Metals and their relationship to measures of physiological stress in common buzzards (Buteo buteo) and sparrowhawks (Accipiter nisus)

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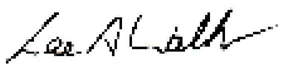
Signed 

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1 Executive Summary

The Predatory Bird Monitoring Scheme (PBMS) and Natural England work collaboratively on a developmental terrestrial chemical monitoring programme for England. The data from this programme have been used by Natural England to advise government on chemicals policy. The data are also used by PBMS and Natural England to service the H4 indicator that is a suite of metrics in the 25-Year Environment Plan (25-YEP) Outcome Indicator Framework, aiming to show the change in the exposure of wildlife to harmful chemicals and the impact of environmental exposure on wildlife. The indicator contributes to measuring whether we are moving towards the goal of ‘managing exposure to chemicals and pesticides’ as given in the Environmental Improvement Plan 2023 (‘the 2023 Plan’).

The Eurasian sparrowhawk (*Accipiter nisus*) has been used as a model species for heavy metal exposure in the terrestrial compartment. However, due to increasing requirements for investigating a wide range of emerging contaminants, the mass of tissue required, liver, may limit the ability to analyse for all contaminants. It therefore becomes difficult to report changes in the time trend of both legacy and emerging contaminants with this small-size raptor samples. Given such limitations on the size of sample mass, there are three needs to address: a) to identify another predatory bird species as a substitute for the sparrowhawk, b) to determine some biological indicators explaining the exposure of organisms to metals, and c) to optimise biological tissues used for chemical analysis of a broad suite of contaminants, including metals.

The common buzzard (*Buteo buteo*) is considered a suitable sentinel raptor for metal exposure, as it is a widely distributed but sedentary raptor in the United Kingdom (UK). However, the sparrowhawk and common buzzard do not have the same diet and feeding behaviour, which could lead to different exposure patterns between these species. Therefore, it is important to assess whether the exposure of the two species to metals differs.

This study, initially conducted before the publication of the H4 interim report 2024 for serving this latest version of the H4 interim report, aims to meet the need and assess whether transitioning from using sparrowhawks to buzzards is pertinent for the H4 indicator. The study will achieve this goal by (1) comparing liver metal concentrations between the two species, (2) building a baseline on exposure to and effects of metals in buzzards, (3) assessing the relationships between the PBMS post-mortem data and metal concentrations, and (4) identifying specific biological parameters that describe and predict heavy metal exposure without using the target organ: the liver.

Under the Predatory Bird Monitoring Scheme (PBMS), 40 sparrowhawks and 40 buzzards were collected across the UK in 2020 and 2021. The concentrations of 14 elements, including the six metals reported in the H4 indicator report 2021: mercury (Hg), lead (Pb), cadmium (Cd), zinc (Zn), copper (Cu), and nickel (Ni), were measured in the liver of these 80 birds. Concentrations of the metals in the liver were statistically compared between the two species and between the years. Metal concentrations were also compared to the threshold values for adverse effects from the two H4 interim reports (2021 and 2024) and the literature. Moreover, by adding the sample data in 2020 and 2021 to the data from 2007 to 2014 mentioned in the H4 interim report 2021, we statistically tested the time trend of the six metals in sparrowhawks. Some biological parameters measured during the post-mortem examination were considered as “health indicators”. Some of them, such as tissue mass, may be affected by harmful effects by

metals, whereas others, such as starvation, may lead to increase metal concentrations in tissue. Although there may be an interaction among such factors, they can potentially be used to predict metal exposure. The relationships between these health indicators and metal concentrations in the liver were also statistically tested.

Our results showed significant differences in the metal composition between sparrowhawks and buzzards. Sparrowhawks showed higher exposure to Hg than buzzards. Mercury concentrations were significantly higher in sparrowhawks than in buzzards, and the proportion of birds exceeding the threshold for Hg was also significantly higher in sparrowhawks. In contrast, concentrations of Pb, Cd, Cu, and Ni in the liver were significantly higher in buzzards than in sparrowhawks.

Our comparisons between the two years showed that concentrations of arsenic (As) were significantly higher in 2021 than in 2020. Concentrations of Cd and Ni were significantly higher in 2021 than in 2020 only in buzzards. In contrast, concentrations of the two metals were not significantly different between years in sparrowhawks. Meanwhile, our over-year time trend analysis showed that no significant trend was observed in the six metals.

Regarding the possibility of modelling exposure by biological parameters, concentrations of Cu and Zn in the liver of both species were explained by the liver weight and fat score: concentrations of Cu and Zn increased when liver weight and the fat score decreased. The liver weight similarly explained Cd in sparrowhawks and Hg, Pb, and Cd in buzzards.

Based on the results of this report, this study demonstrates that (i) it could be challenging to use the buzzard as an alternative species of the sparrowhawk due to the variation in metal concentrations between the two species; (ii) the continuity of the time trends analysis from using sparrowhawks to buzzards needs further assessment of long-term time trend of each metal, such as a current study being conducted by APEM Ltd. on behalf of the Environment Agency using PBMS-generated data; (iii) essential elements, such as Zn and Cu, were closely linked to certain biological parameter, whereas non-essential elements like Cd and Pb were less importantly linked; and (iv) certain biological parameters like fat score and liver weight can be used to estimate concentrations essential metals.

It is therefore concluded that using the buzzard as an alternative for the sparrowhawk, and modelling metal exposure by biological parameters still present challenges as options to resolve the problems of a limited sample mass. Meanwhile, this report identified study areas related to gaps of knowledge for the goals and proposed some recommendations for further studies: Continuing to compare the time trend of metals in the two species, integrating environmental factors into the analysis, and assessing other organs and biological parameters to elucidate the toxicokinetic of metals.

2 Glossary

Arithmetic mean. The sum of a collection of numbers divided by the count of numbers in the collection.

Bioaccumulative (substance). Substance whose concentrations in tissue or organism increase though trophic levels.

Dry weight. Weight of organism or tissue after all the water has been removed.

Essential elements. Elements having a specific biochemical function in organisms, like being a component of enzymes.

Multiple comparisons: Analysis of all possible pairwise comparisons

Non-essential elements. Elements having no specific biochemical function in organisms.

Opportunistic (sampling protocol). Sampling protocol taking advantage of circumstances. In biomonitoring, sampling method using animals found dead or dying in the field.

Post-mortem examination. Examination of a dead body, by dissection, to determine the cause of death and other biological parameters.

P-value. Probability of obtaining the result observed and more extreme results under the assumption that the null hypothesis is correct. A very small p-value means that the observed outcome would be very unlikely under the null hypothesis.

Recovery rate. Percentage of an original substance that is recovered after a chemical analysis, comparing the values in certified reference materials.

Sentinel (animals). Animals used to detect risks to humans or to model exposure to or effects of harmful substances.

Sexual dimorphism. Difference in morphology between sexes.

Starvation. State of a severe deficiency in calorific energy intake or food.

Statistical power. probability that the test correctly rejects the null hypothesis when a specific alternative hypothesis is true.

Target organ. Organ in which chemical contaminants are accumulated.

Wet weight. Weight of organism or tissue containing the water.

3 List of abbreviations and acronyms

Elements

As	Arsenic
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
Fe	Iron
Hg	Mercury
Mn	Manganese
Mo	Molybdenum
Ni	Nickel
Pb	Lead
Se	Selenium
Sr	Strontium
Zn	Zinc

Other contaminants

BDEs	Polybrominated diphenyl ethers
PBT	Persistent, bioaccumulative, and toxic (substance)
PCB	Polychlorinated biphenyls
PFOS	Perfluorooctanesulfonic acid
SGARs	Second-generation anticoagulant rodenticides

Chemical analysis

CRMs	Certified reference materials
DRC	Dynamic reaction cell
dw	Dry weight
ww	Wet weight
LoD	Limit of detection

Health indicators

BW	Body weight
CI	Condition index
FS	Fat score
KW	Kidney weight
LW	Liver weight
RKW	Relative kidney weight
RLW	Relative liver weight

Statistics

ANOVA	Analysis of variance

CIT	Conditional inference trees
CoIA	Co-inertia Analysis
Geo-Mean	Geometric mean
MANOVA	Multivariate analysis of variance
Max	Maximum
Min	Minimum
PCA	Principal component analysis
Q1	First quartile
Q3	Third quartile
SD	Standard-deviation

4 Introduction

4.1 General context

The H4 indicator ‘Exposure and adverse effects of chemicals on wildlife in the environment’ is one of a suite of indicators in the 25-Year Environment Plan (25-YEP) Outcome Indicator Framework (UK Government, 2018). The aim of the H4 indicator is to show how the exposure of wildlife on land or in water to harmful chemicals is changing and to see whether wildlife is impacted by environmental exposure to such chemicals over time (Environment Agency, 2021; 2022). The H4 report covers progress on the development of the H4 indicator, which is based on chemical concentrations found in water and different organisms in three environmental compartments: terrestrial, freshwater, and marine (estuarine, coastal, and offshore). Development of the indicator is being guided by the recommendations from the initial trial of the dashboard approach by Shore et al. (2020) and Shore and Walker (2020).

The H4 indicator tracks changes over time in the exposure of wildlife to various contaminants. In the previous H4 interim report published in 2021, two bird species were used as terrestrial sentinels: heavy metals in the Eurasian sparrowhawk (*Accipiter nisus*) for the period 2000 – 2014 and second-generation anticoagulant rodenticides (SGARs) in the red kite (*Milvus milvus*) in the period of 2015 – 2019, both collected by the Predatory Bird Monitoring Scheme (PBMS) (UK Centre for Ecology and Hydrology; UKCEH). The time trend and threshold assessments were reported for mercury (considered as a persistent, bioaccumulative, and toxic (PBT) substance), lead, cadmium, zinc, copper, and nickel, as well as SGARs in these terrestrial organisms in the H4 interim report 2021 (Figure 1a). Moreover, additional chemicals were included for consideration in the terrestrial compartment as part of the development of the H4 indicator in the latest version of the H4 interim report published in 2024, such as polybrominated diphenyl ethers (BDEs), polychlorinated biphenyls (PCB), and per- and polyfluoroalkyl substances (PFAS) as PBT substances (Figure 1b).

Raptors are considered suitable sentinels for monitoring chemical contaminants due to their widespread distribution in Europe and long lifespan, which allows the assessment of large spatial and long temporal trends (Espín et al., 2016; Furness and Greenwood, 1993; Gómez-Ramírez et al., 2014; Movalli et al., 2019). However, the PBMS biomonitoring protocols are opportunistic and do not allow a systematic sample collection. The sparrowhawk is a relatively small raptor compared to other raptors, with an average biomass of about 150 – 300 g, with the liver, the target organ for many chemical contaminants, weighing between 2 and 8 g. Given the size of the target organ compared to the number of contaminants for which it is intended to monitor changes over time, using sparrowhawk as a model species for the assessments for metals and other contaminants is limited. Therefore, there is a need to explore whether (i) another species could be used as a substitute for the sparrowhawk to develop H4 assessments in terrestrial organisms and/or (ii) whether there are biological parameters which could be used to indicate exposure to metals, conserving biological tissue for analysis of other chemical contaminants.

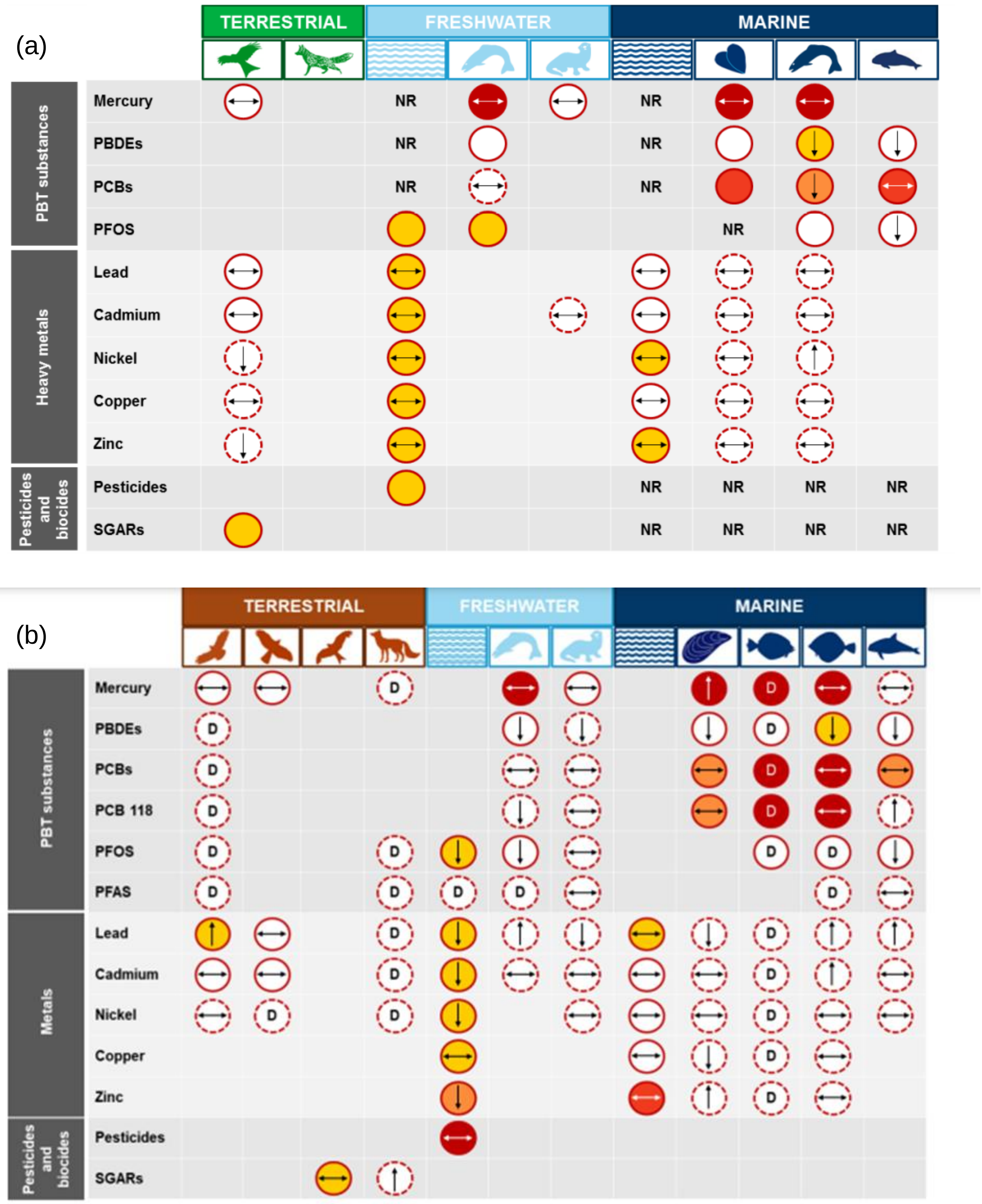


Figure 1. The interim H4 indicator dashboards, taken from the H4 interim report 2021 (a) and from the H4 interim report 2024 (b).

The common buzzard (*Buteo buteo*) is considered a suitable sentinel raptor species, particularly for metals (Badry et al., 2020). Many investigations on metal poisoning of this species have been conducted in the UK (Pain et al., 1995; Taggart et al., 2020) 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). The common buzzard is a widely spread raptor in Europe, like the sparrowhawk, and both bird species are sedentary in the UK. Based on this, the buzzard could be further considered as a replacement to using sparrowhawk.

The two species do not share all important morphological characteristics and ecological traits, such as diet and habitat, that can be related to exposure to chemical contaminants. Buzzards weigh around 700 – 900 g (Tubbs, 1974), although females are slightly bigger than males. Sparrowhawks are much smaller than buzzards and show a remarkable sexual dimorphism: females weigh about 300 g, and males weigh around 150 g (Newton, 1986). The diet of both sparrowhawk and buzzard is different (Table 1). Although both species actively hunt their prey, sparrowhawks mainly feed on avian prey; females can take medium size of prey like fowl and rabbits, whereas males prey on small birds and to a lesser extent rodents. (Götmark and Post, 1996; Newton, 1986; Selås, 1993). Buzzards feed on a range of prey: mainly small mammals but also birds, reptiles, and insects, and show scavenging strategies (Francksen et al., 2016; Graham et al., 1995; Mañosa and Cordero, 1992; Reif et al., 2001). Their preferred habitat types are also different. Sparrowhawks prefer woodland but also nest in city parks or other urban areas (Newton, 1986). In contrast, buzzards frequent all habitats that provide open areas for hunting, including pasture or cultivated farmland (Tubbs, 1974). The difference in the diet and habitat could lead to different exposure patterns between the two species because raptors primarily accumulate non-essential elements in their tissues through their diets (Beyer and Meador, 2011).

Since sparrowhawks had been used so far for assessing the time trend of the exposure in the H4 interim report 2021, using buzzards, which have different exposure scenario and different biological parameters, will provide a different exposure route. Therefore, it is necessary to clarify their different metal exposure patterns to discuss whether a transition from using sparrowhawks to buzzards is pertinent and how such a transition should be adjusted. It is also necessary to assess the relationships between biological parameters and exposure of metals in the two species to discuss the possibility of modelling metal exposure by biological parameters.

Table 1. Ecology of sparrowhawks and buzzards.

	Sparrowhawk (*Accipiter nisus*)		**Comment buzzard** (*Buteo buteo*)
	Females	***Males***	
Feeding trait	Active hunter		Active hunter Facultative scavenger
Diet	Mainly avian prey		Small to medium sized mammals, insects, birds, reptiles.
	Medium sized birds (or mammals)	Small sized birds (or mammals)	
Habitat type	Forest habitats, urban areas		Agricultural areas, forest, and occasionally urban habitats

4.2 Aims of this report

This study reports metal concentrations in the liver of the two predatory bird species: buzzard and sparrowhawk, sampled in 2020 and 2021 across the UK. Although the present report is published after the H4 interim report 2024, this study was initially conducted to meet the need and assess whether transitioning from using sparrowhawks to buzzards is pertinent for the H4 indicator. Based on the data on the metal concentrations in the liver, this report aims to:

1. Compare metal concentrations in the liver of sparrowhawks in 2020 and 2021 to those of buzzards in the same years (i.e., Is the buzzard pertinent as an alternative species of the sparrowhawk for exposure to metals?),
2. Build a baseline on exposure and effects to heavy metals in buzzards (i.e., How should concentrations of metals in the buzzard be adjusted to those in the sparrowhawk for the continuity of the time trends based on the sparrowhawk?),
3. Assess the relationships between some biological parameters (some of the PBMS post-mortem metadata) and concentrations of metals in the two species (i.e., How are biological parameters correlated to concentrations of metals in the species?), and
4. Investigate the potential for using the biological parameters as “health indicators” to estimate heavy metal exposure (i.e., Do some biological parameters predict exposure values? What biological parameters could be used as health indicators? How accurately does these health indicators predict concentration values of metals in the target organs?).

In addition, the over-year time trend of the metals reported in the H4 interim report 2021: Hg, Pb, Cd, Ni, Cu, and Zn, was checked by using the data for sparrowhawks in 2020 and 2021 and the data from 2007 to 2014 taken from the 2021 H4 interim report.

