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Original Research

Environmental Exposure to Metals and Bioaccumulation in the Liver of Three Freshwater Species of Turtles from Two Different Rivers

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Abstract

Exposure to metals remains an important public health concern. The present study is a comprehensive field study to determine the concentrations of Cu, Zn, Ni, Cd, Co, Pb and Cr in water samples and in the livers of deceased freshwater turtles collected from pre-determined sampling sites of the Ravi and Chenab rivers. In total, 35 deceased freshwater turtles were collected from the sampling sites, and liver samples were collected and immediately stored at -20ºC prior to analysis. Heavy metals concentrations of Cu, Zn and Cd (0.073, 0.169 and 0.0042 mg/L^{-1}) in water samples were collected from the Ravi and (0.084, 0.145 and 0.0041 mg/L^{-1}) from the Chenab. Concentrations of Ni, Co, Pb and Cr were significantly lower (0.045, 0.018, 0.06 and 0.121 mg/L^{-1}) in the Ravi while (0.045, 0.018, 0.064 and 0.121 mg/L^{-1}) in the Chenab were significantly higher. Species-wise mean concentrations of all heavy metals were non-significant ($P>0.05$). The mean concentrations of Cu and Ni varied significantly ($P<0.01$) in the liver samples from the Ravi and Chenab. Regardless of the sampling sites, the mean concentrations of Cu and Pb recorded a maximum in *K. Smithi* (26.54±4.53 and 3.85±0.43 mg/Kg^{-1}, respectively) Zn in K. Tecta (42.48±4.06 mg/Kg^{-1}) and Ni, Cd and Co in *L. Punctata*. Among the studied metals, Zn was recorded as most abundant (39.73±2.28 mg Kg^{-1}) and Cd concentration was found to be minimum (0.188±0.017 mg Kg^{-1}) in freshwater turtles collected from both sampling sites.

Keywords: Ravi River, Chenab River, contamination, freshwater turtle, liver; metals

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Introduction

Metal contamination of land and water resources and related human health risks remains a major concern worldwide [1-3]. Heavy metals, including both essential and non-essential elements, are important in ecotoxicology [3] due to their toxicity, bioaccumulation, and bio-magnification in the aquatic ecosystem. Some metals are considered essential or beneficial and are necessary for daily nutrition and physiology [4-5]. Other metals, such as Pb, Hg, Cd and As, are non-essential and can be considered potentially toxic xenobiotic substances. Numerous studies have reported on the bioaccumulation of metals in aquatic organisms in the food chain [6-10]. The contamination of aquatic resources has been hypothesized as a major cause for the decrease in populations of aquatic organisms [11-12]. The trace metal bioaccumulation can be found in fish, turtle, etc. The estimation of the level of these metals in long-living animals, like turtles, may be helpful in the monitoring of the reservoir water quality.

Freshwater turtles are vulnerable to late sexual maturity, low fecundity, and high juvenile mortality due to increased anthropogenic impacts in freshwater bodies and its extremities. Few species of turtles are under serious threat in Pakistan due to the lack of proper freshwater reservoir monitoring [13-16]. The contamination of freshwater reservoirs recently has a major threat and has caused a loss to the population of turtles [16-19]. Eight species of freshwater turtles belonging to two families, Geoemydidae and Trionychidae, are recorded from major rivers of the province of the Punjab, Pakistan [20]. A few eco-toxicological studies have been reported on these freshwater turtles, which have a few advantages as a model organism in monitoring the impact of water pollution compared to many other species. Freshwater turtles are extensively available in major water bodies in Pakistan. Turtles are useful for long-term trends of water pollution due to their long life spans [21-23]. A tremendous increase in human population and urbanization in Pakistan and un-planned industries in the urban areas has resulted in the discharge of untreated industrial and domestic wastes, containing different contaminants through runoff into the Ravi and Chenab rivers [24-25]. These pollutants in the Ravi and Chenab have adversely affected the aquatic fauna and flora and impaired the growth rate of aquatic animals [24-25].

