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Investigation of Selected Heavy Metals Among Tabbed Freshwater Species as Bioindicator of Freshwater Pollution in River Etai in Northern Pakistan

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Abstract

The Accumulation levels of heavy metals for intestine, muscle, gill and liver tissues of two freshwater fishes *Shizothorax Plagiostomus* and *Crossocheilus Diplocheilus* were investigated. The heavy metals such as Cobalt, Zinc, Nickel and Cadmium were determined through atomic absorption spectrophotometer. The aim of current study to evaluate heavy metal concentration in different organs of two freshwater fishes. The concentration of heavy metals is lowest in gills of both species. Metals accumulated in the body of *Schizothorax Plagiostomus* in order of Cd >Zn >Ni >Co. Metals concentration in various organs of *Schizothorax Plagiostomus* was intestine> liver> muscle> gills. Whereas, the concentration of heavy metals in different organs of *Crossocheilus Diplocheilus* was intestine> muscles> liver and gills. Similirly, the accumaltion pattern of heavy metals in the body of *Crossocheilus Diplocheilus* was Cd> NI> Zn> Co. The accumulation pattern of heavy metals was different in both species, while in both species Cd was highest and Co was less accumulated heavy metal. The current study has reported that the concentration of heavy metals Zn, Ni, Cd and Co are more in *Schizothorax Plagiostomus* as compared to *Crossocheilus Diplocheilus*.

Key words: Heavy metals, Freshwater fish, Bioaccumulation.

Introduction

The contamination of water reservoirs− particularly freshwater sources− through heavy metals is increasing because of the expansion of industries, agricultural practices and household wastes (H. Ali & Khan, 2019b). With lacking standardized management practices and pretreatment of water before the release into water bodies; this issue is becoming more severe with time (Wakejo *et al*., 2022). Fish is the direct host for such water and hence becomes the best indicator of aquatic pollution and determination of heavy metals in aquatic ecosystem (Rashed, 2001). So, to study the extent of harmful heavy metals in the body of fish is essential from environmental, public health and ecological point of view (H. Ali & Khan, 2018a). Heavy metals are classified into essential and non-essential metals. When on one side, the fractional concentration of heavy metals is lethal for fish body (Rehman et al., 2015); on other side some of the heavy metals are necessary for the metabolism (i.e. iron, Zn and copper are necessary for metabolism) (Joseph *et al.*, 2012), but for these; too, the optimum concentration is tolerable (Tüzen, 2003). Heavy metals enter into the body of fishes through gills, body surface and digestive tract. However, Gills are the main sites for uptake of heavy metals from aquatic environment (Ali *et al.*, 2014). For sustaining the process of metabolism these metals are taken up by fishes from sediments, food and ambient water (Ali *et al.*, 2014).. The problem arises with the fact that food intake sometimes is not selective. When fish take essential metals, the non-essential metals also taken up by the fish and are accumulated in the tissues (Dallinger *et al*., 1987). This bioaccumulation of heavy metals varies with certain factors including sex, size, life cycle, feeding behavior and capturing season of the fishes (Ullah, Hussain, Mahboob, & Al-Ghanim, 2016). When human consume these contaminated fish which may have a chronic toxic effects (Gale, Adams, Wixson, Loftin, & Huang, 2004). Heavy metals affect the growth rates, reproduction and promotes histopathological changes in fishes and may finally lead to mass mortalities of fishes (Amundsen et al., 1997). (Ahmad, Azizullah, Shama, & Khattak, 2014). Different non-essential heavy metals have different lethal effects on bodies of fishes. The lethality of the heavy metals which we have chosen for our study− Cadmium (Cd), Zinc (Zn), Cobalt (Co) and Nickel (Ni) have been reported in the previous studies. It has been reported in previous studies that Cd has a cumulative polluting effect and could cause serious disturbances in fish metabolism such as abnormal behavior, locomotor anomalies or anorexia (Woo et al., 1994; Bryan et al., 1995). Cadmium may also affect the blood cells (Witeska, 1998). Similarly, Zn accumulation in higher concentration affects growth and survival of fish. The Zn also affect the fish behavior resulting in restless swimming, air guzzling, periods of dormancy and death (Kori-Siakpere & Ubogu, 2008). Cobalt, when present in elevated concentrations in aquatic ecosystems, poses significant risks to fishes and their ecosystems. Cobalt can disrupt fish physiology and impair their growth, reproduction, and overall health. It can accumulate in fish tissues, leading to bioaccumulation up the food chain, ultimately posing a risk to human consumers who consume contaminated fish. Additionally, Cobalt can interfere with enzymatic processes and disrupt the functioning of vital organs, leading to adverse health effects in fish populations (Sfakianakis et al., 2015). Excess intake of nickel causes morphological transformations in numerous cellular systems and chromosomal aberrations (Coen et al., 2001). Nickel and Chromium are the cause of different pulmonary diseases. Intake of copper in high amount cause liver and kidney disorder (Forti et al., 2011). Previously from Pakistan many studies have described the isolation and effect of heavy metals from fresh water bodies like studies of; Ahmed *et al*., 2021, Afzaal *et al.*, 2022, Ali *et al.*, 2020 and Khan *et al.*, 2018. However, none of the studies; to the best of our knowledge, have worked on river Etai in northern Pakistan. The two species were chosen because of their economic importance in the area as a preferred nutrition. Whereas, the studied heavy metals were chosen on the basis of their suspected concentration in research region as these are the effluents of domestic wastes and agricultural run-off. River Etai is a tributary of river Indus and is unexplored in the recent times. Domestic wastes and agriculture effluents are discharged to this river directly or through runoff. Hence, we aimed to study the concentration of the heavy metals in the bodies of fishes, so to estimate its lethality and pollution in the river.