5 Materials and Methods

5.1 Bird sample collection and biological parameters

Forty buzzards and 40 sparrowhawks were used in this study based on a stratified method that avoided a skewed within-year and age distribution. All the specimens were found dead in the wild and collected from across the UK in 2020 and 2021 by the public, birdwatchers, rehabilitation centres, and wildlife managers through bird journals, newsletters, and other communications (Figure 2). Carcasses were sent to the PBMS at the UKCEH Lancaster site. All carcasses were subject to a post-mortem examination conducted by an experienced wildlife ecologist at the UKCEH Lancaster site, and various tissue samples were extracted and stored at -20 °C.

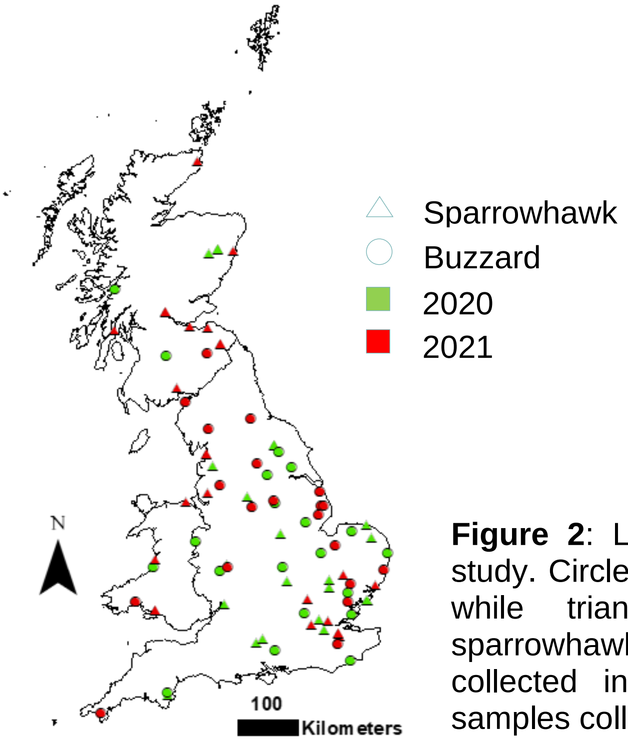


Figure 2: Locations of samples included in the study. Circles represent the locations of buzzards, while triangle represent the locations of sparrowhawks. Green colour represents samples collected in 2020, and red colour represents samples collected in 2021.

The sex of an individual was determined based on the identification of the gonads or bird's size and plumage. The approximate age was determined from plumage characteristics and assigned following the EURING code (EURING, 2016). Specimens were classed into two age classes: young birds collected in the calendar year of hatching (i.e., "first-year individuals") and older birds (i.e., "adults"). The number of birds belonging to each sex and each age class is summarised in Table 2. A probable cause of death was also recorded for all samples, and the majority of them were due to the traffic accident.

Table 2. Number of sparrowhawks and buzzards examined in each demographic group for individuals found dead in 2020 and 2021.

		Females	Males	Unknown	Total
Sparrowhawk	*Adults*	3	7	0	10
	First-year	19	11	0	30
Buzzards	*Adults*	7	3	1	11
	First-year	10	16	3	29
	Total	39	37	4	80

The weights of the liver, kidneys, and whole-body excluding gizzard contents were measured. Relative liver weight (liver weight / body weight) and relative kidney weight (kidney weight / body weight), as well as the ratio between the kidney and liver weights (i.e., (kidney weight)/(liver weight)), were calculated as potential health indicators (cf. below). A fat score was estimated by least visible fat in the sternum and abdomen and recorded as a categorical variable: from 0 (no sign of fat deposits) to 5 (abundant fat deposits) (Walker et al., 2017). A body condition index (Walker et al., 2016), which represents a ratio between the body weight and the size of a bird, based on body weight excluding gizzard contents and a sternum length cubed ((body weight)/(sternum length)^3), was also calculated (Table 3).

Table 3. Morphological and physiological parameters used as potential health indicators in the study.

Health indicator	Abbreviation	Formula / estimation	Note
Body weight	**BW**	The whole-body weight	Excluding gizzard contents. Quantitative variable
Liver weight	**LW**	Weight of the whole liver	Quantitative variable
Kidney weight	**KW**	Weight of the two kidneys	Quantitative variable
Relative liver weight	**RLW**	Liver weight / Body weight	Quantitative variable
Relative kidney weight	**RKW**	Kidney weight / Body weight	Quantitative variable
Ratio between kidney-liver weights	**RKLW**	Kidney weight / Liver weight	Quantitative variable
Fat score	**FS**	Visible fat in the sternum and abdomen	Semi-quantitative variable: from 0 (no sign of fat deposits) to 5 (abundant fat deposits) (Walker et al., 2017)
Condition index	**CI**	(Body weight) / (sternum length)^3	Ratio between the body weight and the size (Walker et al., 2017) Quantitative variable

5.2 Concentrations of elements in livers

The liver of birds was used to monitor exposure to 14 elements. Mercury (Hg), lead (Pb), cadmium (Cd), zinc (Zn), copper (Cu), and nickel (Ni) in the liver of sparrowhawks were reported in the H4 interim report 2021, while only Hg, Pb, Cd, and Ni were reported in the H4 interim report 2024. In addition to the six metals in the H4 interim report 2021, concentrations in the liver of arsenic (As), selenium (Se), iron (Fe), manganese (Mn), molybdenum (Mo), chromium (Cr), cobalt (Co), and strontium (Sr) were also analysed in the present report. Although As and Se are respectively a metalloid and a nonmetal, these 14 elements are hereinafter referred to as “metals”. 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 mL of nitric acid (ROMIL-UpA, Ultra Purity Nitric Acid, 67 – 69%) by the microwave digestion system (Ramp: 45 mins; Temperature: 200 °C; hold: 15 mins; Power: 1600 W). After cooling, the samples were made up to 25 mL 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 three hours. 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; Perkin Elmer Nexion 300D instrument) in standard and reaction (DRC: Dynamic Reaction Cell) mode. Four internal standards (Gallium, Indium, Bismuth and Rhenium, 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 x5 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. There were 28, 23, and 15 samples with concentrations of Ni, Cr, Sr in the liver under the limit of detection (<LoD), respectively. These values <LoD were replaced with half of the maximum value of LoD of the given element, as done in the H4 interim report 2024. The statistics of LoD (minimum, mean, and maximum) and recovery rate for the elements were summarised in Appendix 1.

5.3 Data analysis

5.3.1 Summary for metal concentrations in the liver of the two species and statistical differences between species and years:

5.3.1.1 Descriptive statistics:

The minimum, median, arithmetic mean (referred to as only ‘mean’ hereinafter), geometric mean, and maximum of concentrations were calculated as descriptive statistics by species and by species and year. The distributions of tissue metal concentrations are in general left-hand skewed. Therefore, the median or logarithmically transformed concentrations (using the natural logarithm) were used for further statistical analysis (see each analytical method below).

5.3.1.2 Difference in metal concentrations between species and years:

Metal concentrations were statistically compared between two species and two years by two-way analysis of variance (ANOVA) (i.e., ANOVA using as variables species, year, and an interaction effect between the two predictor variables). When there were significant differences between the four sub-groups of birds (i.e., buzzards in 2020, buzzards in 2021, sparrowhawks in 2020, and sparrowhawks in 2021), Tukey’s test for multiple comparisons was conducted as a post hoc test to assess which of the four sub-groups was significantly different from the other sub-groups. Metal concentrations were logarithmically transformed for these statistical tests. ANOVA requires assumptions such as the normality of residuals and the homoscedasticity. When these assumptions of ANOVA were not met, the non-parametric Kruskal-Wallis test was used to compare metal concentrations between the four sub-groups of birds, followed by Dunn's Kruskal-Wallis multiple comparisons as a post hoc test.

5.3.1.3 Biological thresholds for metal concentrations in the liver:

In this report, several biological thresholds were referred to from the two H4 interim reports (2021; 2024) and the literature and compared with concentrations of certain harmful elements in the liver of the two species (Table 4).

The moisture of samples’ livers was calculated to enable some of the biological thresholds to be reported by wet weight. The mean moisture percentage was 72.1 ± 2.6% (± standard deviation), and the percentage ranged 65.9 – 77.5%. First-year individuals showed significantly higher liver moisture than adults (72.5 ± 2.4 and 70.3 ± 2.7%, respectively; p-value by ANOVA = 0.002), and sparrowhawks showed significantly higher liver moisture than buzzards (72.6 ± 2.8 and 71.4 ± 2.4%, respectively; p-value by ANOVA = 0.047). No statistically significant difference was observed between the sex or the year. Given that the moisture content is around 72%, a factor of 3.6 (100/(100-72)) was used to convert wet weight (ww) concentrations into dry weight (dw).

Table 4. Biological threshold values for metal concentrations in the liver. Thresholds that were not given in the two H4 interim reports (2021; 2024) but added to this report were shaded in the table. When original threshold was given per unit of wet weight, a factor of 3.6 was used to convert the liver element concentrations from per unit of wet weight (ww) to per unit of dry weight (dw).

Metals	Threshold dw Value	*(ww value)*	Species	Effects	references
Hg	7.2 mg/kg	*(2.0 mg/kg)*	Lowest geometric mean in the ring-necked pheasant	Impaired reproduction, predominantly decreased egg hatchability	Shore et al., 2011
Pb	21.6 mg/kg	*(6.0 mg/kg)*	Falconiformes	Clinical poisoning in individual	Franson and Pain, 2011
	6.0 mg/kg		Raptors	Abnormally high exposure	Pain et al., 1995
	20.0 mg/kg		Raptors	Acute exposure causing mortality	
Cd	162 – 259.2 mg/kg	*(45 – 70 mg/kg)*	Adult birds (based on the data on eiders, mallards, Leach’s storm petrels, and starlings)	Adverse physiological effects	Wayland and Scheuhammer, 2011
	3.0 mg/kg		Adult wild freshwater duck species.	Indicative of increased environmental exposure	Scheuhammer, 1987
Ni	3.0 mg/kg		Wild birds (species not specified)	Adverse effects in avian species	Eisler, 1998; Outridge and Scheuhammer, 1993
As	7.2 mg/kg	*(2 mg/kg)*	Avian species (not specified)	Possible dying from acute or subacute poisoning	Eisler, 1994; Goede, 1985
	36.0 mg/kg	*(10 mg/kg)*	Avian species (not specified)	Indicative of arsenic poisoning	
Se	10 mg/kg		Populations of birds using nonmarine habitats.	Reduced adult weight gain lower reproductive success.	Ohlendorf and Heinz, 2011; Outridge et al., 1999
	30 mg/kg		Populations of birds using nonmarine habitats	High risk of embryonic deficiency	

For total Hg concentration in the liver, 2 mg/kg ww was reported as a value associated with impaired reproduction effects, predominantly decreased egg hatchability, based on the lowest geometric mean observed in ring-necked pheasants (*Phasianus colchicus*) (Shore et al., 2011). A concentration of 7.2 mg/kg dw (2 mg/kg ww multiplied by 3.6) was used as a threshold for total Hg concentrations in the liver.

Concentrations of Pb in the liver exceeding 6 mg/kg ww (21.6 mg/kg dw) was used in the two H4 interim reports, as a threshold value associated with clinical poisoning in individual Falconiformes (Franson and Pain, 2011). Meanwhile, a Pb concentration exceeding 6 mg/kg dw in the liver of birds of prey is considered an abnormally high exposure to Pb, and a Pb concentration exceeding 20 mg/kg dw indicates acute exposure causing mortality (Pain et al., 1995).

A cadmium residue in the liver of 45 – 70mg/kg ww (162 – 259.2 mg/kg dw) was used in the two H4 interim reports as a threshold, which may be associated with adverse physiological effects, such as structural and functional damage to kidneys, liver, gut, or salt glands (Wayland and Scheuhammer, 2011). Meanwhile, a concentration in the liver of 3 mg/kg dw is indicative of increased environmental exposure (Scheuhammer, 1987).

Although no threshold for Ni residues was set in the two H4 interim reports, a concentration in the liver of 3 mg/kg dw may be used as the threshold because this value may be associated with adverse effects in avian species (Eisler, 1998; Outridge and Scheuhammer, 1993).

Moreover, a threshold for two other metals was set and compared to concentrations of the measured metals. Arsenic residues in livers in the range of 2 to 10 mg/kg ww were reported as elevated concentrations, and residues > 10 mg/kg ww may be considered indicative of As poisoning (Eisler, 1994; Goede, 1985). Therefore, concentrations of 7.2 and 36.0 mg/kg dw were used as a threshold for As in the liver. A concentration of Se exceeding 10 mg/kg dw may be associated with adverse effects such as reduced adult weight gain or lower reproductive success, while a concentration of Se exceeding 30 mg/kg dw causes a very high risk of embryonic deficiency in populations of non-marine birds (Ohlendorf and Heinz, 2011; Outridge et al., 1999). These values were used as thresholds for concentrations of Se in the liver.

Selenium has been considered to protect against Hg toxicity, and the ratio of Hg/Se may indicate an interaction between these two toxic elements (Cuvin-Aralar and Furness, 1991; Sumino et al., 1977; Yang et al., 2008). A Hg/Se molar ratio approaching 1 suggests the existence of mercuric selenide (HgSe) and is generally considered protective of Hg toxicity. Therefore, the Se/Hg molar ratio was also calculated in this study.

Some thresholds are for effects in individual birds, while others are for mean effects among a sample of birds. Therefore, these thresholds were compared against the geometric mean and the median of concentrations by species and by year. Then, for each threshold value, the proportion of birds whose metal concentrations in the liver exceeded the threshold was compared for each species. The difference in these proportions between the two species was then statistically compared by Fisher's exact test. The difference in Se/Hg molar ratios between the two species was also statistically compared by Wilcoxon Mann-Whitney test.

5.3.1.4 Compositions of metals in the liver between species and years:

Correlations between each metal in the liver were visualised by Principal Component Analysis (PCA). Metal concentrations in the liver were logarithmically transformed and then standardised for this analysis because the range of concentrations is largely different between essential and non-essential metals. The results were projected on the two-dimension plane by the first two principal axes. Samples were represented as points, and the distance between each point represents the difference in the composition of logarithmically transformed metal concentrations. The correlations among elements were represented by arrows. The length of the arrows represents their contribution to the two first axes, and the angle between each pair of the arrows represents the correlation of the given concentrations.

To compare the composition of metals in the liver between sparrowhawks and buzzards and between 2020 and 2021, the centroid and the 95% of confidence ellipse of the centroid of each sub-group were projected on the plot. The significant difference in the composition of metals within the four sub-groups was assessed by multivariate ANOVA (MANOVA).

5.3.2 Metal concentrations in the liver of sparrowhawks in 2020-21 compared to those reported in the H4 interim report 2021:

For the extended temporal trend of metals in sparrowhawks, we used the median values of metal concentrations by year and the median values in previous years mentioned in the H4 interim report 2021. A linear regression model was set for the metals mentioned in the previous report (i.e., Hg, Cd, Pb, Ni, Cu, Zn), and significance of the time trend was then tested by F-test. For Hg and Cd, we calculated the median value of a sub-category of birds defined in the H4 interim report 2021: first-year and non-starved females for Hg and first-year birds for Cd. For other metals (i.e., Pb, Ni, Cu, Zn), the median value of all sparrowhawks was used.

5.3.3 Potential health indicators related to heavy metal exposure

5.3.3.1 Relationships between indicators:

One of the goals of this report is to find which biological parameters could describe exposure to metals. However, determining such parameters is challenging because metallic contaminants affect several organs differently, depending on their body burden (Table 3). For the first step, biological parameters measured during the post-mortem examination and indices based on these parameters were considered as “potential health indicators”, i.e., parameters potentially describing individuals’ health status (i.e., metal exposure levels). The body weight (BW), liver weight (LW), kidney weight (KW), relative liver weight (RLW), relative kidney weight (RKW), ratio between kidney and liver weights (RKLW), fat score (FS), and condition index (CI) were used as potential health indicators in this report (Table 3).

The minimum, median, arithmetic means, standard deviation, and maximum of each parameter were calculated as descriptive statistics by species and year. Significant differences in these potential health indicators between species were checked by ANOVA. The proportion of starvation status (starved / non-starved) was checked by species and sexes, and Fisher's exact test was carried out to test a significant difference in these proportions between species and between sexes by species.

Correlations between these eight potential health indicators were assessed using the Spearman's correlation index. Their significance was checked by Spearman's correlation test (N.B. To assess the correlations, the fat score was considered as a numerical variable). Moreover, MANOVA was conducted to assess whether the indicators significantly differ between sex, age, year, and/or starvation status (starved / non-starved).

5.3.3.2 Relationships between indicators and exposure to metals

The correlations between the potential health indicators and concentrations in the liver of all metals were assessed by each species using the co-inertia analysis (CoIA), which is a type of PCA for two groups of variables. The similarity of the two sets of data (i.e., potential health indicators and metal concentrations) were estimated by the RV coefficient which ranges from 0 (configuration of two matrices is independent) to 1 (configuration of two matrices shows the identical structure). Its significance was tested by a permutation test (Borcard et al., 2018).

The relationship between metal concentrations and biological parameters could be complex, such as a difference in metal concentrations between sub-groups of sparrowhawks (cf. *5.3.2. Metal concentrations in the liver of sparrowhawks in 2020-21 compared to those reported in the H4 interim report 2021*). It is therefore necessary to elucidate potential complex conditions for metal accumulations in birds' tissues. The Conditional Inference Tree (CIT; Hothorn et al., 2006) was applied to concentrations of each metal to identify such complex relationship with potential health indicators. CIT is a recursive binary partitioning analysis that studies significant univariate splits over all possible splitting variables. The splitting variable most significantly associated with response value will be selected, and this step is recursively performed to the two-split data, until no significant difference is observed. Permutation tests are applied for the splitting tests (Strasser and Weber, 1999), and p-values were adjusted by Bonferroni correction. In this study, CIT was performed across the seven potential health indicators (i.e., BW, LW, KW, RLW, RKW, RKLW, FS, and CI), age, sex, year, and starvation status (starved / non-starved).

A linear model was established for each metal to estimate exposure to metals by health indicators. However, there were not enough samples compared with the number of potential health indicators (i.e., the eight biological parameters plus other variables like age, sex, etc.) and their interactions to establish a full model (i.e., integrating all variables and their interactions). Therefore, only the variables identified by CIT as significant parameters were used for a linear model. When there are several explanatory variables identified by CIT, their interaction was also included. The significance of these variables was checked by ANOVA. The final model's coefficient of determination (R^2) was then calculated to estimate the model's performance (i.e., the proportion of the variation explained by the model).

Data were statistically analysed in the R environment version 4.2.1 (R Core Team, 2020) using some packages for specific analysis: "*vegan*", "*FactoMineR*", "*dunn.test*", "*party*", and "*partykit*".

6 Results

The present report primarily focuses on the six metals (Hg, Pb, Cd, Cu, Zn, and Ni) reported in the H4 interim report 2021. The results of As is also described in this report. The statistical results of the other metals are reported in Appendix 2.

