Journal of Elementology ISSN 1644-2296

Gonkowski, S., Könyves, L., Berlinger, B. and Rytel, L. (2024) 'Assessment of cadmium concentration levels in wild bats in Poland by analysis of guano samples', *Journal of Elementology*, 29(1), 277-291, available: https://doi.org/10.5601/jelem.2023.28.4.3252

RECEIVED: 4 December 2023
ACCEPTED: 26 January 2024

ORIGINAL PAPER

Assessment of cadmium concentration levels in wild bats in Poland by analysis of guano samples*

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Abstract

The aim of this study was to investigate cadmium (Cd) levels in wild bats in Poland. Concentration levels of Cd were studied in guano samples collected from 4 bat summer (nursery) colonies of the wildlife greater mouse-eared bat (*Myotis myotis*) located in various parts of Poland. Analysis was made using the inductively coupled plasma optical emission spectrometry (ICP-OES) method. During the present investigation, Cd was noted in all colonies and in all samples included into the study. Cd concentration levels fluctuated from 1.06 mg kg^{-1} to 3.54 mg kg^{-1} (median value 1.905 mg kg^{-1}). Clear differences in Cd concentration levels were noted between particular bat colonies. Moreover, this study has shown that guano sample analysis is a suitable method to evaluate Cd concentration levels in wild bats. The results show that wildlife greater mouse-eared bats in Poland are exposed to Cd. Due to the high toxicity of Cd, even when present in small doses, this metal may be suspected to be a factor influencing the health status of wild bats in Poland

Keywords: cadmium, heavy metals, greater mouse-eared bat (*Myotis myotis*) , wildlife

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* Project financially supported by the Minister of Education and Science under the program entitled "Regional Initiative of Excellence" for the years 2019-2023, Project No. 010/RID/2018/19, amount of funding 12.000.000 PLN".

INTRODUCTION

Pollution of the natural environment with cadmium (Cd), due to its high toxicity and the ability to accumulate in living organisms, is one of the most serious threats to humans and animals (Rahimzadeh et al. 2017, Genchi et al. 2020).

In 2017, the global cadmium production amounted to 31 000 tons (IndexBox 2022). Cadmium is commonly used in various industries and in agriculture (Rahimzadeh et al. 2017, Satarug, 2019, Genchi et al. 2020). Such widespread and multidirectional use of Cd has resulted in the ubiquitous presence of this metal in the natural environment (Rahimzadeh et al. 2017, Satarug 2019, Genchi et al. 2020). Cd has been found in the surface and ground waters, in soil and in the air on all continents (Crea et al. 2017, Satarug 2019). The severity of the problem may be evidenced by the fact that the presence of Cd has even been detected in Antarctica, i.e., an area exposed to little anthropogenic pollution (Zvěřina et al. 2017). Moreover, Cd penetrates and accumulates in plants and animals organisms (Järup, Akesson 2009, Rahimzadeh et al. 2017, Pappuswamy et al. 2021).

Cd penetrates human and animal organisms by two main ways: the digestive tract (with contaminated food and water) and the respiratory system (with contaminated air, dusts and fumes), while the absorption through the skin is vestigial (Satarug 2019, Genchi et al. 2020). Previous studies have shown that Cd is highly toxic and may negatively affect various internal organs and systems (Rahimzadeh et al. 2017, Satarug 2019, Genchi et al. 2020). First of all, Cd disrupts the functioning of the kidneys and liver (Satarug 2019, Gattea Al-Rikabi et al. 2020). It also affects the nervous, gastrointestinal and reproductive systems, impairs immune cells, adversely affects the heart and blood vessels as well as the bones, and shows endocrine-disrupting activity (Satarug 2019, Genchi et al. 2020, Lin et al 2021).

Due to the high toxicity and multidirectional adverse effects demonstrated by Cd, the biomonitoring of this metal in human and animal organisms is very important in current toxicology (Satarug 2019, Genchi et al. 2020). In the light of previous studies, it is known that Cd is commonly found in human organisms, as well as in domestic and wild animals (Satarug 2019, Franzoni et al. 2022). Among the latter, the bats constitute a special group (Zukal et al. 2015).

