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Aquatic pollution assessment using skin tissues of mulley (*Wallago attu*, Bloch & Schneider, 1801) as a bio-indicator in Kalpani river at District Mardan, Khyber Pakhtunkhwa, Pakistan

Iram Alam Sthanadar[1], Aftab Alam Sthanadar[1*,2], Benazir Begum[3], Muhammad Jamal Nasir[4], Imtiaz Ahmad[5], Ali Muhammad[1], Muhammad Zahid[1], Sana Ullah[6]

[1]*Department of Zoology, Islamia College University, Peshawar, Khyber Pakhtunkhwa Pakistan*

[2]*Department of Zoology & Animal Sciences, Post Graduate College Dargai Malakand, Khyber Pakhtunkhwa, Pakistan*

[3]*Department of Physics, Government Girls College Dargai, Malakand Khyber Pakhtunkhwa, Pakistan*

[4]*Department of Geography, University of Peshawar, Khyber Pakhtunkhwa, Pakistan*

[5]*Department of Chemistry, Islamia College University, Peshawar, Khyber Pakhtunkhwa, Pakistan*

[6]*Department of Animal Sciences, Quaid-i-Azam University, Islamabad, Pakistan*

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Abstract

Bioaccumulation profile of five heavy metals including lead (Pb), chromium (Cr), cadmium (Cd), Zinc (Zn) and Nickel (Ni) were investigated in the skin tissue of fresh water fish Mulley, *Wallago attu,* to assess aquatic pollution in Kalpani river at District Mardan, Khyber Pakhtunkhwa Pakistan. Specimens were collected of three different polluted sites of the river during the months of July through October, 2013. The heavy metals concentration was determined by using Perkin Elmer AS 3100 flame atomic absorption spectrophotometer. Pb was not detected in any of the specimens collected from the river while Cd was the highest and Ni was the least accumulated metal for all sampling months and sites. Mean values recorded (wet weight) for Cr, Cd, Zn and Ni were 0.168±0.326 µg g^{-1}, 0.747±1.106 µg g^{-1}, 0.328±0.074 µg g^{-1} and 0.161±0.156 µg g^{-1} respectively. The results showed metals bio accumulation in skin of Mulley in order of Cd>Zn>Cr>Ni, with no detection of Pb. For data validation, statistics of Pearson correlation coefficient matrix was calculated ($r > 0.5$) It was concluded that Cr and Ni were higher than RDA permissible limits and fish skin act as a primary exposed target to aquatic pollutants.

***Corresponding Author:** Aftab Alam Sthanadar ✉ aftabalam.bio@gmail.com

Introduction

Pollution of freshwater aquatic bodies and riverine ecosystems is attributed to heavy metals including some trace metals as well. The current continuously deteriorating pollution scenario is staged by the bulky level of industrial effluents joining aquatic systems without any proper treatment, because of definitely asking more sum of funds and resources. Aquatic pollution is a matter of grave concern around the globe on account of creating many serious and health concerns for aquatic organisms including fish and humans as well (Sthanadar *et al.,* 2013a).

The scenario of aquatic pollution is prevailed at global level 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 Uluözlü, 2007). Among various causes of fresh water and riverine pollution, heavy metals are of considerable importance and consideration (Sthanadar *et al.,* 2013a). Heavy metals are known as high density metallic elements or stable metals with density greater than 5 to 6 g cm^{-3} 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.,* 2012).

Generally water pollution is accredited to the presence of enough harmful or objectionable material that damages its quality. Water pollution has many sources and characteristics such as entrance of bodily wastes produced by humans and other organisms to rivers, lakes, oceans and other surface water. Industries are also playing its prime role by manufacturing new chemicals each year, all of which eventually homes in aquatic systems (Ullah *et al.,* 2014). The aquatic ecosystems are the ultimate recipient of type of pollutants including heavy metals. Aquatic micro flora and micro fauna, which constitute fish food, are capable of incorporating and accumulating heavy metals into their cell from their environment. Small fish become enriched with accumulated heavy metals. Predatory fish generally display higher levels of heavy metals than their prey and eventually man on consuming the predatory fish, suffer from the results of an enrichment having taken place at each tropic level (Obasohan *et al.,* 2006).

