Environment and Ecology 42 (1) : 11—19, January—March 2024
Article DOI: https://doi.org/10.60151/envec/AUYA2089
ISSN 0970-0420

Analysis of Chemical Pollutants using Non-Invasive Approach in Indian Flying Fox (*Pteropus giganteus*) in Selected Regions of Punjab

Gagandeep Kaur, Rajwinder Singh

Received 21 June 2023, Accepted 22 November 2023, Published on 31 January 2024

ABSTRACT

Present study was conducted to estimate heavy metals, macro and micro nutrients along with pesticide residues in fecal pellets of *Pteropus giganteus* fruit bat at three different sites, Chatpat Bani (district Pathankot) site I, village Katani Kalan (district Ludhiana) site II and village Longowal (district Sangrur) as site III. Among heavy metals, Cr predominates (17.35-26.30 ppm) followed by Pb (7.17-10.03 ppm) from three selected sites. Comparatively, higher levels of Cr, Pb, Cd and Ni heavy metals were recorded at site III, whereas As at site II, respectively. Among macro nutrients, K (7653.50-16927.17 ppm) and Mg (3249.33-3516.10 ppm) predominates in three sites. Comparatively higher levels of Mg, Ca and Na macro nutrients were recorded at site III whereas K and P at site I, respectively. Among micro nutrients, Fe (682.88-2069.45 ppm) and Zn (79.18-126.02 ppm predominates in three selected sites. Comparatively higher levels Fe, Zn and Mn micro nutrients were recorded at site II, B at site III and Cu at site I, respectively. Interestingly, contamination of pesticide residues in faecal pellets was found negligible as GLC showed values below detectable limits i.e., <0.05 ppm in all selected locations, which is a positive sign that exposure of bats to different pesticides is negligible in these areas due to less usage of pesticides or excretion by body. In present study, all elements were under permissible limits which are required for growth and reproduction except Mg and K which are good indicator of assessment of environment.

Keywords *Pteropus giganteus*, Macro mutrients, Faecal pellets, Heavy metals, Pesticides.

INTRODUCTION

Bats are nocturnal and placental mammals belonging to second largest order of mammals "Chiroptera" with about 1230 species representing a quarter of total mammalian species found in the world (Roy *et al.* 2020). India has about 11% of world's bat population with more than 90% of bat diversity. *Pteropus giganteus* (Indian flying fox), *Rousettus leschenaulti* (Fulvous fruit bat) and *Cynopterus sphinx* (Short nosed fruit bat) are common and found all over the country of which Indian flying fox is apparent and one of the largest bats in the world while others have their restricted distributions (Singh 2021, Singh *et al.* 2022). There are 8 genera in all, with fourteen species of fruit bats found in the Indian subcontinent (Bates and Harrison 1997). They live in colonies of hundreds

Gagandeep Kaur[1], Rajwinder Singh[2]*

[2]Zoologist
[1, 2] Department of Zoology, Punjab Agricultural University, Ludhiana 141004, India

Email : rajwinder-singh@pau.edu
*Corresponding author

to thousands of individuals and roost in trees like *Dalbergia sisso* (shisham), *Eucalyptus globulus* (safeda), *Tamarindus indica* (tamarind) and *Mangifera indica* (mango). Having frugivorous as well as nectarivorous behavior aids in seed dispersal of around 300 plant species leading to forest regeneration (Singh and Singh 2021). They are key pollinators of economically important Indian plant species like *Durio* sp. (durian), *Bombax ceiba* (silk cotton), *Madhucalati folia* (Mahua), *Syzygium* sp. (wild jamun) and *Musa* sp. (wild banana).

Ecological studies of fruit bats are becoming increasingly important in the Indian subcontinent as their population is declining due to habitat destruction and hunting resulting in local threat to many species (Venkatesan 2007). Bats frequently cohabit with people in urban, industrial and agricultural areas thereby putting them at risk of exposure to a variety of chemical contaminants compromising their health as these chemicals accumulate in their tissues (Russo and Ancillotto 2015). The potential of bats as pollution bio-indicators is twofold : 1), exposure to contaminants such as heavy metals and pesticides contributes to their population decline, 2) contaminants found in bat guano are good indicators of pollution levels in the surrounding areas (Johnson and Vincent 2020). In environment, metal contamination is associated with 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 and vehicular emissions (Pb and other particulates) (Nighat *et al.* 2016). Interestingly, agricultural activities and industrial processes have induced the mobilization of heavy metals in freshwater ecosystems, which raised heavy metal pollution as an important threat to different organisms living in different ecosystems as these metals have potential to harm most organisms at some level of exposure and absorption (Cil *et al.* 2023). Manufacturing of drugs, agrochemicals, plastics, batteries, electroplating and discharge of untreated industrial effluents cause metal pollution (Imlani *et al.* 2022).

