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Monitoring of heavy metals in the environment using bats as bioindicators: first study in Egypt

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Abstract. Two insectivorous bat species, *Taphozous perforatus* and *Rhinopoma cystops*, were collected from two caves located in the Saqqara region, Giza district, Egypt. Liver, kidney and guano samples were subjected to identification and quantification of 14 heavy metals by means of the Inductively Coupled Argon Plasma instrument. Except for cobalt (Co), the other metals were detected in the liver of *T. perforatus* and *R. cystops*, while barium (Ba), cadmium (Cd), chromium (Cr), molybdenum (Mo), nickel (Ni) and lead (Pb) were not detected in the kidney tissues. Livers of female *T. perforatus* contained significantly ($P< 0.05$) higher levels of Ba and Pb than those of males, while an opposite trend was recorded for Ba and Mn in livers of *R. cystops*. The guano samples contained much higher concentrations of the 14 metals than the tissues, generally. There were strong correlations between metal concentrations in liver versus kidney and guano. For example, the Spearman correlation coefficient (r_s) was 0.87 and 0.76, respectively, for Al and Zn in liver *vs* kidney of *T. perforatus*, and 0.91 and 0.97, respectively, for R. *cystops*. It was concluded that accumulation of these contaminants in bat tissues seemed to be affected by factors such as species, gender and sampling time. We suggest bat deposits (guano) to be used for large-scale monitoring programs, at least to avoid stress on the bat species of high conservation concern in the country.

Insectivorous bats, bioindicators, heavy metals, guano, liver, kidneys, Egypt

Introduction

Except Antarctica, bats inhabit all continents, representing the second largest mammal order. They comprise about 20% of mammal species, with the greatest diversity especially in the tropics (Nowak 1994). Insectivorous bats are predators of some insect pests, such as cucumber beetles, June bugs, corn earworm moths, cotton bollworm moths, tobacco budworm moths and Jerusalem crickets, which threaten the yield of important agricultural and forest economic crops (Cleveland et al. 2006). In Indiana, USA, it has been estimated that a single colony of 150 big brown bats (*Eptesicus fuscus*) feed on nearly 1.3 million insect pests each year (Whitaker 1995). Other estimates suggested that a single little brown bat can consume 4–8 g of insects each night during their active season (Kurta et al. 1989). Such voracity possibly contributes to the disruption of population cycles of agricultural pests (Whitaker 1995). The value of bats for the agricultural industry in USA is estimated at 22.9 billion USD / year; including the reduced costs of pesticide applications that are not needed to suppress the insects consumed by bats (Cleveland et al. 2006). On the other hand, the analyses performed by Boyles et al. (2011) suggested that the decline of bat populations in North America could lead to agricultural losses of more than 3.7 billion USD / year. Given the wide distribution and high species richness of bats, these mammals face

Figs. 1–4. 1 – the roosting cave from outside (entrance). 2 – bats captured by net. 3 – Egyptian tomb bat (*Taphozous perforatus*). 4 – lesser mouse-tailed bat (*Rhinopoma cystops*).
Obr. 1–4. 1 – jeskyně s úkrytem netopýrů zvnějšku (vchod). 2 – netopýři odchycení do sítě. 3 – hrobkovec egyptský (*Taphozous perforatus*). 4 – víkonos africký (*Rhinopoma cystops*).

a noticeable array of threats in the early 21st century. Most of these threats are directly related to human population increase, with the greatest pressure occurring especially in tropical countries (Zukal et al. 2015).

Bats often coexist with humans in urban, industrial, and agricultural landscapes (Bartonička & Zukal 2003, Russo & Ancillotto 2015), thereby potentially exposing themselves to different varieties of chemical pollutants such as heavy metals. In foraging areas of bats, the quality of water is very important for the life of these animals. Riparian habitats and other wetlands are prime foraging areas for insectivorous bats as they support large numbers of insects. Wastewater treatment works (WWTWs) are known to provide profitable foraging areas for insectivorous bats because of their association with high abundance of pollution-tolerant dipterans (Vaughan et al. 1996, Fukui et al. 2006). In the Saqqara district of Egypt, ditches and drainage of sewage treatment plants in addition to the Mariotteya canal may be considered as profitable foraging areas for bats. It has been recently reported that the Mariotteya water body at tributaries adjacent to bat roosting caves in Saqqara of Giza, is being subjected to multiple sources of pollution through the dumping of improperly treated organic and inorganic chemical wastes in addition to sewage materials. This caused mass mortalities among a Nile fish, *Oreochromis niloticus* at the Mariotteya stream (Eissa et al. 2013, Mahmoud et al. 2014). Heavy metals (e.g., Cu, Cd, Pb) were found in the Mariotteya stream at concentration levels exceeding the permissible limits, especially for Cd and Pb (Mahmoud et al. 2014). This means that the water bodies associated with the Mariotteya stream are contaminated with heavy metals and other organic pollutants.

Heavy metals are natural components of the Earth's crust and cannot be degraded or destroyed and can easily enter the human body along the food chains, causing detrimental health effects even at very low concentrations. Heavy metals of special concern to human health and environment have been recently summarised by Mansour (2014). Their excessive release in the environment refers mainly to human anthropogenic activities which include mining, waste disposal, fertilizers, pesticides containing metals such as Cu, Zn and Mn, traffic polution (exhaust gases, tire wear, and brake lining wear), industrial effluents, domestic emissions, and atmospheric depositions (Frickel & Elliott 2008). Releasing of large quantities of metals (e.g., arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) into the environment leads to their distribution between environmental media and accumulation in food chains; inducing adverse human health effects at the cellular level (Fulladosa et al. 2002), as well as higher levels (Stewart et al. 2003).

There is always a natural background for heavy metal concentrations in different media (e.g., soils, rocks, sediments, water, and living organisms), with concentration levels varying greatly. However, the normal background values of metal pollution are far less than the anthropogenic pollution. Eleven elements (e.g., arsenic, cadmium, cobalt, chromium, copper, mercury, manganese, nickel, lead, tin and thallium) are recognized as being of greatest wildlife concern (Beyersmann & Hartwig 2008). Lead, mercury, arsenic and cadmium, which are heavy metals, are considered amongst the most hazardous, however, light metals such as aluminum and selenium can be toxic to living organisms at higher concentrations; others are essential elements for a range of normal functions in animals at trace doses (e.g., manganese, nickel, cobalt, copper, iron, and zinc), yet can be toxic at elevated concentrations. Furthermore, free ions of heavy metals can cause many serious problems, including oxidative stress or permanent signaling within the cell; therefore, their levels tend to be strictly controlled at the cellular level (Bremner & Beattie 1990).

Variability in the levels of heavy metals found in bat bodies is certainly influenced by their background environmental levels, which reflect the amounts accumulated. Exposure to contaminants, including heavy metals, has been implicated as a major factor contributing to recent decreases in bat populations (Jones et al. 2009). Heavy metal pollution can affect ecological, genetical, physiological, reproductive, and behavioral parameters, and mortality in severe cases (e.g., Vaughan et al. 1996, Van De Sijpe et al. 2004, Pikula et al. 2010, Zocche et al. 2010, Naidoo et al. 2013).

It is worth mentioning that there has been a growing interest in the study and conservation of bats throughout the world. The literature offers much information about many features of bat life-history and biology that make these organisms a perfect group for monitoring of environmental contaminants including heavy metals. In a recent paper, Zukal et al. (2015) have provided an overview of heavy metal research on wild bat populations to date; pointed out major gaps in our present knowledge; and suggested future directions and approaches for the study of heavy metal contamination and its possible direct adverse effects on bats. According to Zukal et al. (2015), out of 52 studies undertaken on heavy metals in bats, only 2% were carried out in Africa while the majority was carried out in North America (44%) and Europe (34%).

The aim of the present investigation was to quantify heavy metal concentrations in kidney and liver tissues of two insectivorous bat species; namely *Taphozous perforatus* and *Rhinopoma cystops*, as well as in bat deposits (guano) collected from their roost caves, and to assess the use of bats as bioindicators of environmental pollutants in Egypt.