Materials and Methods

Study Area

The study was conducted in the River Etai, a tributary of the River Indus, situated in District Shangla, Northern Pakistan. The geographical coordinates of the study area were approximately 34°10'N to 33°08'N latitude and 72°33'E to 73°01'E longitude. River Etai covers an area of approximately 1,586 square kilometers and is situated at an elevation of 3,164 meters above sea level (Navid et al., 2017). During the months of December, January and February the weather of Shangla is too cold because of heavy snow fall. In July and August weather is moderate and pleasant. The temperature in summer remains between 17 c^o to 30 c^o while in winter the temperature ranges from 0c^o to 20c^o.

Sample Collection

Adult *Schizothorax Plagiostom*us and *Crossocheilus Diplocheilus* species were collected using hand nets from River Etai between May and July. Weekly visits to the sampling site were conducted throughout the study period. Fish of the same size were selected for the dissection process. They were carefully washed with distilled water and dissected to extract specific tissues, including the intestine, gills, liver, and muscle

Sample Preparation

Tissue Collection: A one-gram sample was collected from each tissue (intestine, gills, liver, and muscle) and transferred to separate volumetric flasks (100 mL).

Rinsing: The tissue samples were soaked and rinsed with distilled water to remove any external contaminants.

Drying: After rinsing, the samples were blotted with a blotting factor and dried at 60°C for a few minutes in an oven.

Digestion: Each dried tissue sample was transferred to a volumetric flask, and 55% nitric acid in a ratio of 1 mL per 1 g of chlorine (70%) was added to each flask. The flasks were then covered with aluminum foil and incubated for 12 hours at room temperature.

Heating: After incubation, the flasks were heated to 200-250°C to allow for digestion. The tissue samples were digested for the examination of heavy metals such as Cd, Zn, Co and Ni. A clear solution was obtained after complete digestion. The completion of digestion was indicated by the conversion of brown fumes into dense white fumes.

Dilution: Prior to analysis, the samples were cooled down and diluted with 10 mL of distilled water. The resulting solutions were stored in perfectly washed plastic bottles for further analysis. All the tissue samples stored in bottles were transported to centralized research laboratory in University of Peshawar for the detection of heavy metals.

