# Geochemical landscapes as drivers of trace and toxic element profiles in wild red deer ( Cervus elaphus )

French, Andrew S.; Shaw, David; Gibb, Stuart W.; Taggart, Mark A.

*Published in:*
Science of the Total Environment

*Publication date:*
2017

# Geochemical landscapes as drivers of trace and toxic element profiles in wild red deer (*Cervus elaphus*)

Andrew S French*[1], David Shaw[2], Stuart W Gibb[1], Mark A Taggart[1]

[1]Environmental Research Institute, North Highland College, University of the Highlands and Islands, Castle Street, Thurso, KW14 7JD, UK

[2]UHI Rural Studies Centre, North Highland College, University of the Highlands and Islands, Dale Farm, Halkirk, KW12 6UW, UK

*Corresponding author: Andrew S French; Email: andrewsamuelfrench@gmail.com

Phone: +44 1847 889578

Key words

trace element; wildlife; principal component analysis; tissue contaminant; Scotland

Colour images

NONE

Supplementary files

Supplementary File.docx

# Abstract

Tissue concentrations of essential trace and toxic elements in red deer (*Cervus elaphus*) are associated with the plants, soil and water they ingest. As such, variation in tissue concentrations is associated with variation in local geochemistry and bioavailability of elements. Physiological factors such as liver fluke (*Fasciola hepatica*) infection, breeding status, and in-tissue element interactions may also affect tissue concentrations, though their effects in red deer are not well understood. The primary objective of this study was therefore to survey wild red deer liver element concentrations across a range of geographically distinct populations during the Scottish red deer stalking season; and, in so doing, establish element reference ranges while also exploring geographic and temporal variation and physiological factors. Livers were sampled from carcasses intended for human consumption on nine hunting estates during two seasons (2012-13, 2013-14). Samples were digested and analysed by ICP-OES for essential trace elements (Co, Cu, Fe, Mn, Mo, Se, Zn) and for Cd. Data (n = 787) were modelled against cull location, date, and *F. hepatica* diagnosis. Interactions between elements within liver, and differences in element profiles between estates, were explored by principal component analysis. Our results revealed marked geographic variation in Cd, Cu and Se, where up to four-fold differences in median element concentrations occurred between estates, and, in males, Mn, Mo and Zn declined as the breeding season approached. In both sexes, within-liver associations (Cd-Cu-Se and Mn-Mo-Zn) were found. In females, liver Zn was greater on average in individuals that were not infected with *F. hepatica*. This study is the first to quantify geographic variation in Scottish red deer liver element concentrations; the drivers of which remain to be explored (and may be management related), and, the consequence of which may affect sub-clinical health.

# 1. Introduction

Red deer (*Cervus elaphus*) obtain essential trace and toxic elements through their diet – a portion of which is stored in the liver. In farmed red deer, reference ranges for deficiency (i.e., tissue concentrations of essential trace elements below which clinical symptoms occur) have been proposed by referring to historical data from blood and liver samples from clinically deficient individuals (Wilson and Grace, 2001). Conversely, in wild deer, a lack of data has led to uncertainty surrounding deficiencies, even where low liver element concentrations have been found (e.g., Cu and Co related sicknesses in moose (*Alces alces*) manifesting as muscle weaknesses and behavioural abnormalities; Frank, 1998; Frank et al., 2004). Consequently, sub-clinical deficiencies purported to affect wild populations (e.g., declines in moose populations (*A. alces*); Custer et al., 2004; Murray et al., 2006), and reduced reproductive performance in black-tailed deer (*Odocoileus hemionus columbianus*); (Flueck, 1994) are challenging to validate. To reduce this uncertainty, tissue reference ranges for essential trace elements in wild deer are required; and, monitoring toxic element content in wildlife (and indeed game species in regions where animals are harvested for meat, e.g., in Scotland) is also warranted.

The transfer of elements from soil and subsequent uptake by soil biota and plants in a specific locality is dependent on soil conditions: i.e., redox potential (Eh), pH, organic matter content, clay content and hydrous oxides of Fe and Mn (Husson, 2012; Rieuwerts et al., 1998). As such, wild deer have access to variable quantities of essential trace and toxic elements depending on their locality, and this is reflected by differing tissue element concentrations between herds; e.g., Co, Cu and Se in Norwegian red and roe deer (*Capreolus capreolus*) (Vikøren et al., 2005, 2011) and Cd in Canadian moose (Gamberg et al., 2005).

62 The dominant soil types in the Scottish Highlands are rich in organic matter, and the
63 landscape is a patchwork of peat, gley and podzols (**Fig 1**). As such, the region can be
64 broadly characterised by two prevailing acidic soil conditions that are differentiated by their
65 redox potential (Eh); these are, (i) reducing conditions that associate with soils in lowlands
66 and on shallow slopes that are subject to permanent or partial waterlogging, and (ii) oxidising
67 conditions that associate with drained soils on steep hill slopes. The diet of wild deer in the
68 Scottish Highlands is dominated by plants, but also comprises fresh water, soil minerals
69 (ingested either involuntarily with plant roots or as a natural lick) and seaweed (where
70 accessible (Conradt, 2000). Deer diet may also be punctuated with occasional anthropogenic
71 sources of elements (i.e., road salt, which may promote mobilisation of metals in roadside
72 verge soils (Amrhein et al., 1992), which may subsequently be taken up by plants), trace
73 element-enriched mineral blocks provided for deer and/or livestock, and point sources of
74 contamination from historic or present mining activity and/or local industry). In terms of land
75 use, largely unfenced hunting estates (which dominate the Scottish Highland land area) are
76 principally managed for the maintenance of red deer populations for commercial hunting. As
77 such, the availability of elements to wild red deer in the Scottish Highlands is in the interest
78 of land owners and gamekeepers, but is likely to be primarily associated with the prevailing
79 soil conditions that control element availability for plant uptake. Here, soil pH and Eh affect
80 the solubility of elements in soil solution (Husson, 2012), which (for example) results in low
81 availability of Mn to cereals grown in calcareous (i.e., high pH) soils , which are typical of
82 the far north west Highlands and Islands of Scotland (George et al., 2014).

83 The distribution and storage of elements in red deer tissues varies between organs. As such,
84 physiological factors such as accumulation with age (e.g., Cd in liver and kidney; Wolkers et
85 al., 1994), and antagonism between one or more elements, affect tissue element
86 concentrations through interactions (for example, between Hg and Se; Berzas Nevado et al.,

2012; Patiño Ropero et al., 2016). Such interactions are highly complex and their mechanisms often remain unclear. As such, various correlations within red deer liver (e.g., Zn-Cd, Cu-Cd and Se-Cd; Reglero et al., 2008) have received little interpretation, though (in some cases) might be explained by their joint roles as co-factors in antioxidant enzymes (e.g., copper (Cu) and zinc (Zn), which are both bound by the antioxidant enzyme Cu-Zn superoxide dismutase (SOD)). Likewise, storage of elements might be affected by macro-parasites (e.g., liver fluke (*Fasciola hepatica*)), which occurs in red deer in the Scottish Highlands; French et al., 2016); though negative associations between *F. hepatica* and *Fasciola magna* and Cu in fallow (*Dama dama*) and red deer (Lazarus et al., 2008; Vengušt et al., 2003) remain the only two documented occurrences, respectively. The onset of the breeding season might also affect liver element status, particularly in red deer males, as they become physiologically stressed and develop fatty liver (Zomborszky and Husvéth, 2000); however, temporal related changes in liver element concentrations in relation to breeding stresses have (to our knowledge) not been explored.

In the Scottish Highlands, wild red deer are managed by shooting and fencing to maintain populations for hunting and to maintain forestry (native and commercial). Therefore, through restriction of deer movement and provision of mineral licks (which may or may not promote the availability of some elements at the population level), the availability of elements to wild deer is affected by land management, which could ultimately affect the health of wild deer, for which those managing the deer have a strong interest. The aim of this study was to survey essential trace and toxic element content in Scottish wild red deer (harvested for human consumption); and, in so doing, establish liver element reference ranges for wild (Scottish) red deer. Furthermore, by stratifying data by hunting estate, and considering liver fluke infection and sampling dates, we aimed to identify any marked geographic variation in single element concentrations. In addition, by quantifying associations between elements, we aimed

to: (i) identify patterns in element (co-)accumulation (perhaps driven by physiological mechanisms), and (ii) explore geographic variation in element profiles that may be driven by environmental factors, such as soil conditions (e.g., redox potential).

# 2. Materials and methods

## 2.1 Sample collection, sample digestion and ICP-OES analysis

Sampling took place between August and February 2012-13 and 2013-14. All samples were collected by deer stalkers/gamekeepers (hunters) on privately owned Scottish hunting estates (Alladale (AL), Altnaharra (AT), Applecross Trust (AP), Ardnamurchan (AR), Badanloch (BA), Ben Loyal (BL), Conaglen (CO), Strathconon (ST), and two neighbouring estates North Harris Trust and Aline (NA)) - see Materials and Methods of French et al., (2016) for ethics statement regarding the sampling of wild red deer for this study. In terms of hunting biases, hunters were asked to record the sex, date of cull and age category of each animal. Age category was determined (objectively) from tooth eruption for calves and yearlings and (subjectively) from inspection of tooth wear for young, mature and old animals.

During the 2012-13 and 2013-14 stalking seasons (July 1$^{st}$ to October 20$^{th}$ for males; October 21$^{st}$ to February 15$^{th}$ for females), 793 wild red deer liver samples (of known sex, estimated age category, and sampling date) were collected into 50ml centrifuge tubes and stored frozen at -20ºC at nine Scottish Highland hunting estates (**Table 1**; see Appendix A for breakdown of samples collected stratified by age category and estate). Participating estates were chosen primarily due to their contrasting environments (e.g., soil type) (**Fig 1**). Faecal samples were collected in parallel and 715 of these samples were diagnosed as positive or negative for liver

fluke (*F. hepatica*) using a coproantigen ELISA that was estimated as 96% specific (95% CI, 95% – 100%) and 79% sensitive (95% CI, 73% – 84%) to patent infection (French et al., 2016). Six whole livers, collected on two estates (Altnaharra and Badanloch), were sampled at five points - two samples from the left lobe, two from the right lobe and one from the caudate lobe - and were used to investigate heterogeneity in liver element concentrations.

Samples were digested following an adapted UV-assisted method (Manjusha et al., 2007). Samples were oven-dried at 105ºC to constant weight and 0.30g of dried sample was weighed into 15ml pure quartz vials; 0.30 g of certified reference materials (CRMs; bovine liver BCR®-285R, dogfish liver NRC-CNRC DOLT-4 and lobster hepatopancreas NRC-CNRC TORT-2) and 0.50ml MilliQ® (for procedural blanks) were similarly weighed out. Next, 2ml of $HNO_3$ (TraceMetal™ Grade, Fisher Scientific, UK) was added to each vial and left for 14 hours (overnight) at room temperature to pre-digest. Vials were then placed on an aluminium heating block (Stuart 2 Block SBH130D, Bibby Scientific, UK) that was increased (over one hour to minimise foaming) from 70°C to 120°C. Following cessation of fuming, samples were removed from the block and 0.25 ml of $H_2O_2$ was added twice to each vial; samples were then replaced on the heating block until reactions subsided. Finally, vials were placed in a UV-digester (705 UV Digester Metrohm, Switzerland) for two hours with the addition of 0.25ml of $H_2O_2$ every 30 minutes (total $H_2O_2$ per vial: 1.25ml) until clear, colourless digests remained. Finally, vials were removed from the digester and diluted to 10ml with MilliQ®.

Digested samples were analysed by ICP-OES (Varian 710-ES, Agilent Technologies, USA) for seven essential trace elements (Co, Cu, Fe, Mn, Mo, Se, and Zn) and for Cd. In addition, samples were analysed for contamination by soil (using aluminium (Al) as proxy for soil content) and for lead ammunition (Pb), though we did not have sufficient certified reference material information to include these two elements in our further statistical analyses. A

158 calibration blank and a series of four in-house multi-element instrument calibration standards
159 containing our analytes (Sigma-Aldrich, UK) were used; emission lines and limits of
160 detection (LODs) are shown in **Table S1**. All samples, procedural blanks and CRMs were run
161 over two weeks, in random order with respect to sex, site and season.

162 Recoveries for Cd and all essential trace elements (Co, Cu, Fe, Mn, Mo, Se and Zn) ranged
163 from 91.1 to 108% in all CRMs (**Table S1**); noting that this falls in the acceptable range (80
164 – 110 % recovery) for trace elements measured in foods up to the order of 1000ppb
165 recommended by AOAC International (Murphy et al., 2013). In addition, LODs were
166 exceeded for all measurements of Cd and essential trace elements, with the exceptions of Se
167 and Co where 13.4 and 13.9% of measurements fell below the LOD (0.062µg/g liver (dry
168 weight) for Co; 0.28 µg/g liver (dry weight) for Se), respectively (**Table S1**). Within liver
169 heterogeneity in element concentrations was smallest for Mn, Mo and Zn (mean relative
170 standard deviation (RSD) ranged between 6.0 and 8.0%) and largest for Cd, Co, Cu, Fe and
171 Se (mean RSD ranged from 10.3 to 14.4%) (**Table S2**).

## 172 2.2 Statistical methods

173 All analyses were carried out using R (R Core Team, 2012) and RStudio (RStudio Team,
174 2015). To account for sex sampling biases (i.e., collinearity between sampling date and sex),
175 data were stratified into males and females; thus, capturing data that reflected the sex-specific
176 nature of Scottish red deer herds outside the breeding (Sep - Oct) season.

177 To reduce the effect of hunter bias that originated from the subjectivity of age categorisation
178 based on tooth wear, we chose not to include age as an ordinal predictor variable in any
179 statistical models. Instead, we pooled males from the three age groups that are similar (in
180 terms of size and herding/feeding behaviour; i.e., young, mature and old) and only present

sample numbers for calves and yearlings in **Table 1**. Similar, for females, we pooled a group of similar individuals (in terms of body size and feeding behaviour; yearling, young, mature, old). Female yearlings (~1.5 years old) were retained but not males, because males (at 1 year old) remain with their mother within female herds; hence, habitat use and element intake/requirements for yearling males will not necessarily reflect that typical for adult males.