Bats is a diversified group of mammals, which includes over 1400 species, inhabiting various climatic zones (all continents except Antarctica), and differing in terms of the type of food and lifestyle (Wilson, Mittermeier 2019). Unfortunately, despite legal protection covering the majority of bat species in many countries of the world, the number of these mammals is drastically decreasing (Mariton et al. 2022). Bats are considered to be one of the most endangered groups of mammals globally (Frick et al. 2020). One of the factors significantly contributing to this state of affairs is the anthropogenic influence on the environment (Frick et al. 2020, Mariton et al. 2022). This impact includes the destruction of natural habitats of bats, death of animals as a result infrastructural facilities (for example wind turbines), and contamination of the environment with chemical pollutants. It should be pointed out that higher exposure of bats to environmental pollution is favoured by the fact that they are relatively long-lived animals (for example, *M. Myotis* included into this study can live up to 37 years), they eat large amounts of food (especially insectivorous bats), and often establish colonies close to human settlements (Timofieieva et al. 2021).

Moreover, bats are very sensitive to any changes in the environment and its pollution with chemical toxins (Russo, Ancillotto 2015). Therefore, they are considered to be one of the best bioindicators of environmental quality, including the exposure to heavy metals (Zukal et al. 2015, Timofieieva et al. 2022).

The majority of previous studies concerning bats' exposure to Cd are based on analysis of various matrices, such as various tissues, blood, urine or fur (Zukal et al. 2015, Mina et al. 2019, Timofieieva et al. 2021, 2022). Collection of such samples is often connected to the death of animals (tissue collection) or at best with the exposure of the animals to stressors (blood or fur collection). Many studies are also performed on tissues from animals that have died due to other reasons, which may not fully reflect the exposure of the whole population to toxic factors. Due to the legal protection of most bat species, especially those living in Europe, it is important to be able to study the exposure of these animals to environmental factors without the need to kill or stress them. The analysis of guano samples seems to be a good option in such studies. On one hand, such samples may be easily collected without the need to significantly interfere with the life of a bat colony or individual animal. However, until now, the use of guano samples in studies on exposure of bats to Cd has been used relatively rarely (Hartman 2000, Mansour et al. 2016, Houchens 2020). It may be due to the fact that, unlike feces samples, biological level values for Cd have been established in the more frequently used matrices. These thresholds help us to assess the health risk caused by exposure to toxic substance, and for Cd they have been determined at 5 µg l^{-1} in blood and 5 µg g^{-1} of creatinine in urine (Sapota 2019).

The aim of the present study was to determine the levels of Cd in guano samples collected from colonies of greater mouse-eared bat (*Myotis myotis*), the most popular species of bat in Poland. To the best of our knowledge, this is the first such investigation in Poland and one of few studies using guano analysis tor evaluation of the exposure of wild bats to cadmium in Europe.

MATERIALS AND METHODS

Reagents

In the present study, the following reagents were used: 1. Deionized water obtained from a Purite Select Fusion 160 BP water purification system (SUEZ Water Technologies & Solutions, Trevose, PA USA). The conductivity of the ultrapure water was less than 0.1 ΩS cm^{-1}. 2. Concentrated nitric acid (69 m/m%, Aristar) (HNO_3) and hydrogen peroxide (30% m/m%, AnalaR NORMAPUR) (H_2O_2) purchased from VWR International Ltd. (Leicestershire, UK). Both reagents were of trace analysis quality. 3. Laboratory glassware and plastic tools cleaned by 0.15 M hydrochloric acid (37 m/m%, Aristar) (HCl) solution obtained from VWR International Ltd. (Leicestershire, UK), then rinsed with deionized water). 4. The argon gas used in the ICP-OES measurement was of 4.6 purity and purchased from Messer Hungarogaz Ltd. (Budapest, Hungary).

Sample collection

Guano samples were collected on August-September 2021 in 4 bat summer (nursery) colonies of greater mouse-eared bats (*Myotis myotis*) located in various parts of Poland (Table 1).

Table 1

Characterization of bat colonies included into the study

Colony number	Name of place	Type of place	Industry/ dominant landform	Number of citizens in town	Colony location	Number of animals in colony	Coordinates
1	Brenna	village	none/ forest	6134	school attic	250	49°43′37.4″N 18°53′46.3″E
2	Sliwice	village	none/ agricultural	2464	church tower	450	53°42′19.0″N 18°10′04.4″E
3	Pulawy	city	chemical, pharmaceutical, construction/ industrial	47417	children's foster home attic	250	51°25′19.9″N 21°58′37.4″E
4	Opole Lubelskie	town	food/ small-town	8605	school attic	200	51°09′02.7″N 21°58′24.6″E

Glass litter boxes were put in different parts of the floor of the rooms where bat colonies lived. After 48 hours, the boxes were removed and the guano samples they contained were put into glass containers, frozen and stored at -20°C until further analysis. 80 guano samples (20 from each bat colony) were analyzed. The number of litter boxes set for each colony was the same to standardize the sampling method.