Material and Methods

Samples

Within a study of ecological and toxicological issues in some insectivorous bats in Egypt, the Saqqara archaeological area (29° 52' N, 31° 13' E) which is located on the west bank of the Nile, some 17 km south of Giza, and some 40 km away from Cairo, was selected for bat sampling. The area is surrounded by numerous palm groves and cultivated fields irrigated from the Mariotteya canal, which is located about 3 km from the bat collecting site. Ditches and drainage of sewage treatment plants are found in the area. The Saqqara archaeological site is characterized by the presence of many deserted caves, some of which are used by bats as roosting sites. Two neighboring large caves occupied by a relatively large number of bats were selected for collecting the required specimens. The two caves (Fig. 1) have narrow entrances and are completely dark, with long branching and narrow passages characterized by high atmospheric humidity. Figs. 3, 4 show the roosting bats inside the cave.

For ecological studies, specimens of the two bat species were collected monthly from the caves during a period of 24 months (January 2012 – December 2013). Specimens collected during the second year of study were used in toxicological studies. The bats were captured using a mist net at the entrance to their roosting cave early in the morning (Fig. 2). Bat specimens were occasionally collected by the use of a hand net. The collected bats were transported alive to the laboratory in the Department of Zoology, Faculty of Science, Ain Shams University, Abbasiya, Cairo, Egypt. Based on Dietz (2005), Prof. Sohail S. Soliman (Faculty of Science, Ain Shams University) identified the bats as the Egyptian tomb bat, *Taphozous perforatus* Geoffroy, 1818 (Fig. 3), and the lesser mouse-tailed bat, *Rhinopoma cystops* Thomas, 1903 (Fig. 4). The preceding ecological studies (Soliman et al. 2015) provided accurate differentiation between age and sex of the bats, thus adults of both male and female bats were used for toxicological studies.

Guano samples (bat droplets) were collected from the interior walls of the bat roosting caves by means of a thin spatula, in four seasons of the year 2013 (March, June, September and November). Each guano sample was kept in a glass bottle until analyzed for heavy metal contamination.

Samples of liver and kidneys were dissected out from 91 adult individuals of *T. perforatus* (48 males, 43 females), and 72 adult individuals of *R. cystops* (40 males, 32 females). Tissue samples were then stored at –80 °C for heavy metal analyses, no more than two weeks after collection. Other portions of liver and kidneys were retained frozen for quantification of pesticide residues, the data for which will be reported elsewhere. The bats were euthanized by decapitation, as approved by the University of Ain Shams Animal Ethics Committee, and consistent with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals (Leary 2013).

Analysis

Kidney, liver and guano samples were dried to constant weight at 80 °C. Portions of 0.25 g tissues or 0.02 g of guano were digested by 7 ml of 65% HNO_3 and 1 ml of 30% H_2O_2 using the Microwave Digestion Labstation Closed System (Ethos Pro, Milestone, Italy) for 40 min. Sub-samples of the digest were diluted with de-ionized water so that the analyte was within the calibration range and the final acid content was 10%. Concentrations of heavy metals were measured by means of the Inductively Coupled Argon Plasma, iCAP 6500 Duo (Thermo Fisher Scientific, England), equipped with a Charged Injection Device (CID) detector which enables measuring a wide range of element concentrations in the matrix. Typical instrumental conditions for the analysis (e.g., wave lengths, axial or radial plasma view, interfering element correction especially for Al & Fe, detection limits, quality control assurance, etc.) were applied according to the manufacturer's instructions (Thermo Scientific 2007) and in accordance with the USEPA method 6010b (1996). 1000 mg/l multi-element certified standard solution (Merck, Germany) was used as stock solution for instrument standardization, after calibration of metals at 0.05 ppm. A total of 14 elements (Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Zn) were subjected to detection and determination in the analyzed samples. Usually, each sample was run in 3 replicates and the results were expressed in terms of µg/g dry weight tissue or guano (mean±SE). Certified reference materials of an appropriate matrix type that had detectable metal concentrations in sample masses equivalent to that of bat matrices were not available. Therefore, analytical recoveries were determined from spiked kidney, liver and guano samples and the mean (±SE) recovery generally ranged between 77±3.22% and 112±4.20% for the analyzed metals. The method detection limits (MDL) ranged between 0.01 and 0.10 µg/g for metals such as Cd, Pb, Co, Cr, Ni, Mo; while MDL exceeded 1.0 µg/g for the other metals.

Due to the small weight of liver and kidneys of the studied specimens, and the costs of the analyses, the monthly collected samples were pooled into four portions; each consisted of three-month collections, so that the pooled samples were designated to winter, spring, summer and autumn seasons. Each combined sample was analyzed in three determinations both for males and females. The data obtained were subjected to statistical analyses using GraphPad Prism 5 Demo (www.graphpad.com/downloads/docs/Prism5Regression.pdf), and expressed as means±SE. The paired samples (t) test was used to compare the data for significance at $P<0.05$. Spearman rank correlation coefficients (r_s) for each pair of the data set were calculated at 95% confidence intervals. The analyses were restricted to elements with detectable residues only, as inclusion of animals with non-detected values can result in spurious correlations. Also, the effects of sex and time on renal and hepatic metal concentrations were studied.

Table 1. Mean concentrations of heavy metals (µg/g tissue) in liver and kidney tissues from adult individuals of *Taphozous perforatus* collected in the Saqqara area in 2013; data from the particular seasons were pooled; each value is a mean of 3 determinations; M = males, F = females, nd = not detected

Tab. 1. Průměrné koncentrace těžkých kovů (µg/g tkáně) v tkáních jater a ledvin dospělých jedinců hrobkovce egyptského (*Taphozous perforatus*) kolektovaných nedaleko Sakkary roku 2013; údaje z jednotlivých ročních dob byly spojeny; každá hodnota představuje průměr ze tří měření; M = samci, F = samice, nd = nezaznamenáno

metal	liver / játra								kidney / ledvina							
kov	winter / zima		spring / jaro		summer / léto		autumn / podzim		winter / zima		spring / jaro		summer / léto		autumn / podzim	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Al	47.84 ±1.02	74.18 ±0.88	94.99 ±1.01	57.26 ±0.16	88.53 ±0.99	93.88 ±0.01	79.80 ±0.10	82.44 ±0.96	34.40 ±0.27	25.20 ±0.31	50.40 ±0.10	47.90 ±0.10	82.00 ±0.11	32.10 ±0.25	37.80 ±0.15	40.90 ±0.15
Ba	6.24 ±0.12	18.73 ±0.90	10.41 ±0.01	14.29 ±0.10	11.97 ±0.98	21.67 ±0.11	11.10 ±0.04	11.92 ±0.02	nd	nd	nd	nd	nd	nd	nd	nd
Ca	342.38 ±1.30	448.88 ±0.01	515.99 ±0.01	328.40 ±0.95	422.50 ±0.20	415.53 ±0.80	453.56 ±0.02	541.78 ±0.10	280.80 ±0.39	229.30 ±0.71	258.70 ±0.30	197.50 ±0.49	352.00 ±0.40	278.30 ±0.25	263.40 ±0.20	245.20 ±0.11
Cd	0.10 ±0.03	0.15 ±0.01	0.22 ±0.01	0.22 ±0.01	0.15 ±0.03	0.21 ±0.01	0.18 ±0.02	0.10 ±0.03	nd	nd	nd	nd	nd	nd	nd	nd
Co	nd nd	nd nd	nd nd	nd nd	0.11 ±0.01	0.11 ±0.02	0.10 ±0.01	0.11 ±0.02	nd	nd	nd	nd	nd	nd	nd	nd
Cr	1.54 ±0.01	1.52 ±0.02	2.33 ±0.16	1.92 ±0.08	3.53 ±0.03	3.78 ±0.01	1.48 ±0.01	2.00 ±0.01	nd	nd	nd	nd	nd	nd	nd	nd
Cu	4.91 ±0.08	8.63 ±0.09	6.48 ±0.02	11.44 ±0.02	10.10 ±0.10	5.95 ±0.01	5.99 ±0.01	7.05 ±0.76	4.58 ±0.42	4.17 ±0.02	6.07 ±0.04	5.35 ±0.17	5.50 ±0.02	4.91 ±0.10	5.14 ±0.03	4.74 ±0.03
Fe	365.67 ±0.31	585.98 ±0.22	575.00 ±0.10	525.83 ±0.03	652.41 ±0.01	654.09 ±0.01	479.72 ±0.02	602.63 ±0.01	162.20 ±0.19	160.70 ±0.25	159.00 ±0.10	142.60 ±0.38	216.80 ±0.24	209.10 ±0.14	198.10 ±0.17	207.30 ±0.20
Mg	260.15 ±0.06	223.90 ±0.91	270.77 ±0.21	304.29 ±0.01	297.28 ±0.10	294.10 ±0.1	286.64 ±0.02	281.20 ±0.10	179.00 ±1.50	175.40 ±0.39	202.50 ±0.02	191.60 ±0.29	215.30 ±0.27	176.00 ±0.30	181.70 ±0.16	162.50 ±0.14
Mn	4.89 ±0.11	5.24 ±0.11	6.88 ±0.10	8.82 ±0.02	7.64 ±0.04	7.73 ±0.15	6.66 ±0.02	6.83 ±0.03	0.79 ±0.02	0.72 ±0.03	1.09 ±0.02	0.89 ±0.04	1.06 ±0.07	0.65 ±0.04	0.73 ±0.02	0.64 ±0.02
Mo	0.84 ±0.01	0.95 ±0.05	0.70 ±0.01	0.95 ±0.01	0.99 ±0.01	0.92 ±0.03	0.85 ±0.01	0.79 ±0.01	nd	nd	nd	nd	nd	nd	nd	nd
Ni	0.46 ±0.08	0.44 ±0.01	1.07 ±0.01	0.97 ±0.03	1.09 ±0.01	1.26 ±0.06	0.88 ±0.06	0.86 ±0.03	nd	nd	nd	nd	nd	nd	nd	nd
Pb	0.58 ±0.02	0.75 ±0.04	0.67 ±0.01	1.12 ±0.03	0.65 ±0.01	0.83 ±0.04	0.64 ±0.01	0.75 ±0.01	nd	nd	nd	nd	nd	nd	nd	nd
Zn	25.00 ±0.10	39.16 ±0.07	26.86 ±0.03	63.54 ±0.05	67.67 ±0.135	30.26 ±0.08	31.89 ±0.04	34.22 ±0.13	27.17 ±0.02	26.28 ±0.11	41.54 ±0.04	35.30 ±0.11	43.12 ±0.07	29.65 ±0.02	29.82 ±0.10	30.56 ±0.02