Conservation of bats has grown in popularity globally and the goal of this study was to determine the concentrations and distribution of heavy metals, macro and micro nutrients and pesticides in the faecal pellets of Indian flying fox in light of their increasing contamination, as well as to determine how their deposition levels vary in the faecal pellets of Indian flying fox at different locations of Punjab, India. This study provides a baseline data which might be considered as a precursor to a broad array of issues that concern our environment and health of both humans and biodiversity using bats as bio-indicators and help in conservation measures.

MATERIALS AND METHODS

Selection of sites

The present study was conducted across three locations in Punjab, India. Chatpat Bani, village Kataruchak, district Pathankot was selected as site-I. It is a sacred groove situated at 32.2041°N, 75.5688°E. This site has spread in approximately 30.0 acres area covered under thick forest rich in flora and fauna (Fig. 1A). Village Katani Kalan, district Ludhiana was selected as site-II, situated at 30°84” N, 76°06” E. This village is located at the shores of Sirhind canal along with human settlements (Fig. 1B). Village Longowal, district Sangrur was selected as site-III, situated at 30.2167°N, 75.6833°E located 19.0 km from main city area of Sangrur. The site is surrounded by various tree species along with lake and human settlements (Fig. 1C). All the selected sites have orchards of guava (*Psidium guajava*), ber (*Ziziphus mauritiana*), mango (*Mangifera indica*) and litchi trees (*Litchi chinensis*) which serves as food for the fruit bats.

Collection of *P. giganteus* faecal pellets

To obtain samples of faecal pellets of *P. giganteus,* a transparent polythene sheet was unfurled around the roosting trees of *P. giganteus* having required diameter from all three sites. The required number of samples of faecal pellets were collected after 2-3 days of unrolling the transparent polythene sheet and transported to Biodiversity and Biosystematics laboratory, Department of Zoology, Punjab Agricultural University, Ludhiana in small polythene bags/vials. The samples were air dried and further digested before preparing them for heavy metal analysis (Piper 1944).

Fig. 1. Satellite view of three study regions (A) Chatpat Bani, village Kataruchak, district Pathankot (site-I), (B) Village Katani Kalan, district Ludhiana (site-II), (C) Village Longowal, district Sangrur (site-III).

Digestion and analysis of heavy metals, micro and macro nutrients in *P. giganteus* faecal pellets

For digestion, a total of 12 samples (4 from each site) were analyzed along with blank sample. Faecal pellets were crushed and made into powder using pestle and mortar and each sample was transferred into separate petri-dishes and dried in microwave oven for 1-2 h at 80-90°C. About 0.5 g of each dried sample (3 replicates each) was weighed using electronic weighing balance and transferred into 50 ml conical flask to which 10 ml diacid was added and then kept overnight. The solution appeared dark red in color. A stock/diacid solution (Nitric acid and Perchloric acid) was prepared in the concentration of 3:1 in which samples were to be digested. After 24 h, the conical flasks were put on hot plate to digest the samples (100°C) for 40-45 min until the samples turned colorless and their volume reduced to 1-3 ml. The color of the samples turned from red to orange to yellow and then became colorless. This step was performed outdoors as it involved emission of poisonous fumes of diacid. The digested sample was then diluted with de-ionised water up to 25 ml and filtered to remove any residues using Whatman no. 42 and then transferred to sterilized plastic vials with lid and stored at room temperature prior to analysis.