Heavy metals like, chromium, copper, zinc, nickel, lead etc, are some of the major components of industrial wastes, which along with other products from industrial operations are discharged into the aquatic environment. These substances are toxic to aquatic life (Dutton *et al.,* 1988). Fish are at the high tropic level of food web and may accumulate large amounts of some metals from the water and often in concentrations several times higher than in the ambient water. Heavy metals are taken up through different organs of the fish because of the affinity between them. In this process, many of these heavy metals are concentrated in various amounts in different organs of fish (Rao and Padmaja, 2000; Bervoets *et al.,* 2001). Heavy metals are also potentially accumulated in different parts of marine environments as well including water, sediments and fish. These are subsequently transferred to human beings as fish consumption has been increased among the health conscious due to their high protein supply, low saturated fat and omega fatty acids content that are known to contribute to good health and are handy for various diseases (Copat *et al.,* 2012; Sthanadar *et al.,* 2013c; Ullah and Ahmad, 2014).

Likely, heavy metals like copper, iron and zinc are essential for fish metabolism, while some others such as mercury, cadmium and lead have no known role in biological systems. For normal metabolism the essential metals must be taken up from water or food, but excessive intake of the essential metals can produce toxic effects (Sthanadar *et al.,* 2013b). Studies from the field and the laboratory experiments reveal that accumulation of heavy metals in fish is

mainly dependent upon metals concentration in ambient water and exposure period, although some other factors including water salinity, pH, hardness, water temperature, ecological needs, size and age, life cycle, collection season and feeding habits of fish also play a significant role in metal accumulation (Canli and Furness, 1993).

Heavy metals are indeed of serious concerns, of its toxic and bio-chemically non bio-degradable nature. They ultimately enter into the food chains and results in bioaccumulation or bio-magnification, and notably cause physiological and morphological alterations in the fish as well as in human bodies (Vinodhini and Narayanan, 2008; Bhuvaneshwari *et al.,* 2012). Relevantly, fish is a good bio indicator for the estimation of heavy metals pollution in aquatic environment (Karadede *et al.,* 2004; Yalmaz *et al.,* 2007; Yang *et al.,* 2007; Yousafzai *et al.,* 2010). These metals have enough chemical affinity to bio accumulate in various organs of the aquatic organisms (Karadede *et al.,* 2004; Bhuvaneshwari *et al.,* 2012).

Skin is the most exposed body part of the fish. Skin accumulates metals through absorption. Higher load of contaminants results in higher accumulation of metals through skin. The blood stream transportes the theses metals to various tissues (Van der Putte and Part, 1982). Most of the population consumed via skin and reaches to muscles. Likely, the absorbed metals ultimately approach to human digestive system and results in devastating miseries for most of the times. 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; Abah *et al.,* 2013; Ambedkar and Muniyan, 2011; Tanee *et al.,* 2013).

The present study was aimed to determine the presence of heavy metals in the skin tissue of *Wallago attu,* collected from Kalpani River at District Mardan, Khyber Pakhtunkhwa Pakistan, to further pave the way for health hazards caused by these heavy metals, when such fish muscles are consumed. The present study will open further gateways for future research regarding health issues caused by such polluted white meat.

Materials and methods

Sample collection

To assess the bioaccumulation profile of heavy metals in the skin of fresh water fish Mulley, *Wallago attu*, initially a total of 20 fish samples was collected from three different polluted sites of Kalpani River at District Mardan, 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 and were then washed with distilled water.

Fish identification and dissection

Fish specimens collected were identified using standard keys of Talwar and Jhingran (1991), and Mirza and Sandhu (2007). 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. Weighted portions of desired tissues of skin were separated and were shifted to properly mark 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 skin tissue for heavy metals including lead (Pb), cadmium (Cd), chromium (Cr), zinc (Zn) and nickel (Ni), tissues were digested. 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 done according to Van Loon (1980), and Due Freez and

Steyn (1992). At the same time 10 ml nitric acid (55%) and 5 ml per Chloric acid (70%) were added to each flask. The flasks were then placed on hot plate and allowed to digest at 200°C 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 completion of digestion. 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 skin 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 skin tissues.

Data generalization and Statistics

Data obtained was generalized and the results were expressed as mean ± standard deviation. Pearson correlation coefficient was calculated for each month for the observed Analytes. Statistical analysis of data was carried out using MS Excel 2013.

Results and discussion

The bioaccumulation profile of the studied heavy metals including lead (Pb), chromium (Cr), cadmium (Cd), zinc (Zn) and nickel (Ni) in the skin tissue of fresh water Mulley, *Wallago attu* was analyzed by using Perkin Elmer AS 3100 flame Atomic Absorption Spectrophotometer. The heavy metals profile was recorded in triplicate for each sample. Out of the total collected twenty fish specimens from three different sites in the river, three healthy individuals were selected for carrying out the analysis. Table 1 is showing recorded values of the studied heavy metals for each month of the study period.

