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Relationships between soil and badger elemental concentrations across a heterogeneously contaminated landscape



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HIGHLIGHTS

GRAPHICAL ABSTRACT

- Element concentrations in liver and soil were determined for 448 badgers in England.
- No relationships were seen between liver and soil essential element concentrations.
- Non-essential element liver and soil concentrations were positively correlated.
- Pb and Cd liver concentrations were notably elevated in ex-mining areas.
- Badgers can be used to highlight local non-essential elemental contamination.

Relationships between soil and badger elemental concentrations across a heterogeneously contaminated landscape What are the links between environmental and wildlife elemental concentrations? Badger liver elemental concentrations between environmental and wildlife elemental concentrations? Image: Discrete the links between environmental and wildlife elemental concentrations? Image: Discrete the links between environmental and wildlife elemental concentrations? Image: Discrete the links between environmental and wildlife elemental concentrations? Image: Discrete the links between environmental and wildlife elemental concentrations? Image: Discrete the links between environmental environmental and wildlife elemental concentrations? Image: Discrete the links between environmental environmental elemental concentrations? Image: Discrete the links between environmental element elemental element elements? Image: Discrete the links between elemental element elements? Image: Discrete the links between elemental elements

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ABSTRACT

Understanding the links between environmental and wildlife elemental concentrations is key to help assess ecosystem functions and the potential effects of legacy pollutants. In this study, livers from 448 European badgers (*Meles meles*) collected across the English Midlands were used to investigate the relationship between elemental concentrations in topsoils and wildlife. Mean soil sample concentrations within 2 km of each badger, determined using data from the British Geological Survey's 'Geochemical Baseline Survey of the Environment', were compared to badger liver elemental concentrations, focusing primarily on Ag, As, Cd, Cr, Cu, K, Mn, Pb, Se, Zn. Generally, the badgers appeared to have elemental concentrations comparable with those published for other related animals, though Cu concentrations for most biologically usesential elements, biologically non-essential elements, specifically Pb, Cd, As, and Ag, were positively correlated between soil and badger livers. Lead and Cd, the elements with the strongest relationships between soils and badger livers, were primarily elevated in badgers collected in Derbyshire, a county with a millennia-long history of Pb mining and significant Pb and Cd soil pollution. Cadmium concentrations in badgers were also, on average, almost nine times higher than the local soil concentrations, likely due to Cd biomagnification in earthworms, a dietary staple

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of badgers. While badgers are good models for studying associations between soil and wildlife elemental concentrations, due to their diet, burrowing behaviours, and site fidelity, all flora and fauna local to human-modified environments could be exposed to and impacted by legacy pollutants.

1. Introduction

The elemental composition of soils varies locally and globally due to both geological factors (Garrett, 2013) and anthropogenic pollution, stemming from activities such as mining, smelting, industrial processes and combustion of fossil fuels (Wuana and Okieimen, 2011; Kabir et al., 2012; Tchounwou et al., 2012; Khan et al., 2021). For example, centuries of intensive Pb and Zn mining in Derbyshire in the U.K. has resulted in high metal concentrations in soils across the region (Rawlins et al., 2012). Elemental pollution can remain in the environment for hundreds of years after the responsible processes have ceased operation, so soil elemental compositions can reflect historical, as well as current, anthropogenic activities (Merrington and Alloway, 1994; Alibrahim et al., 2017).

Both the composition and concentration of elements in the soil can affect their uptake by resident animals (Hettiarachchi and Pierzynski, 2004; Scheckel et al., 2009). Transfer into animals depends on the bioavailability of the contaminant, which is affected by the chemical form of the contaminant and soil conditions. Other factors influencing elemental transfer include the nutritional and metabolic function of the contaminant and the animal's level of exposure to the contaminated soil (Hettiarachchi and Pierzynski, 2004; Scheckel et al., 2009; Tchounwou et al., 2012). Many studies have examined elemental (primarily trace metal) concentrations in wild terrestrial mammals and have attempted to correlate these with environmental concentrations (Wren, 1986; Gall et al., 2015; Rainbow, 2018). These studies indicate that mammals can take up elements, particularly non-biologically essential elements, from environmental sources, and that they can accumulate them to potentially toxic concentrations, though elemental toxicity and toxic thresholds are hard to define, particularly when comparing across species) (Rainbow, 2018; McCarty et al., 2020). However, few studies have focused on multiple elemental compositions, and few have been able to correlate soil elemental concentrations directly with levels in individual animals.

The European badger (Meles meles) is a medium-sized mammal commonly found across Europe. Badgers are generalist feeders; in England, they primarily consume earthworms (Lumbricus terrestris) whenever available (Macdonald and Newman, 2022), as well as insects and plant matter, which they obtain mainly by foraging through topsoil (Shepherdson et al., 1990; Cleary et al., 2009; Balestrieri et al., 2019). Lumbricus terrestris have been known to accumulate high elemental concentrations, particularly in metal-polluted sites, and it has been suggested that these concentrations may transfer into their predators (Kennette et al., 2002; Uba et al., 2009; Cooke, 2011). Badgers also have a high site fidelity, as they live in family-based groups in burrow systems (dug by the badgers) with relatively small territories, meaning that they are unlikely to move much further than 4-5 km from their place of birth (Kruuk and Parish, 1982; Gaughran et al., 2018). This combination of high soil exposure and strong site fidelity make badgers an ideal study species when investigating elemental transference from the environment to animals.

