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Hernout, Béatrice V., Arnold, Kathryn E. orcid.org/0000-0002-6485-6065, McClean, Colin J. orcid.org/0000-0002-5457-4355 et al. (3 more authors) (2016) A national level assessment of metal contamination in bats. Environmental Pollution. pp. 847-858. ISSN 1873-6424

https://doi.org/10.1016/j.envpol.2016.04.079

A national level assessment of metal contamination in bats

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Key words: trace elements exposure; tissue metal concentrations; metal bioaccumulation; *Pipistrellus sp.* bats; wildlife ecotoxicology

Highlights

- Metal concentrations were determined in tissues from 193 individual bats
- 7-11% of the bats sampled metal concentrations above Pb toxic thresholds
- Pb posed the greatest risk, followed by Cu, Zn and Cd

  Concentrations of metals in different tissues were generally positively associated
- Metal contamination may represent an environmental stressor for bat populations

Abstract

Many populations of bat species across the globe are declining, with chemical contamination one of many potential stressors implicated in these demographic changes. Metals still contaminate a wide range of habitats, but the risks to bats remain poorly understood. This study is the first to present a national scale assessment of toxic metal (Cd, Pb) and essential trace metal (Cu, Zn) concentrations in bats. Metal concentrations in tissues (kidneys, liver, stomach and stomach content, bones and fur) were measured in 193 *Pipistrellus sp*. in England and Wales using ICP-MS, and compared to critical toxic concentrations for small mammals. The concentrations of metals determined in bat tissues were generally lower than those reported elsewhere. Strong positive correlations were found between concentrations in tissues for a given metal (liver and kidneys for Cd, Cu and Pb; stomach and fur and fur and bones for Pb), suggesting recent as well as long term exposure to these contaminants. In addition, positive correlations between concentrations of different metals in the same tissues (Cd and Zn, Cu and Zn, Cd and Pb, Pb and Zn) suggest a co-exposure of metals to bats. Approximately 21% of the bats sampled contained residues of at least one metal at concentrations high enough to elicit toxic effects (associated with kidney damage), or to be above the upper level measured in other mammal species. Pb was found to pose the greatest risk (with 7-11% of the bats containing concentrations of toxicological concern), followed by Cu (4-9%), Zn (0.5-5.2%) and Cd (0%). Our data suggest that a leaching of metals into our storage matrix, formaldehyde, may have occurred, especially for Cu. The overall findings suggest that metal contamination is an environmental stressor affecting bat populations, and that further research is needed into the direct links between metal contamination and bat population declines worldwide.

Capsule

This national survey showed metal concentrations in tissues of 7-11% of bats sampled were above Pb toxic thresholds for small mammals.

Introduction

During the last decades, declines in bat populations (e.g. including species such as *Pipistrellus sp.*, *Rhinolophus hipposideros*, *Rhinolophus ferrumequinum* and *Myotis myotis*) have been observed across Europe and North America (Dietz et al., 2009; Jones et al., 2009; Stebbings, 1988). These population declines might be attributable to several

stressors including changes in resources such as water and food quantity and quality, roost loss, urbanization and agricultural intensification, exposure to chemicals, the increase in wind turbines, the pressure of diseases such as white nose syndrome, and climate change (Frick et al., 2010; Jefferies, 1972; Jones et al., 2009; Walker et al., 2007; Wickramasinghe et al., 2003). Due to their relatively long life (e.g. up to 40 years old) and their high daily food intake (e.g. up to 0.5 g/gbw/d on a wet basis measured experimentally) (Anthony and Kunz, 1977; Podlutsky et al., 2005), bats can be particularly prone to chemical exposure, especially to contaminants such as metals, that accumulate through the food chain (Hickey et al., 2001). The main exposure routes are the ingestion of contaminated food and water, followed by dermal exposure and inhalation (Allinson et al., 2006; Clark and Shore, 2001; Lilley et al., 2012). Exposure to organic chemicals has been associated with declines in a number of bat species in certain regions. For example: the drastic decline of the greater horseshoe bat population (*Rhinolophus ferrumequinum*) in Germany was linked with the use of lindane and DDT in agriculture and forestry (Dietz et al., 2009). While the effects of organic compounds on bats has received some attention, the literature on the potential impact of metals remains scarce, although metal contamination of ecosystems is widespread (Hickey et al., 2001).

Metal emissions increased during the industrial revolution. In England and Wales, a large number of land sites remain contaminated with metals (Environment Agency, 2009). High metal concentrations have also been observed in many other polluted regions of the world, including mainland Europe and North America (Lado et al., 2008; Shacklette and Boerngen, 1984). Soil-associated metals can be accumulated by invertebrates and plants and can then move along the food chain into species, such as insectivorous mammals and birds (Ma and Talmage, 2001; Fritsch et al., 2012). Consequently, it is likely that bats will be exposed to food items contaminated with metals. Laboratory studies show that exposure of bats to metals can elicit a range of effects including tremors, spasms, general slowness, lack of control in body movement, effects at the physiological and histological levels (e.g. oxidative stress, DNA damage, tissue damage including inclusion bodies, neurochemical alterations), and possibly mortality following exposure to lead, cadmium, and zinc (Clark and Shore, 2001; Hariono et al., 1993; Hurley and Fenton, 1980; Nam et al., 2012; Sutton and Wilson, 1983). While non-essential metals, such as Cd and Pb, could be toxic at low concentrations, essential metals, such as copper and Zn, are tightly regulated at constant concentrations in tissues of mammals and, therefore, mostly present within a narrow range. Essential metals can cause negative effects when present at concentrations outside this range, although

their lower limit is less well documented than their upper limit in tissues of small mammals (Clark and Shore, 2001; Ma and Talmage, 2001; Sheffield et al., 2001). Information on levels of exposure for metals are, however, restricted l to studies with limited sample sizes, areas of study, tissue types and/or metals studied (Carravieiri and Scheifler, 2013; Clark and Shore, 2001). Walker et al. (2007) measured metal residues in bat tissues in a small area of England (Devon and Cornwall), and showed that around 5% of the Pipistrelle samples had renal residues high enough to cause acute Pb poisoning (associated with kidney damage). These data suggest that metal exposure could be a potential environmental stressor that may contribute to the observed population declines in bats. Information on exposure at a national and global scales is, however, non-existent.

To quantify the potential impacts of metals on bat populations, we previously developed and applied a spatially explicit modeling framework to predict the potential exposure of the *Pipistrellus sp*. bat (*Pipistrellus pipistrellus* and *Pipistrellus pygmaeus*) to soil-associated metals (Cd, Cu, Pb and Zn) via their diet (Hernout et al., 2013). The results predicted that 5.9% of the distribution in which *Pipistrellus sp.* resides in England and Wales have Pb levels of concern to bat health, followed by Cu with 2.8%, 0.6% for Cd and 0.5% for Zn (Hernout et al., 2013). This modeling framework was recently applied to 14 insectivorous bat species (Hernout et al., 2015). The overall modeling results indicate that metals could indeed be an environmental stressor affecting populations of multiple bat species in England and Wales.

While the modeling work highlights that metal exposure may be an issue, the approach that has been used is purely predictive. It would therefore be invaluable to complement the model predictions with real data on bat exposure to metals across England and wales. Therefore, here we describe the generation and use of a large and unique national-scale dataset on metal concentrations (Cd, Cu, Pb and Zn) in different bat organs and tissues (kidney, liver, stomach, bones and fur) to establish the toxicological pressure of metal contamination in England and Wales on bats. The data are also used to explore correlations between: concentrations of individual metals in different tissues; different metals in the same tissue; concentrations of metals in tissues and soils where the bats were sampled; and to evaluate the potential for leaching of metals into our preservative medium to provide further information on using specimens stored in formaldehyde, a common preservation method for veterinary and museum samples, for metal analysis. The results provide an important contribution towards efforts to understand the current observed declines in bat populations across the globe.

Materials and methods

Sample collection and processing. The common pipistrelle (*P. pipistrellus*) bat is widely distributed across Europe, including the whole of the UK. Adult males of the *P. pipistrellus* (n=190) species and the sibling species *P. pygmaeus* (n=3) were obtained from sites across England and Wales (Figure 1, Figure S1, Table S1). Only males were selected since females can transfer metals through lactation (Streit and Nagel, 1993) and therefore, they have a better ability to eliminate the metals compared to males. Adult individuals were selected to maximize the chance of detecting concentrations (above the limit of detection LOD), since Cd and Pb accumulation can increase with age (e.g. in bones for Pb) (Goyer 1996; Ma and Talmage, 2001; Rudy, 2009; Sheffield et al., 2001).

All the bats used in this study were selected from an archive of 3,000 bats provided by the Animal Health and Veterinary Laboratory Agency (AHVLA, Surrey, England, UK). Bats were collected and submitted by bat conservation organizations and members of the public, working under license from Natural England where necessary, in 2008, 2009 and 2010 as part of ongoing UK bat lyssavirus surveillance conducted by the AHVLA (McElhinney et al., 2013; Schatz et al., 2013). Bats were either found dead or died during rehabilitation, prior to submission. No bats were culled for the purposes of this study. Bats were identified and after lyssavirus screening (for which samples of brain were collected), carcasses were kept in 40% formaldehyde solution (saturated aqueous solution containing up to 40% pure formaldehyde) by the AHVLA. None of the specimens were stored in ethanol. Metal concentration analysis was conducted in 2012.

We selected the bats for analysis to represent the pollution gradient of metals for England and Wales (Figure S2). 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 NRSI soil data used for this study includes two sets of data: the first set corresponded to samples obtained between 1979 and 1987; and the second for samples obtained between 1994 and 2003. The analytical method of extraction was the same for both datasets. The more recent dataset was used in preference to the older data with the older data only used to fill gaps in the more recent dataset (Hernout et al., 2011). 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). The frequency distribution of the soil concentrations of the locations in which bats were collected and the frequency distribution of soil concentrations across the area of

England and Wales were similar for each metal studied (Figure S2). Bats located in areas with extreme concentrations of metals in soils (high as well as low concentrations) were also included to give a complete spatial coverage across the area of England and Wales (Figure S2).

Individuals were dissected to excise kidneys (n=191), liver (n=191), stomach (with stomach content) (n=168), fur (n=192) and bones (humerus, radius and femurs) (n=192). A small sample of fur was used for this study: 0.14 (±0.19) g of fur (wet weight) shaved using a ceramic scalpel (8.6×10^{-3} ($\pm 5.5 \times 10^{-3}$) g in constant dry weight). We selected the area between the scapulae (approximately a patch 0.5 X 0.5 mm) since this region is usually clipped to allow attachment of the transmitter (see further details in Hernout et al., 2016). Tissues in poor conservation state or missing (previously extracted) were not analyzed. The tissues were oven dried until constant dry weight, and analyzed for metals by inductively coupled plasma mass spectrometry (ICP-MS).

Aliquots of the preservative solution: formaldehyde (0.5 ml) were also taken to quantify any metal that may have leached from the bat body into the preservative (n=100 aliquots of formaldehyde, from 100 bat individuals). In addition, aliquots of fresh formaldehyde (2 ml), similar to the solution used to preserve our samples, were analyzed to verify whether trace metals are contained in the initial preservative solution (n=5) (36.5–38% solution, Copr. Sigma, contains 10-15% methanol). Prior to analysis, the aliquots were evaporated in the oven, until constant dry weight.

Sample analyses.

Quantification of metal concentrations. Prior to analyses by ICP-MS (using an Agilent 7500ce, Cheshire, UK), dried samples (including the evaporated formaldehyde aliquots) were digested on a hot block at 100°C for 1 hour in 1 ml of nitric acid (Aristar grade reagent, 69% w/v), followed by another hour at 100°C following addition of 0.2 ml of hydrogen peroxide (35% wt). 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. A 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).

