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Estimation of Heavy Metals in Indian Flying Fox *Pteropus giganteus* (Brünnich, 1782) from Punjab, Pakistan*

Shagufta Nighat,[1] Muhammad Sajid Nadeem,[1,] Tariq Mahmood,[2]**
Amjad Rashid Kayani,[1] Muhammad Mushtaq[1] and M. Mahmood ul Hassan[3]
[1]Department of Zoology, PMAS-Arid Agriculture University, Rawalpindi 46300
[2]Department of Wildlife Management, PMAS-Arid Agriculture University, Rawalpindi
[3]Department of Zoology and Fisheries, University of Agriculture, Faisalabad

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MSN and MMH conceived and designed the study. SN, TM and ARK collected field data. SN and MSN wrote the article. SN, ARK and MM performed statistical analysis.

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Heavy metals, Fruit bats, *Pteropus giganteus*, metal accumulation.

A B S T R A C T

Bats can be important environmental indicators because they are sensitive to a wide range of environmental stresses to which they respond in predictable ways. We studied the proportions and distribution of heavy metals (Cd, Cu, Pb and Zn) in a fruit bat *Pteropus giganteus.* The concentrations of heavy metals in tissue samples (Liver, Heart, Kidney) of *P. giganteus* were determined from central and northern parts of Punjab Province. Deposition of metals significantly varied in different organs (Liver, Heart and Kidney). However, metal concentrations were non-significant between both sexes. Likewise, central Punjab appeared to be more polluted than northern Punjab but again non-significant difference was observed between two regions. This study provides a baseline data which might be considered as a precursor to a broad array of issues that concern our environment in general and the health of both humans and biodiversity in particular using bats as bio-indicators and pointing out the impacts of heavy metal contamination on bat species and to propose conservation measures.

INTRODUCTION

In urban terrestrial environments metal and metalloid contamination is frequently associated with historic inputs from industrial smelters [Aluminium (Al), Cadmium (Cd), Copper (Cu), Lead (Pb), Zinc (Zn)]; coal-fired power stations [Arsenic (As), Chromium (Cr), Mercury (Hg), Selenium (Se)]; mining activities (numerous metals) and vehicular emissions (Pb and particulates) (Vike, 1999, Roach, 2005; Xie *et al*., 2006). Industrial processes and some agricultural activities have greatly increased the mobilization of many metals in freshwater ecosystems. Therefore, heavy metal pollution has become an important problem in recent years. These metals are potentially harmful to most organisms at some level of exposure and absorption (Güven *et al*., 1999). The manufacture of pigments, drugs, agrochemicals, plastics, batteries, electroplating, and discharge of untreated effluents from different industries cause metal pollution (Raihan *et al*., 1995). Metals like Hg, Cd, Cr, Pb, Ni, Co and Zn are highly toxic to both flora and fauna components of the ecosystem (Burger and Gochfeld, 1997; Gerbersmann *et al.*, 1997; Lee *et al*., 2006). Heavy metals have serious impact on the environment and can threaten the ecosystems (Güven *et al*., 1999; Sanpera *et al*., 2000; Battaglia *et al.*, 2005). Pb and Cd are nonessential heavy metals for organisms and are distributed globally from burning of fossil fuels, road traffic, and industries (Kenntner *et al*., 2003). Dietary exposure to high levels of Pb and Cd can be toxic (Scheuhammer, 1987) and influence the endocrine system (Stoica *et al*., 2000), kidneys and reproduction (Burger *et al.*, 1993), behavioral response (Furness, 1996; Hui, 2002), enzymes involved in hemoglobin formation, and growth rates (Eisler, 1988). Zn is used in alloying, dyeing, and manufacturing electric goods, insecticides, and cosmetics, and causes reduced species diversity and abundance, as well as fainting, nausea, and stomach disorders (Perez-Lopez *et al*., 2008). Copper (Cu) is an essential trace element that is metabolically regulated, but its excesses result in additional stress to birds already living in stressful environmental conditions (Debacker *et al*., 2000).

Environmental pollution has become a worldwide concern as it is likely to affect the ecological system and living organisms. The indiscriminate release of harmful chemicals and heavy metals in the environment by industries and other activities of man may adversely affect the quality of our air, water and food resources (Hamidullah *et al*., 1997; Yousafzai and Shakoori, 2008). They commented that once inside the living organisms through food chain, these chemicals may accumulate in

* Part of Ph.D. thesis of first author
** Corresponding author: sajidnm@uaar.edu.pk
sajidnm@hotmail.com
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higher concentrations and induce various metabolic disorders. Coetzee *et al.* (2002) stated that all metals originate naturally and are integral part of the environment. They are found in varying levels in all ground and surface waters. Gerbersmann *et al.* (1997) found out that among these metals, mercury, cadmium, lead, nickel, cobalt and zinc are quite toxic in nature to both flora and fauna. The rate of heavy metal absorption varies depending on species physiology, metal properties, and bio-availability in the environment. After absorption, metals circulate in the body, are excreted or get deposited in various body tissues, or are sequestered in feathers (Furness *et al*., 1986). Long-term exposure to heavy metals can also cause disruptive behavior and reduction in disease resistance and affect other physiological processes (Dauwe *et al*., 2006).