Particular emphasis was laid on avoiding any disturbance and causing any stress to the bats during sample collection. Sampling was done in compliance with the Act for the Protection of Animals for Scientific or Educational Purposes of 15 January 2015 (Journal of Laws of the Polish Republic U.Dz. 2015, No. 266), binding in the Republic of Poland. Because guano sample collection was a non-invasive procedure, which was not associated with stressing and scaring the bats and did not affect their welfare, neither consent nor approval from the Bioethical Committee for the present study was required.

Sample preparation

0.5 g from each sample was transferred into a CEM MARS6 MARS XPreSS Teflon vessel. 5 mL HNO_3 and 5 mL H_2O_2 were added and the decomposition method was started in the CEM MARS6 microwave digestion system, CEM Corporation, Matthews, North Carolina, USA (ramp 35 min, temp. 200°C, hold 50 min, energy 1700 W). After digestion, the vessels were emptied and washed in 50 mL polypropylene (PP) tubes (Deltalab, Rubí Barcelona, Spain). Then, they were replenished to 25 mL with deionised water, and before analysis diluted 2-fold with deionised water in 12 mL PP tubes (Deltalab, Rubí Barcelona, Spain) after adding 100 µL of internal standard solution containing 100 mg L^{-1} yttrium (Y). Blank and NIST-1577C certified reference material (CRM) quality control (QC) samples were prepared by the same method.

Analytical measurements

Determination of Cd concentrations was carried out on a Perkin Elmer Avio 550 Max (Perkin Elmer, Waltham, MA, USA) ICP-OES apparatus with the following parameters: solid-state RF generator, 40 MHz, free running; RF power: 1500 W; nebuliser type: Meinhard concentric glass, type K1; Spray Chamber type: cyclonic glass; plasma gas flow rate: 12 L min^{-1}; auxiliary gas flow rate: 0.2 L min^{-1}; nebuliser gas flow rate: 0.70 L min^{-1}, and sample flow rate: 1.50 mL min^{-1}. The detection limit (DL) of Cd was 0.05 mg kg^{-1}.

Calibration was performed with the ICP multi-element standard (Perkin Elmer, Waltham, MA, USA). Internal quality control of the measurements was carried out via measuring QC samples of known concentration. After discarding the extremes, the standard deviation of data (*s*) was established, which had to remain within the ±15% of the nominal concentration value in order to accept the QC measurement.

Statistical analysis

The statistical analysis was made with GraphPad Prism version 9.2.0 (GraphPad Software, San Diego, California USA). Descriptive statistics with calculations of minimum and maximum values, arithmetic mean ± standard deviation (SD), geometric mean, median and frequency of detection was used to generally characterize Cd levels in bat guano samples. In turn, the non-parametric Kruskal-Wallis test was used to compare Cd levels between particular colonies, and the differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

The presence of Cd was noted in all bat guano samples included into the study and from all bat colonies investigated (Table 2).

Table 2

Concentration values (mg kg^{-1}) and frequency of detection (%) of cadmium in the analyzed bat guano samples cumulative data

Bat colony number	*n*	Minimum value	Maximum value	Arithmetic mean	Geometric mean	Median	Frequency of detection
1	20	1.18	2.02	1.57±0.20	1.55	1.54	100
2	20	1.06	2.67	2.16±0.37	2.12	2.13	100
3	20	1.60	3.54	2.40±0.52	2.35	2.35	100
4	20	1.28	2.29	1.76±0.26	1.74	1.75	100
Total (all colonies)	80	1.06	3.54	1.97±0.48	1.91	1.91	100

n – number of samples

Considering samples from all colonies included into the study, Cd concentration levels ranged from 1.06 mg kg^{-1} to 3.54 mg kg^{-1}. Arithmetic mean amounted to 1.97±0.48 mg kg^{-1}, geometric mean achieved 1.91 mg kg^{-1} and median value was 1.91 mg kg^{-1} (Table 2).