The digested samples of fruit bats were analyzed for the presence of heavy metals (Cr, Ni, As, Cd and Pb), macro-nutrients (Na, Mg, P, K and Ca) and micro- nutrients (B, Mn, Fe, Cu and Zn) by Inductively Coupled Argon Plasma Atomic Emission Spectrophotometer (ICAP-AES), model ICAP6300 Duo in the Department of Soil Science, PAU, Ludhiana (Sansoni and Pandey 1994). Atomic Emission Spectroscopy uses Inductively Coupled Plasma (ICP) as the energy source. The ICP source consists of three concentric quartz tubes through which Argon flows @ 0.5-1.01/ min and two or three turn gold plated copper coil surrounding the tubes near the upper end. Alternating current at a frequency of 60 Hz and power levels up to several kilowatts is passed through the copper coil. Argon is ionized by the momentary high voltage (200-240 V) discharge. The ionized gas is further passed through the high frequency magnetic field which raises the temperature of the resulting plasma to about 10,000 K. Liquid samples in the form of an aerosol are injected into the high temperature spray chamber. Here, free atoms of the analyte are formed which emit characteristic spectra. The photomultiplier tubes measure the intensity of emitted light which is directly proportional to the concentration of element within the sample.

The readings taken on ICAP-AES were converted into parts per million (ppm= μg/g) as per the following formula :

$$\frac{\text{Reading of sample–Reading of blank}}{\text{Weight of sample}} \times \text{Total volume used}$$

Digestion and analysis of pesticide residues in *P. giganteus* faecal pellets

For the analysis of pesticide residues in the faecal pellets of *P. giganteus*, the samples were collected from all the three sites as described earlier and their analysis was done in the Pesticide Residue Analysis Laboratory, Department of Entomology, Punjab Agricultural University, Ludhiana. Pesticide residues of commonly used organochlorines, organophosphates and synthetic pyrethroids were analyzed in *P. giganteus excreta* by using modified QuEChERS method (Anastassiades *et al.* 2003). About 12 samples (4 from each site) were analyzed. About 5 g of faecal sample was taken in clean plastic vial. To each vial 10 ml of acetonitrile, 2 g of NaCl (sodium chloride) and 2 ml of distilled H_2O was added. The vials were vortexed for 5 min for proper mixing. This was followed by centrifugation @10,000 rpm for 5 min and 6 ml of supernatant was extracted and transferred to fresh plastic vials containing 7 ml acetonitrile and 2g Na_2SO_4 (sodium sulphate). The vials containing supernatant were vortexed for 10-15 sec. An aliquot of 4 ml was taken out and transferred to fresh vials containing 125 mg PSA (primary and secondary amine exchange material) and 750 mg anhydrous $MgSO_4$ (magnesium sulphate). Then, these vials were centrifuged at @10,000 rpm for 5 min. From these vials, 3 ml of supernatant was extracted and transferred to another set of calibration tubes which were then brought to sample evaporation concentration plus. This instrument was set at 45°C and allowed to run for 1-2 h till the sample got completely evaporated. Then, these vials were taken out and rinsed using acetone by filling it up to 1 ml for GLC analysis.

The sample extracts were analyzed using Gas Liquid Chromatograph (GLC), model Nucon 5700 equipped with Electron Capture Detector (ECD) and capillary column of 2 m length × 3 mm id packed with 1.5% OV-17±1.95% OV210. The working conditions of GLC included injector temperature 280ºC, column initial hold temperature 200ºC for 5 min followed by 220ºC for 10 min and detected temperature 300ºC and carrier gas (nitrogen) flow was maintained at 3.0 kg/cm^2. Before use, the column was primed with several injections of standard solution of either OCs/ OPs/SPs pesticide till a consistent response was obtained. Suitable aliquots of cleaned sample were then injected into the ECD mode of the detector. The compound in the sample was identified and quantified by comparisons of the retention time and peak heights of the sample chromatograms with that of standard run under identical operating conditions.

The residue was quantified by using the formula :

$$\text{Residue (ppm)} = \frac{\text{ng of standard} \times \text{Peak area of standard} \times \text{Total volume made}}{\text{Peak area of sample} \times \text{Volume of sample used} \times \text{Weight of sample taken}}$$

Statistical analysis

The data was analyzed using ANOVA. In order to test the significance among various parameters, the data was subjected to percentage (%). All values were expressed as mean±SE and are considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Analysis of heavy metals in *P. giganteus* faecal pellets

The heavy metals studied in faecal pellets of *P. giganteus* fruit bat from different selected locations included Arsenic (As), Cadmium (Cd), Lead (Pb), Nickel (Ni) and Chromium (Cr) along with macro and micro-nutrients (Na, Mg, P, K, Ca, B, Mn, Fe, Cu and Zn). Out of these As, Cd and Pb are included in the category of non-essential nutrients and others like Fe, Cr, Cu, Ni, Zn and Mn are essential nutrients which are needed in the body to perform specific functions. But all these nutrients are toxic when present above threshold levels in human beings and similarly in fruit bat too. In the present study, the concentration of heavy metals varied from location to location.

