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Hernout, Béatrice V, McClean, Colin J orcid.org/0000-0002-5457-4355, Arnold, Kathryn E orcid.org/0000-0002-6485-6065 et al. (3 more authors) (2016) Fur:A non-invasive approach to monitor metal exposure in bats. CHEMOSPHERE. pp. 376-381. ISSN: 0045-6535

https://doi.org/10.1016/j.chemosphere.2015.12.104

Fur: a non-invasive approach to monitor metal exposure in bats

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Key words: trace metal elements; environmental pollution monitoring; bat tissues; less-destructive/invasive sampling; *Pipistrellus pipistrellus/pygmaeus*; wildlife ecotoxicology

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Abstract

This paper presents a novel assessment of the use of fur as a non-invasive proxy to biomonitor metal contamination in insectivorous bats. Metal concentrations (cadmium, copper, lead and zinc) were measured using ICP-MS in tissues (kidneys, liver, stomach and stomach content, bones and fur) obtained from 193 *Pipistrellus pipistrellus/pygmaeus* bats. The bats were collected across a gradient of metal pollution in England and Wales. The utility of small samples of fur as an indicator of metal exposure from the environment was demonstrated with strong relationships obtained between the concentrations of non-essential metals in fur with concentrations in stomach content, kidneys, liver and bones. Stronger relationships were observed for non-essential metals than for essential metals. Fur analyses might therefore be a useful non-invasive proxy for understanding recent, as well as long term and chronic, metal exposure of live animals. The use of fur may provide valuable information on the level of endogenous metal exposure and contamination of bat populations and communities.

1. Introduction

Non-invasive sampling approaches such as fur, feather or claw are invaluable in field-based monitoring studies to understand levels of pollution in wildlife species, particularly for protected species, such as bats (Dauwe et al., 2000; D'Havé et al., 2006). These methods are less damaging and stress inducing than invasive approaches such as blood sampling or the taking of biopsies and allow the monitoring of both levels of pollution and the fitness of a given population over time without significantly affecting the fitness of individuals (D'Havé et al., 2006).

Hair sampling is highly suited as a non-invasive sampling technique as hair is easily accessible, stable, and its storage does not require restricted conditions (Appenzeller and Tsatsakis, 2012). Hair is particularly useful for monitoring metals as the sulfhydryl group of

59 the keratin present in the hair matrix has the ability to bind metal cations (Beernaert et al.,
60 2007). The organism can excrete metals in hair *via* their continuous contact with the
61 bloodstream during growth (Beernaert et al., 2007). Hair analysis can provide reliable
62 information on exposure to non-essential metals (e.g. Cd and Pb) as these are accumulated in
63 storage organs and then be deposited in the hair as well as in the nails, teeth, urine, faeces and
64 sweat (Kales and Christiani, 2005). Unlike bone and stomach content analysis which can
65 provide measures of long and short-term exposure to metals, respectively, hair reflects
66 exposure during the period in which the hair is growing (Kales and Christiani 2005). Hair
67 analysis can indicate metal bioavailability from the environment (McLean et al., 2009; Nolet
68 et al., 1994; Pereira et al., 2004; Marcheselli et al., 2010). Hair has been defined as a
69 biomarker in human studies (Kales and Christiani, 2005) and there is evidence for its utility in
70 mammalian wildlife studies (McLean et al., 2009; Pereira et al., 2006; Beernaert et al., 2007).
71 In wild mammals, positive correlations have been determined between concentrations of
72 metals in hair and blood (Vermeulen et al., 2009) and concentrations in kidneys, liver and
73 muscle (Beernaert et al., 2007; Pereira et al., 2006; D'Havé et al., 2006; Marcheselli et al.,
74 2010).

75 Bats can be particularly exposed to contaminants due to their relatively long life and high
76 food intake (Hickey et al., 2001). In a recent modelling exercise for *Pipistrellus sp*. in
77 England and Wales, we predicted that around 6% of areas where bats reside to have Pb levels
78 that pose a risk to bat health, 3% for Cu, followed by Cd (0.6%) and Zn (0.5%) (Hernout et
79 al., 2013). The exposure risks of soil-associated metals have been predicted to vary across bat
80 species (Hernout et al., 2015). In order to determine the role of metal pollution in bat
81 population declines, monitoring studies combining determination of metal concentrations *via*
82 less-invasive methods and demographic parameters would be useful. It is vital that the assay
83 is sensitive enough such that the sample of hair required is small enough that its collection

84 does not cause lasting harm to the animal, for example, affecting thermoregulation. In bat
85 monitoring programs using radio-tracking approaches, the attachment of a transmitter is
86 usually between the scapulae and the fur in this region (approximately a patch 0.5 x 0.5 mm)
87 is clipped to allow attachment of the transmitter (Womack et al., 2013; Kosonen, 2013).
88 These fur clippings are currently discarded but might be used for further analyses. However,
89 very little is known about the validity of using bat fur as a biomarker of metal exposure,
90 particularly when comparing concentrations determined in fur versus internal organs. A recent
91 study on the utility of bat fur to assess metal exposure using a relative small sample size
92 (Flache et al., 2015). Only one study has correlated Pb concentrations in hair and different
93 tissues of fruit bats (*Pteropus species*), but this was also from a restricted number of
94 individuals and for a limited geographic area (Hariono et al., 1993).

95 This research therefore investigated the suitability of bat fur as a non-invasive bioindicator of
96 exposure to non-essential (i.e. Pb and Cd) and essential (i.e. Cu and Zn) metals. The
97 evaluation was done using a dataset on metal contamination for a large number of bat
98 individuals that were obtained from across a gradient of metal pollution across England and
99 Wales.

