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Bioaccumulation profile of heavy metals in the liver tissues of *Wallago attu* (MULLEY) from Kalpani River Mardan, Khyber Pakhtunkhwa Pakistan

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Abstract

We examined the bio accumulation profile of 05 heavy metals (Pb, Cr, Cd, Ni and Zn) in the liver tissue of fresh water fish Mulley, *Wallago attu* collected from 04 different polluted sites of Kalpani River in Khyber Pakhtunkhwa province Pakistan. The heavy metals concentration recorded in the liver of *Wallago attu* was determined by using Perkin Elmer AS 3100 flame atomic absorption spectrophotometer. Metals bio accumulated in the liver tissue in the order of Cd>Cr>Zn>Ni with no detection of lead (Pb) in any fish sample. Cadmium (Cd) was the highest and nickel (Ni) was the least accumulated metal in the fish liver. Mean concentrations of detected four heavy (Cr, Cd, Ni, Zn) in the liver of *Wallago attu* in all 04 samples were 0.90±0.8118, 3.33±1.3595, 0.04±0.0250, 0.72±0.1937 µg g -1 (wet weight). Overall, cadmium (Cd) was more accumulated in the fish liver as compared to other heavy metals. At present cadmium has crossed the permissible limits of FAO (1983). Our findings suggest that fish liver have comparatively more chemical affinity to bio accumulate cadmium (Cd) as compared to heavy metals like Zn, Cr and Ni.

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Introduction

Aquatic pollution is a globally prevailed scenario and has been a matter of serious concern for the last previous decades, regularly caused by pollutants of multifarious nature, which is not only unsafe for aquatic life but also posing serious health risks for human life as well (Karadede *et al.,* 2004; Mendil and Uluozlu, 2007). Among various causes of fresh water and riverine pollution, heavy metals are of considerable importance and consideration (Sthanadar *et al.,* 2013). Heavy metals are known as high density metallic elements or stable metals with density greater than 05.00 to 06.00 g/cm3, which may have hazardous effects on plant or animal ecosystems when present in higher concentrations than found naturally (Keepax *et al.,* 2011, Tanee *et al.,* 2013). Activities like effluents from industries, agricultural runoff and untreated sewage system are constantly contributing to heavy metals pollution across aquatic environment (Bhuvaneshwari *et al.,* 2012a; Sthanadar *et al.,* 2013).

Metals of such serious concerns are enough toxic and bio-chemically non bio-degradable in nature. They ultimately inter into the food chains and results into bioaccumulation, bio-magnification and notably causing physiological and morphological alterations in the fish as well as in human bodies (Vinodhini and Narayanan, 2008;Terra *et al.,* 2008; *Bhuvaneshwari et al.,* 2012a; Sthanadar *et al.,* 2013).Toxicity of heavy metals as a result of fish contamination has led to many studies in other parts of the world (Chi *et al.,* 2007; Dural *et al.,* 2007; Nesto *et al.,* 2007; Terra *et al.,* 2008; Ambedkar and Muniyan, 2011; Abah *et al.,* 2013; Sthanadar *et al.,* 2013; Tenee *et al.,* 2013).

Relevantly, fish is a good bio indicator for the estimation of heavy metals pollution in aquatic environment (Karadede *et al.,* 2004; Calvi *et al.,* 2006; Yang *et al.,* 2007 and Yilmaz *et al.,* 2007 ; Yousafzai *et al.,* 2010; Sthanadar *et al.,* 2013; Tanne *et al.,* 2013). These metals have enough chemical affinity to bio accumulate in various organs of the aquatic organisms (Karadede *et al.,* 2004; Bhuvaneshwari *et al.,* 2012a), which in turn may enter into the human metabolism through consumption causing serious health hazards (Tanee *et al.,* 2013).

