Environmental Pollution 266 (2020) 115280

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Biological and anthropogenic predictors of metal concentration in the Eurasian otter, a sentinel of freshwater ecosystems☆

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ARTICLE INFO

Article history:
Received 11 December 2019
Received in revised form
17 July 2020
Accepted 21 July 2020
Available online 4 August 2020

This paper is dedicated to the memory of our dear colleague and friend, Richard F. Shore, who recently passed away. Richard hugely contributed to the field of wildlife toxicology and will be greatly missed.

Keywords:
Toxic heavy metals
Nanosilver
Drivers of bioaccumulation
Lutra lutra
pH
Rainfall
Age class

ABSTRACT

Toxic metals have been linked to a range of adverse health effects in freshwater organisms. However, for higher vertebrates, there is little understanding of the large-scale drivers of exposure. We quantified toxic metal/semi-metal concentrations in a sentinel freshwater top predator, the Eurasian otter (*Lutra lutra*), across England and Wales, and determined how this varied with key natural and anthropogenic factors. We related liver concentrations in 278 otters that died between 2006 and 2017 to habitat biogeochemistry, proximity to point source contamination and to biological characteristics (length, sex, condition). Evidence for any positive association with putative anthropogenic sources (mining, human population, known discharges) was weak or lacking in nearly all cases, with the exception of a positive association between lead and human population density. Despite concerns that burgeoning use of nanosilver in consumer products might increase silver concentrations in waste waters, there was no increase over time. Spatial variation in soil/sediment pH, precipitation, and soil calcium oxide are indicated as significant predictors of metal concentrations in otters (higher cadmium and silver in areas with lower pH and higher rainfall, and higher chromium and lead in areas of lower calcium oxide). Liver chromium and nickel concentrations declined significantly over time (Cr 0.030 ± 1.2 to 0.015 ± 1.3 μg/g dry weight, Ni 0.0038 ± 1.2 to 0.00068 ± 1.5 μg/g, between 2006–2009 and 2014–2017), but other metals showed no temporal change. Biotic associations were important, with age related accumulation indicated for mercury and cadmium (as well as interactions with body condition). Our results suggest that larger-scale geochemical and hydrological processes are important in determining metal exposure in otters, and we provide an indication of risk factors that may be of relevance for freshwater vertebrates in other countries with well-developed water pollution management.

1. Introduction

Industrialisation and associated anthropogenic activities have resulted in significant inputs of heavy metals into freshwater ecosystems (Tchounwou et al., 2012; Schwarzenbach et al., 2006). These elements are of concern because they do not degrade and can accumulate in living organisms in which they may exert toxic effects ranging from carcinogenicity and genotoxicity to lethality (Chen et al., 2000; Croteau et al., 2005). Although regulatory interventions in various parts of the world have reduced discharges of toxic metals such as mercury (Hg), historic contamination persists in many places. Furthermore, development and use of new products has also resulted in newer metal inputs to freshwaters. For example, increased use and disposal of electronic products are associated with leaching of lead (Pb) from landfilled waste (Robinson, 2009) and recognition of the antibacterial properties of silver (Ag) has led to increased use in a wide variety of consumer products, particularly in nanoparticle form (Wei et al., 2015). Concerns have been raised over the risks associated with these metals for both human and environmental health (Fabrega et al., 2011).

The risk to biota from metals depends upon their bioavailability

☆ This paper has been recommended for acceptance by Wen-Xiong Wang.
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https://doi.org/10.1016/j.envpol.2020.115280
0269-7491/

and subsequent accumulation. Metals can be present as different species, and form complexes or interact with organic and inorganic ligands. Various geochemical properties influence metal speciation, complexation and bioavailability in waters. These include acidification of freshwater ecosystems, salinisation, changes in base cation concentrations and increased dissolved organic matter (DOM) (Acosta et al., 2011; Winterdahl et al., 2014; Campbel and Stokes, 1985; Carpenter et al., 2011); all can be affected by anthropogenic activities (Winterdahl et al., 2014; Luoma and Rainbow, 2005). Hence, as well as directly discharging metals to the environment, humans also modify geochemical parameters that can alter metal bioavailability and bioaccumulation.

How geochemical conditions impact metal bioavailability, bioaccumulation and toxicity in aquatic animals has been investigated under experimental conditions, and through novel modelling approaches that have combined biokinetic parameters (e.g. ingestion rate) with environmental chemistry (Acosta et al., 2011; Campbel and Stokes, 1985; Cusimano et al., 1986; Gundersen et al., 1994; Peterson et al., 1984; Duman et al., 2007; Winter et al., 2005; Wang and Rainbow, 2008). In contrast, few studies have explored these interactions in natural systems. In the current study, we explore abiotic and biotic factors that might drive metal acquisition in natural freshwaters, using the Eurasian otter (*Lutra lutra*) as a sentinel species. The otter is a non-migratory top predator that largely feeds on fish in freshwater or coastal habitats (Miranda et al., 2008). Otter carcasses have been collected from across Britain for over 20 years for various research purposes (Sherrard-Smith et al., 2009; Kean et al., 2011; Sherrard-Smith and Chadwick, 2010; Pountney et al., 2015), providing a means to assess (through tissue analysis) spatial variation in metal concentrations in this top predator. We determined the concentrations of major toxic metals in the livers of Eurasian otters from across England and Wales and examined the extent to which variation in residues was explained by the geochemical properties of the environment that the otters inhabited.