6.1 Metal concentrations in the liver of buzzards and sparrowhawks

6.1.1 Summary for metal concentrations in the liver of the two species and statistical differences between species and years

Table 5 summarises the descriptive statistics of the concentrations of the seven metals in the liver, and Figure 3 shows graphical representations of each metal by species and year.

Concentrations of many metals in the liver significantly differed between the two species. The geometric mean of concentrations of Hg was significantly higher in sparrowhawks (geometric mean = 2.52 mg/kg dw) than in buzzards (0.91 mg/kg dw) with ANOVA. Concentrations of Hg were 2.77 times higher in sparrowhawks than buzzards. Moreover, concentrations of Hg in the liver were significantly higher in 2021 than in 2020 when both species were included. However, each species showed no significant difference between the years. Concentrations of Cu in the liver of sparrowhawks were significantly higher than those of buzzards (21.00 and 16.39 mg/kg dw, respectively), but no difference between the two years was observed. Concentrations of Pb and Cd in the liver of buzzards were significantly higher (0.73, and 0.59 mg/kg dw, respectively) than those of sparrowhawks (0.15, and 0.39 mg/kg dw, respectively). Concentrations of Pb did not differ between the two years. Concentrations of Cd in the liver of buzzards were significantly higher in 2021 than in 2020 (1.71, and 0.67 mg/kg dw, respectively), while no difference was observed in sparrowhawks. Concentrations of As in the liver did not significantly differ between species, but concentrations were higher in 2021 than in 2020 (0.19, and 0.29 mg/kg dw, respectively) when the species' were combined. Concentrations of Ni were analysed by Kruskal-Wallis test, and concentrations in the liver of buzzards in 2021 (median = 0.04 mg/kg dw) were significantly higher than the other sub-groups (median < 0.01 mg/kg dw in buzzards in 2020, < 0.01 mg/kg dw in sparrowhawks in 2020, and <LoD in sparrowhawks in 2021). No significant difference was observed in concentrations of Zn (geometric mean = 135.99 mg/kg dw in sparrowhawks and 131.80 mg/kg dw in buzzards).

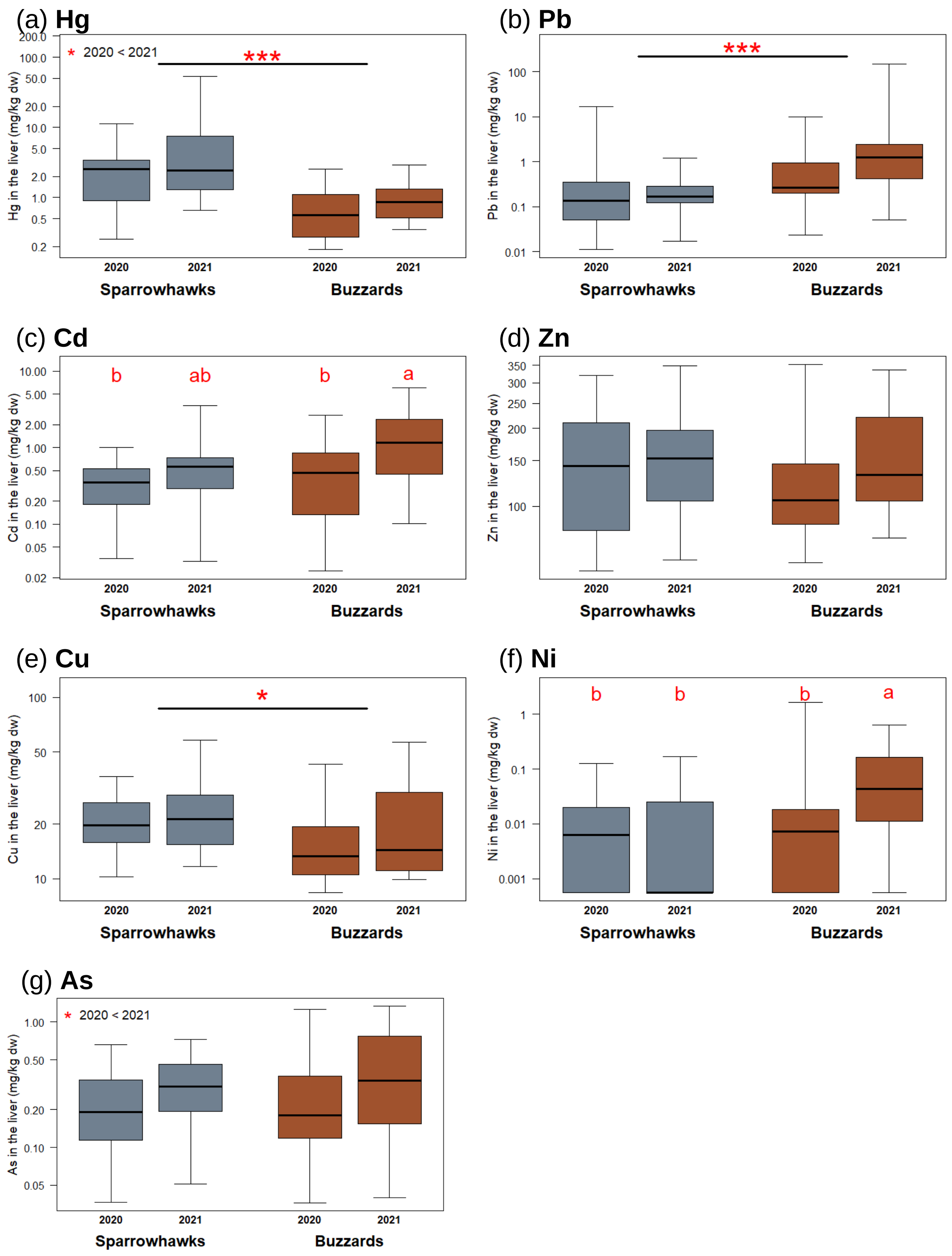


Figure 3: Box and Whisker plots showing median, interquartile range and minimum/maximum range of Hg (a), Pb (b), Cd (c), Zn (d), Cu (e), Ni (f), and As (g) concentrations in sparrowhawks and buzzards collected in 2020 and 2021. Statistically significant differences between the two years or between the species are indicated by asterisks (*: p-value < 0.05; **: p-value < 0.01; ***: p-value < 0.001). When the trend between the years differed between the species, significant differences (p-value < 0.05) are indicated by different letters.

Table 5. The minimum (Min), median, arithmetic means (Mean), geometric mean (Geo-Mean), standard-deviation (SD), and maximum (Max) of concentration of Hg, Pb, Cd, Cu, Zn, Ni, and As (mg/kg dry weight). Values under the limit of detection are indicated as LoD.

		Hg			Pb			Cd		
		Total	2020	2021	**Total**	2020	2021	**Total**	2020	2021
Sparrowhawk	**Min**	**0.25**	0.25	0.66	**0.01**	0.01	0.02	**0.03**	0.04	0.03
	Median	**2.57**	2.57	2.42	**0.17**	0.14	0.17	**0.40**	0.35	0.56
	Mean	**4.89**	2.76	7.01	**0.65**	1.06	0.25	**0.60**	0.41	0.79
	Geo-Mean	**2.52**	1.90	3.33	**0.15**	0.14	0.16	**0.39**	0.31	0.48
	Max	**53.71**	11.34	53.71	**16.88**	16.88	1.22	**3.56**	0.99	3.56
Buzzard	**Min**	**0.18**	0.18	0.35	**0.02**	0.02	0.05	**0.02**	0.02	0.10
	Median	**0.69**	0.56	0.87	**0.62**	0.26	1.26	**0.73**	0.46	1.17
	Mean	**0.91**	0.79	1.03	**8.01**	1.21	14.80	**1.19**	0.67	1.71
	Geo-Mean	**0.71**	0.58	0.86	**0.73**	0.40	1.35	**0.59**	0.37	0.95
	Max	**2.94**	2.55	2.94	**146.68**	10.00	146.68	**6.10**	2.66	6.10

		Zn			Cu			Ni			As		
		Total	2020	2021	**Total**	2020	2021	**Total**	2020	2021	**Total**	2020	2021
Sparrowhawk	**Min**	**56.27**	56.27	62.07	**10.22**	10.22	11.62	**LoD**	LoD	LoD	**0.04**	0.04	0.05
	Median	**153.02**	143.22	153.40	**19.95**	19.76	21.22	**<0.01**	<0.01	LoD	**0.26**	0.19	0.31
	Mean	**153.31**	152.03	154.59	**22.97**	21.13	24.82	**0.02**	0.02	0.02	**0.29**	0.24	0.33
	Geo-Mean	**135.99**	131.71	140.41	**21.00**	19.78	22.29	**<0.01**	<0.01	<0.01	**0.23**	0.19	0.27
	Max	**348.91**	320.45	348.91	**57.95**	36.44	57.95	**0.17**	0.13	0.17	**0.73**	0.66	0.73
Buzzard	**Min**	**60.54**	60.54	75.65	**8.35**	8.35	9.84	**LoD**	LoD	LoD	**0.04**	0.04	0.04
	Median	**114.75**	105.47	131.93	**14.02**	13.33	14.42	**0.02**	<0.01	0.04	**0.25**	0.18	0.34
	Mean	**146.86**	131.49	162.22	**19.00**	17.00	21.00	**0.10**	0.10	0.11	**0.37**	0.29	0.46
	Geo-Mean	**131.80**	118.00	147.22	**16.39**	15.10	17.79	**0.01**	<0.01	0.03	**0.25**	0.20	0.31
	Max	**352.80**	352.80	336.55	**56.76**	42.72	56.76	**1.65**	1.65	0.63	**1.34**	1.26	1.34

Table 6. Difference and ratio of the mean or geometric mean (geo-mean) concentrations in the liver of Hg, Pb, Cd, Cu, Zn, Ni, and As (mg/kg dry weight) between the sparrowhawk (-spa) and buzzard (-buz).

	Formula	Hg			Pb		
		Total	2020	2021	**Total**	2020	2021
Difference of means	**(Mean-buz) - (Mean-spa)**	**-3.98**	-1.97	-5.98	**7.36**	0.16	14.56
Ratio of means	**(Mean-buz) / (Mean-spa)**	**0.19**	0.29	0.15	**12.25**	1.15	59.31
Difference of geo-means	**(Geo-mean-buz) - (Geo-mean-spa)**	**-1.81**	-1.32	-2.47	**0.58**	0.25	1.19
Ratio of geo-means	**(Geo-mean-buz) / (Geo-mean-spa)**	**0.28**	0.31	0.26	**4.85**	2.77	8.48

	Formula	Cd			Zn		
		Total	2020	2021	**Total**	2020	2021
Difference of means	**(Mean-buz) - (Mean-spa)**	**0.59**	0.26	0.92	**-6.45**	-20.54	7.63
Ratio of means	**(Mean-buz) / (Mean-spa)**	**1.99**	1.64	2.17	**0.96**	0.86	1.05
Difference of geo-means	**(Geo-mean-buz) - (Geo-mean-spa)**	**0.20**	0.06	0.47	**-4.19**	-13.72	6.81
Ratio of geo-means	**(Geo-mean-buz) / (Geo-mean-spa)**	**1.53**	1.18	1.98	**0.97**	0.90	1.05

	Formula	Cu			Ni		
		Total	2020	2021	**Total**	2020	2021
Difference of means	**(Mean-buz) - (Mean-spa)**	**-3.97**	-4.13	-3.82	**0.08**	0.08	0.09
Ratio of means	**(Mean-buz) / (Mean-spa)**	**0.83**	0.80	0.85	**4.88**	4.68	5.07
Difference of geo-means	**(Geo-mean-buz) - (Geo-mean-spa)**	**-4.61**	-4.68	-4.51	**0.01**	0.00	0.03
Ratio of geo-means	**(Geo-mean-buz) / (Geo-mean-spa)**	**0.78**	0.76	0.80	**3.93**	1.37	11.33

	Formula	As		
		Total	2020	2021
Difference of means	**(Mean-buz) - (Mean-spa)**	**0.08**	0.04	0.12
Ratio of means	**(Mean-buz) / (Mean-spa)**	**1.29**	1.18	1.36
Difference of geo-means	**(Geo-mean-buz) - (Geo-mean-spa)**	**0.02**	0.01	0.04
Ratio of geo-means	**(Geo-mean-buz) / (Geo-mean-spa)**	**1.09**	1.04	1.13

Table 6 represents both difference and ratio of the mean or geometric mean concentrations in the liver between the two species. Both the differences and the ratios between the two species depended on the metal considered. Zinc, Cu, and As showed relatively constant ratios between the two years (mean Zn in sparrowhawks / mean Zn in buzzards: 0.9 – 1.05; Cu: around 0.8; As: 1.1 – 1.3). For Ni, the ratio of the means was relatively constant (around 5), but the ratio of the geometric means was not (1.4 in 2020 and 11.3 in 2021). This could be due to many concentrations replaced with half of the LoD value in sparrowhawks, which modified the geometric mean. For Hg, the ratio of the geometric mean of Hg was constant between the two years, around 0.3. In contrast, the ratios of Cd and Pb differed between the two years.

6.1.2 Biological thresholds for concentrations of metals in the liver

The geometric means of all seven metals by species did not exceed their respective thresholds. However, some individuals showed a concentration exceeding the thresholds.

Table 7 summarises the proportion of birds whose concentrations in the liver exceeded the thresholds. Six sparrowhawks (15% of all sparrowhawks) had concentrations of Hg higher than the threshold: 8.42, 11.34, 12.15, 13.45, 13.90, and 53.71 mg/kg dw. In contrast, no buzzard showed a Hg concentration higher than the threshold. The proportions of birds were significantly different between the species (p-value = 0.026). Four sparrowhawks (10%) and two buzzards (5%) showed higher Se concentrations than its threshold: 10.29, 12.19, 12.60, and 13.32 mg/kg dw in the four sparrowhawks and 11.54 and 14.43 mg/kg dw in buzzards. The proportion of birds exceeding the threshold was not significantly different between the two species. Seven sparrowhawks (17.5%) showed a Hg/Se ratio exceeding 1: 1.14, 1.40, 1.52, 1.89, 2.48, 2.85, and 5.22, while no buzzard (0%) showed a Hg/Se ratio higher than 1, and the proportions were significantly different (p-value = 0.012).

For Pb, only one sparrowhawk (2.5%) had a higher Pb concentration than the threshold: 16.88 mg/kg dw. In contrast, four buzzards (10%) showed Pb concentrations higher than the threshold of 6 mg/kg dw, and half of them (5%) even showed Pb concentrations exceeding 20 mg/kg dw.: 6.02, 10.00, 123.84, and 146.68 mg/kg dw.

For Cd, only one sparrowhawk (2.5%) and three buzzards (7.5%) showed a Cd concentration higher than the threshold: 3.56 mg/kg dw in one sparrowhawk and 4.33, 4.52, and 6.10 mg/kg dw in buzzards. These proportions did significantly differ between the species. Concentrations of Ni and As in the liver of all birds did not exceed their thresholds.

Table 7. Proportion of birds with metal concentrations in the liver exceeding the biological threshold values (cf Table 4). The results of the Fisher's exact test for the difference in the proportion of birds exceeding these thresholds is also mentioned.

Metal	Threshold value	Proportion of bird > threshold		p-value of Fisher's test
		Sparrowhawk	*Buzzards*	
Hg	> 7.2 mg/kg dw	15.0%	0.0%	0.026
Pb	> 6.0 mg/kg dw	2.5%	10.0%	0.359
	> 20.0 mg/kg dw	0.0%	5.0%	0.494
	> 21.6 mg/kg dw	0.0%	5.0%	0.494
Cd	> 162 mg/kg dw	0.0%	0.0%	1.000
	> 3.0 mg/kg dw	2.5%	7.5%	0.615
Ni	> 3.0 mg/kg dw	0.0%	0.0%	1.000
As	> 7.2 mg/kg dw	0.0%	0.0%	1.000
	> 36.0 mg/kg dw	0.0%	0.0%	1.000
Se	> 10 mg/kg dw	10.0%	5.0%	0.675
	> 30 mg/kg dw	0.0%	0.0%	1.000
Ratio Hg/Se	> 1	17.5%	0.0%	0.012

6.1.3 Overview of the composition of metal concentrations

PCA in this section was carried out using the six metals: Hg, Pb, Cd, Cu, Zn, and As. Another PCA using all metals is reported in Appendix 2. Nickel was excluded from the analysis due to a high proportion of concentrations <LoD. Figure 4 shows the results on the first and second principal components which represent 45.5% and 24.3% of the variation of the data, respectively. Concentrations of all metals in the liver were positively correlated (i.e., acute angles between arrows), except for the correlations between Pb – Hg and Pb – Cu, which were not importantly correlated (i.e., angles between these arrows were almost orthogonal). MANOVA indicated that the composition of these six metals significantly differed between sparrowhawks and buzzards (p-value < 0.05) but not between the two years (p-value > 0.05).

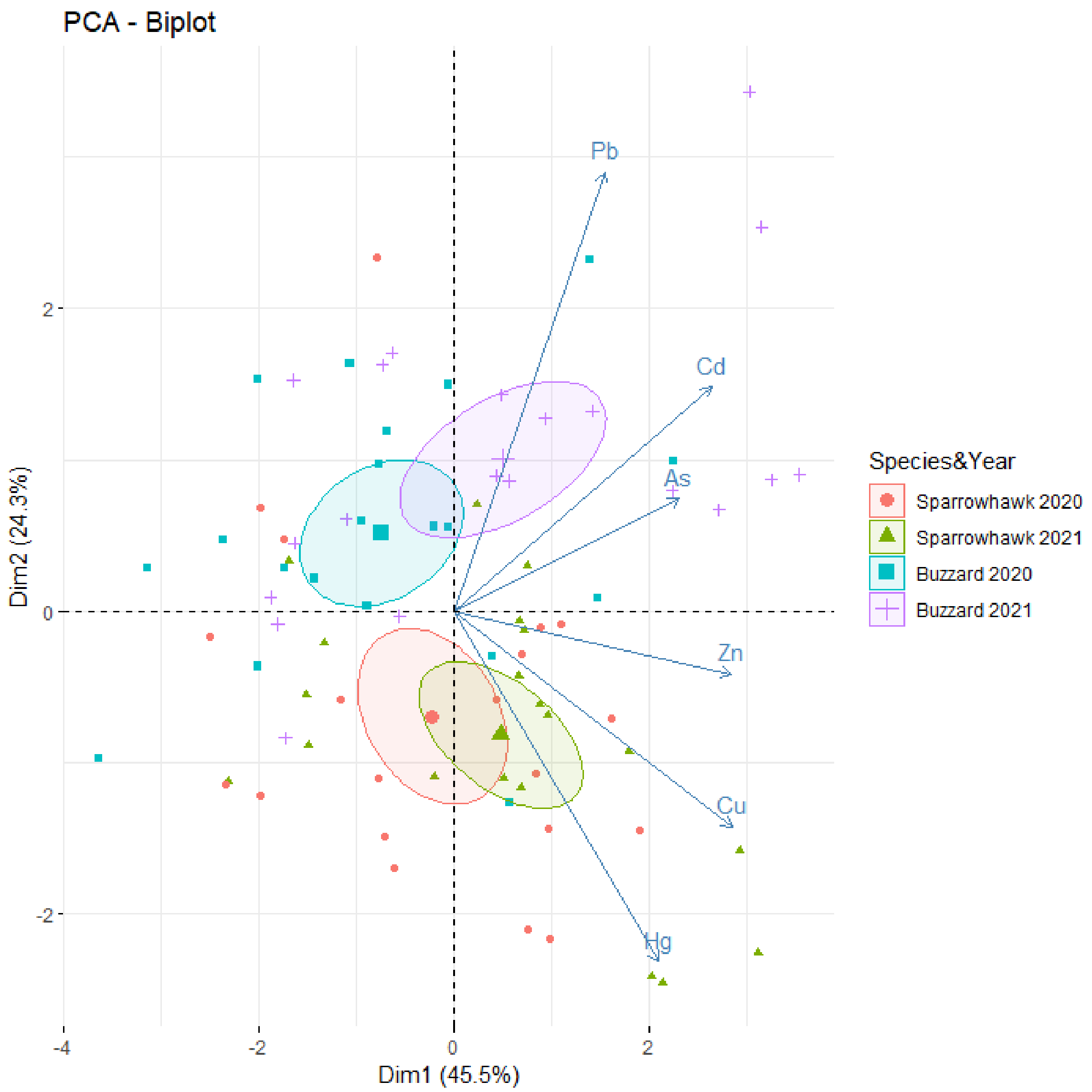


Figure 4: Principal component analysis (PCA) biplot of the data on concentrations of Hg, Pb, Cd, Cu, Zn, and As in the liver of sparrowhawks and buzzards collected in 2020 and 2021. The centroid and the 95% of confidence ellipse of the centroid of the four sub-groups split by two species and two years were represented by different colour and shapes of points. Nickel was excluded from the analysis due to a high proportion of values <LoD.