At site I, heavy metals were present in the faecal pellets of *P. giganteus* in the order Cr>Pb>Ni>Cd>As with concentration values (ppm) as 17.35, 9.55, 4.40, 1.42 and 0.99, respectively. At site II, their order was Cr>Ni>Pb>As>Cd with concentration values (ppm) as 24.87, 7.82, 7.17, 3.70 and 0.64, respectively. At site III, they followed the order Cr>Pb>Ni>As>Cd with concentration values (ppm) as 26.30, 10.03, 9.20, 2.14 and 1.72, respectively (Fig. 2). Higher levels of heavy metals (Cr, Pb, Cd and Ni) were recorded in faecal pellets of *P. giganteus* at site III whereas in case of As, higher levels were recorded at site II. Comparable levels of Cd and As were recorded in all the three locations under study. Concentration levels of toxic heavy metals have been found to be present in faecal pellets of *P. giganteus*. However, very few studies have been conducted on the accumulation of heavy metals and their impact on Chiroptera thus, permissible limits for various heavy metals in different species of Chiropterans have not been well established (Zukal *et al.* 2015). The comparative studies on liver, kidney and faecal pellet samples found that in general, faecal pellet (guano) samples contain higher concentrations of metals as compared to other tissues (Mansour *et al.* 2016). Only 5-10% of orally ingested metals get absorbed and nearly 99.5% of ingested metals are excreted through guano thus, leaving only 0.5% to be deposited in various body tissues (Klaassen 1976). Hernout *et al.* (2016) stated that a positive correlation exists between concentration of Pb and Cd in guano samples which suggest that concentration of one metal can predict the concentration of another metal in the guano sample. Studies on threshold limits of heavy metals in bat guano are limited and thus, studies on various mammalian tissues being used as reference for comparison indicate that in the present study, the concentrations of heavy metals in faecal pellets of *P. giganteus* are below the permissible limits for other tissues.

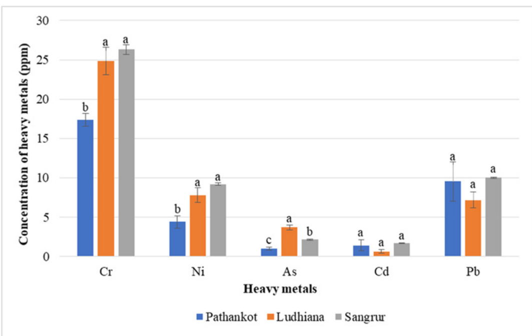

Fig. 2. Concentration of heavy metals in faecal pellets of *P. giganteus* at different locations.

Analysis of macro-nutrients in *P. giganteus* faecal pellets

Along with heavy metals, macro and micro-nutrients were also analyzed from faecal pellets of *P. giganteus.* In Chatpat Bani, district Pathankot, the concentration level of macro- nutrients found in the faecal pellets of *P. giganteus* ranged in the order as K>Mg>P>Ca>Na. The highest concentration levels were recorded in K (16927.17 ppm) and lowest in Na (649.93 ppm) with an increase of 96% in the concentration levels of K as compared to that of Na (Fig. 3). In village Katani Kalan, district Ludhiana, various macro-nutrients were found in the faecal pellets of *P. giganteus* in the order of concentration as K>Mg>Ca>P>Na. The highest concentration levels were recorded in K (9713.31 ppm) while the lowest recorded in Na (602.64 ppm) with an increase of 94% in the levels of K as compared to that of Na. In village Longowal, district Sangrur, the macro-nutrients found in the faecal pellets of *P. giganteus* were in order of concentration as K>Mg>Ca>Na>P. The highest concentration levels

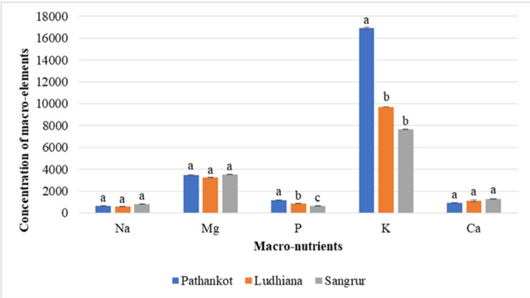

Fig. 3. Concentration of macro-elements in faecal pellets of *P. giganteus* at different locations.

