Research Article

Accumulation of heavy metals (Cu, Zn, Ni, Cd, Co, Pb and Cr) in blood of freshwater Turtles from Balloki Headworks and Trimmu Barrage, Punjab, Pakistan

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Abstract

The present study provides data on heavy metals (Cu, Zn, Ni, Cd, Co, Pb and Cr) accumulation in freshwater turtles species i.e. *Kachuga smithi*, *K. tecta* and *Lissemys punctata* collected from Balloki Headworks and Trimmu Barrage, Punjab, Pakistan. No previous record has been found regarding such studies on freshwater turtles in Pakistan. Blood Samples were taken from freshwater turtles and immediately placed in heparanized vials. Samples were digested by using acids and diluted upto 25 ml volume. Then heavy metals were detected in blood samples by using Atomic Absorption Spectrometer (Aurora AAS, AI-1200, Canada). Data were statistically analyzed by applying Analysis of Variance Technique and Duncan's Multiple Range Test (DMR). Mean concentrations of Cu, Zn, Ni and Cd were highly significant ($P < 0.01$) species-wise and non-significant locality-wise ($P > 0.05$). Mean concentrations of Co, Pb and Cr were species-wise and locality-wise non-significant ($P > 0.05$). Regardless of locality, mean concentrations of all heavy metals were found maximum in *L. punctata* except Pb (0.82 ± 0.15 mg Kg^{-1}) which was high in blood of *K. smithi* and Co (0.87 ± 0.18 mg Kg^{-1}) in *K. tecta*. Among seven heavy metals, Zinc concentrations were found maximum i.e. 8.47 ± 0.64 mg Kg^{-1} and Cd concentration was found minimum 0.038 ± 0.002 mg Kg^{-1} in blood of turtles species.

Key words: Freshwater; Heavy metals; Blood; Turtles; Pollution.

Introduction

Punjab (Pakistan) hosts eight species of freshwater turtles belonging to two families viz., Geoemydidae (Brown River Turtle *Kachuga smithi*; Indian Saw-backed turtle

Kachuga tecta; Brahminy River Turtle *Hardella thurgi*; Spotted Pond Turtle *Geoclemys hamiltonii*) and Trionychidae (Indian Soft-shell Turtle *Aspiderates gangeticus*; Peacock Soft-shell Turtle,

Aspederates hurum; Narrow-headed Soft-shell Turtle *Chitra indica*; Indian Flap-shell Turtle *Lissemys punctata*) [1]. Heavy metals pollution can cause serious loss of wildlife population [2-4]. Turtles own several advantages as biomonitors of environmental contamination as compared to many other species. They are widely distributed and found in almost all freshwater ecosystems in Pakistan. Turtles are identified as a scavenger of water bodies. Their long life spans allow monitoring of long term trends in environmental pollutants [5].

Increasing human population and establishment of industries in the urban areas of the Punjab province (Pakistan) has resulted in the discharge of untreated industrial and sewage wastes, containing heavy metals and their compounds, into the rivers. This polluted water causes serious effects on the aquatic life [6-8]. The bulk quantities of toxic heavy metals are continuously discharged by domestic sewage and industrial effluents in river Ravi which badly affect the growth rate of aquatic animals [9, 10].

Dissimilarities in metal levels can be clearly analyzed among various species. In case of turtles the level of heavy metals toxicity can be well assessed by using blood without causing damage to unique fauna. Blood sampling may be useful for pollution assessment in threatened or endangered populations [11]. Trace metals are most important because many of these metals are biologically essential nutrients when in lower concentrations, however, they become toxic if their concentrations exceed certain thresholds [12-14]. Consequently, some species populations are becoming threatened due to various anthropogenic activities. It is very first time in Pakistan that this research work highlights the accumulation of heavy metals in blood of freshwater turtle as one of the major contributor of their silent depletion.

