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

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Abstract

We examined the bio-accumulation of 04 heavy metals (Cr, Cd, Ni, Zn) in the gills tissue of fresh water fish Mulley, *Wallago attu* collected from 4 different polluted sites of River Kalpani in KPK province Pakistan, during July 2012 to February 2013. The heavy metals (Cr, Cd, Ni, Zn) concentration in the gills tissue of *Wallago attu* was determined by using a graphite furnace atomic absorption spectrometry. Metals accumulated in the order Cd>Zn>Cr>Ni in the gills tissue of *Wallago attu*. Cd was the highest and Ni was the least accumulated metal. Mean concentrations of Cr, Cd, Ni and Zn in Gills of *Wallago attu* in all 04 samples were 0.19± 0.0664, 0.83± 0.8317, 0.05± 0.0281, 0.59± 0.1059 µg g -1 (wet weight). Overall, Cd was more accumulated heavy metal in fish gills as compared to other heavy metals. Our findings suggest that fish gills have comparatively more tendency to bio accumulate Cd as compared to other heavy metals like Zn, Cr and Ni.

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Introduction

The contamination of freshwaters with a wide range of pollutants has become a matter of grave concern over the last few decades, not only because of the threat to public water supplies, but also causing enough damage to the aquatic life (Corbett, 1977; Mance, 1987; Nasreen *et al*., 1995; Jan *et al*., 2002). Which is indeed, ultimately unsafe for humans life as well (Karadede *et al.,* 2004; Mendil and Uluozlu, 2007). Likely, heavy metals from natural sources and anthropogenic activities are continuously added into aquatic systems, causing serious threat because of their toxic nature (Bowlby *et al*., 1988; Bhuvaneshwari *et al.,* 2012a), via bioaccumulation, bio-magnification in food chain and its non bio-degradible nature in ecosystems (Eisler, 1988; USEPA, 1991; Yousafzai and Shakoori, 2008b). The accelerated release of heavy metals is even endangering certain aquatic species and have already caused the extinction of some species in aquatic fauna (IUCN, 1994; Etuk, 1999).

Fish are considered as one of the most indicative factor for the estimation of metals pollution in the aquatic ecosystems (Amaraneni 2006; Yang *et al.,* 2007; Yousafzai *et al.,* 2010). As metals have the tendency to accumulate in various organs of the aquatic organisms, especially in fish (Karadede *et al.,* 2004), which in turn may enter into the human metabolism through consumption causing serious health hazards (Puel *et al*., 1987; USEPA, 1991). Fishes accumulate comparatively high amount of heavy metals as located at the high trophic level in food web. However bio accumulation of heavy metals (Zn, Ni, Cr, Cu, Cd and Pb) varies from organ to organ (Canli & Atli, 2003; Yousafzai, 2004)

Studies carried out on different varieties of fish species have shown that heavy metals ultimately alter the normal mode of physiological activities and biochemical parameters both in tissues and in blood (Tort and Torres, 1988; Canli, 1995; Basa *et al.,* 2003). The toxic effects of heavy metals have been reviewed previously (Waqar, 2006; Yilmaz *et al.,* 2007; Bhuvaneshwari, R. 2012a). In the present study, we investigated the concentration of 04 heavy metals (Cr, Cd, Ni, Zn) in the gill samples of Mulley, *Wallago attu*, a fresh water predatory fish, extremely voracious and carnivorous in nature (Jorgensen and Pedersen, 1994) 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 is found throughout the four provinces of Pakistan including River Kalpani joining River Kabul, a tributary of River Indus. However our study fish is also abundant in D.I Khan, Peshawar, Indus plain and adjoining hilly areas (Yousafzai *et al*., 2010). *Wallago attu* is a fresh water shark, locally known as Mullee, Mulley or Mullai.

Gills were chosen for the present study, based on previously conducted studies. Fish gills are non-edible parts but very important organ due to its main role in the energy release via respiration. Any delicate change in the physiology and anatomy of gills can seriously affect all the vital functions of a fish. Healthy gills easily leads to a healthy fish while retarded growth and weak health of a fish is the clear indication of un healthy gills. Fish with reduced sizes can ultimately lead to the human food shortage.(Burton *et al.,* 1972; Sellers *et al.,* 1975; Bolis *et al.,* 1984; Reid, 1990; Wepener *et al.,* 2001; Burger *et al.,* 2002; Yousafzai and Shakoori, 2008b; Kamaruzzaman *et al.,* 2010; Bhuvaneshwari *et al.,* 2012a). Likely, fish gills are more sensitive organs due to its thin membranous structure and organs of first contact between water born metals and a fish body. As gills are negatively charged that is why positively charged heavy metals are easily accessing to the gills surfaces for deposition at here. (Playle, 1998; Yousafzai and Shakoori, 2008b).