The highest Cd concentration levels were found in bat colonies 3 and 2, where the median value amounted to 2.40±0.52 mg kg^{-1} and 2.16±0.37 mg kg^{-1}, respectively (Figure 1). Differences between these two colonies were not statistically significant, but Cd concentration levels in colonies 2 and 3 were significantly higher than those observed in colonies 1 and 4. (Figure 1). In colony 4, the median value amounted to 1.76±0.26 mg kg^{-1}, and the Cd concentration levels were the lowest in colony 1, so the median value in this colony achieved 1.57±0.20 mg kg^{-1}. Differences between colonies 1 and 4 were not statistically significant (Figure 1).

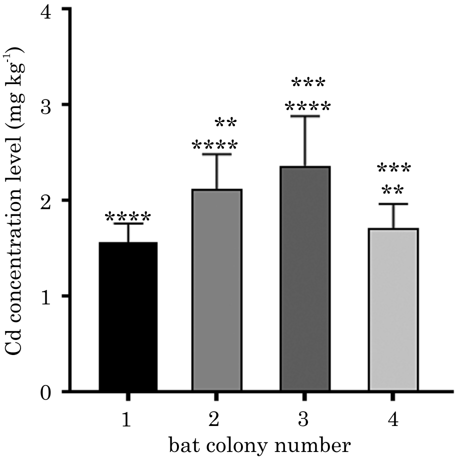

Fig. 1. Mean concentration levels (±SD) of cadmium in the bat guano samples collected in particular colonies: *** indicates statistically significant differences between bat colonies 1 and 2 and between bat colonies 1 and 3, ** indicates statistically significant differences between bat colonies 2 and 4, **** indicates statistically significant differences between bat colonies 3 and 4

DISCUSSION

The presence of Cd in all samples included into this study regardless of where the colony lives clearly indicates that this metal is a common pollutant of the natural environment in Poland. The prevalence of Cd in the Polish natural environment (including water, soil and air), as well as in humans, animals and plants in Poland has also been confirmed by previous studies (Chałabis-Mazurek et al. 2021, Tomska et al. 2021). However, this study is the first description of Cd in wild bats living in Poland although the exposure of bats to a wide range environmental pollutants is increasingly becoming the subject of investigations, because such investigations help to assess the degree of environmental pollution and the resulting dangers for humans.

The exposure of various species of bats to Cd has been evaluated in different parts of the world and in a wide range of matrices (Table 3).

As Table 3 shows, the majority of studies on Cd in bats have been performed using bat tissues, because Cd accumulates in internal organs. However, such studies require the use of carcasses of naturally dead animals or the euthanasia of bats. In the first case, investigations may not fully reflect the Cd levels in the entire population, and in the second case, due to legal protection of many bat species, the possibilities for such studies are limited. Another group of investigations on Cd concentration levels in bats is based on analysis of urine, blood and fur (Table 3). Such studies are less invasive, but involve the capture of animals and subjecting them to stress.

Table 3

Cadmium (Cd) concentration levels in bats
(mg kg^{-1} in solid samples and ml l^{-1} in liquid samples) noted in previous studies

Location	Species	*n*	Matrix	Cd concentration levels	References
Australia	Black Flying Foxes	4	kidney	ND – 8.09 ppm	Skerratt et al. (1998)
		4	liver	<0.03 - 1.36	
		4	brain	ND-<0.03	
	Bent-wing bat	20	liver	0.45	Allison et al. (2006)
	Free-living Grey-headed flying-fox	11	kidney	0.27-219	Pulscher et al. (2020)
		11	liver	0.02-12.6	
		11	fur	<0.01-0.04	
		5	urine	8.93-169	
	Captive Grey-headed flying-fox	14	kidney	0.59-62.8	
		14	liver	0.01-1.06	
		14	fur	0.00-0.02	
		7	urine	6.92-34.6	
	Black flying-fox	9	kidney	4.08-64.1	
		9	liver	0.23-7.58	
		9	fur	0.01-0.03	
Brazil	Velvety free-tailed bat	25	liver	4.07±2.2-4.87±2.7	José Zocche et al. (2020)
	Brazilian free-tailed bat	40	liver	<3.0-<4.05	
	Eptesicus diminutus	32	liver	3.67±2.2	
Christmas Isl.	Christmas Island flying-fox	54	fur	0.02-0.32	Pulscher et al. (2021)
Czech Republic	Mouse-eared bat	33	liver	solitary findings of 0.01	Pikula et al. (2010)
		33	kidney	mean: 0.01	
	Common pipistrelle	23	liver	solitary findings of 0.01	
Egypt	Egyptian tomb bat	91	liver	0.15-0.22	Mansour et al. (2016)
	Egyptian mouse-tailed bat	72	liver	0.07-0.34	
	Non specified	?	guano	0.17-0.21	
Germany	Common pipistrelle	8	fur	<0.01-245	Flache et al. (2015)
	Daubenton's bat	13	fur	<0.01-0.96	
	Common noctule	11	fur	<0.01-0.74	
	Daubenton's bat	21	fur	0.11-0.27	Flache et al. (2006)