Quality assurance and quality control. 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 with an average of 101%, 98%, 99% and 99% for Cd, Cu, Pb and Zn, respectively. The median blank results were below the LOD (Mean of minimum LOD being: 0.009, 0.043, 0.015 and 0.603 µg/d dw 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 had an average variation of 11% (absolute values) of the certified concentrations for all metals (with -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).

Data analyses. Concentrations of Cd, Cu, Pb and Zn determined in bat tissues were expressed as dry weight concentrations. Due to the variation in sample size, the LOD was calculated for each tissue type and metal. Metal concentrations below the LOD were replaced by an estimated value using the log-probit regression method (Helsel 1990; Sinha et al., 2006), implemented by the US EPA in the software ProUCL 5.0.00 (Singh and Maichle, 2013). Around 31, 0.1, 6 and 7% of the data were below DL for Cd, Cu, Pb and Zn, respectively.

Metal concentrations (Cd, Cu, Pb and Zn) in bat tissues, formaldehyde aliquots, and soil were not normally distributed (Shapiro-Wilk test: $p < 0.05$) (Table S2) and were ln-transformed prior to analysis. Thus, concentrations of each metal in different tissues were compared using ANOVA tests and t- tests. Concentrations of different metals across the same tissues were compared using t-tests. To determine the strengths of the associations between metal concentrations in the different tissues; between metal concentrations contained in the formaldehyde aliquot versus the tissues; and between metal concentrations contained in the soil versus the tissues, we used Pearson correlation tests. Concentrations of metals in bat tissues were compared across the years in which the bat were found dead (2008, 2009 and 2010) using ANOVA tests and t- tests, and the concentrations were compared across bat species (*P. Pipistrellus* versus *P. Pygmaeus*) using ANOVA tests. As multiple statistical tests were applied, the p-values were adjusted using the Holm-Bonferroni method. Level of statistical significance was set to 0.05 (after adjustment of the p-values). The number of pairs (n) across the associations was not equal since tissues in a poor conservation state or missing (previously extracted) have not been analyzed. Data analyses were performed with the software R version 2.12.1 (R Development Core team, 2012).

We compared concentrations in liver and kidney with previously derived critical toxic threshold concentrations for toxic metals (Pb and Cd) and with lower and upper level concentrations for essential trace metals (Cu and Zn) (Table 1). To be able to compare our results with previous studies (Lüftl et al., 2003; Pikula et al., 2010), we assumed that concentrations expressed in dry weight were four times higher than wet weight values, as commonly used in the literature (Clark and Shore, 2001). The critical toxic levels for Pb and Cd were associated with structural and functional kidney damage (Chmielnicka et al., 1989; Ma, 2011). Critical toxic threshold in various tissues of wildlife mammals are not well documented and therefore, we focused the comparison with toxicity data on kidney and liver, which are the most illustrated organs regarding toxic thresholds of metals for species of wild mammals. Little is known on the association of tissue residues and the effects on the reproduction and population level effects in small mammals. By definition, there is no critical toxic threshold for essential trace metals, but the upper level of metals in small mammals has been proposed for use in risk assessment. It is important to highlight that upper levels are not to be considered as toxic levels, but can give valuable information to compare our results. The Cu upper range was provided from a review of numerous studies on shrews, voles and mice, whereas the Zn data came from a more limited dataset (Ma and Talmage, 2001; Schleich et al., 2010). There is little information about the health effects on small mammals induced by a deficiency of essential metals (concentrations below the lower range) (Clark and Shore, 2001; Ma and Talmage, 2001; Sheffield et al., 2001).

Results

Metal concentrations. Bats (193 adult male *Pipistrellus sp.*) were obtained from across a pollution gradient in England and Wales between 2008 and 2010 (Figure 1, Figure S1, Figure S2). Median concentrations of the different metals in the analyzed bat tissues were significantly different for kidneys (ANOVA $F(3, 753) = 918.2$, $p < 0.05$), liver ($F(3, 744) = 733.9$, $p < 0.05$), stomach ($F(3, 658) = 929.2$, $p < 0.05$), fur ($F(3, 761) = 774.4$, , $p < 0.05$) and bones ($F(3, 754) = 1018$, $p < 0.05$)), and were highest for Zn, followed by Cu, Pb, and Cd (except for fur and bones for which Pb concentrations were higher than Cu concentrations, Figure 2C and Figure 2B) (Table 1, Figure 2). *Post-hoc* tests revealed significant differences in the concentration of each metal among most pairs of tissue types compared (except for Pb: kidneys and stomach, liver and stomach, bones and fur (Figure 2C); Cu: kidneys and stomach, liver and stomach (Figure 2B); Zn: kidneys and liver, kidneys and stomach, liver and stomach (Figure 2D); Cd: kidneys and liver, kidneys and stomach, liver and stomach

(Figure 2A) (Table 2). For a given tissue, there were significant differences in the concentrations of different metals (Table 3).

Generally, the concentrations of metals in different types of bat tissues were positively correlated with each other for the same metal (except for kidneys and bones, liver and bones for Cd and Cu; kidneys and bones, liver and fur for Pb; and kidneys and stomach, kidneys and fur, liver and stomach, liver and fur for Zn, for which the correlations were not significant) (Table 2). However, for Pb in liver and bones, and for Zn in kidneys and bones, and in liver and bones, the correlations were negative (Table 2). The concentrations of Pb were particularly positively correlated between tissues (Table 2). The strongest associations of concentrations of metals between tissues ($r \geq 0.65$) were between liver and kidneys (for Cd, Cu and Pb), and for Pb between: stomach and fur, and fur and bones (Table 2). The concentrations of different metals were also positively correlated within the same tissue (Table 3). The strongest associations between metals ($r > 0.50$) occurred for Cd and Zn (in kidneys, liver, fur and bones), Cu and Zn (in kidneys, liver, stomach, fur and bones), Cd and Pb (in kidneys, liver and bones), and Pb and Zn (in liver and bones) (Table 3).

While the bats were selected based on a gradient of soil pollution of metals across England and Wales (Figure S2), we did not find significant correlations between concentrations of metals in soil from locations where the bats had been collected and in bat tissues (Table 4, Figure S3). The exception was for Zn with a positive correlation for kidneys, although this association was not strong ($r = 0.16$) (Table 4).

Interspecies differences were found for concentrations levels of Pb in fur (286 ± 1537 versus 5558 ± 7150 µg/g dw for *P. pipistrellus* and *P. pygmaeus*, respectively ANOVA $F(1, 190) = 4.603$, $p < 0.05$) and Zn in kidneys (31 ± 40 versus 79 ± 66 µg/g dw for *P. pipistrellus* and *P. pygmaeus*, respectively; ANOVA $F(1, 189) = 4.234$, $p < 0.05$), although our small sample size of *P. pygmaeus* limits the interpretation of these results. Variations of concentrations Cd in liver and Zn in kidneys and liver were found across time, based on the year in which the bats were collected (ANOVA $F(1, 176) = 4.36$, $p < 0.05$; $F(1, 189) = 12.78$, $p < 0.05$; $F(1, 189) = 5.253$, $p < 0.05$, respectively). *Post-hoc* tests showed differences of concentrations of metals in bat tissues between years: 2008 and 2009 for Cd in liver, Cu in stomach and fur and Zn in liver; 2009 and 2010 for Cu and Zn in stomach; and 2008 and 2010 for Zn in kidneys and liver (Table S3). However, it was not possible to distinguish a trend of variation of metal across time based on our results since the concentrations were increasing as well as decreasing over time (Table S3).

Formaldehyde as a storage solution. Median metal concentrations determined in the preservative solution (40% formaldehyde) after correction for ND values were: 0.02; 21.44; 0.68 and 10.73 μg/L for Cd, Cu, Pb and Zn, respectively (respective ranges: 0.001-4.78, 3.9-51.2, 0.03-590.76, 2.2-70.0) and were significantly different across metals (ANOVA $F(3, 396) = 647.2$, $P < 0.05$). These values were on average 0.7 (±1.8); 8.5 (±59.5); 3.1 (±16.3) and 0.7 (±1.7) fold higher than the concentrations measured in the different tissues, for Cd, Cu, Pb and Zn, respectively (Table 5). Positive relationships were found between the concentrations in the formaldehyde aliquots and the concentrations in all the tissues analyzed for Pb (Table 5). Other positive associations with metal concentrations in the formaldehyde were found for the concentrations of Cd in kidneys, stomach and fur; and for Cu in bones (Table 5). Median concentrations of metals determined in the fresh formaldehyde were: 0.08, 0.96, 3.77, and 291.85 μg/L for Cd, Cu, Pb and Zn, respectively (respective ranges: 0.0-0.34, 0.79-1.33, 3.64-3.95, and 274.85-293.54 μg/L). The concentration of Cu determined in the fresh formaldehyde was significantly lower than in the storage solution (ANOVA $F(1, 103) = 1109$, $p < 0.05$). We did not find significantly higher concentrations of Cu in the formaldehyde containing the bats preserved in 2008 than the bats preserved in 2010, which does not suggest a leaching of Cu across time, but *post-hoc* tests showed higher concentrations for Cd in the bats preserved in 2008 and 2009, compared to bats preserved in 2010 (Table S3). However, the interpretation of our results is limited by the sample size of formaldehyde aliquots of bats preserved in 2009 and 2010 (Table S3). There were no general significant variations in concentrations of metals in the formaldehyde across the years (ANOVA $F(1, 81) = 1.809$, $p > 0.05$; $F(1, 98) = 1.233$, $p > 0.05$; $F(1, 81) = 1.809$, $p > 0.05$; $F(1, 81) = 1.809$, $p > 0.05$, for Cd, Cu, Pb and Zn, respectively).

Metal toxicity in bat tissues. Comparisons of concentrations of metals in liver and kidney to toxicological threshold values or the upper level of metal measured in other mammal species (Table 1), indicate that 21% of the bats analyzed had tissue concentrations above these levels. (Figure 1, Figure 2). Pb was the most toxicologically important metal since 7-11% of the bats had concentrations high enough to illicit structural damage and physiological effects, for kidneys and liver, respectively (Figure 2C). The range of values in bats above the upper limit was 4-9% for Cu and 0.5-5.2% for Zn, for kidneys and liver, respectively (Figure 2B, Figure 2D). Concentrations of Cd in tissues of all bats were well below toxic thresholds (Figure 2A). Comparisons with the lower level of essential metal measured in other small mammals (Table 1)

indicate that 79% and 91% of the bats had concentrations of Cu lower than these levels in kidneys and liver, respectively; and that 94% and 92% had concentrations of Zn lower than these levels in kidneys and liver, respectively.

Discussion:

Metal concentrations. Considering the median values for all metals, our measured tissue concentrations were generally lower than in other studies, except for the concentrations of Pb and Zn reported in bats sampled from the Czech Republic (Table 1). This could be explained by the partial leaching of metals in our storage solution as discussed in further detail below. The commonly observed order of median concentrations of the different trace metals in small mammals is Zn > Cu > Pb > Cd, in particular for kidneys and liver (Cooke, 2011; Ma and Talmage, 2001), was also seen when metals were ranked based on median measured concentrations (based on kidney, liver and stomach samples) (Table 1, Figure 2). The median concentrations of Pb were higher than the concentrations of Cu in fur and bone (Table 1, Figure 2C, Figure2B). Bats obtained from SW England in our study (renal median concentrations of 0.04, 11.30, 0.59 and 15.32 µg/g dw for Cd, Cu, Pb and Zn, respectively, n=5 in our study) also had lower median concentrations than those reported in Walker et al. (2007). Our maximum Pb concentrations were associated with areaswhere soil was highly contaminated with Pb, i.e. the Pennines, which comprises Pb bearing deposits that were extensively mined in the past (Figure S3). The high values of Pb concentrations (>1000 µg/g dw) (Table 1) might have resulted from an external contamination occurring previously or during the analysis (e.g. contact of the scalpel with metal or metal dust in the lab facilities during analyses). However, these high concentrations of Pb found in liver and fur did not occur for the same individuals, and not within the same batch run through the analysis. In addition, our quality assurance provide confidence and assurance of data quality, and we therefore, did not exclude these data from our dataset.

