

# HEAVY METAL ACCUMULATION AMONG DIFFERENT ORGANS OF CULTURED ROHU AND CATLA ALONG WITH EVALUATION OF ENZYMATIC ACTIVITIES IN EXAMINED ORGANS.

### ABSTRACT

**Aims:** The present study was carried out to determine the concentration of heavy metals and to evaluate whether there are any serious toxic effects of the widely exposed heavy metals namely arsenic (As), lead (Pb) and chromium (Cr) in different tissues of two commercially important cultured fish Rohu (*Labeo rohita*) and Catla (*Gibelion catla*)

**Study design:** The study was designed based on Randomized Block Design (RBD) to avoid all kinds of possible biases as well as for ensuring the representation of different fish farms. Three replications were maintained to deal with the possible errors caused by sampling.

**Methodology:** Lead (Pb), Arsenic (As) and Chromium (Cr) were analyzed in a graphite furnace (GBCGF 3000 with Zeeman background corrector) with an auto sampler using Atomic Absorption Spectrophotometer (AAS). ALP (alkaline phosphates) activity of liver, Kidney and muscle was assayed using the standard method stated by Garen and Levinthal (1960) [1]. ATPase enzyme activity was determined to follow the standard method stated by Post and Sen (1967) [2]. During each month, ten individuals of the targeted two species each incorporating with three replications were collected from four intended fish farms, with a total of 240 fishes in the study period.

**Results:** The obtained results stated the highest concentrations of all heavy metals (except arsenic) were recorded in Rohu fish with no significant difference with the other investigated fish species Catla. Organ wise heavy metal concentrations in Rohu (*Labeo rohita*) fish showed that Lead (Pb) concentrations were higher in all organs compared to other investigated heavy metals. The recorded values of Pb in gills, livers, kidneys, and muscles were 0.021, 0.021, 0.028 and 0.008 ppm respectively. In case of Arsenic (As), the highest concentration was recorded in kidneys while the Chromium (Cr) showed higher accumulation in the muscles. While it comes to Catla (*Gibelion catla*) fish, similar results were demonstrated where Lead (Pb) concentration was the highest and Chromium (Cr) concentration was lowest. Among all the three heavy metals, the accumulation trend of Arsenic (As) was found highest in gills of Catla (*Gibelion catla*) but Lead (Pb) and Chromium (Cr) were accumulated in high concentration in kidneys of the same species. The average concentration of different heavy metals in the two investigated fishes were lower than the standard recommended value (0.01ppm) except for Arsenic (As) in Catla (*Gibelion catla*) which was 0.013ppm. Enzymatic activities like ATPase and ALP were recorded highest in kidney tissue and the lowest in muscle in case of both fish species.

**Conclusion:** Even though the average values of Arsenic in Catla (*G. catla*) fish was slightly higher than the recommended value suggested by WHO [3], the concentration is lower in edible part muscle which in turn indicates that the cultured Rohu (*L. rohita*) and Catla fishes in the Chattogram region are safe for human consumption.

*Keywords: Heavy metals, Rohu, Catla, Gill, Liver, Kidney, Muscle, ATPase, ALP activity.*

## 1. INTRODUCTION

Bangladesh is considered as the suitable place for freshwater species farming due to its favorable biophysical resources. The pollution of the aquatic environment with heavy metals has become a worldwide problem during recent years because they are degradable and most of them have toxic effects on many organisms [4]. Different pollutants of environment, metals are of particular concern, due to their potent toxic effects and their ability to bioaccumulate in aquatic ecosystems [5]. In the aquatic ecosystem, fish are staying at the top of the food chain, and they usually can accumulate heavy metals from food, water, and sediment. Some anthropogenic sources including agriculture, shipping, urban and industrial practices, have resulted in disquieting concentrations of heavy metals in aquatic environments. Heavy metal contamination may have demolishing effects on the ecological balance of the recipient environment and a diversity of aquatic organisms [6]. Among animal species, fishes are the inhabitants that cannot escape from the pernicious effects of these pollutants [7]. For the evaluation of the aquatic ecosystems health, fish are widely used because pollutants build up in the food chain and are responsible for ambivalent effects and death in the aquatic systems [8]. Heavy metals may alter the physiological activities and biochemical parameters of various fishes both in tissues and in blood [9]. Accumulation of heavy metals in the tissues of aquatic animals may become toxic when accumulation reaches a substantially high level [10].

