Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print), 2222-3045 (Online)
http://www.innspub.net
Vol. 6, No. 2, p. 74-80, 2015

INNSPUB

RESEARCH PAPER OPEN ACCESS

Bioaccumulation of heavy metals in intestine of mulley (*wallago attu*, bloch & schneider, 1801): a case study of Kalpani river at district mardan, Khyber Pakhtunkhwa, Pakistan

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

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

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

[3]*Department of Zoology & Animal Sciences, Post Graduate 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*

Key words: Bioaccumulation, Heavy metals, Intestine, Kalpani River, *Wallago attu*,

Article published on February 01, 2015

Abstract

The concentration of five heavy metals like lead (Pb), chromium (Cr), cadmium (Cd), Zinc (Zn) and Nickel (Ni) were assessed in the intestine of freshwater fish Mulley (*Wallago attu*), collected from Kalpani river at district Mardan, Khyber Pakhtunkhwa, Pakistan. Sampling was carried out during the months of July to October, 2013. For determining heavy metals concentration, Perkin Elmer AS 3100 flame atomic absorption spectrophotometer was used. Heavy metals concentration was varying among sampling months and sites. Overall mean values recorded (wet weight) for Cr, Cd, Zn and Ni were 0.069±0.068 µg g^{-1}, 0.008±0.004 µg g^{-1}, 0.427±0.100 µg g^{-1} and 0.059±0.032 µg g^{-1} respectively. However, Pb was not detected in any of the collected sample across all the sampling sites. The results showed metals bio accumulation in the intestine of Mulley, in order of Zn>Cr>Ni>Cd with no detection of Pb. Results showed, all the studied heavy metals were falling in the suggested permissible limits. In order to conserve and maintain the water, sediments and fish biodiversity of the river, regular assessment should be carried out and strict environmental policies should be planned by environmental protection agencies. Mass awareness should be initiated. The effluents and other wastes from the industries in the nearby areas should be treated before entering the river.

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

Introduction

In recent years, research regarding fish as well as fish consumption has elevated simultaneously on account of their nutrition worth and therapeutic benefits (Ullah and Ahmad, 2014). Fish is a cheaper and balance source of protein, vitamins, essential minerals and unsaturated fatty acids (Medeiros *et al.,* 2012). AHA (American Heart Association) recommend fish use at least two times per week to insure the daily required intake of omega-3 fatty acids (Kris-Etherton *et al.,* 2002). On the other hand, fish are comparatively positioned at the top of food chain in aquatic environments, consequently they can accumulate various heavy metals from sediments, water and food (Yilmaz *et al.,* 2007; Zhao *et al.,* 2012; Sthanadar *et al.*, 2013a). As several ill effects of heavy metals have been known for longer time, so content of these toxic metals can counter the beneficial effects of fish (Castro-Gonzalez *et al.,* 2008). This may lead to some serious life threating issues such as liver damage, renal failure and cardiovascular diseases (Al-Busaidi *et al.,* 2011; Rahman *et al*, 2012; Sthanadar *et al.,* 2013b). Owing to the situation, many international monitoring programs got establish to evaluate and assess fish quality for human consumption and for monitoring aquatic ecosystem worth (Meche *et al.,* 2010).

In the last few years, heavy metals concentration has been widely studied in fish different tissues all around the world (Elnabris *et al.,* 2013). These studies were focused on bioaccumulation of heavy metals in different parts of edible fish such as muscles, kidneys, liver, gonads, heart, digestive tract, bone and brain (Sthanadar *et al.,* 2013; El-Moselhy *et al.,* 2014). Available literature confirms metals bioaccumulation and their consequent varying distribution inter specifically. These variation in metals uptake is attributed to different factors like feeding behaviour, size, age, sex, reproductive cycle and their geographic locality (Mustafa and Guluzar, 2003; Zhao *et al.,* 2012).

