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

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

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

34

### 35 **Abstract**

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

### 48 **1. Introduction**

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

56 Hair sampling is highly suited as a non-invasive sampling technique as hair is easily  
57 accessible, stable, and its storage does not require restricted conditions (Appenzeller and  
58 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

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

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

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

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

## 137 ***2.2 Quantification of metal concentrations***

138 Metals (Cd, Pb, Cu and Zn) were selected for analysis since they are the most documented  
139 metals in several studies determining concentrations in bat tissues and risks of metals to bats  
140 (Carravieri and Scheifler, 2013; Clark and Shore, 2001; Hernout et al., 2013; Hernout et al.,  
141 2015). Prior to analyses by ICP-MS (Agilent 7500ce, Cheshire, UK), dried samples were  
142 digested on a hot block at 100°C for 1 hour in 1 ml of nitric acid, followed by another hour at  
143 100°C following addition of 0.2 ml of hydrogen peroxide. Digests were made up to a fixed  
144 volume of 10 ml with Millipore water to obtain a final digest containing 10% acid.  
145 Calibration standards were prepared in the same acid matrix.

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

## 154 ***2.3 Data analyses***

155 Metal concentrations in bat tissue and soil (for Cd, Cu, Pb and Zn) were expressed as dry  
156 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



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

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

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

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

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

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

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

299

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309 international, national, and/or institutional guidelines for the care and use of animals were  
310 followed. All procedures performed in studies involving animals were in accordance with the  
311 ethical standards of the institution or practice at which the studies were conducted. This article  
312 does not contain any studies with human participants performed by any of the authors.

313

314 **References**

- 315 Appenzeller BMR, Tsatsakis AM (2012) Hair analysis for biomonitoring of environmental  
316 and occupational exposure to organic pollutants: State of the art, critical review and future  
317 needs. *Toxicol Lett* 210: 119-140. Doi: 10.1016/j.toxlet.2011.10.021
- 318 Beernaert J, Scheirs J, Leirs H, Blust R, Verhagen R (2007) Non-destructive pollution  
319 exposure assessment by means of wood mice hair. *Environ Pollut* 145: 443-451. Doi:  
320 10.1016/j.envpol.2006.04.025.
- 321 Bischoff K, Lamm C, Erb HN, Hillebrandt JR (2008) The effects of formalin fixation and  
322 tissue embedding of bovine liver on copper, iron, and zinc analysis. *J. Vet. Diagn. Invest.* 20:  
323 220-224. Doi: 10.1177/104063870802000213.
- 324 Bush VJ, Moyer TP, Batts KP, Parisi JE (1995) Essential and toxic elements concentrations in  
325 fresh and formalin-fixed human autopsy tissues. *Clin Chem* 41(2) : 284-94.
- 326 Carravieiri A, Scheifler R (2013) Effets des substances chimiques sur les chiroptères:  
327 synthèse bibliographique. *Le Rhinolophe* 19: 1-46.
- 328 Clark DR, Shore RF (2001) Chiroptera. In: Shore RF, Rattner BA, (ed) *Ecotoxicology of wild*  
329 *mammals*, John Wiley & Sons, New York, pp 159-215.
- 330 Cooke JA (2011) Cadmium in small mammals. In: Beyer W, Heinz G, Redmon-Norwood A  
331 (ed) *Environmental contaminants in wildlife interpreting tissue concentrations*. CRC Press  
332 Inc, London, pp 627-642.
- 333 D'Havé H, Scheirs J, Mubiana VK, Verhagen R, Blust R, De Coen W (2006) Non-destructive  
334 pollution exposure assessment in the European hedgehog (*Erinaceus europaeus*): II. Hair and  
335 spines as indicators of endogenous metal and As concentrations. *Environ Pollut* 142: 438-448.  
336 Doi: 10.1016/j.envpol.2005.10.021.
- 337 Dauwe T, Bervoets L, Blust R, Pinxten R, Eens M (2000) Can excrement and feathers of  
338 nestling songbirds be used as biomonitors for heavy metal pollution? *Arch Environ Con Tox*  
339 39: 541-546.
- 340 Dietz C, Von Helversen O, Nill D (2009) *Bats of Britain, Europe and Northwest Africa*. A&C  
341 Black Publishers, London.
- 342 Flache L, Becker N I, Kierdorf U, Czarnecki S, Düring R-A, Encarnaçao JA (2015) Hair  
343 samples as monitoring units for assessing metal exposure of bats: a new tool for risk  
344 assessment. *Mamm Biology* 80(3):178-181. Doi: 10.1016/j.mambio.2015.01.007.
- 345 Fraser EE, Longstaffe FJ, Fenton MB (2013) Moulting matters: the importance of  
346 understanding moulting cycles in bats when using fur for endogenous marker analysis. *Can. J.*  
347 *Zool* 91(8): 533-544. Doi: 10.1139/cjz-2013-0072.
- 348 Fritsch C, Cosson RP, Coeurdassier M, Raoul F, Giraudoux P, Crini N, de Vaufleury A,  
349 Scheifler R (2010) Responses of wild small mammals to a pollution gradient: host factors