Heavy Metal Analysis

For the determination of concentration of heavy metals in the muscle, skin, liver and intestine of fish, atomic absorption Spectrophotometer (Spectra-AA-700) was used. We assessed and made comparison of the mean concentration of Cd, Zn, Co and Ni in muscles, gills, liver and intestine of the *Schizothorax Plagiostomus* and *Crossocheilus Diplocheilus.*

Data Analysis

Descriptive statistics, including means and standard deviations, were calculated to present the results of heavy metal concentrations in the muscles, gills, liver, and intestine of *Schizothorax Plagiostomus* and *Crossocheilus Diplocheilus*. Data analysis was performed using Microsoft

Excel for MS Word version 2010 (a word processing software developed by Microsoft Corporation)

Results

The concentration of heavy metals such as zinc (Zn), cadmium (Cd), nickel (Ni) and Cobalt (Co) were studied in the muscle, gills, intestine and liver of two freshwater fishes *Schizothorax Plagiotomus* and *Crossocheilus Diplochilus* and are presented in (mg/L).

Concentration of Heavy Metals in Different Tissues of *Schizothorax Plagiostomus*:

Cadmium (Cd) Concentrations (mg/L):

Cadmium concentrations were measured in the gills, muscle, liver, and intestine of *Schizothorax Plagiostomus*. The results, presented in Table 1, indicate that Cd concentrations varied among the different tissues. In the gills, muscles, liver and intestine the mean Cd concentration was found to be 0.05 mg/L ± 0.01, 0.05±0.04, 0.05 mg/L ± 0.05 and 0.36 mg/L ± 0.47 respectively.

Zinc (Zn) Concentrations (mg/L):

The result showed that zinc concentrations in the examined tissues varies, indicating potential differences in the bioaccumulation of zinc. The mean concentration of the zinc (Zn) found in gills of *Schizothorax Plagiostomus* was 0.70 mg/L ± 0.11. While the Zn concentration was different in muscles, which had a mean value of 0.88 mg/L ± 0.12. In other tissues such as liver and intestine the Zn concentration was found to be 0.95 mg/L ± 0.25 and 1.31 mg/L ± 0.38 respectively.

Cobalt (Co) Concentrations (mg/L):

Table 1 presents the Cobalt (Co) concentrations in the tissues of *Schizothorax Plagiostomus* Cobalt (Co) concentrations were below the detection limit (0 mg/L) in all examined tissues, including the gills, muscle, liver, and intestine. These findings suggest that Cobalt was not detected in the analyzed tissues.

Nickel (Ni) Concentrations (mg/L):

Nickel (Ni) concentrations were examined in the tissues of *Schizothorax Plagiostomus* and are presented in **Table 1** which indicate variations in nickel concentrations across different tissues. In the gills, Ni concentrations had a mean concentration of 0.01 mg/L ± 0.02. Muscle tissue exhibited mean concentration of Ni as 0.06 mg/L ± 0.01. In the liver tissue, mean Ni concentrations was found to be of 0.01 mg/L ± 0.01. The intestine displayed Ni concentrations of 0.20 mg/L ± 0.12.

Concentration of Heavy Metals in *Crossocheilus Diplocheilus* Tissues:

The concentrations of Cadmium (Cd), Zinc (Zn), Cobalt (Co), and Nickel (Ni) in various tissues of *Crossocheilus diplocheilus* were examined to assess potential heavy metal contamination in the River Etai ecosystem. The tissues analyzed included gills, muscle, liver, and intestine.

Cadmium (Cd) Concentrations (mg/L):

Cadmium levels were assessed across various tissues (gills, muscle, liver, and intestine) in *Crossocheilus diplocheilus*. The data, displayed in **Table 2**, reveals variability in Cd concentrations among these tissue types. The mean Cd concentration in the gills, muscles, liver, and intestine were determined to be 0.05±0.03, 0.06±0.01, 0.06±0.08 and 0.07±0.02 respectively.

Zinc (Zn) Concentrations (mg/L):
The results indicated varying zinc concentrations in the examined tissues. The gills of *Crossocheilus diplocheilus* had an average zinc (Zn) concentration of 0.55±0.17, while the muscles had a different zinc concentration with an average of 0.66±0.15. Additionally, the zinc concentration in other tissues, such as the liver and intestine, was measured as 0.49±0.21 and1.02±0.29, respectively.

Cobalt (Co) Concentrations (mg/L):
In **Table 2**, results for Cobalt (Co) concentrations are displayed, within the tissues of *Crossocheilus diplocheilus*. Interestingly, all examined tissues, including the gills, muscles, liver, and intestine, exhibited Cobalt (Co) concentrations that were consistently below the detection limit (0 mg/L). These findings strongly indicate the absence of detectable Cobalt within the analyzed tissues.