Currently, there is a lack of information regarding metal concentrations and exposure risks to freshwater turtles in the river system. The objective of this study was to measure the concentrations of selected heavy metals (Cu, Zn, Ni, Cd, Co, Pb and Cr) in water and their bioaccumulation in the liver of deceased freshwater turtles collected from Balloki Headworks and Trimmu Barrage in Punjab. The information may be helpful for the environmental monitoring agency to suggest measures to ensure a safe supply of fish for consumption in the human community.

Materials and Methods

Study Area

The pre-determined sampling sites Trimmu Barrage (TB) and Balloki Headworks (BH) were selected from the Chenab and Ravi, respectively (Fig. 1), based on the reported information [24-25].

Sample Collection

A total of 35 deceased freshwater turtles were collected in this study from the selected sites. Many of the turtles were eaten by predators (such as dogs) and their body organs were damaged. All freshwater turtle species collected from BH and TH were identified and their sizes were measured (Table 1). The liver samples were collected after dissection of dead turtles. Liver tissue samples were prepared by the following method of [26]. The concentrations of Cd, Co, Cu, Cr, Pb, Ni, and Zn were estimated in the liver samples by atomic absorption spectrometry (Aurora AAS, Al-1200, Canada) by following the analytical procedure mentioned in AOAC [27].

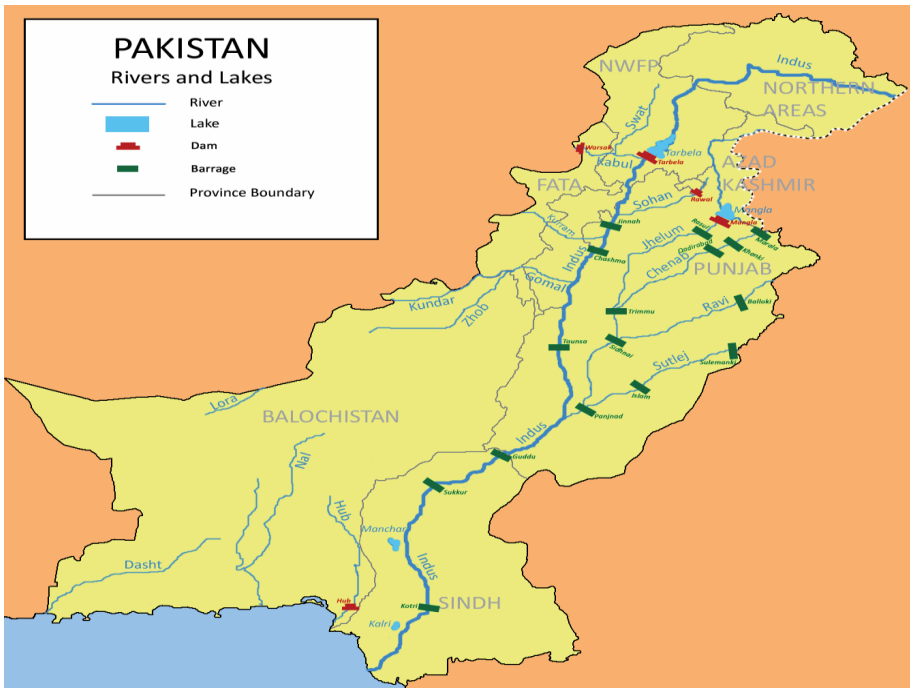
Statistical Analysis

The data thus obtained were statistically analyzed by one-way analysis of variance to find the accumulation differences of heavy metal in the liver samples of freshwater turtles collected from different locations. Means were compared with the help of Duncan's Multiple Range Test ($P<0.05$).

Results and Discussion

Heavy metals concentration in water samples of Cu, Zn and Cd (0.073±0.06, 0.169±0.0248 and 0.0042±0.0005 mg/L^{-1}) collected from the Ravi and (0.084±0.0074, 0.145±0.024 and 0.0041±0.0005 mg/L^{-1}) in water samples from the Chenab. The concentrations of Ni, Co, Pb, and Cr were significantly lower (0.045±0.0044, 0.018±0.0034, 0.06±0.004 and 0.121±0.0066 mg/L^{-1}) in the Ravi and (0.05±0.0049, 0.026±0.0031, 0.056±0.0043 and 0.127±0.0064 mg/L^{-1}) significantly higher in the Chenab (Table 2). The mean concentrations of Cu, Zn, Cd and Co in the water samples from both rivers was lower than the permissible limits, as suggested by [28], whereas the concentrations of Pb and Cr in the water samples from both rivers was higher than the permissible limits, as suggested by [28]. The increase in the concentrations of Ni, Co, Pb and Cr in the water samples of both rivers and the content of these metals in the liver of deceased

a)



b)

c)

Fig. 1. a) water storage reservoirs and barrages of Pakistan,b) satellite image of Trimmu Headworks,c) satellite image of Balloki Headworks.

