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White muscle as a bio-indicator of cadmium (Cd) pollution across Kalpani River Mardan, Khyber Pakhtunkhwa Pakistan

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Abstract

In the present investigation we examined the bio accumulation of 05 heavy metals (Pb, Cr, Cd, Ni, Zn) in the muscle tissue of fresh water fish Mulley, *Wallago attu* collected from 04 different polluted sites of Kalpani River in Khyber Pakhtunkhwa province Pakistan, during July 2012 to February 2013. The heavy metals concentration in the muscle of *Wallago attu* was determined by using Perkin Elmer AS 3100 flame atomic absorption spectrophotometer. Heavy metals accumulated in the order Cd>Cr>Zn>Ni in the muscles tissue of *Wallago attu*, with no detection of lead (Pb) at all. Cadmium (Cd) was the highest and nickel (Ni) was the least accumulated heavy metal in fish white muscle. Mean concentrations of detected four heavy (Cr, Cd, Ni, Zn) in the muscle of *Wallago attu* in all 04 samples were 0.44±0.4201, 0.84±0.8283, 0.15±0.1056, 0.31±0.357 µg g -1 (wet weight). Overall, cadmium (Cd) was more accumulated heavy metal in fish muscle as compared to other heavy metals. Our findings suggest that fish muscle have comparatively more chemical affinity to bio accumulate cadmium (Cd) as compared to heavy metals like Zn, Cr and Ni. That is why, cadmium (Cd) is suggested to be a good bio indicator for cadmium (Cd) pollution in aquatic environment.

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Introduction

It is a matter of serious concern over the last few decades that aquatic resources are constantly polluted by various pollutants. Pollutants of multifarious natures are causing damage not only to aquatic life but also posing threat to public water supplies. Which is ultimately unsafe for human life as well (Jan *et al.,* 2002; Karadede *et al.,* 2004; Mendil and Uluozlu, 2007). Among different pollutants causing water pollution, heavy metals are of considerable importance. Heavy metals from natural sources and anthropogenic activities are constantly added into aquatic systems, posing serious threat because of their toxic nature and non bio-degradible nature via process like bioaccumulation and bio-magnification in the food chains (Terra *et al.,* 2008; Bhuvaneshwari *et al.,* 2012a). The regular and steady influx of heavy metals are even endangering certain aquatic species and have already caused the extinction of certain aquatic fauna across the globe (IUCN, 1994; Etuk, 1999).

Fish are primarily selected as bio indicator for the estimation of heavy metals pollution in aquatic ecosystems (Yang *et al.,* 2007; Yousafzai *et al.,* 2010; Tanne *et al.,* 2013). Heavy metals have chemical affinity to bio accumulate in various organs of the aquatic organisms, likely in fish (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 (Scharenberg *et al.,* 1994; Rao and Padmaja, 2000; Bervoeats *et al.,* 2001; Yousafzai, 2004)

Previous studies carried out on different fish species have shown that heavy metals ultimately alter the normal physiology of fish and considerably alter the biochemical parameters of both inside the different tissues and in blood (Canli, 1995; Basa *et al.,* 2003). Similarly, heavy metals are quite known for its toxic effects over the fish body (Tanee *et al.,* 2013). In the present study, we investigated the concentration of 05 heavy metals in the muscle samples of *Wallago attu*, a fresh water carnivorous fish, extremely voracious in feeding nature (Jorgensen and Pedersen, 1994). *Wallago attu* is locally known as Mulley, Mullee, Mullai belonging to the order *siluriformes* and family *siluridae* (Yousafzai *et al*., 2010), found in India, Sri Lanka, Nepal, Bangladesh, Burma, Thailand, Vietnam, Sumatra and Java (Indonesia) (Talwar and Jhingran, 1991). Across Pakistan, it has been reported in all the four provinces including Kalpani River, Kabul River and River Indus. Interestingly, our study fish is quite abundant across the Khyber Pakhtunkhwa province of Pakistan including D.I Khan, Peshawar, Indus plain and adjoining hilly areas (Yousafzai *et al*., 2010). *Wallago attu* is also known as a fresh water shark.