Nickel (Ni) Concentrations (mg/L):
Nickel (Ni) levels were analyzed in *Crossocheilus diplocheilus* tissues and are summarized in **Table 2**, revealing differences in nickel concentrations among various tissue. In the gills, the average Ni concentration was 0.02±0.01. Muscle tissue exhibited a mean Ni concentration of 0.02±0.05. In the liver tissue, the mean Ni concentration was determined to be 0.04±0.01. The intestine displayed Ni concentrations of 0.15±0.12.

Discussion
For assessing the contamination of water due to effluents from industrialization, agricultural runoff and household drain we investigated the heavy metals accumulation in fish body in river Etai of district Shangla in Northern Pakistan. To the best of our knowledge we are the first to conduct such study on river Etai. We looked at and compared two freshwater fish species for this purpose; *Crossocheilus diplocheilus* and *Schizothorax plagiostomus*. Heavy metals such as Co, Ni, Zn and Cd were examined in following organs; muscles, gills, intestine and liver. All these heavy metals in both the species were recorded within the permissible range according to different health and food agencies like Canadian standard for Zn is 100µg/g, USEPA standard for Ni is 1µg/g and EU/EC standard for Chromium is 0.50mg/L , WHO standard for Cd (5 µg/L) (Ahmad et al., 2014). Bioaccumulation of heavy metals in fish is influenced by a variety of parameters such as eating habits, ambient temperature, water hardness, pH, salinity, age, sex and metal interactions (Hakanson, 1980). Although, we have not considered and studied heavy metals in association with these factors, but our results provide a useful indicator of these factors. In general, all over the world the main source of heavy metals pollution is municipal and industrial effluents (Oguzie & Okhagbuzo, 2010).

Cadmium (Cd):
In our study the observed Cd concentration in the gills of *Schizothorax Plagiostomus* was 0.05±0.01 mg/L. Cd concentration in other tissues like muscles, liver and intestine was observed to be 0.05±0.04 mg/L, 0.05±0.05 and 0.36±0.47 mg/L respectively. Similarly, in *Crossocheilus Diplocheilus*, the concentration of Cd in gills was 0.55±0.17 mg/L, in muscles 0.06±0.01 mg/L, in liver 0.06±0.08 mg/L while intestine had 0.07±0.02 mg/L. These findings show some differences with the earlier studies, like Filazi *et al.,* 2003; Sthanadar *et al.,* 2013 and Zhang *et al.,* 2013; Ambedkar and Muniyan, 2013). Filazi, Baskaya, Kum, & Hismiogullari, 2003 conducted their study in Turkey and recorded the high level of Cd in liver and muscles tissues of Mugil Auralus as 0.15 /0.50 µg/g and 0.10 /0.40 µg/g respectively. Sthanadar, Sthanada, Yousaf, Muhammad, *&* Zahid, *2013* reported Cd in the liver of Wallago Attua was 3.33±1.3595 mg/L. Zhang et al., 2007 reported high amount of Cd in the intestine of *Hemibarbus Labeo* with mean value 0.081µg/g. Ambedkar & Muniyan, 2011 reported higher concentration of Cd in the intestine of *Tilapia Mossambica* 0.88 + 0.01 mg/L. The

concentration of heavy metals in fish tissues were high in these studies because of industrial effluents, plastic industries, domestic effluents and anthropogenic activities. In the current study the amount of Cd was lower than other studies because in our study area there is no industries and factories, so the main sources of heavy metals are mining activities and domestic effluents.

