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Original Article

Bioaccumulation of heavy metals in the tissues of Schizothorax plagiostomus at River Swat

Bioacumulação de metais pesados nos tecidos de Plagiostomo esquizotórax em River Swat

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Abstract

Snow trout (*Schizothorax plagiostomus*) is an economically important freshwater fish, mostly found in northern areas of water reservoirs of Pakistan. The current study was conducted in River Swat to analyze the bioaccumulation of heavy metals (Pb, Cr, Ni, and Zn) in tissues of *Schizothorax plagiostomus*. Tissues were extracted and dissolved in perchloric acid ($HClO_4$) and nitric acid (HNO_3) along with hotplate. The heavy metals, zinc (Zn), lead (Pb), chromium (Cr), and Nickel (Ni) were determined using Perkin Elmer 2380 atomic absorption spectrophotometer. Results shows great variation in the content of the metal related to tissue type and sampling sites. A high concentration of bioaccumulation was reported at Charbagh, whereas lowest at Odigram: Charbagh>Landakai>Odigram. In the same way, Cr was the most accumulated heavy metal followed by lead, nickel, and Zinc:

Keywords: *Schizothorax plagiostomus,* heavy metals, River Swat.

Resumo

A truta das neves (*Schizothorax plagiostomus*) é um peixe de água doce economicamente importante, encontrado principalmente nas áreas ao norte de reservatórios de água do Paquistão. O presente estudo foi realizado em River Swat para analisar a bioacumulação de metais pesados (Pb, Cr, Ni e Zn) em tecidos de *Schizothorax plagiostomus*. Os tecidos foram extraídos e dissolvidos em ácido perclórico ($HClO_4$) e ácido nítrico (HNO_3) com placa de aquecimento. Os metais pesados zinco (Zn), chumbo (Pb), cromo (Cr) e níquel (Ni) foram determinados usando espectrofotômetro de absorção atômica Perkin Elmer 2380. Os resultados mostram grande variação no conteúdo do metal relacionado ao tipo de tecido e locais de amostragem. Uma alta concentração de bioacumulação foi relatada em Charbagh, enquanto a mais baixa, em Odigram: Charbagh > Landakai > Odigram. Da mesma forma, Cr foi o metal pesado mais acumulado, seguido por Pb, Ni e Zn.

Palavras-chave: *Schizothorax plagiostomus*, metais pesados, River Swat.

1. Introduction

Over the past few decades, it is a matter of concern that aquatic resources are at a higher risk of contamination through various contaminants. Such contaminants can seriously affect the ecological balance and diversity of aquatic fauna. Some of these contaminants not only providing harm to aquatic life but also having indirect adverse effects on human life which lead to cause water pollution (Sthanadar *et al*., 2013). In Pakistan, it has been reported that water pollution is increasing day by day, which may be due to the discharge of untreated industrial and domestic wastes into the river and streams. Reportedly only 1% of the industrial wastes in Pakistan are managed before it has

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been discharged to the water resources (Khan et al., 2012). Such contaminants are various in natures in which one of major concern are heavy metals (Batool et al., 2014).

Heavy metals having high density and toxic at low levels above the permissible limit. These metals are potentially toxic, indestructible, and can bio-accumulate in fish body and make them contaminant which in turn shows adverse effects on human health by using seafoods (Khan et al., 2012). Heavy metals include both essential and non-essential metals. Lead, arsenic, cadmium, mercury, chromium are non-essential heavy metals and are toxic at any amount they are present, on the other hand, heavy metals such as nickel, copper, silver, iron, and zinc are essential for the normal metabolism of the body but if their level exceeds from the optimum range, it would also lead to toxicity. These metals are released to the aquatic ecosystem from both natural and anthropogenic sources such as industrialization, soil erosion, agriculture, mining, and discharge of untreated wastewater (Batool et al., 2014).

Bioaccumulation of heavy metals takes place when the rate of ingestion is comparatively high to the rate of egestion (Yousafzai, et al., 2014). Bioaccumulation of these metals adversely affects the liver, muscle, kidney, and other tissues of fish, disturb metabolism and hamper the development and growth of fish (Korai et al., 2008). Heavy metals accumulate in the tissues of the aquatic organisms along the food chain which initiate from aquatic plants and later to the primary consumers and then to secondary and tertiary consumers. Heavy metal concentration in aquatic ecosystems is usually monitored by measuring their concentration in water sediments and biota which generally exist in low levels in water and attain considerable concentration in sediments and biota (Ozturk et al., 2009).

