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## Short Communication

# Detection of Heavy Metals in Three Micro-Bat Species from Central and Northern Punjab

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**Authors' Contributions**
MSN supervised and design the study. SN performed the laboratory work and wrote the manuscript. SIS, AK, AA and MS conducted field surveys and captured bat samples from north and central Punjab. TM analyzed the data.

**Key words**
Bats, Microchiroptera, Heavy metals.

**ABSTRACT**

Metals are elevating in the environment due to rapid industrialization, and other activities like, combustion of fuel, processing of metals, coal mining, automobile, lead-acid batteries, and building material. Toxic metals are bio-accumulated by insectivorous bats due to their foraging habits and high trophic level. There is a growing interest on the conservation of bats throughout the world as they play an important role in controlling insect population. We measured cadmium (Cd), copper (Cu) lead (Pb) and zinc (Zn) concentrations in bat tissues through atomic absorption spectrophotometer. Our findings showed that metals were more concentrated in the liver and kidneys, Zn and Pb showed high mean values as compared to other metals but these values were below the lethal levels. Within the regions and sexes no significant variations were found in metal concentrations; however within three bat species metal concentrations varied significantly. This study provides baseline data for future comparisons and management of bats and metal concentrations in environment.

Majority of micro bats live and roost in caves, tree hollows, rooftops, mines, and sometimes under the bridges. These insectivorous bats are nocturnal and carry out their activities very efficiently at night by using echolocation. They can often be seen, spiraling around street lights and in lawns to capture the insects at night (Jones *et al*., 2003). Diet of micro bats varies from insects to some larger vertebrate species like frogs, rodents, lizards, or even fish (Meyers, 2001).

Bat populations in many countries are declining rapidly. Bioaccumulation of insecticides and other pollutants is thought to be a major cause to this decline. Bats are threatened by chemical treatment of timber, human disturbance and destruction of roost sites (Fenton, 1997). Certain characteristics of bats, such as their small size and high longevity (20 years or more), make them suitable as indicators species of general environmental conditions (Fenton, 1997).

Heavy metals are present everywhere in all types of environments and are highly toxic because they are non-biodegradable, insoluble in water and are very persistent due to having longer half-lives (Burger *et al*., 2007). The indiscriminate release of harmful chemicals and metallic elements in the environment by industries and other activities of man may adversely affect the quality of our air, water and food resources and once inside the living organisms through food chain, these chemicals may accumulate in higher concentrations and induce various metabolic disorders (Kunz and Fenton, 2003). Among the heavy metals arsenic, lead, cadmium, and mercury are considered to have serious health implications on animals. The deposition of these elements in the body of an animal can cause severe damage to intestinal tract, skeletal, central nervous and reproductive systems and mucus tissues (ATSDR, 2007). The manufacturing of pigments, drugs, agrochemicals, plastics, batteries, electroplating and discharge of untreated effluents from different industries cause heavy metal pollution (Burger *et al*., 2007). Insectivorous bats are known to play a major role in minimizing nocturnal insect population by consuming a major population (up to 100 % of their boy mass) in a single night (Kunz and Fenton, 2003). Hemipterans, coleopterans, lepidopterans and trichopterans are among the important dietary elements of insectivorous bats (Agosta and Morton, 2003). A number of insects are predated by bats (Lee and McCracken, 2005), which are devastating pests of many economically important agricultural crops such as corn, cotton, and potatoes

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(Cleveland *et al*., 2007). As micro bats are insectivorous and they are present at higher trophic levels, so they would prove to be best indicators and can reflect the relationship between environmental disturbance and trophic levels in a better way, because bio accumulation and metabolic capacity of diet increases in animals as positions increases in the food chain (Alleva *et al*., 2006). Bats are among the animals which have very slow reproductive rates so the populations recover very slowly if declines. The long-term population changes in bat populations can be monitored easily (Walsh *et al*., 2001). Loss of natural habitat and human activities such as deforestation, use of pesticides, industrial activities are the major causes of population decline globally. 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 few micro bats, keeping in view that heavy metals contamination increasing in different regions of Punjab and to know how deposition levels varies in organs, regions and gender of species.