Patterns of concentrations of metals observed in different tissues in our study may be explained by different physiological and kinetic processes involved with the uptake, accumulation and excretion of different metals. The interpretation of our results could be explained as the different tissues may reflect different exposure time periods. Concentrations of individual metals across tissues were correlated, particularly for Pb (Table 2). Thus, our data suggest that the metal intake by the bats in the UK was due to both recent (e.g. stomach contents), as well long term exposure

(kidneys, liver, fur and bones), especially for Pb which has been shown to reach a steady-state in kidneys in late sub-adult stage of other mammals (e.g. shrews) and has a long half-life in bones (around 10 to 30 years) (Ma, 2011). Kidneys, therefore, represent a valuable indicator for exposure of bats to non-essential metals (Cooke, 2011; Ma, 2011). Fur can represent a valuable less-invasive proxy for endogenous metals contamination monitoring (Hernout et al., 2016; Little et al., 2015), although cautious interpretation is needed since the results provide information about the bat's exposure during the time of formation of the fur. Therefore, the moulting cycle is an important factor to consider (Fraser et al., 2013). A discussion of the use of fur samples as a proxy to monitor metal contamination has recently been reported elsewhere (Hernout et al., 2016). In addition, our positive correlations may be helpful for further analysis with a limited selection of the type of tissues to provide reasonable estimations of the relative concentrations present in other tissues.

Concentrations of the essential metals, which are homeostatically controlled in mammals, measured in our study (Table 1) were lower than the average range observed in kidneys and liver of other mammals such as shrews and moles for Cu (20-30 µg/g dw) and in kidneys and liver for shrews for Zn (71-204 µg/g dw) (Ma and Talmage, 2001). This might have been due to a low dietary intake in bats, as well as some antagonist effect with other metals altering essential metal regulation and absorption or due to leaching into the preservative which is discussed below (Sheffield et al., 2001).

Mean (±sd) metal concentrations in soil for England and Wales were 0.67 (±0.98), 22.4 (±36.8), 73.3 (±281) and 88.48 (±103) µg/g dw for Cd, Cu, Pb and Zn, respectively. The values were two to five times lower than mean concentrations in other European countries and in North America (Lado et al., 2008; Shacklette and Boerngen, 1984; Smith, 2005). These differences in exposure across countries may explain some of the discrepancies between our measured concentrations and those in other studies (Lado et al., 2008; Shacklette and Boerngen, 1984; Smith, 2005). In addition, our comparison between metal concentrations in soil and in bat tissues were not correlated (Table 4). These non-significant linear correlations can be explained by the multiple drivers of contamination through the food chain. Bats forage in multiple habitats and on various prey items, such as: terrestrial and emerging invertebrates (e.g. Diptera, Chironomidae), and are therefore exposed to heterogeneous sources of contamination, such as lake sediments (Currie et al., 1997; Hernout et al., 2013; Hernout et al., 2015). Modeling exercises have shown the importance of prey items in driving the risks of metals to bat health through the food chain (Hernout et al., 2013; 2015). Some studies have investigated the associations between the exposure of metals of wild mammals and the environmental biotic factors influencing the

uptake, such as pH and organic matter content, but only on a gradient from point sources of pollution (Ma and Talmage, 2001; Sheffield et al., 2001). However, such studies are lacking on a larger scale. A recent study has shown associations between freshwater acidity and mercury concentrations contained in bat fur (Little et al., 2015), and such studies on a larger scale are therefore to be encouraged.

Formaldehyde as a storage solution. Our lower levels of metals determined in bat tissue compared to other studies (Table 1) can be partially explained by the leaching of metals into our storage matrix, especially for Cu (Table 5). Based on the comparison of the concentrations in the fresh formaldehyde and the used formaldehyde, leaching may have occurred for Cu, and in a smaller proportion for the non-essential metals. However, the possible leaching of Cu was not confirmed by the comparison of Cu concentrations measured in the aliquots of formaldehyde preserved in 2008, 2009 and 2010 since the bats preserved in 2008 and 2009 did not show higher concentrations of Cu than the bats preserved in 2010 (Table S3).However, our comparisons are based on a small sample size of fresh formaldehyde, and formaldehyde aliquots for bats preserved in 2008 and 2009 (Table S3).

Formaldehyde is a widely used fixative solution for museum collections, and therefore, a large number of specimens are available for endogenous contaminant analysis (Campbell and Drevnick, 2015). These collections have the potential to considerably improve our knowledge on environmental chemistry and the variations of exposure across time (Campbell and Drevnick, 2015). Interestingly, only a few studies have documented the potential effects of the preservative solution on the interpretation of metal analysis. The potential leaching of metals has been illustrated for invertebrate tissues (for Cu), samples of antelope species (for Cu), and human brains (for Cd) (Gellein et al., 2008; Hendrickx et al., 2003; Quan et al., 2002). The leaching was found to be strongly time dependent (Gellein et al., 2008). The leaching is a complex function: tissues containing high levels of metals showed a small leaching process, while other tissues containing low levels of metals readily leached out (Gellein et al., 2008). In our study, whereas positive relationships were found between the concentrations of non-essential elements and Cu in the formaldehyde aliquot and in tissues, it is not possible to derive a correction factor to adjust our metal concentrations in the bat tissues. The potential leaching of Cu could be explained by the binding of Cu to dissolved organic matter, which could be present in the preservative solution (Craven et al., 2012). In addition, metals bind to sulfhydryl proteins, particularly Pb, in biological tissues (Flora et al., 2012). The pH is a factor influencing the dissociation and the binding of metals. Formalin may oxidize to formic acid and

lower the pH of the solution, resulting in an extraction of metals from the biological samples into the preservative medium (Simmons, 2014).

Whereas the potential extraction of metals into the preservative solution may occur, others studies have presented contradictory results regarding the potential effects of the solution. No differences were found between the concentrations of Cu, Zn and Cd samples preserved in formaldehyde and fresh or frozen samples of bovine liver (for Cu and Zn) and human tissues (for Cd, Cu and Zn) (Bischoff et al., 2008; Bush et al., 1995; Theron et al., 1974). Concentrations of Cd, Cu, Pb and Zn increased in samples preserved in formaldehyde compared to frozen specimens in Isopods (for Cd, Pb and Zn) and fish tissues (for Cd and Cu) (Gibbs et al., 1974; Hendrickx et al., 2003). The dissolution of tissues stored in formaldehyde resulted as a decrease of the dry weight of the samples, and thus an increase of metal concentrations was determined in the invertebrate tissues (Hendrickx et al., 2003). However, formaldehyde did not alter the weight and dry weight in invertebrate samples in another study (Knapp, 2012). Further studies are needed on the effects of the preservative solution on chemical analysis, such as analyzing the variations of concentrations of metals among animal tissues and formaldehyde over time, as well as comparing them with fresh tissues. Therefore, caution should be taken while interpreting the results. When possible, fresh or frozen samples are preferred for metal analysis. The concentrations measured in our fresh formaldehyde were lower than 1 mg/L, as reported in Bishoff et al. (2008), where the authors concluded that there is no evidence that the formalin was contaminated. As a conclusion, our metal concentrations may be slightly underestimated due to leaching in the preservative solution, however, we believe that our large dataset provides conservative information comparable with previous studies, such as Walker et al. (2007), and a unique and valuable dataset.

Metal toxicity. The percentage of bats in which concentrations of metals exceeded toxic thresholds suggest that a significant proportion of the bat population in England and Wales may be affected by metal exposure. Laboratory-based studies indicate that the concentrations we observed in bat tissues could cause damage to the kidneys of small mammals (Ma and Talmage, 2001). It is important to highlight that these toxicological data were extrapolated from various rodent test species which might not totally reflect toxicity to bats. For example, other insectivorous mammals, such as shrews, have been shown to be more tolerant to metals than rodents (Ma and Talmage, 2001). While few data are available on the effects of metals in bats in the wild, studies in Australia and France have suggested that Pb exposure

can cause mortality in individual bats, although the direct sources of contamination and the impact on a population level were not shown (Carravieiri and Scheifler, 2013). Lethal effects and reduced body weight associated with renal residues of 225 µg/g dw of Pb have been illustrated on shrews (Pankakoski et al., 1994). These levels were found in two individuals in our study. Our comparison with levels associated with kidney damage suggests that a large number of individuals could have suffered sub-lethal effects.

In considering the toxic effects of metals, it is important to recognize that the bats are exposed to a mixture of metals. Studies of metal mixture interactions for small mammals have produced contradictory results, thus, it is difficult to interpret our data in terms of the potential toxicological effects of the mixtures of metals measured in our bats. For example, different metals can have additive, synergistic and antagonistic interactions depending on a number of variables including the type of metals involved and their relative concentrations (Beyer, 2004; Oestreicher and Cousins, 1985). For example, exposure to Pb and Cd is likely to cause increased kidney damage. However, some metals have antagonistic effects such as that displayed by Cu and Zn (Oestreicher and Cousins, 1985). Essential metal deficiency, which could arise from a low dietary intake or if a non-essential metal out-competes an essential metal for a key binding site in a tissue, can also be associated with health effects, such as: slow growth, anemia, impaired immune response, central nervous system histopathology (Eisler, 2000). For example, Zn can cause toxicity through inducing deficiencies in other essential metals, particularly Cu (Beyer, 2004; Sheffield et al., 2001). The large percentages of bats presenting lower concentrations of essential metals than the lower levels measured in other small mammals may indicate a deficiency. However, our results are in concordance with the study of Lüftl et al. (2003, cited in Carravieiri and Scheifler, 2013) where metal residues in *Pipistrellus pipistrellus* tissues were measured, and showed minimum values of Cu and Zn below the lower levels measured in other small mammals. Further research is needed about the possible deficiency of essential metals in insectivorous bats. The associations found for different metals in the same tissue suggest a co-exposure of metals to bats with highly correlated concentrations of metals: Cd and Pb, Cd and Zn, Cu and Zn, and Pb and Zn (Table 3). These metals may have the same transfer pathways, diet or habitat contamination (Walker et al., 2007).

Importance of metals in the context of declining bat populations. A substantial proportion of the bats contained residues of metals high enough to cause toxicity, in terms of health effects associated with kidney damage. Bats are

exposed to a large range of environmental stressors (e.g. climate change, white nose syndrome in North America) (Sherwin et al., 2013; Blehert, 2012), thus a better understanding of stressor interactions could be beneficial to bat conservation. Further research is needed on the potential connections between metal levels and population declines and the importance of metal pollution among other factors. Alongside other factors, metal exposure could be an additional stressor involved in the continuing decline in bat populations observed in countries with a legacy of mining and heavy industries. Further studies could investigate the importance of metal pollution on bat population dynamics by using long term capture-recapture monitoring dataset. This study has focused on analysis of adult males of only two sibling species. It is likely that metal concentrations (and hence toxicity) will be different in females and juveniles and in other species. Further spatial studies could also investigate whether the habitat could induce differences in metal accumulation and consider various sources of metal pollution, such as water and sediments. Metals may interfere with the normal functioning of the immune system and increase the prevalence of parasites or wildlife infectious diseases (Bichet et al., 2013; Gasparini et al., 2014). Therefore, research on the potential interactions between environmental pollution and bio-contamination are also to be encouraged. In a context of diverse environmental stressors affecting wildlife populations, our analytical studies show the importance of metal contamination as an additional stressor to bat populations.