Initial data exploration revealed extremely high concentrations of Mn (451 - 809µg/g) in four samples and Pb (602 - 1698µg/g) in two samples. The distances between the animals from which these samples were collected and the nearest known possible point sources of contamination (i.e., Pb mine remnants east of Ardnamurchan (British National Grid (BNG) ref: NM 804 659), and east of Strathconon (BNG ref: NH 375 381), and Cu mine remnants east of Applecross (BNG ref: NG 849 432) were in excess of 17km. As such, it is unlikely that the extreme liver element concentrations are directly attributable to feeding around these particular contaminated sites. More likely, the two samples that contained extreme Pb concentrations (602 - 1698µg/g) were probably attributable to Pb ammunition fragments, as these values closely reflect those measured adjacent to bullet tracks in large game carcasses (Dobrowolska and Melosik, 2008). The four samples that contained extremely high Mn concentrations (in excess of 16-fold greater than the next greatest concentrations), also contained aluminium (Al) (11.5 - 192 µg/g). As such, it is likely that these four samples were contaminated by dust and dirt/soil, whereby excessive Al and Mn are recognised indicators of sample contamination in trace element studies of biological samples (Pineau et al., 1993). Data for omitted samples are shown in **Table S3**.

For the 13-14% of the Co and Se data that fell below the LOD (i.e., for Co and Se), a multiplicative random lognormal replacement method was used to impute alternative plausible values (Cohen and Ryan, 1989; Helsel and Hirsch, 1992; Palarea-Albaladejo and

205 Martín-Fernández, 2013). Here, the R library (zCompositions; Palarea-Albaladejo and
206 Martín-Fernández, 2015) was used to fit a lognormal distribution (for Se and Co for each sex)
207 while accounting for the proportion of data below the LOD (i.e., censored data; Cohen,
208 1976). This method is less often used than the conventional substitution of values below the
209 LOD with values equal to half the detection limit; however, its use results in relatively less
210 biased summary statistics (Baccarelli et al., 2005) than conventional substitution, which
211 inherently reduces variance (Palarea-Albaladejo and Martín-Fernández, 2013).

212 Differences in median element concentrations between estates were explored using 95%
213 bootstrap resampling (percentile) confidence intervals. In addition, Spearman's ρ correlations
214 between male and female element concentrations were calculated using the median for each
215 sampling site; i.e., the correlation between males and females was calculated for nine data
216 points.

217 Generalised linear (GLM) and generalised least squares (GLS) models were used to
218 investigate geographic and temporal variation in element concentrations and any associations
219 with *F. hepatica* infection in the liver. Firstly (for the dataset containing *F. hepatica*
220 diagnoses; n = 715), GLMs were constructed using four predictors: estate, days into the
221 stalking season (female 1 – 118 days; male 33 – 112 days), season culled and *F. hepatica*
222 infection status (infected/non-infected). In addition, the relationship between cull date
223 [squared] and element concentrations was explored by visual inspection of GLM partial
224 residuals, and inclusion of a fifth [squared] term in final models was based on a lower
225 Akaike's Information Criterion (AIC) for the more complex model. GLMs were considered
226 valid if visual inspection of graphical illustrations of model residuals revealed no patterns.
227 Where visual inspections revealed non-normality and non-constant error variance of
228 residuals, these were generally remedied by log transformation of element concentrations;

but, where heteroscedasticity remained, variance structures were implemented using GLS (error structures detailed in **Table S5**). Negligible autocorrelation (ACF; ACF < 0.15) was found for element concentrations in temporally and spatially adjacent culled animals; hence, no correlation structures were implemented for any model. In addition, no data were removed as a consequence of leverage, and visual inspection of coplots revealed no relevant interactions between predictors. Following final model validation, models were used in conjunction with least squares means (L-S means) and Tukey pairwise comparisons in R (Lenth, 2016) to identify significant differences in average (often log-transformed) tissue element concentrations between estates, sampling years, and *F. hepatica* and non-infected groups, while accounting for temporal relationships. For comparison, this statistical procedure was also applied to the larger dataset (n = 787; details of error structures are shown in **Table S6**), but omitting the predictor for *F. hepatica* infection status.

Principal component analysis (PCA) was used to identify associations between elements, and to identify differences in element profiles between estates, whereby element concentrations were log transformed (with the exception of Mo, Mn in females and males). Here, the three principal components that explained ~60% of variation in element concentrations were also included as response variables in GLMs; whereby models were constructed following the same procedure as described for the single element GLMs, and comparisons were again made using L-S means (Lenth, 2016).

# 3. Results

## 3.1 Liver element reference ranges for Scottish red deer

In both sexes, liver concentrations of Cd, Co, Cu, Fe and Se followed lognormal distributions, whereas Mn, Mo and Zn tended towards normality (particularly in females).

Variation in both sexes was extensive for Cu (IQ ratio >130%), Cd and Se (IQ ratio >70%) (**Table 2**); however, males also exhibited considerable variation (IQ ratio >70%) in Co, Mn and Mo. Variation in Fe was similar between the sexes (IQ ratio ≈ 45%), whereas variation in Zn was smaller in females (IQ ratio 20%) than males (52%) (**Table 2**).

## 3.2 Geographic variation in red deer liver single element concentrations

Differences in median element concentrations between estates were striking in males for all elements except for Fe (**Fig 2**; **Table S4**). Moreover, (two to three-fold) differences between estates observed in males, were reflected by (two to five-fold) differences between estates in females in Cd, Se and to a lesser degree, Cu (**Fig 2**; **Table S4**). Spearman's ρ correlations between male and female median concentrations on each estate for Cd ($\rho = 0.93$, $p < 0.001$), Se ($\rho = 0.77$, $p = 0.021$) and Cu ($\rho = 0.70$, $p = 0.043$) enumerated these reflections and highlighted marked geographic disparities in the accumulation of these elements. No other significant correlations occurred between males and females.

### 3.2.1 Modelling geographic variation in single element concentrations in relation to *F. hepatica* infection and temporal variation

In females (n = 321), average liver Zn concentration was greater in individuals infected with *F. hepatica* (L-S means (95% CI), 111 (102, 108) µg/g) than non-infected individuals (L-S means (95% CI), 105 (106, 116) µg/g) (**Table S5**). In males (n = 394), no differences in average element concentrations were found between infected and non-infected individuals (**Table S5**). Models that were constructed using the larger dataset (male n = 435; female n = 352) (i.e., containing an additional 72 samples) revealed similar coefficient estimates (**Table**

**S6**). In both sets of models, accounting for temporal variation effectively diluted the magnitude of differences between estates (data shown in **Fig 2**); however, variation remained marked to the extent that at least two significantly distinct estates/groups of estates were identified in at least one sex for all eight elements (**Tables S5** and **S6**). Temporal variation was particularly striking in males, whereby average Mo and Mn concentrations halved during the stalking season (**Fig 3**).

## 3.3 Associations between elements: Principal Component Analysis

Principal component analysis reduced the complexity of our data from eight elements to three principal components that cumulatively explained ~60% of the variation in male and female element profiles. Moreover, two principal components (PC1 in females and PC2 in males) were analogous because they both described variation in Se, Cd and Cu (**Figs 4A** and **4C**); and, the same parallel could be drawn between PC2 in females and PC1 in males (both of which described variation in Mo, Mn and Zn; **Figs 4B** and **4D**). In both sexes, Fe was ostensibly independent from these groups, but in males was associated with Co through PC3 (**Fig 4D**). In females, Co associated with Cd, Cu and Se through PC1 (**Fig 4A**).

### 3.3.1 Modelling geographic variation in element profiles

Stratifying PC1 and PC2 data by estate revealed clusters of estates within which average element profiles were shared (**Figs 4E** and **4F**). After accounting for temporal effects, pairwise comparisons revealed that both sexes in Conaglen had significantly higher Cd-Cu-Se concentrations on average than Altnaharra, Ardnamurchan and Ben Loyal (**Table S7**). Moreover, Conaglen males also had significantly higher Mn-Mo-Zn concentrations than Altnaharra and Ben Loyal.

297 Variations in principal components were also related to the date on which animals were shot,
298 and a similar pattern emerged to the single element models. In males, this translated to an
299 increase in PC1 (which drives a decrease in liver Mo-Mn-Zn; **Table S7**) and an increase in
300 PC2 (which drives a decrease in liver Cd-Cu-Se); and, in females, PC1 increased (increasing
301 Cd-Cu-Se) and PC2 decreased (decreasing Mn-Mo-Zn) (**Table S7**). Reflecting single element
302 results for Cd and Cu, PC2 in males was greatest in 2013-14 (**Table S7**) and PC3 (Fe) in
303 females was greatest in 2012-13 (**Table S7**).

# 304 4.Discussion

305 This study is the first to have extensively quantified element concentrations in wild Scottish
306 Highland red deer livers; and, in so doing, has revealed marked geographic variation in liver
307 element concentrations between hunting estates (in both single element and element profiles).
308 Moreover, we have shown that temporal variation in sampling may substantially influence
309 estimates of average element concentrations in wild red deer. Whilst we have found only a
310 small effect of *F. hepatica* on female liver Zn concentrations, we cannot rule out further
311 effects (on other elements) in livers that are subjected to very heavy fluke burdens; however,
312 the severity of infection was not assessed in this study. Quantifying element profiles revealed
313 two groups of associated elements, and has provided a novel insight into overarching
314 differences between population element profiles. Our observations of some heterogeneity in
315 element concentrations in liver tissue is not great enough to invalidate our inferences
316 surrounding the most marked geographic variation at the population level (i.e., for
317 observations of several-fold differences between hunting estates). Nevertheless, this result
318 does suggests that trace element data should be interpreted with some caution at the
319 individual level if a whole liver average is not provided.

## 4.1 Liver element reference ranges for red deer and geographic and temporal variation in single element concentrations

Prior to our study, data concerning Scottish red deer trace element tissue concentrations were limited to two studies of Cu (data presented in **Table 3**); the largest of which was a comprehensive study of six areas on the Isle of Rum (McTaggart et al., 1981). Data for Rum were expressed as arithmetic means, which (based on the log-normal nature of liver Cu data) inflate their averages; yet, when 95% CIs are considered (**Tables 2** and **S4**), averages from Rum fall modestly within the Cu range for the majority of our study sites. Elsewhere in the literature, liver element data for red deer relate to three distinct environments: (i) relatively pristine (i.e., non-polluted), (ii) polluted (by heavy metal mining and/or agri-industrial activity), and (iii) on farm (**Table 3**). Before discussing our results in depth, it is important to reiterate that during both of our sampling seasons (2012-13 and 2013-14) males were always collected earlier in the year than females (owing to the hunting seasons for red deer in Scotland). As such, date culled and sex are collinear explanatory variables, so it is impossible to disentangle the effects of sex from sampling date. Consequently, direct comparisons in liver element concentrations between males and females are avoided in this discussion.

To our knowledge, Cu is the most widely measured trace element in red deer liver, and population average Cu concentrations are highly variable within countries; for example, in Norway, where municipality averages range from 21.1 to 118 µg/g d.w (Vikøren et al., 2005). Likewise, in the Scottish Highlands, we revealed significant variation between hunting estates, particularly for males (**Fig 2**; **Table S4**). As such, while Scottish male red deer have lower liver Cu overall than their counterparts in the "unpolluted" environments in the

majority of Norway, Poland, New Zealand and Spain, the majority of our study estates exhibit relatively unremarkable male liver Cu (**Tables 3 and S4**). Nevertheless, particularly low levels (< 15 µg/g d.w.), considered marginally deficient in farmed deer (Wilson and Grace, 2001), were found in males in Ardnamurchan and Badanloch. As such, this could (in extreme cases) translate to sub-clinical deficiency, which (for example) is purported in Norwegian deer on the island of Hitra (where liver Cu < 20 µg/g d.w. has been associated with reduced growth rates; (Handeland et al., 2017).

We found Se concentrations in Scottish red deer are higher on average than the vast majority of other red deer ranges. This may be explained by the high Se content in soils of the northwest Scottish Highlands (Shand et al., 2012). As such, even the estate from which we measured the lowest liver Se, Badanloch, had comparable Se concentrations to those in the literature (**Table 3**); indeed, only farmed New Zealand red deer have average Se concentrations significantly in excess of Badanloch. In terms of geographic variation, we found much greater variation in Se (up to three-fold differences between estates) within the Scottish Highlands (for both sexes) (**Table S4**) than has been documented for Norwegian red deer (**Table 3**).

Our data suggest Cd content in Scottish deer liver is lower on average than the majority of other wild populations (**Table 3**). As such, toxic effects of Cd (or indeed disturbances to hepatic storage of other elements, such as Cu and Zn; Durkalec et al., 2017) are unlikely in this region, but may occur in extreme cases. In addition, populations in Poland, Netherlands and Croatia typically have around two to three-fold greater liver concentrations of Cd than Scottish Highland deer, and the highest levels of Cd unsurprisingly associate with agri-industrial and mining areas (**Table 3**). From these findings, it appears that the Scottish Highlands is generally relatively pristine (regarding Cd pollution). Nevertheless, Conaglen

(particularly females) have remarkably high Cd concentrations that mirror more agri-industrially active and metal mining regions (**Tables 3** and S**4**). Whether Conaglen's high accumulation of Cd is a consequence of (i) feeding on particularly Cd rich ground, (ii) a high abundance of *Salix* spp. (which accumulate Cd, which (for example) results in higher concentrations of Cd in upland birds in comparison to lowland birds; Hogstad, 1996), or, (iii) is simply a reflection of culling practices (i.e., a focus on comparatively old females) is unclear. The potentially confounding effect of age is applicable for Cd (which has been found to accumulate in red deer liver; Wolkers et al., 1994). However, age related accumulation (and depletion) may also occur for other elements; for example, high metabolic demand for/depletion of Cu in calves and yearlings has been suggested as a factor underlying differences in liver Cu between age classes in Norwegian red deer (Vikøren et al., 2005). In the present study, we did not have a balanced study design that would have allowed us to estimate rates of accumulation/depletion robustly, even if we used hunter age classifications (**Table 1**). Nonetheless, the distribution of samples stratified by age category, and the similarities between estates, demonstrate that hunters' priorities for culling (of animals destined for human consumption) are at least consistent between our study sites. While this may not translate to consistent average age of culling (precise to a single year), our analyses using data only from independent animals does ensure calculation of representative population averages for "adult" herds.

