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Bioaccumulation of Copper (Cu), in Water, Sediments and in different tissues of *Cyprinus carpio,* from Kalpani Stream Mardan, Khyber Pakhtunkhwa, Pakistan

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Abstract

The study was conducted to analyze copper (Cu) bioaccumulation in water, sediments and in different tissues of common carp*, (Cyprinus carpio),* like muscles, Intestine, skin, liver and gills collected from Kalpani stream Mardan. The results recorded for bioaccumulation of copper was like that, in water samples the mean value for copper concentration was 0.014ppm; in sediment samples mean value for copper concentration was 0.47ppm. Similarly the mean value for copper concentration in Muscle tissue was 0.003ppm; in Intestine tissue was 0.10ppm; in Skin tissue was 0.06ppm; in Liver tissue was 0.03ppm and in Gills tissue was 0.07ppm. Our finding shows that copper concentration on the basis of mean values was high in sediment samples, which is then followed by intestine tissue, gills tissue, skin tissue, liver tissue, water samples and finally muscle tissue. It can be shown as Sediments>Intestine>Gills>Skin>Liver>Water>Muscle. So it's concluded that Copper show more affinity towards Sediments and least affinity toward muscle tissue. When we compare copper concentration in water and sediments so concentration is more in sediments samples as compared to water samples. Copper concentration in the three media was in the order of Sediments>Fish>Water.

Keywords: Metals; Copper; sediments; *Cyprinus carpio*; gills

1. Introduction

The River System of Khyber Pakhtunkhwa constitutes part of Pakistan's River system, which include land locked river system that is Baluchistan, the Indus drainage system and coastal drainage [36]. Among all these three river systems, Indus is the largest drainage system and takes its origin from Kailas range in Western Tibet (China). It has a drainage area of about 1,165500 Km^3 and a total running length of about 2900 kilometers [40]. Kalpani Stream is considered as the life line of part of Nowshera and Mardan Districts. It originates at a height of about 1520 feet in Malakand Agency and Landi Khwar nearby, Chanchanokhat of Districts Mardan. Its direction of flow is from North to South, and has a running length of about 110 kilometers from its origin [2].

The entire fish species of Kalpani Stream belong to South Asian sub region of the Oriental Region however, *Heteropneustus fossilis, Chana punctatus* and species of genus Puntius are from Indus River. Various efforts have been made by researchers to explain the different fish species of Kalpani. The different fish species of Kalpani and its offshoots have been considered and studied at different places and time by different researcher [27, 28]. The physiochemical factors of an area play significant role in the distribution of fish population, its assemblages and output of aquatic environment. The information about the rank of the physiochemical factors is very essential for management and conservation of aquatic ecosystem. There are different research workers who have conducted their research work on Kalpani Stream [3, 44]. Kalpani Stream facilitates diverse services to the local people of Mardan, for example, irrigation, relief from intense heat of summer, fishing, waste bin, disposal of industrial waste, soil formation, ground water renewal, and others. The Kalpani Stream is the main reservoir of the waste disposal as well as industrial effluents in District Mardan. Municipal treatment arrangements along with industrial waste prior to their discarding to Kalpani Stream are meager. The situation has become severe with the increasing anthropogenic activities therefore, Kalpani Stream has been considered as dirtiest streams of Khyber Pakhtunkhwa [16].

Due to rapid industrialization, the number of population and factories has increased rapidly. The pollution of freshwater with a broad range of hazardous material has developed into a serious subject of consideration over the last few decades [15]. The natural aquatic systems have been largely polluted with trace metals which get discharged from factories, household and other anthropogenic behavior [39]. Trace metal concentration has stressful effects on the ecological equilibrium of the recipient environment and a variation of marine biota [5]. Fishes cannot hide from the harmful effects of aquatic pollutants because they are the inhabitants of both fresh water and marine water [32]. The studies conducted upon various fishes have shown that these metals change the biochemical factors and physiological activities in the blood as well as in the tissues [7]. For fish, the gills, skin and digestive tract are the most likely sites of uptake of water borne chemicals [8]. Tawari-Fufeyin and Ekaye have presented that biomagnifications is largely documented to change in depuration period, and uptake for a variety of metals in various fishes [38]. Kargin reported that, biomagnifications of metals in different organs of the fish is mainly concerned with different factors such as season, chemical, and physical properties of aquatic body [26].