100 ## 2. Methods and Materials

101 ### *2.1 Sample selection and processing*

102 Adult males (n=193) of the *Pipistrellus pipistrellus/pygmaeus* species were obtained from
103 across England and Wales (including five confirmed specimens of *Pipistrellus pygmaeus*).
104 Metal can be transferred through lactation in females (Streit and Nagel, 1993) and metal (i.e.
105 Cd) accumulation can correlate with the age of organisms (Rudy, 2009), therefore, we
106 focused only on adult males. The individuals were selected from an archive of 3000 bats,
107 collected in 2008, 2009 and 2010, and provided by the Animal Health and Veterinary

Laboratory Agency (AHVLA) (Surrey, England). Bats that were either found dead or died during rehabilitation, were submitted by bat conservation organizations and members of the public, working under license from Natural England where necessary. The bats were not killed for the purposes of this study. Bats were collected as part of the ongoing UK bat *Lyssavirus* surveillance system (McElhinney et al., 2013; Schatz et al., 2013). Bats were identified and after *Lyssavirus* screening, carcasses were kept in 40% formaldehyde solution by the AHVLA, a common preservation method for veterinary and museum samples, until metal concentration analysis was conducted.

To select the bats for analysis, data on metal concentrations in soils from the locations at which the 3,000 bats were found were acquired from the National Soil Resources Institute (NSRI) soil dataset (5 x 5 km resolution). The subsample of 193 bats was then selected to reflect the frequency distribution of soil metal concentrations across England and Wales (from the NSRI dataset). We first selected the samples to obtain similar frequency distribution from the soil concentrations of the locations in which bat were collected and the soil concentrations across the area of England and Wales, for each metal studied. Next, we purposefully included bats from soils with extreme concentrations of metals in soils to give complete spatial coverage across the area of England and Wales (Figure S1). We then tested for correlations between soil concentrations in the 5 x 5 km grid cell where a bat was collected and the specific tissue concentrations of that individual.

Prior to analysis, individuals were dissected to excise kidneys (n=191), liver (n=191), stomach (and stomach content) (n=168), fur (n=192) and bones (humerus, radius and femurs) (n=192). A small sample of 0.14 (± 0.19) g of fur (wet weight) was shaved using a ceramic scalpel, which corresponds to 8.6×10^{-3} ($\pm 5.5 \times 10^{-3}$) g of fur sample in constant dry weight. Fur samples were rinsed with Millipore water to avoid exogenous sources of contamination from our samples. The tissues were then dried until constant dry weight prior to extraction

and analysis. Tissues in a bad conservation state or missing (previously extracted) have not been analyzed. An aliquot of formaldehyde (0.5 ml) (n=100) was also taken to quantify any metal that may have leached from the bat body into the preservative (See Supplemental information Text S1).

2.2 Quantification of metal concentrations

Metals (Cd, Pb, Cu and Zn) were selected for analysis since they are the most documented metals in several studies determining concentrations in bat tissues and risks of metals to bats (Carravieri and Scheifler, 2013; Clark and Shore, 2001; Hernout et al., 2013; Hernout et al., 2015). Prior to analyses by ICP-MS (Agilent 7500ce, Cheshire, UK), dried samples were digested on a hot block at 100°C for 1 hour in 1 ml of nitric acid, followed by another hour at 100°C following addition of 0.2 ml of hydrogen peroxide. Digests were made up to a fixed volume of 10 ml with Millipore water to obtain a final digest containing 10% acid. Calibration standards were prepared in the same acid matrix.

A constant amount of the internal standards (rhodium and indium) was added to all tubes. Quantification was performed by internal standardization where the analyte signals and the internal standard signals were compared. Then the calibration curve was used to convert the analyte signal into concentration values. This method determines accurate concentrations and corrects for drift (changes in sensitivity over time) and matrix effects (sample-related changes in sensitivity). Standard quality insurance procedures were employed including analysis of method blanks, spiked samples and certified reference materials (bovine liver BCR 185R and spinach NCS ZC73013). (See Supplemental information Text S2).

2.3 Data analyses

Metal concentrations in bat tissue and soil (for Cd, Cu, Pb and Zn) were expressed as dry weight concentrations. These were not normally distributed (Shapiro-Wilk test: $p<0.001$)

157 (Table S1). Due to the variation in sample size between analyses, the detection limit (DL) was
158 calculated for each tissue type and metal. For statistical analyses, metal concentrations below
159 detection limit were replaced by an estimated value using the log-probit regression method
160 (Helsel 1990, Sinha et al. 2006), implemented by the US EPA in the software ProUCL 5.0.00
161 (Singh and Maichle, 2013). Around 31%, 0.1%, 6% and 7% of the data were below DL for
162 Cd, Cu, Pb and Zn, respectively. Tissue and soil concentrations of metal were ln-transformed
163 prior to analysis. To determine the strengths of the associations between metal concentrations
164 contained in fur versus the other tissues, and fur versus soil concentrations, we used Pearson
165 correlation tests. As multiple correlation tests were applied, the p-values were adjusted using
166 the Holm-Bonferroni method. The number of pairs (n) across the associations was not equal
167 since tissues in a poor conservation state or missing (previously extracted) have not been
168 analyzed. The results are presented in the Table 1, the strongest relationships ($r > 0.40$) were
169 selected for representation (Figure 1), and the other relationships ($r < 0.40$) are presented in
170 Figure S2. Further details on the statistical results can be found in Table S1 (Supplemental
171 information). Data analyses were performed with the software R version 2.12.1.
172

173 ## 3. Results and discussion

174 We observed significant positive relationships between concentrations of metals in fur and
175 concentrations in the other internal tissues studied (stomach, kidneys, liver and bones) (Table
176 1, Figure 1, and Figure S2). The exceptions were observed for Zn and liver, for which the
177 relationship was negative and non-significant, and Zn and kidneys, and Pb and liver for which
178 the relationships were non-significant (Table 1, Figure S2). Similar results have been
179 observed for several other mammal species. For example, positive relationships between fur
180 and kidneys, liver and bones Pb concentrations were measured in fruit bats (Hariono et al.,
181 1993). Nolet et al. (1994) showed positive relationships between metal concentrations in

beaver hair and the bark of Salicaceae, which is their main food item. Positive correlations between concentrations in hair have been found with concentrations in kidneys and liver for Pb and Cd on the wood mice (*Apodemus sylvaticus*) (Tête et al., 2014; Beernaert et al., 2007) and for Pb and Cu in the European hedgehog (*Erinaceus europaeus*) (D'Havé et al., 2006). We found stronger positive correlations for non-essential than essential metals (Table 1, Figure 1, Figure S2), which can be due to their regulation by homeostatic mechanisms (D'Havé et al., 2006) and can suggest an effective regulation process of essential metals for bats, as described for mammals (McLean et al., 2009).