In unpolluted environments, Zn content appears highly consistent regardless of other liver element concentrations (**Table 3**). Indeed, average liver Zn falls within a range of 70 to 110 µg/g d.w. for our study and all other studies in unpolluted environments (Durkalec et al., 2017; Falandysz et al., 2005; Jarzyńska and Falandysz, 2011; Lazarus et al., 2008; Reglero et al., 2009, 2008). Interestingly, we have revealed a distinction between males (at the lower end of this range) and females (at the upper), but, without sex specific data from other

studies, it is unclear whether this reflects physiological differences in Zn metabolism between males and females.

We found Co concentrations in the Scottish Highland red deer liver to be around two-fold lower than in Norway and NE Poland (**Table 3**), which to our knowledge are the only documented concentrations of Co in red deer. This lack of data is in part due to a focus on vitamin $B_{12}$ (as opposed to its constituent, Co). Despite the scarcity of data on this element, we note that our observation of ostensibly greater concentrations of liver Co in females than males is reflected by findings for red deer in Norway (Vikøren et al., 2005). It is unknown whether our observations relate truly to sex differences (as opposed to differences that reflect disparate sampling windows). In future, it might therefore be appropriate to quantify vitamin $B_{12}$ content, for which there are more comparable data (Clark et al., 1986; Grace and Wilson, 2002), and with which reproductive performance of farmed deer has been correlated (Audigé et al., 1999).

In terms of temporal variation, we observed steep declines in males (for Mn, Mo and Zn; **Fig 3**). Interestingly, when these declines are taken into account by our GLM/GLS models (**Tables S5** and **S6**), we find that it reduced the apparent marked geographic variation observed in males (**Fig 2**) to more closely resemble the less striking geographic variation seen in females. This lack of striking geographic variation suggests that these elements are in similar supply across the region. In addition, the tendency towards normal distributions of Mn and Zn and the relatively narrow interquartile ratio of Zn in particular (**Table 2**) may indicate that concentrations of these elements are controlled by physiological homeostatic mechanisms rather than local dietary limitation. It is tempting to speculate that dilution caused by increased liver volume (whereby fat deposits mobilised during the breeding season may swell the liver; Zomborszky and Husvéth, 2000) contributes to this decline. However,

owing to a lack of strong decline in other elements (**Tables S5** and **S6** and **Fig 3**), this does not seem to account for our observations. Depletion throughout the stalking season may more likely be indicative of physiological stress associated with breeding. Indeed, in males, breeding stresses could relate to cessation in appetite, which (for example) coincides with increased serum testosterone and depletion in serum Ca, Mg and P in farmed red deer (Kuba et al., 2015; Mysterud et al., 2008).

### 4.1.1 Liver fluke and liver element concentrations

Our observation of lower liver Zn in females infected with *F. hepatica* may have been assisted by the remarkably low variation in liver Zn for females (**Table 2**); i.e., small effects (such as the 6µg/g shift we observed) are inherently easier to detect. If so, the greater variation between individuals exhibited by other elements may indeed mask effects of *F. hepatica*. Given the scarcity of data for deer on this topic (and the marginal difference observed), it is debatable whether we have observed a true effect related to *F. hepatica* infection. If Zn depletion does indeed occur, the underlying physiological mechanism behind it may be related antioxidant Cu-Zn superoxide dismutase - the activity of which is reduced in rats experimentally infected with *F. hepatica* (Kolodziejczyk et al., 2006). Ultimately, our sampling design may have generally impeded our ability to detect the effects of fluke on element concentrations. We sampled from August to February (2012-13 and 2013-14), which coincides with the infection and maturation (and detectability) of *F. hepatica* in the UK. As such, collinearity between cull date and infection status may have increased our chances of type II error. To robustly assess the influence of *F. hepatica* on element concentrations, we therefore suggest sample collection during a shorter sampling window and validation of the extent of fluke burdens through inspection of a sub-sample of carcasses.

## 4.2 Associations between elements and possible factors associated with geographic variation

Two questions arise from the results of our principal component analysis. Firstly, the analogous principal component loadings between males and females (i.e., Cd-Cu-Se and Mn-Mo-Zn; **Fig 4**) tempt the question: What environmental and physiological factors drive the (co-)accumulation of trace elements? Secondly, **Fig 4**, which illustrates overlapping average estate element profiles, tempts the question: What environmental factors that influence element bioavailability are shared between the estates within these groups?

In terms of factors associated with (co-)accumulation of elements, the principal component loadings illustrated in **Fig 4**, are similar to those previously identified in Norwegian red deer (in terms of Cd-Cu-Se grouping; Egeland et al., 2005). Moreover, the Se-Cd-Cu group also reflects positive correlations found within livers of Spanish deer (between Cd-Se and Cu-Se ; Reglero et al., 2008). As such, it appears that red deer accumulate elements in similarly associated groups regardless of their environment. Simplistically, owing to similarities in environmental factors that affect the bioavailability of more than one element (particularly Eh-pH; Husson, 2012), we might therefore expect our observed loadings to reflect Eh-pH associated bioavailability; however, we find this not to be so simple. Here, four of our analytes ($Cd^{2+}$, $Co^{2+}$, $Mn^{2+}$ and $Zn^{2+}$) are theoretically bioavailable under similar Eh-pH conditions (**Fig S2**), but these do not reflect our PCA loadings (**Fig 4**). Likewise, Mo (as $MoO_4^{2-}$) appears available only under high pH conditions, yet it correlates strongly with Mn (**Fig S1**), which is more bioavailable under low pH (**Fig S2**). As such, it seems more likely that physiological (rather than environmental) factors drive the (co-)accumulation we have observed.

462 In terms of drivers of geographic variation, our study sites (**Fig 1**) can be differentiated in
463 terms of redox potential. Waterlogged (reducing) conditions dominate low lying areas and
464 areas covered by blanket bog (i.e., at Altnaharra, Ardnamurchan, Badanloch and to a lesser
465 extent Ben Loyal), whereas free-draining (oxidising) conditions dominate steep mountainous
466 areas (i.e., at Applecross, Conaglen, North Harris, and to a lesser extent Alladale and
467 Strathconon). As such, in terms of principal component analysis (**Fig 4**), it is interesting that
468 estates cluster roughly into groups that share soils of similar redox potential. Moreover, after
469 modelling temporal effects (**Table S7**), significant differences in Cd-Cu-Se remain for both
470 sexes between (montane podzol dominated) Conaglen, and the two most northerly (peat and
471 gley dominated) estates, Ben Loyal and Altnaharra. This result is in agreement with the
472 theory that the oxidising conditions in Conaglen will drive higher bioavailability of Cu and
473 Se (**Fig S2**) than the reducing conditions in Ben Loyal and Altnaharra, but does not explain
474 (co-)accumulation of Cd. As such, geographic variation in liver element concentrations of
475 Scottish Highland red deer is influenced to a substantial degree by local redox conditions;
476 but, correlations between (for example) Cd and Se and Cd and Cu, require further
477 investigation.

# 478 5. Conclusions

479 Ultimately, in the Scottish Highlands, wild red deer numbers are managed for deer stalking
480 and to manage impacts (e.g., on native forest regeneration). The underlying health of deer, in
481 part governed by dietary intake of essential elements, is therefore also partly dependent on
482 anthropogenic influence – i.e., through land management practices (fencing, for example)
483 access to essential elements may be restricted (or promoted) temporally and geographically.
484 As such, if wild deer health, welfare and performance is to be maximised in the region (for
485 multiple benefit), it is desirable to know if or where deer may be susceptible to element

deficiencies/toxicities, which may then have undesirable consequences for their health. This study has revealed that wild deer liver element concentrations are markedly varied across the Scottish Highlands, and, that this is probably driven by major differences in soil type/chemistry across the region. As such, those managing Scotland's wild deer should be aware that management practices (current and future) that restrict deer movement might affect wild red deer element accumulation and therefore have knock-on implications for health. Whilst we have found only weak evidence that liver fluke (*F. hepatica*) infection influences liver element concentrations in red deer at this time, we advocate continued monitoring in light of increasing incidences of *F. hepatica* in UK livestock (APHA, 2016; Skuce and Zadoks, 2014), which, in turn, may be reflected in wild deer. Finally, the results of this work begs two key questions: (i) what are the health implications for red deer (for example) relating to differences in Se, Cu and Cd concentrations between Scottish hunting estates?; and, (ii) what are the principal environmental (potentially management related) and physiological factors driving the geographic and within-tissue variations we have observed?

# 6. Acknowledgements

We would like to thank the game-keeping department of the North Highland College and the deerstalkers of: Alladale, Altnaharra, Aline, Applecross, Ardnamurchan, Badanloch, Ben Loyal, Conaglen, North Harris Trust and Strathconon estates for their diligent sample collection, without whom, this study would not have been possible.

# Author contributions

Conceived and designed the experiments: ASF, MAT. Performed laboratory analyses: ASF, MAT. Analysed and presented the data: ASF. Provided study resources: ASF, DS, SWG,

MAT. Wrote the paper: ASF, MAT. Supervised research activity: MAT, SWG, DS. Acquired funding: MAT.

# Funding sources

ASF's studentship was funded by the Highlands and Islands Scotland European Social Fund 2007-2013 programme. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

# Literature cited

Amrhein C, Strong JE, Mosher PA., 1992. Effect of deicing salts on metal and organic matter mobilization in roadside soils. Environmental Science & Technology 26:703–709. http://dx.doi.org/10.1021/es00028a006

APHA., 2016. GB Emerging Threats Quarterly Report: Small Ruminant Diseases - Oct - Dec 2016. Animal and Plant Health Agency: Penrith; 2016. URL: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/596314/pub-survrep-sr0416.pdf.

Audigé L, Wilson PR, Morris RS., 1999. Reproductive performance of farmed red deer (*Cervus elaphus*) in New Zealand. Animal Reproduction Science 55:239–254. http://dx.doi.org/10.1016/S0378-4320(99)00018-4

Baccarelli A, Pfeiffer R, Consonni D, Pesatori AC, Bonzini M, Patterson DG, Bertazzi PA, Landi MT., 2005. Handling of dioxin measurement data in the presence of non-detectable values: Overview of available methods and their application in the Seveso chloracne study. Chemosphere 60:898–906. http://dx.doi.org/10.1016/j.chemosphere.2005.01.055

Berzas Nevado JJ, Rodríguez Martín-Doimeadios RC, Mateo R, Rodríguez Fariñas N, Rodríguez-Estival J, Patiño Ropero MJ., 2012. Mercury exposure and mechanism of response in large game using the Almadén mercury mining area (Spain) as a case study. Environmental Research 112:58–66. http://dx.doi.org/10.1016/j.envres.2011.09.019

Clark RG, Burbage J, Marshall PM, Valler T, Wallace D., 1986. Absence of a vitamin B12 weight gain response in two trials with growing red deer (*Cervus elaphus*). New Zealand Veterinary Journal 34:199–201. http://dx.doi.org/10.1080/00480169.1986.35349

Cohen AC., 1976. Progressively Censored Sampling in the Three Parameter Log-Normal Distribution. Technometrics 18:99–103. http://dx.doi.org/10.2307/1267922

Cohen MA, Ryan PB., 1989. Observations Less than the Analytical Limit of Detection: A New Approach. JAPCA 39:328–329. http://dx.doi.org/10.1080/08940630.1989.10466534

Conradt L., 2000. Use of a seaweed habitat by red deer (*Cervus elaphus* L.). Journal of Zoology 250:541–549. http://dx.doi.org/10.1111/j.1469-7998.2000.tb00795.x

546 Custer T, Cox E, Gray B., 2004. Trace elements in moose (*Alces alces*) found dead in
547 Northwestern Minnesota, USA. Science of The Total Environment 330:81–87.
548 http://dx.doi.org/10.1016/j.scitotenv.2004.03.019

549 Dobrowolska A, Melosik M., 2008. Bullet-derived lead in tissues of the wild boar (Sus
550 scrofa) and red deer (Cervus elaphus). European Journal of Wildlife Research
551 54:231–235. http://dx.doi.org/10.1007/s10344-007-0134-y

552 Durkalec M, Kolenda R, Owczarek T, Szkoda J, Nawrocka A, Grzegrzółka J, Dzięgiel P, et
553 al., 2017. Expression of metallothionein in the liver and kidneys of the red deer
554 (*Cervus elaphus L.*) from an industrial metal smelting area of Poland. Ecotoxicology
555 and Environmental Safety 137:121–129.
556 http://dx.doi.org/10.1016/j.ecoenv.2016.11.018

557 Egeland B, Fimreite N, Rosef O., 2005. Liver Element Profiles of Red Deer with Special
558 Reference to Copper, and Biological Implications. vol. no. 2/2005. HiT Publication.
559 Telemark University College: Porsgrunn, Norway. ISBN:1503-3767

560 Falandysz J, Szymczyk–Kobrzyńska K, Brzostowski A, Zalewski K, Zasadowski A., 2005.
561 Concentrations of heavy metals in the tissues of red deer (*Cervus elaphus*) from the
562 region of Warmia and Mazury, Poland. Food Additives & Contaminants 22:141–149.
563 http://dx.doi.org/10.1080/02652030500047273

564 Flueck WT., 1994. Effect of Trace Elements on Population Dynamics: Selenium Deficiency
565 in Free-Ranging Black-Tailed Deer. Ecology 75:807–812

566 Frank A., 1998. "Mysterious" moose disease in Sweden. Similarities to copper deficiency
567 and/or molybdenosis in cattle and sheep. Biochemical background of clinical signs
568 and organ lesions. Science of The Total Environment 209:17–26

569 Frank A, McPartlin J, Danielsson R., 2004. Nova Scotia moose mystery – a moose sickness
570 related to cobalt- and vitamin B12 deficiency. Science of The Total Environment
571 318:89–100. http://dx.doi.org/10.1016/S0048-9697(03)00374-7

