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ESTIMATION OF HEAVY METALS (Cd, Cu, Pb, Zn) FROM SIX SPECIES OF VESPER BATS

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ABSTRACT

Metals play an important role in the biological functions of our body, but their bio toxic effects can be harmful and can disturb the normal body functions. Metals are elevating in the environment due to rapid industrialization, and other anthropogenic activities. Toxic metals are bio accumulated by insectivorous bats due to their foraging habits and high trophic level. As the bio-accumulation of toxic pollutants is one of the major reasons of bat decline in the world, this study was carried out to quantify heavy metal concentrations in the kidney, liver, and heart of micro-bats collected from different areas of Punjab, Pakistan. Our findings showed significant difference in Cd, Cu, Pb and Zn concentrations in liver, heart and kidneys. Significant differences were observed for Pb and Zn concentrations in the liver of the six species under observation while Cu and Pb levels in the kidney and heart varied significantly. This study provides a base for future monitoring of metals in bats for management.

Key words: Metals, Bats, Vespertilionidae, Chiroptera.

INTRODUCTION

Vespertilionidae family is believed to be originated from Molossidae in early Eocene epoch. It is largest group of Chiroptera order commonly known as vesper bats or evening bats and comprises about one third bat species of the world (Koopman, 1993) with 48 genera and 407 species (Simmons, 2005). Body length ranges 3-13 cm without tail and generally these bats appear grey in color. Vesper bats are most wide spread and have worldwide distribution except for Antarctica (Feldhamer *et al*., 2007; Hill and Smith, 1992). Some of them consume fish and small passerine birds in diet, otherwise almost all vesper bats are voracious insect eater. In Kansas, individual adult bat is estimated to consume 2.0g insect per night and annual consumption believed to be more than 16 tons (Kunz and Fenton, 2003; Kunz *et al*., 1998, Kurta *et al*., 1989). Many of these insects serve as vectors for various human diseases. They also help to minimize the insect pests on agricultural, garden and ornamental plant through heavy predation and thus hold their economic importance (Agosta and Morton, 2003). Mostly these bats depend on echolocation and roost in caves however some also use tree cavities, rocky crevices, burrows of other animals for roosting.

In Pakistan, Vespertilionidae family is represented by 14 genera and 33 species; none of them exhibits any marked individual characteristic but they are small bats with extensive inter femoral membrane which wholly encloses tail. There is no nose leaf except for one species, always prominent tragus and eyes are small and poorly developed (Roberts, 1997). In this family some of the females bear two pairs of mammae. Generally females establish separate maternal colonies and young ones are without hairs at time of birth (Roberts, 1997). Bat populations in many countries are declining rapidly. Bio-accumulation of insecticides and other pollutants is thought to be a major cause to this decline (Walker *et al*., 2007). Bats are threatened by chemical treatment of timber, human disturbance and destruction of roost sites (Fenton, 1997). The interest in the study and conservation of bats throughout the world has been growing (Stebbings and Griffith, 1986) and present study was aimed to assess the concentrations and distribution of heavy metals viz. cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) in the liver, heart and kidney of vesper bats, keeping in view increasing heavy metals contamination in different regions of Punjab and to know how deposition levels vary in organs, regions and gender of species.

MATERIALS AND METHODS

Study Area: Punjab (31°17′040.6″ N; 72°70′97.16″E) is a heavily populated province of Pakistan that comprises about 53% (https://en.wikipedia.org/wiki/2017_Census_of_Pakistan) of the country's total population. River Indus and its tributaries pass through Punjab. Its land is mostly irrigated and canals can be found over a major part of Punjab. Weather extremes are also prominent. The Pothwar Plateau is present in the northern parts of the province, which is an arid zone while the foothills of the Himalayas are found in the extreme north.

Type of industry in the central Punjab is mainly of electrical fittings and machines, steel products, leather textiles and ceramics, and the industry present in the northern Punjab is of cement and oil, chemicals, furniture and poultry feed etc. The areas of central Punjab and northern Punjab were selected for bat sampling in order to compare metal pollution in both areas as well. The bat samples were collected from different areas of central Punjab (Gujranwala, Faisalabad, Sheikhupura districts) and Northern Punjab (Jhelum, Chakwal, Attock, Rawalpindi districts, and Islamabad Capital Territory) (Table-1).