The aim of the present study was to report upon the heavy metals pollution across the Kalpani River, which has not been previously reported due to its hard and tough access. Futher to determine the presence of the selected heavy metals in the fish gills along with its pattern of accumulation.. The present study area really needs a serious attention due to un checked drainage of industrial effluents over here. Kalpani River is considered as one of the neglected area where the un-cheked water pollution can lead to serious human health hazards.

Materials and methods

Sample collection

For the assessment of bioaccumulation of heavy metals in the gills of fresh water fish Mulee, *Wallagu attu*, twenty 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), Jayaram (1999), 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 gills tissues. Weighted portions of desired tissues of gills 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), 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 (Cr, Cd, Ni, Zn) present in the tissue extracts. Air-acetylene was used as fuel for flame. Heavy metals concentrations of chromium (Cr), cadmium (Cd), nickel (Ni) and zinc(Zn) in the gills tissue of each samples 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 were calibrated against the standard curves to know the concentration of heavy metals present in the tissues.

Data generalization and Statistic:

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 package program (Pakage-12, registered).The obtained data were plotted on simple bar graphs to see their values conveniently.

Results

The bioaccumulation profile of 04 heavy metals (Cr, Cd, Ni, Zn) in the gills tissue of fresh water Mulley, *Wallago attu* was recorded by using Perkin Elmer AS 3100 flame Atomic Absorption Spectrophotometer. The heavy metals profile was recoded in triplicate in each sample. Atleast 03 fish samples were selected for the analysis from each 04 sampling site. Therefore, a total of 12 fish samples were used. For convenience a single mean value was shown 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 more accurate values 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 heavy metals.

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

Analytes			Sites			
	Site 01.	Site 02.	Site 03.	Site 04.	Mean	Standard error of Mean
Cr	0.34	0.07	0.10	0.28	0.19	0.19± 0.0664
Cd	0.00	3.33	0.00	0.01	0.83	0.83± 0.8317
Ni	0.09	0.01	0.01	0.12	0.05	0.05± 0.0281
Zn	0.88	0.51	0.38	0.6	0.59	0.59± 0.1059

Values recorded for Cr, from 04 sampling sites were: 0.34, 0.07, 0.10, 0.28 with a mean value and satandard error of the mean as, 0.19± 0.0664. Similarly values recorded for Cd at all 04 site were 0.00, 3.33, 0.00, 0.01 with mean value and standard error of the mean as 0.83± 0.8317. The values for Ni deposition in the gills tissues at all 04 sampling sites were 0.09, 0.01, 0.01, 0.12 with a mean value and standard error of the mean as 0.05± 0.0281. In the same way, bioaccumulation profile of Zn across the gills tissues recorded in fish samples at all 04 sampling site were 0.88, 0.51, 0.38, 0.6 with a mean value and standard error of the mean as 0.59± 0.1059. 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 gills tissue, Cd was the highest in concentration and Ni was the lowest accumulated heavy metal in gills of Mulley, *Wallago attu*. Out of four heavy metals (Cr, Cd, Ni, Zn) studied in this investigation, the order of concentration of metals accumulation in the gills tissue was cadmium > zinc > chromium> nickel. Each metal concentration was shown on standard bar graphs with their relevant values at the top of each bar, shown in Fig 01.

Discussions

Chromium uptake

In the present study Cr in the gills of *Wallago attu*, Mulley was recorded with a mean value of 0.19±0.0664 μ g / g wet weight. Chromium is comparatively less deposited heavy metal in *Wallago attu* as compared to the concentration of Cd and Zn. Heavy metals deposition in the fish gills was previously reported by Burton *et al.*, 1972; Sellers *et al.*, 1975; Van der Putte *et al.*, 1981a, b; Heath, 1991; Spicer and Weber, 1991; Van den Heever and Frey, 1994; Seymore, 1994; Avenant-Oldewage and Marx, 2000.

