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The importance of in-year seasonal fluctuations for biomonitoring of apex predators: A case study of 14 essential and non-essential elements in the liver of the common buzzard (*Buteo buteo*) in the United Kingdom☆

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A R T I C L E I N F O

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A B S T R A C T

Trace elements are chemical contaminants spread in the environment by anthropogenic activities and threaten wildlife and human health. Many studies have investigated this contamination in apex raptors as sentinel birds. However, there is limited data for long-term biomonitoring of multiple trace elements in raptors. In the present study, we measured the concentrations of 14 essential and non-essential trace elements in the livers of the common buzzard (*Buteo buteo*) collected in the United Kingdom from 2001 to 2019 and investigated whether concentrations have changed during this period. In addition, we estimated the importance of selected variables for modelling element accumulations in tissues.

Except for cadmium, hepatic concentrations of harmful elements in most buzzards were lower than the biological significance level of each element. Hepatic concentrations of certain elements, including lead, cadmium, and arsenic, varied markedly seasonally within years. Their peak was in late winter and trough in late summer, except copper which showed an opposite seasonal pattern. In addition, lead in the liver consistently increased over time, whereas strontium showed a decreasing trend. Hepatic concentrations of cadmium, mercury, and chromium increased with age, whereas selenium and chromium were influenced by sex. Hepatic concentrations of arsenic and chromium also differed between different regions.

Overall, our samples showed a low risk of harmful effects of most elements compared to the thresholds reported in the literature. Seasonal fluctuation was an important descriptor of exposure, which might be related to the diet of the buzzard, the ecology of their prey, and human activities such as the use of lead shot for hunting. However, elucidating reasons for these observed trends needs further examination, and biomonitoring studies exploring the effects of variables such as age, sex, and seasonality are required.

1. Introduction

Trace elements are naturally occurring at low levels. They are now widely spread mainly due to various anthropogenic activities, becoming non-degradable chemical contaminants (Nriagu, 1989, 1979; Walker et al., 2012). Terrestrial wildlife is exposed to these contaminants through different routes; principally the oral route in wild vertebrates (Beyer and Meador, 2011; Shore and Rattner, 2001; Walker et al., 2012).

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Each essential element, like copper (Cu) or zinc (Zn), has a range of optimal intake for maintaining their biological performance. An over-intake of non-essential elements like lead (Pb), cadmium (Cd), and mercury (Hg) above a certain threshold can cause dysfunctions like carcinogenicity, immuno-suppression, poor body condition, and impaired reproduction in wildlife (Walker et al., 2012). Exposure at high dose can cause adverse effects and may be ultimately lethal, while chronic exposure at lower dose to non-essential elements and their subsequent accumulation in tissues could cause a wide range of health effects (Eisler, 2000; Nordberg et al., 2014). Ecotoxicological studies on exposure and effects in wildlife, such as exposure to Pb from ammunition, can provide insights into potential exposure and effects on human health (e.g., Dobrowolska and Melosik, 2008; Knott et al., 2010; Pain et al., 2010). It is of benefit for human health to assess the effects, sources, and temporal trends of chronic exposure of wildlife to environmental contaminants.

Research on environmental pollutants in wildlife has a long history (Rattner, 2009). Such studies have often been initiated to understand the risks from pollutants to individual species. However, biomonitoring has also been conducted for temporal changes in wildlife contamination over a large spatial range. Birds have been used as sensitive indicators of environmental quality, including the presence of contaminants (Furness and Greenwood, 1993). Raptors are especially suitable for monitoring persistent, bioaccumulative, and toxic substances in the environment. They are positioned as apex predators, are relatively long-lived and thus exposed to contaminants over time, and integrate over large spatial areas (e.g., Espín et al., 2016; Movalli et al., 2017, 2008). Non-migratory raptor species are particularly of value for local contamination. They can be a key tool for evaluating the outcomes of long-term exposure to environmental contaminants over large spatial scales. The common buzzard (*Buteo buteo*; hereafter 'buzzard') is widely distributed across Europe. Buzzards living in the United Kingdom (UK) are non-migratory and territorial, inhabiting different habitats (e.g., forests, agricultural zones, mountain regions, and sub-urban areas). They opportunistically prey upon bird and mammal species as well as reptiles, batrachians, and insects and scavenge from avian and mammalian carcasses, including gamebirds (e.g., Francksen et al., 2017, 2016; Graham et al., 1995; Reif et al., 2004, 2001; Swann and Etheridge, 1995). Their relative abundance makes them a favourable species for measuring numerous contaminants over large spatial scales (Badry et al., 2020).

Buzzards have been widely monitored, particularly for their exposure to Pb. The wide use of Pb bullets by hunters is considered as an important source of worldwide Pb contamination (Fisher et al., 2006; Pain et al., 2019). Raptors, particularly scavenging birds like buzzards, suffer Pb poisoning through ingesting ammunition or ammunition fragments embedded in their prey (Krone, 2018; Monclús et al., 2020). Many investigations on buzzard Pb poisoning have been conducted in the UK (e.g., Pain et al., 1995) and European countries (e.g., Battaglia et al., 2005; Carneiro et al., 2014; Jager et al., 1996; Kanstrup et al., 2019; Kitowski et al., 2016). Plus, recent long-term monitoring of buzzards demonstrated a temporal change in Pb in buzzard tissues in the UK (Taggart et al., 2020). Cadmium has been investigated as another of the most potentially harmful elements (e.g., Battaglia et al., 2005; Carneiro et al., 2014; Hontelez et al., 1992; Kanstrup et al., 2019), although there is no research on long-term temporal trends of Cd. In contrast, there are few studies on other non-essential or essential elements in buzzards. For instance, despite a wealth of knowledge on the toxicity of arsenic (As) to wildlife (Eisler, 2000; Nordberg et al., 2014; Shore and Rattner, 2001), published data on As levels in buzzards is sparse (e.g., Carneiro et al., 2014; Naccari et al., 2009; Pérez-López et al., 2008). Studies reporting Hg, nickel (Ni) and chromium (Cr) in buzzards are fewer (e.g., Carneiro et al., 2014; Castro et al., 2011; Kitowski et al., 2016; Komosa et al., 2012). Essential elements like Zn, Cu, and manganese (Mn) in buzzards have hardly been reported (e.g., Komosa et al., 2012; Naccari et al., 2009). Furthermore, to our knowledge, there is no long-term monitoring for the time trend of these elements in buzzard tissues.