Muscles were chosen for the present investigation of 05 heavy metals (Pb, Cr, Cd, Ni, Zn) in fresh water fish Mulley, *Wallago attu,* collected from Kalpani River in Khber Pakhtukhwa province Pakistan. Muscle is the major tissue of interest under routine monitoring of heavy metal contamination because it is widely consumed by people (Tanee *et al.,* 2013). Kalpani River is considered as one of the polluted water body in Khyber Pakhtunkhwa province Pakistan. However due to its hard and tough access it is not thoroughly checked for its status of pollution and heavily polluted by mining, sewage, shipping, industrial effluents and anthropogenic activities. Thus the aim of the present study was to determine the presence and pattern of selected heavy metals in the muscle of *Wallago attu*. Further to report upon the heavy metal pollution level in River Kalpani as the most neglected area due to hard and tough access.

Materials and methods

Sample collection

For the assessment of bioaccumulation of heavy metals in the muscle of fresh water fish Mullee or Mullai, *Wallagu attu*, 12 fish samples were netted from 04 different polluted sites of Kalpani River, Khyber Pakhtunkhwa, Pakistan. The gills nets (Patti) of the specific 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

Fishes were identified according to the methods described by Talwar and Jhingran (1991), Mirza and Sundho (2007). Weight and length of each fish was precisely noted by using measurement tape and digital balance respectively. After morphometric measurement fishes were washed with distilled water and dissected for muscle tissues. Weighted portions of desired tissues of muscle were separated and shifted to properly marked sterilized polythene bags, stored in the freezer at -20 C^o.

Reagents

Concentrated nitric acid (55%) and Perchloric acid (70%) were used for tissue digestion to extract the heavy metals.

Metal extraction

For the analysis of heavy metals, the tissue digestion was carried out. Tissue samples were thawed, rinsed in distilled water and blotted with blotting paper. Then shifted to 100 ml volumetric flasks already washed with distilled water and dried in oven at 60 C^o for a few minutes. Known weight of each tissue was shifted to volumetric flasks. Samples were digested according to the methods described by 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^o until a transparent and clear solution was obtained. The dense white fume from the flasks after brown fumes was an indication of completion of digestion. By this method digestion was completed in 02-04 hours as stated by Van Loon (1980). 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.

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 muscle tissue of each sample was 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 know the concentration of heavy metals present in the muscle 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 data were plotted on simple bar graphs to see their values conveniently.

Results

The bioaccumulation profile of 05 heavy metals (Pb, Cr, Cd, Ni, Zn) in the muscle 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. A total of 12 fish samples were used. 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 was recoded for all 05 heavy metals (Pb, Cr, Cd, Ni, 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: 0.01, 1.70, 0.00, 0.05 with a mean value and standard error of the mean as, 0.44± 0.4201. Similarly values recorded for cadmium (Cd) at all 04 sites were 0.01, 3.33, 0.03, 0.01 with mean value and standard error of the mean as 0.84± 0.8283. The values for nickel (Ni) deposition in the muscle tissues at selected 04 sampling sites were 0.06, 0.47, 0.02, 0.07 with a mean value and standard error of the mean as 0.15±0.1056. Similarly, bioaccumulation profile of zinc (Zn) across the muscle tissues recorded in fish samples collected from 04 sampling site were 0.37, 0.35, 0.21, 0.32 with a mean value and standard error of the mean as 0.31± 0.357. 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 muscle tissue, lead (Pb) was entirely absent. Cadmium (Cd) was the highest in concentration and nickel (Ni) was the lowest accumulated heavy metal in the muscle 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 muscle tissue was cadmium > chromium > zinc > nickel. Each metal concentration was shown on standard bar graphs to see conveniently, as in Fig 01.

Discussion

Muscle is the major tissue of interest under routine monitoring of metal contamination in aquatic environment because of the edible nature of fish white muscle. It is quite important and valuable to investigate fish muscle for the deposition of heavy metals causing different health hazards to humans and other aquatic organisms while consuming fish as a food. Fish muscle was previously studied for recoding the level of heavy metals causing fresh water and riverine pollution across the aquatic ecosystems (IUCN, 1994; Khan *et al.*, 1999a; Akif *et al.*, 2002 ; Yousafzai and Shakoori, 2009; Yousafzai *et al.*,2012; Abah *et al.*, 2013; Tanee *et al.*, 2013).