Sample Collection: Bat surveys were conducted on monthly basis from 2010-2013 for locating different roosting sites. Mist nets were erected at the sampling sites before sunset for bat capturing. Help was also taken from local people in locating the roosting sites of bats. Different stations were mist netted in the districts of central and northern Punjab. Bats were captured following the methods of Kurta *et al*. (1989). The nets were laid in L or V shape preferably near water bodies just before the sunset in order to increase the capturing efficiency. Capturing efforts were not always be successful, so the roosting sites were visited again and again. GPS locations of bat roosts/netting stations were also recorded using Garmin Etrax H (Table-1). We tried to capture only those species which are common in area and their status is 'Least Concern" according to IUCN; bats were not trapped in breeding season.

Procedure and Analysis: After the collection, samples were brought to the laboratory of the Department of Zoology, PMAS-Arid Agriculture University, Rawalpindi in small clean polyethylene bags, or cloth bags and kept frozen before being analyzed. Some basic information like date of collection, locality, sex, and species was noted for each specimen following Walker *et al.* (2003). The collected samples were adults and identified following Bates and Harrison (1997), Roberts (1997) and Mahmood-ul-Hassan *et al*. (2009). The bats were dissected to excise liver, heart and kidney. These organs were thoroughly washed with distilled water, dried, weighed and cut into pieces (2 gm) and transferred into quartz crucibles. Digestion of tissue was carried out with 1 ml of concentrated Nitric acid (HNO_3) and 0.25 ml concentrated Perchloric acid ($HClO_4$). The digestion was initially done at low temperature and then at high temperature using hot plate until a clear straw color solution was formed. These samples were diluted with de-ionized water and the final volume rose up to 10 ml for further processing (Ostapezuk *et al*., 1984; Nighat *et al*., 2013). The estimation of heavy metals was done following Sperling *et al*. (1999) by atomic absorption spectrophotometer (G. B. C. 932 Plus, UK). The precision and performance of the instrument was checked by analyzing the certified standard reference metal solutions (1000 µg/g) before processing samples of bats under study.

Concentrations of metals were not in normal distribution (Shapiro-Wilk test, $P < 0.05$) and data was log-transformed to get the normal distribution. Chi-square test, ANOVA, and Student's t-test were used for data analysis and mean values were compared by LSD (Least significant difference) and Tuckey's HSD (Honestly Significant Difference). The results were presented as mean ± standard deviation. Values of $P < 0.05$ and $P < 0.01$ were considered as significant and highly significant when and where appropriate. All tests were performed in SPSS, version 17.0 (SPSS Inc. Chicago Illinois).

RESULTS AND DISCUSSION

A total of 102 bats (50♂, 52♀) belonging to six species (Table 1) were captured from different areas of Punjab. A high species richness of four was observed in Islamabad, Chakwal and Faisalabad areas during the study. The probable reason for this may be that this area has a large number of fruit orchards, on which a large population of insects is likely to be present. Micro-bats visit these areas to hunt these insects which are major part of their food.