This study is to report long-term biomonitoring of multiple elements in buzzards. We have measured 14 essential and non-essential elements in livers of 72 dead buzzards collected in the UK from 2001 to 2019 to investigate how their concentrations have changed over time. In addition, correlations between elements were assessed, and the importance of variables for biomonitoring of exposure of apex species to metal contaminants was estimated and discussed.

2. Materials and methods

2.1. Buzzard sample collection and preparation

Specimens ($n = 72$) of buzzards found dead or dying in the wild were collected from the UK from 2001 to 2019. Requests were made to the public, birdwatchers, rehabilitation centres, and wildlife managers through bird journals, newsletters, and other communications. Carcasses were sent to the UK Predatory Bird Monitoring Scheme (PBMS) of the UK Centre for Ecology & Hydrology (UKCEH). For each sample, collection location and date were recorded (for the locations of the samples, see Supplementary Information Figure S1). All carcasses were subject to a post-mortem examination conducted by an experienced wildlife ecologist at the UKCEH, and various tissue samples were extracted and stored at −20 °C.

The sex of an individual was determined based on identification of the gonads or bird's size and plumage. Approximate age was determined from plumage characteristics and assigned following the EURING code (EURING, 2016). For the present analysis, we placed specimens into two age classes: young birds collected in the calendar year of hatching (i.e., juvenile) and older birds (i.e., adults). The body and each organ's wet weights were also recorded.

2.2. Determination of concentrations of elements in livers

The liver and kidneys are target organs of non-essential elements (Beyer and Meador, 2011). As the liver is generally resistant to the toxic effects of non-essential elements like Cd (Scheuhammer, 1987), we used the livers of buzzards for measuring their exposure to 14 elements: Pb, Cd, Hg, As, Ni, Cr, selenium (Se), Cu, Zn, iron (Fe), manganese (Mn), cobalt (Co), strontium (Sr), molybdenum (Mo).

For each specimen and three certified reference materials (CRMs), Lobster Hepatopancreas TORT-2, Fish Liver DOLT-5, and Fish Protein DORM-3, 1 g wet weight were individually weighed into MARS Xpress Teflon vessels (CEM MARS5 microwave). The samples, CRMs and two method blanks were then digested with 10 mls of nitric acid (ROMIL-UpA, Ultra Purity Nitric Acid, 67–69%) by the microwave digestion system (Ramp: 45 min; Temperature: 200 °C; hold: 15 min; Power: 1600 W). After cooling, the samples were made up to 25 mls with ultra-pure water. The moisture content of the sample was determined by drying a 1 g sub-sample at 105 °C for a minimum of 3 h. Dry weight concentrations were calculated based on the wet weight concentration of the analysed sample and the gravimetrically determined moisture content of a separate sub-sample.

The samples were analysed using Inductively Coupled Plasma Mass spectrometry (ICPMS; PerkinElmer Nexion 300D instrument) in standard and reaction (Dynamic Reaction Cell –DRC) mode. Four internal standards (Ga, In, Bi and Re, VWR) were added via a T-piece in the sample introduction to correct for sample-specific matrix effects and instrumental drift. The ICPMS analysis was performed at an ×5 dilution, using acid matrix-matched calibration standards for the 14 elements: Mn, Fe, Co, Ni, Cu, Zn, Se, Sr, Mo, Cd, Pb, Cr (DRC), As (DRC), and Hg. Gold (5 mg/L) was added to the standards, CRMs and samples for the stabilisation of Hg in solution. Individual sample values represent a mean of three replicate analyses for each element.

All concentrations are expressed as mg/kg of dry weight. The limit of detection (LoD) was measured for each sample and each element. The statistics of LoD and CRM recovery rate for the elements were summarised in Supplementary Information Table S1. There was one sample with concentrations of Hg below the LoD. Four, six, and 16 samples of Sr, Cr, and Ni concentrations, respectively, were also below the LoD. We replaced these under LoD values with half of the maximum value of LoD of the given element.

2.3. Data analysis

To compare with the results of other studies which used wet weight, we used factor 3.1 to convert the concentration of element in livers from per unit of wet weight (ww) to per unit of dry weight (dw) (Monclús et al., 2020).

2.3.1. Biological thresholds of liver concentrations

We used the biological thresholds of certain harmful elements to assess their potential adverse effects. For the biological threshold of hepatic Pb concentrations of birds of prey, we followed Pain et al. (1995): a liver Pb concentration of more than 6 mg/kg dw is likely to have resulted from abnormally high exposure to Pb; a concentration exceeding 20 mg/kg dw is likely to be potentially lethal exposure. The proportions of buzzards with high levels of Pb have been recorded in previous studies in the UK. Taggart et al. (2020) compared by Fisher's exact test the proportions of abnormally high (8.0%) and potentially lethal exposure (2.7%) in their 220 buzzards sampled in the period 2007–2018 with those in 56 buzzards in the period 1981–1991 of Pain et al. (5.4 and 1.8%, respectively) to compare the rate of birds highly exposed to Pb between the two periods. To strengthen this comparison between different periods of sampling, we used the subset of our birds in the same period as Taggart et al. (i.e., 2007–2018; n = 36) and compared this subset with the proportions of birds with high Pb levels of each study by the Fisher's test. (N.B. Some buzzards used in the study of Taggart et al. (2020) were provided from the UKCEH, but the buzzards in the present study were analysed independently from them.)