Zinc (Zn):

Zinc (Zn) plays a vital role in the physiology of fish and is absorbed from the aquatic environment primarily through their gills, where it enters their bloodstream. Once in circulation, zinc can be distributed to various organs and tissues, with the liver acting as a primary storage site. In the liver, zinc may be involved in detoxification processes, aiding in the removal of harmful substances from the fish's body. While zinc can accumulate to some extent in fish muscles, its concentration there is usually lower than in the liver. Muscles may utilize zinc in metabolic processes and cellular functions. Zinc's importance extends to enzyme function, immune system support, and DNA synthesis in fish. Excess or unneeded zinc is excreted from the body, primarily through feces and urine, with some reabsorption potential in the intestines. The specifics of zinc uptake, distribution, and utilization can vary among fish species and are influenced by environmental factors. Monitoring and managing zinc levels in aquatic ecosystems are critical to ensuring the health and well-being of fish populations (Moore et al., 1988). The result revealed that concentration of Zn was found in abundance in all of the organs of *Schizothorax Plagiostomus*. The content of zinc was higher in all the organs in both species, the concentration of Zn in gills of *Schizothorax Plagiostomus* was 0.70±0.1 and in muscles, liver and intestine the concentration of zinc was observed to be , 0.88±0.12 mg/L, 0.95±0.25 mg/L, 1.31±0.38 mg/L respectively. Whereas, in *Crossocheilus Diplocheilus* the concentration of Zn in gills was 0.55±0.17mg/L, however, Zn concentration in muscles, liver and intestine was observed as 0.66±0.15 mg/L, 0.49±0.21 mg/L and 1.02±0.29 mg/L respectively. These results are in line with some other studies like; Annune and lyaniwura *et al*., 1993; Rasheed, 2001; Marcovecchio, 2004; Zhang *et al.,* 2007 and Sthandar *et al.,* 2013; Ali et al., 2017 who reported higher concentration of Zinc in their studies. Annune & Iyaniwura, 1994 conducted their study in Nigeria and found higher concentration of Zn in the liver of *Oreochromis Niloticus* and *Clarias Gariepinus*. They recorded the concentration of Zn in gills, muscles and liver as; 38.51 ± 2.3, 15.42 ± 1.3 and 80.56 ±2.05 respectively. Rashed, 2001 recorded high level of Zn in the liver of *Talapi Nilotica* fish collected from Naseer lake in Egypt. Marcovecchio, 2004 recorded higher concentration of Zn in the muscles and liver of *Mugil Liza* as 20.5 µg/g and 44.3 µg/g respectively. Similarly, Zhang, He, Li, & Wu, 2007 reported higher concentration of Zn in the intestine 6.222µg/g and muscles 5.907µg/g of *Hemiculter nigromarginis*. A. A. Sthanadar et al., 2013 had recorded Zn in the liver of *Wallago Attu* with mean value 0.72± 0.1937 mg/L. H. Ali et al., 2017 have reported higher Zinc concentration in *Schizothorax Plagiostomus* muscles which has been isolated from river Barandu, river Panjkora and river Swat. The authors have reported the zinc concentrations in muscle of *Schizothorax Plagiostomus* in order of 10.17 ± 4.79 to 18.00 ± 1.88 mg kg^{-1}, 2.72 ± 2.36 to 10.32 ± 2.99 mg kg^{-1}, and 7.53 ± 3.48 to 12.80 ± 1.08 mg $kg^{-1}$for river Barandu, river Panjkora and river Swat. The concentration of Zn was higher in this study because of agriculture wastes having chemical fertilizer and biocides. The concentration of heavy metals in these previous studies were high than that of present study this is due to the contamination of these reservoir from the discharge of industrial effluents, agriculture runoff, wastes from anthropogenic activities and domestic wastes. Moreover, some studies also exists which had revealed lower concentration of Zn than our findings also exists, like study of Shah et al., 2021. They have reported 0.013 ± 0.01, 0.55 ± 0.24, and 0.08 ± 0.00 mg/L concentration of Zinc in the muscle of *Schizothorax Plagiostomus* at Charbagh, Odigram, and Landakai point in River swat, which is lower than the current results. They analyzed Zn concentration in *Schizothorax Plagiostomus* in three different points of the river swat, which is less polluted as compared to our study area (Shah et al., 2021).