Metal Concentrations in Tissues: In comparison of metal concentration, all the four metals were observed to be significantly different among tissues i.e. liver, heart and kidney (Table 2). However, all the concentrations observed, (Table 2) are below the permissible limits for mammals as per WHO (1989); viz Cu-71, Zn-289, Cd-173 and Pb-25 µg/g (dw). The bio-accumulation patterns of metal concentration was recorded as: kidney >liver > heart, and for different metals was as: zinc > lead >copper > cadmium. Spear (1981) and Alleva *et al*. (2006), reported that Zn is present in all tissues of organisms and mostly deposited in bones, liver and kidney in an animal's body. Uysal *et al*. (1986) reported that the accumulation of the metals in living organisms are dependent upon the species, size of the tissues and organs as well as the type of metal itself. Bats eat a variety of food from flower to insects, fish and small mammals but 70% of the bats are insectivorous. Pattern of heavy metals' deposition might be result of the differences in feeding habits, habitats and metabolism of the animals. Méndez and Alvarez-Castañeda (2000) gave comparative analysis in the liver of two species of ichthyophagous bats and reported the heavy metal load as (µg/g in dry weight) 133 (Zn), 27.4 (Cu), 1.25 (Pb), 6.5 (Cd) for *Myotis vivesi* and 57 (Zn), 13.1 (Cu), 0.57 (Pb), 8.0 (Cd) for *Noctilio leporinus.* Heavy metals are non-biodegradable natural resources while some are stable environmental trace components of fresh and marine waters, but their levels have increased now due to domestic, industrial, mining and many agricultural practices (Kalay and Canli, 2000; Yousafzai and Shakoori, 2008). If the concentration levels of heavy metals or the micro contaminants increase beyond a certain level, they can act either rapidly or chronically in toxic manners (Gulfaraz *et al.*, 2001). Analytical recoveries determined by Walker *et al.* (2007) in England from kidney samples of brown long-eared bats were 3.38, and 0.830 ug/g dry mass for Pb and Cd respectively, that are much higher from the values recovered in this study from the kidney of vesper bats (Pb, 1.721± 1.32 µg/g and Cd, 0.378 ± 0.29 µg/g; Table 2). 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. In present study these values were lower in liver i.e. 1.659 µg/g for Pb and 0.754 µg/g for Cu (Table 2).

Regional Variations: Non-significant difference was noted in the level of metal concentrations (µg/g) between regions i.e. northern and central Punjab. It was hypothesized that central Punjab is more industrialized than the northern Punjab hence more metal pollution was expected from this area. However, due to urbanization, perhaps northern Punjab is now equally industrialized in population and pollution therefore non-significant differences in metal levels were observed. In the liver samples of micro-bats collected from both regions of Punjab, all the four metals from central part were found to be relatively more heavily loaded than the samples from northern parts of the provinve. In the heart samples from central region, Zn, Pb and Cu were relatively high with the exception of the Cd in the samples from northern region. As for case of kidney, Zn and Pb were greater in the samples of northern region while Cu and Cd were proportionally high in the kidney samples from central Punjab.

Species Comparison: The metal concentration within six species was calculated for an overall comparison. The results showed that only the concentration levels of Pb and Zn were significantly different among different species of micro-bats in liver (Table 3) but there was no difference in concentrations of Cd and Cu among the species. Pb concentration was highest in *P. pipistrelus* (2.437±2.96 µg/g) and lowest in *P. ceylonicus* (0.703 ± 1.09 µg/g. Similarly, Zn appeared highest in *P. ceylonicus* (5.912 ± 2.65 µg/g) and was lowest in *P. javanicus* (2.650±0.59 µg/g; Table 3). O'Shea *et al.* (2001) reported that insectivorous bats accumulate the elements after feeding on insects that had spent the aquatic phase of their life cycle in contaminated water. Pb is reported to bio-accumulate in the skeleton and wet tissues of mammals and it reduces the reproductive capacity of the animals. Zn is present in all the tissues of all organisms; in general, Zn-specific sites of accumulation in animals are bone, liver and kidney (Spear, 1981). Khan *et al*. (2009) from Pakistan reported that Zn is significant element of automobile components and its presence in the roadside soil showed that vehicular traffic is the major anthropogenic source of pollution. They reported that the largest natural emission of Zn to water results from erosion while natural inputs to air are mainly due to igneous emissions and forest fires.

Cu and Pb were significantly different among species in the kidneys (Table 4). The highest concentration of Cu was recorded in *S. heathii* (1.051± 1.37 µg/g) while lowest in *Hypsugo savii* (0.101± 0.07 µg/g). Likewise, highest Pb was observed (Table 4) in *P. javanicus* (2.120± 0.19 µg/g) and lowest in *H. savii* (0.357± 0.44 µg/g). However, overall Zn was proportionally found in higher concentration in all the six bat species. Walker *et al.* (2007) in England from kidney samples of *Pipistrellus* species (n = 172) recorded Pb and Cd as 2.45 and 1.42 ug/g dry mass which is greater than any of the *Pipistrellus* species in present study. PCRWR (2010) reported that in Pakistan, main contributors to the surface and ground water pollution are the byproducts of various industries such as textile, metal, dying chemicals, fertilizers, pesticides, cement, petrochemical, energy and power, leather, sugar processing, construction, steel, engineering, food processing, mining and others.