cont. Table 3

Location	Species	*n*	Matrix	Cd concentration levels	References
	Common pipistrelle	111	guano	1.6-3.2	Hartman (2000)
		22	kidney	0.0045-14	
		22	liver	0.049-6.5	
	Various species	47	guano	0.80-41	
Great Britain	Brown long-eared	59	kidney	<0.081-12.5	Walker et al. (2007)
	Natterer's bat	13	kidney	median 6.27 (max 18.5)	
	Common pipistrelle	172	kidney	<0.081-29.1	
	Whiskered bat	17	kidney	<0.081-16 (med 1.61)	
	Common pipistrelle	191	liver	0.0015-2.5	Hernout et al. (2016)
Italy	Greater mouse-eared bat	101	fur	<0.001-0.075	Ferrante et al. (2018)
		101	liver	<0.001-1.495	
Mexico	Mexican free-tailed bat	48	liver	<0.20-1.98	Thies and Gregory (1994)
	Mexican free-tailed bat	70	liver	0.08-1.631	Ramos et al. (2020)
	Fish-eating bat	10	liver	4.1-7.9	Méndez and Alvarez-Castañeda (2000)
	Greater bulldog bat	10	liver	1.5-14.4	
Portugal	Various species: Savi's pipistrelle, Lesser noctule, Common pipistrelle, Soprano pipistrelle	56	bone	0.00-0.06	Mina et al. (2019)
			brain	0.01-0.10	
			heart	0.03-1.04	
			liver	0.17-1.77	
			fur	0.02-057	
			wing	0.02-0.24	
South Africa	Banana bat	26	liver	<2.867	Naidoo et al. (2013)
	Egyptian free-tailed bat and Angolan free-tailed bat	11	fur	0.016-0.171	Cory-Toussaint et al. (2022)
		11	blood	0.0001-0.004	
Taiwan	Non specified	6	body	0.05-1.37	Hsu et al. 2006
Ukraine	Kuhl's pipistrelle	54	fur	0.004-1.462	Timofieieva et al. (2022)
			wing	0.001-0.612	
			lung	0.001-1.121	
			liver	0.002-4.291	
			kidney	0.001-13.138	
			bone	0.001-0.092	
			muscle	0.001-1.892	

cont. Table 3

Location	Species	n	Matrix	Cd concentration levels	References
	Serotine bat	20	fur	0.12-3.12	Timofieieva et al. (2021)
		18	wing	0.01-2.93	
		20	lung	0.01-0.12	
		19	liver	0.01-0.69	
		19	kidney	0.03-1.92	
		20	bone	0.001-0.33	
	Common noctule	19	fur	0.059-0.82	
		10	wing	0.02-0.35	
		20	lung	0.003-0.1	
		17	liver	0.065-0.763	
		18	kidney	0.11-1.42	
		20	bone	0.001-0.05	
USA	Grey bat	4	guano	0.59-0.65	Houchens (2020)

n – number of samples, ND-not detected

Therefore, guano analysis seems to be a relatively good way to assess the exposure of protected bat species to toxic elements because such samples are easy to collect without disturbing the animals too much. Moreover, previous studies on various animal species (also on bats) have proven that Cd levels in the feces reflect the degree of exposure to this element (Zukal et al. 2015, Eeva et al. 2020). However, the evaluation of bats to Cd by guano sample analysis has been made relatively rarely (Table 4). Perhaps it is related to some difficulties and limitations during analysis of such samples. Namely, guano samples may be contaminated with substances from the external environment, the analysis covers mainly the degree of oral exposure, and it is impossible to determine the exposure of individual animals.

The comparison of present results with results obtained in previous studies conducted on guano samples (Table 4) shows that concentration levels of Cd noted in the present study are higher than those determined in bats in Egypt or USA. This may result from the fact that previous studies have been performed in completely differ regions, where environmental pollution with Cd may be lower, which in turn is related to the degree of urbanization an industrialization. Namely, it is known that bats living near big cities are more exposed to Cd than bats from rural areas (Timofieieva et al. 2022). The results of the present study seems to confirm this assertion because the highest Cd concentration levels were noted in colony no. 3 located in Puławy, the largest city among those included in the research. Moreover, the chemical industry, which may affect the Cd content in the environment, is located in Puławy.