We specifically hypothesised that variation in liver toxic metal concentrations in otters would be positively correlated with sediment metal concentrations and anthropogenic discharges (such as those from industry, domestic wastewater treatment and mining), and moderated by variation in environmental variables including rainfall, soil and water chemistry. We also examined the importance of biotic factors, such as otter length, in explaining variation in liver metal concentrations. We focused our statistical analyses on elements with no known biological function (Ag, arsenic [As], cadmium [Cd], Hg, and Pb) and also chromium [Cr] and nickel [Ni] which although thought to be essential trace elements (Puls, 1994) have also been reported to have ecotoxicological significance in vertebrates at environmentally relevant concentrations (Nordberg et al., 2014). We also quantified cobalt [Co], copper [Cu], iron [Fe], zinc [Zn], manganese [Mn], molybdenum [Mo], and selenium [Se] residues, and summarise the concentrations found. Special attention was paid to silver since little monitoring is in place to determine whether nanosilver is entering freshwater environments and may be subsequently accumulated through the food chain; we hypothesised that there would be a positive association between measured Ag concentration in otters, and predicted nano-Ag in river systems.

2. Materials and methods

2.1. Otter collection and post mortem examination

Otter carcasses sent in to Cardiff University Otter Project (www.cardiff.ac.uk/otter-project) are submitted by local authorities, environmental organisations and members of the public. For each otter, location (National Grid Reference, to a minimum 6 figures) and date of collection is recorded, and a detailed post-mortem examination is conducted following a standard protocol (Simpson, 2000) that includes recording phenotypic characteristics (e.g. sex, length, weight, age-class). Otters are categorised as juvenile, sub-adult or adult based on their size and developmental features. Length and weight are used to derive a condition score and thereby control for variation in fat level, using Peig and Green's (2009) scaled mass index (SMI). Tissue samples, including liver, are retained from the post-mortem examination and archived at −20 °C prior to any analysis.

For the present study, liver samples were analysed from 278 otters. These otters were a stratified random sub-sample of all animals collected, selected in order to provide a good spatial spread (predominantly from England and Wales, but including three animals found in Scotland; mapped, supplementary material, Fig. S1), and (within the constraints of the samples available) a balanced sex ratio and consistent age-class ratio, across years. Sampling focused on the periods 2006–2009, and 2014–2017 for all metals with the exception of silver, for which sampling was from all years 2008–2017 (see Table S2 for breakdown). Further adjustments to the sample selection were made based on preliminary exploration of spatial data describing the number of consented discharges (Natural Resources Wales, 2018; Environment Agency, 2018) to ensure that there were individuals from areas with high, medium and low anthropogenic discharges. The otters had died from various causes (86% roadkill; 2% emaciation; 1% fighting injuries; 1% respiratory infection; 1% electrocution; 1% separation from mother; 8% unknown).

2.2. Sample preparation & analysis

We determined the liver concentrations of the thirteen elements Ag, As, Cd, Cr, Co, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se and Zn. Sample preparation and analysis was carried out following validated methods for the analysis of trace elements in animal tissue samples. In brief, two 1 g randomly selected liver subsamples were taken and weighed accurately to three decimal places. The first, used for trace element analysis, was acid digested and subsequently analysed using inductively coupled plasma mass spectrometry (ICP-MS). The second was used for determination of dry matter content by oven-drying the subsample for 18 h at 105°C and then re-weighing it. Residue analyses were carried out in 2009, 2011 and 2017 and some refinements to methods were made between years. A detailed description of the methods used, the limits of detection applied, and the % recovery data derived from certified reference materials are provided Tables S3-4. All element concentrations are expressed on a μg/g dry weight basis and are not recovery corrected, because consistent and satisfactory recoveries were obtained between different periods, for most elements. Recoveries for lead (Pb) were insufficient for the samples analysed in 2017, hence those data were excluded from further analysis.

2.3. Sources of spatial data

We collated data describing spatial variation in stream and soil biochemistry, weather, and potential anthropogenic sources of contaminants from a range of sources (Table S5). In addition, a map of predicted nanosilver concentrations in surface water was derived from a model that simulated nanosilver loadings from households to rivers (Dumont et al., 2015), based on data on connectivity to sewerage, sewage treatment efficiency, the spatial distribution of sewage treatment plants, dilution, downstream transport, water evaporation, water abstraction, and nanoparticle sedimentation. All spatial data were mapped in ArcGIS® - ArcMap (Version 10.4.1)

(ESRI, 2016) as continuous raster layers or point data.

The otter's linear home range along water courses varies between 5 and 40 km (Kruuk, 1995; Erlinge, 1967). We assumed that each otter could potentially have ranged across a 20 km diameter circular area (approximate midpoint of linear range), the centre of the circle being where the otter carcass was found. We used the Geospatial Modelling Environment (Version 0.7.4) (Beyer, 2014) isectpolypoly tool to extract data that pertained to each otter 20-km-diameter range. For each area, we extracted raster data for a range of geological and anthropogenic variables (Table S5) that were summarised either as mean values (soil pH, sediment elements, stream pH, rainfall, silver nanoparticle concentrations in surface water, human population density) or sum values (annual mass inputs of metals to controlled waters). The presence or absence of past and present mining sites within the circular area was included as a binary variable. For five otters, one or more of these environmental variables was unavailable.