Fish bio accumulate comparatively high amount of heavy metals as located at the high trophic level in food web. However bio accumulation of different heavy metals (Zn, Ni, Cr, Cu, Cd and Pb) varies from organ to organ (Bervoeats *et al.,* 2001; Yousafzai, 2004; Sthanadar *et al.,* 2013). Fish liver is metabolically active organ and highly active in the uptake and storage of heavy metals, hence it is a good monitor of water pollution with metals since their concentrations are proportional to those present in the environment (Dural *et al.,* 2007; Vinodhini and Narayanan, 2008; Bhuvaneshwari *et al.,* 2012a). Although fish livers are seldom consumed. However fish liver may represent a good biomonitor of metal pollution (Chaffai *et al.,* 1996; Bhuvaneshwari *et al.,* 2012a). Other than liver, heavy metals are known to bio accumulate in other organs viz., fish muscle, intestine, gills, head, bones, intestines, reproductive organs. Out of which, skin is the primary target organ for heavy metals physio-chemical interaction (Zhang *et al.,* 2007; Fonge *et al.,* 2011; Ebrahimi and Taherianfard, 2011; Bhuvaneshwari *et al.,* 2012a; Dahunsi *et al.,* 2012).

The present study aims to determine the presence and pattern of selected heavy metals in the liver tissue of *Wallago attu,* as periodically consumed fish species caught from Kalpani River in Khyber Pakhtunkhwa Pakistan. Further to report upon the heavy metals pollution level across River Kalpani as one of the neglected area.

Materials and methods

Sample collection

To assess the bioaccumulation profile of heavy metals in the liver of fresh water fish Mullee, *Wallagu attu,* initially a total of 20 fish samples were netted from 04 different polluted sites of Kalpani River, Khyber Pakhtunkhwa, Pakistan. The gills nets (Patti) of particular size (40x6ft) were used. The collected samples were brought to the laboratory in an ice box in cold condition and then washed with distilled water.

Fish identification and dissection

Fish were identified according to Talwar and Jhingran (1991), Mirza and Sundho (2007) methods. Weight and length of each fish was precisely noted by using measurement tape and digital balance respectively. After morphometric measurement fish samples were washed with distilled water and dissected for liver tissues. Weighted portions of desired tissues of liver were separated and shifted to properly marked sterilized polythene bags, stored in the freezer at -15 C°.

Reagents

Per chloric acid (70%) and nitric acid (55%) were used for tissue digestion to extract the desired heavy metals.

Metal extraction

To analyze liver tissue for heavy metals like lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni) and zinc (Zn), the process of tissue digestion was carried out. The samples were thawed and rinsed in distilled water, then blotted properly with blotting paper. Samples were then shifted to 100 ml volumetric flasks already washed with distilled water and dried in oven at 60 C° for a few minutes. Known weight of the tissue samples were shifted to volumetric flasks. Digestion was carried out according to Van Loon (1980) and Due Freez and Steyn (1992). Likely added 10 ml nitric acid (55%) and 5 ml per Chloric acid (70%) at the time of digestion to each flask. The flasks were then placed on hot plate and allowed to digest at 200 to 250 C° until a transparent and clear solution was obtained. The dense white fume from the flasks after brown fumes was an indication of digestion completion. After digestion, samples were cooled. The digests were diluted to 10ml with Nano pure distilled water appropriately in the range of standards that were prepared from stock standard solution of the metals (Merck). Samples were stored in properly washed glass bottles until the metal concentration was determined and noted with care.

Instrumentation

Flame Atomic Absorption Spectrophotometer (Perkin Elmer model AS 3100 double beam mode, USA) with multi element hollow cathode lamp was used for the analysis of heavy metals (Pb, Cd, Cr, Ni, Zn) present in the tissue extracts. Air-acetylene was used as fuel for flame. Heavy metals concentrations of lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni) and zinc (Zn) in the liver tissue of each sample were analyzed in triplicate. The results were presented as µg metal/g wet weight. A range of analytical standards for each metal was prepared from E. Merck Stock solution. Standard curves were prepared and the obtained data was calibrated against the standard curves to precisely record the concentration of heavy metals present in the liver tissues.

Data generalization and statistics

Data obtained was generalized and the results were expressed as mean ± standard error of the mean (S.E.M). Statistical analysis of data was carried out using SPSS statistical program (Pakage-12, registered).The obtained figures were plotted on simple bar graphs to see their values conveniently.