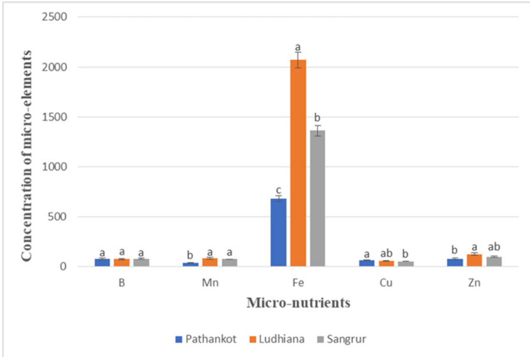

Fig. 4. Concentration of micro-elements in faecal pellets of *P. giganteus* at different locations.

were recorded in K (7653.50 ppm) while the lowest in P (648.08 ppm) with an increase of 91% in the concentration levels of K as compared to that of P.

Among different sites, highest concentration levels of Na (804.47 ppm), Mg (3516.10 ppm) and Ca (1247.80 ppm) were recorded in village Longowal, district Sangrur, followed by Chatpat Bani, district Pathankot and village Katani Kalan, district Ludhiana. No significant difference was found in the concentration levels of Na and Mg in all three locations under study. The highest concentration levels of P (1173.52 ppm) and K (16927.17 ppm) were recorded in Chatpat Bani, district Pathankot, followed by village Katani Kalan, district Ludhiana (869.58 ppm) and village Longowal, district Sangrur (648.08 ppm). All the three locations under study show significant difference in the concentration level of P.

The limits defining toxicity of macronutrients in *P.giganteus* remain unknown. However, nutrient requirement and the concentration of that particular nutrient in excreta can be compared to determine the toxicity of nutrient in mammals as the nutrient excreted out is far greater than that is absorbed within the body. As suggested by National Research Council (1978), Na requirement for growth and reproduction in small mammals is 0.5-l.5 ppm (500-1500 ppm) of food dry weight. In the present study, Na concentration in bat guano ranges from 602-804 ppm, which is below the upper limit for nutrient requirement. Thus, Na absorbed within the body of bat is less and does not cause any toxicity. Similarly, magnesium requirement of small mammals is 400-1000 ppm dry food weight (National Research Council 1978). Studier *et al.* (1991) in their study on big brown bats found that Mg levels in faecal pellets were at least three times higher than the nutritional requirement. Also, Mansour *et al.* (2016) in their study found Mg concentrations in bat guano to be very high i.e., 3562 ppm. In present study, Mg levels are also reported to be high i.e., above 3000 ppm. As nutrient concentration of bat guano reflects their habitat quality. It can be concluded that Mg was high in the environment/food. However, it cannot be said whether the present levels of Mg are toxic to bats or not as relevant studies are not available. Phosphorus levels in the bat guano in our study are also reported to be high. Studies regarding nutritional requirements of P or threshold limits for P toxicity in bats are also not available. In general, P rich guano of bats is commercially used as a natural source of fertilizer. Another essential macronutrient i.e., potassium (K) is required by small mammals in amount ranging from 2000-7000 ppm (National Research Council 1978). In our study, K is also present in very high concentration which indicates its abundance in the environment/food. However, whether the levels are toxic or not to bats cannot be clearly defined. Calcium dietary requirements for growth and reproduction in small mammals are 4000-8000 ppm (National Research Council 1978). Ca levels in our study are quite low comparatively and thus, do not cause any toxicity to fruit bat.

Analysis of micro-nutrients in *P. giganteus* faecal pellets

In Chatpat Bani, district Pathankot, the micro-nutrients found in the faecal pellets of *P. giganteus* ranged in the order Fe>Zn>B>Cu>Mn (Fig. 4). The concentration values of different micro-nutrients like B, Mn, Fe, Cu and Zn were 75.72, 37.88, 682.88, 62.38 and 79.18, respectively. The highest concentration levels were recorded in Fe (682.88 ppm) while the lowest recorded in Mn (37.88 ppm) with an increase of 94% in the concentration levels of Fe to that of Mn. In village Katani Kalan, district Ludhiana, the micro-nutrients found in the faecal pellets of *P. giganteus* ranged in the order Fe>Zn>Mn>B>Cu. The highest concentration levels were recorded in Fe (2069.45 ppm) while the lowest recorded in Cu (57.35 ppm) with an increase of 97% in the concentration levels of Fe to that of Cu. In village Longowal, district Sangrur, the micro-nutrients found in the faecal pellets of *P. giganteus* ranged in the order Fe>Zn>B>Mn>Cu. The highest concentration levels were recorded in Fe (1361.30 ppm) while the lowest recorded in Cu (53.08 ppm) with an increase of 96% in the concentration levels of Fe to that of Cu.