Distance of the otter carcass to the coast was also included to test whether potential differences in the extent of freshwater and marine derived diets affected metal acquisition. To calculate the distance between the otter location and the mouth of the river, otter locations were snapped to the 1:50,000 Watercourse Network layer from the Centre for Ecology & Hydrology (CEH) (Moore et al., 1994) using ArcGIS® - ArcMap (Version 10.4.1) (ESRI, 2016). Snapping tolerance was set to 5000 m. Otters outside this zone ($n = 4$) were excluded from the scoring, due to uncertainty about the relevance of the river catchment. The shortest distance from the otter location to the coast along rivers was calculated using RivEx (Version 10.25) (Hornby, 2017).

The data and supporting documentation were deposited with NERC's Environmental Information Data Centre (EIDC) under the dataset name: Biological characteristics, liver metal concentrations, habitat biogeochemistry and habitat contamination sources of UK otters (2006–2017). The DOI is https://doi.org/10.5285/0fbb2c90-5b54-427a-a083-55c022802a80 (Brand et al., 2019).

2.4. Statistical analysis

All analyses were carried out in R (R Core Team, 2016) via the R Studio (Version 1.0.153) interface. Principal component analysis (PCA) was used to reduce dimensionality among correlated environmental variables, namely soil carbon (soil C), soil pH, river pH, sediment calcium oxide (sedCaO), soil chloride (soil Cl), soil sulphur (soil S) and rain (using a correlation matrix). Five otters were omitted from PCA due to missing environmental data.

Biotic and abiotic drivers of metal concentrations in otter liver were explored using generalised linear models (GLMs). For each model, metal/semi-metal concentration (Ag, As, Cd, Cr, Hg, Ni, Pb) was the dependent variable. All independent variables in the initial model, and all relevant two-way interactions between these variables, are listed in Table S6. Otter sex, length and body condition (SMI) were included to control for potential biotic variation. Length was used rather than age-class because sample sizes differed greatly between age-classes (juveniles were very poorly represented, see Table S1); length is significantly associated with age-class (Fig. S7) and provided a continuously distributed variable. Liver concentration of Se was included in the model for Hg, based on well-known biological interaction (Koeman et al., 1973). Change over time was explored using a categorical term to compare period 1 (2006–2009) with period 2 (2014–2017) for all dependent variables except Ag and Pb. In the model for silver, year was treated as a continuous variable (2008–2017), whereas for Pb change over time could not be tested because data from the latter period were excluded due to poor recoveries. Principle components 1, 2 and 3 were included to describe variation in soil and stream chemistry, and rainfall. Principle Component 1 (positively associated with soil C and rain, negatively associated with soil pH and river pH), Principle Component 2 (negatively associated with sedCaO and soil S) and Principle Component 3 (negatively associated with soil Cl) were also used as independent variables in each GLM (detailed results of the PCA are reported in Table S8 and Figs. S9-10). Otters for which PCs were not available (i.e. the five otters for which environmental data were missing) were initially excluded from GLMs, but were returned to models where model reduction steps led to removal of the PCs (this occurred in models of As and Ni, but not for Ag, Cd, Cr and Hg). Where those individuals could not be returned, models were run with all individuals but without PCs, to ensure that the significance of the other independent variables in the models was not affected. Sediment levels of the relevant metal (i.e. sediment Ag in the model of otter Ag, etc) were included to control for background variation. Human population density, presence/absence of mining sites, and known inputs of the relevant metal to controlled waters, were included to test for association with anthropogenic inputs. For Ag, predicted nanosilver in surface water was also included to test for association between predicted nanosilver concentrations in water and those in otter tissues. Metal concentrations were skewed, therefore preliminary models were fitted using (i) raw data, with Gaussian error family and log link function, (ii) log transformed data, with Gaussian error family and identity link and (iii) raw data, with Gamma error family and log link function. Final models were chosen based on comparisons of model residual normality, homoscedasticity, and absence of leverage, and resulted in selection of log transformed data and a Gaussian error family with identity link function for all models. Models were simplified using stepwise deletion (using AIC values from the drop1 function in R) to maximise model efficiency. Final model checks were made to assess whether the variation of residuals was associated with the year in which the otter was found, the year in which metal analysis was conducted, or with age-class, i.e. whether any remaining variation or bias with these variables was not adequately accounted for by the model. Additionally, we tested whether residual variance differed between regions (using UK Environment Agency regional structures, which are based on river catchments (Environment Agency, 2014), in order to identify potential spatial autocorrelation. No significant association with residuals were found. Model predictions were made using the 'predict' function in R (R Core Team, 2016) based on the final model for each determinand. When predicting the nature of association with each variable in question, all other variables remaining in the relevant model were fixed at their mean, or at "Female" for the variable Sex, "2006–2009" for the variable Year and 'no' for presence of mining sites.