Acknowledgements

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

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Table and figure legend

Table 1: Metal concentrations in bat tissues presented on a dry weight basis. Median and maximum metal concentrations (µg/g dw) measured in kidneys (n=191), liver (n=191), stomach (n=168), fur (n=192) and bones (n=192) of *Pipistrellus sp*. in this study and previous studies. Toxic (for Pb and Cd) and upper range values (for Cu and Zn) (µg/g dw) from other studies are used to compare our tissue concentrations.

Table 2: Differences and relationships between concentrations of metals in the bat tissues (kidneys, liver, stomach, fur and bones) for a given metal. The *post hoc* t-test (F) was used to explore these differences. Pearson's coefficients (r) were used to evaluate the strength of these associations. As multiple statistical tests were applied, the p values were adjusted using the Holm-Bonferroni method. Asterisks (*) indicates significant correlation ($p<0.05$) (after correction); (ns) indicates a non-significant correlation; and (n) indicates the sample size.

Table 3: Differences and relationships between concentrations of the different metals (Cd, Cu, Pb and Zn) for a given tissue. The *post hoc* t-test (F) was used to explore these differences. Pearson's (r) were used to evaluate the strength of these associations. As multiple statistical tests were applied, the p values were adjusted using the Holm-Bonferroni method. Asterisks (*) indicates significant correlation ($p<0.05$) (after correction); (ns) indicates a non-significant correlation; and (n) indicates the sample size.

Table 4: Relationships between concentrations of metals in the soil and bat tissues (kidneys, liver, stomach, fur and bones). Pearson's coefficients (r) were used to evaluate the strength of these associations. As multiple statistical tests were applied, the p values were adjusted using the Holm-Bonferroni method. Asterisks (*) indicates significant correlation ($p<0.05$) (after correction); (ns) indicates a non-significant correlation; and (n) indicates the sample size used for the correlations.

Table 5: Ratios between concentrations in formaldehyde (µg/L) and the concentration in the different tissues (µg/g) and their standard deviations. Relationships between concentrations in the formaldehyde aliquot and other tissues (kidneys, liver, stomach, fur and bones). Pearson's coefficients (r) were used to evaluate the strength of these associations. As multiple statistical tests were applied, the p values were adjusted using the Holm-Bonferroni method. Asterisks (*) indicates significant correlation ($p<0.05$) (after correction); (ns) indicates a non-significant correlation; and (n) indicates the sample size used for the correlations.

Figure 1: Map of England and Wales showing the locations of where the 193 bats analyzed were collected with the Pb concentrations of soil (in µg/g dw) in background. Lead soil concentrations are ranged from 0.63 to 250 (light grey cells), and 250 to 17365 (dark grey cells). The bats presenting toxic residues (for Pb) or residues above the upper level concentrations (for Zn and Cu) (n=41) are represented in black circles, and the others are represented in white circles. The white grid cells represent an absence of data (NSRI dataset).

Figure 2: Metal concentrations and toxic threshold values or upper limit levels. Median metal concentrations (µg/g dw) in kidneys, liver, fur, bones and stomach for Cd (A), Cu (B), Pb (C) and Zn (D). The y axis has been transformed with a root square transformation. Black lines represent critical toxic threshold (for Pb) or maximum upper range level (for Cu and Zn) found in the literature. The upper and the lower whisker extend from the hinge to the highest and the lowest values that are within: 1.5 times the inter-quartile range.

Supporting information captions

Table S1: Geographical coordinates of bats collection points in WGS84 format (degrees minutes).

Table S2: Statistical results of the Shapiro (W) with their p values, applied on our dataset of metal concentrations in the different bat tissues and organs, formaldehyde aliquots and soil concentrations (NSRI dataset).

Table S3: Descriptive statistics (mean and standard deviations) and differences (*post-hoc* t-test) between concentrations (µg/g dw for organs and tissues and µg/L dw for the formaldehyde) of the different metals (Cd, Cu, Pb and Zn) for a given sample type within the different years in which the samples were found. The *post hoc* t-test (F) was used to explore these differences. As multiple statistical tests were applied, the p values were adjusted using the Holm-Bonferroni method. Asterisks (*) indicates significant correlation ($p<0.05$) (after correction) and (n) indicates the sample size.

Figure S1: Maps of England and Wales showing the locations of where the 193 bats analyzed were collected with the soil metal concentrations (in µg/g dw) in background for a) Cd, b) Cu, and c) Zn. The bats presenting toxic residues (for Pb) or residues above the upper level concentrations (for Zn and Cu) (n=41) are represented in black circles, and the others are represented in white circles. The white grid cells represent an absence of data (NSRI dataset).

Cd soil concentrations are ranged from 0.05 to 1.5 (light grey cells) and 1.5 to 41 (dark grey cells). Cu soil concentrations are ranged from 0.04 to 70 (light grey cells), and 70 to 1507.7 (dark grey cells). Zn soil concentrations are ranged from 0.02 to 200 (light grey cells), and 200 to 3648 (dark grey cells).

Figure S2: 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 S3: Maps showing the metal concentrations measured in organs and tissues (in µg/g dw) (kidneys, liver, stomach, fur and bones) of 193 *Pipistrellus sp*. sampled across England in Wales (in µg/g dw) for Cd, Cu, Pb and Zn. The letters indicate the metal and the numbers in subscript indicate the tissue analyzed, as following: a) Cd, b) Cu, c) Pb and d) Zn and 1) kidneys 2) liver 3) stomach 4) fur 5) bones. The toxicological thresholds, and the lower and upper range values of concentrations measured in small mammals were included as break values.

Cd concentrations in tissues are ranged from 3.6 X 10^{-3} to 0.25 (light grey cells), 0.25 to 0.75 (dark grey cells) and 0.75 to 0.8 (black cells) (Map a_1); from 1.5 X 10^{-3} to 0.25 (light grey cells), 0.25 to 0.75 (dark grey cells) and 0.75 to 13 (black cells) (Map a_2); from 1.7 X 10^{-3} to 0.25 (light grey cells), 0.25 to 0.75 (dark grey cells) and 0.75 to 2 (black cells) (Map a_3); from 3.9 X 10^{-3} to 0.5 (light grey cells), 0.5 to 1 (dark grey cells) and 1 to 212 (black cells) (Map a_4); and from 5.0 X 10^{-4} to 0.5 (light grey cells), 0.5 to 1 (dark grey cells) and 1 to 24 (black cells) (Map a_5); in kidneys, liver, stomach, fur and bones, respectively.

Cu concentrations in tissues are ranged from 3.5 X 10^{-2} to 20 (light grey cells), 20 to 30 (dark grey cells) and 30 to 134 (black cells) (Map b_1); from 3.3 X 10^{-2} to 20 (light grey cells), 20 to 30 (dark grey cells) and 30 to 71 (black cells) (Map b_2); from 5.3 X 10^{-1} to 25 (light grey cells), 25 to 100 (dark grey cells) and 100 to 240 (black cells) (Map b_3); from 2.2 to 10 (light grey cells), 10 to 40 (dark grey

cells) and 40 to 103 (black cells) (Map b_4); and from 2.1 $X10^{-1}$ to 3 (light grey cells), 3 to 10 (dark grey cells) and 10 to 25 (black cells) (Map b_5); in kidneys, liver, stomach, fur and bones, respectively.

Pb concentrations in tissues are ranged from 5.2 X 10^{-3} to 15 (light grey cells), 15 to 25 (dark grey cells) and 25 to 367 (black cells) (Map c_1); from 2.4 X 10^{-3} to 5 (light grey cells), 5 to 10 (dark grey cells) and 10 to 5039 (black cells) (Map c_2); from 4.0 X 10^{-3} to 50 (light grey cells), 50 to 100 (dark grey cells) and 100 to 134 (black cells) (Map c_3); from 4.5 X 10^{-2} to 100 (light grey cells), 100 to 350 (dark grey cells) and 350 to 20398 (black cells) (Map c_4); and from 2.8 X 10^{-3} to 100 (light grey cells), 100 to 350 (dark grey cells) and 350 to 708 (black cells) (Map c_5); in kidneys, liver, stomach, fur and bones, respectively.

Zn concentrations in tissues are ranged from 1.3 to 87 (light grey cells), 87 to 274 (dark grey cells) and 274 to 354 (black cells) (Map d_1); from 8.0 X 10^{-1} to 71 (light grey cells), 71 to 465 (dark grey cells) and 465 to 5205 (black cells) (Map d_2); from 1.1 to 200 (light grey cells), 200 to 400 (dark grey cells) and 400 to 1336 (black cells) (Map d_3); from 11.8 to 100 (light grey cells), 100 to 200 (dark grey cells) and 200 to 578 (black cells) (Map d_4); and from 7.0 X 10^{-1} to 250 (light grey cells), 250 to 500 (dark grey cells) and 500 to 1029 (black cells) (Map d_5); in kidneys, liver, stomach, fur and bones, respectively.