350 influence metal and metallothionein levels. Environ. Pollut 158: 827-840. Doi:  
351 10.1016/j.envpol.2009.09.027.  
352  
353 Gellein K, Flaten TP, Erikson KM, Aschner M, Syversen T (2008) Leaching of trace elements  
354 from biological tissue by formalin fixation. Biol Trace Elem Res 121(3): 221-5.  
355  
356 Gibbs RH, Jarosewich E, Windom HL (1974) Heavy metal concentrations in museum fish  
357 specimens: effects of preservatives and time. Science 184(4135):475-7. Doi:  
358 10.1126/science.184.4135.475.  
359  
360 Hariono B, Ng J, Sutton RH (1993) Lead concentrations in tissues of fruit bats (*Pteropus sp.*)  
361 in urban and non-urban locations. Wildlife Res 20: 315-320. Doi: 10.1071/WR9930315.  
  
362 Helsel D, R (1990) Less than obvious: statistical treatment of data below the detection limit.  
363 Environ. Sci. Technol 24:1776-1774. Doi: 10.1021/es00082a001.  
  
364 Hendrickx F, Maelfait JP, De Mayera A, Tackc FMG, Verlooc MG (2003) Storage mediums  
365 affect metal concentration in woodlice (Isopoda). Environ Pollut 121: 87-93. Doi:  
366 10.1016/S0269-7491(02)00203-8.  
  
367 Hernout BV, Arnold KE, McClean CJ, Grimm V, Boxall ABA (2011) Predicting the Threats  
368 of Chemicals to Wildlife: What Are the Challenges? Integr Environ Assess Manage 7(3): 499-  
369 506. Doi: 10.1002/ieam.198.  
  
370 Hernout BV, Somerwill KE, Arnold KE, McClean CJ, Boxall ABA (2013) A spatially-based  
371 modeling framework for assessing the risks of soil-associated metals to bats. Environ Pollut  
372 173: 110-116. Doi:10.1016/j.envpol.2012.08.017.  
  
373 Hernout BV, Pietravalle S, Arnold KE, McClean CJ, Aegerter J, Boxall ABA (2015)  
374 Interspecies variation in the risks of metals to bats. Environ Pollut 206: 209-216.  
375 Doi:10.1016/j.envpol.2015.06.016.  
  
376 Hickey MBC, Fenton MB, MacDonald KC, Soulliere C (2001) Trace elements in the fur of  
377 bats (Chiroptera: Vespertilionidae) from Ontario and Quebec, Canada. B Environ Contam  
378 Tox 66: 699-706. Doi: 10.1007/s001280065.  
  
379 Joint Nature Conservation Committee (2007) Second Report by the UK under Article 17 on  
380 the implementation of the Habitats Directive from January 2001 to December 2006.  
381 Peterborough JNCC. www.jncc.gov.uk/article17. Accessed 15 March 2014.  
382  
383 Kales SN, Christiani DC (2005) Hair and metal toxicity. In: Tobin DJ (ed) Hair in toxicology:  
384 an important biomonitor, Royal Society of Chemistry, Cambridge, MA, pp 125-158.  
  
385 Kosonen E (2013) On the trail of the northern bat A radio-tracking study of the northern bat  
386 (*Eptesicus nilssonii*) colony in southwestern Finland.  
387 <http://julkaisut.turkuamk.fi/isbn9789522163363.pdf>. Accessed 12 January 2014.  
  
388 Little ME, Burgess NM, Broders HG, Campbell LM (2015) Mercury in Little Brown Bat  
389 (*Myotis lucifugus*) Maternity Colonies and Its Correlation with Freshwater Acidity in Nova  
390 Scotia, Canada. Environ Sci Technol 49(4): 2059-2065. Doi: 10.1021/es5050375.