Heavy metals enter from contaminated water into fish body via different routes and accumulate in different organ of fish [7]. The contaminants which entering the aquatic ecosystem may not directly damage the organisms; however, they can be deposited into aquatic organisms through the effects of bioconcentration, bioaccumulation and the food chain process and eventually threaten the health of humans by food consumption [10].

Indigenous and Indian major carp species such as Rohu (*Labeo rohita*), Catla (*Gibelion catla*) and Mrigal (*Cirrhinus cirrhosus*) have become popular in Bangladesh as suitable aquaculture species over the last decade. This study provided the information about accumulation level of As, Cr and Pb in commercially important two fish species- Rohu (*Labeo rohita*) and Catla (*Gibelion catla*) which are cultured in freshwater. Chattogram is situated in the southern part of Bangladesh which is the largest port city and the coastal city of the country. Different types of land drainages and other coastal pollutants enter the inland water bodies during the rainy season which contains many toxic heavy metals. These heavy metals accumulate into the different cultured species. Due to unawareness and lack of proper management systems; severe toxicity can alter ecological balance and heavy metal accumulation may cause an irreversible loss which will eventually encounter in the emerging sector of country's economy. If the people consume fishes containing higher level of heavy metals; can experience serious illness which can even lead them to a considerable life threat. Therefore, realizing this significance of the issue; assessment of the harmful effects and comparative study of heavy metals accumulation at different organs of fishes are needed to carry out along with the enzymatic variation in investigated organ. Consequently, monitoring programs and research on heavy metals in inland water samples have become widely important due to concerns over toxic effects of heavy metals in aquatic organisms and to humans through the food chain [11]. Thus, this study aimed to assess the heavy metals accumulation in liver, kidney, muscle and gill tissues of two commercially important cultured fishes (Rohu and Catla) and draw a comparison between ALP and ATPase activities of those organs of fishes.

## 2. MATERIALS AND METHODS

### 2.1 Study area and sample collection process

The experimental analysis was conducted in the laboratory of Applied Chemistry and Chemical Technology department, Chattogram Veterinary and Animal Sciences University, Chattogram. Ten individuals each with three replications from the two targeted fish species

Rohu (*Labeo rohita*) and Catla (*Gibelion catla*) were collected from the four fish farms (Jashim Fisheries 2- Sadarghat, Chattogram; Omera Fisheries Limited, Patenga, Chattogram; Maccher hat, Sadarghat, Chattogram and Kalu Shah Nagor Fisheries, Port connecting road, Chattogram) which are located on the different places of Chattogram at each month starting from January 2019 to March 2019 and brought to the laboratory. A total of 240 individuals (10 Rohu fish+ 10 Catla fish from each farm with three replications incorporating 60 species from each farm) were collected from the two species. Fish samples were stored in plastic bags at -20° C until dissection. Total length (cm) and weight (g) of fish samples were measured carefully and recorded in every time.

## 2.2 Dissection of fish and preparation of the sample for digestion

Each of the collected fish sample was dissected for collection of its gill, muscle, liver, and kidney tissues and kept into the plastic jar as sample. All sample preparation and analysis method were carried out according to the procedure described by UNEP Reference Methods [12]. For further analysis, the prepared samples were stored in 10% formalin solution for 7 days.

## 2.3 Digestion of the sample

The selected sample tissues were digested with concentrated nitric acid and perchloric acid (2:1 v/v) at 60 °C for 3 days. By using a microwave digester all samples were diluted twice with distilled water. After acid digestion, all samples were analyzed for three heavy metals (As, Pb and Cr) by atomic absorption spectrometry (Phillips AAS with double beam and deuterium background corrector).

## 2.4 Heavy metal analysis

Lead (Pb), Arsenic (As) and Chromium (Cr) were analyzed in a graphite furnace (GBCGF 3000 with Zeeman background corrector) with an auto sampler. For determining each metal, digested samples were analyzed three times. The standard addition method was used to correct for matrix effects (If any). Standard solutions prepared from commercial materials were used to calibrate the instrument. An analytical blank was also run to understand the instinct errors in the sample analysis.