Furthermore, freshwater turtles do not have any legal coverage through any Law or Act therefore they are continuously neglected. No previous record has been found regarding such studies on freshwater turtles in Pakistan.

Materials and Methods

Balloki Headworks and Trimmu Barrage were selected as study sites (Figure 1). Turtles were captured by using either drag net or with help of baited hooks set at various points of left and right marginal banks of study areas and released back after getting blood samples. For species identification Minton (1966) was followed [15]. Blood Samples were taken from the femoral vein of turtles using sterile plastic syringes and needles. During blood extraction, careful cleaning of the femoral region (with ethanol and de-ionized water) prior to sampling was practiced. Blood samples were immediately placed in acid washed heparanized vials. All samples were kept at 4°C. Samples were digested with 5 ml perchloric acid and 15 ml nitric acid on hot plate in laboratory. Samples were cooled at room temperature, diluted upto 25 ml volume by adding de-ionized water and then filtered by using whatman filter paper [16].

Heavy metals i.e. Copper (Cu), Zinc (Zn), Nickel (Ni), Cadmium (Cd), Cobalt (Co), Lead (Pb) and Chromium (Cr) were detected from the blood samples of turtles using Atomic Absorption Spectrometer (Aurora AAS, AI-1200, Canada). Analytical analyses of heavy metals were performed following the conditions described in AOAC [17].

Statistical Analysis

Data were statistically analyzed by applying Analysis of Variance Technique [18] to test the effect of locality on heavy metal concentration in blood of freshwater turtles. Duncan's Multiple Range Test (DMR) was applied to compare species-wise and locality-wise mean concentrations of heavy metals in freshwater turtles at both localities. The significance level was taken as $P < 0.05$.

Figure-1 Water Storage Reservoirs and Barrages of Pakistan

Results

In the present study three freshwater turtle species *i.e.* *Kachuga smithi*, *K. tecta* and *Lissemys punctata* were found at Balloki Headworks and Trimmu Barrage. The blood samples were taken from 40 freshwater turtles of Balloki Headworks (*K. smithi*; 17, *K. tecta*; 2 and *Lissemys punctata*; 21) and 47 freshwater turtles of Trimmu Headworks (*K. smithi*; 20, *K. tecta*; 8 and *Lissemys punctata*; 19). Mean concentrations of Cu, Zn, Ni and Cd were highly significant ($P < 0.01$) species-wise and non-significant locality-wise ($P > 0.05$) (Table 1 and 2). Mean concentrations of Co, Pb and Cr were species-wise and locality-wise non-significant ($P > 0.05$) (Table 2). Regardless of locality, mean concentrations of all heavy metals were found maximum in *L. punctata* except Pb which was high in blood of *K. smithi*. The overall mean concentrations of heavy metals followed the following order of their accumulation in blood of freshwater turtles is as:

$Zn > Cr > Cu > Co \geq Ni > Pb > Cd$

Zn (11.97 ± 1.05 mg Kg⁻¹) was found in maximum concentrations than other metals. Mean minimum concentration of zinc was found in *K. tecta* (5.39 ± 0.59 mg Kg⁻¹). Mean concentration of zinc in the blood of freshwater turtles at Balloki Headworks was high than at Trimmu Barrage (8.50 ± 1.1 , 8.44 ± 0.68 mg Kg⁻¹ respectively). Mean concentration of copper was minimum in *K. tecta* (0.96 ± 0.1) and maximum in *L. punctata* (1.56 ± 0.12 mg Kg⁻¹). Mean concentration of copper in the blood of freshwater turtles at Trimmu Barrage was maximum (1.42 ± 0.100 mg Kg⁻¹) whereas minimum at Balloki Headworks. Mean concentration of nickel was maximum in *L. punctata* (0.87 ± 0.067 mg Kg⁻¹) and minimum in *K. tecta* (0.41 ± 0.10 mg Kg⁻¹). Mean concentration of nickel in the blood of freshwater turtles was high at Balloki Headworks (0.77 ± 0.08 mg Kg⁻¹) where as