572 French AS, Zadoks RN, Skuce PJ, Mitchell G, Gordon-Gibbs DK, Craine A, Shaw D, Gibb
573 SW, Taggart MA., 2016. Prevalence of Liver Fluke (*Fasciola hepatica*) in Wild Red
574 Deer (*Cervus elaphus*): Coproantigen ELISA is a Practicable Alternative to Faecal
575 Egg Counting for Surveillance in Remote Populations. PLOS ONE 11:e0162420.
576 http://dx.doi.org/10.1371/journal.pone.0162420

577 Gamberg M, Palmer M, Roach P., 2005. Temporal and geographic trends in trace element
578 concentrations in moose from Yukon, Canada. Science of The Total Environment
579 351–352:530–538. http://dx.doi.org/10.1016/j.scitotenv.2004.05.033

580 George TS, French AS, Brown LK, Karley AJ, White PJ, Ramsay L, Daniell TJ., 2014.
581 Genotypic variation in the ability of landraces and commercial cereal varieties to
582 avoid manganese deficiency in soils with limited manganese availability: is there a
583 role for root-exuded phytases? Physiologia Plantarum 151:243–256.
584 http://dx.doi.org/10.1111/ppl.12151

585 Grace N, Wilson P., 2002. Trace element metabolism, dietary requirements, diagnosis and
586 prevention of deficiencies in deer. New Zealand Veterinary Journal 50:252–259.
587 http://dx.doi.org/10.1080/00480169.2002.36321

588 Handeland K, Viljugrein H, Lierhagen S, Opland M, Tarpai A, Vikøren T., 2017. Low
589 Copper Levels Associated with Low Carcass Weight in Wild Red Deer (*Cervus*
590 *elaphus*) in Norway. Journal of Wildlife Diseases 53:176–180.
591 http://dx.doi.org/10.7589/2016-02-037

592 Helsel DR, Hirsch RM., 1992. Methods for Data Below the Reporting Limit. Statistical
593 Methods in Water Resources. Studies in environmental science 49. Elsevier:
594 Amsterdam ; New York. pp. 357–376. ISBN:978-0-444-88528-9

595 Hogstad O., 1996. Accumulation of cadmium, copper and zinc in the liver of some passerine
596 species wintering in Central Norway. Science of The Total Environment 183:187–
597 194. http://dx.doi.org/10.1016/0048-9697(95)05060-4

598 Husson O., 2012. Redox potential (Eh) and pH as drivers of soil/plant/microorganism
599 systems: a transdisciplinary overview pointing to integrative opportunities for
600 agronomy. Plant and Soil 362:389–417. http://dx.doi.org/10.1007/s11104-012-1429-7

601 Jarzyńska G, Falandysz J., 2011. Selenium and 17 other largely essential and toxic metals in
602 muscle and organ meats of Red Deer (*Cervus elaphus*) — Consequences to human
603 health. Environment International 37:882–888.
604 http://dx.doi.org/10.1016/j.envint.2011.02.017

605 Kuba J, Błaszczyk B, Stankiewicz T, Skuratko A, Udała J., 2015. Analysis of Annual
606 Changes in the Concentrations of Selected Macro- and Microelements, Thyroxine,
607 and Testosterone in the Serum of Red Deer (*Cervus elaphus*) Stags. Biological Trace
608 Element Research:1–6. http://dx.doi.org/10.1007/s12011-015-0368-6

609 Lazarus M, Orct T, Blanuša M, Vicković I, šoštarić B., 2008. Toxic and essential metal
610 concentrations in four tissues of red deer (*Cervus elaphus*) from Baranja, Croatia.
611 Food Additives & Contaminants: Part A 25:270–283.
612 http://dx.doi.org/10.1080/02652030701364923

613 Lenth R., 2016. Least-Squares Means: The R Package lsmeans. Journal of Statistical
614 Software 69:1–33. http://dx.doi.org/10.18637/jss.v069.i01

615 Manjusha R, Dash K, Karunasagar D., 2007. UV-photolysis assisted digestion of food
616 samples for the determination of selenium by electrothermal atomic absorption
617 spectrometry (ETAAS). Food Chemistry 105:260–265.
618 http://dx.doi.org/10.1016/j.foodchem.2006.11.011

619 McTaggart HS, Lowe VP, Barden PJ, Gellatly JB., 1981. Copper status of red deer on the
620 island of Rhum. The Veterinary Record 109:155–157

621 Murphy CJ, MacNeil JD, Capar SG., 2013. Best Practices for Single-Laboratory Validation
622 of Chemical Methods for Trace Elements in Foods. Part I—Background and General
623 Considerations. Journal of AOAC International 96:190–203.
624 http://dx.doi.org/10.5740/jaoacint.12-204

625 Murray DL, Cox EW, Ballard WB, Whitlaw HA, Lenarz MS, Custer TW, Barnett T, Fuller
626 TK., 2006. Pathogens, Nutritional Deficiency, and Climate Influences on a Declining
627 Moose Population. Wildlife Monographs:1–30. http://dx.doi.org/10.2193/0084-
628 0173(2006)166[1:PNDACI]2.0.CO;2

629 Mysterud A, Bonenfant C, Loe LE, Langvatn R, Yoccoz NG, Stenseth NC., 2008. Age-
630 specific feeding cessation in male red deer during rut. Journal of Zoology 275:407–
631 412. http://dx.doi.org/10.1111/j.1469-7998.2008.00453.x

632 Palarea-Albaladejo J, Martín-Fernández J.A., 2013. Values below detection limit in
633 compositional chemical data. Analytica Chimica Acta 764:32–43.
634 http://dx.doi.org/10.1016/j.aca.2012.12.029

635 Palarea-Albaladejo J, Martín-Fernández JA., 2015. zCompositions — R package for
636 multivariate imputation of left-censored data under a compositional approach.
637 Chemometrics and Intelligent Laboratory Systems 143:85–96.
638 http://dx.doi.org/10.1016/j.chemolab.2015.02.019

639 Patiño Ropero MJ, Rodríguez Fariñas N, Mateo R, Berzas Nevado JJ, Rodríguez Martín-
640 Doimeadios RC., 2016. Mercury species accumulation and trophic transfer in
641 biological systems using the Almadén mining district (Ciudad Real, Spain) as a case
642 of study. Environmental Science and Pollution Research 23:6074–6081.
643 http://dx.doi.org/10.1007/s11356-015-4989-2

644 Pineau A, Guillard O, Chappuis P, Arnaud J, Zawislak R, Lockltch Q., 1993. Sampling
645 conditions for biological fluids for trace elements monitoring in hospital patients: a
646 critical approach. Critical Reviews in Clinical Laboratory Sciences 30:203–222

647 R Core Team., 2012. R: A Language and Environment for Statistical Computing. R
648 Foundation for Statistical Computing: Vienna, Austria. ISBN:3-900051-07-0

649 Reglero MM, Monsalve-González L, Taggart MA, Mateo R., 2008. Transfer of metals to
650 plants and red deer in an old lead mining area in Spain. Science of The Total
651 Environment 406:287–297. http://dx.doi.org/10.1016/j.scitotenv.2008.06.001

652 Reglero MM, Taggart MA, Monsalve-González L, Mateo R., 2009. Heavy metal exposure in
653 large game from a lead mining area: Effects on oxidative stress and fatty acid
654 composition in liver. Environmental Pollution 157:1388–1395.
655 http://dx.doi.org/10.1016/j.envpol.2008.11.036

656 Rieuwerts JS, Thornton I, Farago ME, Ashmore MR., 1998. Factors influencing metal
657 bioavailability in soils: preliminary investigations for the development of a critical
658 loads approach for metals. Chemical Speciation and Bioavailability 10:61–75.
659 http://dx.doi.org/10.3184/095422998782775835

660 RStudio Team., 2015. RStudio: Integrated Development for R. Boston, MA

661 Shand CA, Eriksson J, Dahlin AS, Lumsdon DG., 2012. Selenium concentrations in national
662 inventory soils from Scotland and Sweden and their relationship with geochemical
663 factors. Journal of Geochemical Exploration 121:4–14.
664 http://dx.doi.org/10.1016/j.gexplo.2012.06.001

665 Skuce PJ, Zadoks RN., 2014. Liver fluke - A growing threat to UK livestock production.
666 Cattle Practice 21:138–149

667 Vengušt G, Klinkon M, Bidovec A, Vengušt A., 2003. *Fasciola hepatica*: effects on blood
668 constituents and liver minerals in fallow deer (*Dama dama*). Veterinary Parasitology
669 112:51–61. http://dx.doi.org/10.1016/S0304-4017(02)00413-2

670 Vikøren T, Bernhoft A, Waaler T, Handeland K., 2005. Liver concentrations of copper,
671 cobalt, and selenium in wild Norwegian red deer (*Cervus elaphus*). Journal of
672 Wildlife Diseases 41:569–579

673 Vikøren T, Kristoffersen AB, Lierhagen S, Handeland K., 2011. A comparative study of
674 hepatic trace element levels in wild moose, roe deer, and reindeer from Norway.
675 Journal of Wildlife Diseases 47:661–672

676 Wilson PR, Grace ND., 2001. A review of tissue reference values used to assess the trace
677 element status of farmed red deer (*Cervus elaphus*). New Zealand Veterinary Journal
678 49:126–132. http://dx.doi.org/10.1080/00480169.2001.36219

679 Wolkers H, Wensing T, Groot Bruinderink GW., 1994. Heavy metal contamination in organs
680 of red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*) and the effect on some trace
681 elements. Science of The Total Environment 144:191–199

682 Zomborszky Z, Husvéth F., 2000. Liver total lipids and fatty acid composition of shot red and
683 fallow deer males in various reproduction periods. Comparative Biochemistry and
684 Physiology. Part A, Molecular & Integrative Physiology 126:107–114

# Tables

**Table 1. Number of liver samples collected from red deer (*Cervus elaphus*) collected on nine hunting estates in the Scottish Highlands during the 2012-13 and 2013-14 stalking seasons, stratified by age group (assigned by hunters) and estate.** Numbers in parentheses show the number of samples for which corresponding faecal samples were tested for the presence/absence of liver fluke (*Fasciola hepatica*) antigens by coproantigen ELISA.

| Estate | Sex | Calves | Yearlings | Young | Mature | Old | †Total |
|---|---|---|---|---|---|---|---|
| Alladale | Male | 0 | 0 | **18 (14)** | **51 (46)** | **9 (9)** | **78 (69)** |
| | Female | 25 (24) | **6 (4)** | **21 (19)** | **31 (28)** | **10 (9)** | **68 (60)** |
| Altnaharra | Male | 0 | 0 | **3 (3)** | **55 (51)** | **5 (5)** | **63 (59)** |
| | Female | 0 | **5 (5)** | **6 (6)** | **53 (51)** | **1 (1)** | **65 (63)** |
| Applecross | Male | 0 | 0 | **2 (2)** | **55 (51)** | **6 (5)** | **63 (58)** |
| | Female | 4 (4) | 0 | **3 (3)** | **15 (15)** | **7 (6)** | **25 (24)** |
| Ardnamurchan | Male | 1 (1) | 0 | 0 | **43 (40)** | **2 (2)** | **45 (42)** |
| | Female | 14 (13) | **8 (6)** | **4 (3)** | **14 (12)** | **0** | **26 (21)** |
| Badanloch | Male | 1 (0) | 0 | **1 (1)** | **42 (40)** | **12 (12)** | **55 (53)** |
| | Female | 5 (5) | **3 (3)** | **6 (6)** | **47 (46)** | **3 (3)** | **59 (58)** |
| Ben Loyal | Male | 0 | 13 (12) | **18 (17)** | **22 (21)** | **3 (3)** | **43 (41)** |
| | Female | 6 (6) | **1 (1)** | **9 (9)** | **29 (29)** | **1 (1)** | **40 (40)** |
| Conaglen | Male | 0 | 0 | **1 (0)** | **31 (24)** | **15 (11)** | **47 (35)** |
| | Female | 0 | 0 | **1 (1)** | **28 (20)** | **6 (4)** | **35 (25)** |
| NHT and Aline | Male | 0 | 0 | **3 (3)** | **7 (7)** | **0** | **10 (10)** |
| | Female | 3 (3) | 0 | **5 (5)** | **6 (6)** | **0** | **11 (11)** |
| Strathconon | Male | 0 | 0 | 0 | **23 (20)** | **8 (7)** | **31 (27)** |
| | Female | 9 (9) | **1 (1)** | **4 (4)** | **17 (13)** | **1 (1)** | **23 (19)** |
| | | | | | | Male | **435 (394)** |
| | | | | | | Female | **352 (321)** |
| | | | | | | Total | **787 (715)** |

†Totals show the number of independent individuals in sex specific groups; i.e., (i) males categorised as young, mature and old, and (ii) females categorised as yearling, young, mature and old.