## 2.5 Sample preparation for enzyme analysis

To determine the enzymatic activity different investigated organ including liver, kidney, gill, and muscle were dissected out from the fish. Each of the dissected part was mixed in chilled sucrose solutions (0.25 M) separately with a mechanical tissue homogenizer (WN-AD200LPN, China) to prepare 5% homogenate. The homogenate was centrifuged at 3000 g for 15 min using a laboratory centrifuge (centrifuge 5702R, Germany); the supernatant was collected and stored at -20°C for further analysis within 1–2 days.

### 2.5.1. Alkaline phosphates (ALP) analysis

ALP (alkaline phosphates) activity of liver, Kidney and muscle was assayed using the standard method stated by Garen and Levinthal [1]. For this purpose, a solution was prepared containing 0.1 mL of 0.1 M magnesium chloride, 0.2 mL of HCO<sub>3</sub> buffer, 0.05 ml tissue homogenate, 0.1 mL of 0.1M para-nitrophenyl phosphate and 0.5 mL of distilled water. Water bath is used to incubate the mixture at 37°C for 20 min. Optical density was recorded at 410 nm using UV–visible spectrophotometer (LT2900, Germany).

### 2.5.2. ATPase enzyme analysis

ATPase enzyme activity was determined by following the standard method stated by Post and Sen [2]. A mixture was prepared with 100 mM NaCl, 3 mM magnesium chloride, 0.1 M HCl buffer, 20 mM potassium chloride, 0.1 mL tissue homogenate and 5 mM ATP which was

used. The reaction mixture was incubated for 15 min, and 10% TCA was used to stop the reaction. Optical density was maintained at 660 nm [13].

## 2.6 Statistical analysis

All the data collected during the experimental period and laboratory test were recorded and preserved in computer. Statistical analysis was carried out using SPSS 20. One-way analysis of variance (ANOVA) and least significant difference (LSD) test were used to assess whether metal concentrations varied significantly among species & organs as well as to depict any significance difference in the enzymatic activities. A 5% level of significance was used ( $P < 0.05$ ).

## 3. RESULTS

This study revealed As, Cr and Pb accumulation in different organs (gill, liver, muscle, and kidney) of two freshwater fish species (*Labeo rohita* and *Gibelion catla*) collected from four commercial fish farms in Chattogram district of Bangladesh. These fish species were selected, as they are most consumed and commercially important freshwater fishes in Bangladesh. As, Pb and Cr were chosen because these are common detrimental elements found in industrial discharges. Apart from that, the enzymatic activities (ALP analysis and ATPase) were evaluated to understand the physiological processes of cultured Rohu and Catla fish.

The average of As, Pb and Cr concentration in different organs of the investigated species were presented in Figure 1, Figure 2, Figure 3, and Figure 4 while the enzymatic activities are illustrated in Figure 5 (ATPase) and Figure 6 (ALP). The average concentration of each heavy metal in all organs of investigated fishes are shown in Table 1

### 3.1 Different heavy metals concentration in gills of Rohu fish and Catla fish

The values of heavy metal concentration (As, Pb and Cr) in gills of cultured Rohu and Catla are shown in Figure 1. In the gills of Rohu fish, the highest concentration is recorded for Lead (Pb, 0.021ppm) followed by Arsenic (As, 0.006ppm) and Chromium (Cr, 0.001ppm) where the recorded values of the latter two are significantly different from the first one. But in case of gills of Catla fish, the highest value is recorded for Arsenic (0.021ppm) which has statically insignificant difference with recorded value for Lead (Pb, 0.018ppm). However, the value recorded for Chromium (Cr, 0.002 ppm) is significantly different and far lower than the other two investigated heavy metals in this species (Catla).

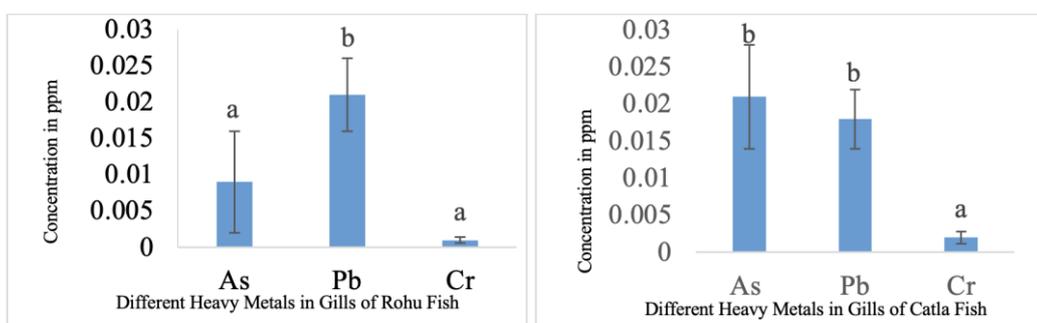


Fig. 1. Different heavy metals concentration in gills of cultured Rohu fish and Catla fish Bars with different letters (a and b) differ significantly ( $P < 0.05$ )