The highest concentration levels of B were reported in village Longowal, district Sangrur (80.05 ppm) followed by Chatpat Bani, district Pathankot (75.72 ppm) and village Katani Kalan, district Ludhiana (75.22 ppm). No significant difference was found in the concentration levels of B in all the three locations. The highest concentration levels of Mn (85.05 ppm), Fe (2069.45 ppm) and Zn (126.02 ppm) were reported in village Katani Kalan, district Ludhiana followed by village Longowal, district Sangrur and Chatpat Bani, district Pathankot. Significant difference was found in the concentration levels of Mn in Chatpat Bani, district Pathankot as compared to other two sites whereas significant difference was observed in the concentration levels of Fe in all the three selected locations. Similarly, significant difference was found between the concentration levels of Zn in Chatpat Bani, district Pathankot and village Katani Kalan, district Ludhiana. In Copper (Cu), highest concentration levels were recorded in Chatpat Bani, district Pathankot (62.38 ppm) followed by village Katani Kalan, district Ludhiana and village Longowal, district Sangrur. Significant difference was found in the concentration levels of Cu in Chatpat Bani, district Pathankot and village Longowal, district Sangrur.

Similar to macro-nutrients, enough studies on micro-nutrient toxicity in bats and mammals are not available. However, in a study conducted by Milatovic and Gupta (2019), it was reported that rats can consume Mn up to 7000 ppm without causing any adverse effects. In the present study, the Mn concentration levels in bat faecal pellets were low i.e., <100 ppm, which suggests that levels of Mn in bat tissues are even lower causing no harm to them as there exists a strong negative correlation (r=–0.91) for occurrence of Mn in tissue and guano of bats (Mansour *et al.* 2016). Although, Mn excess can produce toxic effects, it is often considered to be among the less toxic of the essential trace nutrients to birds and mammals-Subcommittee on Mineral Toxicity in Animals 1980 (National Research Council 1980). Assimilation of iron is poor in bats and thus, its concentration is higher in the guano (Charlton and Bothwell 1983). Toxicity of Fe in bats is observed at very high concentration i.e., >12,000 ppm in tissue as is suggested by Crawshaw *et al.* (1995). In the present study, Fe concentration in faecal pellets ranged from 600-2100 ppm which is much lower than the toxicity limit. As per WHO (1989), the permissible limits of Cu and Zn in mammalian tissues are 71 and 289 ppm, respectively. In the present study, the concentrations of Cu and Zn in faecal pellets of *P. giganteus* were lower than the permissible limits. Thus, from the present study, it can be concluded that presence of heavy metals along with other essential nutrients in bat guano samples serves as a suitable indicator for the assessment of contamination in environment as well as bats without harming them.

Analysis of pesticide residues in *P. giganteus* faecal pellets

Frugivorous bats, being the major pollinators and seed dispersers of our ecosystem, when induced by pesticide exposure, leads to changes in their normal physiology and population status of bats causing direct effect on natural environmental balances. Our present study carried out at three locations of Punjab on the faecal pellets of *P. giganteus* for contamination of pesticides, it was found that pesticide contamination was negligible as the GLC (Gas Liquid Chromatograph) showed values below detectable limits (BDL)

Table 1. Concentration levels of pesticide residues in faecal pellets of *P. giganteus* at different selected locations. Values are expressed as Mean±SE. Different alphabetical superscripts in each row represent significant difference between values (p<0.05). *OCs-Organo Chlorines, *OPs-Organo Phosphates, *SPs Synthetic Pyrethroids, **BDL-Below detectable limit.