3. Results and discussion

Measured metal/semi-metal concentrations in otters varied over some four orders of magnitude (Fig. 1) (for additional descriptive statistics see Table S11). Overall concentrations were broadly similar to those previously reported in Eurasian otters from other parts of the world (Gutleb et al., 1998; Lanszki et al., 2009; Kruuk et al., 1997; Lemarchand et al., 2010; Lodenius et al., 2014). Concentrations of Ag, which can be toxic to mammals, were an order of magnitude lower than those reported in a small sample of Eurasian otters from Belarus ($n = 9$) (Sidorovich, 2000) and in sea otters (*Enhydra lutris*) from the USA ($n = 17$) and Russia ($n = 5$) (Kannan et al., 2006, 2008). The current study presents the first major published dataset for liver total Ag in freshwater otters anywhere in the world and provides a baseline against which to detect potential future increases that may arise in the UK because of increasing use and subsequent discharge of nanosilver.

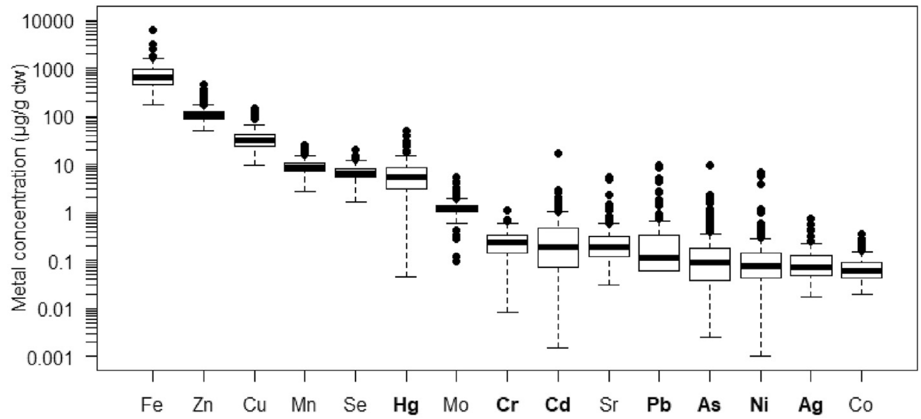

Fig. 1. Median (black band) concentrations (μg/g dry weight) of selected inorganic elements in the livers of otters found dead between 2006 and 2017 in the United Kingdom. Boxes represent quartiles and whiskers are 1.5 × interquartile range, with points beyond that distance plotted as single points. Metals named in bold were the dependent variables in our statistical models. Note that the years in which otters were sampled differs for Ag and Pb (see Table S1 for details) and that for Pb there are fewer data overall, and samples were taken only from years 2006–08.

Concentrations of the elements with known biological functions (Co, Cu, Fe, Zn, Mn, Mo and Se) were generally less variable than the major toxic elements (Fig. 1), likely reflecting homeostatic control of essential trace elements, which would render them less likely to reflect environmental drivers.

Estimation of toxicological significance is challenging because relevant (species-specific) thresholds are lacking. For As, no relevant toxicological threshold is proposed, but Kubota et al. (2001) reported concentrations up to 5.7 μg/g in sea otters (*Enhydra lutris*) with no apparent toxicological consequences. In the present study, concentrations were all <2.27 μg/g, except in one juvenile male with 9.38 μg/g; no gross lesions were observed but no histopathology was undertaken. For Cd and Pb, kidney concentrations exceeding 105 μg/g dry weight (Cd, Shore and Douben, 1994a) and 25 μg/g dry weight (Pb, Ma, 1989) have been related to cellular damage in small mammals. Using reported kidney:liver ratios for these metals (Gutleb et al., 1998; Mason et al., 1986), median kidney concentrations in the otters were estimated, and did not exceed either threshold. Sleeman et al. (2010) report liver Hg levels of 221 μg/g wet weight in northern river otter *Lontra canadensis*, associated with severe pathology, and assumed to be the cause of death. Liver Hg concentrations exceeding 25–30 μg/g wet weight have been linked to adverse effects in non-marine mammals (Shore et al., 2011), in the current study the maximum concentration was 50.3 μg/g dry weight, equivalent to 15.4 μg/g wet weight (based on the % dry matter of the sample). Consistent with this, there was an absence of obvious macroscopic changes in the appearance of the liver and kidneys or any other post-mortem signs suggestive of metal toxicity.

A significant amount of variation in tissue concentrations of Ag, Cd, Cr, Hg, Ni and Pb, but not As, was explained by our models; the explanatory variables differed between metals. Model outputs are provided in full in S6; the nature of all significant associations is discussed here.

A small number of studies have found significant associations between elevated heavy metal concentrations in biota and anthropogenic pollution sources (Wren et al., 1988; Kuklina et al., 2014; Suarez-Serrano et al., 2010; Carrasco et al., 2011). We used human population density, presence/absence of mining, annual reported mass inputs of metals to controlled waters, and (for Ag only) predicted nanosilver in surface waters, to indicate potential anthropogenic pollution. Predicted nanosilver showed no association with Ag concentration in otters, and was dropped from our Ag model. Presence/absence of mining was retained in models for Cd, Cr, Hg and Pb but significance was indicated only for Hg, for which a significant negative association was found ($p = 0.016$), however model predictions show high variation and small differences, with imbalanced comparison and few data in the group with mining sites ($n = 13/197$), and we therefore doubt the validity of this finding. Annual mass inputs of the relevant metal to controlled waters was retained for As, Cd, Hg and Pb (dropped from Ni, and unavailable for Ag and Cr) but significance was indicated only for As (a positive association, p 0.049, as might be expected) but the overall model for As was non-significant so this result is disregarded. Human population density was retained, and significant, in models of Ag, As, Cd and Pb ($p = 0.049$, 0.014, 0.015 and < 0.001) but was dropped from models of Cr, Hg and Ni. Human population density was strongly positively associated with Pb. Unexpectedly, the association was negative in all other cases, however the overall model for As was non-significant, and downward trends for Ag and Cd were weak (Fig. 2). Fewer otters are found in areas of high human population density and it is possible that differences in otter diet, land-use, or waste-water treatment practices are differentially impacting metal concentrations in rural and urban areas; more evidence is needed to clarify the drivers of these associations.