Moreover, differences in Cd concentration levels in bat guano samples between the present study and previous investigations may result from other factors, including bat species, diet, gender, body condition, parasite infections and seasons of the year in which the investigation was performed higher levels of Cd are noted in winter (Walker et al. 2007, Zukal et al. 2015, Pulscher et al. 2020, Timofieieva et al. 2021, Timofieieva et al. 2022). An interesting comparison was made by Zukal et al. (2015). The cited authors calculated that total Cd levels in guano samples collected from insectivorous species fluctuated from 0.03 to 8.5 mg kg^{-1} (mean value 4.13). The levels of Cd observed in this study (greater mouse-eared bat included into this study is an insectivorous species) are slightly lower than the average values calculated by Zukal et al. (2015).

Any comparison of the present results with previous studies on Cd in bats performed on other matrices is practically impossible due to the fact that levels of Cd in various matrices may be completely different, even in bats living on the same area (Table 4). General observations have shown that Cd concentration levels in bat tissues fluctuate from 0.001 to 180 mg kg^{-1} (mean value 1.32) in insectivorous species and from <0.03 to 19.5 mg kg^{-1} (mean value 2.76) in nectarivores/ fructivorous species (Zukal et al. 2015).

Moreover, previous studies have revealed clear differences in Cd levels between particular animals from the same area. For example, Pulscher et al. (2020) have shown that Cd concentration levels in the kidney of bats living in the same area fluctuated from 0.27 to 219 mg kg^{-1}. Such observations may indicate the existence of local, not fully specified factors affecting the bat exposure to this metal. The results obtained during the present study, namely the distinct differences between particular colonies (even those located nearby – colonies 3 and 4) and relatively high levels of Cd in colony 2 (located in a small village without any industry) can also suggest such local, not fully specified factors. On the other hand, the high levels of Cd in colony 2 included into this study may be connected with agriculture, where this metal is commonly used (Satarug 2019).

The presence of Cd in all guano samples included in this study clearly demonstrates the widespread food exposure of wild bats in Poland to this metal. In the light of previous experiments on various species and epidemiological observations on humans, it is relatively well known that even low levels of chronic Cd exposure may result in changes in many internal organs, including the liver, kidney, nervous system and reproductive organs (Johri et al. 2010, Rahimzadeh et al. 2017, Satarug 2019, Genchi et al. 2020). Although information about Cd negative effects on bats is extremely scanty, it has been reported that this metal in bats causes disturbances similar to those observed in other species, and consisting in changes in bones, liver and kidneys (Pulscher et al. 2020). Therefore it can be concluded that exposure to Cd leads to a deterioration of the health condition of bats, and also (taking into account the influence of Cd on the activities of the reproductive system) may cause a reduction in the population size of this animal species. On the other hand, Cd in bat guano can be dangerous to humans. This is all the more likely because numerous greater mouse-eared bat summer colonies are located near human settlements. Two of the colonies included into this study are in schools, one in a church and one at a children's foster home. It is also important to remember about the risk of intoxication during the removal of guano prior to renovation works in attics or when cleaning attics after the summer season, when bats have moved to winter colonies.

CONCLUSIONS

Observations from the present study clearly show that greater mouse-eared bats living in Poland are exposed to Cd. The presence of this metal has been noted in all summer colonies and in all guano samples included into the study, but concentration levels of Cd depended on the location of colonies. Due to high toxicity of Cd, even in small doses, it can be assumed that this metal may be a factor influencing the health status of wild bats in Poland and the size of their population. Moreover, the present study has shown that guano samples analysis is a suitable method to evaluate bat exposure to Cd. The guano sample collection is easy and relatively stress-free for animals, and therefore analysis of such samples is especially useful in the case of protected species.

Author contributions

Conceptualization – S.G and L.R., methodology – L.K. and B.B, sample collection – L.R., writing – original draft preparation – S.G., writing – review and editing – S.G., L.K., B.B, L.R. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest. The authors ensure that they have neither professional nor financial connections related to the manuscript sent to the Editorial Board.

This manuscript contains supplementary material, which is available only online: https://jsite.uwm.edu.pl/issue/view/1-2024/

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