**Table 2. Concentrations (µg/g dry weight) of cadmium (Cd) and seven essential trace elements (cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn)) measured in liver samples (n = 770) of red deer (*Cervus elaphus*) collected on nine hunting estates in the Scottish Highlands during the male (1st July to 20th October) and female (21st October to 15th February) hunting seasons during 2012-13 and 2013-14.**

| | Male (n = 435) | | | | Female (n = 352) | | | |
|---|---|---|---|---|---|---|---|---|
| | Median (95% CI) | Mean (95% CI) | Range | IQ ratio (%) | Median (95% CI) | Mean (95% CI) | Range | IQ ratio (%) |
| Cd | 0.238 (0.216, 0.257) | 0.312 (0.284, 0.343) | 0.0275 - 4.68 | 97.9 | 0.276 (0.254, 0.302) | 0.460 (0.372, 0.569) | 0.0376 - 10.4 | 99.0 |
| Co | 0.093 (0.0839, 0.0998) | 0.101 (0.0962, 0.106) | <0.0622 - 0.498 | 75.1 | 0.152 (0.144, 0.159) | 0.160 (0.153, 0.167) | <0.0622 - 0.735 | 47.4 |
| Cu | 28.7 (25.4, 33.0) | 44.2 (39.8, 48.2) | 2.35 - 309 | 160 | 51.0 (45.1, 59.5) | 66.5 (61.2, 72.1) | 6.42 - 306 | 133 |
| Fe | 457 (441, 473) | 506 (482, 532) | 129 - 3550 | 44.2 | 565 (543, 586) | 653 (607, 703) | 207 - 6350 | 45.7 |
| Mn | 9.29 (8.56, 10.1) | 9.54 (9.12, 9.94) | 2.00 - 23.0 | 74.2 | 14.6 (14.1, 15.1) | 14.9 (14.6, 15.3) | 6.85 - 27.5 | 31.7 |
| Mo | 2.06 (1.89, 2.20) | 2.14 (2.05, 2.24) | 0.404 - 4.84 | 80.8 | 2.66 (2.60, 2.72) | 2.69 (2.63, 2.76) | 1.30 - 5.35 | 32.4 |
| Se | 0.613 (0.561, 0.648) | 0.682 (0.645, 0.719) | <0.277 - 3.18 | 77.3 | 0.654 (0.587, 0.716) | 0.822 (0.753, 0.887) | <0.277 - 3.88 | 98 |
| Zn | 74.7 (71.5, 79.1) | 78.1 (75.2, 81.0) | 25.6 - 282 | 53.3 | 106 (104, 109) | 107 (105, 110) | 55.2 - 344 | 20.2 |

[a]Interquartile ratio (IQ ratio) provides a relative measure of data dispersion between elements, and for each element is defined as the interquartile range divided by the median, all multiplied by 100.

[b]95% CI (confidence intervals) were calculated using a bootstrap resampling (percentile) procedure.

**Table 3. Element concentrations (cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn)) in liver of red deer sampled in polluted, ostensibly non-polluted, and farm environments**.

| | Location (number of sites) | [ε]Sex | n | Cd (95% CI) | Co (95% CI) | Cu (95% CI) | Mn (95% CI) | Se (95% CI) | Zn (95% CI) | [α]Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| **Non-polluted** | NW Scotland (9) | M | 427 | 0.238 (0.216, 0.257) | 0.0926 (0.0836, 0.0990) | 28.7 (25.4, 33.8) | 9.34 (8.7, 10.1) | 0.613 (0.561, 0.650) | 75.7 (71.6, 79.6) | This study [Me DW] |
| | | F | 343 | 0.276 (0.255, 0.310) | 0.152 (0.145, 0.160) | 51.0 (45.1, 59.9) | 14.7 (14.1, 15.1) | 0.649 (0.583, 0.707) | 106 (104, 108) | |
| | W Scotland (6) | M | 87 | | | 53.3 (43.6, 63.0) | | | | (McTaggart et al., 1981) [M DW] |
| | | F | 99 | | | 49.5 (41.2, 57.8) | | | | |
| | Scotland (10) | F | 31 | | | 11.8 (3.70, 19.9) | | | | (Cowie, 1976) [M DW] |
| | SW Norway (5) (Adult only) | MF | 112 | | 0.260 (0.248, 0.272) | 95.7 (82.9, 109) | | 0.330 (0.263, 0.397) | | (Vikøren et al., 2005) [Me WW] |
| | Hitra (various ages) | MF | 45 | | 0.264 (0.252, 0.276) | 21.1 (13.8, 28.0) | | 0.363 (0.290, 0.436) | | |
| | Eid (various ages) | MF | 49 | | 0.231 (0.219, 0.243) | 56.1 (45.7, 66.0) | | 0.231 (0.219, 0.243) | | |
| | Gaular (various ages) | MF | 47 | | 0.264 (0.252, 0.276) | 69.3 (57.1, 82.0) | | 0.363 (0.339, 0.387) | | |
| | Hareid (various ages) | MF | 52 | | 0.264 (0.252, 0.276) | 75.9 (63.7, 88.0) | | 0.330 (0.251, 0.409) | | |
| | Namos (various ages) | MF | 52 | | 0.330 (0.318, 0.342) | 118.8 (104.1, 133) | | 0.264 (0.246, 0.282) | | |
| | NE Croatia(1) | MF | 52 | 0.449 (0.332, 0.565) | | 21.6 (8.03, 35.2) | | [γ]0.380 (0.0370, 0.723) | 93.1 (84.0, 102) | (Lazarus et al., 2008) [Me WW] |
| | NE Croatia (4) | | | | | | | | | (Bilandžić et al., 2009) [M WW] |
| | Virovitica–Podravina | ? | 12 | 0.627 (0.426, 0.828) | | | | | | |
| | Požega–Slavonia | ? | 9 | 0.495 (0.146, 0.844) | | | | | | |
| | Osijek–Baranya | ? | 13 | 0.363 (0.247, 0.479) | | | | | | |
| | Vukovar–Srijem | ? | 11 | 0.660 (0.291, 1.029) | | | | | | |
| | NE Poland (1) | M | 36 | 0.759 (0.554, 0.964) | | 49.5 (40.9, 58.1) | | | 99.0 (93.6, 104) | (Falandysz et al., 2005) [M WW] |
| | | F | 29 | 0.660 (0.492, 0.828) | | 59.4 (52.2, 66.6) | | | 99.0 (91.8, 106) | |
| | NW Poland (1) | M | 10 | | | | | 0.180 (0.0183, 0.342) | | (Pilarczyk et al., 2011) [Me WW] |
| | | F | 13 | | | | | 0.170 [β](ND, 0.345) | | |
| | NE Poland (1) | MF | 20 | 0.550 (0.379, 0.721) | 0.180 (0.156, 0.204) | 49.0 (31.0, 67.0) | 11.0 (9.29, 12.7) | 0.190 (0.146, 0.234) | 100 (90.8, 109) | (Jarzyńska and Falandysz, 2011) [Me DW] |
| | NE Poland (control) (1) | MF | 9 | 0.310 [#](0.182, 0.538) | | 59.1 [#](6.24, 120) | | | 86.8 [#](59.5, 121) | (Durkalec et al., 2017) [Me WW] |
| | New Zealand (various) | MF | 52 | | | 164 (144, 184) | | 0.410 (0.337, 0.483) | | (Tremain-Boon et al., 2002) [M WW] |
| | S Spain (control) (9) | MF | 74 | 0.210 (0.186, 0.237) | | 35.8 (29.3, 43.7) | | 0.162 (0.144, 0.182) | 90.1 (87.2, 92.9) | (Reglero et al., 2009) (M:F ≈ 3.5:1) [GM DW] |
| | S Spain (control)(2) | | | | | | | | | (Reglero et al., 2008) [M WW] |
| | Hornias Bajas | ? | 18 | 0.249 (0.206, 0.292) | | 47.3 (34.8, 60.0) | | 0.193 (0.146, 0.240) | 89.0 (83.7, 94.3) | |
| | Torneros | ? | 20 | 0.226 (0.165, 0.287) | | 55.9 (42.4, 69.0) | | 0.200 (0.190, 0.210) | 95.4 (90.3, 100.5) | |
| | Italy (2) | ? | 201 | 0.231 (0.208, 0.254) | | | | | | (Chiari et al., 2015) [M WW] |
| **Polluted** | Spain (Pb mines) (10) | MF | 87 | 0.274 (0.226, 0.333) | | 61.4 (51.4, 73.2) | | 0.233 (0.208, 0.261) | 91.5 (86.0, 97.3) | (Reglero et al., 2009) (M:F ≈ 3.5:1) [GM DW] |
| | Spain (Pb mines) (2) | | | | | | | | | (Reglero et al., 2008) [M WW] |
| | Minas de Horcajo | ? | 12 | 0.554 (0.378, 0.730) | | 50.8 (35.5, 66.0) | | 0.327 (0.262, 0.392) | 89.9 (82.3, 97.5) | |
| | Navalmartina | ? | 17 | 0.463 (0.328, 0.598) | | 51.7 (33.9, 70.0) | | 0.224 (0.173, 0.275) | 97.6 (82.3, 112.9) | |
| | S Poland (Zn smelter) (1) | MF | 13 | 2.77 [#](0.653, 5.58) | | 4.13 [#](0.528, 14.3) | | | 35.5 [#](20.2, 48.2) | (Durkalec et al., 2017) [Me WW] |
| | E Netherlands (agri-industrial) (1) | ? | 19 | 0.870 (0.586, 1.15) | | 73.0 (47.1, 98.9) | | | 108 (95.9, 120) | (Wolkers et al., 1994) [Me DW] |
| | NW Poland (agri-industrial) (1) | M | 25 | 0.422 (0.314, 0.530) | | 10.4 (6.22, 14.5) | | | 46.4 (36.6, 56.2) | (Wieczorek-Dąbrowska et al., 2013) [Me DW] |
| **Farmed** | E Netherlands (1) | F | 6 | 0.212 (0.158, 0.266) | | | | | | (Wolkers et al., 1994) (female) [Me DW] |
| | New Zealand (various) | MF | 55 | | | 49.7 (30.3, 69.1) | | 0.520 (0.449, 0.591) | | (Tremain-Boon et al., 2002) (both) [M WW] |

95% Confidence intervals (95% CI) were constructed using bootstrap resampling (percentile) procedure for the present study, but were calculated using mean/median/GM mean ± 1.96 × standard errors provided by cited studies.

[α]Reference data averages are indicated by superscripts: mean ([M]), median, ([Me]), geometric mean ([GM]), and sources that were converted from wet weight to dry weight by multiplying by 3.3 are also indicated by superscripts ([WW]).

[β]ND indicates confidence limit below the instrument detection limit (i.e., not detectable).

[ε]Symbol (?) is used where sex is not clearly stated in a reference.

[γ]Twenty liver samples were used to calculate this average.

[#]Range provided (not 95% CI).

# Figures

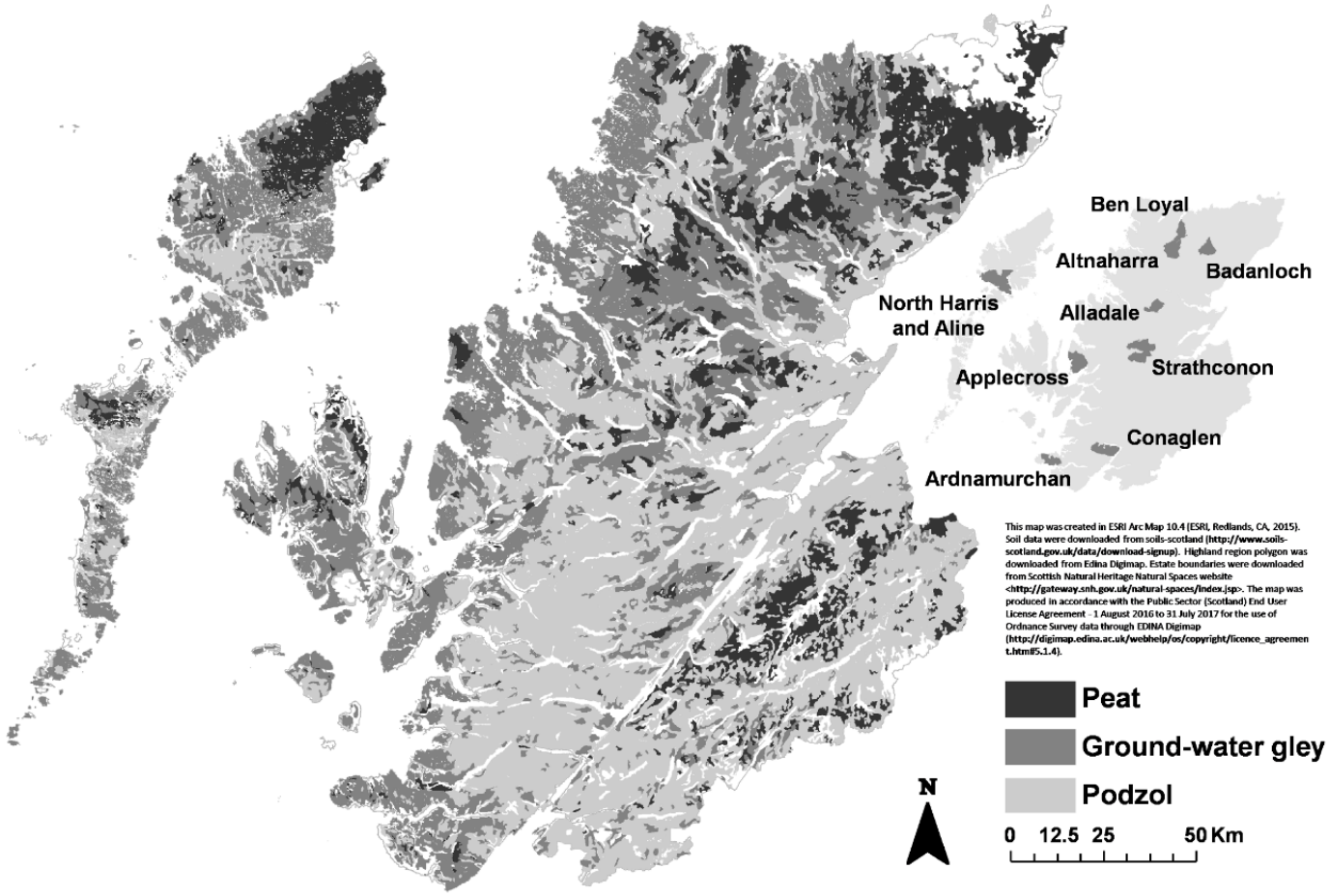


**Fig 1**. **Map of the Scottish Highlands showing the three dominant soil types present in our study sites**. The inset map shows the locations of the nine participating hunting estates.