### 3.2 Different heavy metals concentration in liver of cultured Rohu fish and Catla fish

For Rohu fish, the values of lead were found to be the highest in liver (0.021 ppm) followed by arsenic (0.009 ppm) and chromium (0.001 ppm) respectively. The values recorded for Chromium (Cr) is significantly varied (lower) from those for Lead (Pb) and Arsenic (As). Similar with Rohu fish the values of lead were found to be the highest in liver of *Gibelion catla* (0.020 ppm) with no significant difference with recorded values for Arsenic (0.019 ppm); while the values recorded for Chromium (0.001 ppm) are much lower and statistically different from the other two.

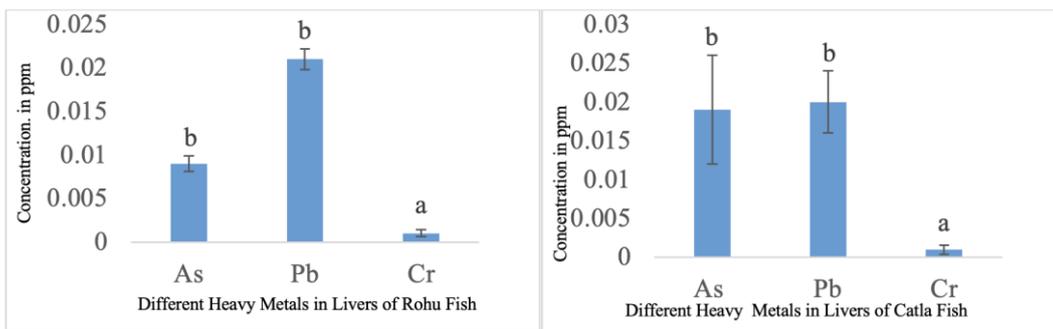
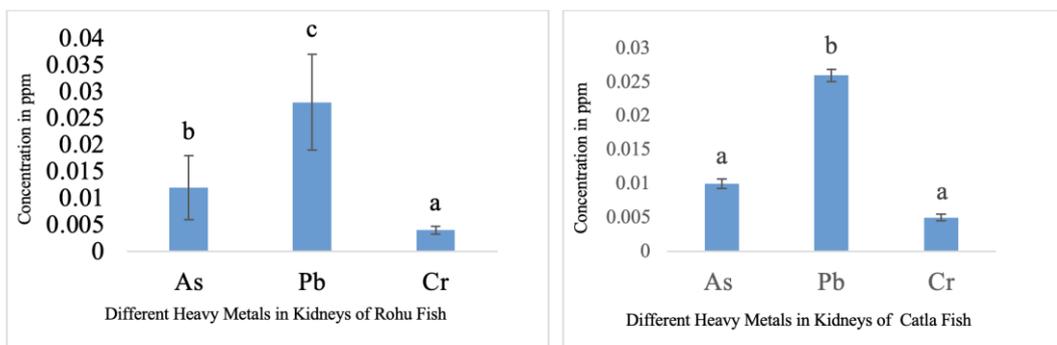


Fig. 2. Different heavy metals concentration in liver of cultured Rohu fish and Catla fish Bars with different letters (a and b) differ significantly ( $P < 0.05$ )