6.2 Concentrations of metals in the liver of sparrowhawks in 2020-21 compared to those reported in the H4 interim report 2021

The medians of Hg and Cd concentrations in the liver were recalculated under the same conditions as the H4 interim report 2021 (Table 8).

Table 8. The minimum (Min), geometric mean (Geo-Mean), arithmetic means (Mean), standard deviation (SD), median, maximum (Max), and first and third quartiles (Q1 and Q3) of concentration of Hg in young and non-starved female sparrowhawks and Cd in sparrowhawks (mg/kg dry weight).

Metal	Conditions	Year	n	Geo-Mean	Mean	SD
Hg	*Young, non-starved females*	2020	9	1.547	2.054	1.346
		2021	6	1.451	1.564	0.619
Cd	*Young birds*	2020	15	0.272	0.360	0.260
		2021	15	0.438	0.797	0.951

Metal	Conditions	Median	Min	Max	Q1	Q3
Hg	*Young, non-starved females*	2.474	0.341	3.591	0.881	3.266
		1.676	0.833	2.161	1.008	2.109
Cd	*Young birds*	0.312	0.035	0.965	0.171	0.464
		0.453	0.033	3.557	0.250	0.738

When the medians of metal concentrations in a year were compared, concentrations of Hg slightly increased from the years 2000 – 2013; the median values did not exceed 1.5 mg/kg dw during this period (Figure 5a). However, a linear regression model showed no significant time trend (p-value = 0.11). In contrast, concentrations of Pb slightly decreased from the years 2007 – 2014, in which the median values were around 0.2 mg/kg dw, except in 2008 (median = 0.11 mg/kg dw) and 2014 (median = 0.16 mg/kg dw) (Figure 5b). However, no significant time trend was observed (p-value = 0.75). The median values of Cd concentrations were similar between 2007 – 2014: around 0.2 – 0.5 mg/kg dw, and no significant time trend was observed (p-value = 0.59; Figure 5c). The median values of Zn concentrations slightly increased from the years 2007 – 2014 (Figure 5d). Although the median values were below 120 mg/kg dw until 2014, the median values exceeded 140 mg/kg dw in 2020 and 2021. However, a linear regression model showed no significant time trend (p-value = 0.28). The median values of Cu concentrations showed a similar trend to Zn (p-value = 0.19; Figure 5e). The median values of Ni concentrations in 2020 and 2021 decreased from the years 2007 – 2014 (Figure 5f). However, the median values of the years 2007 – 2014 were <LoD. The time trend of Ni concentrations was therefore unknown.

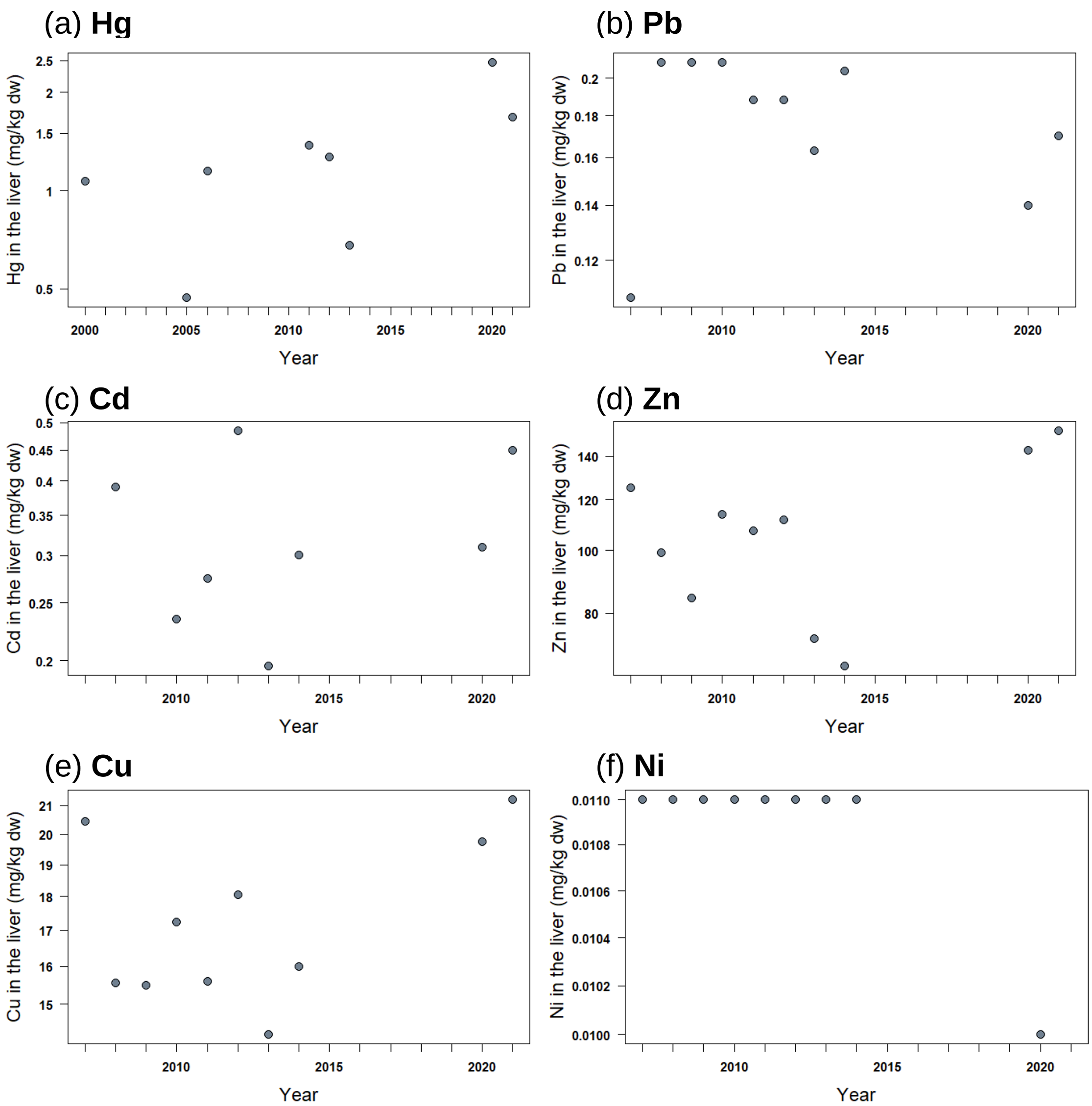


Figure 5: Median value of concentrations of Hg (a), Pb (b), Cd (c), Zn (d), Cu (e), and Ni (f) in the liver of sparrowhawks by year. The median values of Hg concentrations were calculated in first-year and non-starved female sparrowhawks, whereas the median values of Cd concentrations were calculated within first-year sparrowhawks. NB. Median Ni concentrations shown in graphic (f) are all <LOD.

6.3 Potential health indicators related to exposure to metals

Bird samples with unknown BW, LW, RW, and/or sex were excluded from the analysis, and 39 sparrowhawks and 35 buzzards were used in the analysis. The analysis was carried out separately for sparrowhawks and buzzards.

6.3.1 Relationships between potential health indicators

Starved birds represented 24.3% of all birds (Table 9). There was no significant difference in the proportion of starved bird between the species and between the sexes of each species. Table 10 summarises the descriptive statistics of the seven potential health indicators by species and sex. All of them showed significantly higher values in buzzards than sparrowhawks, except for RKW and RKLW that were not significantly different between species.

Table 9. Number of sparrowhawks and buzzards whose cause of death was considered as starvation in 2020 and 2021.

	Starvation status	**Females**	**Males**	**Total**
Sparrowhawk	Starved	4	4	8
	Non-starved	17	14	31
Buzzards	Starved	4	6	10
	Non-starved	12	13	25
Total		37	37	74

Table 10. The minimum (Min), median, arithmetic means (Mean), standard-deviation (SD), and maximum (Max) of concentration of potential health indicators of sparrowhawks and buzzards collected in 2020 and 2021: body weight (BW; g), liver weight (LW; g), relative liver weight (RLW; %), kidney weight (KW; g), relative kidney weight (RKW; %), ratio between kidney and liver weights (RKLW), fat score (0 – 5) and condition index (CI; g/cm^3). Statistically significant differences between the two species are indicated by asterisks (*: p-value < 0.05; **: p-value < 0.01; ***: p-value < 0.001 from ANOVA).

		BW (g) * **			**LW (g) *** **			**RLW (%) ** **			**RKLW**		
		Total	Female	Male	**Total**	Female	Male	**Total**	Female	Male	**Total**	Female	Male
Sparrowhawk	**Min**	**85.71**	143.90	85.71	**1.35**	1.90	1.35	**1.16**	1.16	1.32	**1.90**	1.90	2.13
	Median	**163.99**	200.20	130.44	**3.66**	4.80	3.24	**2.43**	2.29	2.45	**3.33**	3.30	3.57
	Mean	**172.44**	212.50	125.68	**3.91**	4.70	3.00	**2.30**	2.22	2.40	**3.27**	3.13	3.43
	SD	**58.27**	49.25	20.87	**1.48**	1.46	0.86	**0.61**	0.57	0.65	**0.71**	0.56	0.85
	Max	**359.45**	359.40	167.51	**7.08**	7.08	4.77	**3.81**	3.13	3.81	**4.87**	4.05	4.87
Buzzard	**Min**	**458.50**	518.10	458.50	**5.00**	5.00	5.61	**0.82**	0.82	1.06	**1.43**	1.43	1.52
	Median	**782.10**	850.50	651.70	**16.02**	16.27	12.90	**1.95**	1.82	2.00	**2.91**	2.91	2.69
	Mean	**761.20**	879.80	661.40	**14.89**	16.49	13.55	**1.91**	1.84	1.98	**2.97**	3.00	2.95
	SD	**189.90**	173.39	141.57	**6.07**	5.81	6.12	**0.56**	0.48	0.63	**0.99**	0.95	1.04
	Max	**1215.60**	1215.60	911.40	**26.63**	26.63	24.22	**3.26**	2.81	3.26	**5.62**	5.21	5.62

		KW (g) * **			**RKW (%)**			**FS ** **			**CI (g/cm^3) *** **		
		Total	Female	Male	**Total**	Female	Male	**Total**	Female	Male	**Total**	Female	Male
Sparrowhawk	**Min**	**0.53**	1.00	0.53	**0.36**	0.36	0.43	**0.00**	0.00	0.00	**0.07**	2.75	0.07
	Median	**1.10**	1.37	0.88	**0.68**	0.68	0.71	**0.00**	0.00	0.50	**3.13**	3.55	2.87
	Mean	**1.20**	1.47	0.88	**0.71**	0.71	0.72	**0.79**	0.67	0.94	**3.23**	3.77	2.60
	SD	**0.39**	0.30	0.16	**0.15**	0.13	0.18	**1.13**	1.15	1.11	**0.97**	0.80	0.76
	Max	**1.94**	1.94	1.15	**1.04**	0.90	1.04	**3.00**	3.00	3.00	**6.30**	6.30	3.54
Buzzard	**Min**	**1.78**	3.17	1.78	**0.31**	0.31	0.38	**0.00**	0.00	0.00	**7.29**	8.65	7.29
	Median	**5.10**	5.53	4.67	**0.67**	0.61	0.69	**2.00**	2.50	1.00	**12.56**	14.20	10.57
	Mean	**4.98**	5.50	4.55	**0.66**	0.63	0.69	**1.66**	2.19	1.21	**12.15**	13.86	10.71
	SD	**1.31**	1.25	1.22	**0.12**	0.12	0.12	**1.55**	1.56	1.44	**2.81**	2.43	2.28
	Max	**7.36**	7.36	6.75	**0.91**	0.85	0.91	**4.00**	4.00	4.00	**18.77**	18.77	14.67

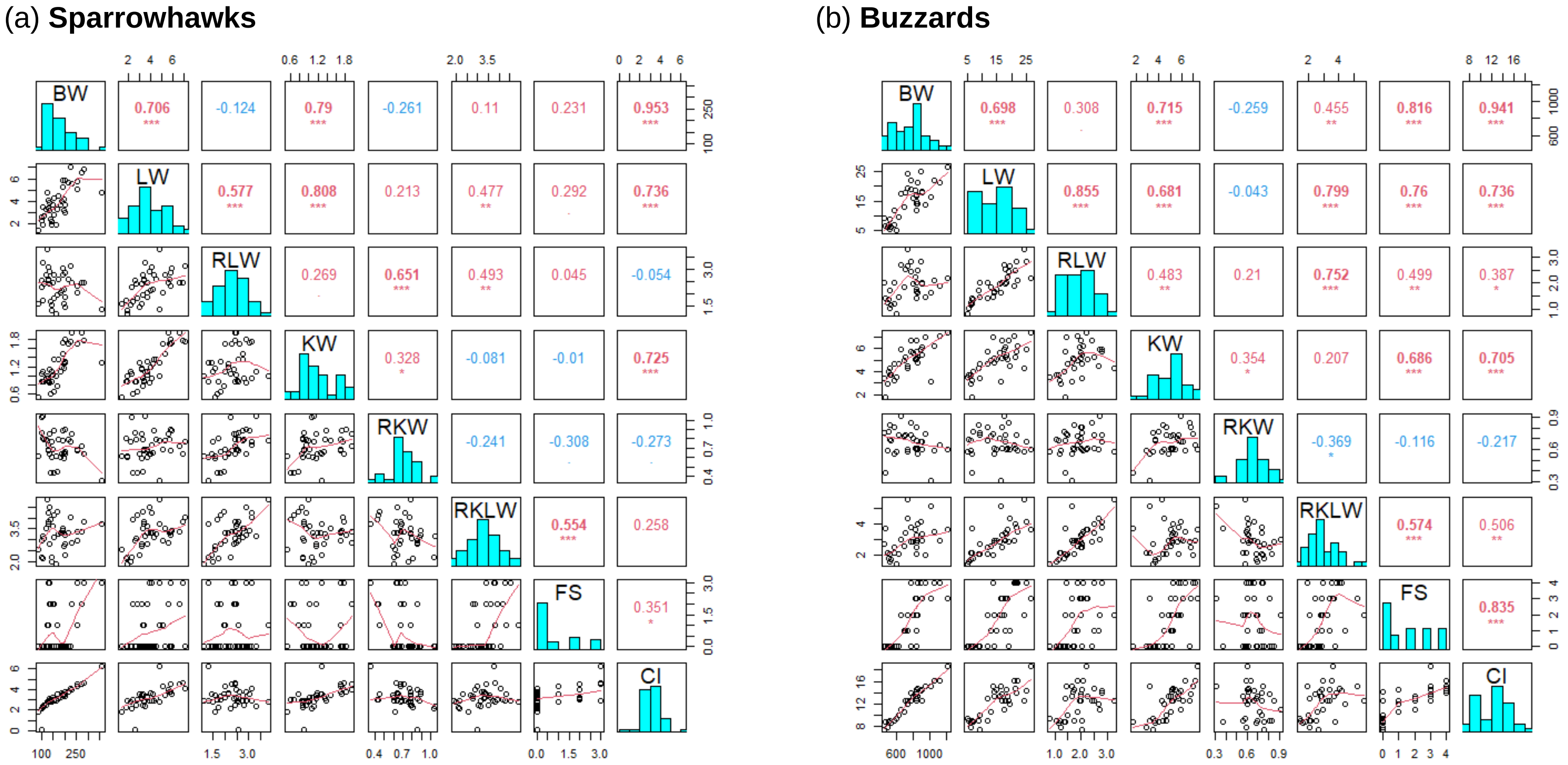


Figure 6. Multipanel display of pairwise relationships between potential health indicators of sparrowhawks (a) and buzzards (b). Diagonal panels represent the histogram of each indicator: body weight (BW; g), liver weight (LW; g), relative liver weight (RLW; %), kidney weight (KW; g), relative kidney weight (RKW; %), ratio between kidney and liver weights (RKLW), fat score (0 – 5; considered as a numerical variable) and condition index (CI; g/cm3). Left-lower panels represent pairwise relationship of each pair of the indicator represented by the lowess line in red. Right-upper panels show Spearman's correlation index of each pair and its significance (*: p-value < 0.05; **: p-value < 0.01; ***: p-value < 0.001).