Table 1. Comparison of means for size (cm) between deceased freshwater turtles of Balloki Headworks (BH) and Trimmu Barrage (TB).

Parameters	N	Mean±SE	t-value	Prob.
Size of dead freshwater turtles of Balloki Headworks	15	303.18±34.49	0.59	0.557
Size of dead freshwater turtles of Trimmu Barrage	20	276.14±29.87		

NS = nonsignificant (P>0.05); * = Significant (P<0.05);
** = Highly significant (P<0.01)
N = Number of observations;
Prob. = Probability

freshwater turtles exhibited a positive relationship. It was due to the discharge of untreated waste from the local industries of paint, dyeing and some food processing industries in the adjacent areas. The highest concentration of these metals in the water samples of the Ravi and Chenab and in the liver is high enough to warrant concern for human consumption of turtles and fish species from these locations.

The 35 deceased freshwater turtles belonging to three species (i.e., *Kachuga Smithi, K. Tecta* and *Lissemys Punctata*) were collected from Balloki Headworks (BH) and Trimmu Barrage (TB). Among the samples, 15 were collected from BH (*K. Smithi*; 4, *K. Tecta;* 4 and *Lissemys Punctata*; 7) and 20 from Trimmu Barrage (TB) (*K. Smithi*; 7, *K. tecta;* 10 and *Lissemys punctata*; 3). The differences in the size of freshwater turtles procured from both locations were insignificant (Table 1). Bioaccumulation of heavy metals was studied species-wise, location-wise and sorption trend of metals regardless of species and locations. The interaction of location and species is also clearly studied (Tables 2 and 3). The location-wise mean concentration of Cu and Ni varied highly (P<0.01) as compared to the other five heavy metals with non-significant variation in relation to localities. Mean maximum concentration of Cu (20.15±2.66 mg Kg^{-1}) was found in the liver of freshwater turtles from TB, while Ni in the liver of turtles collected from BH was 2.92±0.39 mg Kg^{-1}. Regardless of locality and species, the overall mean of heavy metals concentration in livers of freshwater turtles was recorded in the order Zn>Cu>Cr>Pb>Co>Ni> Cd (Tables 2 and 3). Mean Maximum concentration of Zn was found in the liver of freshwater turtles at TB than at BH (42.13±3.62, 37.19±2.68 mg $Kg^{-1,}$ respectively).

The mean concentrations of heavy metals studied among the three species of turtle were insignificant (P>0.05). Maximum and mean concentrations of Cu and Pb were found in liver of *K. smithi* (26.54±4.53 and 4.29±0.51 mg Kg^{-1}, respectively). Mean concentrations of Zn were recorded as 41.71±4.71 and 42.48±4.06 mg Kg^{-1} in *K. smithi* and *K. tecta,* respectively, which were higher than in *L. punctata*. Cadmium, Ni, Co, and Cr

Table 2. Statistical comparison of the concentrations (Mean± SE) of heavy metals estimated from water of Balloki Headworks and Trimmu Barrage with standard values of WHO (2004).

Locality	Cu (mg/l)	Zn (mg/l)	Ni (mg/l)	Cd (mg/l)	Co (mg/l)	Pb (mg/l)	Cr (mg/l)
Balloki	0.073±0.060	0.169±0.0248	0.045±0.0044	0.0042±0.0005	0.018±0.0034	0.06±0.004	0.121±0.0066
Trimmu	0.084±0.0074	0.145±0.0240	0.05±0.0049	0.0041±0.0005	0.026±0.0031	0.056±0.0043	0.127±0.0064
WHO Standards for Pakistan (2004)	2	5	≤0.02	0.01	0.001	≤0.05	≤0.05
t- value when compared with Balloki Headworks	-321.17	-194.80	5.68	-11.60	5	2.5	10.76
Probability	**	**	**	**	**	**	**
t- value when compared with Trimmu Barrage	-258.91	-202.29	6.12	-11.8	8.06	1.39	12.03
Probability	**	**	**	**	**	*	**

* = Significant (P<0.05); ** = highly significant (P<0.01)
Students t-Table value at .05=1.684, at .01=2.423; d.f.= 35

Table 3a. Comparison of means for selected heavy metal concentrations (mean ± Standard Error, mg Kg-1) in liver of deceased freshwater turtles.