### 3.3 Different heavy metals concentration in kidney of cultured Rohu fish and Catla fish

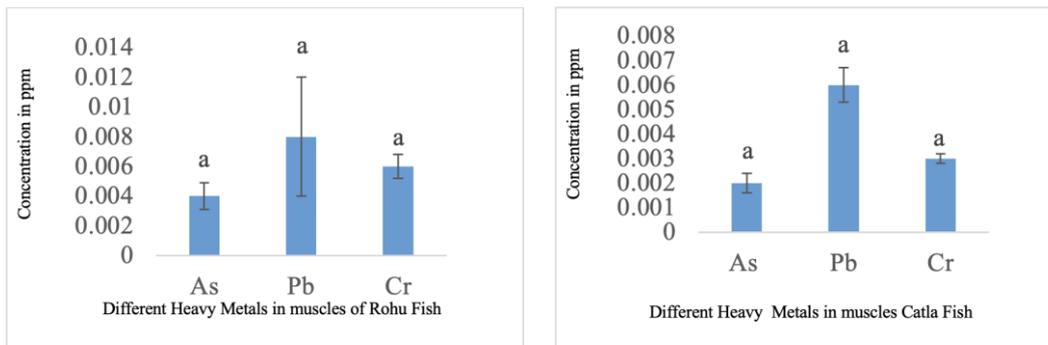
The recorded values are shown in the Figure 3. While Considering the Rohu fish, the concentration of Pb was found to be the highest (0.028 ppm) followed by As (0.012 ppm) and Cr (0.004 ppm) respectively. The obtained values of different heavy metals in kidneys of this species are significantly varied among each other statistically. On the other hand, the following figure (Figure 3) shows that the concentration of lead was the highest (0.026 ppm) with a significantly lower value of Arsenic (0.010 ppm) and chromium (0.005 ppm) for kidneys of Catla Fish. The value of arsenic in kidney is not statistically different from chromium but varied significantly compared to lead.



**Fig. 3. Different heavy metals concentration in kidney of cultured Rohu fish and Catla fish. Bars with different letters (a and b) differ significantly ( $P < 0.05$ )**

### **3.4 Different heavy metals concentration in muscles of cultured Rohu fish and Catla fish**

Among the investigated heavy metals (As, Pb, Cr), lead concentration (0.008 ppm) was the highest in muscles of Rohu fish followed by the concentration of chromium (0.006 ppm) and Arsenic (0.004 ppm) though the values are not significantly varied (Figure 4). Likewise, for Catla Fish, lead concentration (0.006 ppm) was the highest in muscles followed by the concentration of chromium (0.003 ppm) and Arsenic (0.002 ppm) though the values are not statistically significant ( $P < 0.05$ ) (Figure 4).

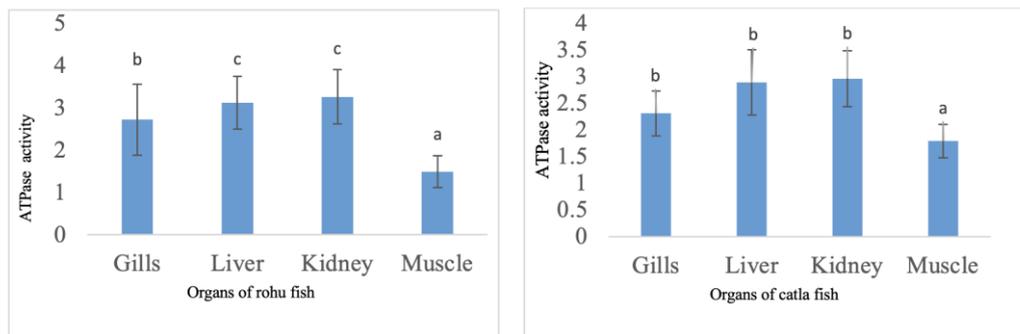


**Fig. 4. Different heavy metals concentration in muscles of cultured Rohu fish and Catla fish. Bars with different letters (a and b) differ significantly ( $P < 0.05$ )**

### **3.5 ATPase activity in different investigated organs of cultured Rohu fish and Catla fish**

The Figure 5 demonstrates the ATPase activities of different examined organs namely gills, liver, kidney, and muscle of Rohu fish and Catla fish. From the findings, it is observed that the ATPase enzymatic activities were found to be the highest in kidneys with insignificant difference for the values obtained in livers in Rohu fish. The figure also suggests that the lowest ATPase activity was recorded in muscle which was significant compared to the above two organs along with the values obtained for gills for this species.

The recorded values of ATPase activities in different organs of cultured Catla fish are quite similar with that obtained for Rohu fish depicting the highest in kidneys followed by liver, gills, and muscles respectively. The values here for gills are not significantly different from liver and kidney. But the lowest value recorded in muscles were significantly varied in comparison with gill, liver, and kidney.