For Cd, a hepatic concentration of 3 mg/kg dw indicates elevated environmental poisoning (Scheuhammer, 1987). Arsenic residues in livers in the range of 2–10 mg/kg ww are considered as elevated concentrations, and residues >10 mg/kg ww is indicative of arsenic poisoning (Eisler, 1994; Goede, 1985). We therefore used 6.2 and 31 mg/kg dw as significant biological concentrations of As. For Ni residues, we used a hepatic concentration >3 mg/kg dw because this value is associated with adverse effects in avian species (Eisler, 1998; Outridge and Scheuhammer, 1993). Total hepatic Hg concentration >2 mg/kg ww (6.2 mg/kg dw) could be associated with adverse effects such as bird reproduction (Shore et al., 2011). Hepatic Se concentrations >10 mg/kg dw are associated with adverse effects such as reduced adult weight gain or lower reproductive success, and Se concentrations exceeding 30 mg/kg dw could be considered harmful to the health of birds (Ohlendorf and Heinz, 2011; Outridge et al., 1999). However, Se has been considered to protect against Hg toxicity, and the ratio of Se/Hg may indicate an interaction between these two elements (Cuvin-Aralar and Furness, 1991; Sumino et al., 1977; Yang et al., 2008). A Se/Hg molar ratio approaching 1 suggests the existence of mercuric selenide (HgSe) and is generally considered to reduce Hg toxicity. We therefore calculated the Se/Hg molar ratio.

2.3.2. Correlations between elements

Correlations between elements, body and liver weights, as well as their ratio (i.e., liver/body weight) were analysed by the Pearson's correlation index and test. Hepatic concentrations of each element were skewed, except Mo (Kolmogorov-Smirnov (KS) tests: p-value <0.05). We therefore applied a log-transformation to each metal, which made them normally distributed (KS tests: p-value >0.05). Nickel was removed from this analysis because of a high proportion of specimens with Ni concentration below LoD (16 specimens). We used 60 of the 72 specimens for this analysis, excluding 12 specimens with unknown body or liver weight.

2.3.3. Statistical modelling and relative importance of variables

The time trend of concentrations of elements in livers was modelled using the multimodel inference based on the information-theoretic approache (Burnham et al., 2011; Burnham and Anderson, 2002). For log-transformed concentrations of each element, we built a set of 40 candidate linear regression models. These models included all combinations of explanatory variables: binary age-class, sex, the interaction of the age-class and sex, time trend over time. The sampling locations were integrated into the analysis as a factor composed of four areas: Scotland, Northern England (North West, North East, Yorkshire & the Humber) Wales & Western England (West Midlands, South West), and Eastern England (South West, East of England, London, South East). Collection date was considered as the mid-point of the month of collection, and time of year within a calendar year was modelled by a sinusoidal function (Naumova et al., 2007; Ramanathan et al., 2020), in which the phase and amplitude of the seasonal relationship were assumed to be the same each year.

Model performance was compared by calculating the second-order Akaike's Information Criterion (AICc) which is used for small sample size (Burnham and Anderson, 2002). The model with the lowest AICc was chosen as the best model of the given element, and the assumptions of this best linear model were checked. We used 68 of the 72 specimens for this analysis, excluding four specimens with unknown sex or age. Plus, for each element, extreme values (i.e., those replaced by half of the maximum LoD) were excluded. The coefficient of determination (R^2) was calculated. We applied the same analysis to body weight and liver weight, which were not logarithmically transformed.

In addition, the relative importance of each model variable was estimated for each element. Estimates of the relative importance of a variable were made based on the multimodel inference approach (Burnham and Anderson, 2002). The probability of the 'best' model over the set of models was estimated by Akaike weight. For each variable, Akaike weights were summed up across all candidate models in which the given variable was included. This sum of Akaike weights indicates the relative importance of the given variable. The relative importance ranges between 0 and 1 and indicates the probability that the given variable would be retained to predict a given data across a set of models. The larger the relative importance, the more critical the given variable is for prediction. Thus, all the variables can be ranked in their importance for being integrated into a predictive model.

All statistical analyses were computed using the statistical software R (ver. 4.2.1) (R Core Team, 2020).

3. Results

3.1. Concentrations of elements in livers and biological thresholds

Descriptive statistics for liver concentrations of each element are summarised in Table 1. The median concentration of Pb in livers was 0.43 mg/kg dw, and the arithmetic mean (hereafter only 'mean') and geometric means were 0.86 and 0.43 mg/kg dw, respectively. Given its log-normal distribution, 95% confidence intervals were calculated for the geometric mean (hereafter 'CI'): 0.25–0.75 mg/kg dw. None of the samples analysed had abnormally high levels of Pb or concentrations associated with potentially lethal exposure, although the maximum Pb concentration (5.996 mg/kg dw) was near the abnormally high Pb level. No significant difference was observed in the proportions of specimens with abnormally high or potentially lethal Pb concentrations between our birds and the birds of both Pain et al. (1995) and Taggart et al. (2020) (Fisher's exact test p-value >0.05).

The median, mean, and geometric mean of liver Cd concentrations were 0.77, 1.60, and 0.63 (CI: 0.33–1.12) mg/kg dw, respectively. Six specimens (8.3% of all specimens) had concentrations related to Cd poisoning. Cadmium concentrations in livers of two specimens among these six reached >19 mg/kg dw. The median, mean, and geometric mean of liver As concentrations were 0.19, 0.52, and 0.23 (CI:

Table 1
The minimum (Min), median, arithmetic means (Mean), geometric mean (Geo-Mean), maximum (Max), and 95% confidence intervals for the geometric mean (95% CI) of concentration of each element (mg/kg dry weight). The number of speciments under the limit of detection (LoD) is given. Values under the LoD are replaced by half of the LoD.N = 72 for ll elements. For other descriptive statistics, see Supplementary Information Table S2.