Cobalt (Co):

Our study revealed 0.06±0.06 mg/L concentration of Co in intestine, while no concentration of Co has been observed in liver, muscles and gills of *Schizothorax Plagiostomus*. Similarly, no concentration of Co was observed in any organ of *Crossocheilus Diplocheilus*. In our study no concentration of Co was observed, except in the intestine of *Schizothorax Plagiostomus*.These results are in contrast to some earlier results, like study of Swaibuh Lwanga, Kansiime, Denny, & Scullion, 2003. They studied Co in fish collected from Lake George, Uganda. The concentration of Co in *O. Niloticus* and *O. Leucostictus* were 11.2 and 7.9 mg/L respectively. The concentration of Co is higher in their area as compared to ours. Our study area had no industries while they have high Co concentration because of mining activities and industrial effluents. These industrial effluents directly flow into water without any pre-treatment, due to these industrial effluents the concentration of heavy metals was higher. Although, to the best of our knowledge, there is not a single study has been reported on bioaccumulation of cobalt in both *Schizothorax Plagiostomus* and *Crossocheilus Diplocheilus.*

Nickel (Ni):

The observed concentration of Ni in gills of *Schizothorax Plagiostomus* was 0.01±0.02 mg/L. In other tissues like muscles, liver in intestine of the *Schizothorax Plagiostomus* had Ni mean concentration of 0.06±0.01 mg/L, liver 0.01±0.01 mg/L and 0.20±0.12 mg/L respectively. Whereas, in *Crossocheilus Diplocheilus* the concentration of Ni in gills, muscles, liver and intestine was observed to be 0.02±0.01 mg/L, 0.02±0.04792 mg/L, 0.04±0.01 mg/L and 0.15±0.12 mg/L respectively. These findings are different from the other studies of Vinodhini and Narayanan, 2008; Yousafzai *et al.,* 2010; Jabeen *et al.,* 2012 and Siraj *et al.,* 2014.Vinodhini & Narayanan, 2008 have conducted experiment on *Cyprinus Carpio* and reported 3.17 ± 0.075 mg/L and 3.750 ± 0.026 mg/L concentration of Ni in gills and liver, respectively. Similarly, Yousafzai, Chivers, Khan, Ahmad, & Siraj, 2010 conducted their study on *Wallago Attu* and *Labeo Dyocheilus*, which was collected from river Kabul, near Peshawar and reported the concentration of Ni in the muscles, liver, gills and intestine in the order of 106.7±6.8, 108.0±19.9 mg/L, 122.7±57.1 mg/L, 95.3±10.4 mg/L and 117.7±33.5 mg/L, 111.7±21.4 mg/L, 152.0±100.5 mg/L and 383.7±108.2 mg/L, respectively. Jabeen, Javed, & Azmat, 2012 studied the Ni concentration in both carnivore and herbivore fish collected from river Ravi. The concentrations of Ni in carnivore and herbivore fish were; 6.31±1.48 and 9.07±1.35 µg/g, respectively. The Ni concentrations in the muscles, liver, gills and intestine of *Aorichthys Seenghala* was studied by Siraj et al., 2014 which was in order of 94.7±33.3, 117.7±13.3, 64.7±12.3 and 118.3±21.5 µg/g. However, the concentration of Ni in these studies were higher in mentioned species as compared to the current study. Because, in these areas the industrial effluents, domestic wastes and waste of anthropogenic activities directly discharge into the river without any pretreatments (H. Ali & Khan, 2019a). In our study area there is no industries and the source of Ni in our research region is Chemical and physical degradations of rocks and soil, natural deposition of nickel-containing particles, and urban wastes. Herein, the comparatively study of heavy metals accumulation in both species have been given. In our study, high concentration of Zinc 1.31±0.38 mg/L in the intestine of *Schizothorax Plagiostomus* has been reported as compared to *Crossocheilus Diplocheilus* 1.02±0.29 mg/L. Also, the zinc concentration in liver, muscle and gills in order of 0.95±0.25 mg/L, 0.88±0.12 mg/L, 0.70±0.11 mg/L, of *Schizothorax Plagiostomus* were high than Zinc concentration in above organs of *Crossocheilus Diplocheilus,* such as muscles 0.66±0.15 mg/L, gills 0.55±0.17mg/L and liver 0.49±0.21 mg/L. The high concentration of zinc may be due to their differences in feeding habits of both *Schizothorax Plagiostomus and Crossocheilus Diplocheilus.* Similarly, the Cd concentration 0.36±0.47 mg/L in the intestine of *Schizothorax Plagiostomus* was high than that of *Crossocheilus Diplocheilus,* which was 0.07 ± 0.02 mg/L. On the other hand, higher concertation of Cd in gills 0.55±0.17 mg/L, muscle 0.06±0.01 mg/L and liver 0.06±0.08 mg/L of *Crossocheilus Diplocheilus* are reported, as compared to *Schizothorax Plagiostomus*, gills 0.05±0.01 mg/L, liver 0.05±0.05 mg/L and muscles of 0.05±0.04 mg/L. In the result of Ni concentration we found high concentration of Ni in intestine and muscles of *Schizothorax Plagiostomus* than *Crossocheilus Diplocheilus*. The Nickel concentration was 0.20±0.12 mg/L in intestine and muscles 0.06±0.01 mg/L of *Schizothorax Plagiostomus*. Although, *Crossocheilus Diplocheilus* species have lower concentration of nickel in both intestine and muscle in order of 0.15±0.12 mg/L and 0.02±0.04792 mg/L. Furthermore, high nickel concentration observed in the gills 0.02±0.01 mg/L and liver 0.04±0.01 mg/L of *Crossocheilus Diplocheilus* that of Ni concentration in the gills and liver of *Schizothorax Plagiostomus* 0.01±0.02 mg/L for both gills and liver.