Results

The bioaccumulation profile of 05 heavy metals (Pb, Cr, Cd, Ni and Zn) in the liver tissue of fresh water Mulley, *Wallago attu* was analyzed by using Perkin Elmer AS 3100 flame Atomic Absorption Spectrophotometer. The heavy metals profile was recoded in triplicate in each sample. At least 03 fish samples were selected for the analysis from each 04 sampling sites, including site 01, 02, 03 and site 04 as in Table 1. Initially a total of 20 fish samples were collected, out of it 12 healthy fish samples were used for onward analysis. For convenience a single mean value was recorded in Table 1 for 03 fish sample collected from site 01. Similarly single mean value was considered after analyzing 03 fish samples collected from site 02, 03 and site 04. For further accuracy of the data, again a mean value with standard error of the mean was calculated for the readings of all 04 sites (site 01, site 02, site 03 and site 04). Single mean values with standard error of the mean were recoded for all 05 heavy metals (Pb, Cr, Cd, Ni, and Zn).

Lead (Pb) was not detected in any of the sample collected from Kalpani River. However the values recorded for chromium (Cr), from 04 sampling sites were: 0.00, 0.24, 0.03, 03.33 with a mean value and standard error of the mean as, 0.9± 0.8118. Similarly values recorded for cadmium (Cd) at all 04 sites were 3.33, 6.66, 3.33, 0.00 with mean value and standard error of the mean as 3.33± 1.3595. The values for nickel (Ni) deposition in the liver tissues at selected 04 sampling sites were 0.11, 0.01, 0.00, 0.03 with a mean value and standard error of the mean as 0.04± 0.0250. Similarly, bioaccumulation profile of zinc (Zn) across the liver tissues recorded in fish samples collected from 04 sampling site were 0.90, 0.81, 1.03, 0.16 with a mean value and standard error of the mean as 0.72± 0.1937. All the obtained results were shown in Fig. 1 in generalized form. The values of each heavy metal recoded were tabulated in Table 1.

Regarding heavy metals bioaccumulation profile in liver tissue, lead (Pb) was entirely absent. Cadmium (Cd) and chromium (Cr) were the highest in concentration and nickel (Ni) was the lowest accumulated heavy metal in the liver of Mulley, *Wallago attu*. Out of 05 heavy metals (Pb, Cr, Cd, Ni, Zn) considered in the present investigation, the order of heavy metals accumulation in the liver tissue was cadmium > chromium > zinc > nickel. Each metal concentration was shown on standard bar graphs to see conveniently, as in Fig 01.

Table 1. Heavy metals concentrations in the liver tissue of *Wallago attu,* Mulley collected from 04 different polluted sites of River Kalpani.

Analytes	Site 01.	Site 02.	Site 03.	Site 04.	Mean	Standard error of Mean
Pb	0.00	0.00	0.00	0.00	0.00	0±0.000
Cr	0.00	0.24	0.03	3.33	0.90	0.90±0.8118
Cd	3.33	6.66	3.33	0.00	3.33	3.33±1.3595
Ni	0.11	0.01	0.00	0.03	0.04	0.04±0.0250
Zn	0.90	0.81	1.03	0.16	0.72	0.72±0.1937

Discussion

Fish liver was previously studied for recoding the level of heavy metals causing fresh water and riverine pollution across the aquatic environment (Vinodhini and Narayanan, 2008; Akan *et al.,* 2009; Yilmaz, 2009; Abdel-Baki *et al.,* 2011; Ebrahimi and Taherianfard, 2011; Bhuvaneshwari *et al.,* 2012a ; Yousaf *et al.,* 2012; Tanee *et al.,* 2013) Fish liver is remarkably active in the uptake and storage of heavy metals across aquatic environment. Though fish livers are seldom consumed, but may act as a good bio-monitor of metal pollution (Chaffai *et al.,* 1996; Dural *et al.,* 2007; Bhuvaneshwari *et al.,* 2012a). Other than liver, heavy metals are known to bio accumulate in organs viz., fish muscle, intestine, gills, head, bones, intestines, reproductive organs. Out of which, liver is the organ of our prime concern in the present investigations. Presently, we examined 05 heavy metals including lead (Pb), chromium (Cr), cadmium (Cd), nickel (Ni) and zinc (Zn) in the liver tissue of *Wallago attu*. The bio accumulation profile of heavy metals showed a highest concentration of cadmium (Cd) deposition in the liver tissue, accordingly followed by chromium (Cr) deposition. However, nickel (Ni) and zinc (Zn) were among the lowest accumulated heavy metals in the fish liver. Our findings exposed a high level of cadmium (Cd) deposition in the fish liver with least accumulation of nickel (Ni) in the liver tissue of Mulley, collected from Kalpani River. Systematically the bioaccumulation profile of the heavy metals in fish liver is followed as:

Bioaccumulation profile of heavy metals in the liver of Mulley, *Wallago attu* (µg/g wet weight).