		Cr	Mn	Fe	Co	Ni	Cu	Zn
Number of <LoD		6	–	–	–	16	–	–
Min		<0.01	5.30	517.62	0.03	<0.001	5.81	57.66
Median		0.03	12.53	1345.93	0.11	0.01	13.89	106.45
Mean		0.04	14.43	1834.01	0.13	0.03	15.53	122.39
Geo-Mean		0.03	12.79	1483.37	0.11	0.01	14.32	112.34
Max		0.28	75.08	16407.80	0.48	0.25	59.67	347.43
95% CI	**(lower)**	0.02	10.45	1150.78	0.08	0.004	12.06	94.21
	(upper)	0.04	15.66	1912.08	0.15	0.030	17.00	133.96
		As	**Se**	**Sr**	**Mo**	**Cd**	**Pb**	**Hg**
Number of <LoD		–	–	4	–	–	–	1
Min		0.03	2.41	<0.001	0.59	0.02	0.03	<0.02
Median		0.19	4.89	0.20	1.81	0.77	0.43	0.51
Mean		0.52	5.93	0.33	1.86	1.60	0.86	0.79
Geo-Mean		0.23	5.14	0.15	1.79	0.63	0.43	0.53
Max		9.57	36.57	2.32	4.03	19.46	6.00	4.50
95% CI	**(lower)**	0.14	4.15	0.07	1.57	0.33	0.25	0.35
	(upper)	0.40	6.38	0.32	2.03	1.12	0.75	0.80

0.14–0.40) mg/kg dw, respectively. Only one specimen (1.4%) showed an elevated As concentration of 9.6 mg/kg dw. Concentrations of Ni and Hg in all specimens were lower than their adverse effect values. Concentrations of Se were >10 mg/kg dw in seven specimens (9.7%), of which one showed concentration of Se >30 mg/kg dw (1.4%). The ratio of Se/Hg was more than one in all livers, ranging from 1.1 (Se/Hg = 4.30/3.90) to 202.4 (Se/Hg = 4.9/0.024 (N.B. the half of LoD)).

3.2. Correlations between elements

Except for Sr, all elements showed positive correlations (Fig. 1; for their ordination in reduced space, see Supplementary Information Figure S2). Most pair-wise correlations were significant. There were two groups within which elements were highly correlated with each other: One group comprises As, Cd, Cr, Hg, Pb, and Se, and the other comprises Cu, Fe, Zn, Mn, and Mo. Body and liver weights were negatively

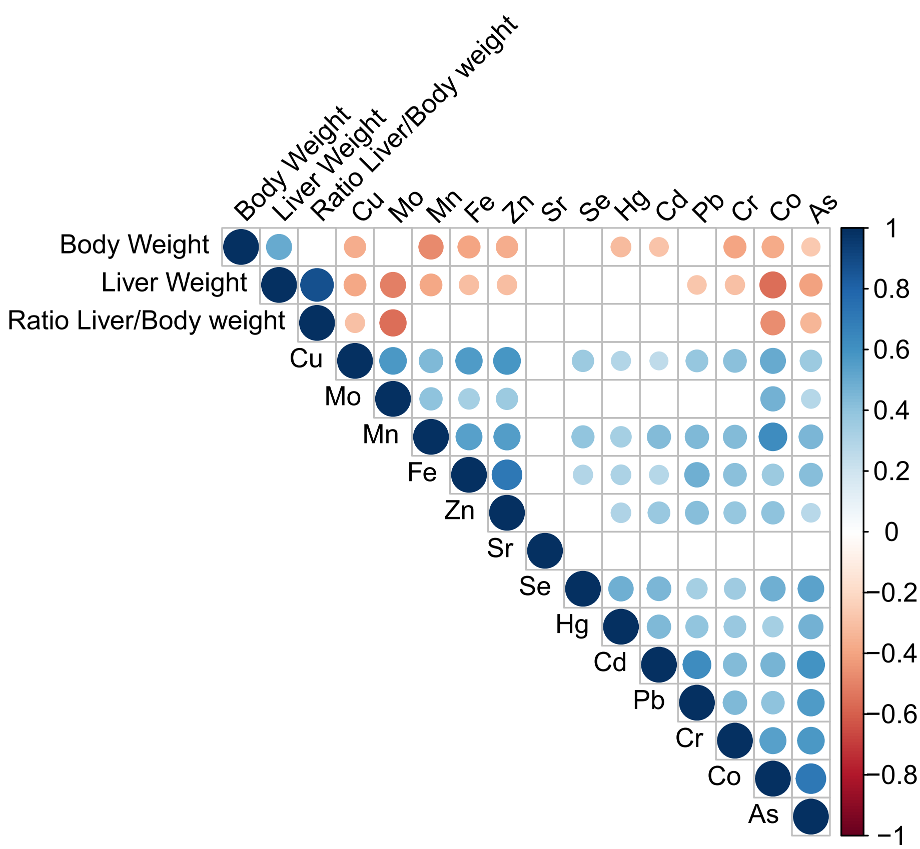

Fig. 1. Significant correlations between metals and biological parameters. The size and the depth of the colour of the circles signify Pearson's correlation coefficients. Orange circles indicate significant and negative correlations, whereas blue circles indicate significant and positive correlations by Pearson's correlation coefficient test. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

correlated with mainly the latter group, as well as Co and As. The ratio of liver/body weight was negatively correlated with Mo, Mn, As, and Co.

3.3. Statistical modelling and relative importance of variables

Table 2 summarises the variables of the best model for each element and R^2 of the model. Regular annual fluctuations, i.e., seasonality, were observed in the models for Pb, Cd, As, Se, Cu, and Co. Peaks of these contaminants were in late winter – early spring, and their troughs were in late summer–autumn, except Cu whose peak was in summer and trough was in winter. (For a graphical representation of seasonality, see Supplementary Information Figure S3 and Figure S4). In addition to seasonality, concentrations of Pb showed a consistent tendency to increase over time ($R^2 = 0.16$; Fig. 2a). Concentrations of Cd were higher in adults than juveniles ($R^2 = 0.32$), and Se were higher in males than in females ($R^2 = 0.13$). Concentrations of As differed between areas: Scotland < East England < the others ($R^2 = 0.34$). R^2 of the models for Cu and Co were 0.11 and 0.24, respectively.

