat. These fish species can adjust at even low levels of oxygen but these are not commonly found in high altitudes (Gul *et al*., 2010).

Lead, copper and zinc are included in very toxic and accessible category of heavy metal. Lead is highly toxic to human and medium toxic to fish. Copper is low medium toxic to human and high toxic to fish. Zinc is low toxic to human and medium toxic to fish. Earlier there was no concern or a little concern about metals because oxides of metals through corrosion were not enough to cause alarming situation. There was the time when some damaging effects of these metals came to be known. Legislation is also being done to control the entry of wastes into water by this industrialization in advanced societies. Compound containing Mercury and Cadmium are not allowed to be disposed off in water except in small concentrations since 1972 (Anon, 1972). Strict control is also required for zinc, copper and leads (Waldichuk, 1974).

There is certain limit of each element in water and toxicity is meant when the amount of these elements cross these limits. According to WHO set limits of these heavy metals are Pb 0.01mg/l, Ni 0.07mg/l, Mn 0.4mg/l, Zn 3mg/l, Cd 0.003mg/l and Cr 0.05mg/l in water while in fish amount of Pb and Ni is 2.0mg/l (WHO, 2011). These heavy metals find their way into human body to some extent through drinking water, food and air. Some heavy metals like copper, zinc and arsenic have their certain role in maintaining metabolism of human body but this happens only when heavy metals are present in trace amounts. Human body requires some heavy metals like cobalt, copper, manganese etc. for proper functioning. As the concentration of these heavy metals crossed that trace amounts limits these become poisonous. Toxic and beneficial nature of heavy metals depends on certain conditions and organism. Heavy metals that have toxic effects on one organism might have beneficiary effects on other organism like vanadium, tungsten and cadmium (Reena *et al*., 2011).

Accumulation of metals is also fish species dependent and different fish species living in same water accumulates different concentration of metals (Zhou et al., 1998). Different fish species can accumulate different amounts of heavy metals in different seasons. Higher uptake levels of metals were noted during summer season which was attributed to increase metabolic rates (Kock *et al*., 1996).

The aims and objectives of this study was to assess heavy metals (Pb, Cr, Cu and Mn) concentration in two fish species and to examine whether fish species from District Bhakkar are suitable for human consumption or not.

MATERIALS AND METHODS

Sample collection

The current study was aimed to evaluate concentration of heavy metals in muscles of two fish species i.e. *Cyrinus carpio* and *Cirrhinus mrigala.* Experiment was performed on 25 fish samples weighing 1kg of each species. These fish species were collected from

District Bhakkar during September 2022-Feburary, 2023. In present study muscles were examined as this part is consumed by human as food.

Samples were collected using cast net (dimension of 100 m × 6 m, having a mesh-size of 60 mm). Then fish samples were brought to University of Sargodha sub campus Bhakkar in plastic bags where the weight and length of samples were noted afterward samples were frozen at as low temperature as -25 ºC.

Preparation of muscles of fish for metal analysis

To get frozen fish ready for muscle removal, fish were left at room temperature for overnight. Then skin of each fish was removed followed by removal of muscles. Muscles were sampled through cut on the same side of the fish. Muscle samples were dried at 50°C, in an oven and then cooled at room temperature. From each sample 2gm of muscle was accurately measured using electric weight balance and proceeded with wet acid digestion technique. For estimation of heavy metals concentration Flame atomic absorption spectrophotometer (FAAS) was used. The liquid sample was aspirated, aerosolized and mixed with combustible gases then a flame of temperature ranging from 2100 ºC to 2800 ºC was used for ignition purposes.

By comparing the results with reference material (CRM IAEA 407) that was supervised by the International Atomic Agency (IAEA), the precision and accuracy of the AAS analysis were verified. Given that the analytical results of the standards and blanks for the metals under study fell within the certified values range of 95–101% recovery, and the heavy metal determination demonstrated an acceptable performance.

Standard solutions: In a volumetric flask, intermediate standards were diluted with 1% nitric acid and kept in plastic bottles. (AOAC, 1990).

Statistical analysis

The results of the experiment were subjected to a statistical analysis using ANOVA, SPSS software (version 20.0). The descriptive statistics of the data were obtained, and the significance level was set at $P<0.05$ (Stell et al., 1980).

RESULTS

This study was conducted to estimate the heavy metal (Cu, Cr, Mn and Pb) concentration in the muscles of two fish species *C. carpio* and *C. mirigala* of river Indus. The found mean values of selected heavy metals was Cu>Pb>Mn>Cr. Results are shown in Table 1.