Sl. No.	Elements	Chatpat Bani (Pathankot)	Village Katani Kalan (Ludhiana)	Village Longowal (Sangrur)
1	*OCs	**BDL	BDL	BDL
2	*OPs	BDL	BDL	BDL
3	*SPs	BDL	BDL .	BDL

i.e., <0.05 ppm (Table 1). This is indeed a positive sign that exposure of bats to pesticides is negligible in these areas. Moreover, it can also be stated that bats in these regions have adapted themselves morphologically not to get effected by pesticide exposure in these areas. Negligible concentrations of organochlorines (OCs) in faecal samples of *P. giganteus* fruit bat can be explained by its negligible application of DDTs or HCHs in their surrounding habitat (agricultural fields, aquatic bodies and domestic areas) for agricultural and vector control purposes. Moreover, the undetectable levels of organophosphates (Ops) indicate that their use in surrounding orchards is also very less. However, some studies reported that bats have been exposed to pesticides and that presence of pesticides in guano is an indication of their presence within the bat tissue as well which leads to metabolic and behavioral changes in particular species if accumulated in higher amount. Kumar *et al.* (2001) carried out their study in 1995 and 1998 and concluded that bats had few residues of OCs and PCBs in them. The study conducted by Eidels *et al.* (2007) detected residues of OPs, pyrethroids and carbamates in bat guano and tissues due to oral exposure in the surrounding environment. Bennett and Thies (2007) reported from their study that the migratory and non-migratory behavior in two species of free tailed bats corresponded to levels of p, p'-DDE in them. This clearly indicates that presence of pesticides in bat guano are related to their presence in tissues of bats which leads to metabolic and behavioral changes in particular species if accumulated in higher amount.

CONCLUSION

Concentration of heavy metals, macro and micro nutrients varied from location to location. Cr, K, Mg, Fe and Zn predominate in three selected sites. Comparatively, higher levels of heavy metals, macro and micro nutrients were reported at village Longowal district Sangrur followed by Village Katani Kalan district Ludhiana and Chatpat Bani, village Kataruchak, district Pathankot, respectively. Interestingly, contamination of pesticide residues in faecal pellets was also found to be negligible in all selected locations, which shows negligible exposure of bats to different pesticides in these areas due to less usage of pesticides or excretion by body. All elements were under permissible limits except Mg and K which are good indicator of assessment of environment.

ACKNOWLEDGMENT

Authors are thankful to Head, Department of Zoology and Department of Entomology, Punjab Agricultural University, Ludhiana, for providing all basic research facilities to carry out this research work. Authors also extend their deep gratitude to DST, Ministry of India for providing the infrastructural facility under DST supported FIST laboratory situated in Department of Zoology, Punjab Agricultural University, Ludhiana.

REFERENCES

Anastassiades M, Lehotay SJ, Stajnbaher D, Schenck FJ (2003) Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J AOAC Int* 86 (2) : 412—431. https://doi.org/10.1093/jaoac/86.2.412.

Bates PJ, Harrison DL (1997) Bats of the Indian Subcontinent, pp 258. Harrison Zoological Museum.

Bennett BS, Thies ML (2007) Organochlorine pesticide residues in guano of Brazilian free-tailed bats, Tadarida brasiliensis Saint-Hilaire, from East Texas. *Bull Environ ContamToxicol* 78(3-4) : 191-94. https://doi.org/10.1007/s00128-007-9089-7.

Charlton RW, Bothwell TH (1983) Iron absorption. *Ann Rev Med* 34 : 55–68.

Cil EA, Uncumusaoglu AA, Ergen SF, Gurbuzer P (2023) Evaluation of water and sediment quality of İnaltı Cave (Northern Türkiye) by using multivariate statistical methods. *Environ Monit Assess* 195 (6) : 658—667. https://doi.org/10.1007/s10661-023-11262-1.

Crawshaw G, Oyarzun S, Valdes E, Rose K (1995) Hemochromatosis (iron storage disease) in fruit bats. *Proc Nutr Advisory Group Am Zool Aquarium Assoc*, *Silver Springs*, *Maryland* 17 : 136—147.

Eidels RR, Whitaker JO, Sparks DW (2007) Insecticide residues in bats and guano from Indiana. In: *Proc Indiana Acad Sci* 116 (1) : 50—57.

Hernout BV, Arnold KE, McClean CJ, Walls M, Baxter M, Boxall AB (2016) A national level assessment of metal contamination in bats. *Environ Pollut* 214 : 847—858. https:// doi.org/10.1016/j.envpol.2016.04.079.