Bones, kidneys and liver are characterized as biomarkers of long-term and chronic exposure of contaminants for small mammals (Cooke 2011; Ma 2011). Our results indicate strong correlations between concentrations in fur and stomach and bones for Cd, and between concentrations in fur and stomach and bones for Pb (Table 1). Stronger positive associations were found between fur and bones than between fur and kidneys or fur and liver concentrations, for Cd and Pb (Table 1, Figure 1, Figure S2). These results are similar to the findings of Hariono et al. (1993) who worked with fruit bats. Kidneys and liver have a great potential to accumulate metals and are therefore, widely used as bioindicators to determined pollution level (Cooke 2011; Ma 2011). In contrast to bones, kidneys and liver can regulate their concentrations via level of metallothionein production and have a detoxification capacity (Shore and Douben, 1994; Ma and Talmage, 2001). In addition, renal concentrations of Pb have shown to reach a steady state in mammals in the later subadult stage of their development (Ma 2011), as shown in life-time exposed shrews (*Sorex araneus*) (Ma and van der Voet, 1993). In contrast to kidneys and liver, non-essential metals that bind to bone matrix are not readily accessible into the bloodstream (Ma, 2011). For example, the half time of Pb is around 10-30 years in bones and it is measured in months for soft tissues (Ma, 2011; Kales and Christiani, 2005). In addition, excessive Zn may be stored in bones and/or interacts with

other elements as suggested in Sanchez-Chardi and Lopez-Fuster (2009). Thus, concentrations in bones indicate a long-term and chronic metal exposure, particularly for non-essential metals (Cd and Pb). Our positive relationships between concentrations in bones, kidneys and liver and fur suggest that bat fur samples can be used as a biomarker of chronic metal exposure for bats, particularly for non-essential metals.

Our positive relationships between ingested food (stomach and stomach content) and hair contamination (Table 1, Figure 1) indicate that concentrations in fur can be used as a biomarker of recent sources of metal exposure, measured on a large number of individuals. The strong positive relationship between metal concentrations, particularly for Pb, followed by Cd, Cu and Zn, in fur and stomach contents can confirm the assumption that food is an exposure route of metal contamination for bats. For humans, consumption of animal-derived foodstuffs such as beef, among several other factors (i.e. drinking water, smoking habits, alcohol conditions and health condition) has also been shown to be the main exposure pathway for metal exposure based on the analysis of human hair (Pereira et al., 2004).

Non-significant positive correlations were observed between Cd concentrations in fur and in the soil of the area where the bat was collected (Table 1, Figure S2). A study on marsupial insectivores and rodents has shown good positive correlations between non-essential metal (Cd and Pb) concentrations in hair and soil sampling in the trapping areas, but not for essential metals (McLean et al., 2009). The strengths of the positive relationships were higher than in our study. This difference could be due to the high mobility and the large home range (which can reach 50 hectares) of *Pipistrellus pipistrellus* (Joint Nature Conservation Committee 2007) compared to the mammalian species studied in McLean et al. (2009). Concentrations in soils may not accurately reflect the environmental exposure and bioavailability of metals for bats, as many environmental and biological factors are playing a role in the exposure of contaminants through the food chain, such as: uptake from media (i.e.

232 soils) into prey items; the environmental factors affecting this uptake (e.g. pH, organic matter
233 content); the oral exposure of the target species; the bioavailability and bioaccessibility of
234 food items into predators; the foraging behavior, age and reproductive stage of the target
235 species, etc. (Hernout et al., 2011). We would advocate that fur is a biomarker of the
236 bioavaible fraction of metal, and a poor indicator of the total metal pollution in media, such as
237 soil. Further spatial analysis could integrate habitat preferences as a potential factor
238 influencing metal bioaccumulation in tissues of bats, as seen in other small terrestrial mammal
239 species (Fritsch et al., 2010).
240 Despite the fact that good positive correlations were obtained between concentrations of
241 metals in fur and several tissues, the correlations were not perfect. The variability might be
242 explained by a range of variables such as age, diet and moulting which can impact the levels
243 of metals contained in fur or hair. For example, Cd accumulation has been associated with age
244 in small mammals (Goyer, 1996; cited by Ma and Talmage, 2001) We did not know the exact
245 age of the individuals, which can reach up to 16 years for *Pipistrellus pipistrellus* (observed
246 maximum age) (Dietz et al., 2009). In our study, only adult males were analyzed, probably
247 reducing the variability in measured concentrations. Radio tracking, as well as OMICS (e.g.
248 genomics, proteomics or metabolomics) and stable isotope studies, combined with hair
249 analyses could provide better information on the foraging habitats and diet intake of bats. As
250 hair bioaccumulates metal during the growing phase, moulting effects could be an important
251 variable. Depending on the moulting stage, higher (before the moult) or lower (after the
252 moult) concentrations could be measured in small mammals (Beernaert et al., 2007; Fraser et
253 al., 2013). For adult bats, the moult occurs once a year, generally in the late summer (summer-
254 fall) (Dietz, 2009). However, moulting cycles can be variable among species, sex and age
255 classes (Fraser, 2013). As it was not possible to identify the month of death of the individual,
256 but only the year, we could not assess the impact of moulting stage on hair metal content.