Heavy metals have gained much consideration among the non-degradable noxious substance owing to their poor consequences on water inhabiting fauna and flora [18]. Heavy metals are the most toxic substances because of their varied effects. Those metals which are highly soluble in water are easily absorbed into the biotic components of an ecosystem. Metals with higher concentration are known to cause harmful effects on blood and organs in fish. They form metal compound when react with enzymes, DNA, RNA and cellular proteins [4]. Copper is one of the common substances and it spreads through the environment by natural processes. It is reddish in color and crystalline in nature. Copper has low tendency of chemical reaction. It forms a greenish surface film when reacts with moist air, which protect the metal from further reactivity and corrosion. The toxicity of copper is also pH dependent. Its toxicity increase with pH below 7.0 and its effects decrease with increase in pH. Copper is an essential micronutrient in random rearrangement of natural proteins and enzyme activity. At somewhat higher but sub lethal concentrations, it causes persistent toxicity to aquatic life. Chronic toxic effects may cause low immune response, shortened life span, poor growth, low fertility and when fishes are subjected to sub lethal application (dose) of copper, it leads to low cardiac activity and reduction in heart rate, changes in appearance and behavior [14, 20]. The typical clinical findings of fish toxicity with copper and copper containing compounds comprise toil breathing, excessive mucus secretion on the body surface, in the gills and under the gill cover. It may also cause suffocation and breakdown of RBCs. When *Cyprinus carpio* was subjected to sub lethal concentration of copper, it showed low Hb level and reduced RBCs count (anemia). This may be due to copper inhibitory enzyme system which is responsible for hemoglobin production. Maximum copper concentration is established in the kidney of some of the species, especially *Carassius auratus*. However, that the highest copper levels were found in liver of *Oreochromus nilotica, Onchorynchus mykiss* and *C. carpio* [29].

Cyprinus carpio has nutritive and commercial as well as economic value, therefore, the present study was conducted to represent some scientific information about the biomagnifications of copper in different tissues (such as liver, gills, intestine, skin and muscle) of common carp, as well as in sediments and water collected from Stream Kalpani Mardan, with the following aims and objectives: To analyze the copper concentrations in water and sediments of Kalpani Stream. To quantify copper concentrations among different organs of fish. To compare the copper concentrations in fish with ambient water and sediments.

2. Methods and Materials

2.1 Samples collection and digestion:

2.1.1 Sample collection:

Water samples were collected in pre-cleaned and acid washed polypropylene bottles. These water samples were filtered through filter paper and conserved by adding 55% concentrated nitric acid (HNO_3). Sediment samples were collected in pre-cleaned, acid washed scoop or grab, transferred to clean polythene bags and transported to the laboratory. These samples were then dried in oven at 110^0C for 5 to 6 hours and ground to powder form in a glass mortar. After that samples were stored in pre-cleaned polythene bags. To get information about contamination and bioaccumulation of heavy metals in common edible fresh water fish species of Kalpani Stream twelve samples were collected from different parts of the Kalpani Stream. Fishing was done with the help of local fishermen. For this purpose Gill net with a cork line or floats at the top and metal line or sinkers with the bottom rope made up of nylon threads were used as fish Gears. Fish samples were preserved in ice container and were carried to the laboratory for further analysis. After morphometric study fish was dissected. Tissue samples were taken from the liver, muscles, skin, gills and intestine. These tissues were washed with distilled water, weighted and shifted to properly mark sterilized polythene bags and stored at -20 ^{0}C for further analysis.

2.1.2 Samples Digestion:

Water samples were digested according to the method described in APHA [1]. According to this method the water samples were taken in separate beakers along with a mixture of 15mL of concentrated HNO_3 and 5mL of $HClO_4$. The beakers were covered with Pyrex glass cover and placed on hot plate for boiling. The samples were allowed to evaporate until 2-3ml volume is left and the dense white fumes after the brown fumes appear which indicate that digestion has been completed. After digestion samples were cooled, filtered, and were diluted to 100ml distilled water by proper rinsing of the digestion beakers. Samples were stored in properly washed glass bottles until the metal concentration could be determined.