Results

The monthly collected samples of liver and kidney tissues were pooled into four combined samples for each organ, representing the seasons of the year. Each combined sample included organs separated from males and females. The analyses conducted in this study considered seasonal, gender and species variations with respect to metal contaminants.

Intra-specific differences in concentration of metals in tissues

Taphozous perforatus: Table 1 shows mean concentrations of heavy metals in the liver of male and female adults of *T. perforatus* in different seasons. Out of 14 elements, cobalt was not detected in winter and spring collections but was measurable at very low concentrations (ca. 0.01 µg/g tissue) in summer and autumn collections. Concentrations of the other metals varied greatly according to the time of collection and gender of the animals. The most dominant elements with high concentrations were Fe, Ca, Mg, and to some extent also Al and Zn. Metals such as Ba, Cu and Mn were found at relatively lower concentrations, but Cr, Cd, Mo, Ni and Pb were detected at very low concentrations. Concentration of Al ranged between 47.8 and 95.0 µg/g in males, and from 57.3 to 93.9 in females. Except in summer, female liver samples contained concentrations of Cu exceeding those estimated in the male ones (Table 1). In kidneys of *T. perforatus*, Ba, Cd, Co, Cr, Mo, Ni and Pb were not detected (Table 1). Ca, Fe, Mg, Al and Zn were found at high concentrations, ranging e.g. between 25.2 and 82.0 µg/g for Al, and between 26.3 and 43.1 µg/g for Zn. Kidneys of male *T. perforatus* showed higher concentrations of Mg than those of females.

Rhinopoma cystops: Occurrence of heavy metals in liver tissues of *R. cystops* during different seasons is depicted in Table 2. Cobalt was not detected in any sample analyzed, and Cd, Mo, Ni and Pb were determined at concentrations mostly less than 1.0 µg/g tissue. Moderate concentrations of Ba, Cr, Cu and Mn and very high concentrations of Ca, Fe and Mg were recorded in the liver of males and females. Liver samples from males contained much higher levels of Al and Ba than those from females. Opposite results were recorded in female specimens in winter, spring and summer collections. In the autumn season, Fe concentration reached 878.17 and 696.1 µg/g in males and females, respectively (Table 2). In kidney tissues of *R. cystops*, the analyzed samples showed undetectable concentration levels of Ba, Cd, Co, Cr, Mo, Ni and Pb (Table 2). Throughout the four seasons, the detectable metals in both sexes ranged between the following values: 41.2–87.8 µg/g (Al); 337.1–497.5 µg/g (Ca); 3.3–6.1 µg/g (Cu); 224.8–296.2 µg/g (Fe); 152.9–194.5 µg/g (Mg); 0.32–0.89 µg/g (Mn); and 24.9–32.9 µg/g (Zn).

Based on the data in Tables 1 and 2, we compared heavy metal concentrations in liver and kidney samples for males and females of both bats species, as presented in Table 3. In case of *T. perforatus*, liver from female specimens contained 16.7 µg/g tissue of Ba; a value which was significantly higher than that recorded for the male specimens (9.9 µg/g; *P*=0.0377). Also, a similar trend was obtained for Pb (*P*=0.0441). Differences between the two sexes with respect to the other determined metals were not significant. Concentration levels of the detected heavy metals in kidneys showed no significant differences between males and females (Table 3).

In case of *R. cystops*, liver from male specimens contained 13.8 µg/g of Ba; a value which was significantly higher than that recorded for female specimens (9.9 µg/g; *P*=0.0496). Also, a similar trend was obtained for Mn (*P*=0.036). Differences between the two sexes with respect to the other determined metals were not significant . Concentration levels of the detected heavy metals in kidneys showed no significant differences between males and females (Table 3).

Table 2. Mean concentrations of heavy metals (µg/g tissue) in liver and kidney tissues from adult individuals of *Rhinopoma cystops* collected in the Saqqara area during 2013; for explanations see Table 1

Tab. 2. Průměrné koncentrace těžkých kovů (µg/g tkáně) v tkáních jater a ledvin dospělých jedinců víkonosa afrického (*Rhinopoma cystops*) kolektovaných nedaleko Sakkary roku 2013; vysvětlivky viz tab. 1