In fishes, waterborne chemicals are mostly accidentally absorbed through gills, skin, and digestive tract. Such absorbed chemicals are then transported by the blood to their respective sites which may be the primary storage site i.e. blood itself or liver where they stay or maybe further transported to other organs such as gills, liver, muscles, kidneys, and gonads (Ullah et al., 2018; Ullah et al., 2019). The concentrations of heavy metals are high in the gills and liver as compared to muscles and gonads (Khan et al., 2012). In general terms, stress is a non-specific response of a body-altering the normal homeostasis of the body. Many factors are responsible for stress that include living factors, non-living factors, and some anthropogenic activities associated with fish rearing and harvesting are also involved (Afridi et al., 2019). The living factors involved in stress are predator, parasitism, and non-living factors associated with stress are temperature, pH, O_2 concentration, and toxic chemicals including heavy metals (Ullah et al., 2016). Prolong exposure to stress result in certain sorts of physiological changes including alteration in blood composition and immune mechanisms (Ullah and Zorriehzahra, 2015). Certain data reveals that intoxication of fish with heavy metals may sometimes cause symptoms similar to that of stress, RBC level may decrease while dealing with heavy metals intoxication but after short exposure, they may increase. Heavy metals decrease WBC count especially of lymphocytes, prolong exposure to heavy metals also causes increase cortisol level which is directly associated with a decrease of WBC particularly lymphocytes and their activity (Witeska, 2005).

Heavy metals also having adverse effects on the nervous system of fishes and are considered as neurotoxins. Heavy metals provoke several disorders mainly on biochemical levels, thus increased lipid peroxidation and depletion in total lipid amount, it would also effect fish behavior, growth, and normal metabolisms of the body (Atchison et al., 1987). Heavy metals also effect embryonic development of fishes because they can also accumulate in the gonads and drastically result in the quantity and quality of gametes. Fish eggshell has not enough power of penetration to show resistance to the entrance of heavy metals. These accumulated heavy metals also result in the delay of hatching, premature hatching, some structural deformations, and even in the death of the hatched larvae.

1.1. Aims and objectives

- To assess the concentration of heavy metal in *Schizothorax plagiostomus*.
- To study the relative bioaccumulation of Pb, Cr, Ni, and Zn.
- To know about the comparative abundance of Pb, Cr, Ni, and Zn in the gills and muscles tissues.

2. Material and Method

2.1. Study area

District Swat is a beautiful valley of Khyber Pakhtunkhwa (Figure 1), which lies between 34° 34" and 35° 55" north latitudes and 72° 08" and 72° 50" east longitudes, surrounded by Chitral and Ghizer on the north, Indus Kohistan and Shangla on the east, Buner and Malakand are situated on to south and Dir protected it from the west and located in the lap of mountainous ranges, which are the offshoots of Hindukush so the larger part of Swat is covered with mountains and hills) (Akhtar et al., 2014). It has the total area of the district is 5337 Km square. The Swat River is perennial in KP Pakistan. The river originates from the Swat Kohistan region of Kalam with the confluence of two main tributaries (Ushu and Gabral) and runs downstream in a narrow gorge up to Baghdheri. Its source lies in the Hindukush Mountains, from where it is fed by the glacial waters throughout the year and flows through the Kalam Valley in a narrow gorge with a rushing speed up to Madyan and lower plain areas of Swat Valley up to Chakdara for 160 km. In the extreme south of the valley, once again the river enters a narrow gorge and joins River Panjkora at Qalangi and finally empties into Kabul River near Charsadda.

2.2. Sample collection

Schizothorax plagiostomus samples were collected from three different sites i.e. Charbagh, Odigram, and Landakai of River Swat. Samples were collected in a triplicate manner from each site with the help of a local lobsterman by using a gills net with specific mesh size. The samples were preserved in 10% formalin.