While a few studies have examined elemental compositions in badgers, they have mainly focused on a relatively small number of individuals and elements (Van den Brink and Ma, 1998; Bilandžić et al., 2012; Ozimec et al., 2015; Mullineaux et al., 2021). A multi-elemental analysis of hundreds of badgers collected across a continuous area with diverse geology and varied historical and current human activities may enable a better understanding of the relationships between soil and animal elemental compositions. The aim of this study was therefore to investigate the relationship between the elemental compositions of topsoil and livers collected from badgers from the English Midlands, examining 25 metals, 3 non-metals, and 2 metalloids. Of these, 10 elements (eight metals: Ag, Cd, Cr, Cu, K,

Mn, Pb, Zn; one metalloid: As; and one non-metal: Se) were explored in more detail to determine possible accumulation variations across different elements, both biologically essential and non-essential, and locations within the region.

2. Methods

2.1. Sample collection and processing

Carcasses of found-dead badgers were collected from an approximately 1.4 million hectare area of England (including the counties of Cheshire, Derbyshire, Nottinghamshire, Leicestershire, Warwickshire, and Northamptonshire) from August 2016 to June 2017, as part of a study of bovine tuberculosis (Fig. 1; Swift et al., 2021). These badger carcasses were primarily collected on roadsides by citizen scientists. Following post-mortem examination (Sandoval Barron et al., 2018; Swift et al., 2021), liver samples, chosen because of their key role in element regulation and accumulation (Kalisińska, 2019), were extracted from 448 of these badgers and freeze dried before being acid digested.

Between 0.1 and 0.2 g of liver, along with 4 mL 70 % HNO_3 and 1 mL H₂O₂, were incubated on a hotplate block digester at 95 °C. After 2 h, the samples were allowed to cool before being dispensed into plastic volumetric flasks, made up to 50 mL with MilliQ water (18.2 M Ω cm; Millipore Corporation, Darmstadt, Germany), and gently mixed. The samples were then left for at least 10 min to allow for heavier material to settle to the bottom of the tube, and 10 mL of the supernatant solutions were decanted and stored at ambient temperature. The solutions were diluted 1-in-10 with MilliQ water prior to elemental analysis using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Model ICAP-Q; Thermo Fisher Scientific, Bremen, Germany). A certified reference material for trace elements in biological samples (BRC-185R Bovine Liver [trace elements]) was run for quality assurance purposes (recovery values for specific elements were: As [74.9 %], Cd [103.9 %], Cu [94.9 %], Mn [99.1 %], Pb [94.2 %], Se [90.1 %], and Zn [101.2 %]). For each element, the operational limit of detection (LOD) was calculated as three times the standard deviation of the concentrations measured in 10 blank digestion samples run alongside the badger livers (Table 1). A value of 0.5*LOD was used in instances where the elemental concentration was lower than the LOD (Kushner, 1976). All concentrations were calculated as mg kg^{-1} dry weight; comparisons with published fresh weight concentrations were performed assuming a liver water content of 72.1 %, as described for European badgers by Kalisińska et al. (2009).

2.2. Data analyses

Soil and sediment elemental concentrations were obtained from the British Geological Survey's *Geochemical BAseline Survey of the Environment* (G-BASE) project (Johnson et al., 2005). These datasets consists of over 40,000 topsoil and 54,000 sediment sampling points across England and Wales where elemental concentrations were measured using X-Ray Fluorescence (XRF) (Johnson et al., 2005). In order to illustrate relative concentrations across the landscape studied, for each element examined, maps were generated in QGIS (QGIS.org, 2022), with soil (or sediment, in the absence of soil) concentrations categorised into four classes: 1) 0 to mean, 2) mean to mean + one standard deviation, 3) mean + one standard deviation to mean + two times the standard deviation, and 4) mean + two times the standard deviation to maximum. All calculations were based on the soil concentrations within the mapped area surrounding the badger collection locations (-3.508178, 51.522730: 0.427985, 53.700807). If no soil



Fig. 1. Map of badger collection locations. Derbyshire is highlighted in green. Satellite imagery map data ©2022 Google.

concentration data was available for a particular element, comparisons were instead based on the sediment concentrations, if available. Any G-BASE concentrations above the BGS's published maximum concentration of the element in soils in England and Wales (Rawlins et al., 2012) were removed to avoid outliers from skewing the scale.