391 Ma WC (2011) Lead in mammals. In: Beyer W, Heinz G, Redmon-Norwood A (ed)  
392 Environmental contaminants in wildlife interpreting tissue concentrations. CRC Press Inc,  
393 London, pp 595-607.

394 Ma WC, Talmage S (2001) Insectivora. In: Shore RF, Rattner BA (ed) Ecotoxicology of wild  
395 mammals. John Wiley & Sons, New York, pp 123-158.

396 Ma WC, van der Voet H (1993) A risk-assessment model for toxic exposure of small  
397 mammalian carnivores to cadmium in contaminated natural environments. Sci Total Environ  
398 Suppl Pt 2: 1701-1714. Doi: 10.1016/S0048-9697(05)80171-8.

399 Marcheselli M, Sala L, Mauri M (2010) Bioaccumulation of PGEs and other traffic-related  
400 metals in populations of the small mammal *Apodemus sylvaticus*. Chemosphere 80: 1247-  
401 1254. Doi: 10.1016/j.chemosphere.2010.06.070.

402 McElhinney LM, Marston DA, Leech S, Freuling CM, van der Poel WH, Echevarria J,  
403 Vázquez-Moron S, Horton DL, Müller T, Fooks AR (2013) Molecular epidemiology of bat  
404 lyssaviruses in Europe. Zoonoses Public Hlth 60(1): 35-45. Doi: 10.1111/zph.12003.

405 McLean CM, Koller CE, Rodger JC, MacFarlane GR (2009) Mammalian hair as an  
406 accumulative bioindicator of metal bioavailability in Australian terrestrial environments. Sci  
407 Total Environ 407: 3588-3596. Doi:10.1016/j.scitotenv.2009.01.038.

408 Nam DH, Yates D, Ardapple P, Evers DC, Schmerfeld J, Basu N (2012) Elevated mercury  
409 exposure and neurochemical alterations in little brown bats (*Myotis lucifugus*) from a site with  
410 historical mercury contamination. Ecotoxicology 21(4):1094-101. Doi: 10.1007/s10646-012-  
411 0864-9. Nolet BA, Dijkstra VAA, Heidecke D (1994) Cadmium in beavers translocated from  
412 the Elbe to the Rhine/Meuse estuary, and the possible effect on population growth rate. Arch  
413 Environ Con Tox 27: 154-161. Doi: 10.1007/BF00214257.

414 Pereira R, Ribeiro R, Gonçalves F (2004) Scalp hair analysis as a tool in assessing human  
415 exposure to heavy metals (S. Domingos mine, Portugal). Sci Total Environ 327: 81-92. Doi:  
416 10.1016/j.scitotenv.2004.01.017.

417 Pereira R, Pereira ML, Ribeiro R, Gonçalves F (2006) Tissues and hair residues and  
418 hispathology in wild rats (*Rattus rattus* L.) and Algerian mice (*Mus spretus* Lataste) from an  
419 abandoned mine area (Southeast Portugal). Environ Pollut 139: 561-575.  
420 Doi:10.1016/j.envpol.2005.04.038.

421 Quan M, Mülders MS, Meltzer DG (2002) The effect of storage conditions on samples for the  
422 evaluation of copper status in blesbok (*Damaliscus pygargus phillipsi*). J S Afr Vet Assoc  
423 73(3): 111-4. Doi: 10.4102/jsava.v73i3.570.

424 Rudy M (2009) Correlation of lead, cadmium and mercury levels in tissue and liver samples  
425 with age in cattle. Food Addit Contam Part A Chem Anal Control Expo Risk Assess  
426 26(6):847-53. Doi: 10.1080/02652030902835747.

427 Sanchez-Chardi A, Lopez-Fuster MJ (2009) Metal and metalloid accumulation in shrews  
428 (Soricomorpha, Mammalia) from two protected Mediterranean coastal sites. *Environ Pollut*  
429 157: 1243-1248. Doi: 10.1016/j.envpol.2008.11.047.

430 Schatz J, Fooks AR, McElhinney L, Horton D, Echevarria J, Vázquez-Moron S, Kooi EA,  
431 Rasmussen TB, Müller T, Freuling CM (2013) Bat rabies surveillance in Europe. *Zoonoses*  
432 *Public Hlth* 60(1):22-34. Doi: 10.1111/zph.12002.

433 Shore RF, Douben PET (1994) The ecotoxicological significance of cadmium intake and  
434 residues in terrestrial small mammals. *Ecotox Environ Safe* 29: 101-113.  
435 Doi:10.1016/0147-6513(94)90035-3.