Studies regarding heavy metals concentration in fish different tissues have been carried out on different rivers of Pakistan. These studies reported occurrence of various heavy metals in bodies of different fish. The present study was conducted on river Kalpani at district Mardan, Khyber Pakhtunkhwa, Pakistan. The bioaccumulation of heavy metals was determind in the intestinal tissue of fresh water fish specie, Mullay (*Wallago attu*), collected from different polluted sites of kalpani River at Khyber Pakhtunkhwa province, Pakistan. Bioaccumulation of heavy metals in fish tissues is a considerable issue across the globe (Sthanadar *et al.*, 2013a), which ultimately leads to human health hazards when white meat is consumed along the accumulated heavy metals. The findings of the present study has attempted to determine the level of heavy metals accumulation in fish intestinal tissue, which will further lead to future gateways of molecular findings regarding the considered issue in the study area in the better interests of biotic as well human beings.

Materials and methods

Sample collection

To assess the bioaccumulation profile of heavy metals in the intestine of fresh water fish Mulley, *Wallago attu*, initially a total of 20 fish samples were collected from 03 different polluted sites of Kalpani River at District Mardan, Khyber Pakhtunkhwa, Pakistan. The gill 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

Samples 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 were separated and were shifted to properly mark sterilized polythene bags, stored in the freezer at -20°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 intestine 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 Preez 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 in the intestinal tissues of each sample were analysed 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 desired intestine 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 and sampling site 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 intestine tissue of fresh water Mulley, *Wallago attu* collected from river Kalpani was analysed. 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 as well as across all the three sampling sites. The heavy metals accumulation took place in the order of Zn>Cr>Ni>Cd.

Lead was not detected in any of the months during study period. The mean values recorded for chromium were 0.03, 0.037, 0.197 and 0.013 for the month of July, August, September and October respectively with a mean value of 0.069±0.086. The mean values recorded for cadmium were 0.007, 0.003, 0.01 and 0.013 for the month of July, August, September and October respectively with a mean value of 0.008± 0.004. The mean values recorded for zinc were 0.283, 0.483, 0.437 and 0.503 for the month of July, August, September and October respectively with a mean value of 0.427±0.100. The mean values recorded for nickel were 0.093, 0.05, 0.02 and 0.073 for the month of July, August, September and October respectively with a mean value of 0.059± 0.031. The heavy metals accumulation took place in an order of Zn>Cr>Ni>Cd. Table 2 is showing month wise concentration of the observed heavy metals in Mulley, *Wallago attu*.

Table 1. Heavy metals concentration in intestine of Mulley.

Analytes	Months	Site 1	Site 2	Site 3	Mean ± SD
Lead					
	July	0	0	0	0.000±0.000
	August	0	0	0	0.000±0.000
	September	0	0	0	0.000±0.000
	October	0	0	0	0.000±0.000
	Mean ± SD	0.000±0.000	0.000±0.000	0.000±0.000	
Cadmium					
	July	0.01	0.01	0	0.007±0.006
	August	0	0.01	0	0.003±0.006
	September	0.01	0.01	0.01	0.010±0.000
	October	0.02	0.01	0.01	0.013±0.006
	Mean ± SD	0.010±0.008	0.010±0.000	0.005±0.006	
Zinc					
	July	0.11	0.59	0.15	0.283±0.266
	August	0.21	0.57	0.67	0.483±0.242
	September	0.54	0.48	0.29	0.437±0.131
	October	0.31	0.62	0.58	0.508±0.169
	Mean ± SD	0.293±0.184	0.565±0.060	0.423±0.243	
Chromium					
	July	0.09	0	0	0.030±0.052
	August	0.11	0	0	0.037±0.064
	September	0.34	0.25	0	0.197±0.176
	October	0.04	0	0	0.013±0.023
	Mean ± SD	0.145±0.133	0.063±0.125	0.000±0.000	
Nickel					
	July	0.03	0.17	0.08	0.093±0.071
	August	0.09	0	0.06	0.050±0.046
	September	-0.04	-0.03	0.13	0.020±0.095
	October	0.07	0.11	0.04	0.073±0.035
	Mean ± SD	0.038±0.057	0.063±0.094	0.078±0.039	

Table 2. Month wise heavy metals concentration in Mulley.