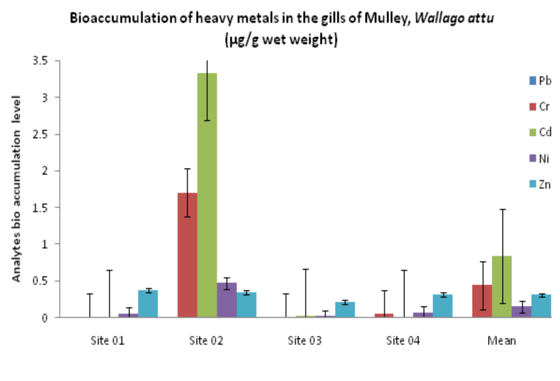

Fig 1. Heavy metals concentrations (μg/g wet weight) in the muscle of *Wallago attu,* Mulley shown on bar graph with bars at their top…

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

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

	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	0.01	1.70	0.00	0.05	0.44	0.44±0.4201
Cd	0.01	3.33	0.03	0.01	0.84	0.84±0.8283
Ni	0.06	0.47	0.02	0.07	0.15	0.15±0.1056
Zn	0.37	0.35	0.21	0.32	0.31	0.31±0.357

At present, 05 heavy metals including lead (Pb), chromium (Cr), cadmium (Cd), nickel (Ni) and zinc (Zn) were investigated in the muscle tissue of fresh water fish Mulley, *Wallago attu* collected from 04 polluted sites of Kalpani River Khyber Pakhtunkhwa Pakistan. The bio accumulation profile of heavy metals showed a highest concentration of cadmium (Cd) deposition across the muscle tissue, positively followed by chromium (Cr) concentration. While nickel (Ni) and zinc (Zn) were low deposited heavy metals in fish muscle. It was interesting to record that cadmium (Cd) was highly deposited heavy metal and nickel (Ni) was the least accumulated heavy metal in the muscle tissue of Mulley, *Wallago attu*. Systematically the bioaccumulation profile of these heavy metals in fish muscle are scrutinized as:

Bio accumulation of Lead (Pb)

In our investigations lead was never detected in any fish sample collected from Kalpani River in Khyber Pakhtunkhwa province Pakistan. However concentration of lead (Pb) deposition is fish muscle was previously recorded by Yap *et al.,* (2005) and Yilmaz *et al.,* (2007), Bhattacharya *et al.,* (2008) and Yousafzai *et al.,* (2010). Regarding lead deposition in fish muscle, Yap *et al.,* (2005) reported a little level of lead (Pb) accumulation in the muscle tissue of Tilapia ,*Oreoclironiis floss*. Similar findings were reported by Yilmaz *et al.,* (2007), Bhattacharya *et al.,* (2008), Yousafzai *et al.,* (2010).

Yilmaz (2007) has recorded a low level of lead accumulation in the muscle tissue of *Leucis cephalus* ranged 0.068-0.874 µg/wet weight, which was quite within the permissible limits of FAO (1983). Contrarily, the findings of Lazorchak *et al.,* (2003) reported the highest level of lead (Pb) in shell fish. The study of Randolph *et al.,* (2004) has also revealed a high level of heavy metals deposition in the muscles tissue of *Lutjanus campechanus*. Likely Yousafzai and Shakoori (2007) also found highest concentration of lead deposition in the muscle tissue of *Tor putitora*. Farkas *et al.,* (2000) has recorded lead (Pb) in Eel (*Anguilla anguilla L*) and Bream (*Abramis brama L*) muscle in 0.93- 0.89 g/ wet weight. Similarly Farkas *et al.,* (2000) recorded lead (Pb) in a Pike- perch (*Stizostedion lucioperca L*) muscle ranged 0.49 – 0.55 g/wet weight and in Eel muscle ranged 0.36 – 0.41 g/ wet weight, which was a highest level of lead (Pb) deposition across the fish white muscle than FAO (1983) permissible limits. But more interestingly Yousafzai *et al.,* (2010) reported a highest level of lead (Pb) in the muscle of *Wallago attu* than in all the tissue of *Labeo dyocheilus*.