Elemental concentrations in badger livers were mapped alongside the BGS soil and sediment maps using QGIS (QGIS.org, 2022). The badger elemental concentrations were categorised into five classes, designed to highlight notably elevated concentrations: 1) 0 to mean, 2) mean to two times the standard deviation, and 3–5) three equal intervals between two times the standard deviation and the maximum concentration of the element found in the badger livers.

To compare soil and badger liver elemental concentrations, mean soil elemental concentrations were determined at the badger collection locations for some selected elements (Ag, As, Cd, Cr, Cu, K, Mn, Pb, Se, Zn). Soil concentrations at the three nearest sampling points, within 2 km, to each badger collection location were averaged. If there were fewer than three soil collection points within 2 km of the badger, no overall soil concentration was determined (see Supplementary Table 1 for numbers of badgers with corresponding soil concentrations for each selected element). Two kilometers were chosen based on badger territory diameters, which are usually between 1 and 2 km, dependent on badger population density and food availability (Kruuk and Parish, 1982). While badgers can disperse over long distances (Byrne et al., 2014), most movements are within territories, so elemental concentrations within a 2 km radius of the collection site should represent likely exposure levels for the badgers.

Relationships between elemental concentrations in the liver and soil were examined through correlations in R (R Core Team, 2018). Due to a lack of normality in the data, identified by Shapiro-Wilk tests, Spearman's

partial correlation coefficients (controlling for when the badger was collected, to account for possible seasonality) were calculated, and the concentrations were log-transformed for any corresponding graphs to more clearly show relationships. To decrease the chance of a type I error, Benjamini -Hochberg corrections were utilised for each statistical test type, with the total number of tests reflecting the number of elements included in each test type (Benjamini and Hochberg, 1995). This was implemented by calculating adjusted *p*-values by multiplying the p-value by the total number of tests and dividing by the p-value's rank (when all relevant p-values were ordered smallest to largest), as described in Yekutieli and Benjamini (1999). Graphs visualizing these statistical tests were generated in R using the ggplot2 package (Wickham, 2016).

3. Results

3.1. Elemental concentrations in badger livers

The elemental concentrations in badger livers varied greatly across the 30 elements investigated (Table 1). Most elemental concentrations, however, appeared to have an approximately normal distribution, but with a long positive tail that prevented the concentrations from being truly normally distributed (Fig. 2, Table 1, Supplementary Fig. 1).

3.2. Relationship between soil and liver concentrations

Elemental concentrations in both soil and badger livers varied across the study area (Fig. 3, Supplementary Fig. 2). The soil elemental concentrations in the study area are known to vary, due to both natural geochemical properties and anthropogenic pollution (Rawlins et al., 2012). Badger liver

Table 1

Summary of badger liver elemental concentrations. All concentrations are in mg kg⁻¹ dry weight. If the minimum value was below the limit of detection, it is reported as "LOD" in this table, and treated as 0.5*LOD for all summary statistic calculations.

Element	Mean	Median	Minimum	Maximum	Standard deviation	Limit of detection
Ag	0.0381	0.0255	0.00154	0.580	0.0424	0.000425
Al	8.37	3.82	LOD	279	22.8	3.22
As	0.0906	0.0737	0.0185	0.683	0.0648	0.00636
В	1.63	0.695	LOD	27.2	3.20	0.00217
Ba	0.487	LOD	LOD	50.3	2.43	0.250
Be	0.0257	LOD	LOD	0.127	0.0173	0.0372
Ca	286	224	65.0	1970	193	0.0500
Cd	3.37	1.74	0.0450	65.7	6.39	0.00317
Co	0.149	0.118	0.0327	1.03	0.105	0.00228
Cr	0.894	0.0640	LOD	12.5	2.14	0.0229
Cs	0.00707	0.00435	LOD	0.111	0.0104	0.00111
Cu	32.0	26.2	2.38	143	19.8	0.115
Fe	1160	1110	298	4990	488	0.442
K	8140	8250	2810	13,400	1670	0.00334
Mg	596	605	201	898	111	0.00176
Mn	16.0	8.59	0.823	122	20.1	0.0517
Mo	2.54	2.57	0.176	4.13	0.609	0.00697
Na	3930	3830	1020	9620	1100	0.0184
Ni	0.145	0.0795	LOD	1.07	0.172	0.0325
Р	9020	9210	2800	13,200	1690	0.00718
Pb	1.38	0.486	0.0744	32.6	3.56	0.0269
Rb	6.45	5.05	0.898	38.7	4.70	0.00596
S	8590	8990	1880	12,900	2000	0.665
Se	3.05	2.92	1.06	15.4	1.13	0.00299
Sr	0.328	0.245	LOD	3.57	0.312	0.0927
Ti	105	76.3	30.7	1550	112	9.28×10^{-6}
T1	0.0143	0.0112	0.000636	0.117	0.0113	7.39×10^{-5}
U	0.00235	LOD	LOD	0.0526	0.00545	0.000684
V	0.282	0.232	0.0278	1.70	0.208	0.00357
Zn	127	117	25.7	674	57.0	2.55

concentrations across the study area appeared to reflect patterns in local soil concentrations for some of the examined elements (such as As and Pb), although not for all (Fig. 3).