257 Further studies could investigate the relationships between contaminants concentrations in fur
258 and other tissues, considering detailed individual factors, such as: aging, moulting phase,
259 gender, habitats and diet, as well as inter-species factors. These information could refine our
260 knowledge on the use of fur as a biomarker and provide the optimal conditions to biomonitor
261 bats for exogenous contamination.
262 Formaldehyde is a widely used fixative solution for Museum's collection, as we have used in
263 our study. The potential leaching of metals (Cu and Cd) has been illustrated in invertebrate,
264 fish and mammals tissues (Gellein et al., 2008; Hendrickx et al., 2003; Quan et al., 2002;).
265 Formalin may oxidize to formic acid and lower the pH of the solution, resulting in an
266 extraction of metals from the biological samples into the preservative medium (Simmons,
267 2014). Whereas the potential extraction of metals into the preservative solution may occur,
268 others studies have presented contradictory results regarding the potential effects of the
269 solution. These studies have not presented differences between the concentrations of metals in
270 samples preserved in formaldehyde versus in fresh or frozen samples (of bovine liver and
271 human tissues) (Bischoff et al., 2008; Bush et al., 2012; Theron et al., 1974). Others studies
272 have presented higher concentrations of Cd, Cu, Pb and Zn in samples preserved in
273 formaldehyde compared to frozen specimens (in arthropod and fish tissues) (Gibbs et al.,
274 1974; Hendrickx et al., 2003). The potential interpretation of these results was the dissolution
275 of tissues stored in formaldehyde resulting as a decrease of the dry weight of the samples, and
276 thus, an increase of metal concentrations was determined in the invertebrate tissues
277 (Hendrickx et al., 2003). Further studies are needed on the effects of the preservative solution
278 on chemical analysis, and caution may be taken while interpreting the results. Therefore,
279 when possible, fresh or frozen samples are preferred for metal analysis.

280 Another methodological bias could be involved by the potential external contamination in our
281 fur samples. Although, the external contamination of metal could have been washed while

stored in the preservative solution, which could have the same effect than a washing using ethanol or acetone solution as used in Little et al., 2015 and Nam et al., 2012. Further studies should investigate the efficiency of the different washing solutions for metal analysis using fur and hair samples. A possible external contamination may have occurred, and therefore, overestimation of our concentrations of metals in fur samples cannot be excluded.

In conclusion, our data suggest that fur is suitable for monitoring long-term, chronic as well as recent exposure, particularly for non-essentials metals (Pb and Cd). Fur was an optimal biomarker for Pb, based on our strong positive relationships of concentrations determined with fur and stomach and bone samples. The use of fur is also strongly recommended to monitor potential accumulation in kidneys (for Pb, Cd and Cu), bones (for Cd and Zn), and stomach for (Cd, Cu and Zn). Despite inter-individual variations, potential underestimation (leaching in the preservative solution), and/or overestimation (external contamination for the fur samples) of our concentrations of metals due to methodological bias, we believe that the results presented here demonstrated that fur analysis could be a useful indicator for understanding the exposure of bat populations to metals. This approach could provide a valuable tool in further developing our understanding of the importance of metals as a driver for some of the observed declines in bat populations seen around the globe.

Acknowledgements

Thanks to the Animal Health and Veterinary Laboratory Agency and the National Museum Liverpool for supplying the bat individuals. The UK bat lyssavirus surveillance program is funded by Defra (grant SV3500). S. Pietravalle and J. Aegerter for their helpful advice, the Environment Risk Assessment Team and the Heavy Metals in Food Team at FERA, Sand Hutton. This research has been financially supported by the European Union under the 7th Framework Programme (project acronym CREAM, contract number PITN-GA-2009-238148). KEA was funded by a Royal Society University Research Fellowship.

Ethical approval: The authors declare that they have no conflict of interest. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. This article does not contain any studies with human participants performed by any of the authors.

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461

462 **Table and figure legend**

463

464 **Table 1:** Relationships between bat fur and internal organs (kidneys, liver, stomach and
465 stomach content, bones) and soil concentrations. Pearson's coefficients (r) were used to
466 evaluate the strength of these associations. As multiple correlation tests were applied, the p
467 values were adjusted using the Holm-Bonferroni method. Asterisks (*) indicates significant
468 correlation ($p<0.05$); (ns) indicates a non-significant correlation; and (n) indicates the sample
469 size.

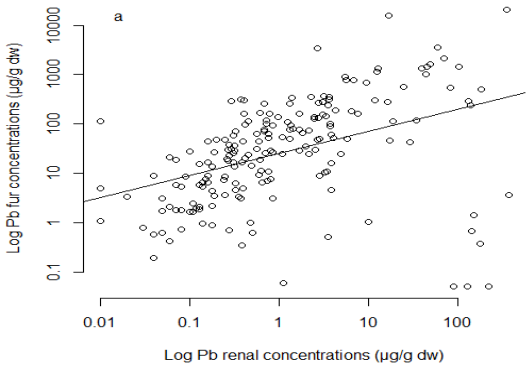
470

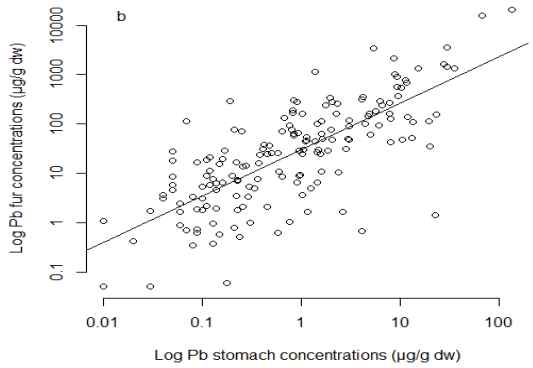
471 **Figure 1:** Relationships between log-transformed metal concentrations in bat fur and kidneys
472 (Pb), stomach and stomach content (Pb, Cd and Cu) and bones (Pb and Cd) (µg/g dw). The
473 trend line indicates the linear regression.