Table 1. Month wise heavy metal concentrations in the skin of *Wallago attu* collected from different sites of River Kalpani, District Mardan Khyber Pakhtunkhwa Pakistan.

Analytes	Jul	Aug	Sep	Oct	Mean ± St. Deviation
Pb	0	0	0	0	0± 0.0000
Cr	0.61	0.07	0.04	0.11	0.20± 0.1349
Cd	0.004	6.66	0.01	0.01	1.67± 1.6633
Ni	0.34	0.07	0.02	0.20	0.15± 0.0717
Zn	0.38	0.30	0.39	0.23	0.32± 0.0375

Lead was not detected in any of the collected sample. The mean values recorded for chromium were 0.617, 0.147, 0.157 and 0.063 with a mean value of 0.168± 0.326. Mean cadmium ranged from 0.004 to 2.223 with a mean value of 0.747±1.106. The mean values for nickel deposition across the sampling sites were 0.525, 0.09 and 0.34 with a mean of 0.161±0.156. The mean values of Zinc ranged from 0.233±0.390 for all sampling sites. The heavy metals accumulation took place in an order of cadmium > zinc > chromium > nickel. Table 2 is showing month wise as well as site wise results for the studied parameters.

The strongest correlations ($r > 0.5$, $p = 0.001$) with Cadmium across all sampling sites included Nickel (0.776) in the month of July, Zinc (0.997) in the month of August, Chromium (0.998) in the month of September and Nickel (0.891) in the month of October. The strongest correlations ($r > 0.5$, $p = 0.001$) with Zinc across all sampling sites was shown by Nickel in the months of July (0.979), August (0.886) and September (0.771). The strongest correlations ($r > 0.5$, $p = 0.001$) with Chromium across all sampling sites included Cadmium (0.667) followed by Zinc (0.604) in the month of August,

Cadmium in the month of September (0.998) and October (0.822). The strongest correlations (r > 0.5, p = 0.001) with Nickel across all sampling sites included Cadmium (0.979) followed by Zinc (0.776) in the month of July, Zinc (0.886) in the month of August, Chromium (0.925) followed by Cadmium (0.901) in the month of September and Chromium (0.991) followed by Cadmium (0.891) in the month of October. Tables 3 to 6 are showing Pearson correlation coefficient matrix of the studied Analytes for the month of July, August, September and October respectively.

Table 2. Site Wise heavy metals' concentrations in the skin of *Wallago attu* collected from different sites of River Kalpani, District Mardan Khyber Pakhtunkhwa Pakistan.

Analytes	Months	Site 1	Site 2	Site 3	Mean ± St. Deviation
Pb	July	0	0	0	0 ± 0.000
	August	0	0	0	0 ± 0.000
	September	0	0	0	0 ± 0.000
	October	0	0	0	0 ± 0.000
Cd	July	0	0.001	0.01	0.004 ± 0.006
	August	0	0.01	6.66	2.223 ± 3.842
	September	0	0.01	0.03	0.013 ± 0.015
	October	0.03	0.01	0.01	0.017 ± 0.012
Zn	July	0.4	0.68	0.07	0.383 ± 0.305
	August	0.4	0.43	0.09	0.307 ± 0.188
	September	0.41	0.32	0.44	0.390 ± 0.062
	October	0.29	0.12	0.29	0.233 ± 0.098
Cr	July	1.74	0	0.11	0.617 ± 0.974
	August	0.23	0.12	0.09	0.147 ± 0.074
	September	-0.37	-0.23	0.13	-0.157 ± 0.258
	October	-0.07	0.05	0.21	0.063 ± 0.140
Ni	July	0.08	0.22	0.73	0.343 ± 0.342
	August	0.08	0	0.15	0.077 ± 0.075
	September	-0.04	-0.07	0.17	0.020 ± 0.131
	October	0.09	0.21	0.31	0.203 ± 0.110

Table 3. Correlation coefficient matrix of the studied Analytes for the month of July.

Analytes	Cd	Zn	Cr	Ni
Cd	1			
Zn	**-0.889**	1		
Cr	-0.450	0.250	1	
Ni	**0.979**	**-0.776**	**-0.623**	1

Bold r-Values >0.500 are significant at p < 0.05.

Table 4. Correlation coefficient matrix of the studied Analytes for the month of August.