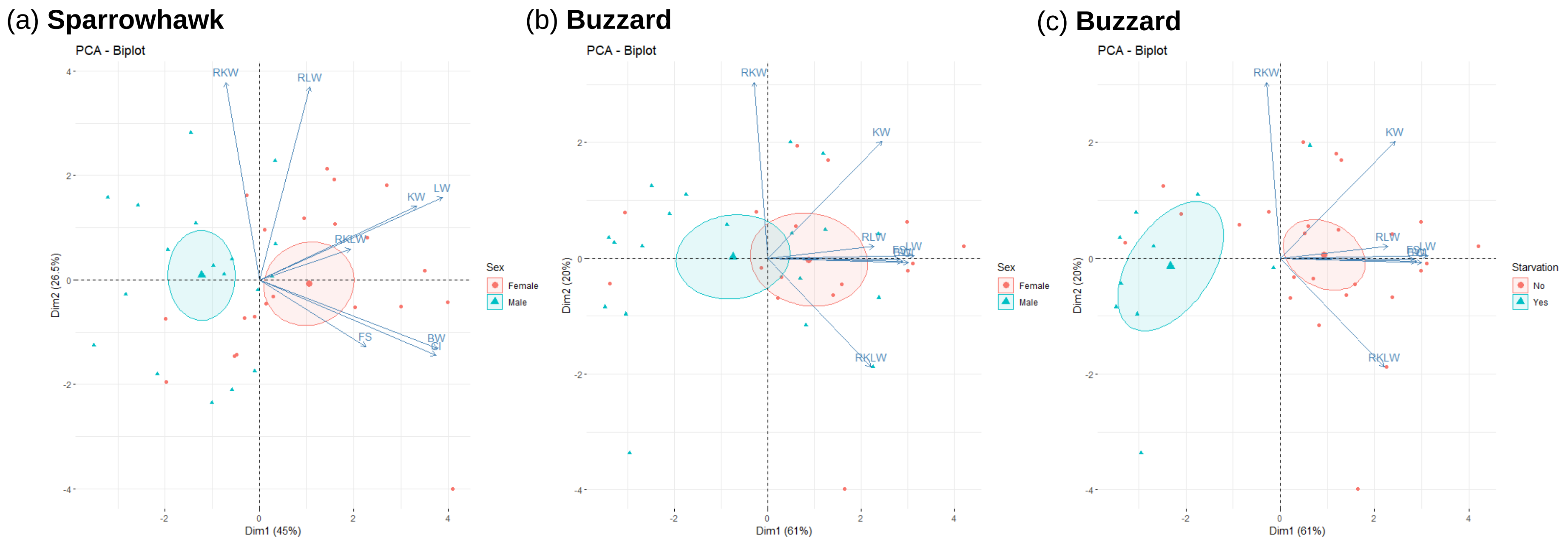


Figure 7. Principal component analysis (PCA) biplot of potential health indicators of sparrowhawks (a) and buzzards (b, c) collected in 2020 and 2021: body weight (BW; g), liver weight (LW; g), relative liver weight (RLW; %), kidney weight (KW; g), relative kidney weight (RKW; %), ratio between kidney and liver weights (RKLW), fat score (0 – 5; considered as a numerical variable) and condition index (CI; g/cm3). The factors significantly affected these indicators were tested by MANOVA. The centroid and the 95% of confidence ellipse of the centroid of the sub-groups of the significant factors (p-value < 0.05) were projected on the plot (sparrowhawk: sex (a); buzzard: sex (b) and starvation (c)).

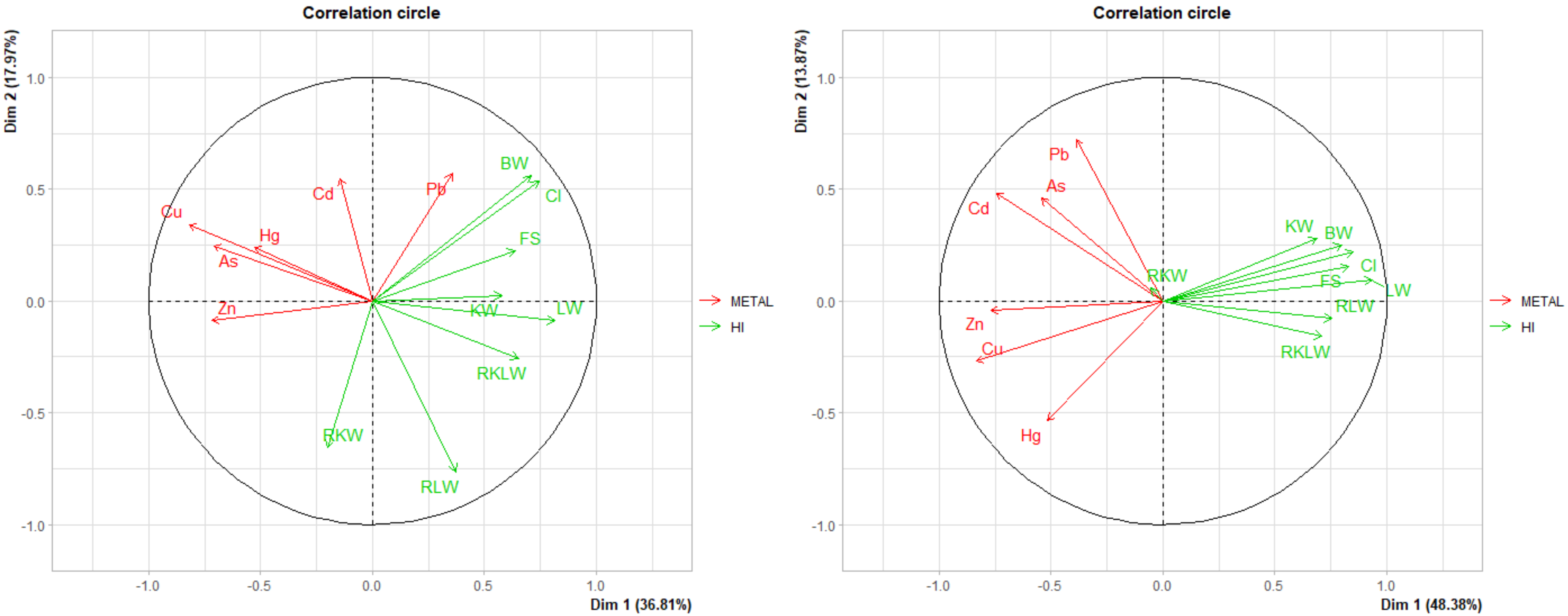
Figure 6a shows the correlations between these potential health indicators by species. In sparrowhawks, there were significant positive correlations between BW, LW, and KW. RLW was significantly and positively correlated with LW, while RKW was significantly and positively correlated with KW. RLW and RKW were also positively correlated. RKLW was positively correlated with LW and KW. FS was significantly and positively correlated with RKLW. CI was significantly and positively correlated with BW, LW, KW and FS, but not with RLW and RKW. Figure 7a represents the ordination of these seven potential indicators by the first two principal component axes that represent 49.4% and 30.2% of the variation, respectively. The composition was significantly distinguished by sex. BW, KW, LW, and CI were higher in females than in males.

In buzzards, there were significantly positive correlations between BW, LW, and KW (Figure 6b). RLW were significantly and positively correlated with LW and RKW, while RKW was correlated with no other potential health indicators except for RLW. RKLW were positively correlated with BW, LW, RLW, and FS, but negatively correlated with RKW. FS and CI were significantly and positively correlated with all other indicators except for RKW. The composition of the seven potential indicators were significantly distinguished by the starvation status and the sex, as represented in Figure 7b and 7c, which represent 64% and 20.2% of the variation by the first and second principal component axes, respectively. BW, CI, and FS were higher in females than males, while all but RKW were higher in non-starved buzzards than starved buzzards.

6.3.2 Relationships between potential health indicators and exposure to metals

In both sparrowhawks and buzzards, potential health indicators and concentrations of metals were significantly correlated (p-values < 0.05). The RV coefficient was 0.43 for sparrowhawks and 0.57 for buzzards, meaning that potential health indicators and metal concentrations were slightly but more importantly correlated in buzzards than in sparrowhawks. Figure 8a represents the ordination of the two datasets (metals and potential health indicators) for sparrowhawks, whereas Figure 8b represents the ordination for buzzards. In sparrowhawks, each biological parameter was correlated to different metals to different degrees, among which LW was negatively correlated to Cu. In buzzards, all parameters except for RKW were negatively correlated to the metals, particularly to Zn and Cu.

(a) **Sparrowhawk** (b) **Buzzard**



RV= 0.43; p-value <0.001 *RV= 0.57; p-value <0.001*

Figure 8: Co-inertia analysis (CoIA) biplot representing the correlations between concentrations of Hg, Pb, Cd, Cu, Zn, and As (group 'METAL') in the liver of sparrowhawks (a) and buzzards (b) collected in 2020 and 2021 and their potential health indicators ('HI'): body weight (BW; g), liver weight (LW; g), relative liver weight (RLW; %), kidney weight (KW; g), relative kidney weight (RKW; %), ratio between kidney and liver weights (RKLW), fat score (0 – 5; considered as a numerical variable) and condition index (CI; g/cm^3). Ni, Cr, and Sr were removed from the analysis because of a high number of their values <LoD. The RV coefficient, which represents the similarity of the two sets of data, and the p-value of the RV coefficient by a permutation test are shown in the graphics.

The results of CIT on sparrowhawks and buzzards showed that FS and LW, as well as RLW, were significantly related to concentrations of metals in the liver. Concentrations of Zn in sparrowhawks significantly differed by FS (Figure 9a). Concentrations of Zn were significantly lower in the liver of sparrowhawks with high FS than in the sparrowhawks with low FS (p-value < 0.001). Concentrations of Cu in the liver of sparrowhawks were significantly related to both FS and LW (Figure 9b). Concentrations of Cu were significantly lower in the sparrowhawks with low FS than in the sparrowhawks with high FS (p-value < 0.001). High Cu concentrations in the liver of sparrowhawks with low FS value (FS ≤1) were also related to LW (p-value = 0.005): sparrowhawks with low LW showed significantly higher Cu concentrations than sparrowhawks with high LW.

Many metals in buzzards were related to potential health indicators. Concentrations of Hg significantly differed by LW (Figure 10a): Hg concentrations were significantly higher in the liver of buzzards with low LW. (p-value < 0.012). Concentrations of Pb and Cd were significantly related to RLW (Figure 10b and 10c): both Pb and Cd were significantly higher in the liver of buzzards with low RLW (p-value = 0.01 and < 0.001, respectively). Concentrations of Zn were significantly lower in the liver of buzzards with high FS than in the buzzards with low FS (p-value < 0.001; Figure 10d), and concentrations of Cu were high when LW was low (p-value < 0.05; Figure 10e).

In both species, As was significantly explained by no biological parameters and no other factors like the sex, age class, starvation status, etc. Only the starvation status significantly explained concentration of Se in the liver of buzzards (Appendix 2).

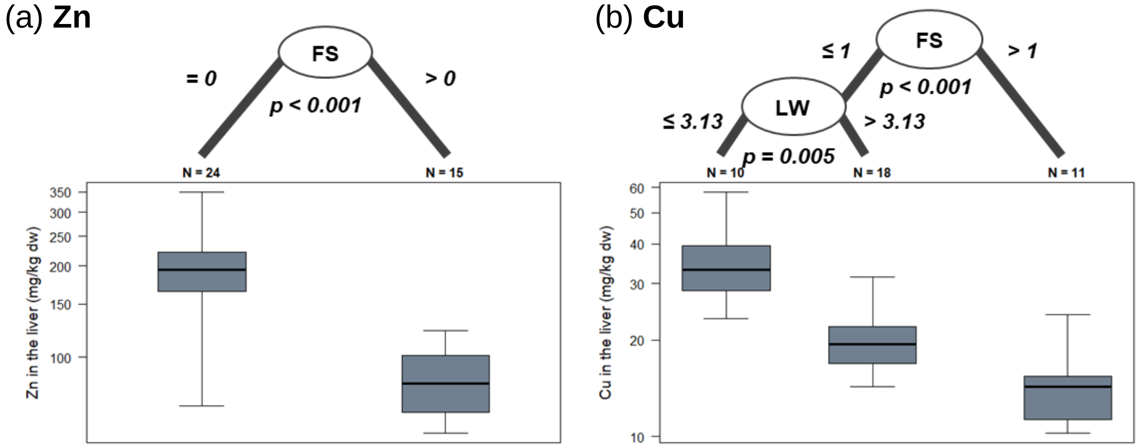
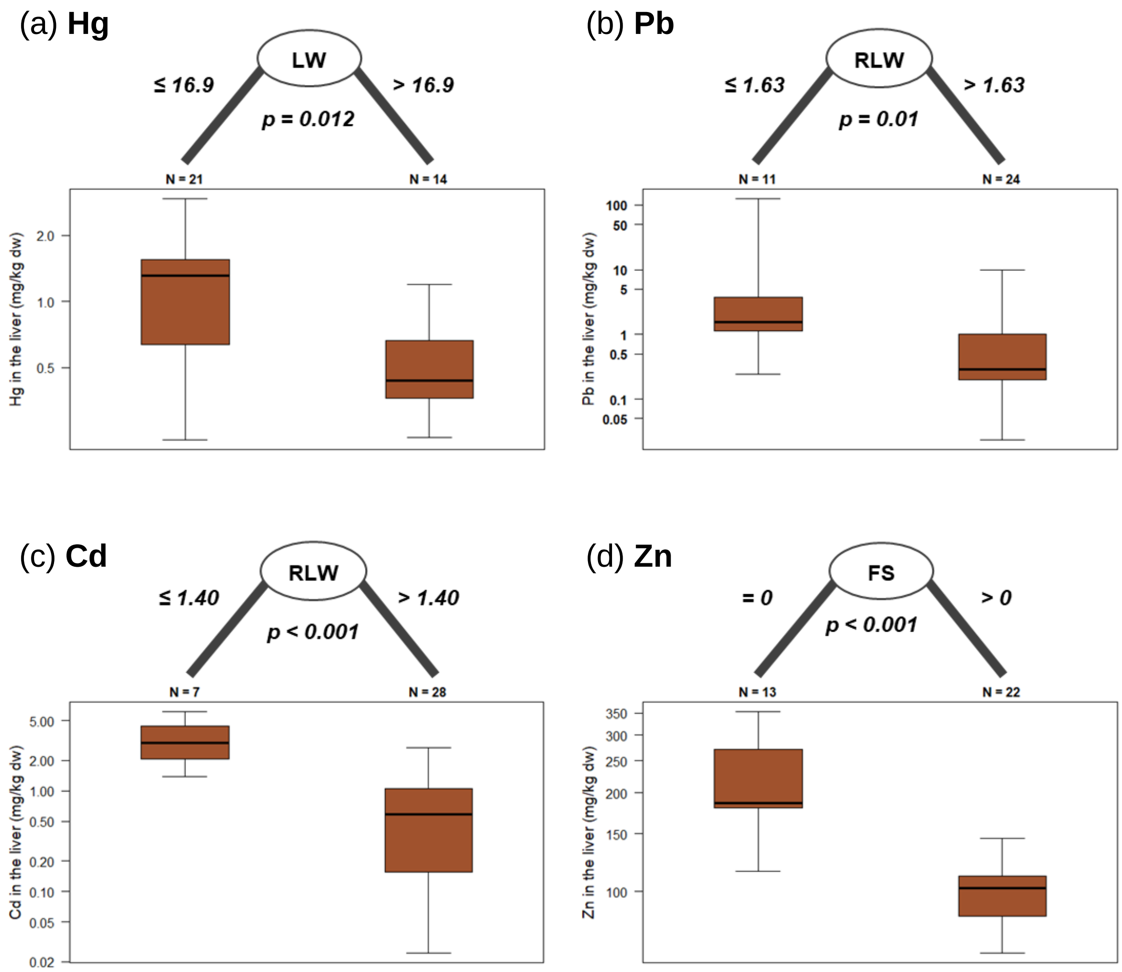


Figure 9. Conditional inference tree (CIT) representing significant factors that influence concentrations of Zn (a) and Cu (b) in the liver of sparrowhawks collected in 2020 and 2021. Partitioning variables significantly selected for splits are indicated in ovals with its p-value, and splitting criteria are listed beside the lines connected to the ovals. Boxplots in the lower part describe metal concentrations of each sub-group with its sample size.



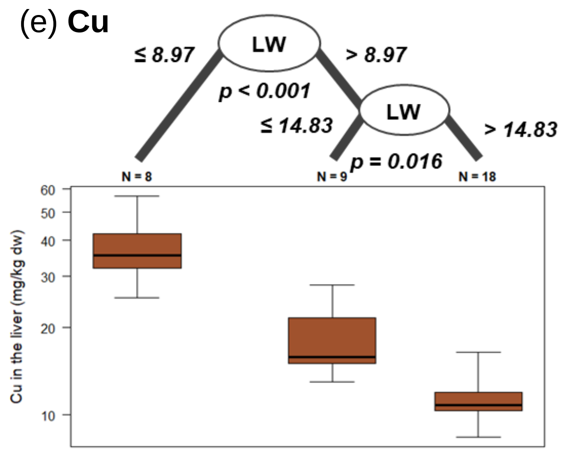


Figure 10. Conditional inference tree (CIT) representing significant factors that influence concentrations of Hg (a), Pb (b), Cd (c), Zn (d), and Cu (e) in the liver of buzzards collected in 2020 and 2021. Partitioning variables significantly selected for splits are indicated in ovals with its p-value, and splitting criteria are listed beside the lines connected to the ovals. Boxplots in the lower part describe metal concentrations of each sub-group with its sample size.

Given the results of CIT, it is supposed that FS, LW, and RLW could be health indicators related to metals in the liver of the two species. Table 11 shows the parameters of the linear model for each metal.

Table 11. Linear model for the relationship between concentrations of metals in the liver and the potential health indicators selected from the results of CIT: FS, LW, and RLW, as well as the interactions between variables when several variables were identified by CIT. Blank means that no explanatory variable was identified by CIT.

Species	Metal	Model	R^2
Sparrowhawk	**Hg**		
	Pb		
	Cd		
	Zn	*=exp(5.211), when **FS**=0* *=exp(4.674), when **FS**=1* *=exp(4.434), when **FS**=2* *=exp(4.182), when **FS**=3*	0.649
	Cu	*=exp(3.817) - 0.170 **LW**, when **FS**=0* *=exp(2.994) + 0.006 **LW**, when **FS**=1* *=exp(3.094) - 0.091 **LW**, when **FS**=2* *=exp(2.196) + 0.064 **LW**, when **FS**=3*	0.678
	As		
Buzzard	**Hg**	*=exp(0.672 - 0.065 **LW**)*	0.317
	Pb	*=exp(2.854 - 1.707 **RLW**)*	0.328
	Cd	*=exp(2.786 - 1.702 **RLW**)*	0.501
	Zn	*=exp(5.342), when **FS**=0* *=exp(4.643), when **FS**=1* *=exp(4.697), when **FS**=2* *=exp(4.506), when **FS**=3* *=exp(4.523), when **FS**=4*	0.694
	Cu	*=exp(3.895 - 0.073 **LW**)*	0.713
	As		

In sparrowhawks, there were significant relationships between concentrations in the liver of essential metals, Cu and Zn, and health indicators FS and LW (p-value < 0.001). The linear models explained a high proportion of the variation of Cu and Zn in the liver (R^2 = 0.649 and 0.678, respectively; Table 11). The model for Cu included FS and LW, as well as the interaction between FS and LW: concentrations of Cu decreased when FS increased (Figure 11a). Similarly, concentrations of Cu generally decreased when LW increased, but the slope of negative relationships between Cu and LW differed between FS (Figure 11b, c). Concentrations of Zn were explained by the model only by FS: Zn decreased when FS increased (Figure 11b).

In buzzards, there were significant relationships between concentrations in the liver of both essential and non-essential metals and health indicators (p-value < 0.001). There was only one explanatory variable in each model. The models for essential metals Cu and Zn showed higher R^2 (0.713 and 0.694, respectively; Table 11) than the models for non-essential metals Hg, Pb, and Cd (R^2 = 0.317, 0.328 and 0.501, respectively; Table 11). Concentrations of Hg in buzzard decreased when LW increased (Figure 12a). Likewise, concentrations of both Pb and Cd decreased when RLW increased (Figure 12b, c). Like sparrowhawks, concentrations of Zn in buzzards decreased with an increase of FS (Figure 12d). Concentrations of Cu were significantly explained by only LW in buzzards. Cupper in buzzards decreased with an increase of LW (Figure 12e).