Cu and Pb were also appeared significant among all the species in the heart (Table 5). Cu concentration dominated in *P. tenuis* (1.27± 1.09 µg/g) and lowest in *H. savii* (0.32± 0.53 µg/g). Similarly, maximum Pb was deposited in *P. javanicus* (1.84± 0.59 µg/g) and it was minimum (0.57± 0.72 µg/g) in *H. savii* (Table 5). Like liver and kidneys, in heart Zn accumulation was higher in all the species of bats while Cd levels were generally lower in all species. Cd usually concentrates in the internal organs of animals rather than in muscle or fats. Cd accumulates typically higher in kidney than in liver, and also higher in liver than in muscles. Cd is a non-essential heavy metal with toxic effects; it may accumulate in humans from food chain and its deposition usually increases with age (WHO, 1992).

Gender Variations: Non-significant difference was observed in the pooled data in metal concentrations between males and females of all micro-bats. However, when we analyzed male and female concentrations separately in liver, kidneys and heart, it showed a significant difference of Cd and Cu in kidneys only (Table 6) while all metals were found non-significant in liver and heart in both sexes. Level of metal accumulation was slightly different in both sexes, though non-significant. Female bats showed a slight higher heavy metal accumulation as compared to the male bats. The accumulation level of different metals in different tissues of both sexes may be influenced by a combination of factors, such as dietary preferences, physiological metabolism in relation to stage in the reproductive cycle or foraging behavior (Alquezar *et al*., 2006). Khan *et al*. (1995) and Komarnicki (2000) reported gender differences with respect to bio-accumulation patterns of nonessential metals in several species of mammals including humans. They reported that Zn tends to deposit and increase in the soft tissues of females of some mammals. Clark (1979) has reported that Pb in mammals has been found at higher levels in females as compared to males except for the big brown bat where concentrations in males were significantly higher than females by about 1.5 times. Males evidenced with significantly high mean values for Pb and Co in liver at a polluted site of Spain but non-significant sex-dependent variation was detected for kidneys, males and females showed a similar pattern of Cd accumulation in livers (Pankakoski *et al*., 1993; Clark *et al*., 1992: Komarnicki, 2000). In another study non-significant difference of any metal on renal concentrations due to sex or age was observed in the brown long-eared bats in south west England (Walker *et. al.,* 2007).

Table 1. Study areas (main stations) of central and northern Punjab for capturing of bats.

		Latitude	Longitude	Sample No.
Central Punjab	Gujranwala	N32°8′60.00″	E74°10′60.00″	11
	Faisalabad	N31° 25′ 4.8″	E73° 4′ 44.4″	13
	Sheikhupura	N31°74′30.00″	E73° 95′ .52″	15
Northern Punjab	Rawalpindi	N33°40′38.00″	E72°51′21.00″	14
	Chakwal	N32°56′0.63″	E73°43′14.57″	14
	Jhelum	N33° 54′ 26″	E72° 18′ 40″	13
	Attock	N33°43'4.749"	E73'36.36.36"	10
	Islamabad	N 33° 42'0.55"	E73'25.10.7"	12
		Total		102

Table 2. Comparisons of metal concentrations (µg/g) in the liver, heart and kidney of the bats (mean ± standard deviation).

	Cadmium	Copper	Lead	Zinc	P value
Liver	0.236 ± 0.25^{a}	0.754±1.04a	1.659 ± 1.51^{b}	4.163 ± 2.38^{c}	0.000
Heart	0.490 ± 0.65^{a}	$0.958\pm1.15^{a\,b}$	1.292 ± 0.72^{b}	4.075 ± 2.05^{c}	0.012
Kidney	0.378 ± 0.29^{a}	0.827 ± 0.91^{a}	1.721 ± 1.32^{b}	3.983 ± 2.78^{c}	0.000

Similar super scripts show non-significant differences (P > 0.05).