*Materials and methods*

Bats samples were collected (2010-2013) from Central (Sheikhupura, Faisalabad) and Northern (Chakwal, Jhelum, Rawalpindi, Islamabad) Punjab. 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. Bat roosts were located through surveys and with the help of local people. Mist nets were erected in L or V shape preferably near water bodies just before the sunset in order to increase the capturing efficiency following Kurta and Kunz (1989). Capturing efforts were not always be successful, so the roosting sites were visited again and again. 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. All animal handling/capturing/sacrifice were approved by the Ethical Committee of the University (PMAS-AAUR/2646).

After the collection, samples were brought to the laboratory in small clean polyethylene bags, or cloth bags and kept frozen before being analyzed. Following the Walker *et al*. (2003), some basic information like date of collection, locality, sex, and species was noted for each specimen. The collected samples were adults and identified following Bates and Harrison (1997). 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). 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-Wik test, $P < 0.05$) and data was log-transformed to get the normal distribution. ANOVA, and Student t-test were used for data analysis. The results are 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 49 samples (26 ♂, 23 ♀) of three species were collected from study districts. Naked-rumped Tomb bat (*Taphozous nudiventris*) was widely distributed and captured (n=32; 18 ♂, 14 ♀) from all five districts of Punjab as well as Islamabad Capital Territory. False Vampire bat (*Megaderma lyra*) was captured only from Rawalpindi district (n=06; 4 ♂, 2 ♀) and similarly Greater Mouse-tailed bat (*Rhinopoma microphyllum*) was captured (n=11; 4 ♂, 7 ♀) only from Islamabad Capital Territory area. In all three bat species metal concentrations were differed significantly from one another (one way ANOVA, F=6.73, $P<0.05$). However, levels of heavy metals were under the permissible limits for mammals as per WHO (1989); *viz*. copper-71, .zinc-289, cadmium-173, and lead-25 µg/g (dw).

In *M. lyra*, liver and kidneys were more heavily loaded with metals. In liver Zn accumulation was 4.22± 2.18 µg/g, Pb was 2.18± 0.43 µg/g and Cd 0.42± 0.52 µg/g. It was observed that Cd accumulated more than Cu in liver of this species (Table I). Heart has highest value for Zn 3.41± 1.74 µg/g followed by Pb, Cu and Cd. In the kidney mean metal load was observed as Pb (4.71± 3.60 µg/g), Zn (4.27± 1.39 µg/g) followed by Cu and Cd. In this bat Pb was greater than Zn in the kidney. This species was captured only from Rawalpindi region. It showed that the concentration of Pb is higher in this area (Table I). Pb is reported to bio accumulates in the skeleton and wet tissue of mammals and it reduces the reproductive capacity of the animals. Several studies have reported the Pb poisoning and potential Pb-mediated effects on reproduction in bats (Hariono *et al*., 1993).

**Table I.- Concentration of heavy metals (µg/g) in *Megaderma lyra* (n=6), *Rhinopoma microphyllum* (n=11) and *Taphozous nudiventris* (n=32).**

| | Liver | Heart | Kidney |
|---|---|---|---|
| ***Megaderma lyra*** | | | |
| Cadmium | 0.42 ± 0.52 | 0.09 ± 0.14 | 0.07 ± 0.06 |
| Copper | 0.31 ± 0.25 | 1.01 ± 0.75 | 0.46 ± 0.34 |
| Lead | 2.18 ± 0.43 | 2.21 ± 0.18 | 4.71 ± 3.60 |
| Zinc | 4.22 ± 2.18 | 3.41 ± 1.74 | 4.27 ± 1.39 |
| ***Rhinopoma microphyllum*** | | | |
| Cadmium | 0.10 ± 0.11 | 0.03 ± 0.09 | 0.05 ± 0.12 |
| Copper | 0.22 ± 0.29 | 0.46 ± 0.47 | 0.62 ± 0.64 |
| Lead | 1.32 ± 1.06 | 0.96 ± 0.60 | 0.82 ± 1.15 |
| Zinc | 6.11 ± 3.26 | 6.11 ± 3.26 | 4.10 ± 2.24 |
| ***Taphozous nudiventris*** | | | |
| Cadmium | 0.04 ± 0.09 | 0.03 ± 0.12 | 0.06 ± 0.13 |
| Copper | 0.68 ± 0.50 | 1.05 ± 1.44 | 0.89 ± 0.74 |
| Lead | 0.89 ± 0.85 | 0.86 ± 0.75 | 0.80 ± 0.90 |
| Zinc | 3.45 ± 2.17 | 4.32 ± 2.17 | 4.01 ± 2.55 |