436 Simmons JE (2014) *Fluid preservation: a comprehensive reference* (ed) Rowman &  
437 Littlefield, Lanham.

438 Singh A, Maichle R (2013) USA EPA, User Guide ProUCL Version 5.0.00. Statistical  
439 software for environmental applications for data sets with and without nondetect observations.  
440 (EPA/600/R-07/038).

441 Sinha P, Lambert MB, Trumbull VL (2006) Evaluation of statistical methods for left-censored  
442 data with nonuniform detection limits. *Environ Toxicol Chem* 25: 2533-2540. Doi:  
443 10.1897/05-548R.1.

444 Streit B, Nagel A (1993) Heavy metal transfer by lactation in a bat colony. *Fresen Environ*  
445 *Bull* 2: 168-173.

446 Tête N, Afonso E, Crini N, Drouhot S, Prudent A, Scheifler R (2014) Hair as a noninvasive  
447 tool for risk assessment: Do the concentrations of cadmium and lead in the hair of wood mice  
448 (*Apodemus sylvaticus*) reflect internal concentrations? *Ecotox Environ Safe* 108: 233-241.  
449 Doi: 10.1016/j.ecoenv.2014.07.010.

450 Theron PF, Rimmer R, Nicholls HA, Ehret WJ (1974) Causes of variation of copper, iron,  
451 manganese, zinc and magnesium levels in bovine livers. 2. The dispersion of trace metals in  
452 bovine livers and the effects of formalinized storage on mineral concentrations and moisture  
453 content. *J S Afr Vet Assoc* 45(1): 73-6.

454 Vermeulen F, D'Havé H, Mubiana VK, van den Brink NW, Blust R, Bervoets L, De Coen W,  
455 (2009) Relevance of hair and spines of the European hedgehog (*Erinaceus europaeus*) as  
456 biomonitoring tissues for arsenic and metals in relation to blood. *Sci Total Environ* 407: 1775-  
457 1783. Doi: 10.1016/j.scitotenv.2008.10.039.

458 Womack KM, Amelon SK, Thompson III FR (2013) Resource selection by Indiana bats  
459 during the maternity season. *J Wildlife Manage* 77(4): 707-715. Doi: 10.1002/jwmg.498.  
460 ProUCL 5.0.00 User Guide document (EPA/600/R-07/038)  
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.