Concentrations of Sr, Hg, and Cr did not seasonally fluctuated. However, Sr showed a decreasing tendency over time with no seasonal fluctuation ($R^2 = 0.05$; Fig. 2b). Mercury concentrations were higher in adults than juveniles ($R^2 = 0.11$; Supplementary Information Figure S5). Chromium concentrations were higher in males than females, in adults than juvenile, but lower in Scotland than the other areas ($R^2 = 0.24$; Figure S5).

The body weight was explained by age and sex ($R^2 = 0.28$); adults were heavier than juveniles, and females were heavier than males. In contrast, no variable explained the liver weight of buzzards.

The relative importance of the variables for each element is given in Table 3. For many metals, seasonality was more important than the other variables. The relative importance of seasonality was quite high for As and Co (>0.90), followed by Pb (0.85), Cd (0.82), and Cu (0.75), and a moderate level for Se (0.58). The age class was quite important for Cd and Hg (>0.90). The area was highly important for As (0.86), but also for Cr (0.64) and Pb (0.55). The relative importance of sex was not high compared to the other variables, except for Cr (0.72) and Se (0.67). The importance of the interaction between sex and age class was low for all elements (<0.13). The time trend showed a moderate level of importance for Pb (0.50), Sr (0.64), and Mo (0.40).

4. Discussion

4.1. Concentrations of elements and their time trend

4.1.1. Lead

The median concentration of Pb in our UK buzzard livers between 2001 and 2019 was lower than those in the previous studies: Pain et al. (1995) between 1981 and 1992 (median = 1.3 mg/kg dw) and Taggart et al. (2020) between 2007 and 2018 (median and geometric mean = 0.72 and 0.80 mg/kg dw, respectively). If we assume that the median Pb value are similar to the geometric mean because of a frequent trend of log-normal distributed hepatic metal concentrations, hepatic Pb concentrations in the 90s were much higher than the upper limit of the CI for our geometric Pb mean. In contrast, Pb in buzzards from the more recent monitoring was around this upper limit. However, the proportions of birds with abnormal or potentially lethal Pb levels were not significantly different from these two studies. Taggart et al. (2020) pointed out a similar level of hepatic Pb concentrations and no significant difference in the proportions between the two periods. Our results were broadly in line with it but suggested slightly lower hepatic Pb. Further analyses in depth are required to compare the results from these studies.

Buzzards from some other European countries showed higher liver Pb concentrations than ours. For instance, buzzards from Poland showed a mean of 2.0 (Komosa et al., 2012) or a geometric mean of 1.2 mg/kg dw (Kitowski et al., 2016). Buzzards from Italy showed a median of 1.0 (Battaglia et al., 2005), a mean of 2.1 (Zaccaroni et al., 2008), or a mean of 14.5 mg/kg dw (Naccari et al., 2009). Buzzards from the Netherlands showed medians of 0.7 (Hontelez et al., 1992) and 1.9 mg/kg dw (Jager et al., 1996). However, our median value was exceeded by those in Portugal (median of 0.28 mg/kg dw; Carneiro et al., 2014) and Denmark (median of 0.28 mg/kg dw; Kanstrup et al., 2019). The results of the studies in Spain were variable; buzzards analysed by Pérez-López et al. (2008) showed a lower mean liver Pb concentration (4.2 mg/kg dw) compared to ours, but concentrations observed in Castro et al. (2011) and García-Fernández et al. (1997) showed the opposite pattern: medians of 0.32 and 0.39 mg/kg dw, respectively. Liver Pb concentrations of buzzards vary between countries and between studies.

Our model showed a clear seasonal variation of liver Pb concentrations within years. Previous studies mentioned the high risk of lead poisoning in scavenging and predatory birds during the hunting season (e.g., Berny et al., 2015; Carneiro et al., 2014; Gangoso et al., 2009; Mateo et al., 1999). These birds are highly exposed to Pb when they ingest Pb shotgun pellets or fragments embedded in prey bodies (Finkelstein et al., 2012; Pain et al., 2019). Therefore, exposure of buzzards to Pb can significantly increase during autumn and winter when they scavenge shot game animals or hunt animals with embedded shot fragments, which is in line with our model for Pb. In addition, Taggart et al. (2020) demonstrated by an isotopic analysis that much Pb in the livers of buzzards showing potentially lethal exposure to Pb was consistent with that derived from Pb shotgun pellets.

Our model also revealed an increasing temporal trend of exposure to Pb over time. This result differs from the systematic review by Monclús et al. (2020), which found a marginally significant decrease (p-value = 0.06) in hepatic Pb concentrations between the periods 2000–2009 and 2010–2019 across Europe. In the UK, Pb shot was banned over wetlands but is legal for most terrestrial shooting (Mateo and Kanstrup, 2019; Stroud, 2015). Plus, a recent study revealed that the proportion of ducks

Table 2
Comparison of the performance of the best model for each element in the liver of buzzards in the UK. For each element, the included variables (X: included; blanc: excluded) and its coefficient of determination (R^2) are given. The interaction between sex and age is excluded from the table because no model included it. If the variables sex, age or area are included in the model, difference within their classes are given. We excluded Mn, Fe, Ni, Zn, and Mo from the table because their best model was null model (i.e., with no variable). (Mal: males; Fem: females; Adu: adults; Juv: juveniles, Scot: Scotland; N. Eng: North England; E. Eng: East England; W. Eng: Wales & West England.)