Heavy metals	Cu			Zn			Ni		
Species	TB	BH	Overall Means	TB	BH	Overall Means	TB	BH	Overall Means
K. Smithi	22.32±5.21	33.93±8.03	26.54±4.53	36.23±5.39	51.31±7.31	41.71±4.71	1.95±0.30	1.89±0.25	1.93±0.20
K. tecta	20.20±2.87	8.93±1.67	16.98±2.50	47.12±4.98	30.89±0.84	42.48±4.06	2.28±0.21	1.95±0.27	2.19±0.17
L. punctate	4.52±0.00	12.71±5.45	11.89±4.94	33.64±0.00	33.71±1.80	33.70±1.61	4.93±0.00	3.81±0.59	3.92±0.54
Overall Means	20.15±2.66	16.81±4.07	18.53±2.39	42.13±3.62	37.19±2.68	39.73±2.28	2.30±0.23	2.92±0.39	2.60±0.23

TB: Trimmu Barrage; BH: Balloki Headworks

Table 3b. Comparison of means for selected heavy metal concentrations (mean ± Standard Error, mg Kg-1) in liver of deceased freshwater turtles.

Heavy metals	Cd			Co			Pb			Cr		
Species	TB	BH	Overall Means	TB	BH	Overall Means	TB	BH	Overall Means	BH	TB	Overall Means
K. Smithi	0.156±0.038	0.172±0.057	0.162±0.030	3.10±0.61	3.38±1.13	3.20±0.53	4.13±0.77	4.58±0.57	4.29±0.51	5.88±0.80	5.08±0.33	5.59±0.52
K. tecta	0.192±0.043	0.196±0.018	0.193±0.030	2.42±0.54	1.32±0.77	2.10±0.45	4.06±0.57	3.33±0.56	3.85±0.43	5.69±0.19	5.48±0.07	5.63±0.14
L. punctate	0.229±0.000	0.208±0.029	0.210±0.026	4.17±0.00	3.96±0.90	3.98±0.81	1.37±0.00	1.67±0.39	1.64±0.35	5.97±0.00	6.18±0.30	6.16±0.27
Overall Means	0.180±0.028	0.197±0.020	0.188±0.017	2.78±0.39	3.20±0.61	2.98±0.35	3.93±0.45	2.75±0.40	3.36±0.31	5.78±0.31	5.76±0.21	5.77±0.19

TB: Trimmu Barrage; BH: Balloki Headworks

showed greater accumulation in *L. punctata*. None of the heavy metals showed noticeable higher accumulation in *K. tecta*. The mean concentration of Pb and Cr was higher in turtles collected from TB compared to BH. On the other hand, Cd (0.197±0.02 mg Kg^{-1}) and Co (3.20±0.61 mg Kg^{-1}) concentrations were higher in the liver of turtles from BH.