low at Trimmu Barrage (0.58 ± 0.061). Mean concentration of cadmium in blood of *L. punctata* (0.047 ± 0.003 mg Kg⁻¹) was maximum whereas mean minimum concentration of cadmium was in *K. tecta* (0.030 ± 0.006 mg Kg⁻¹) and *K. smithi* (0.03 ± 0.003 mg Kg⁻¹). The mean concentration of cadmium in blood of freshwater turtles at Balloki Headworks was 0.041 ± 0.0030 and Trimmu Barrage was 0.035 ± 0.003 mg Kg⁻¹. Mean concentration of cobalt was high in *K. tecta* (0.87 ± 0.18 mg Kg⁻¹) and low in *K. smithi* 0.54 ± 0.06 mg Kg⁻¹.

The mean concentration of cobalt in blood of freshwater turtles at Balloki Headworks was 0.64 ± 0.08 . Mean concentration of lead was high in *K. smithi* (0.83 ± 0.152) and low in *K. tecta* (0.43 ± 0.147 mg Kg⁻¹). The mean lead concentration in blood of *L. punctata* (0.61 ± 0.081 mg Kg⁻¹). The mean concentration of lead in blood of freshwater turtles at Trimmu Barrage was high 0.77 ± 0.12 mg Kg⁻¹ than at Balloki Headworks 0.58 ± 0.08 mg Kg⁻¹. The mean concentration of Chromium was high in *L. punctata* (1.85 ± 0.18 mg Kg⁻¹) and low in *K. smithi* (1.44 ± 0.16 mg Kg⁻¹). The concentration of chromium in blood of *K. tecta* was (1.56 ± 0.27 mg Kg⁻¹). The mean concentration of chromium in blood of freshwater turtles at Balloki Headworks was 1.68 ± 0.17 mg Kg⁻¹ and Trimmu Barrage was 1.61 ± 0.51 mg Kg⁻¹.

Discussion

This study reports selected heavy metals values in blood of a wild population of freshwater turtles. It is important to emphasize the lack of ecotoxicological research in freshwater turtles and that a very few studies published provide just a glimpse of the complexity of toxic metals and their accumulation in organisms' tissues. Ley-Quinonez *et al.* [19] found that Zn was the most prevalent metal in blood (41.89 µg g⁻¹) of loggerhead turtles.

Table-1. Comparison of mean concentrations of Cu, Zn and Ni (mg Kg⁻¹) in blood of freshwater turtles (Species-wise variations and Locality-wise variations).

Heavy metals	Cu			Zn			Ni		
Species	Trimmu Barrage	Ballkoi Headworks	Overall Mean Conc.	Trimmu Barrage	Ballkoi Headworks	Overall Mean Conc.	Trimmu Barrage	Ballkoi Headworks	Overall Mean Conc.
<i>K. Smithi</i>	1.20±0.08	1.05±0.11	1.13±0.06B	6.48±0.92	4.36±0.32	5.51±0.54B	0.42±0.08	0.62±0.13	0.51±0.07B
<i>K. tecta</i>	1.01±0.13	0.74±0.07	0.96±0.11C	5.60±0.67	4.57±1.59	5.39±0.59B	0.33±0.10	0.73±0.22	0.41±0.1C
<i>L. punctata</i>	1.82±0.19	1.32±0.12	1.56±0.12A	11.7±0.94	12.22±1.83	11.97±1.05A	0.84±0.09	0.90±0.10	0.87±0.07A
Overall Mean Conc.	1.42±0.10	1.18±0.08	1.31±0.07	8.44±0.68	8.50±1.15	8.47±0.64	0.58±0.06	0.77±0.08	0.66±0.05

Table 2 Comparison of mean concentrations of Cd, Co, Pb and Cr (mg Kg⁻¹) in blood of freshwater turtles (Species-wise variations and Locality-wise variations).