Analytes	July	August	September	October	Mean ± SD
Pb	0	0	0	0	0.000±0.000
Cd	0.007	0.003	0.01	0.013	0.008±0.004
Zn	0.283	0.483	0.437	0.503	0.427±0.100
Cr	0.03	0.037	0.197	0.013	0.069±0.086
Ni	0.093	0.05	0.02	0.073	0.059±0.031

Lead was not detected in any of the sampling sites during study period. The mean values recorded for chromium were 0.145, 0.063 and 0.000 for site 1, site 2 and site 3 respectively with a mean value of 0.069±0.073. The mean values recorded for cadmium were 0.01, 0.01 and 0.005 for site 1, site 2 and site 3 respectively with a mean value of 0.008±0.003. The mean values recorded for zinc were 0.293, 0.565 and 0.423 for site 1, site 2 and site 3 respectively with a mean value of 0.427±0.136. The mean values recorded for nickel were 0.038, 0.063 and 0.078 for site 1, site 2 and site 3 respectively with a mean value of 0.060±0.020. The heavy metals accumulation took place in an order of Zn>Cr>Ni>Cd. Table 3 is showing sampling sites wise concentration of the observed heavy metals in Mulley, *Wallago attu* collected of river Kalpani.

Table 3. Site wise heavy metals concentration in Mulley.

Analytes	Site 1	Site 2	Site 3	Mean ± SD
Pb	0.000	0.000	0.000	0.000±0.000
Cd	0.01	0.01	0.005	0.008±0.003
Zn	0.293	0.565	0.423	0.427±0.136
Cr	0.145	0.063	0.000	0.069±0.073
Ni	0.038	0.063	0.078	0.060±0.020

The strongest correlations ($r > 0.5$, $p = 0.001$) with Cadmium across all sampling sites included Chromium (0.826) and nickel (0.786). The strongest correlations ($r > 0.5$, $p = 0.001$) with Zinc across all sampling sites included Nickel (0.598). The strongest correlations (r > 0.5, p = 0.001) with Chromium across all sampling sites included Nickel (0.998). Table 4 is showing Pearson correlation coefficient matrix for the observed heavy metals across sampling sites.

Table 4. Pearson correlation coefficient matrix for the measured heavy metals across sampling sites.

Analytes	Cd	Zn	Cr	Ni
Cd	1			
Zn	0.025	1		
Cr	**0.826**	**-0.543**	1	
Ni	**-0.786**	**0.598**	**-0.998**	1

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

The strongest correlations (r > 0.5, p = 0.001) with Zinc across all sampling months included nickel (0.504). The strongest correlations (r > 0.5, p = 0.001) with Chromium across all sampling months included nickel (0.884). Table 5 is showing Pearson correlation coefficient matrix for the measured heavy metals across all sampling months.

Table 5. Pearson correlation coefficient matrix for the measured heavy metals for sampling months.

Analytes	Cd	Zn	Cr	Ni
Cd	1			
Zn	0.207	1		
Cr	0.159	0.033	1	
Ni	0.007	**-0.504**	**-0.844**	1

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

The strongest correlations (r > 0.5, p = 0.001) with Cadmium across all sampling sites included Chromium (0.500) in the month of July, Nickel (0.945) in the month of August, Chromium (0.705) in the month of September and Chromium (0.999), followed by Zinc (0.993) 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 (0.960), by Chromium in the months of August (0.978), September (0.999) and October (0.993). The strongest correlations (r > 0.5, p = 0.001) with Chromium across all sampling sites included Zinc in the month of July (0.564) and August (0.978), by Zinc (0.999) and Cadmium (0.705) in the month of September and by Cadmium (0.999) and Zinc (0.993) October. The strongest correlations (r > 0.5, p = 0.001) with Nickel across all sampling sites included Zinc (0.960) followed by Chromium (0.773) in the month of July, Cadmium (0.945) followed by Chromium (0.756) in the month of August, Zinc and Chromium (0.984) in the month of September followed by Cadmium (0.545). Tables 6 to 9 are showing Pearson correlation coefficient matrix of the studied Analytes for the month of July, August, September and October respectively.

Table 6. Pearson correlation coefficient matrix for the measured heavy metals during the month of July.

Analytes	Cd	Zn	Cr	Ni
Cd	1			
Zn	0.434	1		
Cr	**0.500**	**-0.564**	1	
Ni	0.163	**0.960**	-0.773	1

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

Table 7. Pearson correlation coefficient matrix for the measured heavy metals during the month of August.