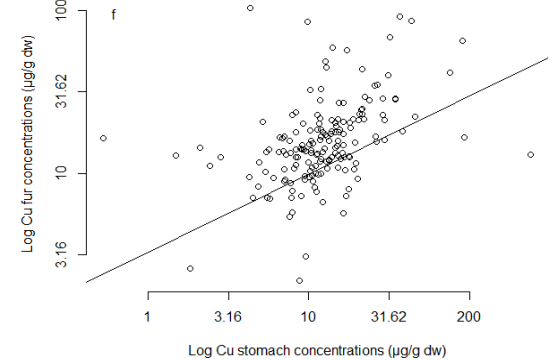
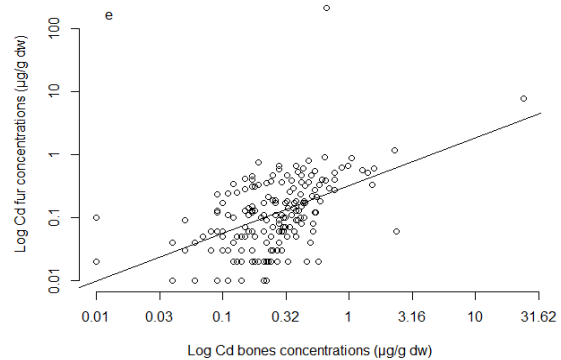
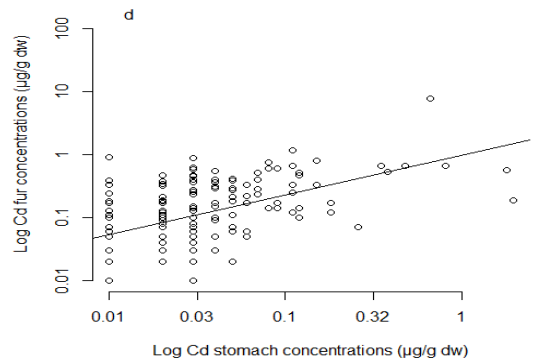
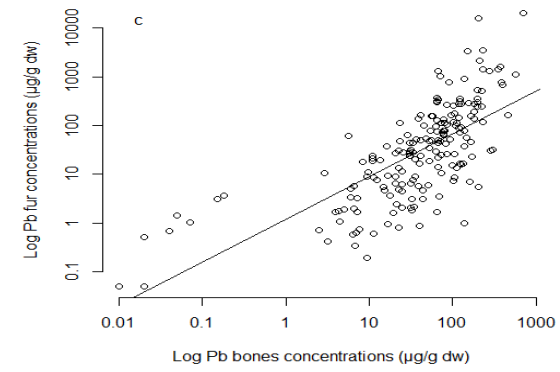
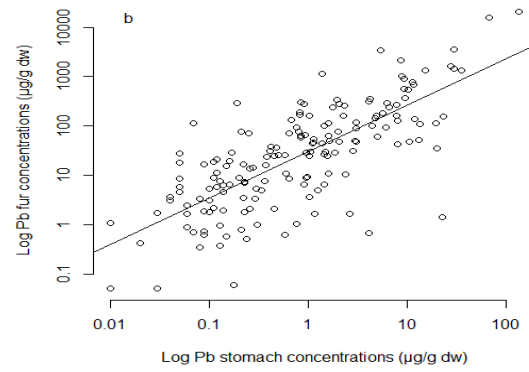
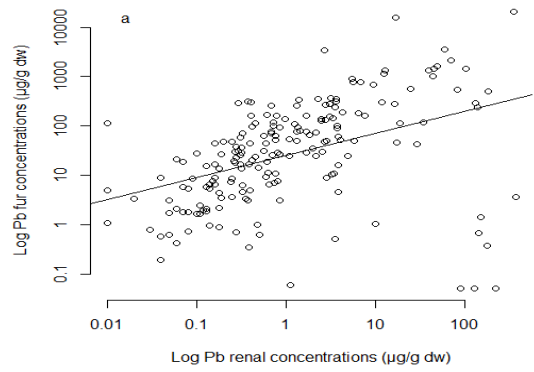
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471 **Figure 1:** Relationships between log-transformed metal concentrations in bat fur and kidneys  
 472 (Pb), stomach and stomach content (Pb, Cd and Cu) and bones (Pb and Cd) (µg/g dw). The  
 473 trend line indicates the linear regression.

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



1 **Supplemental information**

2 ***Text S1. Formaldehyde aliquot analyzes***

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

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

9  
10 Each analytical batch contained 1 spike, 4 blanks and 2 certified reference materials (bovine  
11 liver BCR 185R and spinach NCS ZC73013). Results for the spike sample showed a good  
12 recovery, blank concentrations were below detection limits. The reference material results  
13 were within the acceptable range for Pb and Zn and had an average variation of 11% (absolute  
14 values) of the certified concentrations for all metals.

15 The average spike recovery were 101, 98, 99 and 99% for Cd, Cu, Pb and Zn, respectively.  
16 The median blank results were below detection limits (Mean of minimum LOD being: 0.009,  
17 0.043, 0.015 and 0.603 for Cd, Cu, Pb and Zn, respectively). The reference material results  
18 were within the acceptable range for Pb for NCS ZC73013 and Zn for BCR 185R. The  
19 average percentage of variation from the certified concentrations were -7, -10, -15 and -0.2 for  
20 Cd, Cu, Pb and Zn, respectively for BCR 185R and 22, -6, 2 and 24 for Cd, Cu, Pb and Zn,  
21 respectively for NCS ZC73013.