Samples of *R. microphyllum*, were captured only from Islamabad area. Liver deposited greater load of metals i.e. Zn 6.11±3.26 µg/g, Pb 1.32± 1.06 µg/g, Cu 0.22±0.29 µg/g and Cd 0.10 ± 0.11 µg/g. Heart and kidney have a high mean concentration of Zn followed by Pb, Cu and Cd (Table I). Zn is present in all the tissues of all organisms but Zn-specific sites for accumulation in animals are bones, liver and kidney (Spear, 1981). The acute gastrointestinal effects and headaches, impaired immune function, changes in lipoprotein and cholesterol levels, reduced copper status, and zinc iron interactions all are due to higher Zn accumulation (DRI, 2006).

In *T. nudiventris*, like other two species Zn was found higher in heart (4.32± 2.17 µg/g) followed by Pb and Cu. Similarly Zn was high (3.45± 2.17µg/g) in the liver; than Pb (0.89± 0.85(µg/g) and Cu (0.68 ±0.50 µg/g). Concentration of Cd was lowest (0.04± 0.09µg/g) in liver. The pattern of metal concentration was as Zn> Cu > Pb > Cd in the kidney samples of this bat (Table I). High levels of Pb and Cd in diet can be toxic to animal species (Scheuhammer, 1987) and influence the endocrine system (Martin *et al*., 2003), kidneys (Nordberg, 1971), reproduction (Buerger *et al*., 1986), behavioral response (Hui, 2002), molting, migration (Honda *et al*., 1986), enzymes involved in hemoglobin formation, and growth rates (Eisler, 1988). Cu is an essential trace element but its excesses may cause stress to animals (Debacker *et al*., 2000).

In gender variations, no significant difference was observed between male and female in the metal accumulation however, level of metal deposition was varied in both sexes. In female bats proportionally higher heavy metal concentration was observed as compare to the male bats. The deposition level of various metals in different tissues of both sexes may be influenced by many factors, such as dietary preferences, physiological metabolism in relation to stage in the reproductive cycle or foraging behavior (Alquezar *et al*., 2006). Gender variations in bioaccumulation pattern of nonessential metals in many species of mammals including human were also documented by Khan *et al*. (1995) and Komarnicki (2000). They observed that Zn tends to deposit and increases in the soft tissues of females of some mammals. Similarly, Pb concentration in mammals was observed higher in females as compared to males with exception of big brown bat in which male concentrations were significantly higher than females by about 1.5 times. In Spain, males appeared with high mean values in liver for Pb and Co but no significant sex-dependent variation was detected for kidneys; both sexes showed a similar trend of Cd deposition in liver (Komarnicki, 2000). In the brown long-eared bats in south west England, no significant variations of any metal on renal concentrations was observed due to sex or age (Walker *et al*., 2007).

Level of metal concentrations between regions *i.e.* Northern and Central Punjab was non-significant. 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 recent urbanization, perhaps Northern Punjab is now equally industrialized in population and pollution therefore no significant difference in metal levels observed; Zn was proportionally high which revealed that Zn is rising due to industrial activities. In the liver of micro-bats collected from both regions, all the four metals from Central Punjab were found to be relatively more heavily loaded than the samples of the Northern Punjab. Zn, Pb and Cu were relatively higher in the heart samples from Central region, while Cd was higher in the samples of Northern region. Zn and Pb were greater in the renal samples of Northern region while Cu and Cd were proportionally high in the samples of Central Punjab.

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*Statement of conflict of interest*

Authors have declared no conflict of interest.

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