There was evidence of a significant decline in liver Cr and Ni concentrations between the two study periods ($p = 0.017$ and 0.004 respectively), with model predicted mean (±SE) concentrations of Cr falling from 0.030 ± 1.2 μg/g dry weight in 2006–2009, to 0.015 ± 1.3 μg/g in 2014–2017, while concentrations of Ni fell from 0.0038 ± 1.2 μg/g to 0.00068 ± 1.5 μg/g during the same period. A nationwide study of metal ambient concentrations between 1980 and 2005 reported significant decreases in ambient concentrations of metals in the UK since the 1980s and predicted ambient concentrations would continue to fall or level off as “background” levels are approached (Brown et al., 2008). Chromium has a wide range of industrial uses and commercial wastewater is an important source of chromium entering waste water treatment plants. Decline in otters is likely to reflect the introduction of alternative products (following environmental concern and regulatory reviews in Europe) which have resulted in decreases in industrial chromium use as textile dye (in the form of sodium dichromate) and wood preservative (in the form of chromated copper arsenate) in the UK (Zarogiannis, 2005). Declines in nickel correspond with known reductions in ambient concentration in the UK since the 1980s. The transition from coal-burning to natural gas, advances in clean technology and greater regulation of industrial processes have played an important part in this reduction (Bruckmann, 2001;

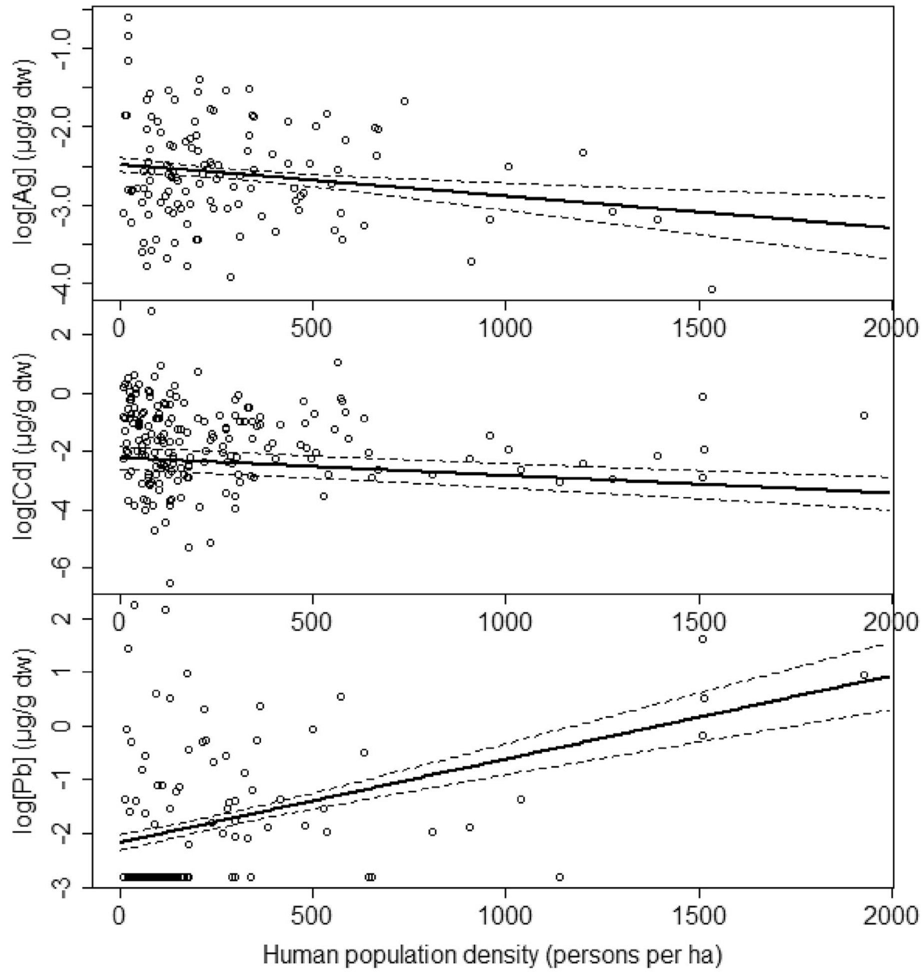

Fig. 2. Association between human population density and liver silver (Ag), cadmium (Cd) and lead (Pb) concentrations in otters (µg/g dw). Solid lines indicate model predicted liver tissue metal concentrations, while other significant variables in each model (see Table S6) were statistically controlled. Dashed lines indicated standard error around the predicted concentrations.

Maggs and Moorcroft, 2000), and the increasing substitution of heavy fuel oil (and more recently, coal) with orimulsion has also contributed (Bruckmann, 2001). Although year was retained also in models for Ag, As, and Hg, there was no evidence for any significant association. It seems possible that concentrations of these elements have reached their nadir, following earlier reported declines (Brown et al., 2008).