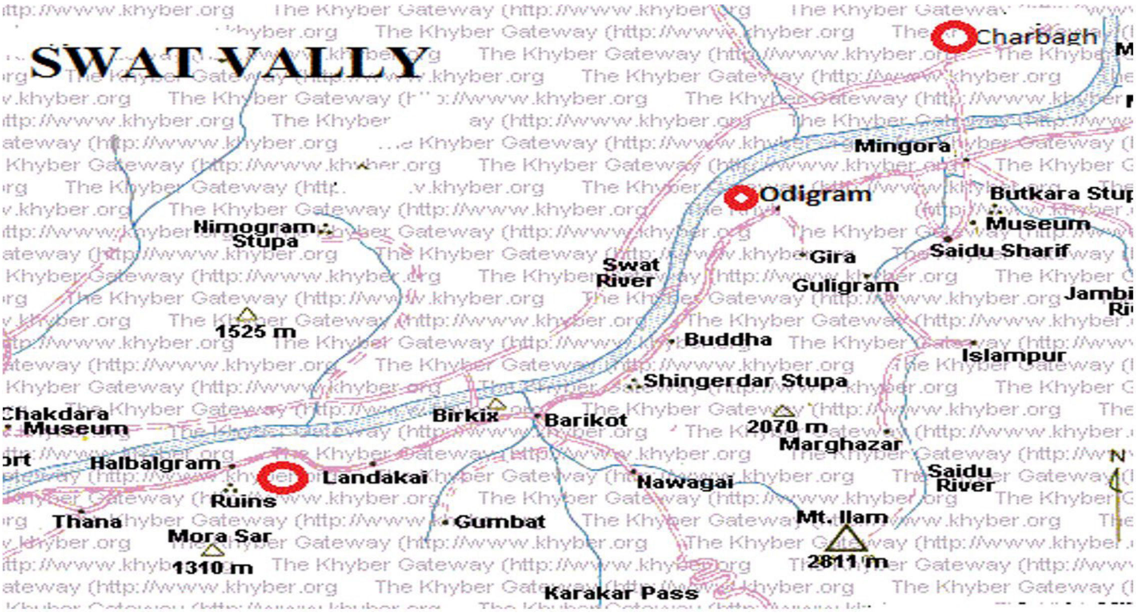

Figure 1. Sampling sites: Charbagh, Odigram, and Landakai of River Swat (Google map, 2017).

2.3. Fish identification

Fish samples were identified with the help of taxonomic keys i.e. Talwar and Jhingran (1991) and Mirza and Sandhu (2007).

2.4. Dissection

The same size of fishes was selected for dissection. The fish samples were washed with distilled water and dissected to extract muscle and gill tissues. One gram (1gm) of each tissue was and shifted to volumetric flasks (Figures 2).

2.5. Tissue dissolution

The extracted tissues were digested to analyze the concentration of heavy metals i.e. lead (Pb), chromium (Cr), nickel (Ni), and zinc (Zn). First of all, the samples were thawed and rinsed in distilled water, and then samples were blotted properly with blotting paper. 100 ml volumetric flasks were washed with distilled water and then, flasks were dried in an oven at 600C for a few minutes. Samples were then shifted to these volumetric flasks. Digestion was carried out by adding 5 ml nitric acid (55%) and 1 ml perchloric acid (70%) to each flask. Then the flasks were covered up and placed it for 12 hours at room temperature, after this, the flasks were then placed on a hot plate and were allowed to digest at 200-250 C until a transparent and clear solution was obtained. The conversion of brown fumes to the dense white fume from the flask was the indication of digestion completion. Samples were then cooled after digestion. The digested samples were diluted to 50 ml with distilled water appropriately in the range of standards that were prepared from the stock standard solution of the metals. Properly washed glass bottles were used for storing samples until the metal concentration was determined and noted with care.

Figure 2. The collected samples of the *Schizothorax plagiostomus* species from Charbagh, Odigram and Landakai of River Swat.

2.6. Atomic absorption spectrophotometer

Perkin-Elmer 2380 atomic absorption spectrophotometer was used for the analysis of heavy metals like zinc, lead, chromium, and nickel present in the extracted tissues of muscles and gills. Air acetylene was used as a fuel for the flame. For analysis of different heavy metals, the atomic absorption was set up for each metal by giving that metal wavelength and standard (Figures 3).