22

23

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

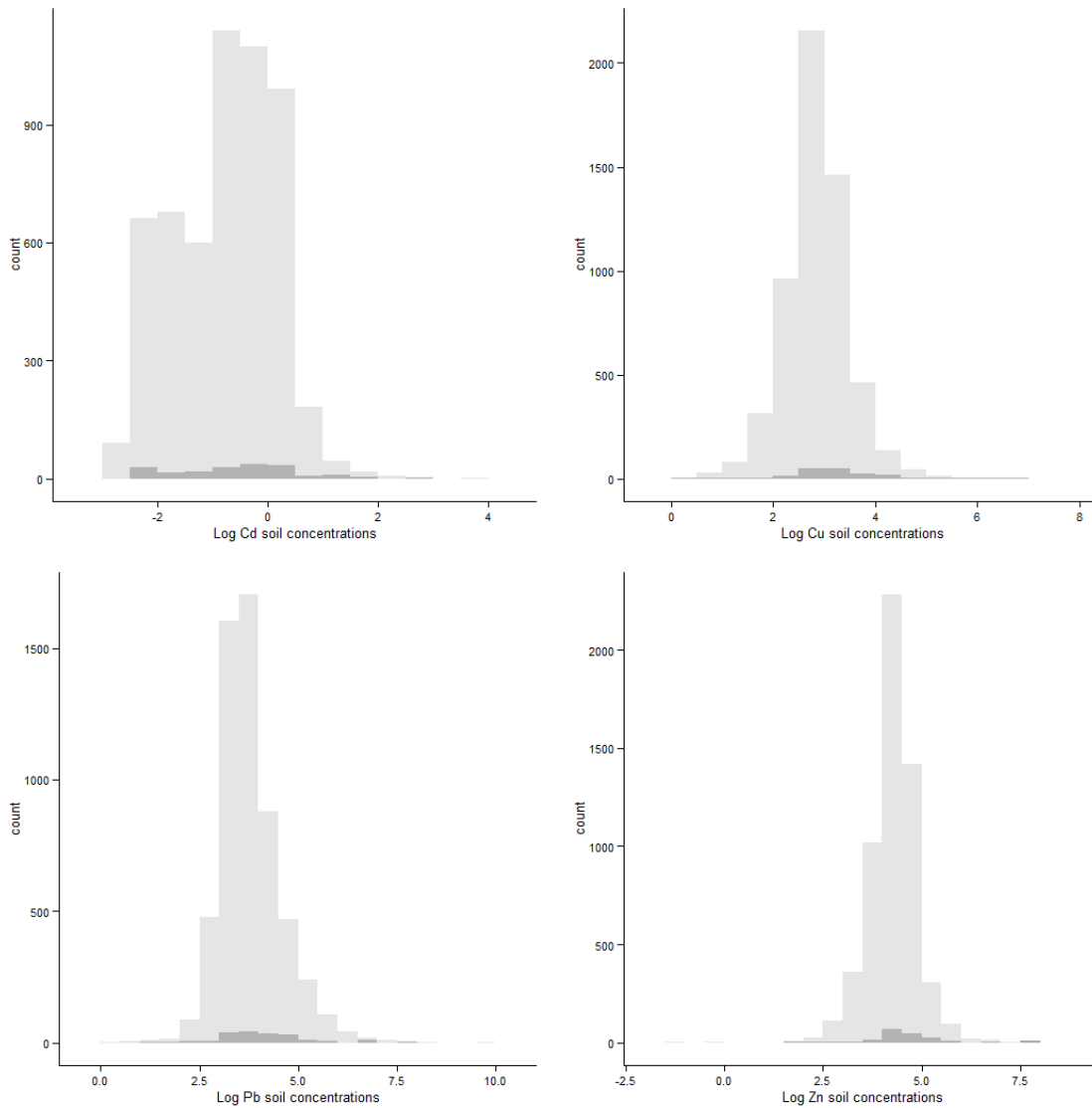
Metal	Statistical test	Kidneys	Liver	Stomach	Bones	Soil	Fur
Cd	Shapiro W P value	0.46 < 2.2e-16	0.15 < 2.2e-16	0.27 < 2.2e-16	0.13 < 2.2e-16	0.46 < 2.2e-16	0.05 < 2.2e-16
	Pearson r p value	0.37 4.058e-07	0.23 0.002257	0.54 5.358e-13	0.53 2.887e-14	0.10 0.1846	-
	Bonferroni Adjusted p value	1.2174e-06	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	-