Figure 1

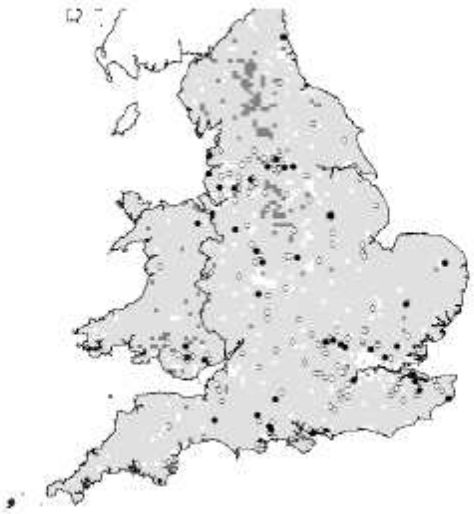

Figure 2

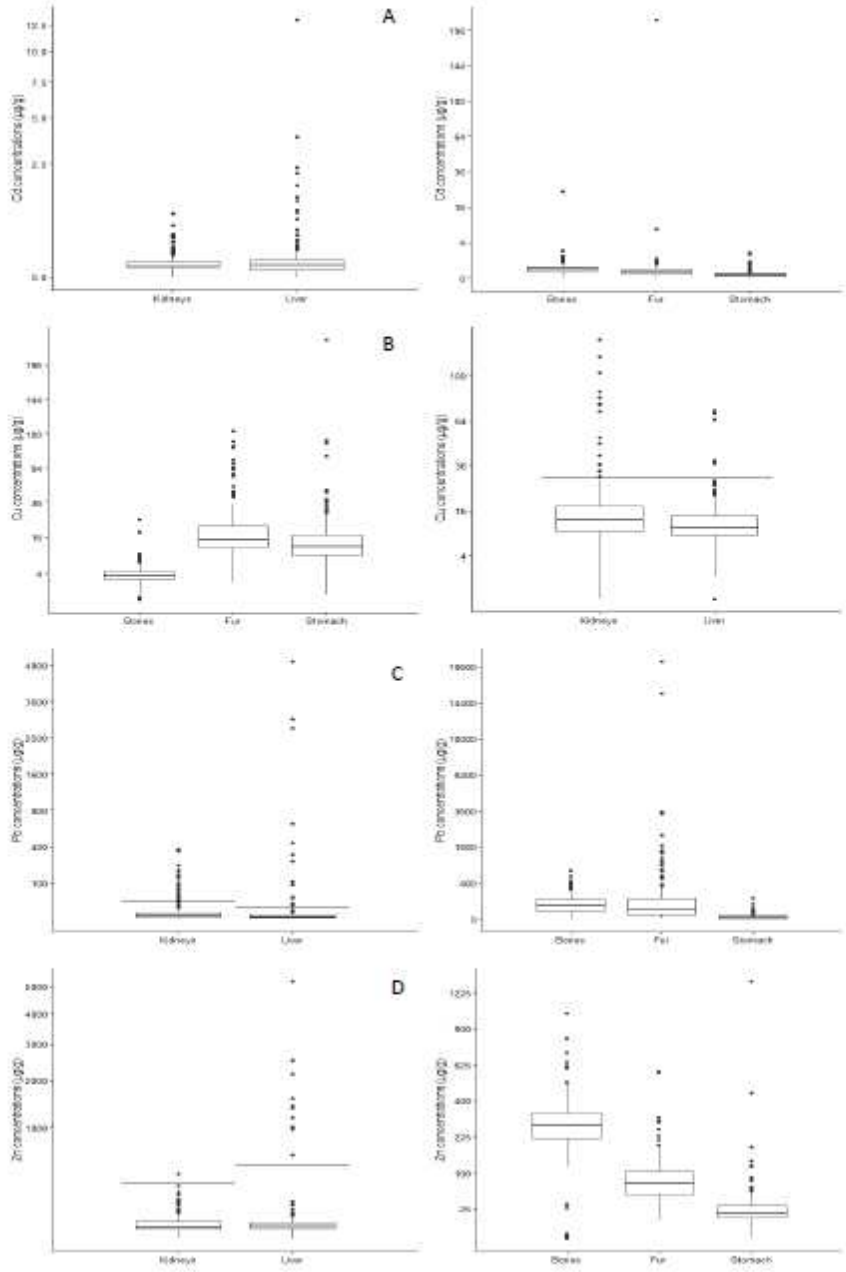

Table 1:

Metal	Tissue	Statistics	Metal concentrations measured in *Pipistrellus sp.* tissues (µg/g dw). Sample size and the country are indicated in brackets, References are indicated in subscripts.		Toxic threshold (for Cd and Pb) and lower and upper range values (for Cu and Zn) (µg/g dw). References are indicated in subscripts.
			This study	Literature data	
Cd	Kidneys	Median	0.02 (n191)	1.42 (n172) (E) $_{i}$; 0.59 (n43) (A) $_{c}$	105.00 $_{a}$
		Range	3.6 X 10^{-3} - 0.79 (n191)	29.1 (n172) (E) $_{i}$; 11.27 (n43) (A) $_{c}$	-
	Liver	Median	0.03 (n190)	1.53 (n14) (G) $_{h}$	-
		Range	1.5 X 10^{-3} - 12.98 (n190)	-	-
	Stomach	Median	0.03 (n168)	-	-
		Range	1.7 X 10^{-3} - 1.97 (n168)	-	-
	Fur	Median	0.10 (n192)	0.81 (n8) (G) $_{b}$	-
		Range	3.9 X 10^{-3} - 212.38 (n192)	245(n8) (G) $_{b}$	-
	Bones	Median	0.24 (n192)	-	-
		Range	5.0 X 10^{-4} - 24.01 (n192)	-	-
Cu	Kidneys	Median	12.89 (n191)	33.72 (n43) (A) $_{c}$	20 $_{e}$ - 30.00 $_{e}$
		Range	3.5 X 10^{-2} - 134.05 (n191)	144.72 (n43) (A) $_{c}$	-
	Liver	Median	10.69 (n190)	-	20 $_{e}$ - 30.00 $_{e}$
		Range	3.3 X 10^{-2} - 70.99 (n190)	-	-
	Stomach	Median	11.90 (n168)	-	-
		Range	5.3 X 10^{-1} - 239.53 (n168)	-	-
	Fur	Median	14.96 (n192)	36.2 (n8) (G) $_{b}$	-
		Range	2.2 - 103.33 (n192)	176 (n8) (G) $_{b}$	-
	Bones	Median	3.61 (n192)	-	-
		Range	2.1 X 10^{-1} - 25.22 (n192)	-	-
Pb	Kidneys	Median	0.70 (n191)	2.45 (n172) (E) $_{i}$; 3.83 (n43) (A)$_{c}$; 0.52 (n23) (C) $_{f}$	25.00 $_{d}$
		Range	5.2 X 10^{-3} - 367.22 (n191)	69.7 (n172) (E) $_{I}$; 63.42 (n43) (A) $_{c}$	-
	Liver	Median	0.33 (n190)	1.32 (n23) (C) $_{f}$; 2.95 (n14) (G) $_{h}$	10.00 $_{d}$
		Range	2.4 X 10^{-3} - 5039.93 (n190)	-	-
	Stomach	Median	0.82 (n168)	-	-

	Fur	Range	4.0 X 10^{-3} - 133.50 (n168)	-	-
		Median	28.80 (n192)	34.2 (n8) (G) $_{b}$	-
	Bones	Range	4.5 X 10^{-2} - 20398.88 (n192)	519 (n8) (G) $_{b}$	-
		Median	53.15 (n192)	-	-
		Range	2.8 X 10^{-3} - 707.67 (n192)	-	-
Zn	Kidneys	Median	18.05 (n191)	212.40 (n43) (A) $_{c}$; 1.88 (n23) (C) $_{f}$	87 $_{e}$ - 274.00 $_{g}$
		Range	1.3 - 354.17 (n191)	1760.0 (n43) (A) $_{c}$	-
	Liver	Median	18.79 (n190)	1.12 (n23) (C) $_{f}$	71 $_{e}$ - 465.00 $_{g}$
		Range	8.0 X 10^{-1} - 5205.31 (n190)	-	-
	Stomach	Median	19.02 (n168)	-	-
		Range	1.1 - 1336.89 (n168)	-	-
	Fur	Median	72.97 (n192)	383 (n8) (G) $_{b}$	-
		Range	11.8 - 578.36 (n192)	1155 (n8) (G) $_{b}$	-
	Bones	Median	275.61 (n192)	-	-
		Range	7.0 X 10^{-1} - 1029.70 (n192)	-	-

References :

Subscripts letters : a : Chmielnicka et al., 1989 ; b : Flache et al., 2015 ; c : Lüftl et al.,2003 ; d : Ma, 1996 ; e : Ma and Talmage, 2001 ; f : Pikula et al., 2010 ; g : Schleich et al., 2010 ; h : Streit and Nagel, 1993 ; i : Walker et al., 2007.

Country indicated by the letters: (A): Austria, (C): Czech Republic, (E): England, (G): Germany.

Table 2:

Metal			Kidneys	Liver	Stomach	Fur
Cd	Liver	t - df r n	-1.6239 – 313.404 (ns) 0.70* 189			
	Stomach	t - df r n	-0.5899 – 320.193 (ns) 0.54* 167	1.0328 – 327.586 (ns) 0.53* 168		
	Fur	t - df r n	-10.7976* – 338.615 0.37* 191	-7.756* – 361.836 0.23* 190	-9.5702* – 344.584 0.54* 167	
	Bones	t - df r	-21.4048* – 365.977 0.15 (ns)	-15.4467*– 311.972 0.09 (ns)	-18.7665* – 319.01 0.41*	-6.7671* – 337.259 0.53*

		n	190	190	167	191
Cu	Liver	t - df r n	2.8401* – 370.772 0.65* 189			
	Stomach	t – df r n	1.1811 – 356.578 (ns) 0.30* 167	-1.6155 – 344.165 (ns) 0.20 (ns) 168		
	Fur	t - df r n	-2.4769* – 357.687 0.39* 191	-5.9639* – 377.117 0.24* 190	-3.8504* – 329.875 0.41* 167	
	Bones	t - df r n	19.7537* – 345.294 0.08 (ns) 190	18.4637* – 370.009 0.05 (ns) 190	18.7826* – 317.026 0.42* 167	26.0241* – 380.026 0.25* 191
Pb	Liver	t – df r n	2.8084* – 377.745 0.75* 189			
	Stomach	t - df r n	1.3757 – 355.981 (ns) 0.56* 167	-1.5488 – 353.612 (ns) 0.34* 168		
	Fur	t - df r n	-13.575* – 379.694 0.42* 191	-16.1651* – 378.594 0.10 (ns) 190	-15.3704* – 355.476 0.75* 167	
	Bones	t - df r n	-16.4676* – 370.642 0.04 (ns) 190	-19.2813* - 365.724 -0.27* 190	-18.6552* – 347.777 0.43* 167	-1.3734 – 364.41 (ns) 0.72* 191
Zn	Liver	t - df r n	-1.6211 – 348.039 (ns) 0.50* 189			
	Stomach	t - df r n	0.0886 – 349.789 (ns) 0.15 (ns) 167	1.6496 – 347.473 (ns) -0.05 (ns) 168		
	Fur	t - df r n	-16.4471* – 345.921 0.19 (ns) 191	-11.341* – 288.205 -0.07 (ns) 190	-15.6927* – 297.012 0.39* 167	
	Bones	t - df r n	-21.6834* – 341.226 -0.28* 190	-17.7395* – 380.292 -0.74* 190	-21.1718* – 343.426 0.30* 167	-10.9714* – 282.076 0.36* 191

Table 3:

		Kidneys (n=191)			Liver (n=190)			Stomach (n=168)			Fur (n=192)			Bones (n=192)			Formaldehyde (n=100)		
		Cd	Cu	Pb	Cd	Cu	Pb	Cd	Cu	Pb	Cd	Cu	Pb	Cd	Cu	Pb	Cd	Cu	Pb
Cu	t df r	-68.58* 357.082 0.28*			-51.13* 254.114 0.41*			-60.02* 277.361 0.29*			-47.45* 262.781 0.34*			-33.46* 299.881 0.16*			-44.34* 110.291 0.09 (ns)		
Pb	T df r	-20.01* 255.393 0.51*	14.92* 235.255 0.23*		-13.82* 306.708 0.75*	17.42* 219.504 0.38*		-18.39* 255.981 0.34*	17.32* 209.139 0.27*		-27.71* 298.087 0.44*	-2.67* 215.258 0.24*		-32.14* 273.953 0.51*	-16.07* 220.879 0.40*		-17.89* 195.889 0.31*	16.62* 108.177 0.01 (ns)	
Zn	t df r	-70.25* 368.001 0.55*	-4.90* 376.503 0.54*	-17.12* 244.538 0.47*	-49.29* 351.763 0.77*	-8.12* 300.501 0.52*	-20.39* 280.677 0.75*	-60.48* 309.065 0.47*	-5.72* 320.79 0.59*	-19.84* 229.728 0.39*	-61.35* 266.71 0.51*	-23.92* 381.702 0.67*	-5.72* 216.641 0.44*	-60.61* 353.493 0.59*	-42.32* 267.063 0.75*	-10.95* 321.87 0.73*	-35.86* 160.845 0.40*	6.13* 130.456 0.29*	-11.89* 151.16 0.33*

Table 4:

Metal	Kidneys (n=191)	Liver (n=191)	Stomach (n=168)	Bones (n=192)	Fur (n=192)
Cd	0.14 (ns)	0.09 (ns)	0.01 (ns)	0.03 (ns)	0.10 (ns)
Cu	0.006 (ns)	0.009 (ns)	0.15 (ns)	0.06 (ns)	0.09 (ns)
Pb	0.08 (ns)	0.08 (ns)	0.04 (ns)	-0.08 (ns)	0.04 (ns)
Zn	0.16*	0.004 (ns)	0.06 (ns)	0.08 (ns)	0.04 (ns)

Table 5:

Metal		Kidneys (n= 99)	Liver (n= 99)	Stomach (n= 79)	Fur (n= 99)	Bones (n= 99)
Cd	Ratio	0.7 (±1.3)	1.2 (±2.3)	0.6 (±1.1)	0.3 (±0.9)	0.6 (±3.3)
	r	0.32*	0.27 (ns)	0.43*	0.34*	0.15 (ns)
Cu	Ratio	16 (±143)	17 (±151)	2 (±1)	1 (±1)	6 (±2)
	r	-0.40*	-0.24 (ns)	0.21 (ns)	-0.14 (ns)	0.29*
Pb	Ratio	7 (±53)	5 (±15)	3 (± 11)	0.3 (±2)	0.1 (±1)
	r	0.63*	0.72*	0.73*	0.66*	0.58*
Zn	Ratio	1.0 (±2.5)	1.3 (±4.1)	0.8 (±1.6)	0.2 (±0.2)	0.05 (±0.05)
	r	0.29 (ns)	0.09 (ns)	0.06 (ns)	0.09 (ns)	0.04 (ns)

Supplementary Information

Bat Ref	**Longitude**	**Latitude**	**Bat Ref**	**Longitude**	**Latitude**	**Bat Ref**	**Longitude**	**Latitude**
1	-1°50'17.153	50°50'04.202	**41**	0°47'34.041	51°19'12.428	**81**	-0°30'50.582	51°44'19.531
2	-0°35'28.763	51°46'09.942	**42**	-0°57'18.441	52°42'10.217	**82**	-2°26'50.598	53°36'45.749
3	-1°52'29.646	50°52'42.995	**43**	0°47'38.038	51°41'29.567	**83**	-2°21'41.814	51°41'14.475
4	-1°34'15.926	53°43'24.16	**44**	-2°15'07.347	53°48'23.498	**84**	-2°50'02.179	50°56'09.257
5	-3°00'27.05	53°38'49.564	**45**	1°06'20.441	51°16'37.752	**85**	0°30'20.316	51°08'02.933
6	-1°06'11.956	50°47'29.755	**46**	-2°13'02.951	53°35'04.5	**86**	-1°11'10.975	53°37'53.229
7	-0°43'56.189	53°11'26.398	**47**	-2°57'26.916	51°36'43.47	**87**	-3°41'32.571	50°25'51.835
8	-2°16'15.533	53°26'36.232	**48**	-2°07'53.985	52°41'47.693	**88**	-0°37'04.096	51°11'03.484
9	-0°21'59.641	51°22'11.841	**49**	-3°30'43.708	51°39'26.985	**89**	0°45'23.955	51°20'23.508
10	-0°21'59.641	51°22'11.841	**50**	-1°59'44.608	50°39'46.099	**90**	-1°27'47.963	51°48'22.572
11	-3°22'03.362	51°45'16.497	**51**	-2°04'40.091	51°31'24.026	**91**	0°04'32.329	51°46'29.221
12	-1°42'00.941	53°40'17.737	**52**	-1°06'59.586	53°21'14.757	**92**	-0°09'43.804	52°52'13.142
13	-0°09'25.299	52°46'52.421	**53**	-2°48'58.985	54°10'04.92	**93**	-1°53'59.748	53°43'42.791
14	0°26'08.247	51°19'28.193	**54**	-3°00'22.038	51°39'23.857	**94**	-0°58'41.603	51°26'58.842
15	-1°52'20.071	50°48'20.597	**55**	-2°12'07.291	53°30'36.021	**95**	-0°39'32.947	51°32'01.276
16	-3°24'30.608	51°37'31.851	**56**	-1°58'10.509	53°49'16.209	**96**	-1°47'24.674	50°44'52.976
17	-0°51'25.757	51°48'06.988	**57**	-3°00'28.261	53°51'07.18	**97**	-3°37'16.962	50°51'56.057
18	0°16'16.436	51°11'34.439	**58**	-1°47'00.079	53°42'08.399	**98**	-0°07'39.384	52°44'28.345
19	-0°07'31.786	51°55'22.581	**59**	-1°34'46.256	52°24'36.085	**99**	-0°02'45.61	51°42'37.169
20	-2°46'34.794	53°24'57.489	**60**	-1°49'55.624	50°54'48.897	**100**	-3°21'05.533	51°36'16.57
21	-4°09'20.698	50°25'59.86	**61**	0°36'37.473	52°11'51.324	**101**	-0°45'45.097	51°23'24.179
22	-2°22'22.949	53°45'08.217	**62**	-1°54'02.756	52°04'05.488	**102**	-2°06'45.86	52°49'04.663
23	-3°20'28.774	51°36'04.043	**63**	-1°15'09.044	51°34'23.294	**103**	-1°59'22.6	52°41'22.063
24	-1°10'09.691	50°49'37.615	**64**	1°18'31.525	51°07'46.051	**104**	-0°09'54.507	51°54'49.222
25	-1°21'08.593	52°03'26.866	**65**	-2°05'14.978	52°48'29.141	**105**	-2°57'06.461	52°34'36.695
26	0°17'35.039	51°36'09.261	**66**	1°20'55.393	52°37'59.29	**106**	-0°53'02.664	51°24'55.977
27	-1°52'09.871	50°48'14.111	**67**	-2°09'13.366	53°54'32.967	**107**	-1°35'36.183	55°10'03.579

28	-2°05'20.874	50°40'34.539	**68**	-3°00'30.05	53°52'21.589	**108**	-2°17'12.248	53°06'06.248
29	0°23'27.449	51°15'57.819	**69**	-1°02'24.874	54°12'17.012	**109**	-0°39'12.53	51°55'12.984
30	-1°40'08.457	51°14'45.421	**70**	0°37'04.245	51°51'01.047	**110**	-0°26'19.582	54°00'47.752
31	-0°09'58.735	53°34'43.744	**71**	-1°46'26.266	51°10'20.873	**111**	-0°07'33.777	52°44'34.73
32	-0°54'04.369	51°43'10.697	**72**	-3°01'46.536	51°36'28.325	**112**	-1°42'48.732	53°43'22.294
33	-1°55'05.323	52°08'14.759	**73**	-1°41'05.363	53°42'43.205	**113**	-1°36'47.676	53°45'08.061
34	-2°31'33.767	53°29'43.93	**74**	-0°51'31.722	51°40'43.575	**114**	1°11'37.915	51°13'31.101
35	-1°30'50.147	52°45'30.913	**75**	-2°30'17.158	53°21'12.977	**115**	-0°34'29.512	51°27'45.196
36	-2°08'46.841	53°32'32.804	**76**	-3°14'44.59	51°38'33.506	**116**	-2°04'47.2	51°00'06.488
37	-0°12'21.654	51°48'00.37	**77**	1°06'21.75	51°16'57.146	**117**	-1°52'24.498	50°52'33.117
38	-0°28'58.596	51°11'42.792	**78**	-1°43'57.726	53°48'26.615	**118**	-3°20'10.934	51°37'44.598
39	-1°50'02.688	50°45'41.792	**79**	-3°01'39.468	50°44'53.543	**119**	-1°47'52.329	53°50'04.146
40	0°18'41.414	51°13'31.394	**80**	-0°08'42.485	52°46'54.99	**120**	-0°11'23.054	51°46'19.118

Table S1: Geographical coordinates of bats collection points in WGS84 format (degrees minutes).

Bat Ref	Longitude	Latitude	Bat Ref	Longitude	Latitude	Bat Ref	Longitude	Latitude
121	-2°03'35.064	50°56'06.972	**146**	-2°46'50.891	53°54'17.711	**171**	-0°33'06.83	51°27'37.708
122	-2°02'50.638	52°46'16.536	**147**	-1°32'22.169	53°25'22.792	**172**	-2°07'19.948	53°32'42.61
123	-0°00'15.358	52°09'49.44	**148**	-2°46'31.999	51°16'31.158	**173**	-1°54'52.279	53°15'30.457
124	-2°27'51.85	53°30'36.624	**149**	-1°07'09.651	51°53'32.325	**174**	0°04'43.809	51°44'58.373
125	-1°02'39.404	51°27'23.508	**150**	-2°26'54.36	53°34'07.177	**175**	-0°43'23.38	51°02'52.476
126	-0°27'31.28	51°50'00.097	**151**	-1°54'27.161	52°19'02.049	**176**	-2°37'07.359	53°38'52.437
127	-0°42'36.35	52°52'52.068	**152**	-0°28'52.172	51°20'10.975	**177**	-1°46'06.217	51°42'39.816
128	-0°58'10.16	51°27'14.753	**153**	0°25'38.31	51°44'11.651	**178**	-3°20'05.734	51°37'44.657
129	-0°42'33.487	53°00'28.372	**154**	-0°58'39.823	50°49'28.9	**179**	-0°42'56.968	51°49'29.136
130	-2°43'08.678	51°08'49.535	**155**	-2°07'17.884	52°48'54.923	**180**	-1°24'49.619	53°02'35.18
131	-2°27'02.275	53°29'28.859	**156**	0°06'54.217	53°17'01.609	**181**	-1°40'16.621	54°39'55.978
132	-1°40'16.621	54°39'55.978	**157**	-1°20'53.776	52°44'16.853	**182**	-1°10'41.6	50°38'43.869

133	1°03'50.162	51°20'21.907	**158**	-2°59'27.614	53°50'48.27	**183**	-2°55'30.584	53°12'06.712
134	-1°40'39.56	53°51'24.093	**159**	-3°35'56.597	50°27'40.198	**184**	-1°24'59.169	53°43'37.826
135	-2°26'00.39	52°32'18.733	**160**	-2°04'02.953	52°20'26.26	**185**	-1°45'57.922	53°48'39.809
136	-1°50'53.714	53°46'04.969	**161**	-0°38'07.788	51°12'54.302	**186**	-2°29'37.576	53°03'05.816
137	-2°11'09.066	53°36'15.881	**162**	0°47'58.979	51°14'43.082	**187**	-0°34'13.512	51°14'09.202
138	-1°00'10.047	51°46'28.177	**163**	-2°46'16.014	53°37'34.827	**188**	-3°11'31.694	53°06'41.017
139	-2°12'12.935	52°53'26.364	**164**	-3°19'22.139	51°39'35.212	**189**	0°12'45.429	51°36'40.722
140	0°43'27.825	51°10'59.591	**165**	-0°48'27.088	51°48'50.531	**190**	-3°00'16.543	53°50'34.919
141	-4°14'17.286	53°18'53.278	**166**	-0°48'12.795	51°05'01.797	**191**	-1°33'46.052	52°45'44.543
142	-3°50'01.785	52°38'08.575	**167**	0°43'18.908	51°20'07.007	**192**	-2°48'39.728	51°20'10.42
143	-2°27'02.275	53°29'28.859	**168**	-1°57'17.282	53°26'37.147	**193**	-3°40'07.662	51°47'21.687
144	0°27'22.323	51°24'50.418	**169**	-2°47'23.822	53°30'04.588			
145	-0°46'45.176	51°50'29.832	**170**	-1°00'35.279	51°27'12.766			

Table S2: Statistical results of the Shapiro test (W) with their p values, applied on our dataset of metal concentrations in the different bat tissues and organs, formaldehyde aliquots and soil concentrations (NSRI dataset).

Metal	Statistical test	Kidneys (n=191)	Liver (n=190)	Stomach (n=168)	Bones (n=192)	Fur (n=192)	Formalin (n=100)	Soil (n=193)
Cd	W	0.46	0.15	0.27	0.13	0.05	0.10	0.46
	P value	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16
Cu	W	0.56	0.63	0.40	0.63	0.68	0.78	0.40
	P value	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16	4.718e-11	< 2.2e-16
Pb	W	0.35	0.13	0.33	0.73	0.17	0.14	0.41
	P value	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16
Zn	W	0.55	0.21	0.19	0.88	0.66	0.66	0.37
	P value	< 2.2e-16	< 2.2e-16	< 2.2e-16	3.833e-11	< 2.2e-16	8.426e-14	< 2.2e-16

Table S3: Descriptive statistics (mean and standard deviations) and differences (*post-hoc* t-test) between concentrations (µg/g dw for organs and tissues and µg/L dw for the formaldehyde) of the different metals (Cd, Cu, Pb and Zn) for a given sample type within the different years in which the samples were found. The *post hoc* t-test (F) was used to explore these differences. As multiple statistical tests were applied, the p values were adjusted using the Holm-Bonferroni method. Asterisks (*) indicates significant correlation ($p<0.05$) (after correction) and (n) indicates the sample size.