Spatial variation in metal concentrations was complex, and in addition to the putative anthropogenic drivers discussed above, we hypothesised that a range of other environmental variables including rainfall, and soil/sediment/water geochemistry, and proximity to the coast, might also be associated with metal concentrations in otters. Because many of these variables were correlated with one another it was not always possible to individually test for associations, and so we used ordination (PCA) to reduce dimensionality.

PC1 (which negatively represented soil pH, river pH, and positively represented soil C and rainfall), was positively associated with liver metal concentrations of Ag and Cd ($p = 0.009$ and 0.002 respectively) (Fig. 3a and b). A positive association between metal concentration and PC1 suggests that higher liver concentrations are found in areas with lower pH (i.e. more acidic conditions). Metals are more readily mobilised under more acidic conditions (John and Leventhal, 1995) and increased metal accumulation has been reported in organisms from low pH waters (Meyer et al., 1995; Scheuhammer and Blancher, 1994; Wren and Stephenson, 1991). The pH range over which rapid change in sorption capacity occurs is generally higher for Cd and Ag (pH 5–8) than for other metals such as Pb and Cu (pH 3–6) (Davis and Leckie, 1978; John and Leventhal, 1995; Saha et al., 2002; Smith, 1999). Thus, within the pH range of the rivers in England and Wales (in our data, min 6.1 max 8.1, mean 7.5), Cd and Ag may be the metals most likely to desorb from the surface of particulate matter, become available for incorporation in biological processes, and result in elevated liver concentrations in otters where pH is lowest, thus explaining why these metals show this association. For Cr, Ni and Pb, PC1 was dropped from the models, and for As and Hg although retained, the association with PC1 was non-significant.

A positive association with PC1 also suggests higher metal concentration in areas of high rainfall. This might result both from increased deposition of airborne contaminants, and washout of metal from soils into waterways. Positive associations have previously been demonstrated between rainfall and otter liver Cd (Mayack, 2012), but not (as far as we are aware) for Ag. The increased use of Ag nanoparticles in domestic products has resulted in higher (airborne) emissions of silver (Quadros and Marr, 2011; Sung et al., 2008; Reidy et al., 2013) which can undergo long-range transport (Kimbrough and Suffet, 1995; Reidy et al., 2013). The association between Ag in otters and PC1 (and therefore potentially with rainfall) is consistent with the concept that Ag availability is