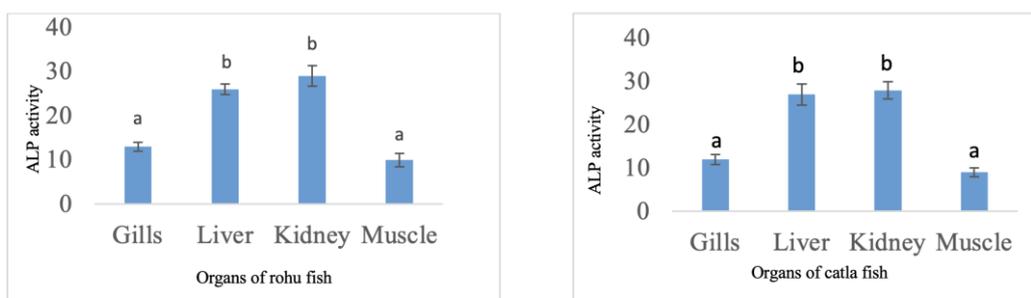


**Fig. 5.** ATPase activity as  $\mu\text{g}$  of phosphorus  $\text{mg protein}^{-1}$  per min ( $37^\circ\text{C}$ ) in different organs of cultured **Rohu** fish and **Catla** fish. Bars with different letters (a and b) differ significantly ( $P < 0.05$ )

### 3.6 ALP activity in different organs of cultured Rohu fish and Catla Fish

For Rohu Fish, the concentration of ALP activity was found highest in kidney (29 ppm) with insignificant difference for that of liver (26 ppm). The lowest value was observed in muscles (10 ppm) with significant difference from kidney and liver whereas the recorded values in gills are not statistically different from muscle (10 ppm).

The concentration of ALP activity was found highest in kidney (28 ppm) with insignificant difference for that of liver (27 ppm) in **Catla** fish. The lowest value observed in muscles (09 ppm) and gills (12 ppm) with significant difference from above mentioned two organs whereas the recorded values in muscle and gills are not statistically different among themselves.



**Fig. 6.** ALP activity as n moles of para-nitrophenol  $\text{mg protein}^{-1}$  ( $37^\circ\text{C}$ ) in different organs of cultured **Rohu** fish and **Catla** fish. Bars with different letters (a and b) differ significantly ( $P < 0.05$ )

### 3.7 Average concentration of different heavy metals in organs of cultured **Rohu** and **Catla** fish

The data values of Table 1 show that for Rohu Fish, concentration of Arsenic (As) with a mean value of 0.008 ppm which is lower than the recommended value of 0.01 ppm [3, 14]. The average value of lead (Pb) was observed (0.0195 ppm) which is also lower than the standard value of 0.3 ppm [3, 14]. The mean average value of Chromium (Cr) was the least

among the three investigated heavy metals (0.003 ppm) which is far lower than the standard value (0.1 ppm) of Chromium [3, 14].

The same table (Table 1) also narrates the values in **Catla** which demonstrates that the higher concentration is observed in case of Arsenic (As) with a mean value of 0.013 ppm which is higher than the recommended value of 0.01 ppm [3, 14]. The average value of lead (Pb) was observed (0.0175 ppm) which is also lower than the standard value of 0.3 ppm [3,14]. The mean average value of Chromium (**Cr**) was the least among the three investigated heavy metals (0.00275 ppm) which is far lower than the standard value (0.1 ppm) of Chromium [3,14]

**Table 1. Average concentration of different heavy metals in organs of cultured Rohu and Catla fish with recommended limit**

Heavy metals	Rohu fish	Catla fish	WHO/European Commission limit
As	0.008 ppm	0.013 ppm	0.01 ppm
Pb	0.0195 ppm	0.0175 ppm	0.3 ppm
Cr	0.003 ppm	0.00275 ppm	0.1 ppm

#### 4. Discussion:

Heavy metals persist in the environment long time because these are non-biodegradable substance and may become concentrated up to the food chain [15]. Accumulation of heavy metals occurred and stored faster in living organisms when they are taken up than they are broken down (metabolized) or excreted. They enter the water body through industrial and consumer materials, or even from acidic rain, breaking down of soils and release of heavy metals into different water body [16]. The levels of heavy metals in fish vary species to species and also by the nature of habitats and the concentration of these metals varied among different organs of a species as well as between species [17].