Table 3. Comparison of metal concentrations (ug/g) in liver (mean ± standard deviation) among different species.

Metals	***Scotophilus heathii*** **n= 42**	***Pipistrellus javanicus*** **n= 6**	***Pipistrellus tenuis*** **n= 10**	***Pipistrellus ceylonicus*** **n= 13**	***Pipistrellus Pipistrellus*** **n= 24**	***Hypsugo savii*** **n= 7**	**P. value**
Cadmium	0.035±0.09	ND	0.104±0.17	0.025±0.06	0.217±0.04	0.001±.004	0.119
Copper	1.050±1.67	0.323±0.11	0.602± 0 .37	0.288±0.27	0.515±0.67	0.751±0.67	0.254
Lead	1.086±0.91	1.830±0.56	1 .408± 0.76	0.703±1.09	2.437± 2.96	1.696±0.93	0.023
Zinc	3.836±1.91	2.650±0.59	4.552±3.37	5.912±2.65	3.045±2.75	3.040±0.95	0.009

Sig. P<0.05; ND-not detected.

Table 4. Comparison of metal concentrations (ug/g) in kidney (mean ± standard deviation .) among different species of bats.

Metals	***Scotophilus heathii*** **n= 42**	***Pipistrellus javanicus*** **n= 6**	***Pipistrellus tenuis*** **n= 10**	***Pipistrellus ceylonicus*** **n= 13**	***Pipistrellus Pipistrellus*** **n= 24**	***Hypsugo savii*** **n= 7**	**P. value**
Cadmium	0.104±0.22	0.300±0.23	0.1200±0.16	0.043±0.15	0.155±0.34	ND	0.240
Copper	1.051±1.37	0.456±0.32	0.632± 0 .51	0.279±0.35	0.455±0.43	0.101±0.07	0.029
Lead	1.104±1.01	2.120±0.19	1 .738± 0.94	1.598±0.76	1.598± 0.76	0.357±0.44	0.004
Zinc	3.54±1.61	2.303±2.12	3.43±4.42	5.23±2.86	4.787±4.21	3.431±1.13	0.182

Sig. P<0.05; ND-not detected

Table 5. Comparison of metal concentration (ug/g) in heart (mean ± sd.) among different species.

Metals	*Scotophilus heathii* n= 42	*Pipistrellus javanicus* n= 6	*Pipistrellus tenuis* n= 10	*Pipistrellus ceylonicus* n= 13	*Pipistrellus Pipistrellus* n= 24	*Hypsugo savii* n= 7	*P. value*
Cadmium	0.03 ± 0.09	ND	0.47± 0.96	0.04 ± 0.11	0.13 ± 0.39	ND	0.060
Copper	0.56 ± 0.80	0.96 ± 0.48	1.27 ± 1.09	0.37 ± 0.55	0.80 ±1.55	0.32 ± 0.53	0.040
Lead	0.85 ± 0.86	1.84 ± 0.59	1.40 ± 0.82	0.67 ± 0.80	1.32 ± 0.70	0.57 ± 0.72	0.012
Zinc	4.05 ± 1.98	4.06 ± 2.29	2.14 ± 1.01	4.59 ±1.40	3.88 ± 2.22	3.46 ± 1.70	0.094

Sig. P<0.05; ND-not detected

Table 6. Comparison of metal concentrations (ug/g) in liver and kidney (mean ±standard deviation)between male and female bats.

	Liver		**P value**	**Kidney**		**P value**
	Male ♂ n=50	**Female ♀** n=52		**Male ♂ n=50**	**Female ♀ n=52**	
Cadmium	0.03±0.08	0.08±0.17	0.061	0.03±0.09	0.20±0.31	0.000
Copper	0.70±1.51	0.74±0.73	0.876	0.39±0.47.	0.93±0.1.25	0.005
Lead	1.14±1.02	1.79±2.16	0.053	1.28±0.88.	1.39±.1.08	0.606
Zinc	3.85±2.88	3.87±1.98	0.973	4.21±2.74	3.71±3.16	0.394

Sig. P<0.05; ND-not detected

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