metal	liver / játra								kidney / ledvina							
kov	winter / zima		spring / jaro		summer / léto		autumn / podzim		winter / zima		spring / jaro		summer / léto		autumn / podzim	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Al	69.85 ±0.10	25.18 ±0.01	51.40 ±0.40	21.84 ±0.04	76.60 ±1.00	41.17 ±0.02	63.38 ±0.10	49.30 ±0.30	58.10 ±0.10	48.10 ±0.11	41.20 ±0.22	62.00 ±0.30	87.83 ±0.08	65.60 ±0.31	83.50 ±0.41	56.40 ±0.27
Ba	14.08 ±0.98	9.95 ±0.01	12.27 ±0.77	9.80 ±10	10.92 ±0.01	8.79 ±0.02	17.90 ±0.10	11.07 ±0.02	nd	nd	nd	nd	nd	nd	nd	nd
Ca	334.78 ±1.09	297.33 ±0.10	218.11 ±0.08	28.32 ±0.10	249.71 ±0.01	300.85 ±0.10	541.16 ±1.09	298.83 ±0.03	354.00 ±0.10	359.80 ±0.20	340.70 ±0.25	337.10 ±0.01	572.63 ±0.70	366.20 ±0.21	497.50 ±0.31	370.60 ±0.39
Cd	0.06 ±0.01	0.16 ±0.01	0.05 ±0.01	0.09 ±0.01	0.57 ±0.01	0.11 ±0.01	0.32 ±0.01	0.20 ±0.01	nd	nd	nd	nd	nd	nd	nd	nd
Co	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cr	3.03 ±0.02	1.67 ±0.10	1.21 ±0.01	4.48 ±0.04	7.97 ±0.13	2.70 ±0.02	3.01 ±0.01	2.58 ±0.01	nd	nd	nd	nd	nd	nd	nd	nd
Cu	6.46 ±0.08	7.60 ±0.30	7.64 ±0.10	6.04 ±0.02	6.64 ±0.04	4.87 ±0.01	12.40 ±0.40	8.62 ±0.01	3.27 ±0.30	3.63 ±0.03	4.76 ±0.04	3.70 ±0.04	6.09 ±0.10	5.30 ±0.70	3.72 ±0.01	3.65 ±0.01
Fe	477.01 ±0.01	695.23 ±0.10	418.57 ±0.40	680.69 ±0.20	508.83 ±0.03	695.01 ±0.01	878.17 ±0.10	696.10 ±0.05	226.70 ±0.25	258.00 ±0.10	253.90 ±0.69	224.80 ±0.28	285.89 ±0.30	296.20 ±0.18	274.40 ±0.49	288.50 ±0.41
Mg	219.05 ±0.04	215.41 ±0.07	221.95 ±0.25	229.27 ±0.11	263.90 ±0.20	236.70 ±0.50	221.97 ±0.13	227.32 ±0.02	171.80 ±0.29	152.90 ±0.10	159.60 ±0.20	178.40 ±0.37	204.22 ±0.25	165.50 ±0.30	194.50 ±0.20	161.80 ±0.20
Mn	3.28 ±0.10	3.29 ±0.20	5.06 ±0.06	3.58 ±0.03	5.04 ±0.03	2.55 ±0.40	5.80 ±0.02	3.48 ±0.07	0.32 ±0.02	0.38 ±0.02	0.53 ±0.03	0.42 ±0.01	0.89 ±0.01	0.40 ±0.01	0.58 ±0.01	0.42 ±0.01
Mo	0.57 ±0.01	0.51 ±0.01	0.82 ±0.03	0.78 ±0.09	1.08 ±0.02	0.50 ±0.10	0.62 ±0.01	0.50 ±0.01	nd	nd	nd	nd	nd	nd	nd	nd
Ni	0.39 ±0.01	0.53 ±0.03	0.38 ±0.02	0.46 ±0.01	0.90 ±0.01	0.49 ±0.01	0.46 ±0.03	0.71 ±0.01	nd	nd	nd	nd	nd	nd	nd	nd
Pb	0.74 ±0.04	0.78 ±0.02	0.82 ±0.02	1.08 ±0.03	1.04 ±0.03	1.09 ±0.04	0.96 ±0.06	1.06 ±0.02	nd	nd	nd	nd	nd	nd	nd	nd
Zn	42.97 ±0.04	42.50 ±0.41	63.75 ±0.04	42.85 ±0.02	50.44 ±0.04	60.24 ±0.10	31.86 ±0.05	68.11 ±0.31	27.06 ±0.17	24.90 ±0.11	32.90 ±0.20	28.45 ±0.03	38.29 ±0.10	26.41 ±0.14	29.22 ±0.04	27.45 ±0.03

Inter-specific differences in concentrations of metals in tissues

In this respect, we computed concentration of each metal throughout the whole year. This was done by considering each of the 4 season's values (without sex differentiation) as one "replicate", then calculating minimum, maximum and mean ±SE values for each element, where n=4. The tabulated data in Table 1 were used to calculate metal concentrations in liver and kidney tissues of *T. perforatus*, and those in Table 2 for the same procedure for *R. cystops*. Comparing paired values (t-test) enabled us to assess significance levels between values of the analyzed metals.

Liver: Table 4 shows results of the comparison of concentration levels of some heavy metals in liver tissues from the two insectivorous bats. Out of the 14 elements subjected to assessment in the present study, we focused on 7 which proved to occur in both bat species. All liver specimens obtained from *T. perforatus* were found to contain Al at concentrations of 61.0–91.2 µg/g with a mean value of 77.4 µg/g. For *R. cystops*, such values were 36.6–58.9 and 49.8 µg/g, respectively. This means that the concentration of Al in *T. perforatus* liver samples was significantly ($P<0.05$) higher than that in *R. cystops* liver samples. Also, the mean concentration of Mn in *T . perforatus* liver was highly significant (P<0.01) as compared with that in *R. cystops* liver ($P=0.007$). The mean concentration of Cu was nearly equal in the liver of the two bat species (ca. 7.5 µg/g). Mean concentration values of Cd, Cr, Pb and Zn were generally higher in *R. cystops* liver than in *T . perforatus* liver but without significant differences(Table 4).

Kidneys: Comparison of concentration levels of some heavy metals in kidney tissues of the two studied insectivorous bats is presented in Table 5. Data are given for 7 metals which showed dominance in both bat species. The mean concentration of Mn was 0.823 µg/g in *T . perforatus* kidney compared with 0.495 µg/g in *R. cystops* kidney; the differences between the two species were significant ($P=0.0108$). The opposite was obtained for Ca and Fe where the mean values recorded for *R. cystops* kidney were 399.8 and 263.5 µg/g, respectively, compared with 263.2 and 182.0 µg/g for *T . perforatus* kidney; the differences were highly significant (P<0.01). The mean values of Al, Cu, Mg and Zn concentration did not differ significantly between the two species (Table 5).

Metals in guano

Samples of guano from the caves inhabited by the two insectivorous bats were collected four times during the year 2013 and were subjected to heavy metal analyses, giving the results summarised in Table 6. Concerning mean values, Ca, Mg, Fe, Al and Zn were found at very high concentrations; such as 8763, 3562, 1315, 703 and 443 µg/g dry wt., respectively. The other measured metals were detected at lower concentrations. It was observed that concentrations of most metals reached a peak in the winter season (e.g., Al, Fe, Mg and Cu reaching 1114, 1835, 3866 and 38 µg/g dry wt., respectively), however that of Ca was highest in Autumn (10,390 µg/g dry wt.).

Relation between metal concentrations between liver and kidney

We statistically analyzed correlation (r_s) of heavy metals in liver versus kidney for the two insectivorous bat species, as shown in Table 7. In the case of *T. perforatus*, there were strong positive correlations for Al (r_s=0.87), Cu (r_s=0.87), Zn (r_s=0.77), Fe (r_s=0.68) and Mn (r_s=0.66), and a moderate correlation for Mg (r_s=0.56). Calcium showed a weak negative correlation for liver *vs* kidney (r_s= –0.13). In the case of *R. cystops*, a very strong correlation was obtained for Al (r_s=0.91), Zn (r_s=0.97) and Mg (r_s=0.86). A moderate correlation was obtained for Fe (r_s=0.58) and Cu (r_s= –0.62), while a weak correlation was found in Ca and Mn (r_s=0.33 and 0.31, respectively).

Table 3. Concentrations (µg/g tissue) of heavy metals in liver and kidney tissues from adult male and female individuals of *Taphozous perforatus* and *Rhinopoma cystops* collected in the Saqqara area during four successive seasons of the year 2013; data refer to Tables 1 for *T. perforatus* and to Table 2 for *R. cystops*; each value in this table expresses a general mean for a complete year (n=4); for other explanations see Table 1

Tab. 3. Koncentrace (µg/g tkáně) těžkých kovů v tkáních jater a ledvin dospělých samců a samic hrobkovce egyptského (*Taphozous perforatus*) a víkonosa afrického (*Rhinopoma cystops*) kolektovaných nedaleko Sakkary během čtyř následných ročních dob roku 2013; údaje odpovídají tab. 1 u hrobkovce a tab. 2 u víkonosa; každá hodnota v tabulce vyjadřuje průměr za celý rok (n=4); vysvětlivky viz tab. 1