The relationships between soil and badger elemental concentrations were explored in greater depth for ten elements: eight metals (Ag, Cd, Cr, Cu, K, Mn, Pb, Zn), one metalloid (As), and one non-metal (Se). These elements were chosen because of relatively complete G-BASE sampling for these elements in the soils in the study area, and because of the high degree of variability in both the soil and badger liver concentrations. Furthermore, the group was specifically chosen to include both biologically essential and non-essential elements, to assess whether this factor affects accumulation in badgers. Of these ten elements, there were significant correlations between concentrations in badger livers and local soils for five of the elements: Ag, As, Cd, Pb, and Se (Fig. 4). These correlations varied from slight (As: $\rho = 0.130$, Se: $\rho = 0.177$) to moderate (Ag: $\rho = 0.353$, Cd: $\rho = 0.366$), with the strongest relationship being between Pb in soils and livers ($\rho = 0.457$). The concentrations of the other five elements (Cr, Cu, K, Mn, and Zn) were not significantly correlated between livers and soils.

All five elements that were correlated between badger livers and soils were also significantly correlated with each other in badger livers (Table 2). In particular, Se concentrations were moderately correlated with all other metal concentrations (Ag: $\rho = 0.350$; As: $\rho = 0.334$; Cd: $\rho = 0.339$; Pb: $\rho = 0.277$), while Pb was strongly correlated with Cd ($\rho = 0.560$), and moderately correlated with Ag ($\rho = 0.292$) (Table 2). Cd and Se liver concentrations also significantly varied between juveniles and adults (Cd: z = -4.00, p = 0.000324; Se: z = 2.74, p = 0.00616), with Cd higher in adults than juveniles, and Se higher in juveniles than adults. However, Ag (z = 1.99, p = 0.0775), As (z = -0.355, p = 0.723), and Pb (z = 0.483, p = 0.723) liver concentrations did not significantly vary between juveniles and adults.

All five elements were also correlated with each other in the soils from around the badger collection points, with the exception of As and Cd, which were not significantly correlated to each other (Table 2). Selenium was strongly correlated with Pb ($\rho = 0.622$) and As ($\rho = 0.543$), and Pb, Cd, and Ag were all moderately correlated with each other (Table 2). Despite being positively correlated in the badger livers, Ag and As had a slight negative correlation in soils at badger collection points ($\rho = -0.176$) (Table 2).

4. Discussion

4.1. Implications of badger liver elemental concentrations

Overall, most of the badgers collected during this study appeared to have hepatic elemental concentrations broadly similar to those found in prior studies examining wild European badgers or other wild mustelids (Bilandžić et al., 2012; Ozimec et al., 2015; Kosik-Bogacka et al., 2019; Pilarczyk et al., 2019; Mullineaux et al., 2021), and to established ranges for healthy individuals of domestic species (primarily bovine, porcine, and ovine) (Eisler, 1998; WVDL, 2015). However, there are no published 'thresholds' for concentrations associated with acute or chronic toxicity in badgers, and few published expected concentrations for mustelids, so contextualizing these concentrations is difficult. The mean Cd concentration $(3.37 \pm 6.39 \text{ mg kg}^{-1} \text{ dry weight})$ was elevated above concentrations found in wild badgers in Croatia in prior studies (geometric mean of 1.92 mg kg⁻¹ dry weight [converted] across three badgers, Bilandžić et al., 2012; mean of 1.42 mg kg⁻¹ dry weight [converted] across 29 badgers, Ozimec et al., 2015). Furthermore, four individuals had Cd liver concentrations indicative of possible toxicity (35.8 mg kg^{-1} dry weight [converted] in vertebrate livers, Eisler, 1985), while eight individuals had Pb concentrations indicative of possible toxicity (17.9 mg kg⁻¹ dry weight [converted] in mammal livers, WVDL, 2015).

The Cu concentrations detected in the badger livers were relatively low compared to prior studies. A quarter of the sampled badgers had liver Cu concentrations that were indicative of Cu deficiency in mustelids (2.86–17.9 mg kg⁻¹ dry weight [converted]; WVDL, 2015). Copper deficiency can have long-term health effects, including slow growth, emaciation, impaired immune response, and reduced survival in mammals (Eisler, 1998). However, there have been few studies investigating Cu concentrations and deficiency thresholds for mustelids, so it is unclear whether the badgers were truly Cu deficient, or just had naturally relatively low Cu concentrations. Copper concentrations in livers are known to vary widely, even across similar species and areas (Łanocha-Arendarczyk and Kosik-Bogacka, 2019). When compared to previous studies on wild badgers, the mean liver Cu concentration (32.0 \pm 19.8 mg kg⁻¹ dry weight) was lower than those found in Northern Ireland (mean of 77.6 mg kg⁻¹ dry weight across 57 badgers; Mullineaux et al., 2021) and Croatia (geometric mean of 54.5 mg kg^{-1} dry weight [converted] across three badgers; Bilandžić et al., 2012). As the liver Cu concentrations did not correlate with local soil concentrations, and as the badgers with low liver Cu concentrations were distributed throughout the whole of the study area, the significance and possible causes of these low concentrations are unclear, though Cu deficiencies are usually linked to insufficient Cu in diets (Fisher, 1975).