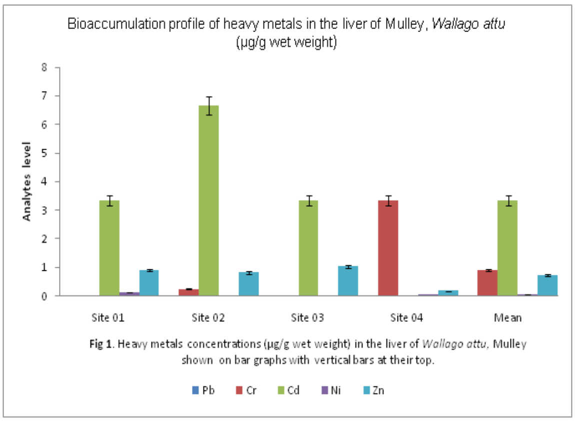

Fig. 1. Heavy metals concentrations (µg/g wet weight) in the liver of Wallago attu, Mulley shown on bar graphs with vertical bars at their top.

Bio accumulation of Lead (Pb) and Chromium (Cr)

The lead (Pb) deposition across the fish liver was previously reported by Vinodhini and Narayanan (2008), Akan *et al.,* (2009), Yilmaz (2009), Yousafzai *et al.,* (2009) and Tanee *et al.,* (2013). Sources of lead (Pb) are refineries and industrial untreated effluents. In Kalpani River lead (Pb) was not detected due to the absence of lead (Pb) contributing sources. But it is highly imperative to periodically check the level of lead (Pb) in the aquatic environment. Although lead (Pb) is one of the non-essential but life threatening heavy metal and can easily lead to human health hazards like chronic damage to human nervous system (Neuro toxicity), comma, mental retardation, kidney failure (Nephrotoxicity), lungs cancer and even death (Garacia-Leston *et al.,* 2010; Al- Busaidi *et al.,* 2011; Sthanadar *et al.,* 2013). The increased level of industrialization is continuously increasing the risk and damages of lead (Pb) to humans via different types of food chains. It is quite considerable that industrial effluents and domestic sewage should approach the main flow after its proper treatment (Sthanadar *et al.,* 2013).

In the present investigations, chromium (Cr) in the liver tissue of Mulley, *Wallago attu* was recorded with a mean value of 0.90±0.8118 µg/ wet weight. Chromium (Cr) travels across the different organs of the fish but does not normally accumulate in fish body and hence low concentrations of chromium were reported even from highly polluted parts of the world.

However chromium (Cr) uptake is only higher in young fish but not in adult fish due to its rapid elimination from fish body (Dara, 1995; Sthanadar *et al.,* 2013).

Chromium (Cr) deposition in the fish liver was previously recorded by Vinodhini and Narayanan (2008), Akan *et al.,* (2009), Yilmaz (2009), Yousafzai *et al.,* (2009), Abdel-Baki *et al.,* (2011) and Bhuvaneshwari *et al.,* 2012a. Studies have reported different level of chromium (Cr) deposition in fish liver. Vinodhini and Narayanan (2008) have reported a low level of chromium (Cr) deposition in the fish liver with a mean value of 0.863 ± 0.015 (μg/g.d.wt). Contrarily, Yousafzai (2004) has previously recorded a high level of chromium (Cr) in *Tor putitora* liver with a mean value of 3.2± 0.05 μg/wet weight of the body. This was in fact a high level of chromium (Cr) in the fish liver. Similarly Rauf *et al.,* (2009) has recorded a high level of Chromium in the liver of fish species, *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* caught from the River Ravi in Pakistan. The findings of Yousafzai *et al.,* (2010) also reported a high level of chromium (Cr) in the fish liver.Our findings have reported a very low level of chromium (Cr) in the fish liver of Mulley, *Wallago attu*. Our findings are in agreement with the findings of Avenant-Oldewage and Marx (2000), who recorded a low level of chromium in the liver of *Clarias gariepinus*. The findings of Yousafzai *et al.,* (2012) also support our data. On the other side our figures are not in the agreement with the findings of Yousafzai (2004) and Yousafzai *et al.,* (2010). A raised level of chromium deposition in fish body is really worthy of future investigations for hepatic responses.