metal	*Taphozous perforatus*						*Rhinopoma cystops*					
kov	liver / játra			kidney / ledviny			liver / játra			kidney / ledviny		
	M	F	*P*	M	F	*P*	M	F	*P*	M	F	*P*
Al	77.79 ±10.46	76.94 ±7.704	0.95	51.15 ±10.84	36.53 ±4.97	0.27	65.31 ±5.36*	34.37 ±6.52*	0.01	67.66 ±10.99	58.03 ±3.81	0.44
Ba	9.93 ±1.27*	16.65 ±2.19*	0.04	nd	nd	nd	13.79 ±1.52*	9.90 ±0.47*	0.05	nd	nd	nd
Ca	433.60 ±36.09	433.60 ±44.09	1.00	288.70 ±21.62	237.60 ±16.81	0.11	335.90 ±72.71	295.80 ±3.25	0.60	441.20 ±56.38	358.40 ±7.45	0.19
Cd	0.17 ±0.03	0.17 ±0.03	0.93	nd	nd	nd	0.25 ±0.12	0.14 ±0.02	0.39	nd	nd	nd
Cr	2.22 ±0.48	2.31 ±0.50	0.91	nd	nd	nd	3.81 ±1.45	2.86 ±0.59	0.57	nd	nd	nd
Cu	6.87 ±1.13	8.27 ±1.19	0.43	5.32 ±0.31	4.79 ±0.24	0.23	8.29 ±1.40	6.78 ±0.83	0.39	4.46 ±0.63	4.07 ±0.41	0.62
Fe	518.20 ±61.90	592.10 ±26.43	0.31	184.00 ±14.07	179.90 ±16.74	0.86	570.60 ±104.20	691.80 ±3.70	0.29	260.20 ±12.99	266.90 ±16.27	0.76
Mg	278.70 ±8.24	275.90 ±17.96	0.89	194.60 ±8.66	176.40 ±5.95	0.13	231.70 ±10.75	227.20 ±4.41	0.71	182.50 ±10.23	164.70 ±5.29	0.17 0.17
Mn	6.52 ±0.58	7.16 ±0.76	0.53	0.92 ±0.09	0.73 ±0.06	0.13	4.79 ±0.54*	3.23 ±0.23*	0.04	0.58 ±0.12	0.41 ±0.01	0.19
Mo	0.84 ±0.06	0.90 ±0.04	0.43	nd	nd	nd	0.77 ±0.12	0.57 ±0.07	0.19	nd	nd	nd
Ni	0.87 ±0.15	0.88 ±0.17	0.98	nd	nd	nd	0.53 ±0.12	0.55 ±0.06	0.91	nd	nd	nd
Pb	0.64 ±0.02*	0.86 ±0.09*	0.04	nd	nd	nd	0.89 ±0.07	1.01 ±0.07	0.31	nd	nd	nd
Zn	41.17 ±9.14	41.80 ±7.47	0.96	35.41 ±4.04	30.45 ±1.86	0.31	47.26 ±6.69	53.43 ±6.41	0.53	31.87 ±2.46	26.80 ±0.76	0.09

Table 4. Concentrations (µg/g tissue) of selected heavy metals in liver tissues of two insectivorous bat species collected in the Saqqara area during four successive seasons of the year 2013 (without sex differentiation); data are computed from those in Table 1; means not designated by superscript in the same row are not significantly different, means designated by superscript (^{a}) in the same row are significantly different at *P*≤0.05, means designated by superscript (^{b}) in the same row are highly significantly different at *P*≤0.01
Table 4. Koncentrace (µg/g tkáně) vybraných těžkých kovů v tkáních jater dvou hmyzožravých netopýrů kolektovaných nedaleko Sakkary během čtyř následných ročních dob roku 2013 (bez rozlišení pohlaví); hodnoty jsou spočteny z údajů v tab. 1; hodnoty průměru neoznačené indexem v tomtéž řádku nejsou statisticky významně rozdílné, hodnoty označené indexem (^{a}) jsou významně rozdílné při *P*≤0.05, hodnoty (^{b}) jsou významně rozdílné při *P*≤0.01; mean = průměr; SE = směrodatná odchylka

metal	*Taphozous perforatus*			*Rhinopoma cystops*			*P*
	min	max	mean±SE	min	max	mean±SE	
Al	61.01	91.21	77.37±6.29^{a}	36.62	58.89	49.84±0.02^{a}	0.01
Cd	0.15	0.22	0.18±0.02	0.07	0.34	0.19±0.06	0.85
Cr	1.53	3.66	2.26±0.48	2.35	5.34	3.33±0.68	0.25
Cu	6.52	8.96	7.57±0.57	5.76	10.51	7.53±1.03	0.98
Mn	5.07	7.85	6.84±0.64^{b}	3.29	4.64	4.01±0.30^{b}	0.01
Pb	0.67	0.90	0.75±0.05	0.76	1.07	0.95±0.07	0.06
Zn	33.06	48.97	41.48±3.52	42.74	55.34	50.34±2.77	0.09

Relation between metal concentrations between liver/kidney and guano

Taphozous perforatus: Correlation between occurrence of heavy metals in liver and guano is presented in Table 8. In most cases there was a negative correlation, very strong for Pb, Mg, Mn and Ni (r_s= –0.99, –0.95, –0.87, and –0.80, respectively), strong for Cu (r_s= –0.75), Al (r_s= –70), and weak for Cr (r_s= –0.45) and Fe (r_s= –0.48). A positive correlation was obtained for Ba, Ca, Cd, Mo and Zn (r_s=0.41, 0.78, 0.73, 0.11, and 0.43, respectively). Concerning the concentration of heavy metals in kidney and guano, there was a weak positive correlation for Ca (r_s=0.36), and a negative correlation with a different degree of strength for Al, Cu, Fe, Mg, Mn and Zn. Specifically, Cu and Al achieved strong correlation of –0.97 and –0.70 (Table 8).

Rhinopoma cystops: Correlation between occurrence of heavy metals in liver and guano is presented in Table 8. Only Al and Ca showed weak positive correlations (r_s=0.11 and 0.37). The

Table 5. Concentrations (µg/g tissue) of selected heavy metals in kidney tissues of two insectivorous bat species collected in the Saqqara area during four successive seasons of the year 2013 (without sex differentiation); data are computed from those in Table 1; for other explanations see Table 4
Table 5. Koncentrace (µg/g tkáně) vybraných těžkých kovů v tkáních ledvin dvou hmyzožravých netopýrů kolektovaných nedaleko Sakkary během čtyř následných ročních dob roku 2013 (bez rozlišení pohlaví); hodnoty jsou spočteny z údajů v tab. 1; vysvětlivky viz tab. 4

metal	*Taphozous perforatus*			*Rhinopoma cystops*			*P*
	min	max	mean±SE	min	max	mean±SE	
Al	29.80	57.05	43.84±5.92	51.60	76.72	62.84±6.22	0.07
Ca	228.1	315.20	263.20±18.43^{b}	338.90	469.40	399.80±31.05^{b}	0.01
Cu	4.37	5.71	5.06±0.28	3.45	5.70	4.26±0.50	0.22
Fe	150.80	213.00	182.00±15.23^{b}	239.40	291.00	263.50±13.26^{b}	0.01
Mg	172.10	197.10	185.50±6.40	162.40	184.90	173.60±4.96	0.19
Mn	0.69	0.99	0.82±0.07^{a}	0.35	0.65	0.49±0.06^{a}	0.01
Zn	26.73	38.42	32.93±2.71	25.98	32.35	29.34±1.39	0.28

Table 6. Heavy metal concentrations (µg/g) in guano collected in the study area during four seasons of the year 2013
Tab. 6. Koncentrace těžkých kovů ve vzorcích trusu (µg/g) sebraných ve studované oblasti během čtyř ročních dob roku 2013; SE = směrodatná odchylka

metal kov	winter zima	spring jaro	summer léto	autumn podzim	mean±SE průměr±SE
Al	1114.00	464.30	657.20	577.40	703.20±142.50
Ba	5.15	4.54	4.88	4.36	4.73±0.18
Ca	6410.00	8448.00	9803.00	10390.00	8763.00±883.40
Cd	0.17	0.21	0.17	0.15	0.18±0.01
Co	1.82	0.91	1.13	1.18	1.26±0.20
Cr	4.77	3.28	3.48	3.45	3.75±0.34
Cu	38.19	32.29	35.31	34.98	35.19±1.21
Fe	1835.00	906.60	1259.00	1123.00	1315.00±192.20
Mg	3866.00	3459.00	3462.00	3404.00	3562.00±101.30
Mn	69.21	57.43	61.58	58.88	61.78±2.62
Mo	1.72	1.57	1.45	1.38	1.53±0.07
Ni	4.16	1.95	2.83	2.60	2.89±0.46
Pb	7.55	5.90	7.08	7.55	7.02±0.39
Zn	455.60	430.20	456.20	428.50	442.6±7.67

other tested metals showed a negative correlation, which was very strong for Mn (r_s= –0.91) and very weak for Cu and Ni (r_s= –0.023 and –0.030). A positive strong correlation between metals in kidney and guano was found in Ca (r_s=0.75), and a strong negative correlation in for Mg (r_s= –0.75). Except Mn, the other tested metals showed a weak negative correlation between metals in kidney versus those in guano (Table 8).