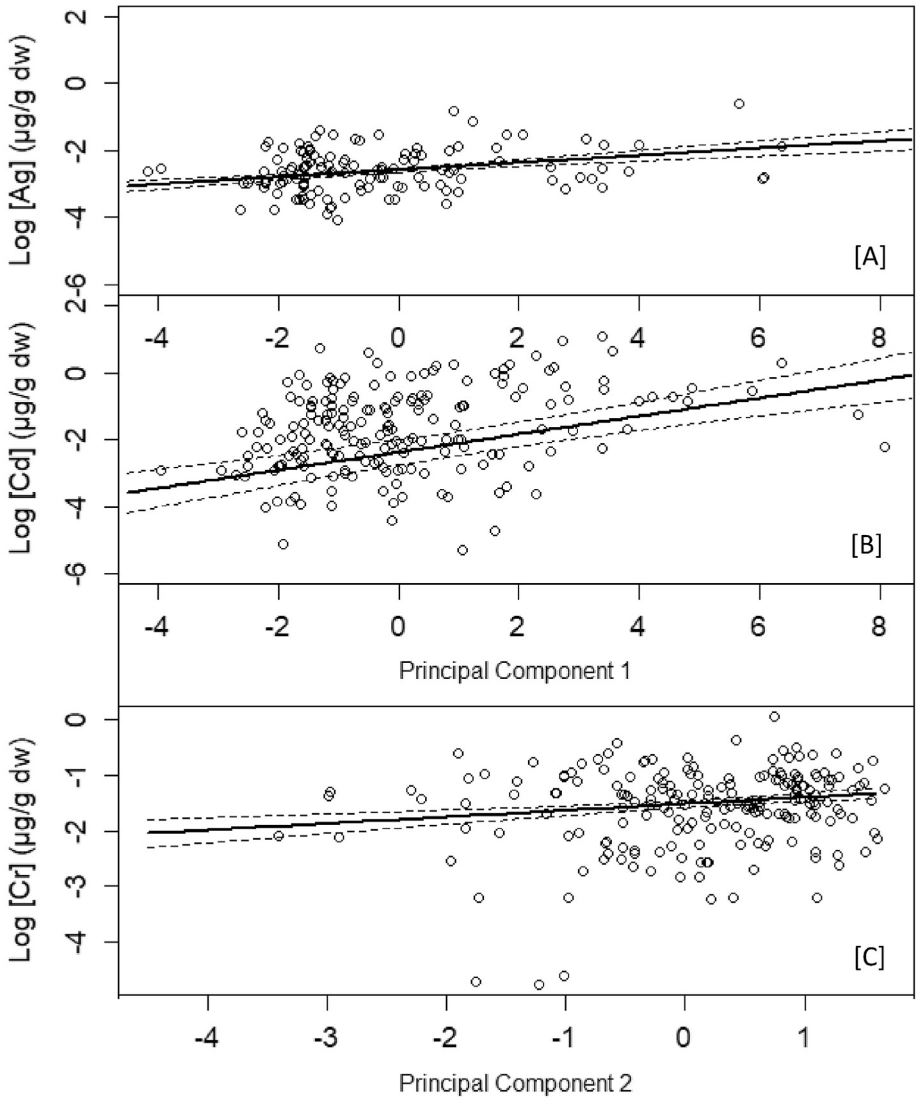

Fig. 3. Association between environmental variables and metal concentration in otters (μg/g dw). [A] and [B] represent associations between PC1 and Ag, and Cd, respectively while [C] represents the association between PC2 and Cr. PC1 is based on principal components analysis of Soil C, Soil pH, River pH and Rainfall; high PC values indicate high Soil C and Rainfall, and low Soil pH and River pH. PC2 is based on sediment CaO and soil S; high PC values indicate low sediment CaO and low soil S. Solid lines indicate model predicted liver tissue metal concentrations, while other significant variables in each model (see Table S6) were statistically controlled. Dashed lines indicated standard error around the predicted concentrations.

associated with atmospheric Ag deposition, while the absence of any association with predicted nanosilver in surface waters suggests that releases from wastewater treatment plants may be less important. More detailed modelling of the behaviour of Ag in different environments is needed in order to elucidate potential future exposure of and risks to biota.

The third factor influencing PC1, soil C, seems unlikely to be a key driver of either liver Ag or Cd in otters. Metals are complexed by organic matter, and their bioavailability to organisms is thus reduced by increasing organic matter content (Winter et al., 2005; Hogstrand and Wood, 1998; McGeer et al., 2002; Voets et al., 2004; Gorski et al., 2008). Further exploration of our GLMs, using soil C as a discrete variable in each GLM (instead of PC1), indicated a highly significant negative association between soil C and liver Cd ($F_{1,183} = 11.050$, $p < 0.001$), and no association between soil C and liver Ag, but because of collinearity in our dataset we cannot separate a potential association with rainfall and pH from that with soil C.

PC2 (which negatively represented sediment CaO and soil S) was positively associated with Cr ($p = 0.025$)(Fig. 3c). Although retained in models of Ag, As and Hg, there was no evidence for a significant association, and it was dropped from models of Cd, Ni and Pb. A positive association between metal concentration and PC2 suggests higher liver concentrations in areas of low sediment CaO, and low soil S. Based on their geochemistry we suggest that sediment CaO is the more likely driver of this association. CaO is alkaline, and a metal-removing sorbent (Bhakta and Munekage, 2013), meaning that metals are more likely to be bound to sediment high in CaO, and thus less available to freshwater foodchains. Conversely, elemental sulphur decreases soil pH, which would contribute to metal mobilisation. Further exploration of our Cr model, using soil S and SedCaO, in turn, as discrete variables (instead of PC2), indicated that soil S was not significant, whereas SedCaO was significant ($F_{1,176} = 3.259$, $p = 0.04$). PC3 (which was negatively associated with soil Cl) was retained in models for Ag, As, Hg and Pb but showed no significant association with metal concentrations.

We also explored whether variation in otter liver metal concentrations reflected spatial variation in average sediment concentrations of the same metal within a 20 km diameter circular area around the carcass location, or distance to the coast. We found no significant associations between tissue metal residues and metal concentrations in sediment. In contrast, associations between metal concentrations in biotic samples and corresponding soils have been shown for Cd and Pb in various terrestrial mammals (Grawé et al., 1997; Pankakoski et al., 1993; Ma, 1989; Ma et al., 1991; Shore, 1995), and may be the result of a closer direct contact with (including ingestion of) soil and vegetation in terrestrial habitats. A previous study of Eurasian otters reported a correlation between bone Pb concentrations and both Pb emissions and Pb in stream sediments in Britain (Chadwick et al., 2011). However, lead concentrations tend to be higher in bone than in liver and reflect lifetime accumulation as opposed to more recent (approximately a month) exposure (Shore and Douben, 1994a). It is possible that associations between tissue and sediment Pb may in fact only become manifest over a prolonged exposure period in otters; liver residues may vary with shorter-term, local scale fluctuations in exposure to environmental Pb concentrations. Consistent with this is that associations with long term industrial activity and atmospheric deposition of pollutants were found for bone, but not for liver, in aquatic mustelid species in Canada (Wren et al., 1988b). It should be noted no association with sediments was found for As, Cr, Ni or Pb, whereas for Ag, Cd and Hg no suitable data describing sediment concentrations were available, so this was not tested. Distance from the coast was not retained in models for Cd, Cr, or Ni, and showed no significant association for Ag, As, Hg or Pb.

A number of caveats should be noted with regard to the environmental data used in our models. Although we included soil and water pH, known to affect metal bioavailability, we could not utilise more advanced modelling approaches (such as the Windermere Humic Aqueous Model (WHAM) (Tipping, 1994)) because the environmental input parameters for our models were not appropriate for WHAM. We were unable to include data on river flow, a potentially important factor (Jobling et al., 2006; Ji et al., 2002) because of a lack of spatially/temporally explicit data. It should also be noted that relatively few data were available from sites with low soil and river pH, or sites with high soil carbon and rainfall (Figure S4). Spatial (and individual) variation in otter diet might also contribute to differences in metal exposure, but controlling for this is beyond the scope of the current study.