3. Results

The current study was conducted to analyze the bioaccumulation pattern of heavy metals i.e. zinc (Zn), lead (Pb), chromium (Cr), and nickel (Ni) in the different tissues such as the muscles and gills of a freshwater fish *Schizothorax plagiostomus* at River Swat. The samples were collected from three different sites of River Swat such as "Charbagh, Odigram, and Landakai". The concentration Cr was highest in muscles and gills. The concentration of metals in the above

Figure 3. Atomic absorption spectrophotometer used for the analysis of heavy metals i.e zinc, lead, chromium and nickel present in the extracted tissues of muscles and gills.

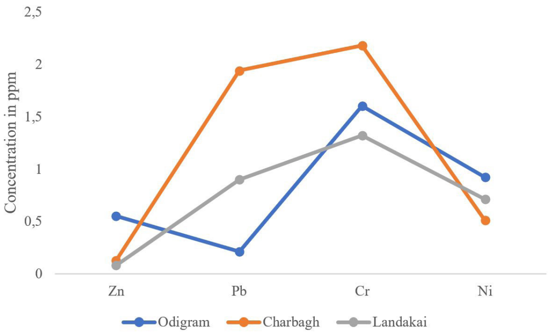

Figure 4. Concentration of heavy metals in muscles of *Schizothorax plagiostomus*

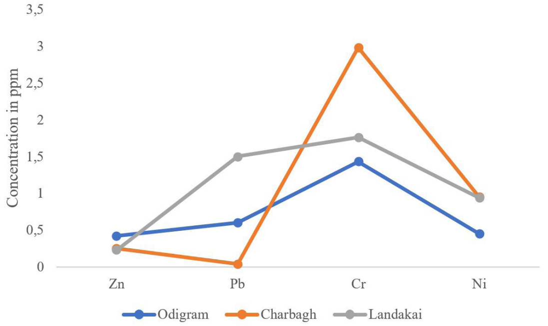

Figure 5. Concentration of heavy metals in gills of *Schizothorax plagiostomus*

three sites were in the order of *Charbagh>Landakai>Odigram*. The order of accumulation of metals at *Odigram, Charbagh, and Landakai* were Cr>Ni>Zn>Pb, Cr>Pb>Ni>Zn, and Cr>Pb>Ni>Zn respectively. A high concentration of zinc was recorded at Odigram site and a low concentration at the Landakai site. The order of accumulation of zinc was *Odigram>Charbagh>Landakai* (Figures 4 and 5).

The mean concentration of Zn in the muscles of *Schizothorax plagiostomus* at Odigram, Charbagh and Landakai was 0.55 ± 0.24, 0.013 ± 0.01 and 0.08 ± 0.00ppm respectively and that in gills was 0.42 ± 0.12, 0.25 ± 0.07 and 0.23 ± 0.06ppm respectively. The lead was highly accumulated at the Charbagh site and the least at the Odigram site. The order of accumulation at Odigram was Charbagh>Landakai>Odigram. The mean concentration ± standard error of lead in the muscle of *Schizothorax plagiostomus* at Odigram, Charbagh, and Landakai was 0.21 ± 0.01, 1.94 ± 0.44, and 0.90 ± 0.16ppm respectively, and that in gills was 0.60 ± 0.31, 0.40 ± 0.02, and 1.50 ± 0.48ppm respectively. Along with this high accumulation of Cr was at the Charbagh site and least was at the Odigram site. The order of accumulation was Charbagh>Landakai>Odigram. Chromium was the most accumulated metals at all three sites and tissues. The mean concentration ± standard error of Cr in the muscle of *Schizothorax plagiostomus* at Charbagh, Odigram, and Landakai was 1.60 ± 0.40, 2.18 ± 0.29, and 1.32 ± 0.30ppm respectively, and that in gills was 1.43 ± 0.20, 2.98 ± 0.41, and 1.76 ± 0.04ppm respectively. Similarly, the high accumulation of Ni was at Landakai and low was at Odigram. The order of accumulation was Landakai>Charbagh>Odigram. The mean concentration ± standard error of Ni in the muscle of *Schizothorax plagiostomus* at Odigram, Charbagh and Landakai was 0.92 ± 0.38, 0.51 ± 0.09 and 0.71 ± 0.10ppm respectively and that in gills was 0.45 ± 0.25, 0.95 ± 0.05 and 0.94 ± 0.10ppm respectively. At Charbagh and Odigram sites, high accumulation of metals occurred in muscles followed by gills whereas in Landakai high accumulation occurred in gills followed by muscles. The overall order of accumulation was Cr>Pb>Ni>Zn (Table 1 and Figure 6). The overall heavy metals concentrations (ppm) in muscle and gills of *S. plagiostomus* at Odigram, Charbagh, and Landakai site (Figure 7).