Previous study showed that gills, liver, kidney, and muscle of *Gibelion catla*, *Labeo rohita* and *Cirrhinus cirrhosus* when examined for the accumulation of metals; are significantly different due to their ability to concentrate heavy metals. However, Catla showed significantly higher tendency for the accumulation of all metals in its body than other two fish species [18].

El-Shahawi and Al-Yousab [19] reported that in fish, the levels of nickel, cobalt, lead, and chromium was found to follow the order: liver > skin. Metal levels found in liver and skin followed the sequence: chromium > lead > nickel > cobalt. However, during this study the **Rohu** fish organs accumulated Arsenic and Lead by following the order: kidney > liver > gills > muscle while Chromium showed: muscle > kidney > liver > gill. In Catla fish organs, Arsenic accumulation occurred following the order: gills > liver > kidney > muscle, while for lead accumulation in this fish the order is kidney > liver > gill > muscle. The Cr concentration followed the order: kidney > muscle > gill > liver.

It is very difficult to compare the metal concentrations even between the same tissue in different species because of many factors such as aquatic environments, the type of species investigated, the level of water pollution, feeding habits, and level of fish presence in water, whether pelagic or benthic fish etc. [20]

Heavy metal concentrations were found to decrease in sequence of the *Cyprinus carpio* samples, in the muscle and stomach-intestine as  $Fe > Cu > Pb > Ni > Cr > Cd$ ; in the gill, heart and liver as  $Fe > Cu > Ni > Pb > Cr > Cd$  and in the air sac as  $Fe > Cu > Ni > Pb > Cd > Cr$  [21]. Kidney, together with the gills and intestine, are responsible for excretion and the maintenance of the homeostasis of the body fluids and besides producing urine, act as an excretory route for the metabolites of a variety of xenobiotic to which the fish may be exposed. It was evident from our study that heavy metal concentration in kidney of Rohu and Catla fish as  $Pb > As > Cr$ . In liver of both fish metal concentration found in the order of  $Pb > As > Cr$  and in the muscle of both fish it found that  $Pb > Cr > As$ .

Gills are the potential site for contaminant uptake in fish because of their anatomical and physiological properties that maximize absorption efficiency from water [22]. However, it was evident from our study that, in general kidney and liver were the sites of maximum accumulation for the Pb and As while gill was the overall site of least metal accumulation in both species. This might be due to the storage of heavy metals mainly occurred in internal organs like livers and processed via the excretory organs like kidneys [23, 24]. A similar result has been obtained by Siddiqua et. al. [25] where they found that the Lead and Arsenic are accumulated in higher concentration in kidneys and livers of commercially important marine fishes in the Chattogram coast of Bangladesh. The metal concentrations in the gills of Rohu and Catla fish occurred in the decreasing order of  $Pb > As > Cr$  and  $As > Pb > Cr$  respectively.

Between two fish species, the variation of metals concentration in the muscle of Rohu and Catla fish are found in the order of  $Pb > Cr > As$  and  $Pb > Ar > Cr$  respectively. In average Cr concentrations have found to be lower in Rohu fish gills and liver than other two organs so do in Catla where Cr also found lower in liver and gills. The low concentrations of all metals were found both species' muscle relatively.

In Catla fishes, arsenic overpassed the recommended value provided by WHO or European Commission [3,14] whereas Lead and Chromium are far lower than the guideline value. In case of Rohu fish, experimented value of As, Pb and Cr are within the recommended value provided by WHO or European Commission [3,14]. Bashir et al. [23] found that muscle contained the minimum concentrations of trace metals among all the tissues which support our findings. Among different organs, highest concentration of metals was observed in kidney tissues but the lowest concentration in muscles was found which is a positive finding as we mainly consume the muscles portion of fish widely. The concentration of heavy metals is recorded lowest in muscles compared to kidneys and livers in the commercially important marine fishes of Bangladesh [25] which is also found true for freshwater species in this study.

However, all these concentrated metals in different parts of fish body could be concentrated into human body, if they are consumed. In Bangladesh, water sources are getting more polluted day by day and thus these heavy metals (HMs) from polluted water bodies are getting more concentrated in the fishes living in those areas. The HMs can also be concentrated in fish body through the feed they are reared with. Therefore, the potential limitation of this study is we didn't take into consideration about the HMs sourcing from the artificial feeds of the farmed fishes.