Discussion

The literature offers much information about many features of bat life-history and biology that make these organisms a perfect group for monitoring of environmental contaminants including

Table 7. Correlations (r_s) between heavy metal concentrations in liver *vs* kidney tissues in *Taphozous perforatus* and *Rhinopoma cystops* collected in the Saqqara area throughout the year 2013; Spearman rank correlation coefficients (r_s) for each pair of the data set at 95% confidence intervals
Tab. 7. Korelace (r_s) mezi koncentracemi těžkých kovů v tkáních jater a ledvin u hrobkovce egyptského (*Taphozous perforatus*) a víkonosa afrického (*Rhinopoma cystops*) kolektovaných nedaleko Sakkary během roku 2013; korelační koeficienty Spearmanova ranku (r_s) jsou pro každý pár souboru údajů na 95% intervalu spolehlivosti

metal / kov	*Taphozous perforatus*	*Rhinopoma cystops*
Al	0.87	0.91
Ca	–0.13	0.33
Cu	0.87	–0.62
Fe	0.68	0.58
Mg	0.56	0.86
Mn	0.66	0.31
Zn	0.76	0.98

Table 8. Correlations (r_s) between heavy metal concentrations in liver *vs* guano and kidney *vs* guano in *Taphozous perforatus* and *Rhinopoma cystops* collected in the Saqqara area throughout the year 2013; Spearman rank correlation coefficients (r_s) for each pair of the data set at 95% confidence intervals; nd = not detected

Tab. 8. Korelace (r_s) mezi koncentracemi těžkých kovů v játreach a trusu, resp. ledvinách a trusu u hrobkovce egyptského (*Taphozous perforatus*) a víkonosa afrického (*Rhinopoma cystops*) kolektovaných nedaleko Sakkary během roku 2013; korelační koeficienty Spearmanova ranku (r_s) jsou pro každý pár souboru údajů na 95% intervalu spolehlivosti; nd = nezaznamenáno

metal kov	*Taphozous perforatus*		*Rhinopoma cystops*	
	liver *vs* guano játra a trus	kidney *vs* guano ledviny a trus	liver *vs* guano játra a trus	kidney *vs* guano ledviny a trus
Al	–0.70	–0.70	0.11	–0.27
Ba	0.41	nd	–0.51	nd
Ca	0.78	0.36	0.37	0.75
Cd	0.73	nd	–0.66	nd
Cr	–0.45	nd	–0.41	nd
Cu	–0.75	–0.97	–0.02	–0.28
Fe	–0.48	–0.10	–0.15	–0.19
Mg	–0.95	–0.32	–0.50	–0.75
Mn	–0.87	–0.41	–0.91	–0.54
Mo	0.11	nd	–0.20	nd
Ni	–0.80	nd	–0.03	nd
Pb	–0.99	nd	–0.20	nd
Zn	0.43	–0.25	–0.25	–0.04

heavy metals. Therefore, bats have been extensively used as ecological indicators of habitat quality (Wickramasinghe et al. 2003, Park 2015). Bats, similarly as human beings, may be exposed to heavy metals through three main pathways including direct ingestion, inhalation through mouth and nose, and dermal absorption through skin exposures. Surface waters have long been recognized as sources of heavy metals contamination at levels that may pose health risks through ingestion and dermal absorption (Wu et al. 2009). Therefore, insects, water and air are considered as main sources of heavy metal accumulation in insectivorous bats. In South Africa, riparian habitats and wastewater treatment works (WWTWs) are prime foraging areas for insectivorous bats as these ecosystems support large numbers of insects (Vaughan et al. 1996, Fukui et al. 2006). However, bats that feed on these insects may also accumulate metals such as cadmium and copper in their tissues, with acute or chronic effects on their health. Metal concentrations in the kidney, liver and muscle tissue of *Neoromicia nana* (Vespertilionidae), caught at the sites, were found to contain (copper, zinc and iron) in all tissue samples, but the toxic metals cadmium, chromium and nickel were mostly present in tissue of bats at wastewater-polluted sites. Thus, although WWTWs provide an optimal food resource to bats in the short-term, it may pose serious long-term health risks for these top predators (Naidoo et al. 2013).

As mentioned above, heavy metals (e.g., Cu, Cd, Pb) were found in the Mariotteya stream at concentration levels exceeding the permissible limits, especially for Cd and Pb (Eissa et al. 2013, Mahmoud et al. 2014). Water bodies associated with the Mariotteya stream, which is considered the nearest foraging place for the bats of the Saqqara caves, are thus likely to act as a probable source of heavy metal contamination to the studied bats.

According to Zukal et al. (2015), the number of studies confirming direct adverse effects and toxicity of heavy metals on bats is low; however, some impacts and cases of poisoning have been documented. Moreover, results of previous studies suggest that bat populations under natural environmental conditions are frequently exposed to multiple anthropogenic stressors at the same time (Pikula et al. 2010, Zukal et al. 2015). On the other hand, Zukal et al. (2015) reported that extremely high heterogeneity in the published data on heavy metals in bats make it impossible to carry out a statistical analysis, and thus comparison can be based only on general variability, such as mean values and ranges. In this respect, we try to compare our results with some other studies.

In bat species studied by Walker et al. (2007) in Britain, it was reported that there was no significant variation in renal Pb or Cd concentration between male and female bats. In *T. perforatus* (Table 3), females showed significantly higher Pb concentration in liver than males ($P<0.05$). A similar trend was obtained for Barium (Ba), but no significant differences related to sex were obtained for the other analyzed metals, neither in liver nor kidneys of *T. perforatus*. In *R. cystops* (Table 3), Ba and Mn concentrations in liver were significantly higher in males, and there were no significant differences related to sex for the other analyzed metals, neither in liver nor kidneys of *R. cystops*. On the other hand, our results coincide with those of Pikula et al. (2010) who found that the liver Pb concentration was approximately two times higher in females than in males of *Myotis myotis*. A similar gender difference was found also for kidney Zn concentration in *Pipistrellus pipistrellus*. In the present investigation, Zn concentrations in liver and kidney showed insignificant differences between males and females, both in *T. perforatus* and *R. cystops* (Table 3).

If we compute the total mean concentrations of shared metals (e.g, Al, Cd, Cr, Cu, Mn, Pb and Zn) in liver of the two studied insectivorous bat species, the result will be 136.5 µg/g dry wt for *T. perforatus* and 116.2 µg/g dry wt for *R. cystops*, respectively (Table 4). A similar calculation of the total mean concentrations of shared metals (e.g., Al, Ca, Cu, Fe, Mg, Mn and Zn) in kidneys will result in 713.4 µg/g dry wt for *T. perforatus* and 933.8 µg/g dry wt for *R. cystops*, respectively (Table 5).

In the study by Walker et al. (2007), renal concentrations of heavy metals (e.g., Hg, Pb and Cd) differed among four species of insectivorous bats. The median concentrations in pooled data without sex and age differentiation ranged between 1.16–4.05 µg/g dry wt for Pb and 0.83–6.27 µg/g dry wt for Cd. The results of the present investigation revealed undetectable concentrations of both Pb and Cd in the kidneys of the two studied insectivorous bats, but their livers contained 0.75 and 0.18 µg/g dry wt of Pb and Cd, respectively for *T. perforatus*, and 0.95 and 0.19 µg/g dry wt of Pb and Cd, respectively for *R. cystops* (Table 4). Our results coincide with those of Walker et al. (2007) regarding variation in metal concentrations among different bat species, but they may give an indication of lower contamination levels of Pb and Cd in the studied area. However, the absence of Pb in kidneys of the two studied insectivorous bats suggests that reductions in petrol Pb emissions have not significantly affected the bioavailability of Pb to bats in the region of our study.

Also, our findings reveal a strong positive relationship between liver and kidney in the concentration of heavy metals in both studied bat species (Table 7). This suggests that individuals exposed to high levels of a metal are likely to accumulate high levels of this metal in both above mentioned organs. It seems that transfer rates (and possibly pathways) differ not only between bat species but also between toxicants (Walker et al. 2007). Our results are supported by a previous study by Clark et al. (1986) who found that levels of cadmium in liver and kidney were positively correlated in both the Gainesville bats ($r=0.837$) and bats from the Judges Cave ($r=0.852$) in the Jackson County, Florida, USA.

The above findings may reflect specificity in accumulation of some heavy metals with respect to organ and sex of the two insectivorous bat species used in the present investigation, and can be attributed to differences in dietary exposure. *T. perforatus* bats tend to be nocturnal hunters and their normal prey consists of moths, butterflies, and termites (Rydell & Yalden 1997). *R. cystops* feed primarily on beetles, neuropterans and moths (Whitaker & Yom-Tov 2002). Therefore, heavy metals are transferred from sediments/water/soil/plants or other sources to insect larvae and adults, and finally to the bats themselves (Reinhold et al. 1999, Hernout et al. 2013). Not only will pathways differ within the food-chain but they may also differ depending upon the type of toxicant. Nevertheless, the water and vegetation fauna prevailing in the Mariotteya ecosystem contributed to such differences in heavy metal accumulation in the two studied bat species. Also, we have to consider other factors, such as foraging habits, flying activities and bat metabolism which are known to significantly influence accumulation of toxicants in bat tissues (Bayat et al. 2014).