4. Discussion

In the present study *Schizothorax plagiostomus* was selected for the study based on the nutritive, economical, and consumption values. Whereas the metals for the

Table 1. Accumulation of heavy metals in muscles and gills of *Schizothorax plagiostomus*.

Location	Tissues	The concentration of metals (Avg ± SE)			
		Zn	**Pb**	**Cr**	**Ni**
Odigram	Muscle	0.55 ± 0.24	0.21 ± 0.01	1.60 ± 0.40	0.92 ± 0.38
	Gills	0.42 ± 0.1	0.60 ± 0.31	1.43 ± 0.20	0.45 ± 0.25
Charbagh	Muscle	0.13 ± 0.01	1.94 ± 0.44	2.18 ± 0.29	0.51 ± 0.09
	Gills	0.25 ± 0.07	0.04 ± 0.02	2.98 ± 0.41	0.95 ± 0.05
Landakai	Muscle	0.08 ± 0.00	0.90 ± 0.16	1.32 ± 0.30	0.71 ± 0.10
	Gills	0.23 ± 0.06	1.50 ± 0.48	1.76 ± 0.04b	0.94 ± 0.10

study were selected based on their concentrations in the study area. In the current study, heavy metals such as Zn, Pb, Cr, and Ni in the different tissues muscles, and gills *of Schizothorax plagiostomus* were within the permissible limit according to different health and food agencies like Canadian standard for Zn is 100µg/g, UK standard for Pb is 1µg/g, USEPA standard for Ni is 1µg/g and EU/EC standard for Chromium is 0.50mg/L (Ahmad et al., 2014). The high accumulation of Cr in the present-day study in fish tissues as compared to other metals is due to the abundance of the metal in water and sediment. The bioaccumulation of Zn, Pb in the tissues of *Schizothorax plagiostomus* at river Panjkora were 0.04-1.19, 0.01-0.09 µg/g respectively. The concentrations of heavy metals like Pb and Zn in the liver of *Tor putitora* from river Kabal were 93.66-136.8, 1694.0-1935.5 µg/g respectively, and that in gills were 219.3-321, 1993.9-2414 µg/g respectively. Some recent analysts revealed the mean concentration of Pb, Ni, Cr in the fish tissues at Kayseri Turkey were 0.82-1.40, 1.23-3.67, and 5.01-5.97 µg/g respectively (Duran et al., 2014). The accumulations of Cr, Zn, and Ni in the liver of wallago at Kalpani river Mardan were 0.901, 0.72, and 0.04 µg/g respectively (Sthanadar et al., 2013). The accumulation pattern of heavy metals like Zn and Ni in the muscles of *Cirrhinus mrigala* from river Ravi was in a range of 58.74-77.86 and 1.33-2.19 µg/g respectively. The concentrations of heavy metals like Pb in the tissue of grey mullet at the black sea were 0.38mg/kg (Stancheva et al., 2013). The accumulations of heavy metals like Zn, Pb, Ni, and Cr in the tissues of some edible fishes at Bangshi river Dhaka Bangladesh were 168.97, 4.64, 2.59, and 1.12 mg/kg respectively. The results of the recent study of heavy metals such as Cr, Pb, and Zn in the tissues of fishes at Yangtze river 0.239, 0.811, 7.55 mg/kg respectively (Yi and Zhang, 2012). The accumulations of heavy metals like Pb, Cr in the tissues of fishes at the Mediterranean Sea were 0.18, 0.003 µg/g respectively (Copat et al., 2011). The accumulation of heavy metals such as Pb, Zn, Ni, and Cr in the tissues of *L. rohita* in a freshwater lake in Bhopal were 0.21-1.77, 0.182-1.29, 0.1-1.63, and 0.08-1.31 µg/g and in C. idella were 0.8-1.77, 0.8-3.17, 0.35-1.44, and 1.64, 0.25-1.01µg/g respectively (Malik et al., 2010). The bioaccumulation patterns of Pb, Ni and Cr in the different tissues of fish at Avasar dam lake turkey were in a mean of; in muscle 3.11, 3.52, and 1.18 mg/kg, in gills were 3.11, 3.52, and 1.61mg/kg and in the liver were 3.39, 58.85 and 0.83 mg/kg respectively (Ozturk et al., 2009). The concentration of Zn, Pb, and Cr in the muscles of *C. punctatus* in the river Ganges were 0.42-0.96, 0.056-0.072, and 1.86-2.89 ppm, and that in *A. oar* were 0.80-120, 0048-0.092, and 2.46-3.89 ppm respectively (Gupta et al., 2009). The concentration of Pb in the muscles, gills, and liver of *Catla catla* from Keenjhar 0.71-2.31, 0.74-2.25, and 0.89-2.68 µg/g respectively (Korai et al., 2008). The results obtained from the bioaccumulation of Pb, Ni, and Cr in the liver of cyprinus carpio were in a mean of 2.00, 0.973 and 1.693 µg/g respectively similarly that in gills were 1.400, 1.043, and 1.883 µg/g respectively (Vinodhini and Narayanan, 2008). The contents of heavy metals in fish tissues in these previous studies were high than that of the present-day study and this is probably due to the contamination of these reservoirs from the discharge of domestic and industrial wastes. Surface water pollution due to sewage-disposal has been declared as a major factor