27

28

29

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



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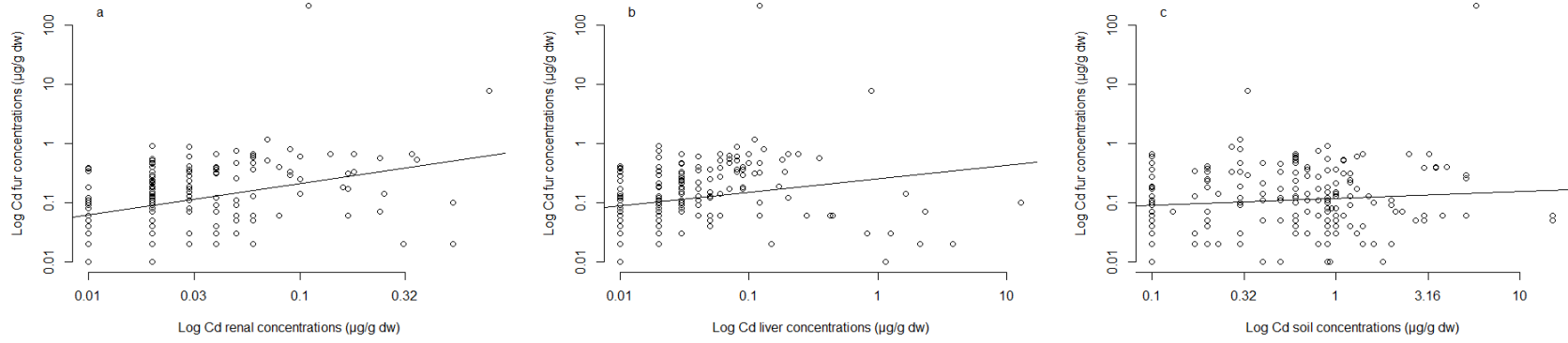
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37 **Figure S2: Relationships between log-transformed metal concentrations in bat fur and**  
 38 **kidneys (Cd, Cu and Zn), liver (Cd, Cu, Pb and Zn), stomach and stomach content (Zn),**  
 39 **bones (Cu and Zn), and soil (Cd, Cu, Pb and Zn) ( $\mu\text{g/g dw}$ ). The trend line indicates the**  
 40 **linear regression.**