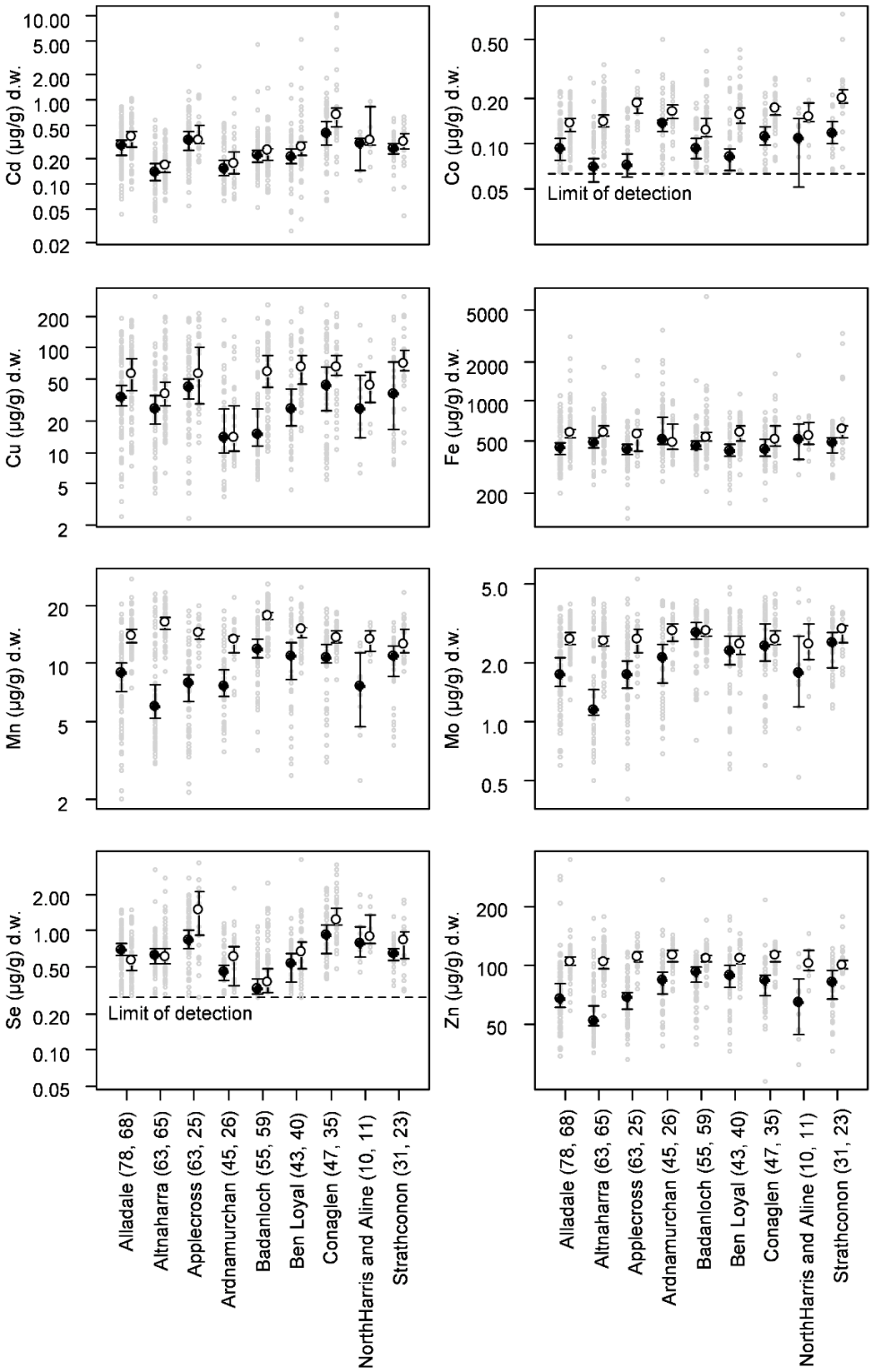


**Fig 2. Median liver element concentrations in red deer (*Cervus elaphus*) on nine hunting estates in the Scottish Highlands.** Filled (black) circles represent males, and unfilled (white) circles represent females. Vertical lines represent 95% bootstrap resampled confidence intervals for each median. Light grey circles illustrate all measured concentrations. Number of sampled males and females are indicated in parentheses ($N_{Male}$, $N_{Female}$). Note, however that the extent of geographic variation for Mn, Mo and Zn for males illustrated in this figure decreases when temporal variation is accounted for by generalised linear/least square models that include sampling/cull date as an explanatory variable.

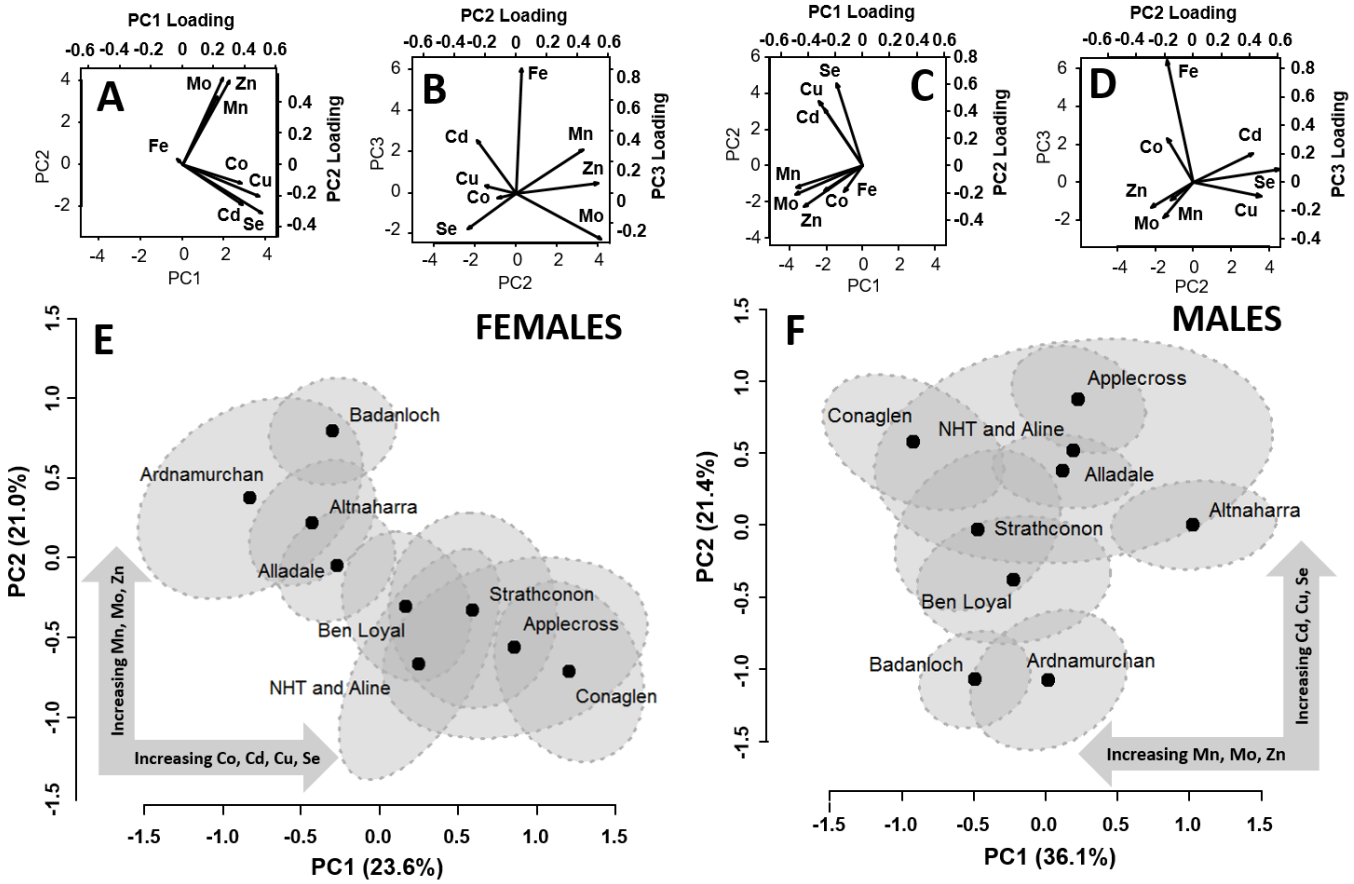


**Fig 3. Principal component analysis for liver element profiles in male and female Scottish red deer.** Loadings (A), (B), (C) and (D) illustrate associations between elements in red deer liver tissue. In females, there are two groups i) Cd, Se, Cu and Co, ii) Mn, Mo and Zn, and apparent independence of Fe. In males, there are three groups: i) Cd, Cu and Se ii) Mn, Mo and Zn, and iii) a weak association between Fe and Co. For females (E) and males (F) in PC1-PC2 space, mean and 95% (s.e. C.I.) ellipses for liver element profiles for each estate illustrate differences in population average element profiles.

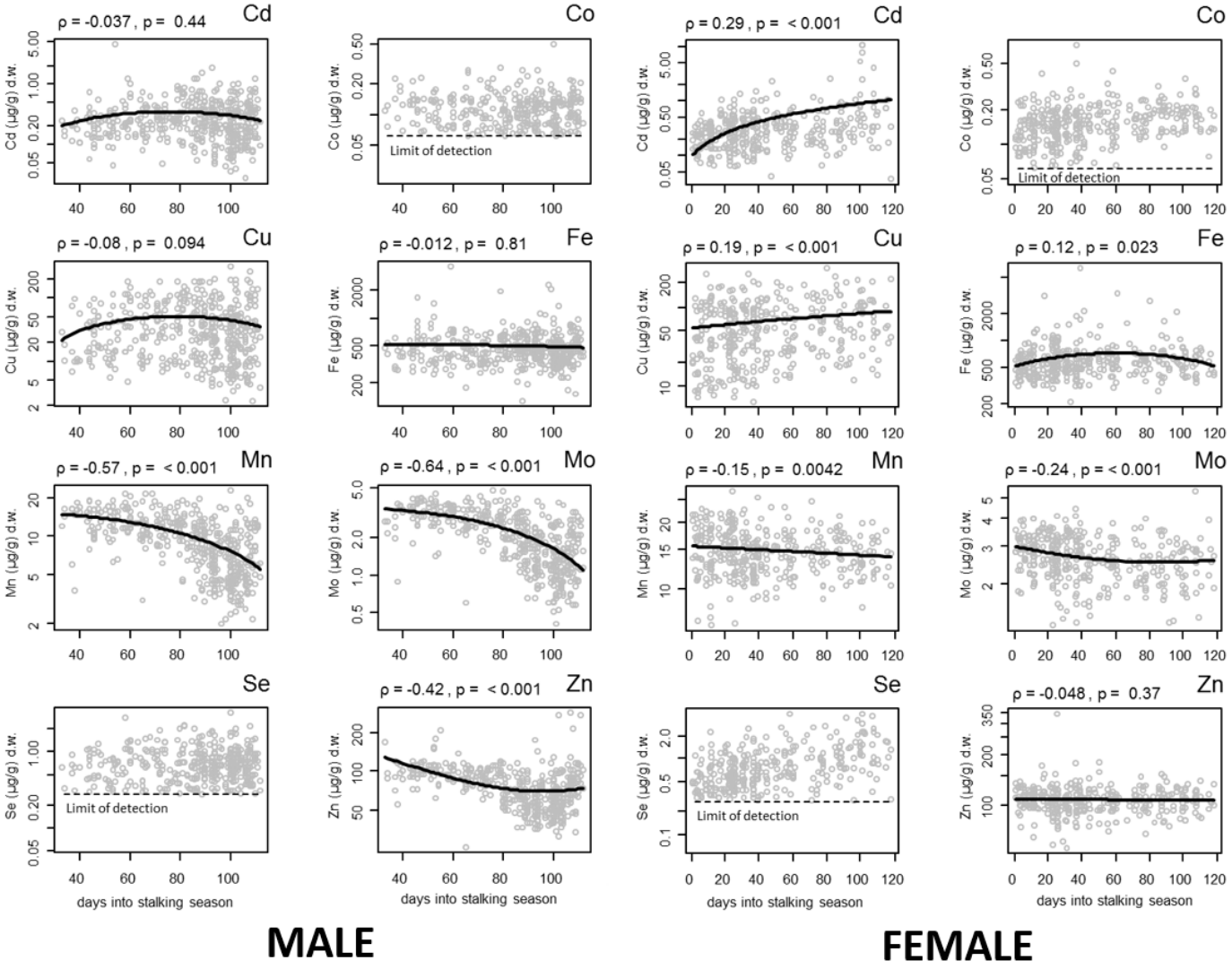


**Fig 4. Temporal trends in element concentrations (cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn)) in liver samples collected from nine hunting estates in the Scottish Highlands during the male (n = 435) and female (n = 352) red deer (*Cervus elaphus*) stalking seasons (2012-13 and 2013-14).** The stalking season for males runs from 1st July to 20th October; the stalking season for females runs from 21st October to 15th February. Note all vertical axes are on the logarithmic scale and correlation coefficients are Spearman's ρ. Illustrative quadratic regression lines (males: Cd, Cu, Fe, Mn, Mo, Zn; females: Fe and Mo) and linear regression lines (males: Co, Se; females: Cd, Co, Cu, Mn, Se, Zn) (created using the LOESS smoothing function in R) do not show model coefficients.