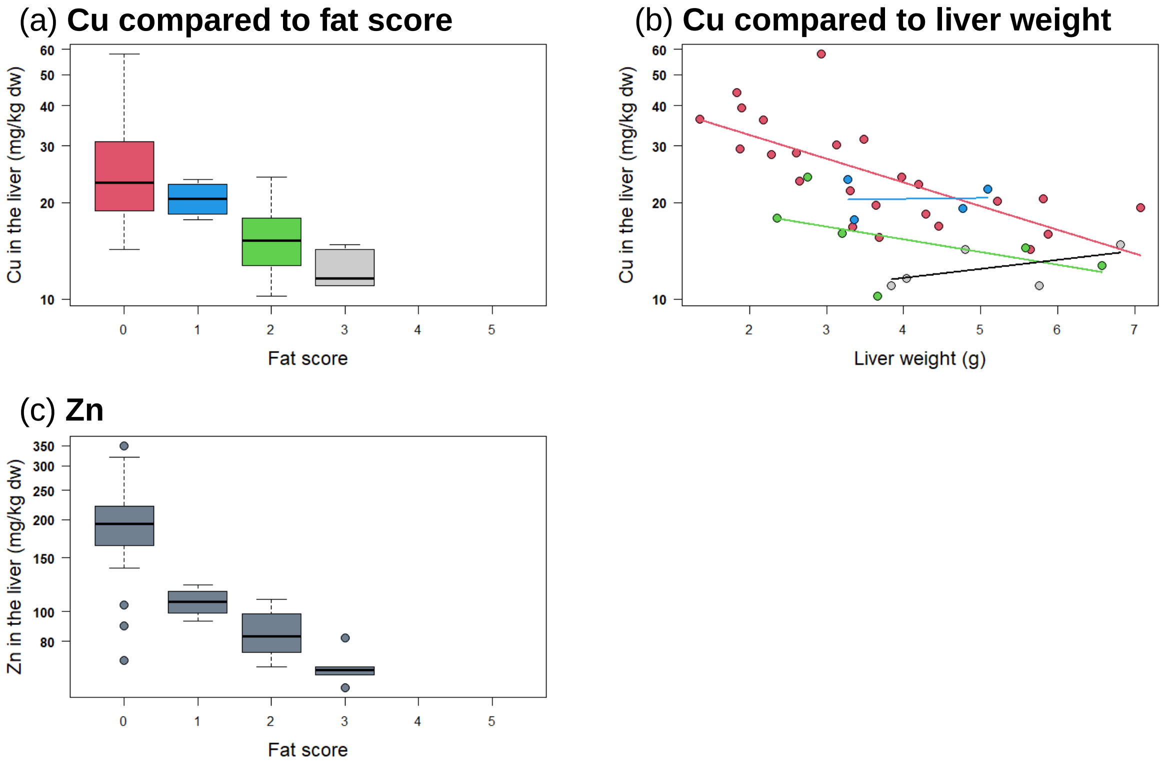


Figure 11. Linear models for metal concentrations in the liver of sparrowhawks collected in 2020 and 2021: concentrations of Cu compared to (a) fat score and (b) liver weight, and (c) Zn compared to fat score. In Figure 11b birds with different fact score represented by the different colours used in Figure 11a.

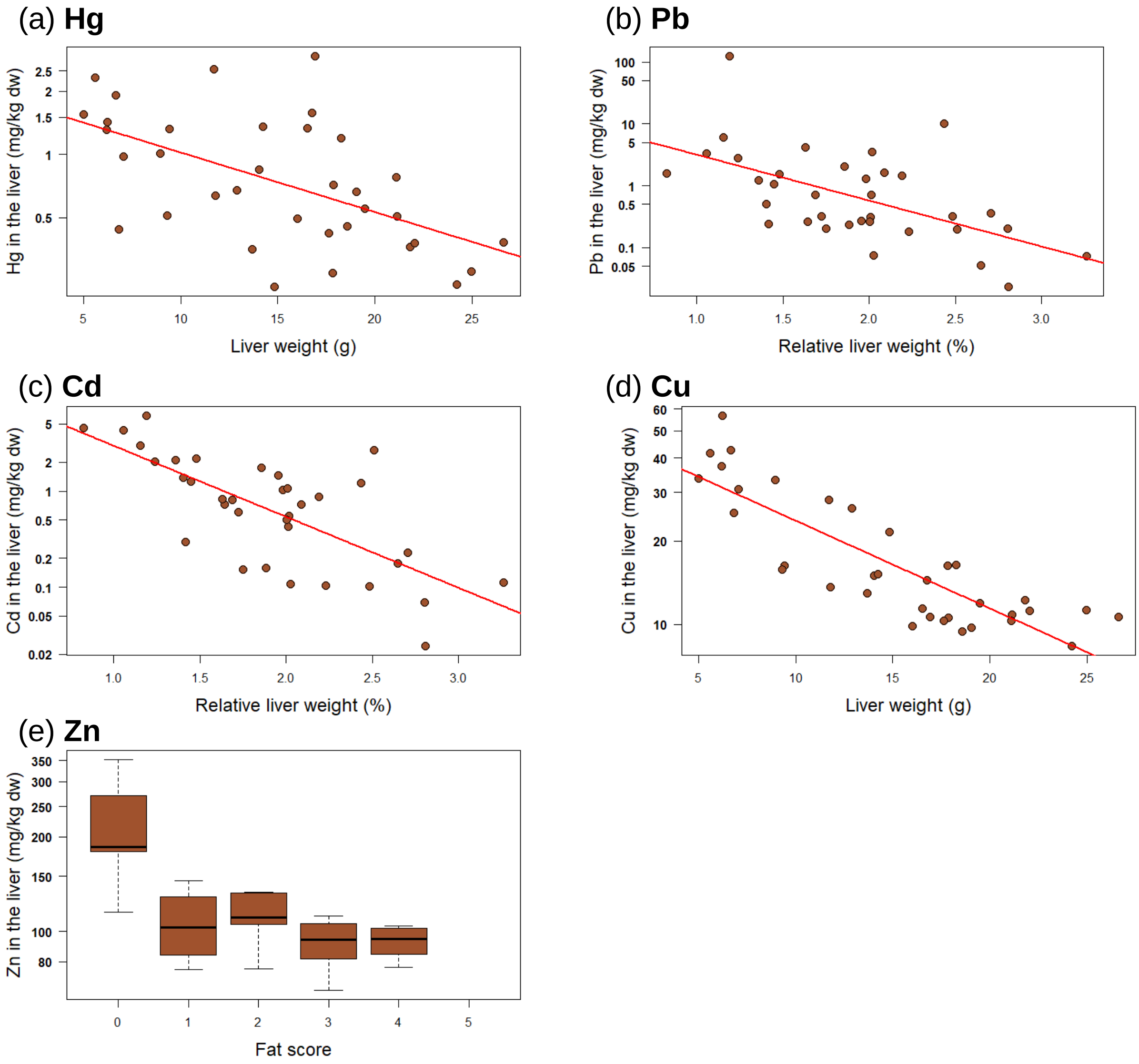


Figure 12. Linear models for metal concentrations in the liver of buzzards collected in 2020 and 2021: concentrations of Hg compared to liver weight (a), Pb compared to relative liver weight (b), Cd compared to relative liver weight (c), Cu compared to liver weight (d), and Zn compared to fat score (as a categorical factor) (e)

7 Discussion

7.1 Concentrations of metal in the liver of the two species

Our results showed a significant difference in concentrations of metals between sparrowhawks and buzzards.

Concentrations of many metals were higher in buzzards than in sparrowhawks. Although the proportion of birds with a metal concentration exceeding the threshold of effect was not significantly different between buzzard and sparrowhawks, some buzzards showed higher Pb and Cd concentrations than known thresholds, which might cause adverse effects. Moreover, two buzzards showed extremely high Pb concentrations in the liver that exceeded 100 mg/kg dw. Such high liver Pb concentrations have been reported in the UK (Pain et al., 1995; Taggart et al., 2020). Although any specific analysis was conducted in this study, it might be due to direct ingestion of lead shot ammunition that causes high exposure to Pb. Meanwhile, no sparrowhawk showed such a high Pb concentrations. These results indicate that buzzards are more exposed to these two harmful metals than sparrowhawks and that some of them might be at risk of harmful effects. This could be because of their diet and human activities. For example, scavenging 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). Taggart et al. (2020) demonstrated by an isotopic analysis that much Pb in the livers of buzzards was derived from Pb shotgun pellets. For Cd, Kitowski et al. (2016) reported higher liver Cd concentrations in small mammal-eating raptors than raptors with other diets. The results of these studies concur with the result of this report.

In contrast, concentrations of Hg showed the opposite pattern. Concentrations of Hg in the liver were higher in sparrowhawks than in buzzard. An important exposure of sparrowhawks to Hg was also evident from our assessments of the threshold: A high proportion of sparrowhawks with a liver Hg concentration exceeding the threshold for adverse effects on reproduction. The results of the Hg:Se ratio indicate that some sparrowhawks might be seriously exposed to Hg and at risk of harmful effects caused by Hg. Higher concentrations of Hg in sparrowhawks could also be due to their diet and habitat. For instance, Kitowski et al. (2016) showed that the consumption of small birds, such as passerines, can be a source of Hg contamination of predatory birds. Meanwhile, Cristol et al. (2008) demonstrated that Hg could bioconcentrate in terrestrial bird species living near contaminated water sources, meaning that wetlands could be a source of Hg contamination. This report did not focus on the environmental factor modulating exposure scenario (i.e., diet and habitat). To switch model species, it is necessary to consider these environmental factors related to exposure patterns.

Zinc and Cu are considered essential elements for organisms, and no clear threshold values for their residues in birds' liver has been reported. Nonetheless, a range of 187–323 mg/kg dw was considered an acute Cu poisoning concentration in the liver of Canada Geese (*Branta canadensis*) (Henderson and Winterfield, 1975). 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. Although biological thresholds differ between species, concentrations of Zn and Cu observed in our study were lower than the values expected to cause harmful effects on organisms in the literature. Regarding Ni, no sparrowhawk and buzzard showed Ni concentrations higher than the thresholds of effect, and Ni concentrations of some birds were even <LoD. Therefore, it is considered that the health risk of exposure to Zn, Cu, or Ni is currently low for both sparrowhawks and buzzards.

7.2 Time trend of concentrations of metals in the liver

In this report, no explicit time trend in exposure of sparrowhawks to metals was observed from 2006 to 2021. Only concentrations of Hg, Cd, As, and Ni increased from 2020 to 2021. The comparison with the data from the H4 interim report 2021 demonstrates no significant time trends. Plus, when we focused on concentrations in the liver of sparrowhawks, Hg, Pb, Cd, Zn, Cu, and Ni did not differ between 2020 and 2021. This result is not in line with the results from the H4 interim report 2021, in which a negative trend was observed in Zn and Ni by Spearman's correlation test and Kruskal-Wallis test in the period of 2007 – 2014. Potential reasons for this discordance could differ between Zn and Ni. For Ni, this discordance could be due to the difference in statistical methods used. In this report, only the median value in a year for the time trend was used, while the previous report used all data on sparrowhawks collected during the monitoring period. The statistical power of the analysis in this report is lower than in the previous report, and the results from our analysis on the time trends could not provide strong evidence. Moreover, many Ni concentrations were <LoD in the H4 interim report 2021 and in the individuals in 2020 and 2021 of this study. The high proportion of Ni concentrations <LoD makes the analyse of the time trend of Ni difficult. In contrast, the reason for the different results of Zn is due to an increase of concentrations of Zn in the liver in 2020/2021 compared to the period of 2007 – 2014. The similar trend was observed in Cu. Although the concentrations of Zn and Cu in our study were far from their adverse effects, observation for their time trend is required.

Our results on buzzards showed an increasing trend of some metals from 2020 to 2021, like Cd and Ni. Although not statistically significant, Pb concentrations in buzzards were also higher in 2021 than in 2020. However, Pb concentrations in this study were within a range of previous data for buzzards in the UK. Pain et al. (1995) reported 1.3 mg/kg dw as the median of Pb concentration in the liver of buzzards collected between 1981 – 1992, whereas Taggart et al., (2020) reported 0.72 mg/kg dw as the median of Pb concentration in the liver of buzzards collected between 2007 – 2019. Moreover, high risk of exposure to Pb has been reported in scavenging predatory birds during the hunting season due to ingestion of shotgun pellets or fragments embedded in prey bodies. (e.g., Berny et al., 2015; Carneiro et al., 2014; Gangoso et al., 2009; Mateo et al., 1999). Taggart et al., (2020) demonstrated seasonal fluctuation of exposure of buzzards to Pb, which could be related to the hunting season. Likewise, Ozaki et al. (2023) observed seasonal fluctuation of other metals in the liver of buzzards, such as Cd and Cu. Their observation indicated that seasonal fluctuation might occur in other metals whose sources are not just linked to hunting. The reasons for the seasonality of the other metals are still unclear, but it is potentially valuable to understand metal annual variability.

For both sparrowhawks and buzzards, a clear pattern of increasing or decreasing time trends was not found for any metals. Further studies are required for solid evidence on the time trend of exposure to metals by taking into account the statistical power of analysis and other environmental factors influencing exposure, like seasonality.

7.3 Potential health indicators related to exposure of raptors to metals

In this study, eight parameters were assessed as potential health indicators of exposure to metals. Among them, the fat score and the liver weight significantly explained concentrations of Cu and Zn in the liver of the two species. The (relative) liver weight also explained concentrations of Hg, Pb, and Cd in the liver of buzzards, although these harmful metals were less importantly explained by the liver weight than the two essential elements.

The relationships between these parameters and concentrations of metals in the liver varied between species. On the one hand, pattens of metal concentrations varied between the two species. On the other hand, relationships between these parameters differed according to species. The body, liver and kidney weights were highly correlated in both species. The condition index, calculated by body mass and weight, was also positively correlated with the three weights. However, the fat score was independent of the other parameters in sparrowhawks, while the fat score was positively correlated with the other parameters in buzzards. The relationship between each metal and each biological parameter might be unique to each species.

The sex and starvation status affected some parameters like the body and liver weights, but their influence on the biological parameters differed between species. Sex significantly influenced these parameters in both species, but the difference in the biological parameters between two sexes was more contrasted in sparrowhawks than in buzzards. The results certainly reflect the marked sexual dimorphism of sparrowhawks: females are 60 – 100% heavier and 15% larger in leg and wing length than males (Moss, 1979). In contrast, adult males are slightly smaller than females in buzzards (Tubbs, 1974). The starvation status significantly affected the body and liver weights, as well as the fat score, in buzzards. Such a significant difference in biological parameters by starvation was not observed in sparrowhawks, which means that the body condition measures in buzzards might be more sensitive to the starvation status than sparrowhawks. The results from CIT showed that both the sex and the starvation status did not explain or less explained concentrations of metals than some other parameters. Therefore, the liver weight and/or the fat score are, at least among the eight parameters, the most suitable biological parameters to predict concentrations of metals in the liver.

The reasons for the significant relationships between these biological parameters and concentrations of metals remain unclear. One of the possible reasons is the harmful effects of metals on body conditions. For example, the toxic effects of Cu can decrease body and tissue weight and feather growth, whereas high levels of Zn affect body conditions, decreasing body mass (Koivula and Eeva, 2010). However, many sparrowhawks and buzzards in this study did not show a high level of concentrations of these metals related to their harmful effects. Another possible reason is a relative increase in metal concentrations due to liver weight loss. In the study of Esselink et al. (1995), the barn owl (*Tyro alba*) showed an increase in concentrations of Cu 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 (i.e., body weight loss) by starvation was negatively correlated with concentrations of metals, such as Cu, Zn and

Fe, in common guillemots (*Uria aalge*). However, concentrations of harmful metals did not change in these two studies, which does not concur with our results. Our results might be due to a combination of several causes, but further studies are required to elucidate this point. Therefore, it is necessary to continue to study potential health indicators related to the exposure of raptors to metals by species.

8 Conclusion

8.1 Summary of the overall results

This report measured concentrations of metals in the liver of sparrowhawks and buzzards collected across the UK in 2020 and 2021. To assess whether it is pertinent to use the buzzard as a substitute for the sparrowhawk for the H4 indicator, the metal concentrations in the liver were compared between the two raptor species. The time trends of metal concentrations were also checked, and it was investigated whether biological parameters measured during the post-mortem examination could be used as potential health indicators explaining exposure to metals.

The composition of metals in the liver differed between the two species due to significant differences in concentrations of some metals. In addition to the difference in metal concentrations, exposure pattern from 2020 and 2021 was also different between the two species, such as Cd in the liver, which significantly increased from 2020 to 2021 in buzzards only. These differences between the species might be mainly due to the difference in their diet and habitat. Regarding a long-term exposure change assessment for sparrowhawks, there was no clear evidence for significant time trends of the exposure. This means that the results for Zn and Ni in this report differed from the H4 interim report 2021. Meanwhile, many birds did not show a high level of metal concentrations potentially causing harmful effects, except Hg in some sparrowhawks in 2020/2021. Some biological parameters, such as the liver weight and fat score, explained concentrations of metals, particularly essential metals such as Zn and Cu. However, the relationships between these parameters also significantly varied between species, possibly due to their morphology and ecology differences.

8.2 Answer to the main aims

1. **Is the buzzard pertinent as an alternative species of the sparrowhawk for exposure to metals?**
    - From the study of this report, it could be challenging to use the buzzard as an alternative species of the sparrowhawk. First, the variation of concentrations between the two species differed among metals. For instance, liver Pb concentrations were higher in buzzards than sparrowhawks, and some buzzards might ingest lead shot ammunition, which may be not the case for sparrowhawks. In contrast, liver Hg concentrations were higher in sparrowhawks than buzzards. Second, the time trend from 2020 to 2021 of certain metals varied between the two species (e.g., Cd increased from 2020 to 2021 only in buzzards). These discrepancies in metal concentrations could be due to their morphological difference and their ecology, such as their diet and habitat. It would not be possible to predict metals concentration in one species based on the concentrations in the other species. Therefore, a change to monitoring metals in buzzards would require the establishment of baseline data. An advantage of switching to using the buzzard as a sentinel species for metals is that the larger liver mass available from this species would allow the measurement of multiple contaminant groups in the same individual, which could provide cost efficiency during sample analysis. A further advantage of

using the buzzard is that this bird is considered as a relevant sentinel species at the EU level (Badry et al., 2020).

2. **How should concentrations of metals in the buzzard be adjusted to those in the sparrowhawk for the continuity of the time trends based on the sparrowhawk?**
   - Given the discrepancies mentioned above, it is important to assess how a long-term time trend of each metal differs between the two species to adjust these discrepancies (i.e., whether the two birds show a similar trend or different time trend). This assessment is being carried out in a study funded by the Environment Agency as part of H4 reporting development (APEM, 2025). It is also important to consider and include potential environmental and biological factors influencing exposure to metals in the time trend analysis to correct the difference in the exposure pattern between the two species. Once these assessments are completed, it may be possible to estimate exposure in one species based upon data generate from the other species.