4.2. Essential element accumulation in badgers

Of the 10 elements examined in more detail, Cr, Cu, K, Mn, Se, and Zn are considered biologically essential elements, as they perform key biochemical and physiological functions necessary for life (Tchounwou et al., 2012). Their concentrations are normally highly regulated in the body, and excretion methods are in place to remove excessive concentrations (Tchounwou et al., 2012; Kalisińska and Budis, 2019; Łanocha-Arendarczyk and Kosik-Bogacka, 2019). While these mechanisms can be overwhelmed, this normally occurs during acute exposure, as opposed to chronic, environmental exposure (Kalisińska and Budis, 2019; Łanocha-Arendarczyk and Kosik-Bogacka, 2019). The concentrations of the essential elements in the soil at the badger collection locations varied from the bottom 20 % to the top 10 % of the respective elemental concentrations



Fig. 2. Histograms of badger liver concentrations of 10 key elements (Ag, As, Cd, Cr, Cu, K, Mn, Pb, Se, Zn). The red line represents the mean, while the blue line represents two standard deviations from the mean.

found overall in English and Welsh soils (Rawlins et al., 2012). Despite this wide environmental variation, Cr, Cu, K, Mn, and Zn concentrations in the badger livers remained relatively consistent across the study area, and there was no relationship between their concentrations in the soil and the badger livers.

Essential elements are not usually accumulated from the environment and sequestered into bodies, primarily due to active regulation of these elements. Previous studies on Cr, Cu, and Mn have found little evidence of accumulation in terrestrial mammals related to environmental exposure (Kalisińska and Budis, 2019; Kośla et al., 2019; Łanocha-Arendarczyk and Kosik-Bogacka, 2019). Potassium accumulation has not been studied in depth, as few studies examine K in wildlife (Vengušt and Vengušt, 2004), but, as a key essential element, its lack of accumulation in badger livers is unsurprising. Accumulation of Zn has been reported, but depends on the species in question, as well as the tissue examined (Kosik-Bogacka and Łanocha-Arendarczyk, 2019). Generally, while Zn seems to accumulate in



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Fig. 4. Badger liver metal log₁₀ concentration versus soil metal log₁₀ concentration of 10 key elements (Ag, As, Cd, Cr, Cu, K, Mn, Pb, Se, Zn). Metals that correlate between liver and soil concentrations are on the left, while metals with no correlation between liver and soil are on the right. The reported *p*-values are adjusted following Yekutieli and Benjamini (1999).

Fig. 3. Maps of badger collection sites, showing badger liver concentration and soil concentrations of 10 key elements (Ag, As, Cd, Cr, Cu, K, Mn, Pb, Se, Zn). Soil concentrations are derived from 2,300,000 scale BGS Digital Data under Licence No. 2021/086 British Geological Survey @ and Database Right UKRI. All rights reserved. Soil concentrations are grouped into four classes: 1) 0 – mean, 2) mean – mean + standard deviation, 3) mean + standard deviation – mean + two times the standard deviation, and 4) mean + two times the standard deviation – maximum. Badger liver concentrations are grouped into five classes: 1) 0 – mean, 2) mean – two times the standard deviation, and 3–5) three approximately equal intervals between two times the standard deviation and the maximum concentration of the element found in the badger livers.

Table 2

Significant correlations between Ag, As, Cd, Pb, and Se. On the bottom left of the table, shown in grey, are correlations between the soil concentrations found at the badger collection sites, while on the top right of the table, shown in white, are the correlations between the badger liver concentrations. Non-significant correlation R values are not reported, and are replaced by "–". The reported p-values were adjusted following Yekutieli and Benjamini (1999). All correlations were calculated using the Spearman's correlation coefficient.

		Ag	As	Cd	Pb	Se
Ag	R		0.213	0.139	0.292	0.350
	р		< 0.0001	0.00353	< 0.0001	< 0.0001
As	R	-0.176		0.0995	0.157	0.334
	р	0.00111		0.0353	0.00108	< 0.0001
Cd	R	0.424	_		0.560	0.339
	р	< 0.0001	0.577		< 0.0001	< 0.0001
Pb	R	0.336	0.267	0.430		0.277
	Р	< 0.0001	< 0.0001	< 0.0001		< 0.0001
Se	R	0.138	0.534	0.360	0.622	
	р	0.0104	< 0.0001	< 0.0001	< 0.0001	

ruminants living in Zn-polluted environments, this trend has not been strongly detected in carnivorous mammals (Dip et al., 2001; Kosik-Bogacka and Łanocha-Arendarczyk, 2019; Ziętara et al., 2019). In some studies, Cu and Zn concentrations have been found to vary between mustelids in control and Cu—Zn contaminated sites (Van den Brink and Ma, 1998; Sanders et al., 2020). However, these variations could be attributed to dietary differences, which are known to affect Cu and Zn concentrations in mustelids (Brzeziński et al., 2014), or to sampling in discrete areas or areas with a clear contamination gradient, as opposed to a heterogeneously contaminated landscape, such as the area sampled during this study.