Element	n	Temporal trend	Sex	Age	Area	Seasonality	Peak	Trough	R^2
Pb	68	Increasing				X	Feb.	Sep.	0.16
Cd	68			Adu.>Juv.		X	Feb.	Aug.	0.32
As	68				Scot. < E. Eng. < N. Eng. < W. Eng.	X	Feb.	Aug.	0.34
Hg	67			Adu.>Juv.					0.11
Se	67		Mal.>Fem.			X	Apr.	Oct.	0.13
Cu	68					X	Jul.	Jan.	0.10
Co	68					X	Mar.	Sep.	0.14
Cr	60		Mal.>Fem.	Adu.>Juv.	Scot. < W. Eng. < N. & E. Eng.				0.24
Sr	64	Decreasing							0.05

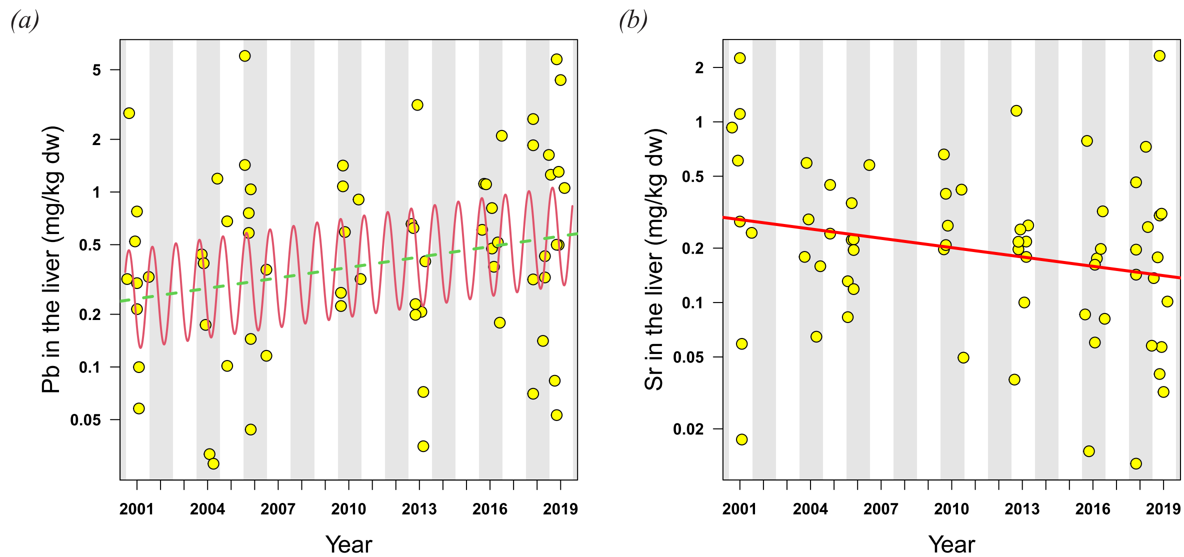

Fig. 2. Concentrations of lead (a) and strontium (b) in the liver of buzzards collected in the UK from 2001 to 2019 in relation to their collection date (i.e., the mid-point of the month of collection). Each point represents a concentration of the given element of an individual. For lead, the continuous lines represent modelled values, and the bold dashed line represents the temporal trend.

Table 3
Comparison of the relative importance of variables (%) for each element: trend over the year (Trend), sex, age, the interaction between sex and age (Sex:Age), area, and the Seasonality.

Element	N	Relative importance					
		Trend	*Sex*	*Age*	*Sex:Age*	*Area*	*Seasonality*
Pb	68	0.50	0.28	0.48	0.06	0.55	0.85
Cd	68	0.26	0.41	>0.99	0.13	0.15	0.82
As	68	0.22	0.23	0.23	0.01	0.86	>0.99
Hg	67	0.32	0.33	0.94	0.08	0.07	0.19
Se	67	0.35	0.67	0.60	0.12	0.21	0.55
Ni	52	0.25	0.27	0.26	0.03	0.15	0.11
Zn	68	0.33	0.29	0.30	0.03	0.04	0.10
Cu	68	0.36	0.30	0.29	0.03	0.10	0.75
Fe	68	0.30	0.39	0.28	0.04	0.22	0.15
Mn	68	0.27	0.28	0.26	0.02	0.34	0.17
Mo	68	0.40	0.32	0.32	0.10	0.11	0.25
Co	68	0.26	0.28	0.25	0.02	0.08	0.94
Cr	60	0.34	0.72	0.56	0.10	0.64	0.26
Sr	64	0.64	0.26	0.26	0.02	0.07	0.13

illegally killed by lead shotgun pellets remained high (about 70%) throughout a period of about 20 years (Stroud et al., 2021). Lead is stable under most environmental conditions, and the decomposition of particulate lead takes tens or hundreds of years (Rooney et al., 2007; Scheuhammer, 1996). The observed increasing trend of Pb in buzzards can imply that remaining Pb residues in the environment, potentially due to both legal and illegal shooting, might also be a non-negligible source of exposure which gradually imposes an important Pb burden on raptors.

4.1.2. Cadmium

Compared to Pb, the proportion of potential Cd poisoned birds was elevated (8.3% of specimens). In many other European countries, median values of hepatic Cd concentrations in buzzards exceeded the median value and the upper limit of the CI, such as Portugal (median = 0.18 mg/kg dw, Carneiro et al., 2014) and Italy (median = 0.01 mg/kg dw; Battaglia et al., 2005). Buzzards from Denmark (Kanstrup et al., 2019) showed a higher median concentration (1.2 mg/kg dw), but a lower mean concentration (1.3 mg/kg dw) than our study. Similarly, buzzards from the Netherlands showed both higher and lower median values: 1.2 (Jager et al., 1996) and 0.6 mg/kg dw (Hontelez et al., 1992). Exposure of buzzards to Cd was generally higher in the UK than in European countries.

Our model revealed a seasonal fluctuation within years and, despite recent declines in anthropogenic Cd release (Mahler et al., 2006), liver Cd concentrations remained over time. Buzzards are generalist predators but also opportunists specialising in prey such as rabbits or voles, where or when they are numerous (e.g., Graham et al., 1995; Reif et al., 2004, 2001; Swann and Etheridge, 1995). Kitowski et al. (2016) reported that higher liver Cd concentrations were observed in small mammal-eating raptors compared to raptors with other diets. They argued that an increase in liver Cd concentrations of buzzards could be directly related to their feeding preferences for small mammals, particularly voles. In Britain, the number of certain small mammals such as bank voles (*Myodes glareolus*), field voles (*Microtus agrestis*), or wood mice (*Apodemus sylvaticus*) in general peaks in late summer-early winter and troughs in spring-summer (Harris and Yalden, 2008). Given the synchronisation between the peak abundance of these small mammals, we suppose that small mammals could be an important source of exposure to Cd. Although potential reasons for this high exposure in the UK are unknown, seasonal change in buzzard diet may be one possible reason for the fluctuation of Cd within years.