It is well known that biotic variables such as age can have a significant impact on accumulation for some metals (Shore and Rattner, 2001), and we therefore examined how these might account for variation in metal concentration in otters (significant associations are plotted in Figs. S12-14). We found significant associations with length (in interaction with sex and/or body condition) for both Cd and Hg, which we presume reflects accumulation with age. For Cd, both sexes accumulated higher liver Cd concentrations with length but the rate of accumulation was steeper in females (Length:Sex interaction $p < 0.001$). Accumulation of Cd with age in the liver and particularly the kidney has been observed in a wide variety of mammals (Shore and Rattner, 2001; Shore and Douben, 1994b; Glooschenko et al., 1988) including otters (Hyvärinen et al., 2003; Lanszki et al., 2009), but the cause for the sex difference in accumulation is unclear; it may reflect physiological or dietary differences between males and females (Lanszki et al., 2014; Moorhouse-Gann et al., 2020). We also found an interaction with body condition, such that for otters in typical or good condition (scaled mass index of 0 or greater) there was a steeper association between length and Cd concentration, whereas for otters in poorer condition (SMI <0) the increase in concentration was less marked (Sex:SMI interaction, $p = 0.013$). For Hg, we found a similar (although more extreme) pattern whereby overall the data suggest a positive association between body length and liver Hg for typical and good condition otters (presumably reflecting accumulation with age), but not in otters that were in poor condition ($p < 0.001$). Age-related accumulation of liver Hg in mammals has not been widely observed (Shore and Rattner, 2001; Lodenius et al., 2014) and non-linear, varying trends have been described in otters (Kruuk et al., 1997; Mierle et al., 2000). Younger otters are more likely to forage on crustaceans (Ben-David et al., 2001) while older otters feed more on large piscivorous fish (Kruuk, 1995; Moorhouse-Gann et al., 2020) that occupy a relatively high trophic position and accumulate more Hg than omnivorous and planktivorous species (Zhou and Wong, 2000). Surprisingly, we also found significant but negative associations between length and concentration of Ni ($p = 0.002$), and Pb ($p = 0.002$), which we are unable to explain. Interactions with condition are difficult to interpret, as it is not possible to discriminate from a correlative association whether, for example toxic metal contamination might drive poor condition (e.g. Lanszki et al., 2009; Kruuk et al., 1997; Bremle et al., 1997), or whether liver wastage induced by starvation causes elevated liver pollutant concentrations (Wienburg and Shore, 2004). Previous studies in otters have found associations between metal concentrations and body condition but these have not been consistent. Mercury in river otters (*Lontra canadensis*) from Canada was positively correlated with body fat (Evans et al., 2000) whereas a negative correlation was found in river otters (*Lutra lutra*) from Scotland (Kruuk et al., 1997).

We also found a highly significant positive association between liver Hg and Se concentrations ($p < 0.001$) and the Hg:Se molar ratio was close to or below 1 in all of the otters we examined (Fig. S15). A similar equimolar ratio in liver has been described in other species that feed at a high tropic level (Koeman et al., 1973; Palmisano et al., 1995) and reflects a detoxification mechanism in which Se complexes Hg in a 1:1 M ratio (Koeman et al., 1973).

4. Conclusions

Liver concentrations of toxic metals and semi-metals in otters in British rivers between 2006 and 2017 were broadly similar to levels measured in other studies (Lanszki et al., 2009; Lemarchand et al., 2010; Kruuk et al., 1997; Hyvärinen et al., 2003; Gutleb et al., 1998) and were below levels likely to be associated with adverse effects. Over a broad spatial scale, concentrations were not predominantly driven by point source inputs of anthropogenic contamination but were associated with environmental variables such as pH and rainfall, and biotic factors such as age and sex. Overall, our data describe the current key drivers of metal acquisition in a key freshwater sentinel species at a national scale, and provide a comprehensive dataset against which future change (e.g. from increased usage, or release of metals from sediment contaminated by past mining activities (Hudson-Edwards et al., 2008) can be benchmarked.

CRediT authorship contribution statement

Anne-Fleur Brand: Formal analysis, Investigation, Writing - original draft. **Juliet Hynes:** Resources, Formal analysis, Data curation. **Lee A. Walker:** Investigation. **M. Glória Pereira:** Investigation. **Alan J. Lawlor:** Formal analysis, Investigation, Methodology. **Richard J. Williams:** Investigation, Resources. **Richard F. Shore:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition. **Elizabeth A. Chadwick:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition, Project administration.

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.

Acknowledgements

This research was supported in part by a grant from the Esmée Fairbairn Foundation (grant number 14-1146). The CEH contribution was supported the Natural Environment Research Council award number NE/R016429/1 as part of the UK-SCaPE programme delivering National Capability. The authors of this article thank Sarah Thacker and Elaine Potter for their support with various aspects of laboratory work. This study contains model estimates of topsoil properties [Countryside Survey] and British Geological Survey data owned by © NERC as well as Ordnance Survey data owned by © Crown copyright and database right 2007. Thanks also to members of the public, Wildlife Trusts, and environmental organisations including the Environment Agency and Natural Resources Wales, for the reporting and collection of otter carcasses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.115280.

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