According to Mulec et al. (2013), the idea of detecting heavy metals in guano is appealing for several reasons, including: (a) guano is found in close proximity to bat roosts and is likely to be a mirror of bat exposure to environmental pollutants, (b) sampling of guano is less invasive than direct sampling of bats and may be conducted at times when bats are absent from hibernacula, (c) the chemical and physical characteristics of guano make it a suitable substrate for measuring chemical and biological contaminants.

Already in the 1970s, Petit & Altenbach (1973) proposed the analysis of stratigraphically dated guano deposits, as this could provide a chronological record of selected heavy metals in the food chain of bats in a given area. In the migratory free-tailed bat *Tadarida brasiliensis*, the authors found a correlation between annual fluctuations of mercury in bat guano with annual copper production in a nearby smelter. Early studies conducted by Clark et al. (1986) have shown that heavy metals in guano collected from four bat caves contained very high concentrations of Pb, Cr, Zn and Cd; reaching 3.4–7.1, 0.54–5.0, 340–640, and 0.3–2.3 ppm dry wt., respectively. The authors referred guano contamination to metals escaped from a battery salvage plant at Steel City, Jackson County, Florida, USA, in addition to other sources related to human activities in the area. Also, high concentrations of cadmium have been reported in the guano of gray bats *Myotis grisescens* in the USA (Clark 1988a, b).

In the present study, the guano samples were found to contain the same heavy metals detected in the analyzed bat tissues. Except calcium (Ca), all the other detected metals (Table 6) were previously reported by Zukal et al. (2015). Based on the values of heavy metal concentrations recorded in liver and kidneys of the two insectivorous bats (Tables 4, 5), we can calculate the general mean of selected metals (Al, Cu, Zn and Mn) with respect to their values in liver and kidney of the two bat species. Such estimation results in 58.5, 6.1, 40.4 and 3.1 µg/g dry wt, respectively, for the selected metals in samples representing one complete year (not tabulated). On the other hand, the mean concentration values in guano were 703.2, 35.2, 443.0 and 61.8 µg/g dry wt, respectively, in samples representing one complete year (Table 6). This indicates that contamination of guano was much higher than that of bat tissues; results go parallel with those reported by Zukal et al. (2015). Strong correlations (positive or negative) were found between metal concentrations in liver or kidney and those in guano, both for *T. perforatus* and *R. cystops* (Table 8). It has been previously reported that the elemental composition of bat guano probably reflects heavy metal residues present in the undigested portion of ingested prey species, and as such may provide some clues to the location of contaminants in the environment (Martin 1992).

As far as we are aware, there are no other time-trend data for heavy metals in bats, insectivorous mammals or birds in Egypt, and so it is impossible to assess whether the trend in the studied bats is typical for other bat species or other insectivores. Concentrations of metals such as Cd or Pb in the studied bats were much lower than those associated with adverse effects in mammals (105 µg/g dw for Cd, Chmielnicka et al. 1989; and 25 µg/g dw for Pb, Ma 1996).

Conclusions

This study is the first evaluation of heavy metal concentrations in bats in Egypt. Our results suggest that accumulation of the highly toxic metals such as Pb, Cd and Cu in individuals is very low, and far of acute toxicity. In the wild, bats are frequently exposed to multiple anthropogenic stressors at the same time, which may show antagonistic or, more frequently, combined or synergic effects. Such stressors may include natural toxins, anthropogenic pollutants such as heavy metals, and infectious agents, however the combined effects of such stressors remain practically unexplored (Skerratt et al. 1998). The present study shed light on feasibility of using bats as bioindicators of environmental pollutants. So, continued monitoring of the long-term trends in metal accumulation and associated health status of bats, together with exploration of the level ofcontamination in individuals from different industrialized regions, is merited. The findings may also encourage the use of bat deposits (guano) in a large-scale monitoring program, at least to avoid stress on bat populations. On the other hand, the study urges the need to establish education programmes for the public and school children about bat conservation, and the importance of relevant legislation whichshould be enacted and enforced.

Souhrn

Sledování těžkých kovů v životním prostedí za pomoci netopýrů jako bioindikatorů: první studie v Egyptě. Pro sledování těžkých kovů v tkáních byly použity dva druhy hmyzožravých netopýrů, hrobkovec egyptský (*Taphozous perforatus*) a víkonos africký (*Rhinopoma cystops*); ty byly kolektovány ve dvou jeskyních nedaleko Sakkary v provincii Giza v severním Egyptě. Vyhodnocení přítomnosti a objemu 14 těžkých kovů za pomoci hmotnostní spektrometrie s indukčně vázaným argonovým plazmatem (Inductively Coupled Argon Plasma Instrument) byla podrobena játra, ledviny a vzorky trusu (guano). V játrech hrobkovce a víkonosa byly nalezeny všechny těžké kovy s výjimkou kobaltu (Co), avšak v tkáni ledvin těchto netopýrů nebylo nalezeno baryum (Ba), kadmium (Cd), chrom (Cr), molybden (Mo), nikl (Ni) a olovo (Pb). Játra samic hrobkovců obsahovaly statisticky významně ($P< 0.05$) vyšší hladiny barya a olova než játra samců, ale opačný trend byl zaznamenán pro baryum a mangan v játrech víkonosů. Vzorky trusu obsahovaly obecně mnohem vyšší koncentrace všech 14 kovových prvků. Byla nalezena silná korelace mezi koncentracemi kovů v játrech oproti ledvinám a trusu. Například, koeficient Spearmanovy korelace (rs) činil 0.87 a 0.76 pro srovnání objemu hliníku a zinku v játrech a ledvinách hrobkovce a 0.91 a 0.97 u víkonosa. Z těchto výsledků je souzeno, že hromadění těchto prvků v tkáních netopýrů se zdá ovlivněno druhem, pohlavím a obdobím roku, kdy je hodnocení prováděno. Závěrem bylo navrženo, aby se pro široce pojaté programy monitorování těžkých kovů v prostředí využívaly uloženiny trusu (guano), aby přinejmenším se zabránilo zbytečnému stresování netopýrů, kteří patří k ochranářsky významným organismům Egypta.

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References

Bartonička T. & Zukal J., 2003: Flight activity and habitat use of four bat species in a small town revealed by bat detectors. *Folia Zoologica*, **52**: 155–166.

Bayat S., Geiser F., Kristiansen P. & Wilson S. C., 2014: Organic contaminants in bats: trends and new issues. *Environmental International*, **63**: 40–52.

Beyersmann D. & Hartwig A., 2008. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. *Archives of Toxicology*, **82**: 493–512.

Boyles J. G., Cryan P. M., McCracken G. F. & Kunz T. H., 2011: Economic importance of bats in agriculture. *Science Magazine*, **332**(6025): 41–42.

Bremner I. & Beattie J. H., 1990: Metallothionein and the trace minerals. *Annual Reviews of Nutrition*, **10**: 63–83.

Chmielnicka J., Halatek T. & Jedlinska U., 1989: Correlation of cadmium-induced nephropathy and the metabolism of endogenous copper and zinc in rats. *Ecotoxicology and Environmental Safety*, **18**: 268–276.

Clark D. R. Jr., 1988a: How sensitive are bats to insecticides? *Wildlife Society Bulletin*, **16**: 399–403.

Clark D. R. Jr., 1988b: Environmental contaminants and the management of bat populations in the United States. Pp: 409–413. In: Szaro R. C., Severson K. S. & Patton D. R. (eds.): *Management of Amphibians, Reptiles, and Small Mammals in North America. Proceedings of the Symposium, July 19–21, 1988, Flagstaff, Arizona. General Technical Report RM-166*. US Department of Agriculture Forest Service, Fort Collins, CO, 458 pp.

Clark D. R. Jr., Wenner A. S. & Moore J. F., 1986: Metal residues in bat colonies, Jackson County, Florida, 1981–1983. *Florida Field Naturalist*, **14**(2): 38–45.