4.1.3. Mercury and selenium

No buzzards in our study showed liver Hg concentrations associated with adverse effects. Studies of mercury in buzzard livers in Europe suggest a slight decrease in median Hg concentrations from 2.0 mg/kg dw in Spain during 1997–2005 (Castro et al., 2011), 1.2 mg/kg dw in Portugal 2007–12 (Carneiro et al., 2014), to 1.0 mg/kg dw in Denmark 2013–2016 (Kanstrup et al., 2019). Both median values and the upper limit of the CI for Hg concentrations in our buzzards were lower than those from other countries. Moreover, Se/Hg ratios calculated in livers always exceeded 1. However, the median liver Se concentration of our buzzards was higher than those from Denmark 2013–2016 (median = 3.7 mg/kg dw) (Kanstrup et al., 2019). Seven of our birds had high liver Se concentrations associated with adverse effects, and one of the seven specimens had a concentration considered harmful to health. It is possible that our buzzards were protected against Hg toxicity by high Se concentrations, but high Se levels in some buzzards might cause toxicity.

4.1.4. Other elements (As, Cu, Zn, Mn, Fe, Ni, Cr, Sr, Co, and Mo)

Mean hepatic concentrations of Cu, Zn, and Mn in our birds were within a similar range to the values reported by Komosa et al. (2012) and Naccari et al. (2009): mean = 10.1–39.5 (Cu), 121.5–137.5 (Zn), and 5.3–9.1 mg/kg dw (Mn), respectively. Interestingly, buzzards' body and liver weights were negatively correlated to Cu, Fe, Mn, Mo, and Zn in our study. These elements are considered essential elements, but high concentrations can cause harmful effects on birds. For example, the toxic effects of Cu can decrease egg production, body and tissue weight, and feather growth, whereas high levels of Zn affect body conditions, decreasing body mass (Koivula and Eeva, 2010). Henderson and Winterfield (1975) reported 187–323 mg/kg dw as an acute Cu poisoning concentration in the livers of Canada Geese (*Branta canadensis*). Although accepted limits of these elements vary between species (Mateo and Guitart, 2003; Taggart et al., 2006), concentrations of Cu in our specimens were lower than these critical values. Clinical signs of Zn poisoning were observed in mallards with liver concentrations of 473–1990 mg/kg dw (Levengood et al., 1999). A mean liver Zn concentrations of 440 mg/kg dw (Beyer et al., 2004) and a range of 700–1830 mg/kg dw (Sileo et al., 2003) were reported in wild waterfowl from contaminated sites, whereas Gómez et al. (2004) considered 122 mg/kg dw as equivalent to a liver Zn concentration in passerine and waterfowl from uncontaminated sites. The mean Zn concentration in our study was similar to the value of birds from uncontaminated sites.

The reasons for the significant relationships between biological parameters and hepatic metal concentrations remain unclear. One of the possible causes is a relative increase in hepatic metal concentrations due to liver weight loss. In the study of Esselink et al. (1995), barn owls (*Tyro alba*) showed an increase in hepatic Cu concentrations with liver weight loss because total Cu content in the liver was not affected by body conditions. Debacker et al. (2000) also observed that cachexia by starvation was negatively correlated with hepatic concentrations of essential elements, such as Cu, Zn and Fe in common guillemots (*Uria aalge*) recovered from Belgian beaches. The negative correlations in our study may be due to a decrease in buzzard body and liver weights, such as starvation, leading to an increase in essential element concentrations in livers. However, hepatic concentrations of harmful metals did not change by body or liver weight loss in these previous studies, which does not concur with our results. Our results might be due to several causes, but further studies are required to elucidate this point.

Mean liver As concentrations in our specimens was higher than the mean reported in Carneiro et al. (2014) (0.10 mg/kg dw) but lower than the means in Naccari et al. (2009) and Pérez-López et al. (2008) (1.3 and 5.8 mg/kg dw, respectively). Although one specimen showed an elevated liver As concentration, we suggest that most of our specimens were not poisoned by As. Interestingly, exposure to As clearly fluctuated within years in a similar way to Pb and Cd. Although Pain et al. (1992) did not show a relationship between high tissue Pb concentrations (i.e., Pb shot ingestion) and As levels in the liver, As is present in small amounts in Pb shot (Hall and Fisher, 1985). The lead shot could be a source of As in buzzard tissues. Concentrations of As also varied between the four areas. There are several natural and anthropogenic sources of As, such as fossil fuels, nonferrous metal mining and smelting, and pesticide production and application (Wang and Mulligan, 2006). It is difficult to identify the causes of the variation in As exposure because of a wide surface of area. However, the different pattern of As exposure than Pb might suggest other possible sources of As than the lead shot.

The geometric mean of Ni concentrations in buzzard livers from Poland, 0.25 mg/kg dw (Kitowski et al., 2016), was much higher than the upper limit of the CI in our study. Two previous studies in Poland reported much higher mean Cr concentrations (0.47 and 0.55 mg/kg dw) than our study (Kitowski et al., 2016; Komosa et al., 2012). Kitowski et al. (2016) showed that consumption of passerines is correlated to elevated concentrations of Cr, and the buzzards studied in the Białowieza National Park, Poland, were mainly feeding on birds (Jędrzejewski et al., 1994). Thus, these high Cr concentrations might be explained by a difference in the diet of buzzards between the UK and Poland.

To our knowledge, there is no reference value for the other elements (Fe, Mo, Co, Cr, and Sr). Jager et al. (1996) showed a range of hepatic Fe concentrations in buzzards from the Netherlands similar to ours (range = 475.8–11562.9 mg/kg dw). Kanstrup et al. (2019) measured and provide concentrations of these elements in the livers of buzzards: mean concentrations of 485.61 (Fe), 0.63 (Mo), 0.02 (Co), 0.02 (Cr), and 0.08 (Sr) mg/kg dw. Except for Cr, the specimens showed higher mean liver concentrations for these elements than buzzards from Denmark from 2013 to 2016. Concentrations of Sr in our buzzards showed a decreasing trend. Although the biological thresholds of this element are not clarified, its impact on wildlife might be less important in the future.