# Supplementary materials

**Geochemical landscapes as drivers of trace and toxic element profiles in wild red deer (*Cervus elaphus*)**

Andrew S French*[1], David Shaw[2], Stuart W Gibb[1], Mark A Taggart[1]

[1]Environmental Research Institute, North Highland College, University of the Highlands and Islands, Castle Street, Thurso, KW14 7JD, UK

[2]UHI Rural Studies Centre, North Highland College, University of the Highlands and Islands, Dale Farm, Halkirk, KW12 6UW, UK

*Corresponding author: Andrew S French; Email: andrewsamuelfrench@gmail.com

Phone: +44 1847 889578

**Table S1: Quality control information for eight elements measured (using ICP-OES) in three certified reference materials used to validate element concentrations measured in red deer liver samples.** Limits of detection and quantification were calculated from 174 procedural blanks. Recoveries were calculated from three CRMs: Bovine Liver BCR®-285R, n = 66; Dogfish Liver NRC-CNRC DOLT-4, n = 56; and Lobster Hepatopancreas NRC-CNRC TORT- 2, n = 46.

| | Bovine Liver BCR®-285R | | | | Dogfish Liver NRC-CNRC DOLT-4 | | | | Lobster Hepatopancreas NRC-CNRC TORT- 2 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Emission line** | **Measured** | **Cert.** | **Recov.** | **RSD** | **Measured** | **Cert.** | **Recov.** | **RSD** | **Measured** | **Cert.** | **Recov.** | **RSD** | **LOQ** | **% < LOQ** | **LOD** | **% < LOD** |
| Cd 214.439 | 0.54 | 0.54 | 99.25 | 3.75 | 23.55 | 24.30 | 96.92 | 2.52 | 25.95 | 26.70 | 97.19 | 1.58 | 0.06 | 5.43 | 0.02 | 0.00 |
| Co 228.615 | 0.27 | | | 7.56 | 0.25 | | | 11.19 | 0.48 | 0.51 | 94.92 | 3.93 | 0.21 | 90.65 | 0.06 | 13.90 |
| Cu 327.395 | 279.13 | 277.00 | 100.77 | 5.40 | 33.90 | 31.20 | 108.67 | 9.85 | 106.13 | 101.07 | 100.12 | 1.61 | 0.73 | 0.00 | 0.25 | 0.00 |
| Fe 259.940 | 189.74 | | | 9.99 | 1792.19 | 1833.00 | 97.77 | 3.13 | 103.44 | 105.00 | 98.51 | 5.64 | 3.93 | 0.00 | 1.38 | 0.00 |
| Mn 259.372 | 11.86 | 11.07 | 107.17 | 3.42 | 12.63 | | | 4.00 | 13.45 | 13.60 | 101.12 | 2.12 | 1.80 | 0.00 | 0.55 | 0.00 |
| Mo 202.032 | 4.03 | | | 4.33 | 1.02 | | | 5.74 | 0.96 | 0.95 | 101.16 | 2.86 | 0.26 | 0.00 | 0.09 | 0.00 |
| Se 196.026 | 1.53 | 1.68 | 91.12 | 7.93 | 7.78 | 8.30 | 93.76 | 3.29 | 5.55 | 5.63 | 98.56 | 2.51 | 0.89 | 71.81 | 0.28 | 13.37 |
| Zn 202.548 | 143.13 | 138.60 | 103.27 | 4.04 | 123.57 | 116.00 | 106.52 | 2.34 | 185.66 | 180.00 | 103.15 | 1.61 | 3.63 | 0.00 | 1.27 | 0.00 |

Element concentrations are expressed in µg/g. Recoveries and relative standard deviations (RSD) are expressed in %.

**Table S2: Element homogeneity within red deer (*Cervus elaphus*) liver samples collected on nine hunting estates in the Scottish Highlands during August to February 2012-13 and 2013-14.** Mean %RSD was calculated from %RSDs from six whole livers (three females, two from Altnaharra and one from Badanloch; and three males, all from Badanloch) – each tested at five locations on the liver.

| | Conc. homogeneity in liver | |
|---|---|---|
| **Element** | **Mean RSD (%)** | **Range** |
| **Cd** | 10.30 | (4.58, 21.40) |
| **Co** | 14.29 | (7.16, 28.06) |
| **Cu** | 10.37 | (2.03, 20.19) |
| **Fe** | 10.59 | (4.94, 19.05) |
| **Mn** | 6.01 | (3.88, 9.70) |
| **Mo** | 7.97 | (3.22, 15.84) |
| **Se** | 14.37 | (4.64, 31.60) |
| **Zn** | 6.51 | (3.43, 9.09) |

**Table S3: Extreme outlying data for liver element concentrations (for cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn), lead (Pb) and aluminium (Al) measured in red deer (*Cervus elaphus*) collected across three hunting estates in the Scottish Highlands during the 2012-13 and 2013-14 stalking seasons (1st July – 20th October for males; 21st October – 15th February for females).**

| Estate | Cd | Co | Cu | Fe | Mn | Mo | Se | Zn | Pb | Al | Sex | Age | Lat | Long |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strathconon | 0.09 | 0.16 | 85.8 | 469 | 13.9 | 2.61 | 0.686 | 127 | 1698 | 1.84 | F | calf | 57.51 | -4.95 |
| Alladale | 0.26 | 0.08 | 38.6 | 829 | 608 | 1.28 | 0.466 | 113 | 0.727 | 31.9 | F | calf | 57.87 | -4.78 |
| Alladale | 0.17 | 0.24 | 29.8 | 710 | 451 | 1.34 | 0.424 | 60.3 | 0.403 | 192 | F | mature | 57.89 | -4.75 |
| Strathconon | 0.10 | 0.13 | 58.5 | 511 | 809 | 1.62 | 0.892 | 72.9 | 0.370 | 11.5 | F | mature | 57.58 | -4.87 |
| Ardnamurchan | 0.28 | 0.32 | 32.2 | 397 | 572 | 3.85 | 0.320 | 152 | 1.177 | 102 | M | mature | 56.73 | -6.10 |
| Strathconon | 0.07 | 0.20 | 5.94 | 656 | 7.18 | 1.82 | <0.277 | 54.2 | 602 | 0.867 | M | mature | 57.50 | -4.93 |

**Table S4. Summary statistics for element concentrations (for cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn)) measured in red deer (*Cervus elaphus*) liver collected on nine hunting estates in the Scottish Highlands during August to February 2012-13 and 2013-14.**

| | | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Element | Estate | n | min | max | median | 95% CI | n | min | max | median | 95% CI |
| Cd | Alladale | 78 | 0.0437 | 0.83 | 0.288 | (0.222, 0.336) | 68 | 0.088 | 1.05 | 0.372 | (0.266, 0.421) |
| | Altnaharra | 63 | 0.0362 | 0.583 | 0.139 | (0.112, 0.17) | 65 | 0.0632 | 0.445 | 0.163 | (0.137, 0.179) |
| | Applecross | 63 | 0.0558 | 1.26 | 0.328 | (0.257, 0.423) | 25 | 0.183 | 2.54 | 0.337 | (0.277, 0.484) |
| | Ardnamurchan | 45 | 0.0635 | 0.516 | 0.151 | (0.128, 0.188) | 26 | 0.0672 | 1.07 | 0.17 | (0.125, 0.242) |
| | Badanloch | 55 | 0.053 | 4.68 | 0.224 | (0.179, 0.256) | 59 | 0.0803 | 1.38 | 0.246 | (0.19, 0.265) |
| | Ben_Loyal | 43 | 0.0275 | 1.55 | 0.213 | (0.177, 0.282) | 40 | 0.0376 | 5.19 | 0.278 | (0.214, 0.32) |
| | Conaglen | 47 | 0.0624 | 1.85 | 0.393 | (0.309, 0.562) | 35 | 0.131 | 10.4 | 0.649 | (0.491, 0.784) |
| | NHT & Aline | 10 | 0.112 | 0.422 | 0.304 | (0.145, 0.351) | 11 | 0.162 | 0.951 | 0.337 | (0.289, 0.845) |
| | Strathconon | 31 | 0.0877 | 0.612 | 0.261 | (0.226, 0.309) | 23 | 0.0423 | 0.633 | 0.324 | (0.301, 0.395) |
| Co | Alladale | 78 | < 0.0622 | 0.222 | 0.0933 | (0.0771, 0.107) | 68 | < 0.0622 | 0.277 | 0.138 | (0.12, 0.148) |
| | Altnaharra | 63 | < 0.0622 | 0.257 | 0.0702 | (0.0551, 0.08) | 65 | 0.0708 | 0.339 | 0.139 | (0.129, 0.155) |
| | Applecross | 63 | < 0.0622 | 0.175 | 0.0717 | (0.0582, 0.0858) | 25 | < 0.0622 | 0.309 | 0.186 | (0.158, 0.199) |
| | Ardnamurchan | 45 | < 0.0622 | 0.498 | 0.135 | (0.125, 0.143) | 26 | 0.079 | 0.256 | 0.162 | (0.146, 0.182) |
| | Badanloch | 55 | < 0.0622 | 0.189 | 0.0926 | (0.0796, 0.109) | 59 | < 0.0622 | 0.414 | 0.123 | (0.102, 0.147) |
| | Ben_Loyal | 43 | < 0.0622 | 0.276 | 0.0807 | (0.067, 0.0923) | 40 | 0.0663 | 0.432 | 0.154 | (0.136, 0.171) |
| | Conaglen | 47 | < 0.0622 | 0.218 | 0.112 | (0.099, 0.131) | 35 | 0.0765 | 0.273 | 0.173 | (0.155, 0.179) |
| | NHT & Aline | 10 | < 0.0622 | 0.173 | 0.109 | (0.0508, 0.146) | 11 | 0.0816 | 0.27 | 0.152 | (0.139, 0.187) |
| | Strathconon | 31 | < 0.0622 | 0.214 | 0.118 | (0.101, 0.139) | 23 | 0.0701 | 0.735 | 0.202 | (0.188, 0.231) |
| Cu | Alladale | 78 | 2.39 | 192 | 33.3 | (28.4, 42.6) | 68 | 7.46 | 184 | 55.8 | (39.3, 79.9) |
| | Altnaharra | 63 | 4.02 | 309 | 25.6 | (18.7, 34.2) | 65 | 6.42 | 198 | 35.5 | (27.8, 46.9) |
| | Applecross | 63 | 2.35 | 188 | 42.5 | (31.7, 49.7) | 25 | 12.3 | 212 | 56.5 | (29.3, 107) |
| | Ardnamurchan | 45 | 3.74 | 184 | 14 | (9.98, 25.4) | 26 | 7.46 | 129 | 13.6 | (9.95, 26.6) |
| | Badanloch | 55 | 5.39 | 190 | 15 | (11.6, 27.6) | 59 | 10.2 | 260 | 58 | (42.9, 83.3) |
| | Ben_Loyal | 43 | 4.41 | 178 | 25.4 | (18.1, 39.3) | 40 | 15.9 | 241 | 65.6 | (44.4, 82.1) |
| | Conaglen | 47 | 5.48 | 261 | 42.9 | (24.9, 67.4) | 35 | 10.8 | 216 | 64.8 | (53.7, 85.9) |
| | NHT & Aline | 10 | 6.35 | 164 | 26.1 | (12.2, 54.4) | 11 | 15.1 | 120 | 43.1 | (29.9, 58.5) |
| | Strathconon | 31 | 7.58 | 201 | 36.6 | (14.7, 59.6) | 23 | 12.3 | 306 | 70.6 | (61.6, 94.1) |
| Fe | Alladale | 78 | 197 | 1100 | 445 | (402, 486) | 68 | 314 | 3090 | 570 | (534, 621) |
| | Altnaharra | 63 | 232 | 863 | 478 | (445, 530) | 65 | 295 | 1860 | 578 | (541, 657) |
| | Applecross | 63 | 129 | 721 | 431 | (390, 464) | 25 | 324 | 2040 | 561 | (413, 618) |
| | Ardnamurchan | 45 | 242 | 3550 | 511 | (467, 729) | 26 | 325 | 1000 | 480 | (436, 667) |
| | Badanloch | 55 | 273 | 1430 | 452 | (417, 498) | 59 | 207 | 6350 | 523 | (490, 586) |
| | Ben_Loyal | 43 | 168 | 816 | 418 | (381, 461) | 40 | 274 | 1140 | 574 | (502, 657) |
| | Conaglen | 47 | 179 | 827 | 433 | (378, 517) | 35 | 296 | 1630 | 507 | (461, 653) |
| | NHT & Aline | 10 | 275 | 2240 | 513 | (360, 673) | 11 | 354 | 778 | 546 | (472, 689) |
| | Strathconon | 31 | 257 | 713 | 479 | (410, 531) | 23 | 375 | 3300 | 615 | (536, 653) |
| Mn | Alladale | 78 | 2 | 20.2 | 8.86 | (7.12, 10.1) | 68 | 7.43 | 27.5 | 14 | (12.8, 15) |
| | Altnaharra | 63 | 3.07 | 23 | 6.03 | (5.33, 7.81) | 65 | 9.21 | 23.3 | 16.3 | (15.1, 17.2) |
| | Applecross | 63 | 2.18 | 18.6 | 7.95 | (6.42, 8.75) | 25 | 9.61 | 19.9 | 14.3 | (13.3, 15) |
| | Ardnamurchan | 45 | 3.51 | 18.5 | 7.61 | (6.7, 9.04) | 26 | 6.85 | 21.7 | 13.3 | (11.4, 13.8) |
| | Badanloch | 55 | 3.57 | 20.6 | 11.9 | (10.7, 13.3) | 59 | 10.9 | 25.7 | 17.5 | (16.8, 18.2) |
| | Ben_Loyal | 43 | 2.66 | 21.4 | 11 | (8.22, 12.7) | 40 | 9.03 | 24.7 | 14.9 | (13.3, 15.5) |
| | Conaglen | 47 | 3.11 | 19.1 | 10.6 | (10.1, 12.5) | 35 | 8.18 | 18.4 | 13.5 | (12.8, 14.8) |
| | NHT & Aline | 10 | 2.5 | 12.7 | 7.66 | (4.71, 11.3) | 11 | 10.3 | 16.3 | 13.2 | (11.6, 14.7) |
| | Strathconon | 31 | 3.8 | 16.4 | 10.9 | (8.56, 12.2) | 23 | 9.11 | 23.2 | 12.5 | (11, 15.1) |
| Mo | Alladale | 78 | 0.606 | 3.82 | 1.74 | (1.54, 2.1) | 68 | 1.3 | 3.99 | 2.63 | (2.44, 2.82) |
| | Altnaharra | 63 | 0.508 | 4.19 | 1.16 | (1.08, 1.36) | 65 | 1.34 | 3.97 | 2.57 | (2.45, 2.71) |
| | Applecross | 63 | 0.404 | 4.19 | 1.75 | (1.49, 2.03) | 25 | 1.35 | 5.35 | 2.62 | (2.25, 2.97) |
| | Ardnamurchan | 45 | 0.686 | 4.13 | 2.09 | (1.56, 2.49) | 26 | 1.83 | 3.99 | 2.88 | (2.55, 3.18) |
| | Badanloch | 55 | 0.802 | 4.11 | 2.85 | (2.65, 3.18) | 59 | 1.4 | 3.67 | 2.87 | (2.7, 2.96) |
| | Ben_Loyal | 43 | 0.573 | 4.84 | 2.29 | (1.97, 2.75) | 40 | 1.41 | 3.66 | 2.48 | (2.18, 2.7) |
| | Conaglen | 47 | 0.596 | 4.26 | 2.44 | (1.97, 3.1) | 35 | 1.84 | 4.44 | 2.62 | (2.49, 2.91) |
| | NHT & Aline | 10 | 0.527 | 4.7 | 1.76 | (1.19, 3) | 11 | 1.34 | 4.1 | 2.47 | (2.07, 3.15) |
| | Strathconon | 31 | 1.18 | 3.79 | 2.5 | (1.88, 2.99) | 23 | 1.75 | 3.58 | 2.94 | (2.54, 3.15) |
| Se | Alladale | 78 | < 0.277 | 1.48 | 0.681 | (0.62, 0.77) | 68 | < 0.277 | 1.47 | 0.562 | (0.487, 0.624) |
| | Altnaharra | 63 | < 0.277 | 3.18 | 0.613 | (0.523, 0.702) | 65 | < 0.277 | 2.75 | 0.591 | (0.532, 0.711) |
| | Applecross | 63 | < 0.277 | 2.76 | 0.836 | (0.703, 0.958) | 25 | < 0.277 | 3.69 | 1.47 | (0.912, 2.09) |
| | Ardnamurchan | 45 | < 0.277 | 1.14 | 0.452 | (0.389, 0.517) | 26 | < 0.277 | 2.25 | 0.594 | (0.361, 0.734) |
| | Badanloch | 55 | < 0.277 | 1.08 | 0.321 | (0.295, 0.4) | 59 | < 0.277 | 2.47 | 0.374 | (0.277, 0.471) |
| | Ben_Loyal | 43 | < 0.277 | 1.24 | 0.52 | (0.369, 0.641) | 40 | < 0.277 | 3.88 | 0.65 | (0.478, 0.805) |
| | Conaglen | 47 | < 0.277 | 2.22 | 0.926 | (0.645, 1.09) | 35 | 0.48 | 3.55 | 1.21 | (1.1, 1.66) |
| | NHT & Aline | 10 | 0.447 | 1.99 | 0.768 | (0.603, 1.26) | 11 | 0.658 | 1.92 | 0.892 | (0.766, 1.33) |
| | Strathconon | 31 | < 0.277 | 1.32 | 0.645 | (0.561, 0.711) | 23 | < 0.277 | 1.82 | 0.825 | (0.567, 0.971) |
| Zn | Alladale | 78 | 34 | 282 | 67.1 | (61.2, 79.2) | 68 | 57.9 | 344 | 104 | (100, 109) |
| | Altnaharra | 63 | 35.5 | 173 | 52.3 | (49, 61.8) | 65 | 55.2 | 176 | 103 | (95.9, 108) |
| | Applecross | 63 | 33.3 | 116 | 68.9 | (60.1, 73.8) | 25 | 75.1 | 145 | 109 | (103, 116) |
| | Ardnamurchan | 45 | 38.5 | 271 | 82.6 | (73.7, 95) | 26 | 73.7 | 143 | 111 | (104, 120) |
| | Badanloch | 55 | 39.5 | 116 | 91.9 | (82.3, 97.7) | 59 | 60.9 | 171 | 108 | (104, 111) |
| | Ben_Loyal | 43 | 36.3 | 177 | 87.8 | (76.5, 98.9) | 40 | 67.8 | 146 | 107 | (100, 112) |
| | Conaglen | 47 | 25.6 | 213 | 82.9 | (69.1, 89.1) | 35 | 77.5 | 131 | 112 | (103, 118) |
| | NHT & Aline | 10 | 30.9 | 115 | 64.3 | (46.4, 86.1) | 11 | 74.5 | 146 | 102 | (94.1, 118) |
| | Strathconon | 31 | 36.2 | 121 | 81.1 | (66.5, 93.4) | 23 | 77.5 | 178 | 99.1 | (96.6, 107) |