In order to study the effects of heavy metals among different organs, it was felt important to select a group of animals that are sensitive to a wide range of environmental stresses to which they respond in predictable ways. According to Alleva *et al*. (2006) bats are exactly such a group of mammals and can be important environmental indicators. Additionally, the wide range of food sources exploited by bats allows them to be used as indicators for a wide range of environmental stressors. Walsh *et al*. (2001) stated that Bats can reflect the status of the plant populations on which they feed and pollinate as well as the productivity of insect communities. Bats have enormous potential as bio indicators: trends in their populations can be monitored, short- and long term effects on populations can be measured and they are distributed widely around the globe.

The present study has been conducted, keeping in view the significance of levels of contamination of heavy metals and their adverse effects on animals. Interest in the study and conservation of bats throughout the world has been growing because bats are regarded as threatened animals in various regions, so need protection and conservation (Stebbings and Griffith, 1986). They have been relatively less studied animals because they are more difficult to study than the terrestrial small mammals as they require different experience and specialized equipment (Fenton, 1997). There is no published data available on the potential impacts of heavy metals on bats in Pakistan. So this study will provide baseline data on the concentrations of Cd, Cu, Pb and Zn in the organs of Indian flying fox.

MATERIALS AND METHODS

Sampling

Samples of Indian flying fox were collected from different regions of Punjab Province, namely Faisalabad, Gujranwala, Lahore (central Punjab); and Attock, Chakwal, Jhelum, Islamabad, Rawalpindi (northern Punjab) (Table I).

The surveys for locating roosting sites and collection of samples of Indian flying fox (*Pteropus giganteus*) with the help of local people was carried from 2010 to 2012. Dead bat specimens were collected from roosting sites, orchards and houses with fruit plants (Table I). Retaliatory bat killing is common in orchards and house-based small fruit cultivation. Total 94 samples of Indian flying fox were collected from which 66 were males and 28 were females. GPS (Global Positioning System) locations of specimens were recorded using Garmin GPS. The samples were brought to the laboratory, in small clean polyethylene bags, or cloth bags. Date of collection, locality and gender (basic information) was noted down for each species. Samples were dissected to excise liver, heart and kidney, which were then dried at 80°C for 12 h in an oven (Saeki *et al*., 2000). The organs were cut into pieces, weighed (3 g of dry weight) and transferred into quartz crucibles and digested in 1.0 ml of nitric acid and 0.25 ml perchloric acid. Organs were digested using hot plate initially at low temperature (70°C) and then at higher temperature (200°C) to make it a clear straw colour solution. Then dilutions were made using de-ionised water and the final volume was raised up to 10 ml. Samples were analysed using atomic absorption spectroscopy (G.B.C. 932 Plus, UK) following Fransion (1981). Certified standard solutions of the elements (1000 µg/g) were used for the preparation of element’s working solutions.

Statistical analysis

The concentrations of metals (Cd, Cu, Pb, Zn) were not in normal distribution (Shapiro-Wilk test, $P<0.05$), therefore, data was log-transformed to get the normal distribution. In qualitative comparison metal load is given in %age to show the prevalence while in quantitative comparison the exact metal concentration is given in µg/g (dw). ANOVA was used to compare the metal concentrations within the organs and regions and mean values were compared by LSD (Least Significant Difference) and Tukey’s HSD (Honestly Significant Difference) test; Chi-square was used to check the qualitative difference among organs and regions. In order to compare metal toxicity levels in both regions each of the four metals were pooled to get their overall effect in the region. Results are presented as Mean±Standard error. Value of $P<0.05$ was considered significant when and where appropriate. All tests were performed in SPSS, version 17.0 (SPSS Inc.).

Table I.- Sampling stations of the Indian Flying Fox and the number of samples collected from study area.