4.2. Important variables for predictive models

Seasonality was the most important variable for predicting exposure to many elements among the variables used in this study. Exposure to Pb and Sr also showed a constant trend over time. Age was noteworthy mainly for two non-essential elements, Cd and Hg. Sex was retained for Se and Cr. In contrast, the interaction between age and sex was not essential for any element. The area was important for As and Cr. Although our final model did not include it, the area was as important as the time trend over time for predicting exposure to Pb.

The seasonal variation of Pb is synchronised with the shooting season in the UK. Taggart et al. (2020) argued that an increase in liver Pb concentration of buzzards from summer to winter is consistent with the shooting season for buzzard prey, such as common pheasant (*Phasianus colchicus*; October–January), partridge (*Perdix perdix* and *Alectoris rufa*; September–January), ducks and geese (Anatidae; September–January), red grouse (*Lagopus lagopus*; August–December), and common woodpigeon (*Columba palumbus*) which are most frequently shot in winter. Foraging on these carrion or game animals probably causes elevated lead accumulation, which is in line with our findings. On the other hand, the seasonal variation of other elements, like Cd, could be linked to a variation of certain kinds of prey in the field, such as voles or birds. Raptors primarily accumulate non-essential elements in their tissues through their diets (Beyer and Meador, 2011). Many studies have demonstrated that the diet of buzzards varies both spatially and temporally with changes in the local abundance of prey, including small mammals, birds, amphibians, reptiles, and insects (Francksen et al., 2017, 2016; Graham et al., 1995; Reif et al., 2004, 2001; Swann and Etheridge, 1995). The high relative importance of seasonality for exposure to elements probably reflects the importance of temporal variation in diet.

Several reasons for the importance of area could be assumed, such as possible differences in the soil or environmental contamination level of these elements, in the diet of buzzards, and/or in the effectiveness of the lead shot regulations between these four areas. Each area covered a wide surface of the UK, whereas there is no data for the environmental contamination of elements across the UK in high resolution and for the diet of our buzzards. It is therefore difficult to identify the reasons for the importance. Nonetheless, our results suggest the importance of the location samples, which should be integrated into monitoring for certain metals.

The importance of age for Cd agrees with many previous studies where higher liver Cd concentrations were reported in adults than in juveniles (e.g., Battaglia et al., 2005; Carneiro et al., 2014). With continued exposure, Cd accumulates throughout the life span due to its long biological half-life and slow tissue elimination (Scheuhammer, 1987; Wayland and Scheuhammer, 2011). Hepatic Hg concentrations were explained by age in our study, but the proportion of variation explained by this factor was low ($R^2 = 0.11$). Carneiro et al. (2014) showed that age influenced blood Hg concentrations but not hepatic and renal Hg concentrations. Despite its relative importance as a predictive variable on liver Hg concentrations, the difference between adults and juveniles was relatively small compared to the overall variance within the sample. Consequently, this difference between age classes for Hg is unlikely to be of toxicological significance.

The lack of differences between sexes, except for Se and Cr, is probably due to similar dietary habits. Females can sequester some heavy metals in eggs (Burger, 1994). In the study of Burger and Gochfeld (1996), both Se and Cr were higher in eggs of Franklin's gull (*Larus pipixcan*) than in the feathers of parents. But there was no significant difference between the sexes in their study. Similarly, Zaccaroni et al. (2003) did not observe significant differences in liver Cr concentrations between the sexes of little owls (*Athene noctua*). In our results, age and the interaction between age and sex were not important for these elements, which means that the reason for high Se and Cr in males is not apparent yet. Nonetheless, sex could be examined in further studies because some of our specimens showed high Se concentrations associated with adverse effects.

5. Conclusion

Our study has shed light on the concentrations of many essential and non-essential elements in the liver of the common buzzard as a sentinel species. Most of our samples showed a low risk of harmful concentrations compared to the significant biological concentrations and to previous studies, except for Cd. Exposure of buzzards to many elements was related to seasonal fluctuation. This seasonality could be linked to the bird's diet, which is itself determined by their feeding ecology, availability of its prey, and seasonal variation in human activities like hunting. These factors related to the diet should be considered in further studies on the exposure of apex species to contaminants. Moreover, we have also revealed that exposure to Pb has increased over time in the UK. The reasons for these increasing trends could be related to a lead ammunition use but is still uncertain. Further biomonitoring could help elucidate the source and consequences of exposure to these non-essential elements. The importance of the variables assessed in our study can inform the choice of variables in further biomonitoring schemes.

Credit author statement

S. Ozaki: Formal analysis – Statistical analysis, Visualization, Writing – Original draft preparation. **P. Movalli**: Conceptualization, Methodology, Writing – Review & Editing, Funding acquisition. **A. Cincinelli**: Writing – Review & Editing. **N. Alygizakis**: Writing – Review & Editing. **A. Badry**: Writing – Review & Editing. **J. S. Chaplow**: Software Validation, Data curation, Writing – Review & Editing. **D. Claßen**: Writing – Review & Editing. **R. W. R. J. Dekker**: Writing – Review & Editing, Funding acquisition. **B. Dodd**: Resources, Investigation, Writing – Review & Editing. **G. Duke**: Writing – Review & Editing. **J. Koschorreck**: Writing – Review & Editing. **M. G. Pereira**: Formal Analysis – Chemical analysis, Writing – Review & Editing. **E. Potter**: Resources, Investigation, Data Curation, Writing – Review & Editing. **J. Slobodnik**: Writing – Review & Editing. **S. Thacker**: Formal Analysis – Chemical analysis, Writing – Review & Editing. **N. S. Thomaidis**: Writing – Review & Editing. **G. Treu**: Writing – Review & Editing. **L. Walker**: Conceptualization, Funding acquisition, Methodology, Writing – Review & Editing, Supervision, Project administration.

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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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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