Enzyme's activities including Adenosine triphosphates (ATPase) and alkaline phosphatase (ALP) were evaluated in different important fish organs (liver, kidney, gills, and muscle) in present study. By knowing the enzymatic activities in an organism, we can easily identify disturbances in its metabolism. Adenosine triphosphate (ATP) is a complex organic chemical that provides energy to drive many processes in living cells which includes contraction of muscle, propagation of nerve impulse, and synthesis of chemical. ATP is referred to as the "molecular unit of currency" of intracellular energy transfer in all form of life. ATP is a precursor to DNA and RNA and is also used as a coenzyme. These enzymes release energy and helps in osmoregulatory activities [26]. All living cell contain ATP, and it should

be possible to look for changes in ATP levels in water as an indication of biological contamination. Adenosine triphosphate (ATPase) determination in fish in liver and muscle tissue could be an important biomarker to evaluate the condition of fish in stressed condition [27]. In the stress condition, ATPase activity reduced in fish [28].

In the present study, The ATPase activity in different organs of Rohu fish decreasing in the order: kidney > liver > gill > muscle. On the other hand, ATPase activity in different organs of Catla fish is in the order: kidney > liver > Gill > Muscle. In both fishes, we found that ATPase activity was highest in kidney and lowest in muscle. The ATPase activity is found to be higher in internal organs (liver) in *Heteropneustes fossilis* compared to muscles while exposed in several salinities level in cultured condition which agrees with the result of this study. [29] This is because kidney mitochondria have a greater capacity for ATP hydrolysis and enzymes are stored in livers of fish. Enzyme analysis in different organs can be a vital indicator about the physiological processes of the organisms concerned as well as for the environment they inhabit. Disruption in physiological and biochemical processes can occur when enzyme activities change due to the effects of toxicant in fishes [30]. As the values of recorded ATPase activities are mostly normal, the effects of toxicants i.e., heavy metals are not considered lethal which is also evident from the recorded values of HMs in different organs in this study.

Alkaline phosphatase is an enzyme found throughout the whole body, but it is mainly found in the bones, kidneys, liver, and digestive system. ALP catalyzes the hydrolysis of a wide variety of physiologic and non-physiologic phosphoric acid esters. It is a ubiquitous plasma membrane-bound enzyme which employed to assess the integrity of plasma membrane and endoplasmic reticulum. The liver and biliary tracts are the sources of alkaline phosphatase [31]. ALP is found more in liver than other organs of fish, but ALP production inhibited due to stress condition [32]. ALP used as potential stress indicator. Reduction in ALP activity in liver may happen due to sudden changes in metabolism as there is a need to supply additional energy in hyper osmotic condition.

The activities of ALP enzyme in different organs of Rohu were in the decreasing order of kidney > liver > gill > muscle. The liver tissue produces more ALP than other organs of fish, but the production of ALP is inhibited in stress condition [32]. In case of Catla fish, the activities of ALP enzyme in different organs were in the decreasing order of kidney > liver > gill > muscle. Kidney was found to be an organ in with the highest ALP activity. The ALP activities are also recorded lowest in the muscles in cultured *Heteropneustes fossilis* which eventually decreases with the stressed (salinity) conditions [29]. So, our findings are consistent with previous studies as we found Kidney produces more ALP than liver, gill, muscle respectively.

Enzymes are biochemical macromolecules that control metabolic processes of the organisms, ion transport, regulate cell volume [33]. They are the vital biochemical components which control metabolic process of organisms and for this reason, a slight variation of enzyme activities in organisms might affect severely. Therefore, it is important to monitor the activities of this enzyme and their levels in various organs of fish. The recorded values of different heavy metals mostly lied in the acceptable range which was also reflected in the normal obtained results of the enzymatic activities.

#### 4. CONCLUSION

The present study revealed that Arsenic concentration is higher in Catla fish than Rohu fish. The concentration of other heavy metals lies within the permissible limit which indicates the commercially cultured Rohu and Catla is safe for human consumption. Despite that a value greater than the recommended values has been recorded for Arsenic in Catla fish; their accumulation in muscle tissues for the same fish are less than the permissible value. This is an important advantage since the muscles are the most edible parts by the human being. The enzymatic activities reveal a comparative picture of the cultured environment which can

be concluded sound based on the obtained values. But, for ensuring the continuous sustainability of the economic sector of the freshwater aquaculture industry, it is now very important to pay attention for environmentally friendly discharge of heavy metals and pollutants.

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