		Kidneys (µg/g dw)			**Liver (µg/g dw)**			**Stomach (µg/g dw)**			**Fur (µg/g dw)**			**Bones (µg/g dw)**			**Formaldehyde (µg/L dw)**		
		2008 (n=103)	**2009 (n=49)**	**2010 (n=39)**	**2008 (n=102)**	**2009 (n=49)**	**2010 (n=40)**	**2008 (n=90)**	**2009 (n=43)**	**2010 (n=35)**	**2008 (n=103)**	**2009 (n=49)**	**2010 (n=40)**	**2008 (n=103)**	**2009 (n=48)**	**2010 (n=41)**	**2008 (n=69)**	**2009 (n = 11)**	**2010 (n = 20)**
Mean ± SD	**Cd**	0.1 ± 0.2	0.2 ± 0.3	0.1 ± 0.1	0.1 ± 0.3	0.5 ± 1.9	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.5	0.3 ± 0.3	4.7 ± 30.0	0.2 ± 0.3	0.3 ± 0.3	0.8 ± 3.4	0.3 ± 0.4	0.1 ± 0.6	0.1 ± 0.1	0.01 ± 0.01
	Cu	18.2 ± 21.0	15.9 ± 12.4	19.0 ± 15.4	12.0 ± 7.7	15.0 ± 13.0	12.6 ± 5.5	16.0 ± 13.2	10.6 ± 5.0	24.3 ± 39.8	22.2 ± 18.2	15.8 ± 9.4	19.0 ± 14.3	4.1 ± 2.6	3.4 ± 1.5	4.1 ± 2.5	21.5 ± 8.6	23.4 ± 4.3	22.0 ± 4.1
	Pb	15.9 ± 48.8	20.5 ± 63.5	8.2 ± 20.7	35.8 ± 276.6	186.5 ± 826.0	7.6 ± 39.0	4.9 ± 15.2	2.4 ± 4.6	5.4 ± 11.8	427.6 ± 2060.9	99.0 ± 150.1	531.6 ± 2443.2	86.0 ± 112.0	85.9 ± 94.5	75.2 ± 76.4	13.9 ± 72.7	1.1 ± 1.2	1.5 ± 2.8
	Zn	24.8 ± 27.8	36.4 ± 54.7	42.2 ± 46.3	55.0 ± 198.7	306.5 ± 893.2	41.2 ± 87.6	41.5 ± 140.0	20.0 ± 18.3	43.4 ± 70.7	82.1 ± 63.3	88.0 ± 93.5	102.6 ± 59.1	302.9 ± 107.2	256.5 ± 184.2	264.9 ± 105.6	14.1 ± 13.6	21.7 ± 12.2	13.7 ± 6.8
		2008-2009	**2009-2010**	**2008-2010**	**2008-2009**	**2009-2010**	**2008-2010**	**2008-2009**	**2009-2010**	**2008-2010**	**2008-2009**	**2009-2010**	**2008-2010**	**2008-2009**	**2009-2010**	**2008-2010**	**2008-2009**	**2009-2010**	**2008-2010**
t df	**Cd**	-1.6182 81.447	1.0865 79.661	-0.57143 93.004	-2.9602* 64.738	1.5415 75.448	-1.6809 74.304	0.59521 63.637	-1.8501 62.833	-1.6431 46.35	0.18305 67.825	-0.17885 81.614	-0.021073 71.193	-0.094278 56.32	0.55694 75.933	0.71649 61.346	-1.8985 10.089	3.314* 9.475	3.1234* 56.4
	Cu	-0.095505 120.16	-1.7151 85.865	-1.9213 115.89	-1.8274 126	0.50027 85.779	-1.5164 128.94	2.9262* 73.829	-3.5872* 72.198	-1.5544 54.595	2.5212* 114.07	-1.0247 76.844	1.0444 73.984	1.9868 71.07	-2.0278 78.347	-0.23951 89.152	-1.8046 25.416	0.79444 20.083	-1.2436 67.078
	Pb	-1.1936 100.26	1.1391 84.583	0.11089 80.428	-1.6747 73.591	0.87813 83.917	-0.87747 77.826	0.81362 90.958	-1.3276 67.615	-0.74603 61.32	0.63636 99.157	-0.39039 82.829	0.14785 73.332	0.14422 69.665	-0.0084695 84.682	0.14723 68.271	1.0484 17.158	0.43664 23.857	1.7113 36.344
	Zn	-1.3609 83.678	-1.9535 85.917	-3.9663* 76.98	-2.6306* 66.857	1.21 66.345	-2.4484* 108.57	2.0879 78.486	-3.6065* 75.906	-2.1452 68.987	0.71666 72.554	-1.9197 86.19	-1.7156 59.553	2.3071 57.549	-1.3081 79.587	1.1529 60.716	-2.3561 13.797	1.5796 14.759	-1.2555 50.849

Figure S1: Maps of England and Wales showing the locations of where the 193 bats analyzed were collected with the soil metal concentrations (in µg/g dw) in background for a) Cd, b) Cu, and c) Zn. The bats presenting toxic residues (for Pb) or residues above the upper level concentrations (for Zn and Cu) (n=41) are represented in black circles, and the others are represented in white circles. The white grid cells represent an absence of data (NSRI dataset).

Cd soil concentrations are ranged from 0.05 to 1.5 (light grey cells) and 1.5 to 41 (dark grey cells). Cu soil concentrations are ranged from 0.04 to 70 (light grey cells), and 70 to 1507.7 (dark grey cells). Zn soil concentrations are ranged from 0.02 to 200 (light grey cells), and 200 to 3648 (dark grey cells).

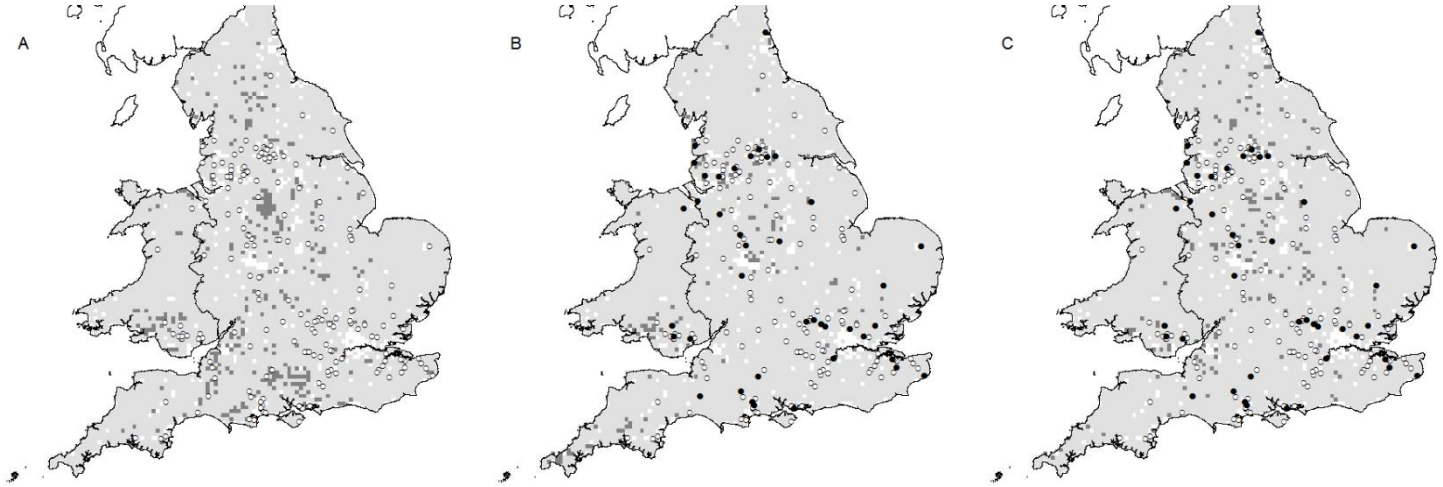

Figure S2: Histograms presenting the distributions of the soil concentrations of metals determined in the whole range of England and Wales (light grey) and the locations where the bat samples have been collected (dark grey). The soil concentrations of metals values were extracted from the NSRI dataset.

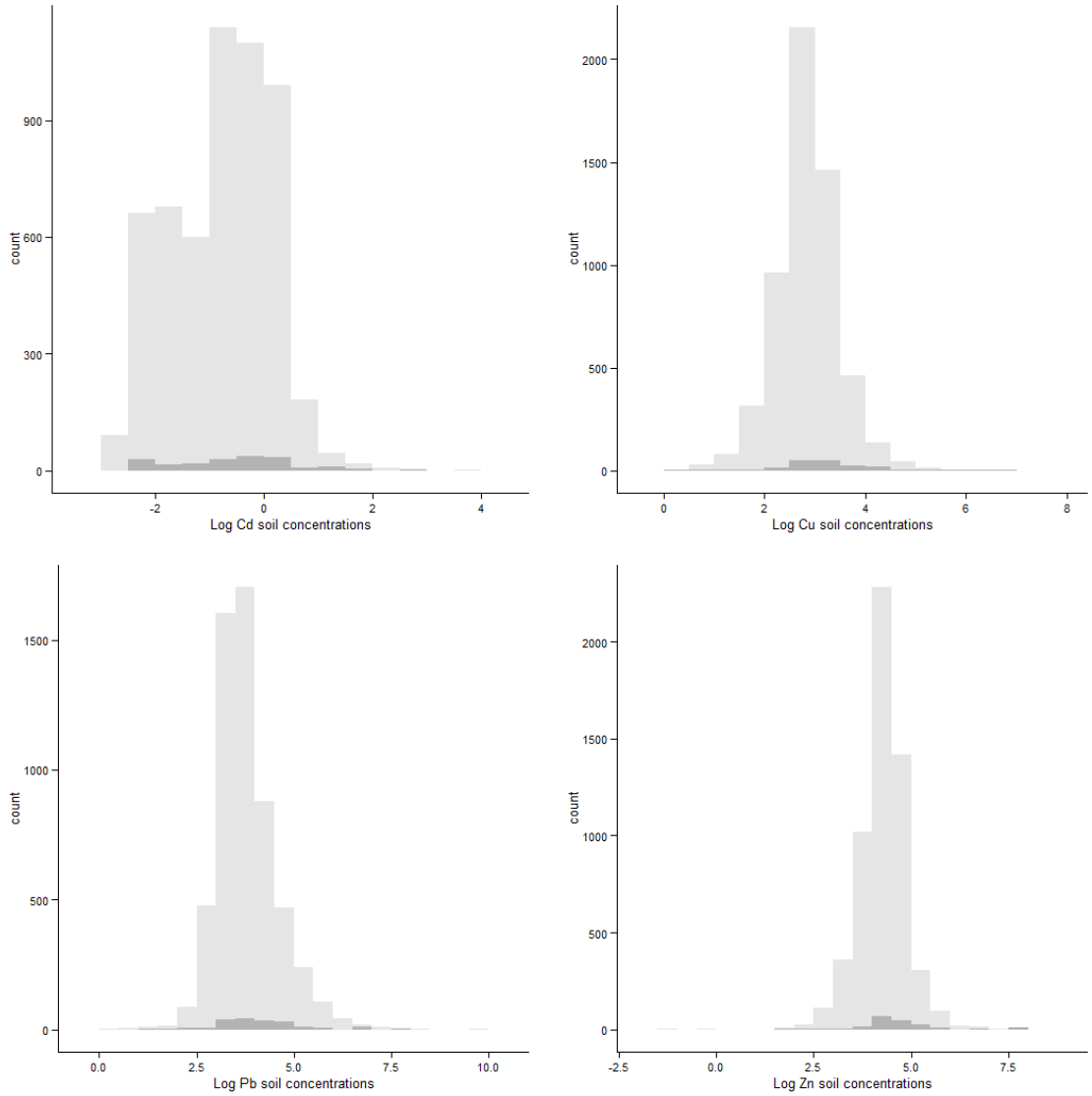

Figure S3: Maps showing the metal concentrations measured in organs and tissues (in µg/g dw) (kidneys, liver, stomach, fur and bones) of 193 *Pipistrellus sp*. sampled across England in Wales (in µg/g dw) for Cd, Cu, Pb and Zn. The letters indicate the metal and the numbers in subscript indicate the tissue analyzed, as following: a) Cd, b) Cu, c) Pb and d) Zn and 1) kidneys 2) liver 3) stomach 4) fur 5) bones. The toxicological thresholds, and the lower and upper range values of concentrations measured in small mammals were included as break values.