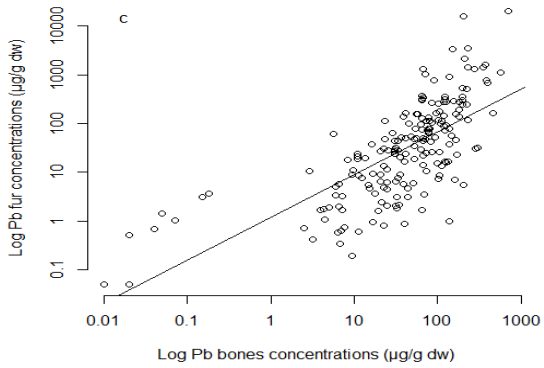
474 **Table 1**

Metal	Kidneys (n=191)	Liver (n=190)	Stomach and stomach content (n=167)	Bones (n=191)	Soil (n=192)
Cd	0.37*	0.23*	0.54*	0.53*	0.10 (ns)
Cu	0.39*	0.24 *	0.41 *	0.25*	0.09 (ns)
Pb	0.42*	0.10 (ns)	0.75*	0.72*	0.04 (ns)
Zn	0.19 (ns)	-0.07 (ns)	0.39*	0.36*	0.04 (ns)

475


a
Log Pb fur concentrations (µg/g dw)
0.1
1
10
100
1000
10000
0.01
0.1
1
10
100
Log Pb renal concentrations (µg/g dw)


b
Log Pb fur concentrations (µg/g dw)
0.1
1
10
100
1000
10000
0.01
0.1
1
10
100
Log Pb stomach concentrations (µg/g dw)


c
Log Pb fur concentrations (µg/g dw)
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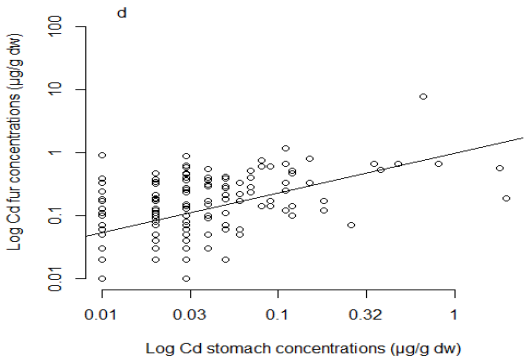
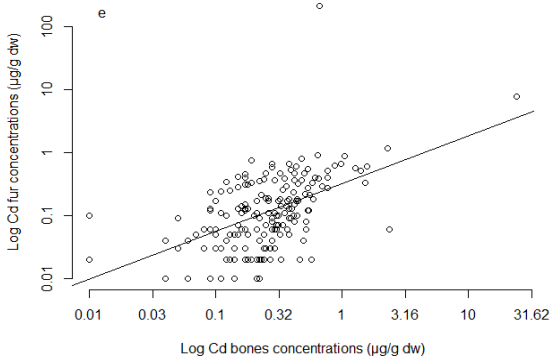
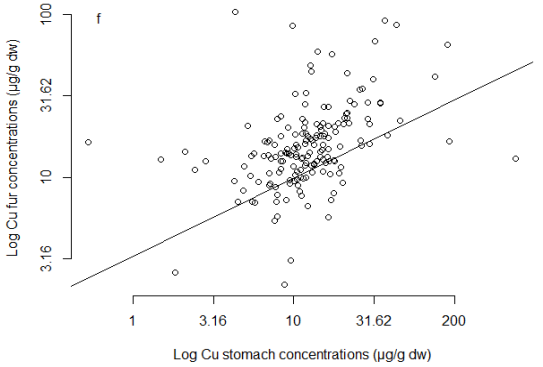
1
10
100
1000
10000
0.01
0.1
1
10
100
1000
Log Pb bones concentrations (µg/g dw)


d
Log Cd fur concentrations (µg/g dw)
0.01
0.1
1
10
100
0.01
0.03
0.1
0.32
1
Log Cd stomach concentrations (µg/g dw)


e
Log Cd fur concentrations (µg/g dw)
0.01
0.1
1
10
100
0.01
0.03
0.1
0.32
1
3.16
10
31.62
Log Cd bones concentrations (µg/g dw)


f
Log Cu fur concentrations (µg/g dw)
3.16
10
31.62
100
1
3.16
10
31.62
200
Log Cu stomach concentrations (µg/g dw)

Supplemental information

Text S1. Formaldehyde aliquot analyzes

Median trace metal concentrations determined in the preservative solution (40% formaldehyde) were: 0.43; 630.43; 21.60 and 351.07 µL/L for Cd, Cu, Pb and Zn, respectively (n= 100 aliquots of formaldehyde from 100 different bat individuals). It may therefore be the case that certain concentrations were underestimated, although a correction based on a quantitative value cannot be defined.

Text S2. Quality assurance and quality control (QA-QC)

Each analytical batch contained 1 spike, 4 blanks and 2 certified reference materials (bovine liver BCR 185R and spinach NCS ZC73013). Results for the spike sample showed a good recovery, blank concentrations were below detection limits. The reference material results were within the acceptable range for Pb and Zn and had an average variation of 11% (absolute values) of the certified concentrations for all metals.

The average spike recovery were 101, 98, 99 and 99% for Cd, Cu, Pb and Zn, respectively. The median blank results were below detection limits (Mean of minimum LOD being: 0.009, 0.043, 0.015 and 0.603 for Cd, Cu, Pb and Zn, respectively). The reference material results were within the acceptable range for Pb for NCS ZC73013 and Zn for BCR 185R. The average percentage of variation from the certified concentrations were -7, -10, -15 and -0.2 for Cd, Cu, Pb and Zn, respectively for BCR 185R and 22, -6, 2 and 24 for Cd, Cu, Pb and Zn, respectively for NCS ZC73013.

Table S1: Statistical results of the Shapiro (W), Pearson correlation (r) and holm Bonferroni tests, with their p values, applied on our dataset of metal concentrations in the different bat tissues and soil concentrations (NSRI dataset).