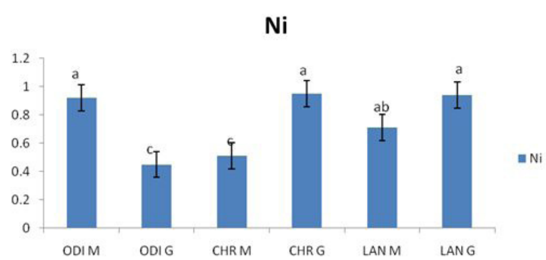

Figure 6. Concentration of Nickel (ppm) in the muscles and gills of *S. plagiostomus.*

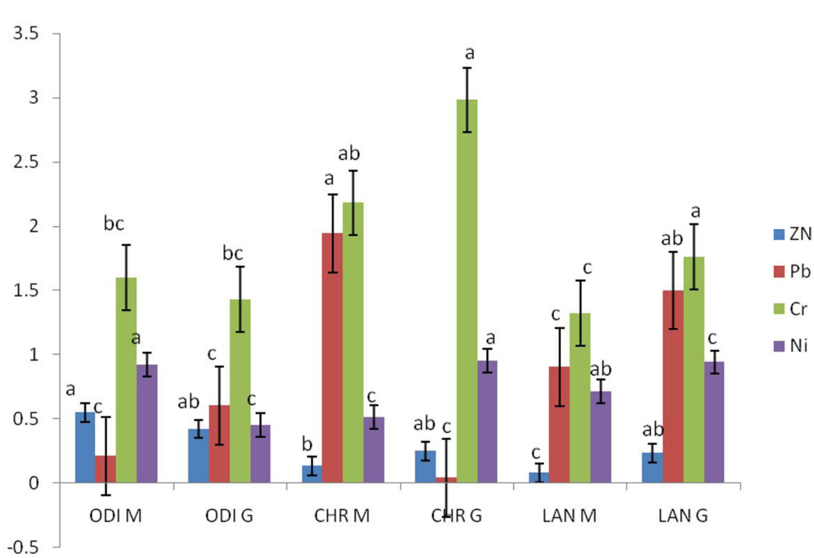

Figure 7. Heavy metals concentrations (ppm) in muscle and gills of *S. plagiostomus* at Odigram, Charbagh, and Landakai site.

responsible for heavy metals contamination. The comparison of the present study with the previous studies describes that river swat is less polluted as compared to the rest of the river and seas of the world. All the available levels of metals in the tissues were within the permissible limits and are safe for human consumption. The present-day study revealed that now there is no risk to fish and humans but in the future it may be a matter of concern due to the rapid enhancement in the establishment of industries and also their effluent direct discharge to reservoirs without any treatment. Therefore there is a need for proper check and balance for the management of river swat to prevent it from future deterioration.

5. Conclusion

It has been concluded that the accumulation of Cr was highest in gills and muscles of *Schizothorax plagiostomus.*

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