Findings about lead (Pb) bioaccumulation across the fish muscles are quite conflicting. Our data has provided no figures about lead (Pb) accumulation in fish muscles. Further investigations will clarify the exact bioaccumulation status of lead (Pb) in fish white muscle. The main 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 lead (Pb) must be thoroughly checked in aquatic ecosystems as it is one of the non essential and 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;). The increased level of industrialization is continuously increasing the risk and damages of lead to humans via different types of food chains. It is highly imperative that industrial effluents and domestic sewage should access the sea after its proper treatment.

Bio accumulation of Chromium (Cr)

In the present study chromium (Cr) in the white muscle tissue of Mulley, *Wallago attu* was recorded with a mean value of 0.44± ±0.4201 µg/ wet weight. Chromium (Cr) accumulation in the fish tissue can easily lead to the fish deaths (Robinoson *et al.,* 2004). Heavy metal like chromium (Cr) travels across the different organs of the fish body and finally reaches to human body by consuming fish muscles which ultimately results into human health hazards like damage to kidney, liver and nervous system (Robinoson *et al.,* 2004).However chromium does not normally accumulate in fish body and hence low concentrations of chromium were reported even from the industrialized part of the world. Its uptake is only higher in young fish but not in adult fish due to its rapid elimination from fish body (Dara, 1995).

Chromium (Cr) accumulation in fish muscle was previously reported by Ahmad and Naim (2008), Xie *et al.,* (2010). Their studies have reported low level of chromium (Cr) deposition in fish muscle. However amazingly, Yousafzai *et al.,* (2010) has recently reported bio accumulation level of chromium (Cr) in Torki, *Labeo dyocheilus* ranged 64.7-105.1 and in Mulee, *Wallago attu* raged 533.3-3206.1 µg/wet weight of the body. Which was in fact a very highest level of chromium (Cr) across the fish muscle. Our findings has reported a low level of chromium (Cr) in the fish muscle of Mulley, *Wallago attu*. Our findings are in agreement with the findings of Ahmad and Naim (2008), Xie *et al.,* (2010). On the other side our data figures are not in the agreement with the findings of Yousafzai *et al.,* (2010). Such a high level of chromium deposition in fish body is really questionable and worthy of future investigations.

Although cromium (Cr) is an essential trace element and play an important role in fish metabolism (Mendil and Uluozlu 2007). Presently the mean concentration of chromium (Cr) in the muscle tissue (0.44± ±0.4201 µg/ wet weight) was quite within the permissible limit (01.00 µg g-1) of FAO (1983) across the Kalpani River in Pakistan.

Bio accumulation of Cadmium (Cd)

Cadmium (Cd) is a non-essential and toxic element among heavy metals. It is considered as an element capable of producing chronic toxicity even when present at concentration below 01.00 µg g-1. However the permissible limits set for cadmium (Cd) is 02.00 µg g-1 (FAO, 1983). Exposure to cadmium (Cd) increases the formation of kidney stones, excretion of calcium in urine and skeletal deformities when long term human exposure occurs to it (Dural *et al.,* 2006; Fianko *et al.,* 2007).

Yilmaz *et al.,* (2007) has recorded a low level of cadmium (Cd) in the muscle tissue of *Oreochromis niloticus* with a mean value of 0.12± 0.02 and in *Mugil cephalus* 0.12± 0.01 from Lake Mugla in Turkey. In the present investigation cadmium (Cd) was recorded 0.84±0.8283 µg/wet weight in the muscle tissues of Mulley, *Wallago attu* collected from Kalpani River. Similarly a low level of cadmium (Cd) in the fish muscle was reported by Mendil *et al.,*(2005), Yilmaz *et al.,* (2007) . Our recorded figure for the bioaccumulation of cadmium (Cd) deposition in the muscle tissue in *Wallago attu* is in agreement with the figure recorded by Yilmaz *et al.,* (2007). Similarly in another study, Yilmaz *et al.,* (2007) also reported a low level of cadmium (Cd) in the muscles of *Leuciscus cephalus* and *Lepomis gibbosus* with a mean value of 0.008± 0.005 and 0.142± 0.064 respectively.