Sediments samples were digested according to the method described in APHA [1]. According to this method, oven-dried samples of sediments were passed through a 2mm sieve. The aqua regia method involving15mL of conc. HNO_3 and 5mL of $HClO_4$ (APHA [1]) was used as the standard method to digest the sample. 2gm of oven dried and marter grounded sample was placed in a beaker by adding 15ml of concentrated HNO_3 and 5ml of $HClO_4$. The beaker is then placed on hot plate and mixture is heated at 80–90 ^{0}C in a fume hood until the dark color disappears. The dense white fume from the beaker after brown fumes is the indication of completion of the digestion process. When the residue is completely dissolved, the container is cooled, and the solution is diluted and transferred quantitatively to a 100 mL volumetric flask by adding distilled water and passing through (Watman 42) filter paper. Samples were stored in properly washed glass bottles until the metal concentration could be determined. For heavy metals estimation the tissue digestion was carried out in the PCSIR Laboratory complexes Peshawar. Tissue samples were thawed, rinsed in distilled water and blotted with blotting paper. After blotting the samples were shifted to 100 volumetric flasks. The entire flasks were washed properly and rinsed with distilled water, before transferring the tissue samples. Then the known weight of each tissue was transferred to these volumetric flasks. Sample digestion was carried out according to the methods presented by Due freez and Steyn [17]. A slight modification was made in the procedure Yousafzai and Shakoori instead of putting 10ml nitric acid (55%) and 5ml per chloric acid (70%) at the time of digestion, 5ml nitric acid (55%)and 1ml per chloric acid (70%) were added to each flask and the flask were then kept for overnight [47]. Next day a second dose of 5ml nitric acid (55%) and 4ml per chloric acid (70%) was added to each flask. The flask were kept on hot plate, covered with Pyrex glass cover and allowed to digest at 200C^o to 250C^o until a clear transparent solution was observed. Initially dark brown fumes appeared followed by white fumes. The dense white fumes from the flask, after brown fumes was an indication of completion of the digestion process. By this method digestion was accomplished in about 30 minutes instead of 3 to 4 hours as described by Van Loon 1980. After digestion, samples were cooled, filtered through (Watman 42) filter paper and diluted to 100 ml with distilled water by proper rinsing of the digestion beakers. Samples were stored in properly washed glass bottles until the metal concentration could be determined.

2.1.3 Estimation:

Determination of heavy metals was done through atomic absorption spectrophotometer in the PCSIR Laboratory Complex Peshawar. Atomic Absorption Spectrophotometer (Spectra AA 2000) was used for determination of heavy metals concentration like copper, in water, sediments and tissue samples of fish. A range of analytical standards for metal was prepared from E Merck stock solution. Standard curves were prepared and the ODs obtained were calibrated against the standard curves to know the concentration of heavy metal, copper, present.

3. Results

The study was conducted from the month of July to October 2012. During this study, heavy metal, that is Copper (Cu), was analyzed for bioaccumulation in water, sediments and in different tissues of Common carp*, Cyprinus carpio,* like muscle, Intestine, skin, liver and gills collected from Kalpani Stream Mardan. All the samples of water, sediments and fish tissues were taken in triplicate. The results recorded for bioaccumulation of copper in water samples was like that; in July concentration was 0.01ppm, in August was 0.03ppm, in September was 0.01ppm and in October was 0.006ppm. So mean value for copper concentration in water samples was 0.014ppm. The concentration of Copper in sediments samples was like that; in July concentration was 0.47ppm, in August was 0.40ppm, in September was 0.48ppm and in October was 0.56ppm. So mean value for copper concentration in sediments samples was 0.47ppm. The concentration of copper in muscle tissue was like that; in July concentration was 0.006ppm, in August was 0.00ppm, in September was 0.00ppm and in October was 0.006ppm. So mean value for copper concentration in muscle tissue was 0.003ppm .The concentration of copper in intestine tissue was like that; in July concentration was 0.11ppm, in August was 0.10ppm, in September was 0.11ppm and in October was 0.09ppm. So mean value for copper concentration in intestine tissue was 0.10ppm. The concentration of copper in skin tissue was like that; in July concentration was 0.06ppm, in August was 0.06ppm, in September was 0.05ppm and in October was 0.08ppm. So mean value for copper concentration in skin tissue was 0.06ppm. The copper concentration in liver tissue was like that; in July concentration was 0.04ppm, in August was 0.00ppm, in September was 0.07ppm and in October was 0.02ppm. So mean value for copper concentration in liver tissue was 0.03ppm. The copper concentration in gills tissue was like that; in July concentration was 0.07ppm, in August was 0.07ppm, in September was 0.09ppm and in October was 0.05ppm. So mean value for copper concentration in gills tissue was 0.07ppm. Our finding shows that copper concentration on the basis of mean values was high in sediment samples, which is then followed by intestine tissue, gills tissue, skin tissue, liver tissue, water samples and finally muscle tissue. It can be shown as Sediments>Intestine>Gills>Skin>Liver>Water>Muscle. So it's concluded that copper show more affinity towards Sediments and least affinity toward muscle tissue. When we compare copper concentration in water and sediments so concentration is more in Sediments samples as compared to water samples. Copper concentration in the three media was in the order of Sediments>Fish>Water. All the results of Copper concentration in different concerned parameters are shown table.1.