Although chromium (Cr) is an essential trace element and play an important role in fish metabolism (Sthanadar *et al.,* 2013). Presently the mean concentration of chromium (Cr) in the liver tissue (0.90±0.8118 μg/ wet weight) is quite within the permissible limit (01.00 μg g-1) of FAO (1983) across the Kalpani River in Pakistan.

Bio accumulation of Cadmium (Cd)

In the present study cadmium (Cd) is highly accumulated in the liver tissue of *Wallago attu* with a mean value of 3.33±1.3595 μg/g wet weight. Previously Yousafzai *et al.,* 2010 recorded a high level of cadmium (Cd) deposition in the liver tissue of *Wallago attu* collected in River Kabul. In a previous study James *et al.,* (2000) pointed out that liver is a major organ where cadmium (Cd) deposition occurs in high level due to continuous accumulation of Cd in the liver throughout life time. Filazi *et al.,* (2003) also reported a high level of cadmium (Cd) in the liver of *Mugil auralus*. Recently Yilmaz (2009) also reported an elevated level of Cd in the fish liver as 3.32 μg/g in *Anguilla anguilla*. Rauf *et al.,* 2009 reported 4.26± 1.57 μg/g high level of Cd in the fish liver. Ebrahimi and Taherianfard (2011) also reported high level of Cd in the fish liver. Contrarily, Farkas *et al.,* (2002) has recorded cadmium (Cd) level lower than the permissible limits in the fish liver in Lake Balaton. In the present investigation cadmium (Cd) was recorded above the permissible limits of FAO (1983). Our data is in agreement with the findings of Yilmaz (2009), Yousafzai *et al.,* (2010); Ebrahimi and Taherianfard (2011) and Filazi *et al.,* (2003). Sources of (Cd) in the River Kalpani are burning fuels and untreated effluents from industries.

Cadmium (Cd) is a non-essential and toxic element. It can easily cause chronic toxicity even present in low amount, below 01.00 μg g-1. The permissible limit set for cadmium (Cd) is 02.00 μg g-1 (FAO, 1983). However it is lethal even below its permissible level. Exposure to cadmium (Cd) increases the formation of kidney stones, excretion of calcium from body in urine and skeletal deformities. Cadmium exposure also causes coronary disorder, hypertension, emphysema and chronic pulmonary diseases in humans (Dural *et al.,* 2006; Fianko *et al.,* 2007). All these findings strongly support that cadmium (Cd) is a lethal element. Its level should be thoroughly checked after specific period of time in order to avoid the its life threatening effects.

Bio accumulation of Nickel (Ni)

In the present investigation nickel (Ni) was recorded in the liver tissue of *Wallago attu* with a mean value of 0.72±0.1937 µg/g wet weight. Commonly nickel (Ni) is known for causing renal problems, lung cancer and birth defects (Forti *et al.,* 2011; Sthanadar *et al.,* 2013). However nickel exists normally at a very low level in the environment and causes a variety of health hazards when reaches to human body, including fibrosis, emphysema and different types of tumors (Forti *et al.,* 2011; Sthanadar *et al.,* 2013). The presence of nickel (Ni) in the fish body is known for respiratory failure and ultimately leading to fish death (Palaniappan *et al.,* 2003; Sthanadar *et al.,* 2013). In the present study nickel (Ni) was recorded in the liver tissue in a level below the permissible limits (10 µg g^{-1}) of FAO (1983).

The level of nickel (Ni) in the fish liver was previously reported by Karadede *et al.,* (2004), Yousafzai (2004), Mendil *et al.,* (2005), Vinodhini and Narayanan (2008), Yousafzai *et al.,* (2009), Yilmaz *et al.,* (2009).. Presently we recorded a low level of Ni in the liver of *Wallago attu*. The low level of nickel (Ni) deposition in the fish liver is supported by the findings of Karadede *et al.,* 2004 and Mendil *et al.,* 2005). Karadede *et al.,* (2004) recorded Ni as 1.2-3.4 µg g-1 in the liver of *Silurus triostegus*, from fish of Tokat lakes. Similarly Mendil *et al.,* (2005) recorded a low level of Ni in the liver of *Liza abu* from Ataturk Dam Lake as 0.56-1.06 µg g-1. However interestingly Yilmaz *et al.,* (2009) has reported absence of Ni in the liver of *Leuciscus cephalus* and *Lepomis gibbosus* caught from Saricay, South-West Anatolia. On the other side a high level of metal deposition across the liver is strongly supported. Liver is known as a target organ of heavy metals accumulation (Javed, 2003; Jabeen and Javed, 2011). Studies have shown that heavy metals are generally accumulated in metabolically active organs like liver. Where the stored heavy metals are detoxificated by producing metallothioneins (Carpene & Vasak, 1989; Sunlu *et al.,* 2001; Karadede *et al.,* 2004; Jabeen and Javed, 2011). The higher accumulation in liver may alter the levels of various biochemical parameters in this organ. This may also cause severe liver damage (Mayers and Hendricks, 1984; Ferguson, 1989; Nayaranan and Vinodhini, 2008). At present our findings are not in agreement with the high level of Ni in the fish liver. This really needs further investigations.

