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A national level assessment of metal contamination in bats☆

Béatrice V. Hernout [a, b, *], Kathryn E. Arnold [a], Colin J. McClean [a], Michael Walls [b], Malcolm Baxter [b], Alistair B.A. Boxall [a]

[a] Environment Department, University of York, Heslington, York, YO10 5NG, UK
[b] The Food and Environment Research Agency Sand Hutton, Fera Science Ltd (Fera), National Agri-Food Innovation Campus, Heavy Metals in Food & Feed National Reference Laboratory, Sand Hutton, York, YO41 1LZ, UK

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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 -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 associations 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 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.

1. 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

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* Corresponding author. Present address: Department of Environmental Toxicology, The Institute of Environmental and Human Health (TIEHH), Texas Tech University, 1207 Gilbert Drive, Box 41163, TX 79409-3290, Lubbock, TX, USA.
E-mail address: beatrice.hernout@gmail.com (B.V. Hernout).

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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 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 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 and stomach content, 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.

2. Materials and methods

2.1. 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 (Fig. 1, Fig. 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 3000 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 (Fig. S2). Data on metal concentrations in soils, from the locations at which the 3000 bats were found, were acquired from the National Soil Resources Institute (NSRI) soil dataset (5 × 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 (Fig. 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 (Fig. 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