Table 1: Mean values of heavy metals

	Mean (mg/kg ± SEM) (n=25)			
	Cr	**Pb**	**Cu**	**Mn**
C. carpio	7.85±1.48	16.05±2.46	25.62±3.23	11.72±1.76
C. mirigala	2.14 ±0.23	22.62±1.76	26.19±2.78	8.87±2.4

In *C. carpio,* Cu was found in highest concentration with mean content value of 25.62 mg/kg with standard error mean value of 3.23 mg/kg. The metal with 2nd highest concentration in *C. carpio* was Pb. It's mean concentration value calculated, was 16.05mg/kg with standard error mean 2.46 mg/kg. The metal whose concentration was lower than Cu and Pb was Mn. Mean content value of 11.72 mg/kg and standard error mean of 1.76 mg/kg. Of all studied metals, Cr concentration was lowest in *C. carpio* as compared with other three studied metals (Cu, Pb and Mn). In *C. carpio*, mean Cr content was 7.85 mg/kg with standard error mean of 1.48.

Same trend was observed in *C. mirigala* as Cu was found in highest concentration. Mean content of Cu was 26.19mg/kg with standard error mean value 2.76 mg/kg (Table 1). Pb was found as 2nd highest concentration in *C. mirigala* after Cu. Mean Pb contents found in *C. mirigala* were 22.62 mg/kg with standard error mean value 1.762 mg/kg. Lower concentration of Mn as compared with Cu and Pb was found in *C. mirigala*. The concentration of Mn was higher than Cr yet. For Mn contents in *C. mirigala* statistics showed mean value of Mn contents was 18.87mg/kg with standard error mean as 2.48mg/kg. Among all studied metals (Cu, Pb, Mn and Cr), concentration of Cr was lowest as recorded in *C. mirigala*. That was 2.14 mg/kg and standard error mean was 0.23 mg/kg. Above mentioned result showed that despite the variations in concentration of metals in muscles of two fish (*C. carpio* and *C. mirigala*), overall trend of metals accumulation in two species was same. Both the fish species have accumulated Cu in highest and Mn in lowest concentration.

Heavy metals concentration (mg/kg) in *C. carpio* and *C. mirigala*

Fig. 1 shows the results of comparative concentration of selected heavy metals in both spices under study. It was reported that same trend of heavy metals concentration was observed in the muscles of both species copper being the most accumulated metal and chromium as least accumulated one.

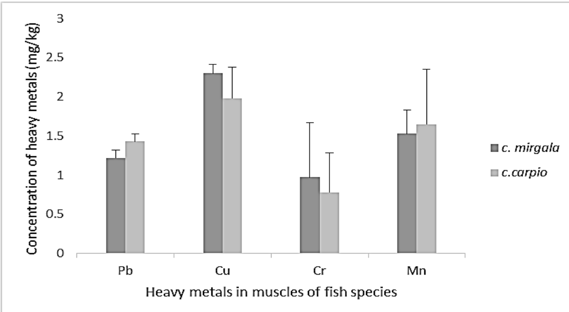

Figure 1: Heavy metals concentration (mg/kg) in *C. carpio* and *C. mirigala*

DISCUSSION

Rapid and yet increasing population of human has led scientists to think about different sources of food production and consumption. Fish comprise an important part in supply of protein to human, so demand of fish and fish products have also increased. Recently the consumption and demand of fish have rapidly increased (Wim *et al*., 2007). With the increase in demand and consumption of fish, it has become more important to know about heavy metals levels in fish in order to make sure that it is safe and healthy for human use. The study of heavy metals concentration is essential because fish beside being part of human food, is also the major part of many food chains, and heavy metals have their polluting effects on these fish species when these are accumulated in huge concentrations. These damaging effects ultimately transferred to consumers of fish when it is consumed (Burger and Gochfeld, 2005).

Among rivers, Indus River is the largest river of Pakistan and is the habitat of numerous edible fish species. This study was conducted to evaluate that whether the use of fish collected from Dera-Bhakar bridge as food is good for human or not.

The goal of the study is to estimate the heavy metal concentration in muscle of *C. mrigala* and *C. carpio*. Fish muscles were selected for study of heavy metals due to the reason that fish is one of the major sources of diet for many people of our country and muscles are the edible portion of fish that is being consumed. Fish species are considered as biomarkers of environment and the study of these fish species can give a good idea of level of environmental degradation. Biomarkers are considered as more valuable than bio indicators as these provides direct measure of changes in organisms (Lafontaine *et al*., 2000).

Our data revealed that the mean concentration of Cr in *C. carpio* and *C. mirigala* was 7.85 ± 2.14 mg/kg respectively. A study carried on Tilapia obtained from river Usuma, Nigeria showed Cr concentration of 0.56 mg/kg (Ugwu *et al*., 2012). Cr concentration of 2.37-5.47mg/kg was recorded in African catfish samples that were collected from Nile river (Osman and Kloas, 2010). *C. carpio* muscles showed concentration of 1.19±0.31mg/kg when sampled from Mogan lake, Turkey (Benzer *et al*., 2013). Mean Cr concentration of 1.18±.73mg/kg was recorded in muscles of Common carp obtained from lake Avsar dam. In Tilapia, of lake Hashenge of Ethopia, the concentration of Cr was found to be 0.37 ± 0.14 mg/kg (Asgedom *et al*., 2012).