Conclusion

The current study was conducted for the comparison of heavy metals (Zn, Ni, Cd and Co) in gills, muscle, intestine and liver in *Schizothorax Plagiostomus* and *Crossocheilus Diplocheilus* caught from river Etai district Shangla. During observation of metals load from river Etai showed that *Schizothorax Plagiostomus* accumulated considerably high amount of heavy metals as compared to *Crossocheilus Diplocheilus.* Our study also showed that higher concentration of Zn, Ni, Cd and Co were accumulated in the intestine of both species. Because the intestine is the most active organ in digestive system of both species. Similarly, the concentration of cobalt was not observed in any organ of *Crossocheilus Diplocheilus* except the intestine of *Schizothorax Plagiostomus*.

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Table1. Concentration of Heavy Metals indifferent Tissues of *Schizothorax Plagiostomus*

Organ	Cd (mg/L)				Zn (mg/L)				Co (mg/L)				Ni (mg/L)			
	S1	S2	S3	Mean (mg/L) ± SD	S1	S2	S3	Mean (mg/L) ± SD	S1	S2	S3	Mean (mg/L) ± SD	S1	S2	S3	Mean (mg/L) ± SD
Gills	0.042	0.053	0.066	0.05±0.01	0.642	0.830	0.630	0.70±0.11	**0**	**0**	**0**	0	0.005	0.031	**0**	**0.01±0.02**
Muscle	0.054	0.051	0.045	0.05±0.04	0.862	0.774	1.029	0.88±0.12	**0**	**0**	**0**	0	0.019	**0**	**0**	**0.06±0.01**
Liver	0.052	0.060	0.053	0.05±0.05	0.657	1.096	1.111	0.95±0.25	**0**	**0**	**0**	0	0.004	**0**	0.028	**0.01±0.01**
Intestine	0.123	0.091	0.053	0.36±0.47	1.396	1.655	0.904	1.31±0.38	**0**	0.005	0.014	0.06±0.06	0.325	0.215	0.085	**0.20±0.12**

Organ	Cd (mg/L)				Zn (mg/L)				Co (mg/L)				Ni (mg/L)			
	S1	S2	S3	Mean (mg/L) ± SD	S1	S2	S3	Mean (mg/L) ± SD	S1	S2	S3		S1	S2	S3	Mean (mg/L) ± SD
Gills	0.063	0.058	0.057	0.05±0.03	0.702	0.608	0.362	0.55±0.17	0	0	0		0.040	0.036	0.010	0.02±0.01
Muscle	0.050	0.070	0.061	0.06±0.01	0.570	0.566	0.844	0.66±0.15	0	0	0		0	0.083	0	0.02±0.05
Liver	0.054	0.067	0.071	0.06±0.08	0.721	0.293	0	0.49±0.21	0	0	0		0.028	0.056	0.038	0.04±0.01
Intestine	0.048	0.074	0.088	0.07±0.02	0.793	0.928	0	1.02±0.29	0	0	0		0.039	0.134	0.282	0.15±0.12

Table 2. Concentration of Heavy Metals indifferent Tissues of Schizothorax diplocheilus