3. **How are biological parameters from the PBMS post-mortem metadata correlated to concentrations of metals in the species?**
   - The biological parameters were significantly correlated to the metal concentrations in this study. Correlation patterns differed between species. However, in both species, essential elements Zn and Cu were linked to certain parameters like liver weight. In contrast, non-essential elements were not or less importantly linked to the biological parameters used in this study. The mechanisms for such correlations are still unclear. Although the threshold analysis showed that only a few samples had concentrations possibly causing harmful effects on organisms, no particular analysis was conducted to assess if metals in the liver affected, directly or indirectly, biological parameters or if specific biological parameters modulated exposure or accumulation.

4. **Do some biological parameters predict exposure values? What biological parameters could be used as health indicators? How accurately does these health indicators predict concentration values of metals in the target organs?**
   - Given the correlations between biological parameters and metal concentrations, certain biological parameters can be used to estimate concentrations of some metals, particularly essential metals, with a high level of accuracy. However, given the discrepancies in the relationships between biological parameters used in the study and concentrations of metals, it is difficult to use only these biological parameters to estimate concentrations of all metals in the liver.

8.3 Recommendations for H4 indicator dashboard

The H4 indicator dashboard represents the results of both the time trend and threshold assessments. In the case of metals in the sparrowhawk, the threshold assessment is based on monitored concentrations for the most recent year. In this report, the results of both trend and threshold assessments for some metals were slightly different from the H4 indicator dashboard in the H4 interim report 2021.

Given that only a small number of birds were used in this analysis (20 sparrowhawks in 2021) and that a different statistical method was applied for the trend and threshold assessment, there is insufficient evidence to change the H4 indicator dashboard. However, the points mentioned below should be considered in further studies:

Mercury – No significant time trend was observed in this report. However, concentrations of Hg slightly increased from the period of 2007-2014. Plus, In this report, 25% of individuals had a Hg concentration exceeding its threshold (Table 12). This threshold is not indicative of effects in individual birds. It should be compared to the mean, geometric mean, or median values of all samples (i.e., samples including males, starved individuals, etc.), all of which were below this threshold of 7.2 mg/kg dw. However, the mean value was similar to this value, and the maximum Hg concentration is 7.5-hold higher than it. Therefore, it is recommended to recheck the current Hg values using more samples.

Lead – No difference between the results of this report and the H4 indicator dashboard. However, the proportion of birds with a Pb concentration exceeding the threshold differed between sparrowhawks and buzzards, which indicates that the difference in the magnitude of exposure between species should be taken into account for the threshold assessment, when an alternative species is chosen as a solution for limited sample mass. Particularly, due to their facultative scavenging feeding habits, some buzzards might ingest lead shot ammunition, which is unlikely to be the case for sparrowhawks. These results may indicate that (a) exposure of buzzards to Pb may reflect acute exposure risk of wildlife due to ingesting lead shot ammunition, whereas (b) Pb concentrations in sparrowhawks may reflect broader environmental contamination but not reflect exposure due to ingested on Pb shot.

Zinc – No significant time trend was observed in this report, whereas the H4 dashboard in the H4 interim report 2021 demonstrated a “decreasing” trend. This study used only the median value of each year for the time trend. It is therefore recommended to recheck the time tend of Zn using the whole or more samples.

Table 12. Proportion of birds in 2021 (20 sparrowhawks and 20 buzzards) with metal concentrations in the liver exceeding the threshold values for the H4 dashboard.

Metal	Threshold value for the H4 dashboard	Proportion of bird > threshold	
		Sparrowhawk	*Buzzards*
Hg	> 7.2 mg/kg dw	25.0%	0.0%
Pb	> 21.6 mg/kg dw	0.0%	10.0%
Cd	> 162 mg/kg dw	0.0%	0.0%

8.4 Recommendations for further studies

One of the main issues related to the H4 indicator is a limited sample mass of certain species, such as the sparrowhawk, for measuring many chemical contaminants. It needs to explore whether (i) the buzzard could be used as a substitute for the sparrowhawk and/or (ii) whether exposure to metals could be estimated by biological parameters to retain the sample mass of the target organ, the liver, for other chemical contaminants than metals.

Given the results of this report, both two goals are difficult to choose as options to resolve the problems of a limited sample mass at the moment. There are still gaps of knowledge for adjusting discrepancies in metal exposure between the sparrowhawk and the buzzard. However, this report identified two study areas related to these discrepancies in metal exposure:

1. **Environmental factors filtering exposure to metals.**
    - In the discussion section, it was hypothesised that the discrepancies in toxic metal exposure between the two species would be due to environmental factors such as the diet, landscape (i.e., habitats), and seasonal changes in bird and/or human activities. These factors could be a filter differently influencing the transfer of contaminants from the environment to birds (Figure 13).

2. **Interactions between physiological/morphological parameters/indices and exposure/toxicokinetic.**
    - This report analysed the possibility of modelling metal exposure by biological parameters. However, adverse physiological and morphological effects of high levels of metal exposure may cause feedback modifying both the intake of contaminated food and the distribution of metals within organs (Figure 13).

Note that the two study areas are not independent, given that the environment determines animals' physiology, morphology, and ecology.

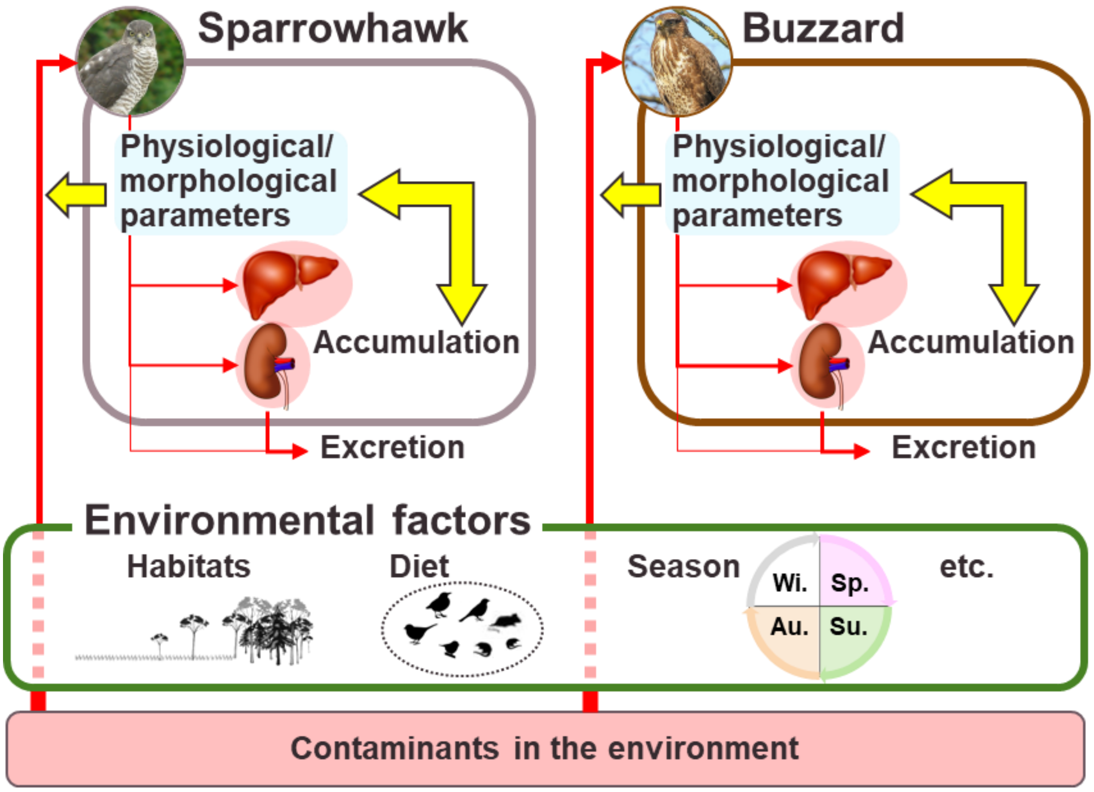


Figure 13. Scheme describing the exposure pathways of wild birds to metals from the environment and the toxicokinetic of metals modulated by biological factors.

Further studies, therefore, need to tackle the two areas primarily. Based on the overall results and discussion, the present report proposes as recommendations for further studies:

- **Continuing to compare the time trend of metals in the two species.**
  It is required to follow the time trend of metal exposure of the two species to check whether their time trends are similar or different. It is also required to use more sample numbers to obtain a stronger evidence base.

- **Integrating environmental factors into the analysis**
  The difference in their ecology should be integrated into the comparative assessment of their metal exposure. For example, the habitats and the season of each individual can be estimated by the location and the day (or month) of finding the dead bodies, assuming that the diet may be associated with the habitats.

- **Assessing other organs and biological parameters to elucidate the toxicokinetic of metals**
  The relationships between biological parameters and concentrations of metals in tissues should be analysed in depth, which requires an assessment of the effect of metals on physiological and morphological parameters. It is also recommended to analyse concentrations of metals in different tissues, such as kidneys. This will provide information on the relationships between the distribution of metal and biological parameters and the possibility of using alternative organs to resolve the problem of small sample mass.

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11 Appendix

11.1 Limit of detection of the 14 elements and their recovery rate

Table 13. Statistics (minimum, mean, and maximum) of the limit of detection (LoD) of the 14 elements (mg/kg dw) and their recovery rate (%).

LoD	Hg	Pb	Cd	Zn	Cu	Ni	As
Min	35.86	0.72	0.72	358.60	7.53	0.72	2.51
Mean	50.67	1.11	0.90	448.70	9.42	0.90	3.14
Max	515.42	9.78	1.11	554.70	11.65	1.11	3.88
Recovery rate (%)	110.28 ± 8.77	90.73 ± 9.81	103.83 ± 6.48	104.19 ± 5.19	92.88 ± 4.25	102.42 ± 4.49	105.3 ± 12.00

LoD	Fe	Mn	Mo	Se	Cr	Co	Sr
Min	358.60	8.61	1.79	35.14	7.53	0.72	0.72
Mean	448.70	10.77	2.24	43.97	9.42	0.90	0.90
Max	554.70	13.31	2.77	54.36	11.65	1.11	1.11
Recovery rate (%)	97.11 ± 4.52	99.60 ± 4.59	104.34 ± 5.99	112.74 ± 0.91	104.23 ± 3.75	100.70 ± 10.86	102.70 ± 4.99

11.2 Statistical results of the elements other than the six metals reported in the previous H4 reports: Se, Fe, Mn, Mo, Cr, Co, Sr

Table 14. The minimum (Min), median, arithmetic means (Mean), geometric mean (Geo-Mean), standard-deviation (SD), and maximum (Max) of concentration of Se, Fe, Mn, Mo, Cr, Co, and Sr (mg/kg dw). Values under the limit of detection are indicated as LoD.

		Se			Fe			Mn		
		Total	2020	2021	**Total**	2020	2021	**Total**	2020	2021
Sparrowhawk	**Min**	**1.82**	1.82	3.10	**272.0**	272.0	594.2	**2.01**	2.01	5.28
	Median	**4.40**	4.30	4.66	**964.1**	958.3	964.1	**11.16**	10.86	11.16
	Mean	**5.38**	4.54	6.22	**1055.3**	1069.4	1041.1	**11.24**	10.79	11.69
	Geo-Mean	**4.88**	4.31	5.53	**975.8**	968.0	983.7	**10.09**	9.27	10.97
	Max	**13.32**	7.99	13.32	**2491.8**	2491.8	1950.0	**27.39**	27.39	18.07
Buzzard	**Min**	**2.89**	2.89	3.14	**651.8**	651.8	779.8	**4.63**	4.63	7.32
	Median	**5.09**	4.90	5.16	**1734.4**	1424.7	1944.2	**13.45**	12.35	14.23
	Mean	**5.76**	5.72	5.80	**2554.2**	1964.3	3144.1	**14.82**	14.47	15.17
	Geo-Mean	**5.40**	5.37	5.43	**1992.5**	1620.8	2449.5	**13.57**	12.93	14.24
	Max	**14.43**	14.43	11.54	**9299.2**	5203.5	9299.2	**31.06**	31.06	27.29

		Mo			Cr			Co			Sr		
		Total	2020	2021	**Total**	2020	2021	**Total**	2020	2021	**Total**	2020	2021
Sparrowhawk	**Min**	**0.10**	0.10	1.58	**LoD**	LoD	LoD	**0.02**	0.03	0.02	**LoD**	LoD	LoD
	Median	**2.28**	2.20	2.35	**LoD**	0.01	LoD	**0.08**	0.09	0.08	**0.03**	0.09	<0.01
	Mean	**2.29**	2.22	2.36	**0.03**	0.04	0.02	**0.09**	0.10	0.09	**0.07**	0.11	0.03
	Geo-Mean	**2.12**	1.93	2.32	**0.01**	0.02	0.01	**0.08**	0.08	0.08	**0.01**	0.03	<0.01
	Max	**3.54**	3.54	3.05	**0.30**	0.30	0.13	**0.22**	0.22	0.18	**0.50**	0.50	0.17
Buzzard	**Min**	**0.94**	1.14	0.94	**LoD**	LoD	LoD	**0.02**	0.02	0.02	**0.04**	0.04	0.05
	Median	**1.80**	1.79	1.83	**0.04**	0.03	0.04	**0.10**	0.08	0.15	**0.21**	0.17	0.26
	Mean	**1.90**	1.86	1.95	**0.15**	0.12	0.17	**0.13**	0.10	0.16	**0.38**	0.28	0.48
	Geo-Mean	**1.84**	1.82	1.87	**0.05**	0.04	0.06	**0.10**	0.08	0.13	**0.25**	0.20	0.30
	Max	**3.22**	2.52	3.22	**1.67**	1.67	0.94	**0.49**	0.41	0.49	**1.86**	0.74	1.86

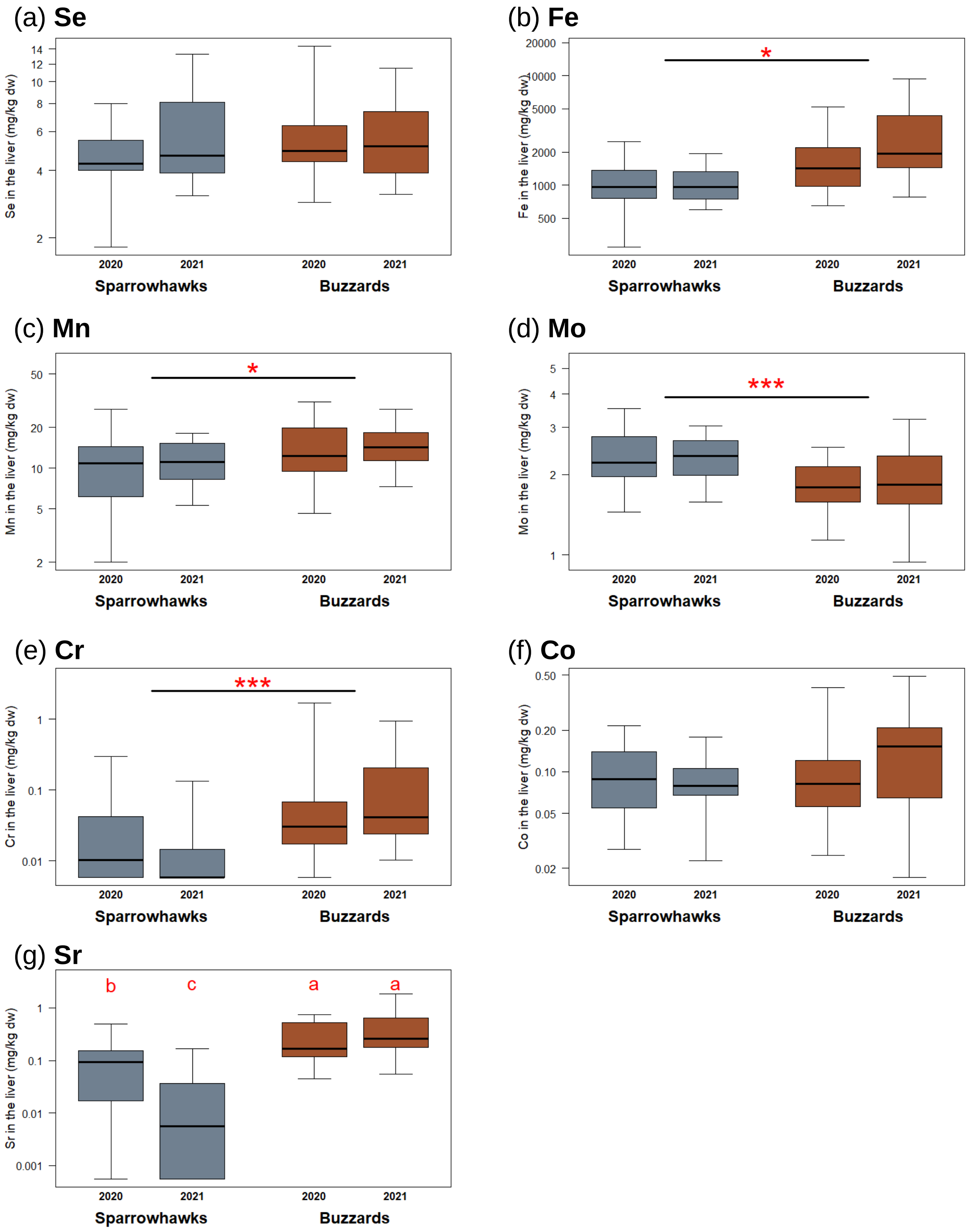


Figure 14: Box and Whisker plots showing median, interquartile range and minimum/maximum range of Se, Fe, Mn, Mo, Cr, Co, and Sr concentrations in sparrowhawks and buzzards collected in 2020 and 2021. Statistically significant differences between the two years or between the species are indicated by asterisks (*: p-value < 0.05; **: p-value < 0.01; ***: p-value < 0.001). When the trend between the years differed between the species, significant differences (p-value < 0.05) are indicated by different letters.