Metal	Statistical test	Kidneys	Liver	Stomach	Bones	Soil	Fur
Cd	Shapiro W P value	0.46 < 2.2e-16	0.15 < 2.2e-16	0.27 < 2.2e-16	0.13 < 2.2e-16	0.46 < 2.2e-16	0.05 < 2.2e-16
	Pearson r p value	0.37 4.058e-07	0.23 0.002257	0.54 5.358e-13	0.53 2.887e-14	0.10 0.1846	-
	Bonferroni Adjusted p value	1.2174e-06	4.5140e-03	2.1432e-12	1.4435e-13	1.8460e-01	-
Cu	Shapiro test W P value	0.56 < 2.2e-16	0.63 < 2.2e-16	0.40 < 2.2e-16	0.63 < 2.2e-16	0.40 < 2.2e-16	0.68 < 2.2e-16
	Pearson r p value	0.39 2.066e-08	0.24 0.0009522	0.41 5.346e-08	0.25 0.0004402	0.09 0.2359	-
	Bonferroni Adjusted p value	1.0330e-07	1.9044e-03	2.1384e-07	1.3206e-03	2.3590e-01	-
Pb	Shapiro test W P value	0.35 < 2.2e-16	0.13 < 2.2e-16	0.33 < 2.2e-16	0.73 < 2.2e-16	0.41 < 2.2e-16	0.17 < 2.2e-16
	Pearson r p value	0.42 1.367e-09	0.10 0.1934	0.75 < 2.2e-16	0.72 < 2.2e-16	0.04 0.5742	-