Table 1: Results of Copper concentration in different concerned parameters

S. No	Area of Observation	July	August	September	October	Mean
1	**Water**	0.01ppm	0.03ppm	0.01ppm	0.006ppm	0.014ppm
2	**Sediments**	0.47ppm	0.40ppm	0.48ppm	0.56ppm	0.47ppm
3	**Muscles**	0.006ppm	0.00ppm	0.00ppm	0.006ppm	0.003ppm
4	**Intestine**	0.11ppm	0.10ppm	0.11ppm	0.09ppm	0.10ppm
5	**Skin**	0.06ppm	0.06ppm	0.05ppm	0.08ppm	0.06ppm
6	**Liver**	0.04ppm	0.00ppm	0.07ppm	0.02ppm	0.03ppm
7	**Gills**	0.07ppm	0.07ppm	0.09ppm	0.05ppm	0.07ppm

4. Discussion

Fishes are famous for their capability to accumulate heavy metals in their tissues. The metals occur most probably as cationic complexes and concentrate in the internal organs of fish. Copper is commonly a natural element in sediment and water. This metal is non soluble in aqueous media, while lots of its salts are really soluble. It is known that metals accumulate on sediment surface, planktonic organisms, in benthic living things and other living matter which is enhanced

through food chain. Fish can biomagnified toxic substances, particularly those which are least soluble in water because they are in close contact with the source that brings these toxic substances in suspension or solution form. Besides this, fish use water soluble oxygen by filtering the massive quantity of water through their gills. Kargin reported that, biomagnifications of metals in different organs of the fish is mainly concerned with different factors such as season, chemical, and physical properties of aquatic body [26].

Cooper determined that, industrial development, livestock manure, fertilizers, air pollution, mining and increase use of pesticides have led to increasing range of heavy metals in water body [10]. Nnabuife has pointed out that if all industrial wastes are not treated according to the WHO rule and regulations; they may pose several complications and issues in future [31, 41]. Exploitation of fresh water habitation may occur from any activity that changes the chemical and physical characteristics of a river or stream [11]. Roesijadi and Robinson mentioned that the heavy metals such as Zn and Cu accumulation pattern have shown much variation in the fish organs [35]. High level of Zn and Cu in metabolically active organs that is liver and kidney represented much accumulation due to strapping of these metals to metallothionine which acts as detoxifying agent. On the other hand metabolically less active tissues such as muscle showed low magnitude of accumulation. Zyadah and Chouiki demonstrated that the accumulation behavior of heavy metals copper, zinc, cadmium and lead concentration in flesh, gills and gonads of three commercial fish *Mullus barbarus, Merlucaus merlucaus and Boobs boobs* have been studied [48]. Zinc, Lead and Copper concentration were found to be low, while cadmium in gonads was found to be high. The concentration of copper in liver presented high level. On the other hand high concentration of cadmium was recorded in liver and gonads. Gills also revealed high levels of lead as compared to other fish organs. High accumulation of heavy metals was shown by the organs of *Boops boops* fish as compared to other fishes. Carvalho et al., studied that trace metals are the major hazardous and wide spread pollutants in aquatic environment and their effect on various components of environment particularly fish is a well recognized phenomenon [13]. Copper is an essential micronutrient in random rearrangement of natural proteins and enzyme activity. At somewhat higher but sub lethal concentrations, it causes persistent toxicity to aquatic life. Exposure of fishes to sub lethal concentrations of copper leads to low cardiac activity and reduction in heart rate [20].