Analytes	Cd	Zn	Cr	Ni
Cd	1			
Zn	0.310	1		
Cr	**-0.500**	**-0.978**	1	
Ni	**-0.945**	**-0.604**	**0.756**	1

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

Table 8. Pearson correlation coefficient matrix for the measured heavy metals during the month of September.

Analytes	Cd	Zn	Cr	Ni
Cd	1			
Zn	**0.686**	1		
Cr	**0.705**	**0.999**	1	
Ni	**-0.545**	**-0.984**	**-0.984**	1

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

Table 9. Pearson correlation coefficient matrix for the measured heavy metals during the month of October.

Analytes	Cd	Zn	Cr	Ni
Cd	1			
Zn	**-0.993**	1		
Cr	**0.999**	**-0.993**	1	
Ni	-0.082	0.200	-0.082	1

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

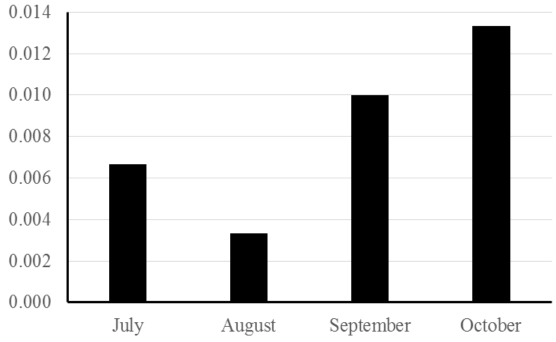

Fig. 1. Month wise Cadmium concentration in Mulley.

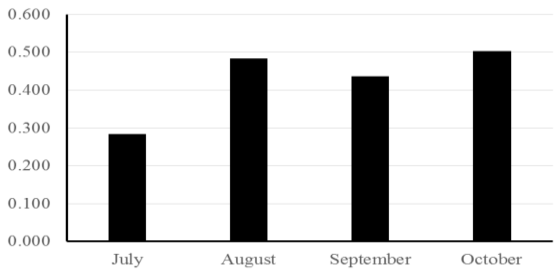

Fig. 2. Month wise Zinc concentration in Mulley.

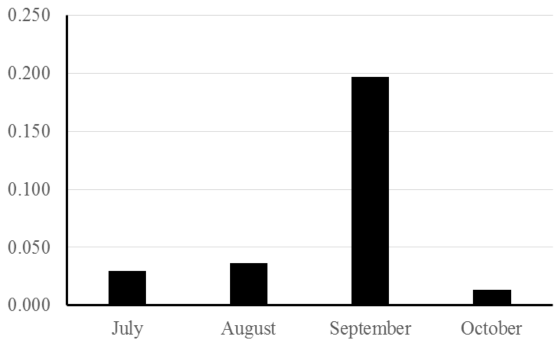

Fig. 3. Month wise Chromium concentration in Mulley.

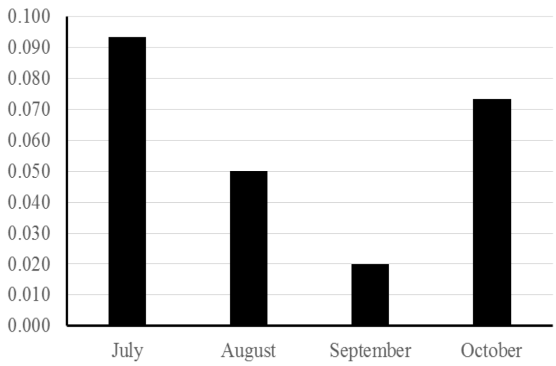

Fig. 4. Month wise Nickel concentration in Mulley.

Conclusion

The study confirms the presence of four heavy metals (Cr, Cd, Ni, and Zn), with the entire absence of Pb in the intestine tissue of *Wallago attu* collected from Kalpani River. All the recorded heavy metals were within the suggested permissible limits. The metals accumulation in the intestine was in order of Zn>Cr>Ni>Cd. Regular assessment of these heavy metals is encouraged. Environmental protection agencies must be vigilant in order to protect and conserve water quality and biodiversity of river Kalpani as it is the main dumping site for the adjoined areas.