	Bonferroni Adjusted p value	4.101e-09	3.868e-01	1.100e-15	1.100e-15	5.742e-01	-
Zn	Shapiro test W P value	0.55 < 2.2e-16	0.21 < 2.2e-16	0.19 < 2.2e-16	0.88 3.833e-11	0.37 < 2.2e-16	0.66 < 2.2e-16
	Pearson r p value	0.19 0.007064	-0.07 0.346	0.39 2.301e-07	0.36 2.24e-07	0.04 0.5378	-
	Bonferroni Adjusted p value	0.02119200	0.69200000	0.00000112	0.00000112	0.69200000	-

Figure S1: Histograms presenting the distributions of the soil concentrations of metals determined in the whole range of England and Wales (light grey) and the locations where the bat samples have been collected (dark grey). The soil concentrations of metals values were extracted from the NSRI dataset.

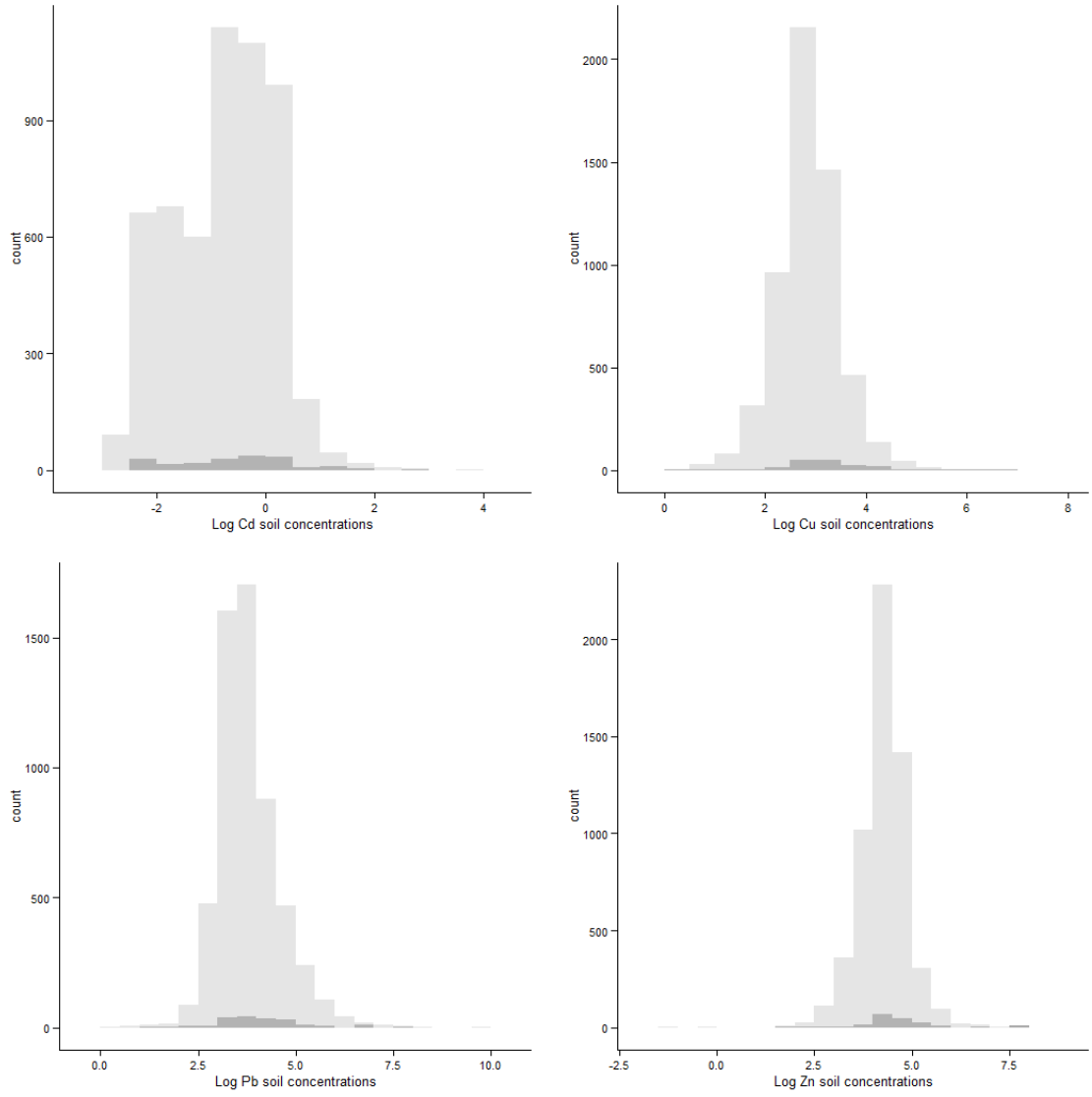
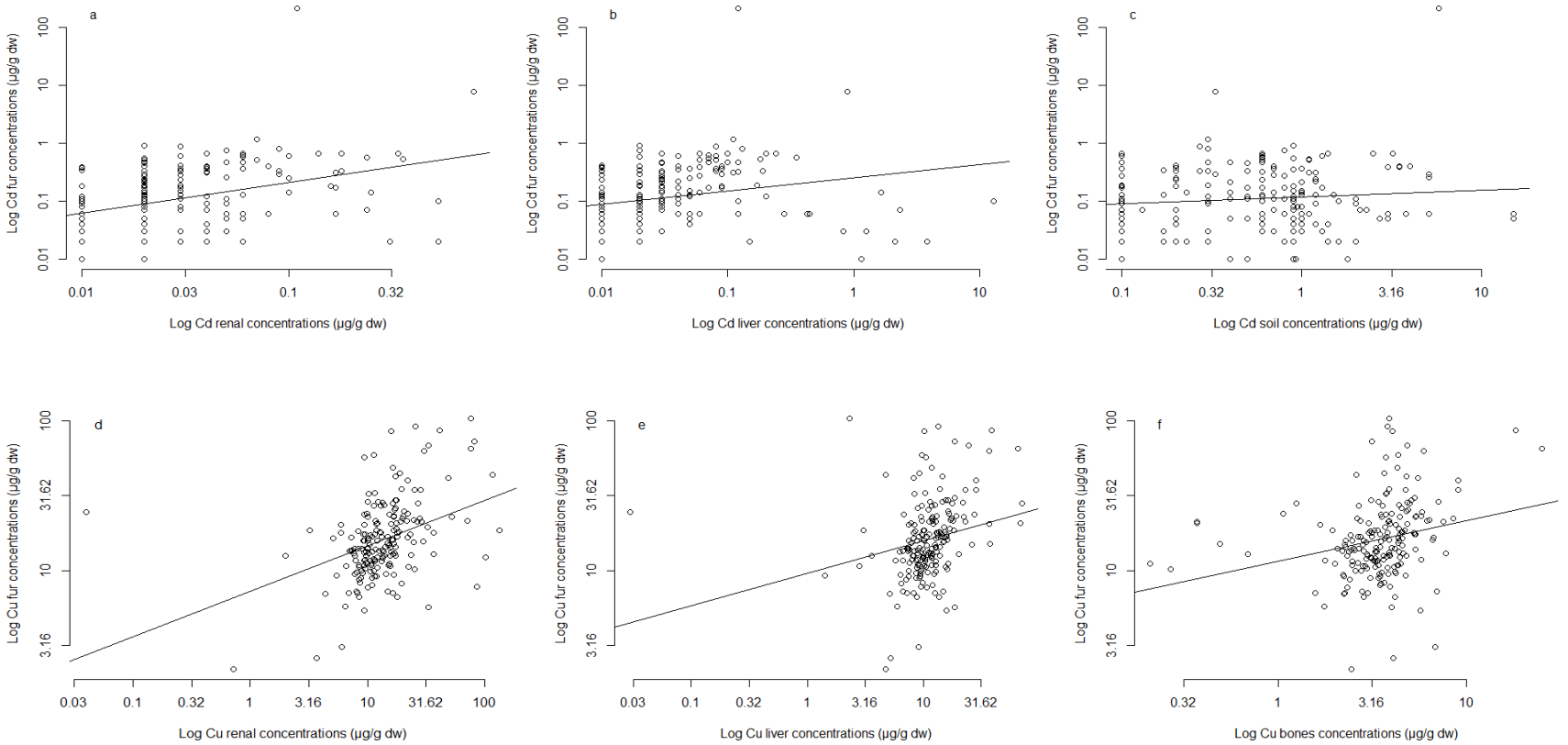