Analytes	Cd	Zn	Cr	Ni
Cd	1			
Zn	**-0.997**	1		
Cr	**-0.667**	**0.604**	1	
Ni	**0.845**	**-0.886**	-0.166	1

Bold r-Values >0.500 are significant at p < 0.05.

Table 5. Correlation coefficient matrix of the studied Analytes for the month of September.

Analytes	Cd	Zn	Cr	Ni
Cd	1			
Zn	0.419	1		
Cr	**0.998**	0.472	1	
Ni	**0.901**	**0.771**	**0.925**	1

Bold r-Values >0.500 are significant at p < 0.05.

Table 6. Correlation coefficient matrix of the studied Analytes for the month of October.

Analytes	Cd	Zn	Cr	Ni
Cd	1			
Zn	**0.500**	1		
Cr	**-0.822**	0.082	1	
Ni	**-0.891**	-0.052	**0.991**	1

Bold r-Values >0.500 are significant at p < 0.05.

Skin is the most exposed part in the fish body. Metals in the skin accumulated through adsorption followed by their absorption. When there are more contaminants in the water, higher amount of metals will be accumulated. Most of the world populations consume fish with skin. From contaminated water metals are ingested via the skin. Blood stream transports it to various tissues (Van der Putte and Part, 1982). In aquatic system once heavy metals enter, its accumulation may occur in fish which also expose metal contaminated water. In the present study fish skin were analyzed for the estimation of Pb, Cr, Cd, Ni and Zn from River Kalpani.

In the present study lead was not detected in the skin of *Wallago attu*. Our findings are quite different from the findings of Yilmaz, 2003. He has recorded Pb with a mean value of 37.39 µg g^{-1} and 4.78 µg g^{-1} (wet weight) in the skin of *Mugilcephalus* and *Trachurus mediterrenuus* caught at Iskenderum Bay Turkey. Yousafzai and Shakoori (2006) also recorded high level of Pb in the skin of *Tor putitora* caught from

river Kabul. He has recorded it with a mean value of 217.9±1.93 µg g^{-1} wet weight, which may be due to high load of lead in river Kabul. Skin is an important excretory organ as metals accumulation is low in the skin. In the present study Pb was not detected in the skin of *Wallago attu* as the main source of Pb is road dust and River Kalpani is far away of road and other transporting links. Eisler (1988) also recorded Pb concentrations in the guppy fish and attributed it to road dust.

Mean Cadmium recorded in the skin of *Wallago attu* was 1.68 µg g^{-1} (wet weight). Cd is either inhaled or ingested from contaminated food. It can cause serious complication of gastro intestinal tracts and kidneys in mammals even if taken in a meagre amount of 200 ppm (Bremner, 1978). Our findings are in agreement with the findings of Yilmaz (2003) and Raynders *et al.* (2008) who has also recorded (Cd) in the skin of *Mugil cephalus* from Iskenderun Bay in Turkey, and in the skin of *Mughil cephalus* nete River system in Belgium respectively. In the present study Cd is highly accumulated metal in the skin of *Wallago attu* followed by Zn in River Kalpani. The sources of Cd are fuel burring, oil emissions and batteries used in industries and in urban area situated in nearby riverine areas. Fig. 1 is showing Cd contents of the skin across all sampling months.

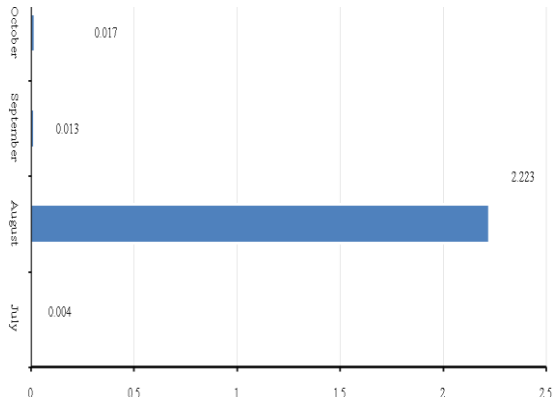

Fig. 1. Cadmium concentrations (mg g^{-1} wet weight) in skin of *Wallago attu* collected from different sites of River Kalpani.

Zn was recorded with a mean value of 0.328 µg g^{-1} (wet weight). In the skin of *Wallago attu* Zn ranked as second highest accumulated metal after Cd. Our findings are similar to that of Yilmaz (2003). The present result is also in agreement with the findings of Hondal *et al.,* (1983) worked on Antarctic fish *Pagotheni aborchgrerinki* collected from Antarctica. Yousfzai *et al.,* 2010 also reported Zn as a highly accumulated metal in the skin of *Cyprinus carpio*. Presence of Zn in river Kalpani may be due to grease and motor oils entrance from industries and urban areas. Fig. 2 is showing Nickel contents of the skin across all sampling months.