Bio accumulation of zinc (Zn)

Previously zinc (Zn) accumulation in the liver tissue was reported (Nemcsok *et al.,* 1987; Van Den Heever and Frey , 1994; Yousafzai *et al.,* 2009, Yousafzai *et al.,* 2010, Yousafzai *et al.,* 2012; Tanee *et al.,* 2013). In the present study zinc (Zn) was recorded in the liver of Mulley, *Wallago attu* with a mean value of 0.72± 0.1937 µg/ wet weight. Primarily, zinc (Zn) enters the fish body through respiratory channel while passing through gills. However zinc (Zn) entry to the fish body is also made via chemical affinity of heavy metals with the fish skin (Sthanadar *et al.,* 2013). Annune and lyaniwura *et al.,* (1993) reported the higher concentrations of Zn in the fish livers of *Oreochromis niloticus* and *Clarias gariepinus* .The elevated level of zinc (Zn) was also reported by Nemcsok *et al.,* (1987), Van Den Heever and Frey (1994). Similarly Rasheed (2001) reported a high level of zinc (Zn) deposition in the liver tissue of *Talapi nilotica* from Nasser Lake, Egypt. Elevated level of zinc deposition in the liver tissue was also supported by the findings of Masoud *et al.,* (2007) and Yang *et al.,* (2007). Young *et al.,* (2007) reported high level of Zn in fish liver from lake Lhasa River in Tabetan Plateau. High level of zinc deposition across the fish liver was also reported by Tanee *et al.,* 2013. High values for the bioaccumulation of zinc (Zn) in the liver tissues are important for onward considerations.

Contrarily, on the other side, Yousafzai *et al.,* (2010) recorded a low level of zinc (Zn) deposition in the liver tissue of *Wallago attu*. Our findings are in agreement with the findings of Yousafzai *et al.,* (2010). Our study reported a low level of zinc in the fish liver. Which is quite within the permissible limits set by FAO (1983). However it is known that zinc (Zn) is an important element for body metabolism and associated with the activities of nearly 100 enzymes involved in lipid, protein and carbohydrate and nucleic acid metabolism in all organisms (Elinder, 1986; Sthanadar *et al.,* 2013). This clearly justifies the body needs for zinc (Zn). Despite of the body needs, zinc (Zn) level should not exceed than the permissible limits (FAO, 1983). A normal fish body needs 50 µg g-1 of zinc. Any exceeding level will definitely result into health hazards of fish as well as for human body on consuming such fish. Sources of zinc in River Kalpani are industrial influents. In the present study heavy metals like zinc (Zn) concentration in the liver tissue of *Wallago attu* was within the limits of FAO (1983).

Conclusions

The study confirms the presence of 04 heavy metals (Cr, Cd, Ni, and Zn) out of 05, with the entire absence of lead (Pb) in the liver tissue of *Wallago attu* collected from Kalpani River. Lead (Pb) was all around absent in the lever tissue. At present no heavy metal has crossed the permissible limits of FAO (1983) except cadmium. The metal accumulation in the liver tissue was in order of Cd>Cr>Zn>Ni. Presently, cadmium (Cd) was proceeding in deposition than Cr, Zn and Ni inside the lever tissue of Mulley, *Wallago attu*. Main causes of the present heavy metals pollution were agricultural runoff, shipping and mining activities occurring in the study area throughout the year. Among heavy metals, cadmium (Cd) is the one, which can cause toxicity even in its low concentration. Further sampling is waiting to periodically check the status of such heavy metals posing serious health hazards at biological level.

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