The results clearly showed the differences between heavy metal bioaccumulation in freshwater turtle species-wise and locality-wise. Among the species', the role of shell composition and structure is noticeable. *L. punctata* has more tendencies to absorb heavy metals than *K. tecta* and *K. simithi* turtles. Both sites are contaminated by untreated agricultural and industrial effluents from point and non-point sources. Trimmu Barrage (TB) receives effluents from sugar cane mills, the textile sector, rice mills, agricultural zones and multiple small and commercial, industrial units near and around Jhang, Jehalum and Chenab cities. Balloki Headworks (BH) receives polluted water of Lahore, Sialkot and Faisalabad cities. One thing is in common with BH and TB in that both receive water from the Chenab, which gets mixed with the Ravi River at Balloki and the Jehlum River at Trimmu [29]. Metals from polluted water move to an organism's body and accumulate in various organs according to accumulation preferences. Other factors, like difference at species level, gender, age, location, seasonal variation, etc., also affect toxicity and accumulation levels in organisms [30]. In the present study, Cu concentrations in the liver of freshwater turtles from BH (16.81±4.07) were similar to Ganges turtles from Rasul Barrage (16.24±2.54) and dissimilar to Ganges turtles from BH (21.23±2.36). In comparison, in the present study Cu concentrations in the liver of the soft shell turtle *L. punctata* from BH (12.71±5.45 mg kg^{-1}) were much lower than those found in Ganges softshell turtles (21.23±2.36) [2, 31]. Zinc is an essential element for reproduction; it accumulates in the bones and carapace from the blood [32-33]. The present findings for Zn are upheld by [32], who estimated the concentration of zinc (38.63±3.53) in the liver of freshwater turtles (*Aspideretes gangeticus*) from BH. The current reported values for Ni concentrations in turtles from both sites are greater than the findings of [33]. The noticeable dissimilarities in the concentrations of Ni may be due to the variations in the level of contamination, environment, and metabolic processes associated with turtle species and with their habitat [30]. [33] No turtles had Cd greater than the minimum detection limit in the liver, while in the present study, a considerable concentration of Cd was detected in the liver of *L. punctata* at both localities. The environmental Cd level (i.e., 1 μg g^{-1}) may influence the gonadal development in *Trachemys scripta* during the postnatal and embryonic stages. The higher concentration of Cd may impair reproductive processes later in life [34]. Among the three species of freshwater turtles, higher concentrations of Co were detected in *L. punctata* compared to the other two species. One strong reason behind this difference may be its soft shell, which may effectively pass on Co to liver by absorption. A higher concentration of Co (2.78±0.39 and 3.20±0.61 mg Kg^{-1}) in the liver of turtles was recorded from TB and BH, respectively. Our findings were in line with the results reported in green turtles by [2, 32] from Yaeyama Islands, Japan. In this study the level of Pb in the liver of freshwater turtles was similar to Olive Ridley turtles (3.32 μg/g) in Mexico [35]. The results of the present study were also in line with [36], who reported higher Pb concentrations of 2.75 μg/g in the liver of Loggerhead turtles from southwestern Mediterranean, Spain.

Cr is an essential element to organisms for carbohydrate metabolism in trace amounts [36]. However, Cr intoxication damages the liver [31]. The concentration of Cr in the present study was quite higher than in *Aspideretes gangeticus* (0.39 μg/g) from two barrages, i.e., BH and Rasul [37-38]. [33] also reported a maximum accumulation of Cr in the liver (3.32±0.559) than in other organs, though this concentration of Cr is less in the freshwater turtles from BH (5.78±0.31 mg Kg^{-1}) and TB (5.76±0.21 mg Kg^{-1}). Information on the toxicity of Cd on reptiles is insufficient, so predicting the consequences of the detected Cd residues on three freshwater species of turtle population health is presently not possible. The accumulation of higher concentrations of metals in the liver of these freshwater species of turtles indicates the pervasiveness in these rivers, and its accumulation up in the food chain [38]. The reptile population throughout the world is currently under threat because of increasing levels of contamination in the freshwater ecosystem, and there is a dire need to determine the level of contamination and potential risk to the turtle population [38].

Conclusion

The overall mean concentrations of Cu, Zn, Ni, Cd, and Cr were high in *L. punctata* as compared to *K. smithi* and *K. tecta*. The mean concentrations of Co and Pb were high in *K. tecta* and *K. Smithi*, respectively. Locality-wise the mean concentrations of Cu and Pb were high in freshwater turtles at Trimmu Barrage, while the mean concentrations of Zn, Ni, Cd, Co, and Cr were high in freshwater turtles at Balloki Headwork. There are chances of heavy metals biomagnifications in top consumers, resulting in serious health hazards. It is the need of time that more research work shall be continued, and law-enforcement agencies collectively should come forward to save the populations of freshwater turtles.

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Conflict of Interest

The Authors declare no conflict of interest.

Ethical Guidelines

The standard guidelines for animal experiments prepared by the department and the university were followed in this study.

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