	Name of district	Coordinates		No. of samples
		Latitude (N)	Longitude (E)	
Central Punjab	Lahore	31°32′36.47″	74°20′18.66″	08
	Gujranwala	32°8′60.00″	74°10′60.00″	21
	Faisalabad	31° 25′ 4.8″	73° 4′ 44.4″	10
Northern Punjab	Rawalpindi	33°40′38.00″	72°51′21.00″	16
	Chakwal	32°56′0.63″	73°43′14.57″	09
	Jhelum	33° 54′ 26″	72° 18′ 40″	04
	Attock	33°43'4.7496"	73'36.36.36"	11
	Islamabad	33° 42' 0.55"	73'25.10.7"	15

Table II.- Qualitative comparison of all metals in liver, heart and kidney of Indian Flying Fox

Organ	Cadmium	Copper	Lead	Zinc	P value
Liver (%)	100	100	92.5	98.8	***0.002****
Heart (%)	94.6	97.8	100	96.8	***0.037****
Kidney (%)	92.6	95.7	94.7	93.6	*0.810**

*Pearson Chi square 2 sided significance

Table III.- Comparisons of metal concentrations (µg/g) in liver, heart and kidney of Indian Flying Fox (mean ± standard Error).

Organs	Cadmium	Copper	Lead	Zinc	*P- value*
Liver	0.781 ± 0.11^{A}	1.792 ± 0.12^{B}	3.613 ± 0.56^{C}	4.422 ± 0.27^{C}	*0.000*
Heart	0.696 ± 0.06^{A}	2.855 ± 0.16^{B}	2.289 ± 0.19^{C}	3.761 ± 0.27^{D}	*0.000*
Kidney	2.287 ± 0.21^{A}	1.666 ± 0.12^{B}	2.158 ± 0.14^{AB}	3.503 ± 0.29^{C}	*0.000*

Similar superscripts in each row shows non-significant difference ($P > 0.05$),

RESULTS AND DISCUSSION

Variations among organs

In the qualitative comparison liver and heart showed significant difference for all the metal determined in Indian flying fox (Table II). When mean concentration values in different organs *i.e.*, liver, heart and kidneys were compared, they showed significant difference for all of the metals ($P<0.05$) but the deposition levels were lower than the toxicity values recommended for mammals. The highest or maximum value for Zn was in liver samples (4.422±0.27 µg/g) and lowest in kidney (3.503±0.29 µg/g). Pb was highest in liver and lowest in the kidney (Table III). Cu was high in the heart samples and low in kidney while Cd was highest in the kidney but lowest in the heart. In liver Cd, Cu, Pb and Zn were significantly different but there was no difference between Pb and Zn. In heart all the four metals were significantly different. Mean values showed significant difference for all metals in kidney but lead was not found significantly different from Cd and Cu (Table III). The mean metal concentration however, resulted with significant difference among three organs. Cd was observed relatively in minimum concentration in all of the four metals analyzed.

In fruit bats, the comparable data about heavy metals is not available; however, Walker *et al.* (2007) reported heavy metal levels in micro-bats of south west England from kidney samples as Hg 101±3.12% (n=23), Pb 112±5.22% (n=23) and Cd 96.2±4.01% (n=22) which are much higher than our recorded levels in fruit bat. This greater level is perhaps difference is diet composition of both micro and fruit bats. Streit and Nagel (1993) reported, in adults of the insectivorous bat *Pipistrellus pipistrellus,* levels of Pb in the liver between 2.95 and 38.5 mg/g dry mass, and Cu levels between 15.7 and 32.0 mg/g dry mass and these values are also much higher than present study. The study revealed that levels of heavy metals were under the permissible limits for mammals as per WHO (1989); *viz.,* copper-71, zinc-289, cadmium-173, and Pb-25 µg/g (dw).

All of the four metals showed different tendencies to accumulate in different organs and it has been observed to depend on physiology of the organs in addition to other factors. The accumulation levels of the metals in living organisms are dependent to species, the size of individuals, tissues and organs as well as the type of metal (Uysal *et al.*, 1986). Zn is a trace element present in all tissues of all organisms, in general, Zn-specific sites of accumulation in animals are bone, liver and kidney (Spear, 1981). If the concentration of Zn as well as other trace element/micronutrients increased beyond the level required, they can act in either acutely or chronically toxic manner (Gulfaraz *et al.*, 2001).

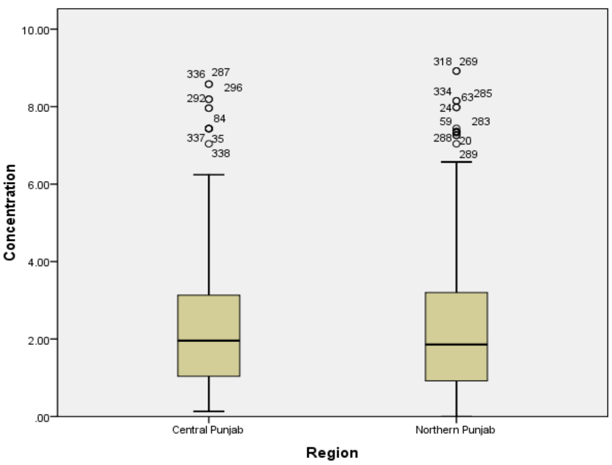

Fig. 1. Organ related comparison of metal (Cd, Cu, Pb, Zn) concentrations (µg/g of dry weight) between the Indian flying fox of central and northern Punjab.