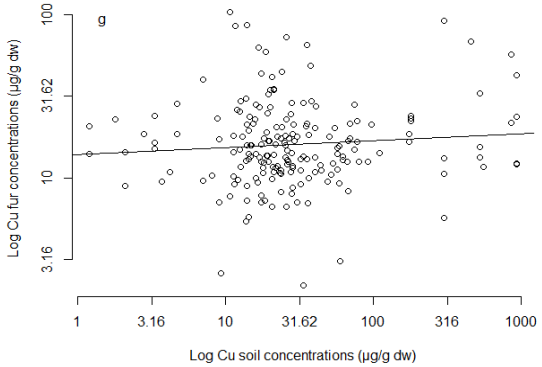
Figure S2: Relationships between log-transformed metal concentrations in bat fur and kidneys (Cd, Cu and Zn), liver (Cd, Cu, Pb and Zn), stomach and stomach content (Zn), bones (Cu and Zn), and soil (Cd, Cu, Pb and Zn) (µg/g dw). The trend line indicates the linear regression.

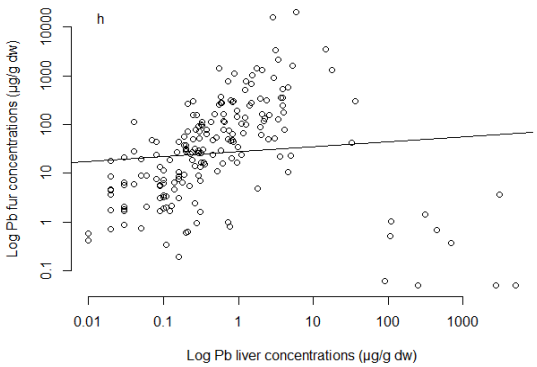
41

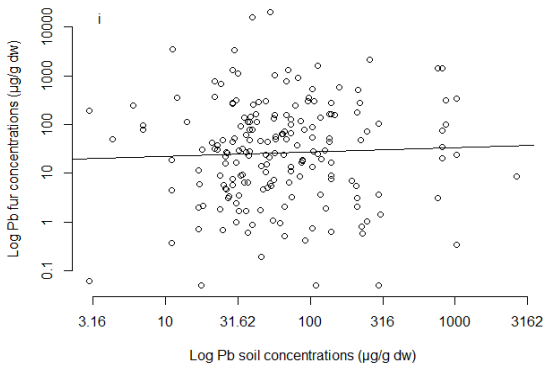

a
Log Cd fur concentrations (µg/g dw)
0.01
0.1
1
10
100
0.01
0.03
0.1
0.32
Log Cd renal concentrations (µg/g dw)
b
Log Cd fur concentrations (µg/g dw)
0.01
0.1
1
10
100
0.01
0.1
1
10
Log Cd liver concentrations (µg/g dw)
c
Log Cd fur concentrations (µg/g dw)
0.01
0.1
1
10
100
0.1
0.32
1
3.16
10
Log Cd soil concentrations (µg/g dw)
d
Log Cu fur concentrations (µg/g dw)
3.16
10
31.62
100
0.03
0.1
0.32
1
3.16
10
31.62
100
Log Cu renal concentrations (µg/g dw)
e
Log Cu fur concentrations (µg/g dw)
3.16
10
31.62
100
0.03
0.1
0.32
1
3.16
10
31.62
Log Cu liver concentrations (µg/g dw)
f
Log Cu fur concentrations (µg/g dw)
3.16
10
31.62
100
0.32
1
3.16
10
Log Cu bones concentrations (µg/g dw)

42

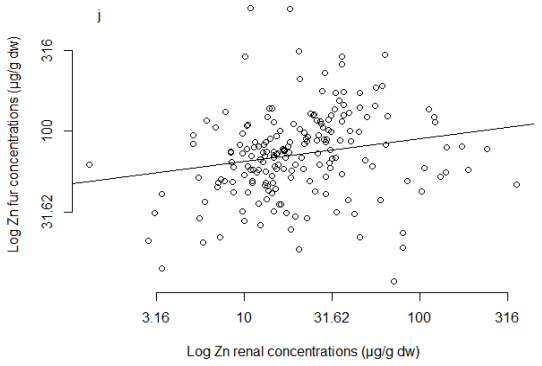
43

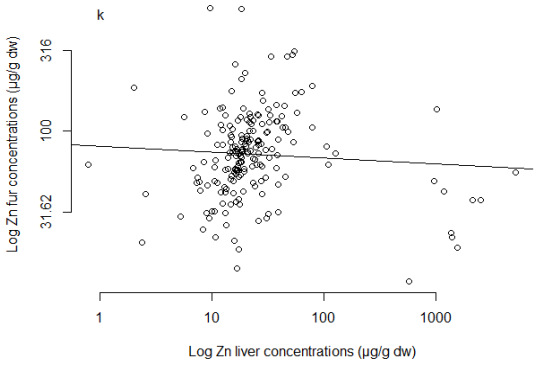

g
Log Cu fur concentrations (µg/g dw)
3.16
10
31.62
100
1
3.16
10
31.62
100
316
1000
Log Cu soil concentrations (µg/g dw)

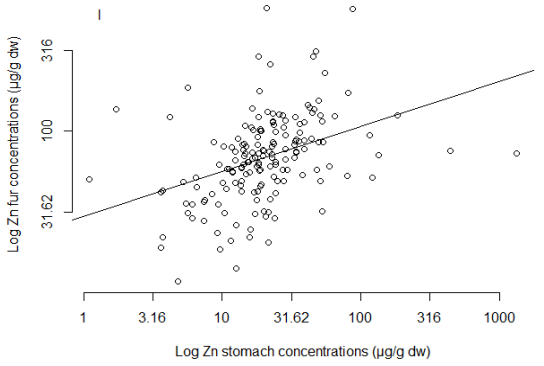

h
Log Pb fur concentrations (µg/g dw)
0.1
1
10
100
1000
10000
0.01
0.1
1
10
100
1000
Log Pb liver concentrations (µg/g dw)


i
Log Pb fur concentrations (µg/g dw)
0.1
1
10
100
1000
10000
3.16
10
31.62
100
316
1000
3162
Log Pb soil concentrations (µg/g dw)

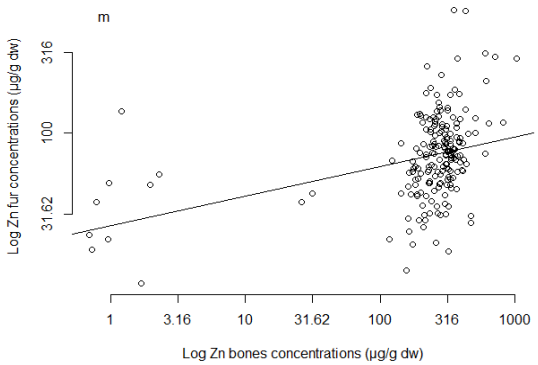
44

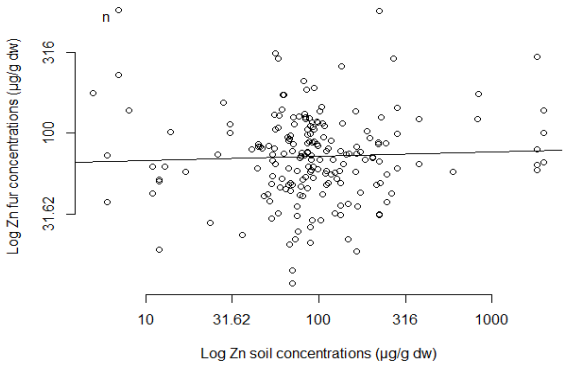

j
Log Zn fur concentrations (µg/g dw)
31.62
100
316
3.16
10
31.62
100
316
Log Zn renal concentrations (µg/g dw)


k
Log Zn fur concentrations (µg/g dw)
31.62
100
316
1
10
100
1000
Log Zn liver concentrations (µg/g dw)


l
Log Zn fur concentrations (µg/g dw)
31.62
100
316
1
3.16
10
31.62
100
316
1000
Log Zn stomach concentrations (µg/g dw)

45


m
Log Zn fur concentrations (µg/g dw)
316
100
31.62
1
3.16
10
31.62
100
316
1000
Log Zn bones concentrations (µg/g dw)


n
Log Zn fur concentrations (µg/g dw)
316
100
31.62
10
31.62
100
316
1000
Log Zn soil concentrations (µg/g dw)

46

47