Of the essential elements explored in more detail during this study, only Se soil and liver concentrations were positively significantly correlated, albeit only slightly ($\rho = 0.177$). This agrees with prior studies, as Se, unlike most other essential elements, has been found to accumulate in terrestrial mammals based on their environment (Pilarczyk et al., 2019). Selenium accumulation has been observed in rodents exposed to contaminated sediments, food, or water (Clark, 1987; Stoewsand et al., 1990; Levengood and Heske, 2008). One study, focusing on a floodplain contaminated with a number of elements, including Se, Zn, and Cu, found that Se in particular accumulated to potentially toxic concentrations in white-footed mouse (*Peromyscus leucopus*) livers (Levengood and Heske, 2008). In mustelids, higher Se concentrations in river otters (*Lontra canadensis*) have been linked to local environmental Se pollution (Sanders et al., 2020).

Selenium has also been known to vary across juveniles and adults, normally accumulating to higher concentrations with age (Pilarczyk et al., 2019). This is the opposite pattern to that observed in this study, where adults had significantly, but only slightly, lower liver Se concentrations than juveniles. It is possible that badgers accumulate Se only until they are sexually mature, as has been hypothesised for raccoons (Clark et al., 1989), or that the differences observed in this study are affected by season (Pilarczyk et al., 2019) and when the juveniles were born (Malzahn and Lang, 1980). It is also possible that comparing Se concentrations across only two age classes, as opposed to exact ages, affected the observed pattern.

4.3. Non-essential element accumulation in badgers

The other four elements examined more closely during this study (Ag, As, Cd, and Pb) are non-essential and potentially toxic at relatively low concentrations (Tchounwou et al., 2012), so their presence in badgers could potentially adversely affect badger health and population dynamics. The concentrations of all four of these non-essential elements were correlated between soils and badger livers, indicating that the badgers accumulated these elements at least partly based on their environmental exposure.

Lead, the element with the strongest soil-liver correlation ($\rho=0.459)$ in this study, is known to accumulate in mammalian livers when animals

are exposed to lead pollution from human mining or industrial activities (Baranowska-Bosiacka et al., 2019). Cd, which also had a notable soilliver correlation ($\rho = 0.368$), has similarly been known to accumulate in the tissues of terrestrial animals living in Cd-contaminated environments (Tomza-Marciniak et al., 2019). Elevated Pb and Cd concentrations have been found in badgers and other mustelids in previous studies on wildlife living in human-modified landscapes (Harding et al., 1998; Alleva et al., 2006; Ozimec et al., 2015; Goretti et al., 2018; Sanders et al., 2020). While earthworms accumulate various elements within their tissues, Cd in particular is highly bioavailable to earthworms when present in the soil, so insectivores focusing on earthworms, such as badgers, are particularly at risk of Cd accumulation (Spurgeon and Hopkin, 1996; Van den Brink and Ma, 1998; Cleary et al., 2009; Tomza-Marciniak et al., 2019). Badgers in Great Britain are known to have a diet rich in earthworms, focused mainly on Lumbricus terrestris (Kruuk and Parish, 1982; Shepherdson et al., 1990; Goszczyński et al., 2000; Cleary et al., 2009), so it seems likely that this was the principal Cd exposure route for the sampled badgers. Cadmium concentrations have also been known to increase with age in wild mustelids (Harding et al., 1998; Sanders et al., 2020), as was observed in this study, suggesting that the badgers are accumulating higher concentrations of Cd in their livers over time.

Both Ag and As concentrations were also correlated between soils and badger livers, though the relationships (Ag: $\rho = 0.353$, As: $\rho = 0.130$) were weaker than those observed with Pb and Cd. Furthermore, unlike Pb and Cd, Ag and As accumulation due to environmental exposure has not been thoroughly studied in terrestrial mammals. Few studies have examined Ag in the livers of wild terrestrial mammals (Eisler, 1996), but those that have found evidence of Ag accumulation, dependent on its chemical structure (Strużyńska, 2019). Studies on As accumulation have mainly focused on small mammals, and have found indications that As accumulates in rodents living in contaminated areas, albeit at low ratios (Erry et al., 2000; Drouhot et al., 2014; Binkowski, 2019). Like Cd, As has also been found to accumulate in earthworms living in contaminated sites (Yang et al., 2018), making badgers particularly vulnerable to As accumulation.