Our findings are in agreement with the findings of Mendil *et al.,*(2005). However Agusa *et al.,* (2004) reported high level of cadmium (Cd) in 05 Caspien sturgeon species. Similarly Kotze *et al.,* (2006) and Senthil *et al.,*(2008) has reported an elevated level of cadmium (Cd) deposition the fish muscles. Ivan *et al.,* (2011) also reported a high level of cadmium (Cd) in fish muscles. More interestingly, Alibabic *et al.,* (2007) has reported a very alarming level of cadmium (Cd) in the fish muscle. All these findings strongly support that cadmium (Cd) in fish white muscle can be used as bio-indicator of heavy metals pollution across water.

Sources of (Cd) are untreated influents of industries. High level of cadmium (Cd) may be of storage role played by body muscles of fish. Which needs future endures to be confirmed. At present, in our findings, cadmium (Cd) was recorded as least accumulated heavy metal in the fish muscle of Mulley, *Wallago attu* with a mean value of 0.84±0.8283 µg/ wet weight. However a highest level of cadmium (Cd) deposition in fish muscles was known. Why cadmium (Cd) accumulation pattern is different, really needs further investigations. Anyhow, it can be assumed that biosequestering or excretion of the cadmium in carnivorous fish like Mulley, *Wallago attu* may be slow as compared to omnivorous fish. Overall high concentration of cadmium (Cd) in the muscle tissues may be due to increased mining activities on the banks of the river or due to the slow rate of excretion of this heavy metal (Heath,1991), or due to more chemical affinity of cadmium (Cd) for muscle tissue

Bio accumulation of Nickel (Ni)

Nickel normally occurs at a very low level in the environment and can cause a variety of health hazards when reached to human body including lungs inflammation, fibrosis, emphysema and tumors (Forti *et al.,* 2011). The presence of nickel (Ni) in fish body is known for respiratory failure and ultimately leading to fish death (Palaniappan *et al.,* 2003). In the present study nickel (Ni) was recorded in the muscle tissue with a mean value of 0.15± µg/ wet weight of the body. Which is extremely below the permissible limit (10 µg g^{-1}) of FAO(1983). The presence of nickel (Ni) was previously investigated by Karadede *et al.,* (2004) , Yilmaz *et al.,* (2007), Yousafzai *et al.,* 2010.

Yilmaz *et al.,* (2007) has reported that nickel (Ni) was not found in the muscle tissues of *Leuciscus cephalus* and *Lepomis gibbosus*. Licata *et al.,* (2003) recorded bioaccumulation level of zinc (Zn) in fish muscle in Lake Faro, in Italy. The concentration of Ni in the muscle of mullet, *M. cephalus* was 3.5 µg g-1. Chandrasekar *et al.*, (2003) also reported a low level of nickel (Ni) (0.18 µg g-1) in the muscle tissue of *O. mossambicus* from kolleru lake, India. Karadede *et al.,* (2004) reported nickel (Ni) as 1.2-3.4µg/wet weight in fish of Tokat Lakes in Turkey. All these studies reported a low level of nickel (Ni) in fish muscles. However Yousafzai *et al.,* (2010) reported a very high level of nickel (Ni) deposition in the muscle tissue of Mulee, *Wallago attu* with a mean value of 106.6 ± 6.8 (µg/g wet weight). Our findings are in line with the findings of Licata *et al.,* (2003), Chandrasekar *et al.*, (2003), Karadede *et al.,* (2004), Yilmaz *et al.,* (2007). On the other side, figure provided by Yousafzai *et al.,* 2010 is really a highest level of nickel (Ni) deposition across the fish muscle. Which needs further studies that why such a highest level of nickel found across the fish muscle. Questions like, Is nickel (Ni) in such a highest level can be found ever found in other fish organ, needs further investigations. At present we found nickel (Ni) in the white muscle tissue of Mulley, *Wallago attu* with a mean value of 0.15±0.1056 µg/ wet weight. Which is quite inside the permissible limits of FAO (1983).