Zia *et al.,* (1994) have reported elevated levels of Cd, Zn, Cu in gills of rainbow trout, *Oncorhynchus mykiss* with heavy metals burden up to 50% of the total metal burden. The concentration of Cr was reported to be higher in gills of *Tor putitora* (Yousafzai and Shakoori, 2006, 2007). The micro-environment of the gill surface consists of a thin, delicate and porous epithelial membrane which primarily contains phospholipids covered by a mucous layer (Bolis *et al.*, 1984; Van de Winkel *et al.*, 1986). According to Reid (1990) the gill surface is negatively charged and thus provides a potential site for gill-metal interaction sites for positively charged metals (Reid and McDonald, 1991). The main sites of heavy metals uptake in fish according to Lovegrove and Eddy (1982), Annune and Iyaniwura (1993) and Wepener *et al*., (2001) are the gills tissue.

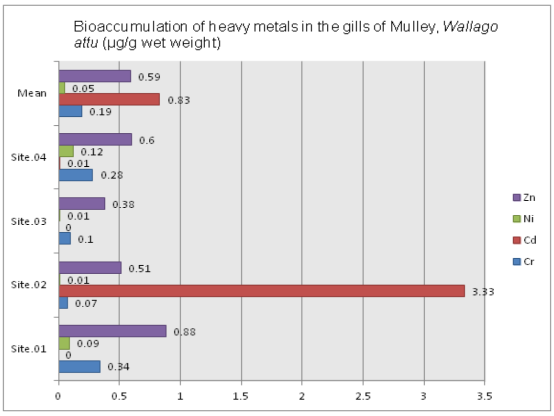

Fig. 1. Heavy metals concentrations (µg/g wet weight) in the gills of *Wallago attu,* Mulley shown by bars with relevant values at their top.

Olaifa *et al.,* (2004) has recorded low level of (Cr) in the gills of *Clarias gariepinus* from fish farm in Ibadan, Nigeria. While Yousafzai and Shakoori, (2006, 2007) have reported high concentrations of (Cr) in the gills of *Tor putitora*. Our findings are same with the findings of Olafia *et al.,* (2004), who has recorded low level of (Cr) in the gills tissue of *Clarias gariepinus*. Source of (Cr) in Kalpani River are air conditioning coolants, engine parts and oil sewages from industries and urban areas. That's why in trace amount it was detected in the gills of *Wallago attu*. However our study revealed that cadmium was the highest in concentration, while nickel was the lowest in gills tissue of *Wallago attu*. Next to Ni in lowest concentration was the bioaccumulation of Cr in the gills tissue of *Wallago attu*. Activities like mining, agricultural activities, city sewage and anthropogenic activities are the possible causing ways of water pollution in the study area in general.

Cadmium uptake

In our present investigation of heavy metals in gills of *Wallago attu*, Mulley, Cd was recorded in highest concentration as compared to other heavy metals. Studies have suggested that in aquatic food chain heavy metals enter through gills of the fish and digestive tract (Burger *et al.,* 2002). Due to physiological active role of gills heavy metals were highly accumulated in gills (Masoud *et al.,* 2007). Yousafzai *et al.,* (2010) also recorded higher metal concentrations of heavy metals in gills. Reid (1990), Reid and Mcdonald (1991) recorded that gills are negatively charged and there is strong potential site for positive charged metals. In the past study Masoud *et al.,* (2007) have recorded high level of (Pb), (Zn), (Cr) in the gills of two different fishes. High levels of heavy metals were also recorded by Yilmaz *et al.,* (2007) in the gills tissue of fish.

Findings of Yilmaz (2008) have reported highest amount of Pb in the gills of *Oreochromis niloticus* , *Leucisus cephalus* and in *Leporrus gibbosus*.. However the findings of Farkas *et al.,* (2000) are contrary. As a low concentration of Pb in the gills of Eel, Bream had ebbn reported by Farkas *et al.,* (2000). Our findings are indirectly in agreement with the findings of Farkas *et al.,* (2000). Sources of lead are boat oil and break emissions in River Kalpani. Pb was not detected in the gills of *Wallago attu*, Mulley, that is why its details were not included over here.

In the present study Cd was detected in the gills of Wallago attu in highest concentration with a mean value of 0.83±0.8317 µ g / g wet weight as compared to other heavy metals deposition in the gills tissue. Contrarily Farkas *et al.,* (2000) recorded low level of (Cd) in Bream, Pike perch and Eel from Lake Balaton Tihaney Hungary. Likely Yilmaz *et al.,* (2007) also recorded low level of (Cd) in the gills of *leuciscus cephalus* and *Lepomis gibbosus*. However Rauf. A *et al.,* (2009) recorded high level of (Cd) in the gills of *Catla Catla, labeo rohito and Cirrtuna mrigala* from the River Ravi Pakistan. In the past study Dural *et al.,* (2007) recorded high level of cadmium in fish gills. Ploetz *et al.,* (2007) also reported (Cd) in gills in high level in *Scomberomorus cavalla, Sparus aurata, Dicentractus labrax, and Mugil cephalus.* Our findings are in line with the findings of Rauf *et al.,* (2009) and Dural *et al.,* (2007), who has also recorded high level of (Cd) in fish gills. Sources of (Cd) in River Kalpani are fuel burning, tire burning and use of batteries.