4.4. Anthropogenic pollution

Metal mining pollution is a global problem, acutely affecting ecosystem function, public health and societal stability (Bebbington et al., 2008; Luckeneder et al., 2021). The UK has a long-standing, extensive legacy of elemental pollution, primarily from mining and smelting, but also from a wide range of other industrial activities (Rainbow, 2018). In the study area, the most notable metal-polluted area is Derbyshire, which has a more than two thousand year long history of Pb and Zn mining and smelting (Fig. 1) (Rawlins et al., 2012; Rainbow, 2018). While all metal mines in the county have now been shut down, and the mine areas now form part of a National Park (Hose, 2017), the soils in areas surrounding historical metal mines and smelters in Derbyshire still contain elevated levels of Pb, Zn, Cd, As, and Ag (Li and Thornton, 1993).

Though most of the badgers used in this study were collected from locations with soil concentrations below the British Geological Survey's Normal Background Concentration (NBC) for 'Principal Domains' (areas not affected by urbanisation or mining activities) (Johnson et al., 2012), 12.7 % of the badgers were collected from sites with soil Pb concentrations in the 90th percentile of the soil Pb concentrations found in England and Wales (Rawlins et al., 2012). The vast majority (89.5 %) of these high Pb badger collection sites were in Derbyshire, with the others collected in a neighbouring county (Nottinghamshire). A similar trend was observed for Cd, though only 16 badgers were collected from sites with soil Cd concentrations in the 90th percentile of the soil Cd concentrations found in England and Wales (Rawlins et al., 2012), of which 14 were found in Derbyshire.

Lead, Cd, and Ag soil concentrations from around the badger collection sites were correlated with one other, mirroring the anthropogenic pollution association between these metals (Table 2) (Li and Thornton, 1993; Rawlins et al., 2012). High concentrations of As are also commonly found near metal mines or smelters (Li and Thornton, 1993), but, in this study, while As was correlated with Pb, it was not correlated with Cd, and had a negative correlation with Ag (Table 2). This is likely due to high natural As concentrations found along an ironstone band in the south-eastern portion of the study area, a region without significant Pb, Cd, Ag, or Zn pollution (Fig. 3) (Rawlins et al., 2012). Soil Zn concentrations near the badger collections sites were also correlated with soil Pb (p < 0.0001, $\rho =$ 0.237), Cd (p = 0.0125, $\rho = 0.164$), and As (p = 0.00887, $\rho = 0.156$), though not Ag (p = 0.570).

Lead, Cd, Ag, and As concentrations in badger livers were all correlated with concentrations in soils, indicating that the badgers were accumulating these elements within their livers based at least in part on local environmental concentrations. Despite the presence of Zn in the soil, and its correlations with the other elements within the soil, Zn concentrations did not correlate between soil and badger livers, likely due to active regulation of this essential element. The mean bioaccumulation factors (BAFs) for Ag, As, and Pb (calculated as BAF = concentration of element in liver/concentration of metal in soil, following Ali and Khan [2018]) were low (Ag: BAF = 0.111 \pm 0.118; As: BAF = 0.00688 \pm 0.00597; Pb: BAF = 0.0129 \pm 0.0120), suggesting that only relatively small amounts of these elements were transferred from the soil into the badger livers. However, since this transfer is proportional, if the soil element concentration is sufficiently elevated, badgers could theoretically accumulate high, potentially toxic amounts of the element.

Unlike the other three non-essential elements, Cd had a notably high mean BAF (9.85 \pm 10.9), indicating that the badgers were accumulating cadmium in their livers at a higher concentration than was found in the environment. This is likely due to the badgers' diet rich in earthworms (Shepherdson et al., 1990), which are known Cd accumulators (Spurgeon and Hopkin, 1996; Van den Brink and Ma, 1998; Cleary et al., 2009; Tomza-Marciniak et al., 2019). High Cd concentrations have frequently been observed in insectivorous small mammals, even in noncontaminated areas, and attributed to the consumption of earthworms and other surface-soil dwelling invertebrates (Cooke, 2011). In contaminated landscapes, small insectivorous mammals have been known to accumulate over twenty times more Cd in their livers than herbivorous or omnivorous species, with a BAF value of over 15 (Sánchez-Chardi and Nadal, 2007; Sánchez-Chardi et al., 2009; Cooke, 2011). In badgers, high kidney Cd concentrations have been recorded, in particular near contaminated floodplains where earthworms were known to have high Cd concentrations (Van den Brink and Ma, 1998). While these high Cd concentrations may pose a health risk, small insectivorous mammals are able to tolerate high Cd concentrations, and it may be that badgers are similarly adapted (Cooke, 2011).