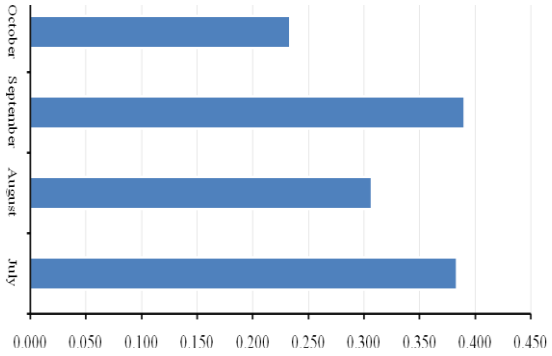

Fig. 2. Zinc concentrations (mg g^{-1} wet weight) in skin of *Wallago attu* collected from different sites of River Kalpani.

Chromium in the skin of *Wallago attu* was with a mean value of 0.168±0.326. Sources of Cr include air conditions, coolants, engine parts and brake emissions. Higher amount of Cr in River Kalpani may be due to the presence of untreated effluents from industries and urban area. Yilmaz (2003) recorded higher amount of Cr 3.22 and 10.90 (µg g^{-1} wet weight) in the skin of Mugilcephalus and Treacherous Mediterranean respectively collected from Iskenderum Bay in Turkey, which may be attributed to higher dumping of these effluents to the Bay. Fig. 3 is showing Cr contents of the skin across all sampling months.

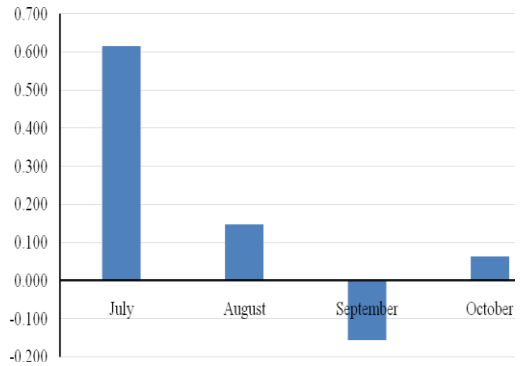

Fig. 3. Chromium concentrations (mg g^{-1} wet weight) in skin of *Wallago attu* collected from different sites of River Kalpani.

In the present study, Ni in the skin of *Wallago attu* is least accumulated with a mean value of 0.161 µg g^{-1} (wet weight). Sources of Nickel are mining activities and industrial wastes (Pyle *et al.,* 2002). Our findings are same to the results of Tjalve *et al.,* (1988) while different from the findings of Yilmaz (2003). Tjalve *et al.,* also recorded Ni as the least accumulated metal in skin. They concluded that it may be due to omnivorous nature of the fish species they studied. Fig. 4 is showing Nickel contents of the skin across all sampling months.

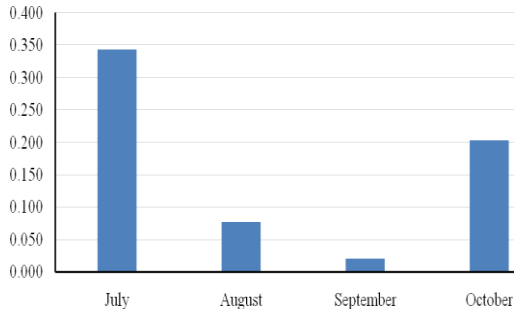

Fig. 4. Nickel concentrations (mg g^{-1} wet weight) in skin of *Wallago attu* collected from different sites of River Kalpani.

Conclusion

The study confirms the presence of four heavy metals (Cr, Cd, Ni, and Zn), with the entire absence of Pb in the skin tissue of *Wallago attu* collected from Kalpani River. In the present investigation Cd and Ni have exceeded the permissible limits suggested by RDA (Adeye, 1993), while Cr and Zn were in permissible limits. The metals accumulation in the skin tissue was in order of Cd >Zn >Cr >Ni. The higher level of heavy metals may be attributed to the presence of grease and motor oil in river water, dumped from industries and urban areas situated in the study area. Regular assessment of these heavy metals is recommended; as the current scenario can be worsen if ignored. Environmental protection agency should play its role for protection and conservation of the river, with respect to water quality and biodiversity.

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