References

Al-Busaidi M, Yesudhason P, Al-Mughairi S, Al-Rahbi WAK, Al-Harthy KS, Al-Mazrooei NA, Al-Habsi SH. 2011. Toxic metals in commercial marine fish in Oman with reference to national and international standards. Chemosphere **85(1),** 67-73.

Castro-Gonzalez MI, Mendez-Armenta M. 2008. Heavy metals: implications associated to fish consumption. Environmental Toxicology and Pharmacology **26,** 263-271.

Du Preez HH, Steyn GJ. 1992. A preliminary investigation of the concentration of selected metals in the tissues and organs of the tiger fish (*Hydrocynus vittatus*) from the Olifants River, Kruger National Park, South Africa. Water **18**, 131-136.

El-Moselhy KM, Othman AI, El-Azem HA, El-Metwally MEA. 2014. Bioaccumulation of heavy metals in some tissue of fish in the Red Sea, Egypt. Egyptian Journal of Basic and Applied Sciences **1,** 97-105.

Elnabris KJ, Muzyed SK, El-Ashgar NM. 2013. Heavy metal concentrations in some commercially important fishes and their contribution to heavy metals exposure in Palestinian people of Gaza Strip (Palestine). Journal of Association of Arab Universities for Basic and Applied Sciences **13,** 44-51.

Kris-Etherton P, Harris W, Appel L. 2002. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation **106,** 2747-2757.

Meche A, Martins MC, Lofrano BESN, Hardaway CJ, Merchant M, Verdade L. 2010. Determination of heavy metals by inductively coupled plasma-optical emission spectrometry in fish from the Piracicaba River in Southern Brazil. Microchemical Journal **94**, 171-174.

Medeiros RJ, dos Santos LM, Freire AS, Santelli RE, Braga AMCB, Krauss TM, Jacob SC. 2012. Determination of inorganic trace elements in edible marine fish from Rio de Janeiro State, Brazil. Food Control **23(2),** 535-341.

Mirza MR, Sandhu AA. 2007. Fish of the Punjab, Pakistan. Polymer Publishers, Rahatmarket,Urdu Bazar.

Mustafa C, Guluzar A. 2003. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environment and Pollution **121,** 129-136.

Rahman MS, Molla AH, Saha N, Rahman A. 2012. Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, Savar, Dhaka, Bangladesh. Food Chemistry **134(4),** 1847-1854.

Sthanadar IA, Sthanada AA, Yousaf M, Muhammad A, Zahid M. 2013a. Bioaccumulation profile of heavy metals in the gills tissue of *Wallago attu* (MULLEY) from Kalpani River Mardan, Khyber Pakhtunkhwa Pakistan. International Journal of Biosciences **3**, 165-174.

Sthanadar IA, Sthanadar AA, Shah M, Asmat P, Yousaf M, Muhammad A, Zahid M. 2013b. White muscle as a bio-indicator of cadmium (Cd) pollution across Kalpani River Mardan, Khyber Pakhtunkhwa Pakistan. International Journal of Biosciences **3**, 105-116.

Sthanadar AA, Sthanadar AA, Muhammad A, Ali PA, Shah M, Zahid M, Begum B, Yousaf M. 2013. Bio accumulation profile of heavy metals in the liver tissues of *Wallago attu* (MULLEY) from Kalpani River Mardan, Khyber Pakhtunkhwa Pakistan. International Journal of Biosciences **03** (11), 92-103.

Talwar PK, Jhingran AGK. 1991. Inland fishes of India and adjacent countries, CRC Press.

Ullah S, Ahmad T. 2014. Nutritional and medical importance of fish: A mini review. Reviews of Progress **2(2),** 1-5.

Van Loon JA. 1980. Analytical atomic absorption spectroscopy: selected methods, Elsevier, New york, USA.337

Yilmaz F, Ozdemir N, Demirak A, Tuna AL. 2007. Heavy metal levels in two fish species *Leuciscus cephalus* and *Lepomis gibbosus*. Food Chemistry **100,** 830-835.

Zhao S, Feng C, Quan W, Chen X, Niu J, Shen Z. 2012. Role of living environments in the accumulation characteristics of heavy metals in fishes and crabs in the Yangtze River Estuary, China. Marine Pollution Bulletin **64,** 1163-1171.