Cleveland C. J., Frank J. D., Federico P., Gomez I., Hallam T. G., Horn J., Lopez J., McCracken G. F., Medellin R. A., Moreno V. A., Sansone C., Westbrook J. K. & Kunz T. H., 2006: Economic value of the pest control service provided by Brazilian free-tailed bat in south-central Texas. *Frontiers in Ecology and Environment*, **4**: 238–243.

Dietz C., 2005: *Illustrated Identification Key to the Bats of Egypt*. Walacea & Egyptian BioMAP Project, London, 36 pp.

Eissa A. E., Tharwat N. A. & Zaki M. M., 3013: Field assessment of the midwinter mass kills of trophic fishes at Mariotteya stream, Egypt: Chemical and biological pollution synergistic model. *Chemosphere*, **90**: 1061–1068.

Frickel S. & Elliott J. R., 2008: Tracking industrial land use conversions: a new approach for studying relict waste and urban development. *Organic Environment*, **21**: 128–147.

Fukui D., Murakami M., Nakano S. & Aoi T., 2006: Effect of emergent aquatic insects on bat foraging in a riparian forest. *Journal of Animal Ecology*, **75**: 1252–1258.

Fulladosa E., Delmas F., Jun L., Villaescusa I. & Murat J. C., 2002: Cellular stress induced in cultured human cells by exposure to sludge extracts from water treatment plants. *Ecotoxicology and Environmental Safety*, **53**: 134–140.

Hernout B. V., Somerwill K. E., Arnold K. E., McClean C. J. & Boxall A. B. A., 2013: A spatially-based modelling framework for assessing the risks of soil-associated metals to bats. *Environmental Pollution*, **173**: 110–116.

Jones G., Jacobs D. S., Kunz T. H., Willig M. R. & Racey P. A., 2009: Carpe noctem: the importance of bats as bioindicators. *Endangered Species Research*, **8**: 93–115.

Kurta A., Bell G. P., Nagy K. A. & Kunz T. H., 1989: Energetics of pregnancy and lactation in free-ranging little brown bats (*Myotis lucifugus*). *Physiology and Zoology*, **62**: 804–818.

Leary S. (ed.), 2013: *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition*. American Veterinary Medical Association, Schaumburg, 102 pp.

Ma W. C., 1996: Lead in mammals. Pp.: 281–296. In: Beyer W., Heinz G. & Redmon-Norwood A. (eds.): *Environmental Contaminants in Wildlife. Interpreting Tissue Concentrations*. CRC Press Inc, London, 758 pp.

Mahmoud N. E., Badawy M. F. M. & Fahmy M. M., 2014: Investigations on mass mortalities among *Oreochromis niloticus* at Mariotteya stream, Egypt: Parasitic infestation and environmental pollution impacts. *Journal of Aquatic Research Development*, **5**: 219.

Mansour S. A., 2014: Heavy metals of special concern to human health and environment. Pp.: 213–233. In: Bhat R. & Gómez-López V.M. (eds.): *Practical Food Safety: Contemporary Issues and Future Directions*. John Wiley & Sons, London, 632 pp.

Martin D. B., 1992: *Contaminant Studies on Endangered Bats in Northeastern Oklahoma*. U.S. Fish and Wildlife Service, Ecological Services, Tulsa, Oklahoma, 16 pp.

Mulec J., Covington E. & Walochnik J., 2013: Is bat guano a reservoir of *Geomyces destructans*? *Open Journal of Veterinary Medicine*, **3:** 161–167.

Naidoo S., Vosloo D. & Schoeman M. C., 2013: Foraging at wastewater treatment works increases the potential for metal accumulation in an urban adapter, the banana bat (*Neoromicia nana*). *African Zoology*, **48**: 39–55.

Nowak R. W., 1994: *Walker's Bats of the World*. Johns Hopkins University Press, Baltimore, 287 pp.

Park K., 2015: Mitigating the impacts of agriculture on biodiversity: bats and their potential role as bioindicators. *Mammalian Biology*, **80**: 191–204.

Petit M. G. & Altenbach J. S., 1973: A chronological record of environmental chemicals from analysis of stratified vertebrate excretion deposited in a sheltered environment. *Environmental Research*, **6**: 339–343.

Pikula J., Zukal J., Adam V., Bandouchová H., Beklová M., Hájková P., Horáková J., Křížek R. & Valentiková L., 2010: Heavy metals and metallothionein in vespertilionid bats foraging over aquatic habitats in the Czech Republic. *Environmental Toxicology and Chemistry*, **29**: 501–506.

Reinhold J. O., Hendriks A. J., Slager L. K. & Ohm M., 1999: Transfer of microcontaminants from sediment to chironomids and the risk for the pond bat *Myotis dasycneme* (Chiroptera) preying on them. *Aquatic Ecology*, **33**: 363–376.

Russo D. & Ancillotto L., 2015: Sensitivity of bats to urbanization: a review. *Mammalian Biology*, **80:** 205–212.

Rydell J. & Yalden D. W., 1997: The diets of two high-flying bats from Africa. *Journal of Zoology*, *London*, **242:** 69–76.

Skerratt L. F., Speare R., Berger L. & Winsor H., 1998: Lyssa viral infection and lead poisoning in black flying foxes from Queensland. *Journal of Wildlife Diseases*, **34**: 355–361.

Soliman S., Mansour S. A. & Soliman K. M., 2015: Population studies on two sympatric bat species: *Taphozous perforatus* and *Rhinopoma hardwickii* (Chiroptera: Microchiroptera) from Egypt. *Egyptian Journal of Zoology*, **64**: 13–32.

Stewart M. A., Jardine P. M., Brandt C. C., Barnett M. O., Fendorf S. E., McKay L. D., Mehlhorn T. L. & Paul K., 2003: Effects of contaminant concentration, aging, and soil properties on the bioaccessibility of Cr (III) and Cr (VI) in soil. *Soil and Sediment Contamination*, **12**: 1–21.

Thermo Scientific, 2007: *US- EPA SW-846 Method 6010B Using an iCAP 6500 Duo*. Thermo Fisher Scientific Inc. AN40836_E 07/07C (www.thermo.com).

USEPA, 1996: *USEPA SW-846 Method 6010b*, *Revision 2*, *December 1996*.

Van De Sijpe M., Vandendriessche B., Voet P., Vandenberghe J., Duyck J., Naeyaert E., Manhaeve M. & Martens E., 2004: Summer distribution of the pond bat *Myotis dasycneme* (Chiroptera, Vespertilionidae) in the west of Flanders (Belgium) with regard to water quality. *Mammalia*, **68**: 377–386.

Vaughan N., Jones G. & Harris S., 1996: Effects of sewage effluent on the activity of bats (Chiroptera: Vespertilionidae) foraging along rivers. *Biological Conservation*, **78**: 337–343.

Walker L. A., Simpson V. R., Rockett L., Wienburg C. L. & Shore R. F., 2007: Heavy metal contamination in bats in Britain. *Environmental Pollution*, 148: 483–490.

Whitaker J. O. Jr., 1995: Food of the big brown bat, *Eptesicus fuscus*, from maternity colonies in Indiana and Illinois. *American Midland Naturalist*, **134**: 346–360.

Whitaker J. Jr. & Yom-Tov Y., 2002: The diet of some insectivorous bats from northern Israel. *Mammalian Biology*, **67**: 378–380.

Wickramasinghe L. P, Harris S., Jones G. & Vaughan N., 2003: Bat activity and species richness on organic and conventional farms: impact of agricultural intensification. *Journal of Applied Ecology*, **40**: 984–993.

Wu B., Zhao D.Y., Jia H.Y., Zhang Y., Zhang X. X. & Cheng S.P., 2009: Preliminary risk assessment of trace metal pollution in surface water from Yangtze River in Nanjing section, China. *Bulletin of Environmental Contamination and Toxicology*, **82**: 405–409.

Zocche J. J., Leffa D. D., Damiani A. P., Carvalho F., Mendonca R. A., dos Santos C. E. I., Boufleur L. A., Dias J. F. & de Andrade V. M., 2010: Heavy metals and DNA damage in blood cells of insectivore bats in coal mining areas of Catarinense coal basin. *Brazilian Environmental Research*, **110**: 684–691.

Zukal J., Pikula J. & Bandouchová H., 2015: Bats as bioindicators of heavy metal pollution: history and prospect. Review. *Mammalian Biology*, **80**: 220–227.

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