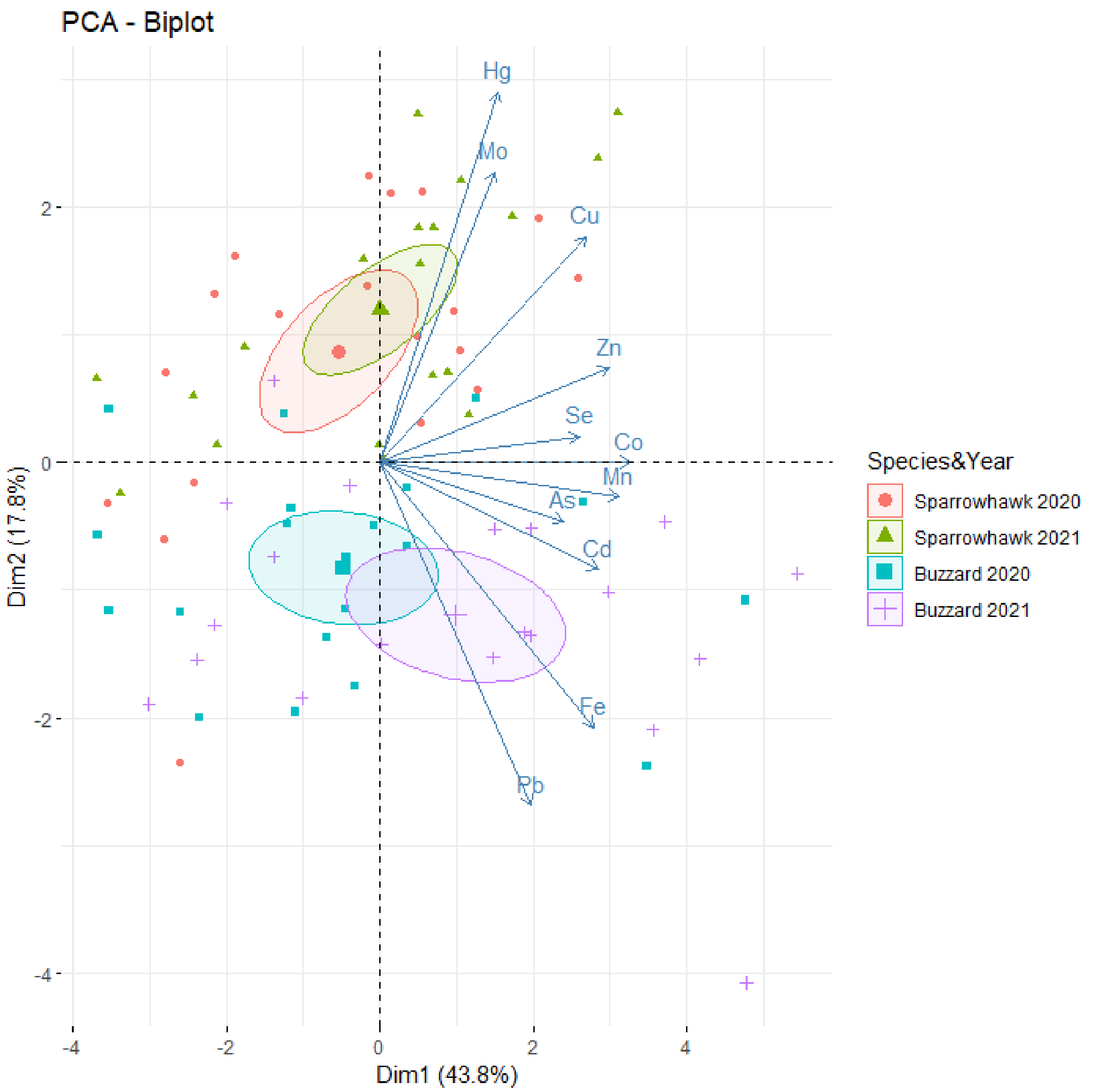
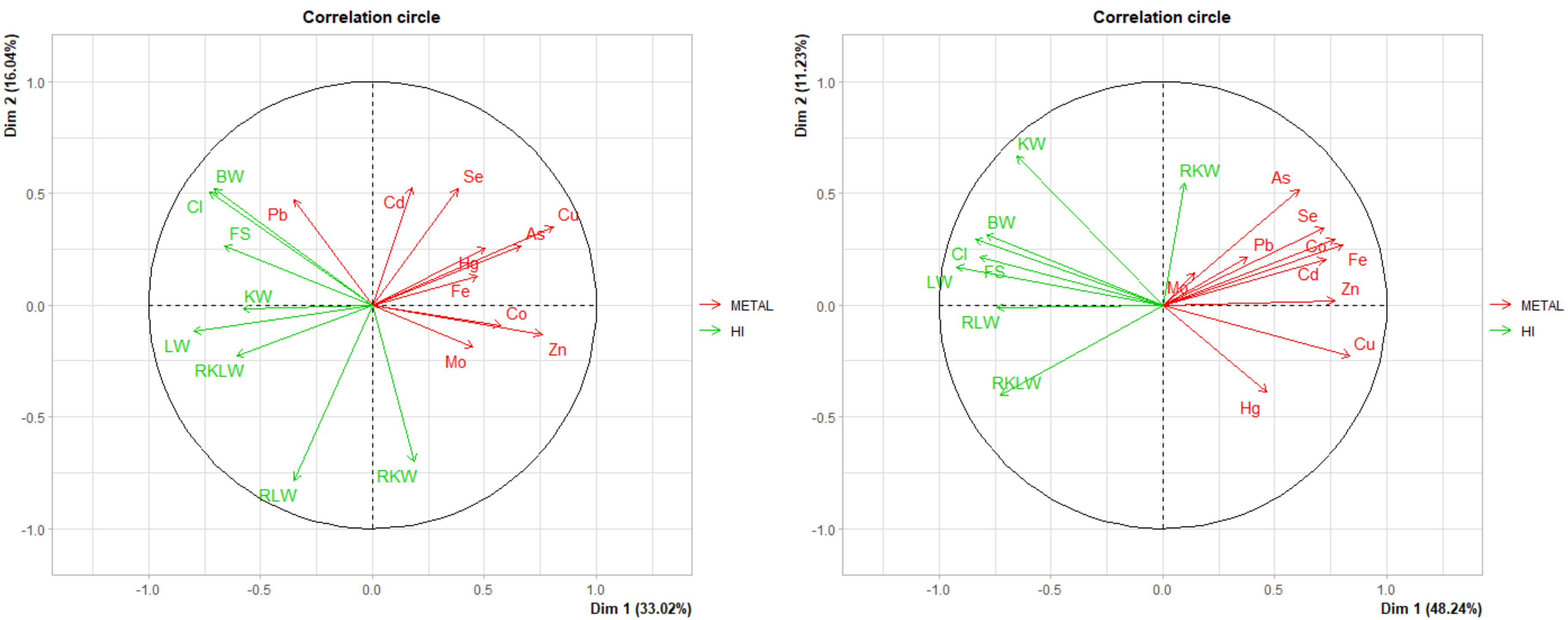


Figure 15: Principal component analysis (PCA) biplot of the data on concentrations of Hg, Pb, Cd, Cu, Zn, As, Se, Fe, Mn, Mo, and Co in the livers of sparrowhawks and buzzards collected in 2020 and 2021. Ni, Cr, and Sr were removed from the analysis because of the number of their values <LoD. The centroid and the 95% of confidence ellipse of the centroid of the four sub-groups split by two species and two years were represented by different colour and shapes of points.

(a) **Sparrowhawk** (b) **Buzzard**



RV= 0.37; p-value <0.001 *RV= 0.55; p-value <0.001*

Figure 16: Co-inertia analysis (CoIA) biplot representing the correlations between concentrations of Hg, Pb, Cd, Cu, Zn, As, Se, Fe, Mn, Mo, and Co in the liver of sparrowhawks (a) and buzzards (b) collected in 2020 and 2021 and their health indicators: body weight (BW; g), liver weight (LW; g), relative liver weight (RLW; %), kidney weight (KW; g), relative kidney weight (RKW; %), ratio between kidney and liver weights (RKLW), fat score (0 – 5; considered as a numerical variable) and condition index (CI; g/cm^3). Ni, Cr, and Sr were removed from the analysis because of the number of their values <LoD. The RV coefficient, which represents the similarity of the two sets of data, and the p-value of the RV coefficient by a permutation test are shown in the graphics.

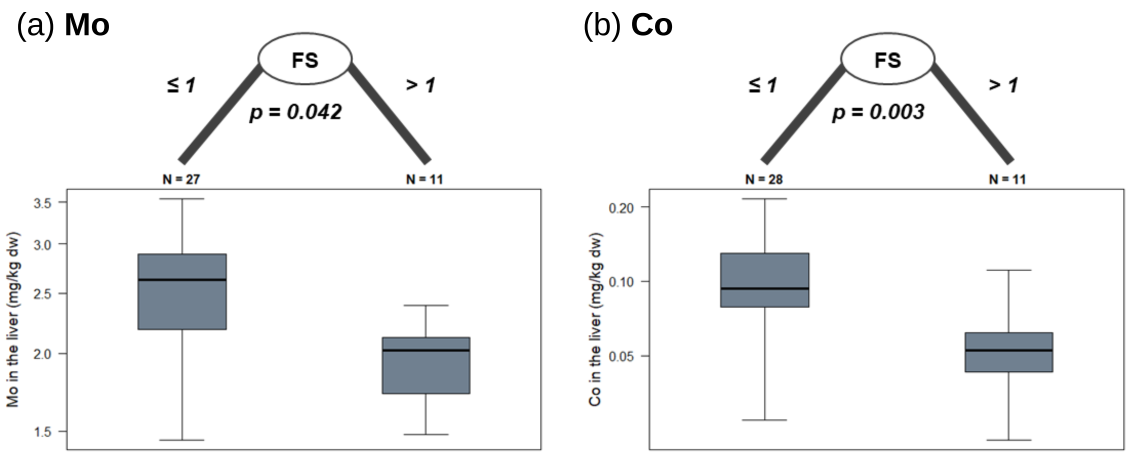


Figure 17: Conditional inference tree (CIT) representing significant factors that influence metal concentrations of Mo (a) and Co (b) in the liver of sparrowhawks collected in 2020 and 2021. Partitioning variables significantly selected for splits are indicated in ovals with its p-value, and splitting criteria are listed beside the lines connected to the ovals. Boxplots in the lower part describe metal concentrations of each sub-group with its sample size.

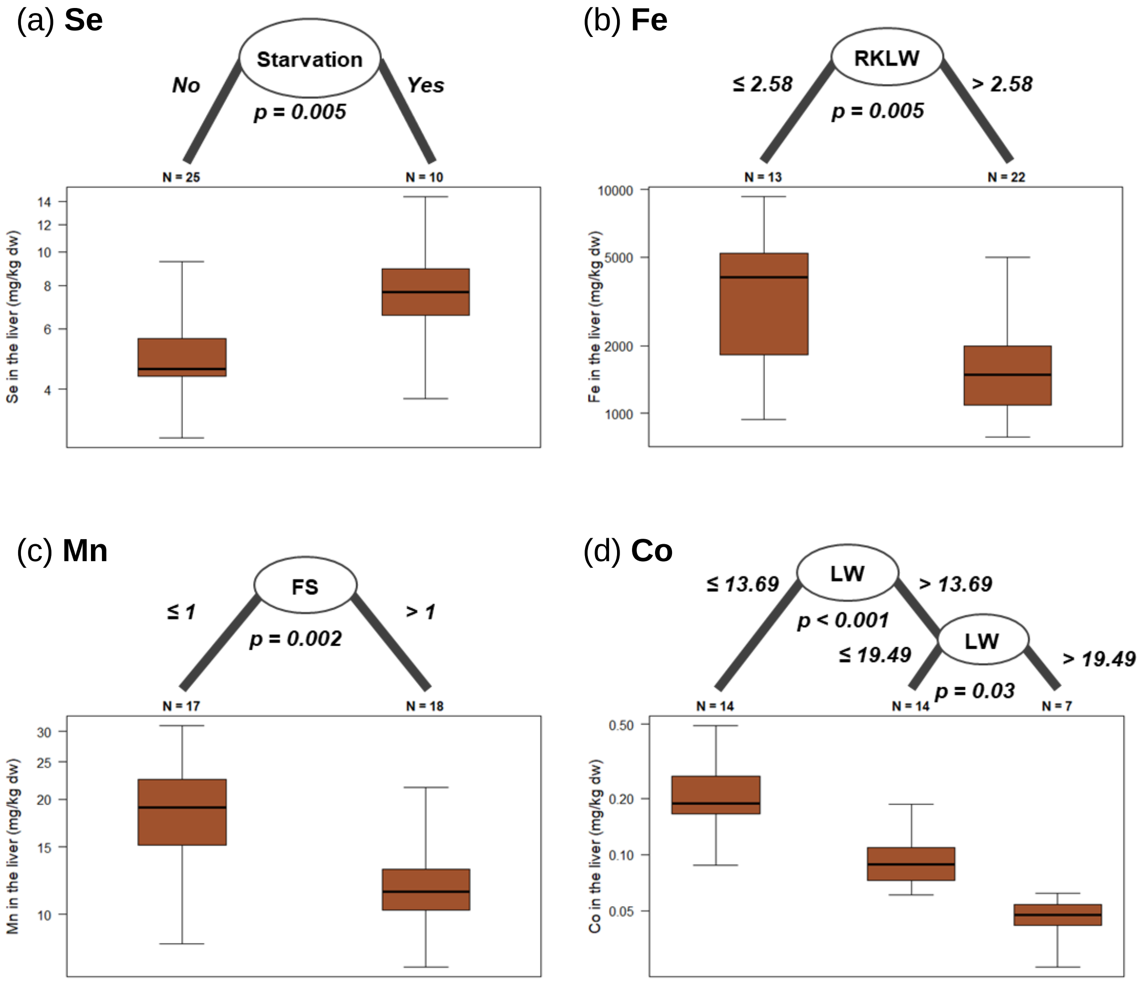


Figure 18: Conditional inference tree (CIT) representing significant factors that influence concentrations of Se (a), Fe (b), Mn (c), and Co (d) in the liver of buzzards collected in 2020 and 2021. Partitioning variables significantly selected for splits are indicated in ovals with its p-value, and splitting criteria are listed beside the lines connected to the ovals. Boxplots in the lower part describe metal concentrations of each sub-group with its sample size.

Table 15. Linear model for the relationship between metal concentrations and the potential health indicators selected from the results of CIT: FS, LW, RLW, RKLW, and starvation status. Blank means that no explanatory variable was selected by CIT.

Species	Metal	Model	R^2
Sparrowhawk	**Se**		
	Fe		
	Mn		
	Mo	*=exp(0.895), when **FS**=0* *=exp(0.954), when **FS**=1* *=exp(0.711), when **FS**=2* *=exp(0.587), when **FS**=3*	0.276
	Co	*=exp(-2.310), when **FS**=0* *=exp(-2.337), when **FS**=1* *=exp(-2.840), when **FS**=2* *=exp(-3.153), when **FS**=3*	0.375
Buzzard	**Se**	*=exp(1.585), when **starved*** *=exp(2.055), when **non-starved***	0.369
	Fe	*=exp(8.839 - 0.414 **RLW**)*	0.368
	Mn	*=exp(2.937), when **FS**=0* *=exp(2.748), when **FS**=1* *=exp(2.577), when **FS**=2* *=exp(2.282), when **FS**=3* *=exp(2.382), when **FS**=4*	0.476
	Mo		
	Co	*=exp(-0.771 - 0.096 **LW**)*	0.685

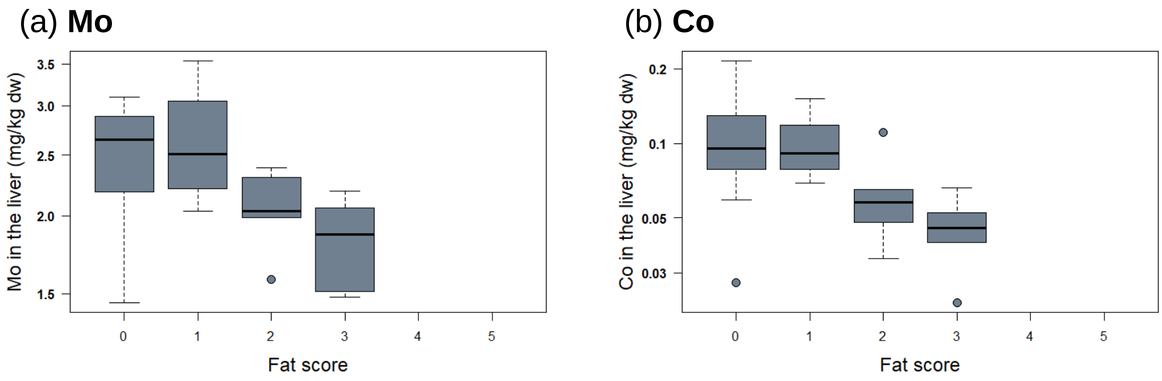


Figure 19: Linear models for metal concentrations in the liver of sparrowhawks collected in 2020 and 2021: concentrations of Mo (a) and Co (b) compared to fat score (as a categorical factor).

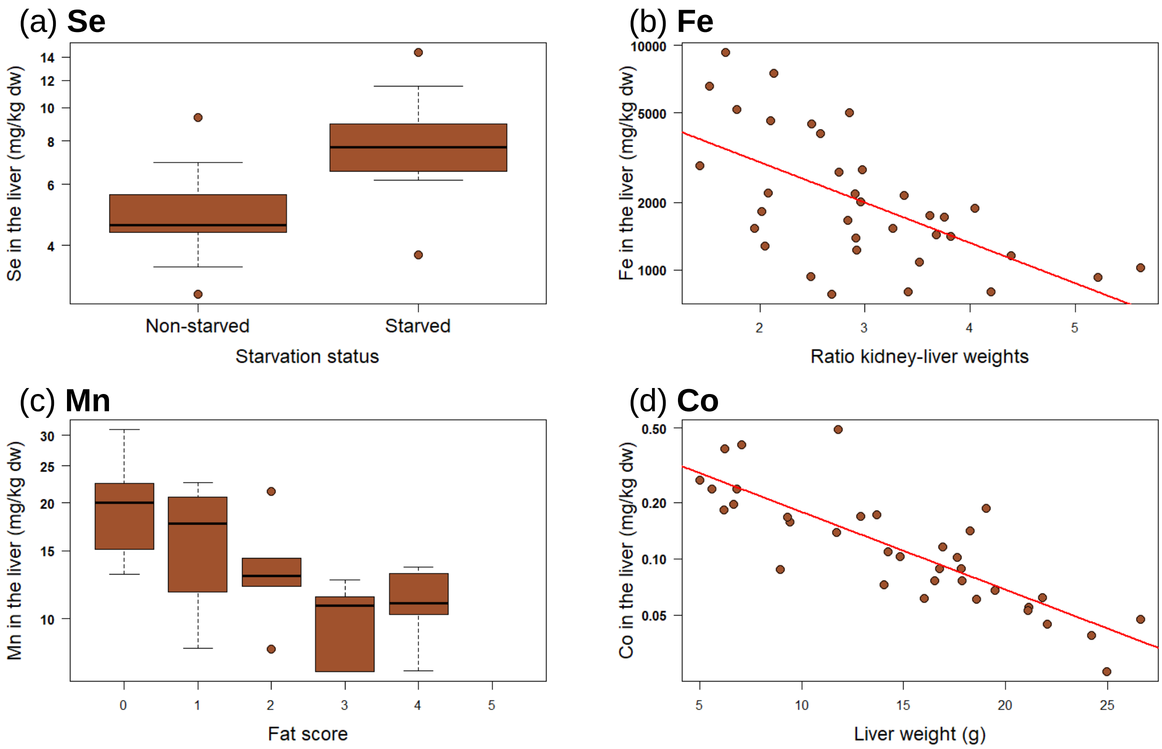


Figure 20. Linear models for metal concentrations in the liver of buzzards collected in 2020 and 2021: concentrations of Se by starvation status, Fe compared to ratio between kidney-liver weights (b), Mn compared to fat score (as a categorical factor) (c), and Co compared to liver weight (d).

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