Heavy metals	Cd			Co			Pb			Cr		
Species	Trimmu Barrage	Ballkoi Headworks	Overall Mean Conc.	Trimmu Barrage	Ballkoi Headworks	Overall Mean Conc.	Trimmu Barrage	Ballkoi Headworks	Overall Mean Conc.	Ballkoi Headworks	Trimmu Barrage	Overall Mean Conc.
<i>K. Smithi</i>	0.029±0.004	0.030±0.005	0.029±0.003C	0.46±0.08	0.62±0.10	0.54±0.06	0.95±0.25	0.67±0.15	0.82±0.15	1.48±0.24	1.41±0.21	1.44±0.16
<i>K. tecta</i>	0.028±0.007	0.042±0.011	0.031±0.006B	0.92±0.22	0.66±0.17	0.87±0.18	0.31±0.10	0.90±0.63	0.43±0.15	1.17±0.52	1.66±0.32	1.56±0.27
<i>L. punctata</i>	0.045±0.004	0.050±0.003	0.047±0.003A	0.69±0.12	0.65±0.12	0.67±0.09	0.77±0.13	0.47±0.09	0.61±0.08	1.90±0.25	1.81±0.28	1.85±0.18
Overall Mean Conc.	0.035±0.003	0.041±0.003	0.038±0.002	0.63±0.07	0.64±0.08	0.63±0.05	0.77±0.12	0.58±0.08	0.68±0.08	1.68±0.17	1.61±0.15	1.64±0.11

Such high concentration of Zn was not reported in other parts of the world and they concluded that levels correlate to exposure of the metals in populations of sea turtles same is true for freshwater turtles populations. Freshwater turtles showed higher concentrations of Zn ($11.97 \pm 1.0 \text{ mg Kg}^{-1}$) and minimum concentrations of Cd ($0.047 \pm 0.003 \text{ mg Kg}^{-1}$) in their blood. Present results are also supported by Paez-Osuna *et al.* [20] who investigated trace metals in blood of *Lepidochelys olivacea* Oaxaca, Mexico. They reported that Zn concentrations were highest in blood ($58.4 \pm 4.7 \text{ } \mu\text{g g}^{-1}$) while other heavy metals also showed the similar trend i.e. Cu ($2.28 \pm 0.40 \text{ } \mu\text{g g}^{-1}$) following zinc. Cadmium ($0.45 \pm 0.20 \text{ } \mu\text{g g}^{-1}$) was in minimum concentrations in blood of *L. olivacea*. Goye and Clarkson [21] described that Zn can stay in blood and bones for several days prior to being fixed or excreted that is the reason behind high burden of zinc in blood. Findings of present study are in line with Morrissey [22] who has also reported Pb accumulation at low concentrations (0.02 mg/l) in the blood of *C. mydas*. Yu, *et al.* [23] did not compare Cr with other studies and being essential element its threshold concentration was not known in reptiles. Similar results were found by Guirlet *et al.* [24]. They reported the elevated Zn concentrations $44.4 \pm 1.12 \text{ } \mu\text{g g}^{-1}$ while minimum loads of Cd $0.32 \pm 0.12 \text{ } \mu\text{g g}^{-1}$ in blood of *Dermochelys coriacea* of French Guiana.

Conclusion

Among freshwater turtle species the overall mean concentrations of Cu, Zn, Ni, Cd and Cr were high in *L. punctata* as compared to *K. smithi* and *K. tecta*. Mean concentration of Co and Pb was 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 headworks. Turtles are used as food items and in medicines in most part of the world. Their smuggling is done from different parts of the world, even from Pakistan. The presence of heavy metals has added fuel to the fire. Therefore, there are chances of heavy metals biomagnifications in top consumers resulting in serious health hazards. Population of freshwater turtles is decreasing day by day due to fishing and illegal trade. It is need of the time that more research investigations and the law enforcement agencies collectively should come forward to cope the issue faced to freshwater turtles.

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