Samir and Ibrahim have reported 2.80ppm of Cu in the muscle of fish, *Oreochromis niloticus* collected from the Northern Delta lakes of Egypt [37]. Previously Yilmaz has also reported 1.45 and1.29ppm of Cu in the muscles of *Mugil cephalus and Trachur mediterraneus* respectively collected from Iskenderm Bay Turkey [42]. While Olaif has recorded 0.05 and 0.07ppm of Cu in the muscles of *Clarias gariepinus* fish collected from Eleiyele Lake and Zartech pond in Ibandan, Nigeria respectively, this value is in accordance to our observed values [32]. Javed and Usmani have recorded high concentration of copper i.e. 22.7 1.9±3ppm in the muscle of fish, *Chana punctatus* [24]. While in the present study, mean value for accumulation of copper in muscle tissues was 0.003ppm.

Intestine appears to be the major organ for copper accumulation [6]. Previously, Ishaq et al., have reported 2.26ppm of copper in the intestine of fish *Clarias gariepinus* collected from Nigeria River [22]. Similarly Olaifa has recorded 0.16 and 0.21ppm of Cu in the intestine of *Clarias gariepinus* fish collected from Eleiyele Lake and Zartech pond in Ibandan, Nigeria respectively, which is similar to values observed during this study [33]. While in the present study, mean value for accumulation of copper in intestine tissues was 0.10ppm.

In the previous study Samir and Ibrahim have reported 4.24ppm of Cu in the liver of fish, *Oreochromis niloticus* collected from the Northern Delta lakes of Egypt which exceeds the values observed during our study [37]. Kalay et al., have reported high copper concentration in the liver of fish, *Mugil cephalus* [24].Similarly Ruelas and Osuna have recorded 48.6ppm of copper in the liver of sperm whale fish [34]. High concentration of copper i.e 2.54ppm was also recorded in the liver of leaping grey mullet, *Liza saliens* [19]. While in the present study, mean value for accumulation of copper in liver tissues was 0.03ppm.

Previously, Carvalho et al., have recorded high copper concentration in the skin of *Delphius delphius* collected from the Portugeese coast of the Atlantic Ocean [13]. Yilmaz has also reported 5.36 and 3.33ppm of Cu in the skin of *Mugil cephalus* and *Trachur mediterraneus* respectively collected from Iskenderm Bay Turkey [42]. In the past study Yousafzai has also represented high level of copper i.e. 76.02 ± 8.55ppm in the skin of *Tor putitora* collected from River Kabul [44]. While in the present study, mean value for accumulation of copper in skin tissues was 0.06ppm.

Ishaq et al., have reported 2.07ppm of copper in the gills of fish *Clarias gariepinus* collected from Nigeria River [22]. Olaifa has recorded 0.0125 and 0.012ppm of

Cu in the gills of *Clarias gariepinus* fish collected from Eleiyele Lake and Zartech pond in Ibandan, Nigeria, which is close to our observed values [33]. On the other hand, high concentration of copper i.e. 74.35 and 63.69ppm was found in the gills of fingerlings and adult *Mystus vittatus*. Similarly, Samir and Ibrahim have reported 154.43ppm of Cu in the gills of fish, *Oreochromis niloticus* collected from the Northern Delta lakes of Egypt [37]. In the previous study, Olaifa have reported 2.89ppm of copper in the gills of fishes Tilapia, *Oreochromis mossambicus* captured at a pond of Kelana Jaya [33]. While in the present study, mean value for accumulation of copper in gills tissues was 0.07ppm.

Copper can combine with other chemical components such as, zinc and mercury to produce an additive hazardous consequence to fish. Accumulation of heavy metals such as, nickel, cadmium, lead and copper was determined in the muscle tissue of *Catla catla* fish which were below detectable value during all the season of the study [9]. During monsoon, the higher level could be due to the influx of metals rich runoff, which may contain industrial, domestic, and agricultural waste. Goldstein and Weese pointed out that *Cyprinus carpio,* Common carp, were subjected for analysis of heavy metals like cadmium, arsenic, copper, lead, chromium, nickel, zinc and Selenium concentrations showed much differences among muscle, liver and whole body [21]. The concentrations of trace elements were the maximum in liver, while the whole bodies showed greater concentration than those in muscle for heavy metals such as nickel, lead, cadmium, zinc and copper. Jorgensen and Pedersen pointed out that several fishes are among the top consumers in the aquatic environment thus metals accumulation in fish tissue may acts as bio indicators to explain the circumstances of the environments [24]. The permissible limit for Cu set by WHO is 30ppm [41]. Similarly the U.S Recommended daily dietary Allowance for Cu supplied by a 100g of fish muscle is 2000-3000 µg, so the mean value of copper recorded in the present study falls within the permissible limits.

6. References

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