Cd concentrations in tissues are ranged from 3.6 X 10^{-3} to 0.25 (light grey cells), 0.25 to 0.75 (dark grey cells) and 0.75 to 0.8 (black cells) (Map a_1); from 1.5 X 10^{-3} to 0.25 (light grey cells), 0.25 to 0.75 (dark grey cells) and 0.75 to 13 (black cells) (Map a_2); from 1.7 X 10^{-3} to 0.25 (light grey cells), 0.25 to 0.75 (dark grey cells) and 0.75 to 2 (black cells) (Map a_3); from 3.9 X 10^{-3} to 0.5 (light grey cells), 0.5 to 1 (dark grey cells) and 1 to 212 (black cells) (Map a_4); and from 5.0 X 10^{-4} to 0.5 (light grey cells), 0.5 to 1 (dark grey cells) and 1 to 24 (black cells) (Map a_5); in kidneys, liver, stomach, fur and bones, respectively.

Cu concentrations in tissues are ranged from 3.5 X 10^{-2} to 20 (light grey cells), 20 to 30 (dark grey cells) and 30 to 134 (black cells) (Map b_1); from 3.3 X 10^{-2} to 20 (light grey cells), 20 to 30 (dark grey cells) and 30 to 71 (black cells) (Map b_2); from 5.3 X 10^{-1} to 25 (light grey cells), 25 to 100 (dark grey cells) and 100 to 240 (black cells) (Map b_3); from 2.2 to 10 (light grey cells), 10 to 40 (dark grey cells) and 40 to 103 (black cells) (Map b_4); and from 2.1 X10^{-1} to 3 (light grey cells), 3 to 10 (dark grey cells) and 10 to 25 (black cells) (Map b_5); in kidneys, liver, stomach, fur and bones, respectively.

Pb concentrations in tissues are ranged from 5.2 X 10^{-3} to 15 (light grey cells), 15 to 25 (dark grey cells) and 25 to 367 (black cells) (Map c_1); from 2.4 X 10^{-3} to 5 (light grey cells), 5 to 10 (dark grey cells) and 10 to 5039 (black cells) (Map c_2); from 4.0 X 10^{-3} to 50 (light grey cells), 50 to 100 (dark grey cells) and 100 to 134 (black cells) (Map c_3); from 4.5 X 10^{-2} to 100 (light grey cells), 100 to 350 (dark grey cells) and 350 to 20398 (black cells) (Map c_4); and from 2.8 X 10^{-3} to 100 (light grey cells), 100 to 350 (dark grey cells) and 350 to 708 (black cells) (Map c_5); in kidneys, liver, stomach, fur and bones, respectively.

Zn concentrations in tissues are ranged from 1.3 to 87 (light grey cells), 87 to 274 (dark grey cells) and 274 to 354 (black cells) (Map d_1); from 8.0 X 10^{-1} to 71 (light grey cells), 71 to 465 (dark grey cells) and 465 to 5205 (black cells) (Map d_2); from 1.1 to 200 (light grey cells), 200 to 400 (dark grey cells) and 400 to 1336 (black cells) (Map d_3); from 11.8 to 100 (light grey cells), 100 to 200 (dark grey cells) and 200 to 578 (black cells) (Map d_4); and from 7.0 X 10^{-1} to 250 (light grey cells), 250 to 500 (dark grey cells) and 500 to 1029 (black cells) (Map d_5); in kidneys, liver, stomach, fur and bones, respectively.


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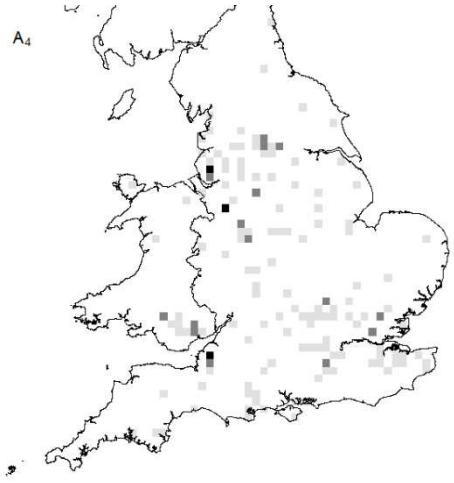

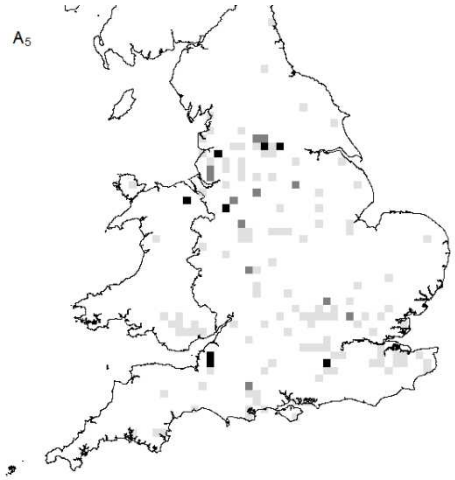

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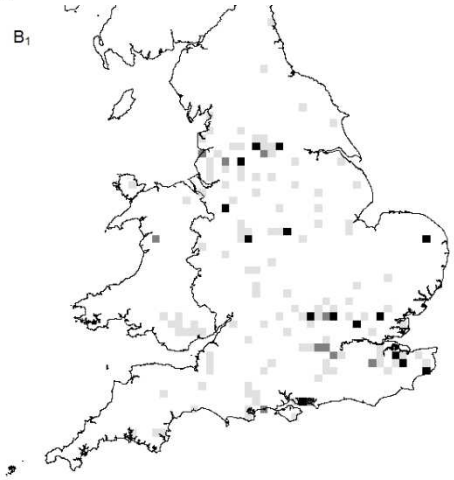


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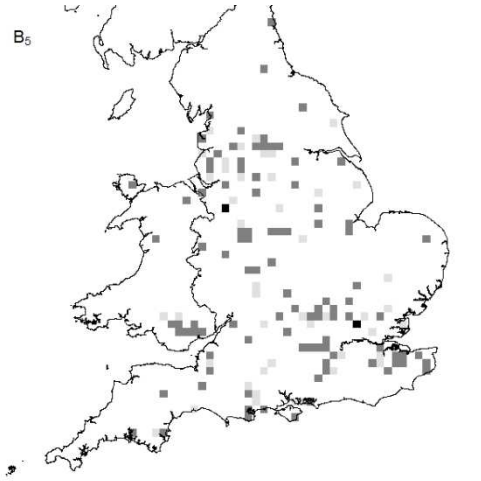

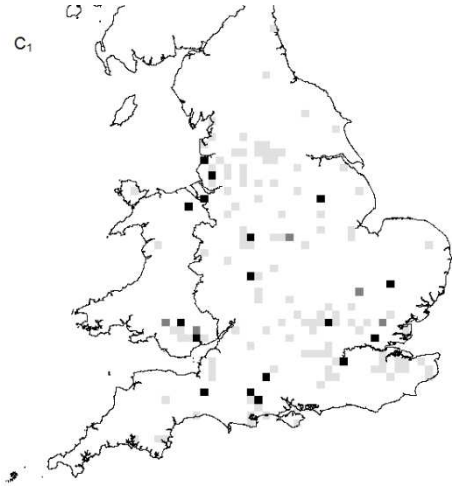

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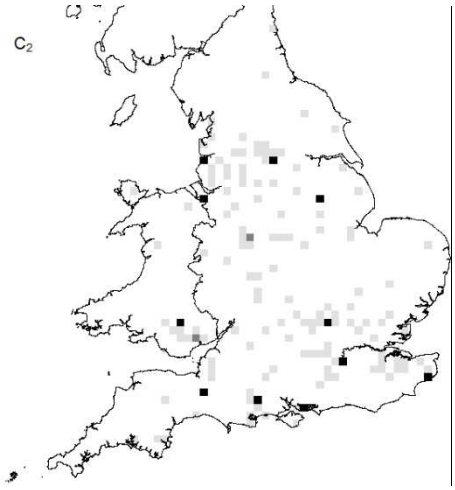

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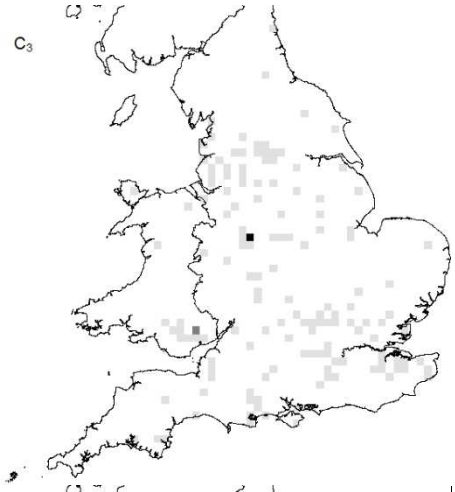

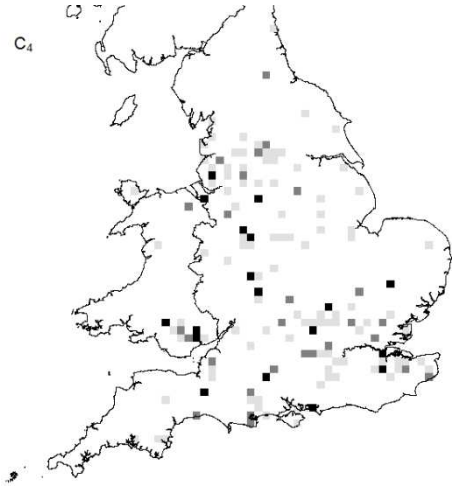

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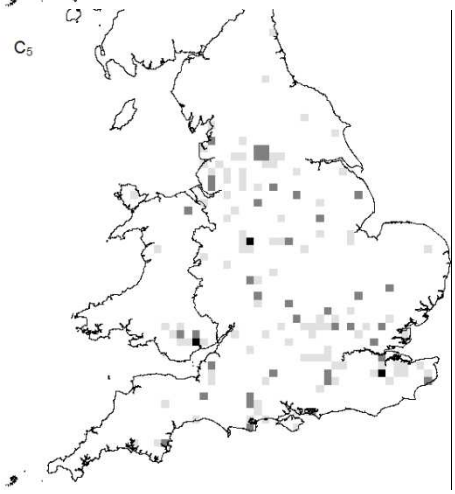

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D1
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