Bio accumulation of Zinc (Zn)

In the present study zinc (Zn) was recorded in the muscles of Mulley, *Wallago attu* with a mean value of 0.31±0.357 µg/ wet weight. Previously zinc (Zn) accumulation was recorded across the different tissues of fish (Badsha and Goldspin, 1982; Karadede *et al.,* 2004; Olaifa *et al.,* 2004; Fernandes *et al.,* 2007; Yilmaz *et al.,* 2007; Yousafzai *et al.,* 2010 ; Yousafzai *et al.,* 2012). 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 (Bhuvaneshwari *et al.,* 2012a).

Fernandes *et al.,* (2007) reported the higher concentrations of Zn in the gills (114 µg g-1) tissue. Similarly Yousafzai *et al.,* (2010) also reported a very high level of zinc (Zn) deposition in the muscle tissue of *Wallago attu* with a mean value of 649.0±107.0µg/ wet weight and *in Labeo dyocheilus* with range of 88.30±185.3 µg/ wet weight. The value (649.0±107.0µg/ wet weight) reported by Yousafzai *et al.,* (2010) for the bioaccumulation of zinc (Zn) in muscle tissue of Mulley, of *Wallago attu* was quite high and quite important to be considered for onward studies. Because it was extremely above the permissible limits of FAO (1983). Which is 50 µg g-1 for zinc (Zn).

Contrarily, on the other side, Yilmaz *et al.,* (2007) recorded zinc (Zn) in the muscles of *Leucisum cephlaus* and *Lepomis gibbosus* with a mean value of 6.50 ±1.237 and 7.870 ± 1.236 respectively. Likely a low level of zinc (Zn) deposition in fish muscle was also reported by Olaifa *et al.,* (2004) with a mean value of 0.66 µg / wet weight in the muscle of *Clarias* *garpienus*. Karadede *et al.,* (2004) also recorded a low concentration of Zn (10.9 µg g-1) in the muscle tissue of cat fish *S. glanis* from the Ataturk Dam Lake (Euphrates), Turkey. Others studies like Badsha and Goldspin, (1982) also suggested that zinc (Zn) is generally low accumulating metal in the white muscle of fish. Our findings are in agreement with the findings of (Badsha and Goldspin, (1982), Olaifa *et al.,* (2004), Yilmaz *et al.,* (2007). Their studies recorded low level of zinc (Zn) in fish muscle which were within the permissible limits set by FAO (1983). However studies conducted on zinc (Zn) accumulation in fish muscle suggests that Zn is an important element for body metabolism. As associated with the activities of nearly 100 enzymes involved in lipid, protein and carbohydrate and nucleic acid metabolism in all organisms (Elinder, 1986). Which shows the the body needs for zinc (Zn). Despite of the body needs for zinc (Zn) still the findings of Yousafzai et al., (2010) are quite notable and questionable as well. Sources of zinc in River Kalpani are industrial influents. In the present study heavy metals like zinc (Zn) concentration in the muscle tissue of *Wallago attu* was within the limits of FAO (1983).

Conclusions

The study confirms the presence of 04 heavy metals (Cr, Cd, Ni, Zn) load across the Kalpani River. There were investigated 05 heavy metals (Pb, Cr, Cd, Ni, Zn) in the muscle tissue of *Wallago attu*. Four out of 05 heavy metals were detected with the exception of lead (Pb). Lead was all around absent in Kalpani River. Presently no heavy metal has crossed the permissible limits of FAO(1983). The metal accumulation in the tissue was in the order Cd>Cr>Zn>Ni. At present, Cd was proceeding in deposition than Cr, Zn and Ni inside the muscle tissue of Mulley, *Wallago attu.* Main causes of the present riverine pollution were agricultural runoff, shipping and mining activities occurring in the study area throughout the year. Further sampling is required to investigate further water pollutants in Kalpani River.

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