Regional variations

Samples collected from central and northern Punjab, assuming central Punjab as heavily industrialized having type of industries as electrical fittings, chemicals, steel products, textiles, sugar, garments, food, ceramics, electrical machines, domestic machines, leather etc. and northern Punjab as less industrialized having predominantly type of industry as chemicals, engineering, poultry feed, furniture, cement, oil. In order to compare metal toxicity levels in both regions all of the four metals were pooled to get their overall effect in the region. It was assumed that central Punjab is heavily populated so it will be more polluted with heavy metals as compared to the northern Punjab but when they were compared for heavy metal load, no difference was found in heavy metal concentration in both regions on qualitative as well as quantitative basis (Table IV, Fig. 1). The possible explanation for this seems the recent industrial development both in the Central as well as Northern Punjab to meet the requirements of expanding human population. The distribution pattern of different metals in the samples of Indian flying fox collected from these regions were as Zn was greater (%age-wise) in the liver, heart and the kidney samples of northern Punjab than in the central Punjab. Zn is essential for human health but pancreas can be damaged due to very high levels of Zn and it can disturb the protein metabolism (Goyer, 1997). Zn is rising in nature due to industrial activities, such as mining coal, waste combustion, steel processing etc. Zn is used in dye casting, automobile and rubber industries too. Zn is also used in cosmetics, photocopier paper, wall-paper, printing ink etc. (Goyer, 1997).

Table IV.- Organ wise qualitative comparison of metal prevalence between Indian flying fox from central and northern Punjab.

Organ	Metal	Central Punjab (n = 39)	Northern Punjab (n = 55)	P value
Liver	Cadmium	100%	100%	1.00
	Copper	100%	100%	1.00
	Lead	97.3%	89.3	0.143
	Zinc	97.3%	98.2	0.648
Heart	Cadmium	94.7%	94.6%	0.679
	Copper	94.7%	100%	0.161
	Lead	100%	100%	1.00
	Zinc	92.1%	100%	0.063
Kidney	Cadmium	94.7%	91.1%	0.406
	Copper	97.4%	94.6%	0.466
	Lead	97.4%	92.8%	0.324
	Zinc	92.1%	94.6%	0.465

*Pearson Chi square 2 sided significance

Environmental challenges of Pakistan are primarily associated with an imbalanced economic and social development in recent decades. All major cities of Pakistan face haphazard, unplanned expansion due to a shift of population from rural to urban areas which worsen the situation to cope up with this challenge. Since the municipal authorities or other utility service providers have limited resources, haphazard urban congestion is the prime reason for deterioration of natural resources like air, water and soil quality (WWF, 2008; PCRWR, 2010).

Gender variations

When mean values (µg/g) were compared between male and female samples (2.41±0.08 ♂ and 2.43±0.12

♀), it appeared to be non significant for both sexes. Khan (1995) and Komarnicki (2000) reported gender differences and the bioaccumulation pattern of nonessential metals in several species of mammals including human. They reported that Zn tends to increase in soft tissues in females of some mammals such as the mole. Generally speaking, the gender differences of essential elements (Co, Fe, Zn, and Mo) may be associated with differences in the metabolic profile of metals involved in the activity of sexual hormones, the uptake of metals, nutritional requirements or interactions between elements (Goyer, 1997). Walker *et al.* (2007) in south west England reported that there was no significant difference observed of sex or age of any metal on renal concentrations in the brown long-eared bats.

CONCLUSIONS

No previous studies are available to compare the heavy metal concentration in Indian flying fox from Pakistan. This study confirm the presence of heavy metals in this species but it is lower than values recommended for mammals. This study provided a baseline data to compare metal concentrations in future studies in the bat fauna. As results have indicated more accumulation of Zn and Pb in the body tissues which could be considered as the precursor to the issues that concern our environment in general and the health of both humans and the biodiversity in particular (recommended bio toxic values for Zn is 14-82 μg/g and for Pb 10 μg/g of dry weight of samples). It is recommended that continuous monitoring is required to assess the metal accumulation which may ultimately lead to the decline in bat population.

Statement of conflict of interest
Authors have declared no conflict of interest.

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