Nickel uptake

In our present study, bioaccumulation of Ni was recorded in the least amount with a mean value of 0.05±0.0281 µ g / g wet weight as compared with the concentration of other heavy metals like Cd, Zn and Cr. Nickel accumulation in gills was previously studied by Vos and Hovens(1986), Tjälve *et al.* (1988), Seymore (1994), Barnhoorn (1996), Coetzee (1996), (Kotze (1997), Yousafzai and Shakoori (2006, 2007, 2008b). Regarding bioaccumulation of heavy metals in the fish body was confirmed by Vos and Hovens (1986), Tjalve *et al.*, (1988). Nickel was considered as highly depositing heavy metals in fish body as compared to its concentration in ambient water. Similar findings were supported the work of Seymore (1994), Barnhoorn (1996), Coetzee (1996), Kotze (1997). The work of Tjalve *et al.*, (1988) reported that fish species have accumulated Ni in all the tissues, but the data indicated that the gills contained the highest levels of Ni, followed by the liver, muscle and skin. It is possibly due to Ni uptake via the gills as a result of its closed blood-water contact. Likely Yousafzai and Shakoori (2006, 2007) have confirmed a highest concentration of Ni in the gills followed by in liver and muscles. In another study Yousafzai and Shakoori (2008b) have reported exactly the same findings in their work over an endangered South Asian Freshwater Fish *Tor putitora* in River Kabul, Pakistan. However our findings are not in line with the findings of the previous works carried over Ni deposition in gills tissue of fish. However if our findings about Ni concentration in the gills tissue compared with the Ni concentration in the ambient water, It was assumed that Ni concentration may be more in fish body as found inside the ambient water. It needs further work to be done. Further sources of Ni are not properly accessing the River Kalpani like other heavy metals sources.

Zinc uptake

Dethloff *et al.* (1999) studied *bioaccumulation* of Zn in the gills of *Oncorhynchus mykiss*, Rainbow trout. The bioaccumulation of Zn in the fish gills was previously reported by Sellers *et al.*, (1975), Avenant-Oldewage *et al.* (2000) in *Clarias gariepinus,* Coetzee *et al.* (2002), Yousafzai and Shakoori (2008b) in *Tor putitora*. Regarding Zn concentration in the gills, a significantly variable amount was reported in fish gills of *Oncorhynchus mykiss* (Dethloff *et al.*, 1999). However studies have suggested that Zn was accumulated comparatively in high concentration as compared to accumulation in other organs (Sellers *et al.*, 1975). High amount of Zn concentration in gills of *Clarias gariepinus* was also reported by Avenant-Oldewage *et al.* (2000). Our findings are in line with the findings of Dethloff *et al.* (1999), Sellers *et al.*, (1975) and Avenant-Oldewage *et al.* (2000). Coetzee *et al.* (2002) studied the bioaccumulation of heavy metals Pb, Cr, Ni, Mn, Al, Fe and Zn in the skin, muscle, liver and gill tissue of *Clarias gariepinus* and *Labeo umbratus.* Both fish study clearly showed that the highest concentrations were found in the gills and liver followed by the skin and muscle. Likely our work is supported by Coetzee *et al.*, (2002) work. The only difference in our work was found that gills of *Wallago attu,* Mulley accumulated Cd in highest concentration in gills tissue then followed by highest concentration of Zn. Which needs further investigations that why Cd is exceeding than Zn. On the basis of our findings it was assumed that Cd was comparatively deposited more than Zn inside the gills tissue. However such findings are awaited to be highlighted by other investigations.

Conclusions

The study confirms the presence of heavy metals load in the River Kalpani like the other South Asian rivers. There were investigated 04 heavy metals (Zn, Ni, Cd, Cr) in the gills tissue of *Wallago attu.* Presently the concentrations of Ni and Cr have crossed the limits of RDA, while Cd and Zn are still within the limits. The metal accumulation in the tissue was in the order Cd>Zn>Cr>Ni. Presently, Cd is depositing more than Zn inside the gills tissue.

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