Imlani A, Tastan Y, Tahiluddin AB, Bilen S, Yumah YU (2022) Preliminary determination of heavy metals in sediment, water and some macro invertebrates in Tawi-Tawi Bay, Philippines. *Marine Sci Technol Bull* 11 (1) : 113—122. https://doi.org/ 10.33714/masteb.1070711.

Johnson J, Vincent M (2020) Tracing heavy metals in urban ecosystems through the study of bat guano-a preliminary study from Kerala, India. *J Threat Taxa* 12 (10) : 16377— 16379. https://doi.org/10.11609/jott.6225.12.10.16377-16379.

Klaassen CD (1976) Biliary excretion of metals. *Drug Meta Rev* 5 (2) : 165—196. https://doi. org/ 10.3109/0360253760-9029977.

Kumar R, Prasad DN, Elangovan V (2001) Diurnal reproductive behavior of Indian flying fox *Pteropus giganteus*. *Asian J Animal Sci* 12(2) : 134—137.

Mansour SA, Soliman SS, Soliman KM (2016) Monitoring of heavy metals in the environment using bats as bioindicators : First study in Egypt. *Vespertilio* 18 : 61—78.

Milatovic D, Gupta RC (2019) Antioxidants in prevention and treatment of diseases and toxicity. In: Nutraceuticals in Veterinary Medicine, pp 205—213. Springer, Cham. https:// doi.org/10.1007/978-3-030-04624-8_14.

National Research Council (1978) Nutrient Requirements of Laboratory Animals. NoIO., 3rd Revised Edition, National Academies Press, National Academy of Sciences, Wash ington, DC.

National Research Council (1980) Mineral tolerance of domestic animals. National Academies Press, National Academy of Sciences, Washington, DC.

Nighat S, Nadeem MS, Mahmood T, Kayani AR, Mushtaq M, Hassan MM (2016) Estimation of heavy metals in Indian flying fox *Pteropus giganteus* (Brunnich 1782) from Punjab, Pakistan. *Pak J Zool* 48(6) : 1780—1787.

Piper CS (1944) Soil and Plant Analysis. New York: Inter science Publishing, Inc, pp 362. Laboratory technique in plant and soil analysis.

Roy K, Saha GK, Mazumdar S (2020) Seasonal influence on the diurnal roosting behavior of free-ranging Indian flying fox *Pteropus giganteus* in an urban landscape, India. *Biologia* 16 : 1—7.

Russo D, Ancillotto L (2015) Sensitivity of bats to urbanization: A review. *Mammal Biol* 80(3) : 205—212. https:// doi.org/10. 1016/j.mambio.2014.10.003.

Sansoni B, Panday VK (1994) Sample treatment of human biological materials. In: Techniques and Instrumentation in Analytical Chemistry. *Elsevier* 15 : 21—52.

Singh J, Singh R (2021) Roosting preference and relation of abiotic factors on population of *Pteropus giganteus* (Chirop tera: Pteropodidae) in Ludhiana. *Agric Res J* 58(6) : 1108-1115. https://doi.org/10.5958/2395-146x.2021.00155.1.

Singh J, Singh R, Singh B (2022) Yield loss assessment and control of fruit bat (*Pteropus.giganteus*) in litchi orchards using artificial light method. *J Appl Hortic* 24 (2) : 219—223.

Singh R (2021) Mammalia : Chiroptera (Bats). In: Faunal Diversity of Agroecosystem in India. Published by Zoological Survey of India (ZSI), Government of India), pp 753–766.

Studier EH, Viele DP, Sevick SH (1991) Nutritional implications for nitrogen and mineral budgets from analysis of guano of the big brown bat *Eptesicus fuscus* (Chiroptera : Vespertilionidae). *Camp Eiochem Physiol* 100 (4) : 1035—1039.

Venkatesan A (2007) Status of the Indian flying fox (*Pteropus giganteus*) in Bengaluru. *BAT NETCCINSA Newsl* 8 (1) : 13—15.

WHO (1989) Lead-environmental aspects: Environmental Health Criteria 85. World Health Organization, International Program on Chemical Safety (IPCS), Geneva.

Zukal J, Pikula J, Bandouchova H (2015) Bats as bioindicators of heavy metal pollution: History and prospect. *Mammal Biol* 80(3) : 220—227. https://doi.org/10.1016/j. mambio. 2015.01.001.