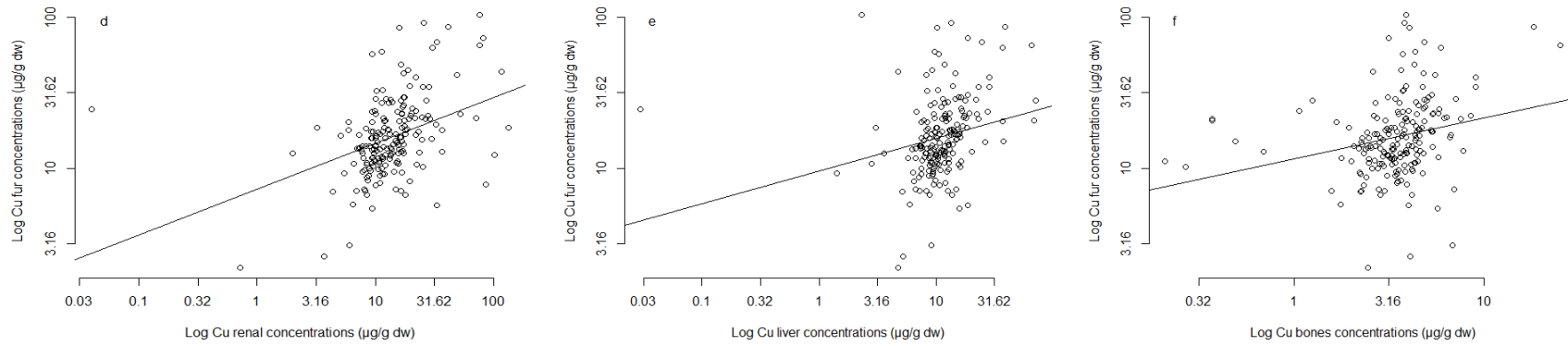




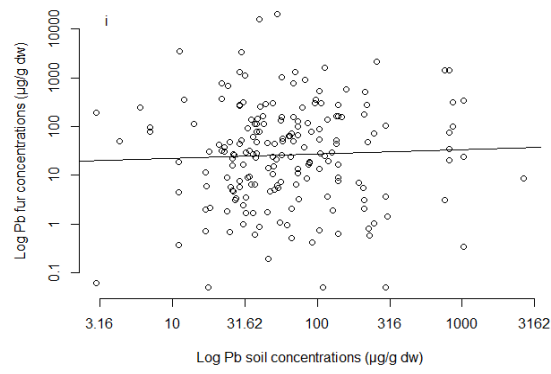
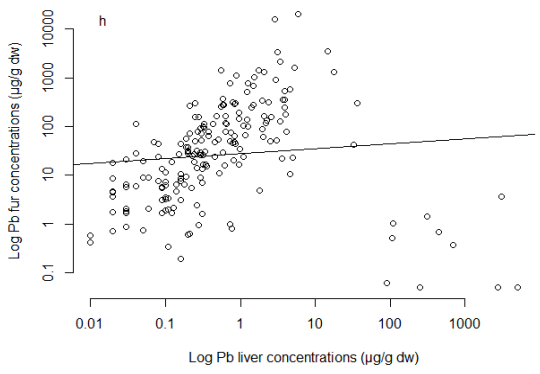
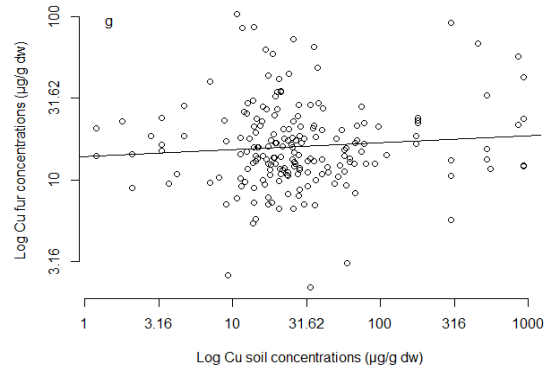
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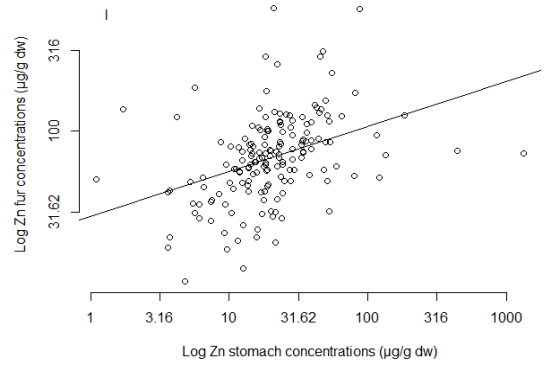
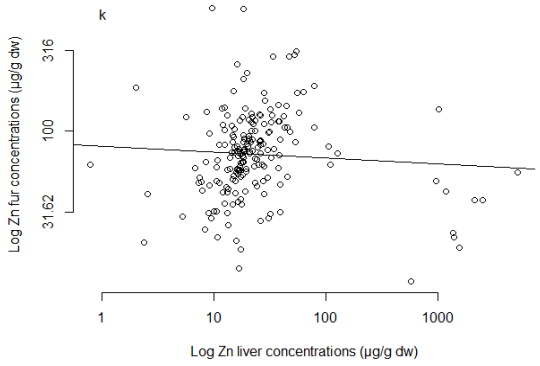
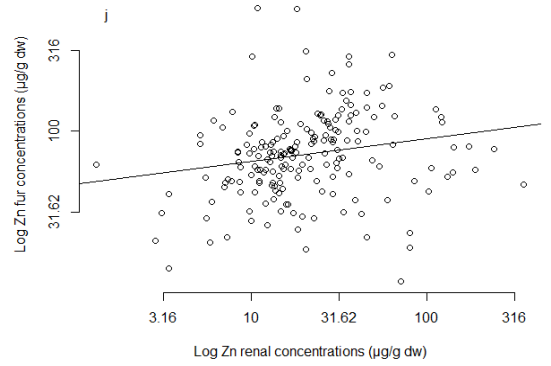
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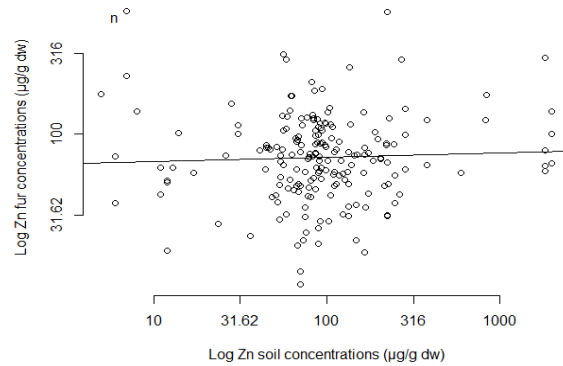
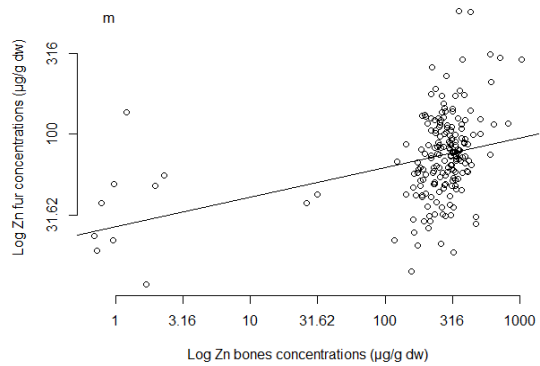


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