4.5. Badgers as bioindicators

Based on the results of this study, it appears that non-essential elements accumulate in badger livers, depending at least partially on concentrations in local soil. While these local soil concentrations were estimated, based on a radius of two km from the badger collection location, since badgers have high site fidelity and strongly defined territories, these estimations should roughly reflect the soil concentrations to which the badgers are exposed. However, there are potentially other routes of elemental exposure for badgers that are more localised and were therefore not fully investigated during this study. Dietary composition is known to affect metal concentrations in mustelids, and, due to the badgers' exploitation of human-provided food resources, the elemental composition of their diets could vary greatly, even within areas with similar elemental concentrations in soil (Cleary et al., 2009; Brzeziński et al., 2014; Balestrieri et al., 2019). Badgers could also be affected by more localised industrial pollution, agricultural activities, or the use of cattle mineral licks (Ward et al., 2010), all of which would not necessarily be reflected in soil elemental concentrations. Furthermore, as most of the badgers were collected at roadsides, they could have been exposed to legacy Cd, Cu, Pb, Zn, and other

contaminants associated with fossil fuels and vehicle pollutants (Akbar et al., 2006). Collecting at roadsides may have also introduced sampling biases, though prior studies have found that road fatality risk is uniform across adult badgers (Clarke et al., 1998), and spatial biases, though there is significant road coverage in the sampled area (Sandoval Barron et al., 2018; Swift et al., 2021). Badger road fatalities also vary based on season, with peaks in the spring and autumn (Clarke et al., 1998), which could affect elemental studies, as some elemental tissue concentrations are known to fluctuate across seasons (Kalisińska and Budis, 2019; Kosik-Bogacka and Łanocha-Arendarczyk, 2019; Pilarczyk et al., 2019; Tomza-Marciniak et al., 2019). However, in this study, seasonality was controlled for when comparing badger liver concentrations to local soil concentrations, so the observed relationships stand true, regardless of when the badgers were collected.

As long as possible local exposure routes are kept in mind, the results of this study indicate that badgers can be used as bioindicators for environmental non-essential element contamination, particularly for Pb and Cd. Badgers are ideal mammalian bioindicator species, due to their high site fidelity, soil-based foraging, and their frequent road traffic mortality events, which result in a potentially large supply of badger carcasses (Clarke et al., 1998). As was demonstrated in this study, citizen scientists can aid in the collection of badger carcasses, allowing for elemental concentrations, as well as other environmental pollutants, to be compared across a wide geographic area. Establishing further badger baseline elemental concentrations would be helpful for comparison in future studies to determine whether badger elemental concentrations are deficient, normal, or elevated.

5. Conclusions

The links between environmental and wildlife elemental concentrations need to be further understood to both allow for a better assessment of ecosystems, and to determine whether raised environmental concentrations could pose a risk to wildlife. In this study, the relationships between elemental concentrations in topsoils and badger livers were explored using one of the largest collections of wildlife for which elemental concentrations were determined. While there are few clear thresholds for expected badger liver concentrations, the badgers generally appeared to have elemental concentrations within normal levels, though Cu concentrations were lower than expected in mustelids. While the concentrations of most essential elements in badger livers did not appear to be affected by local soil concentrations, non-essential elements, specifically Pb, Cd, Ag, and As, did accumulate in the badger livers at least partially depending on soil concentrations. These elements are common legacy pollutants, found at particularly high concentrations near metal mines or industrial areas, and can be toxic at low concentrations, so their accumulation in badger livers can pose a potential health risk. Cadmium in particular accumulated in badger livers to concentrations on average almost nine times higher than the concentrations found in local soils, likely due to the badgers' consumption of Cd-bioaccumulating earthworms. While badgers in particular can be heavily exposed to these elements, due to their diet rich in earthworms and their high levels of soil contact and ingestion, other animals living in the same areas, including domestic animals and humans, could also be exposed to similarly high concentrations of these elements through normal behaviours. As local soil elemental concentrations are expected to change over time, due both to increases in anthropogenic pollution (Van den Brink and Ma, 1998) and element re-distributions due to more frequent flooding as a result of climate change (Jentsch and Beierkuhnlein, 2008), continued monitoring of environmental and corresponding wildlife elemental concentrations is pivotal to both the further understanding of ecosystems and the monitoring of wildlife health risks.

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CRediT authorship contribution statement

Andrea Sartorius: Conceptualization, Funding acquisition, Methodology, Formal analysis, Investigation, Data curation, Visualization, Writing – original draft, Writing – review & editing. Molly Cahoon: Investigation, Methodology, Writing – review & editing. Davide Corbetta: Investigation, Resources, Writing – review & editing. Llorenç Grau-Roma: Investigation, Resources, Writing – review & editing. Matthew F. Johnson: Supervision, Funding acquisition, Writing – review & editing. Elsa Sandoval Barron: Conceptualization, Investigation, Resources, Data curation, Writing – review & editing. Matthew Smallman-Raynor: Methodology, Writing – review & editing. Benjamin M.C. Swift: Investigation, Resources, Data curation, Writing – review & editing. Lisa Yon: Supervision, Funding acquisition, Writing – review & editing. Scott Young: Supervision, Funding acquisition, Conceptualization, Methodology, Investigation, Resources, Formal analysis, Data curation, Writing – review & editing.

Data availability

The data that support the findings of this study are openly available in the Nottingham Research Data Management Repository at https://doi.org/10.17639/nott.7237.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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