In fish bioaccumulation of Cr occurs differently in different tissue. As per previous studies results showed that gills are the preferred site for Cr accumulation followed by kidney and liver. Seasonal changes along with morpho-chemical properties of water are the primary ground for bioaccumulation of Cr in various parts of fish (Sadiq, 1992). It is basically the combination of biotic and abiotic factors that determine the toxicity level of Cr in aquatic ecosystem. Chief biotic factors include age, and type of species while abiotic factors are temperature, dissolved oxygen, salinity, alkalinity and hardness of water etc. concentration of chromium that is found to be in lakes and rivers is 1-10 ug/L which is less than proposed EPA values of chromium (50-100 ug/L). Lethal effects of chromium in fish include renal lesion, anemia, eosinophilia and lymhocytosis. Higher concentrations of chromium might result in gills damage of fish (Sadiq, 1992).

During the study it was expressed that highest concentration of Cu 25.62 ±26.19 mg/kg was found in both studied fish species (*C. carpio* and *C. mirigala)* as compared with other metals (Pb, Mn and Cr). Flesh of common carp and Tilapia fish from lake Hashenge of Ethopia were studied by Asgedom et al., (2012) for evaluation of Pb contents showed concentration as 1.40 mg/kg and 0.85 mg/kg respectively. Similarly, another study by Anemi et al., (2013) reported that mean Cu concentration was 0.045 mg/kg in Tillapia muscle samples. *Oreochromis niloticus* collected from

Northern delta lakes of Egypt showed Cu concentration 2.8-48.84 mg/kg (Saeed and Shaker, 2008). Catfish obtained from Rasalganj fish markete of India showed Cu concentration as 15.0 ± 10.09 mg/kg (Javed and Usmani, 2011). Muscles of catfish of river Nile, Egypt showed Cu concentration of 1.01_5.48 mg/kg (Osman and Kloas, 2010). Anim et al., (2011) worked on African catfish of Nsawam in Densu river, Ghana and found Cu concentration of 45.60 ± 0.74 mg/kg. Recent studies have unfolded the range of effects of Cu over aquatic organism from molecular to structural level. Toxicity level of copper is dependent on pH and hardness of water (Taha, 2004).

Studies have revealed that chronic exposure of Cu can result in reduced body size loss of reproduction potential and survival of juveniles in fish. Sense of smell (olfaction) which is very important for fish as it helps fish to find food and avoid predators, is also badly affected by Cu. With the decrease in olfaction, there is decrease in appetite and availability of food for fish which in turn will affect the growth of fish (Baldwin *et al.,* 2003). Because of presence of Cu in water egg production in fish also decreases, early hatching of eggs, with increased abnormalities in newly hatched fish. Elevating concentration of Cu in pesticides also adds up in the increasing concentration of Cu in water bodies (Taub, 2004).

In present study it was concluded that among all studied four metals (Cr, Pb, Cu and Mn) in *C.carpio* and *C. mirigala* the concentration of Pb was 2nd highest after Cu that is 16.05± 22.62. But it was also noted that the estimated level of Pb in species under study were under the permissible limits of WHO. In comparison with previous studies these concentrations were also lower to show that the Pb contamination in Indus river water is not as much high to cause heavy metals accumulation in fish.

The concentration of Pb was recorded as 1.24 ± 0.20 mg/kg in *C. carpio* of lake Hashenge of Ethopia (Asgedom *et al*., 2012). In river Nile, Egypt, the concentration of Pb in *C. carpio* was recorded as 5.895 ± 14.51 mg/kg (Osman and Kloas, 2010). Lower concentration of Pb in two studied fish was obtained in our study as compared with the *C. carpio* of Avsar dam lake, Turkey where concentration of Pb was 2.14 ± 2.09 mg/kg (Oztirrk *et al*., 2009). Pb concentration (0.08 ± 0.01 mg/kg) in African catfish and 0.34 ± 0.01 mg/kg was recorded in *Tillapia zilli,* this study work was carried out in Densu river, Ghana (Anim *et al.,* 2011). Another study work carried out in Afikpo freshwater of Ghana on *Tillapia zilli* showed concentration of Pb as 0.31 ± 0.01 mg/kg (Nawani *et al*., 2010).

In metals (Cr, Pb, Cu and Mn) accumulation trend, Mn was at number three after Cu and Pb in both fish species (*C. carpio* and *C. mirigala)*. The concentration of Mn was also under the permissible limits of Mn in fish and its products. Resultant concentrations of Mn of present study were also below as compared with earlier studies. Mn level of 1.79 ± 0.53 mg/kg and 1.01 ± 0.32 mg/kg were recorded for *C. carpio* and *Oreochromis niloticus* respectively in fish sampled from Hashenge Lake of Ethopia (Asgedom et al., 2012). Muscles of *C. carpio* of lake Mogan of Turkey also showed Mn concenteration of 1.00 ± .24 mg/kg (Benzer et al., 2013).

CONCLUSION

This study concluded that fish conservation, fisheries development, and safe human consumption would all greatly benefit from an understanding of the harmful effects of heavy metals in freshwater organisms and the acceptable concentrations of these metals in the aquatic environment.

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