**Table S5. Summary of GLS[φ] model coefficient estimates for cadmium (Cd) and seven essential trace element (cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn)) concentrations measured in male (M; n = 394) and female (F; n = 321) red deer (*Cervus elaphus*) liver samples collected on nine hunting estates in the Scottish Highlands during July to February 2012-13 and 2013-14, in relation to sampling date and *Fasciola hepatica* infection status (diagnosed by coproantigen ELISA).**

| Element | Sex | [ε] Estates ( L-S means) | Days into the stalking season (±SE) | [β] Days into the stalking season squared (±SE) | [γ] Season culled (L-S means) | F. *hepatica* status (L-S means) | [ε]Error variance structure |
|---|---|---|---|---|---|---|---|
| **Cd** | **M** | $AT^{a}$, $AR^{ab}$, $BL^{bc}$, $BA^{bc}$, $ST^{bc}$,$NA^{abcd}$, $AL^{cd}$, $AP^{cd}$, $CO^{d}$ | 0.0295 (0.0123) | -0.000198 (0.0000811) | 13-14 > 12-13 | n.d. | varExp(Days into season) |
| | **F** | $AT^{a}$, $AR^{ab}$, $BA^{bc}$, $ST^{abcd}$, $BL^{abcd}$, $AL^{d}$, $AP^{bcde}$, $NA^{cde}$, $CO^{e}$ | 0.00378 (0.00170) | - | n.d. | n.d. | varIdent(Estates) + varPower(Days into season) |
| **Co** | **M** | $AT^{a}$, $AP^{a}$, $BL^{ab}$, $BA^{ab}$, $AL^{ab}$, $NA^{abc}$, $CO^{bc}$, $ST^{bc}$, $AR^{c}$ | -0.00297 (0.00130) | - | n.d. | n.d. | - |
| | **F** | $BA^{a}$, $AT^{a}$, $BL^{ab}$, $AL^{a}$, $CO^{ab}$, $NA^{ab}$, $AP^{ab}$, $AR^{b}$, $ST^{ab}$ | 0.00337 (0.000929) | - | 12-13 > 13-14 | n.d. | varIdent(Estates) |
| **Cu** | **M** | $BA^{a}$, $AR^{ab}$, $AT^{abc}$, $BL^{abc}$, $ST^{abc}$, $NA^{abc}$, $CO^{bc}$, $AL^{c}$, $AP^{c}$ | 0.0540 (0.0182) | -0.000386 (0.0000120) | 13-14 > 12-13 | n.d. | varExp(Days into season) |
| | **F** | $AR^{a}$, $NA^{ab}$, $AT^{ab}$, $AP^{ab}$, $CO^{b}$, $BL^{b}$, $AL^{b}$, $BA^{b}$, $ST^{b}$ | 0.00489 (0.00225) | - | n.d. | n.d. | - |
| **Fe** | **M** | $AP^{a}$, $BL^{a}$, $CO^{a}$, $AL^{a}$, $BA^{a}$, $AT^{ab}$, $ST^{ab}$, $NA^{ab}$, $AR^{b}$ | 0.0111 (0.00653) | -0.0000740 (0.0000427) | 12-13 > 13-14 | n.d. | varIdent(Estates) |
| | **F** | n.d. | 0.0103 (0.00278) | -0.0000690 (0.0000221) | 12-13 > 13-14 | n.d. | varIdent(Estates) + varPow(Days into season) |
| **[α]Mn** | **M** | n.d. | 0.00482 (0.0699) | -0.000828 (0.000456) | n.d. | n.d. | varIdent(Estates) |
| | **F** | $AR^{a}$, $NA^{ab}$, $ST^{ab}$, $AL^{ab}$, $CO^{abc}$, $AP^{abc}$, $BL^{abc}$, $AT^{bc}$, $BA^{c}$ | -0.0110 (0.00893) | - | n.d. | n.d. | varExp(Days into season) |
| **[α]Mo** | **M** | $AT^{a}$, $ST^{ab}$, $BL^{ab}$, $AP^{ab}$, $AL^{ab}$, $BA^{b}$, $AR^{b}$, $CO^{ab}$, $NA^{ab}$ | 0.000744 (0.0141) | -0.000210 (0.0000926) | 13-14 > 12-13 | n.d. | varIdent(Estates) |
| | **F** | n.d. | -0.0154 (0.00503) | 0.0000886 (0.0000411) | 13-14 > 12-13 | n.d. | varExp(Days into season) |
| **Se** | **M** | $BA^{a}$, $AR^{ab}$, $BL^{abc}$, $AT^{bcd}$, $ST^{bcde}$ $AL^{cde}$, $CO^{de}$, $AP^{e}$, $NA^{de}$ | 0.000239 (0.00156) | - | n.d. | n.d. | varExp(Days into season) |
| | **F** | $BA^{a}$, $BL^{ab}$, $AL^{abc}$, $AT^{abc}$, $AR^{abcd}$, $ST^{bcd}$, $AP^{cd}$, $NA^{d}$, $CO^{d}$ | 0.00677 (0.00132) | - | 12-13 > 13-14 | n.d. | varIdent(Estates) + varPower(Days into season) |
| **[α]Zn** | **M** | $AT^{a}$, $ST^{ab}$, $CO^{ab}$, $AP^{ab}$, $BA^{ab}$, $BL^{ab}$, $NA^{ab}$, $AL^{ab}$, $AR^{b}$ | -0.00633 (0.00532) | -0.0000190 (0.0000362) | n.d. | n.d. | varIdent(Estates) + varConstPower(Days into season) |
| | **F** | n.d. | -0.0470 (0.0514) | - | n.d. | pos > neg | varPower(Days into season) |

[α] Female Zn data and male and female Mn and Mo data were not log transformed prior to modelling.

[ε] Estate abbreviations: Alladale (AL), Altnaharra (AT), Applecross Trust (AP), Ardnamurchan (AR), Badanloch (BA), Ben Loyal (BL), Conaglen (CO), Strathconon (ST), and two neighbouring estates North Harris Trust and Aline (NA). Within the sexes, significant differences between estate Least-Square means (L-S means) (at the 5% level) are denoted by compact letter descriptors; estates that share a common letter did not have significantly different element content. L-S means were used to estimate differences between sampling estate means (log transformed unless stated), while accounting for temporal variation modelled by the GLM or GLS. Similarly, L-S means were used to estimate differences between sampling season means and between means for *F. hepatica* infected and non-infected groups, while accounting for geographic and within season temporal variation.

[β] Parameter estimates show the direction of temporal associations, but are of the log transformed scale unless stated. Dashed lines (-) indicate that temporal parameters were not included in the model, as AIC increased with their inclusion.

[γ] n.d. indicates that no differences in concentrations were found between estates, sampling seasons (2012-13 and 2013-14); nor were differences in liver element concentrations found between *F. hepatica* infected and non-infected individuals.

[ε]Error variance structures: All structures (varExp, varConstPower and varPower) indicate that error variance increases as the stalking season progresses.

**Table S6. Summary of model coefficient estimates for cadmium (Cd) and seven essential trace element (cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn)) concentrations measured in male (M; n = 435) and female (F; n = 352) red deer (*Cervus elaphus*) liver samples collected on nine hunting estates in the Scottish Highlands during July to February 2012-13 and 2013-14 in relation to sampling date.**

| | | [ε] Estates (lowest to highest L-S means) | [β] Days into the stalking season (±SE) | [β] Days into the stalking season squared (±SE) | [γ] Season culled (L-S means) | [ε] Error variance structure |
|---|---|---|---|---|---|---|
| **Cd** | **M** | $AT^{a}$, $AR^{ab}$, $BL^{bc}$, $BA^{bc}$, $ST^{bc}$, $NA^{bcd}$, $AL^{cd}$, $AP^{cd}$, $CO^{d}$ | 0.0333 (0.0119) | -0.000216 (0.0000787) | 13-14 > 12-13 | varIdent(Estates) + varConstPower(Days into season) |
| | **F** | $AT^{a}$, $AR^{ab}$, $BL^{abc}$, $BA^{bc}$, $ST^{bc}$, $AP^{bcd}$, $AL^{cd}$, $NA^{bcd}$, $CO^{d}$ | 0.00460 (0.00163) | - | n.d. | varIdent(Estates) + varPower(Days into season) |
| **Co** | **M** | $AT^{a}$, $AP^{ab}$, $BL^{abc}$, $BA^{abc}$, $AL^{bc}$, $NA^{abcd}$, $CO^{cd}$, $ST^{cd}$, $AR^{d}$ | -0.00300 (0.00123) | - | n.d. | - |
| | **F** | $BA^{a}$, $BL^{ab}$, $AT^{a}$, $AL^{a}$, $CO^{ab}$, $AP^{abc}$, $NA^{abc}$, $AR^{bc}$, $ST^{c}$ | 0.00381 (0.000857) | - | 12-13 > 13-14 | varIdent(Estates) |
| **Cu** | **M** | $BA^{a}$, $AR^{ab}$, $AT^{abc}$, $ST^{abc}$, $BL^{abc}$, $NA^{abc}$, $CO^{bc}$, $AL^{c}$, $AP^{c}$ | 0.0522 (0.0176) | -0.000378 (0.000116) | 13-14 > 12-13 | varExp(Days into season) |
| | **F** | $AR^{a}$, $NA^{ab}$, $AT^{ab}$, $AP^{ab}$, $CO^{ab}$, $BL^{b}$, $AL^{b}$, $BA^{b}$, $ST^{b}$ | 0.00546 (0.00215) | - | n.d. | - |
| **Fe** | **M** | $BL^{a}$, $AP^{a}$, $CO^{a}$, $AL^{a}$, $BA^{a}$, $AT^{ab}$, $ST^{ab}$, $NA^{ab}$, $AR^{b}$ | -0.000329 (0.000891) | - | 12-13 > 13-14 | varIdent(Estates) + varExp(Days into season) |
| | **F** | n.d. | 0.0110 (0.00268) | -0.0000740 (0.0000215) | 12-13 > 13-14 | varIdent(Estates) + varPower(Days into season) |
| **[α]Mn** | **M** | n.d. | 0.00115 (0.0675) | -0.000840 (0.000438) | n.d. | varIdent(Estates) |
| | **F** | $AR^{a}$, $NA^{ab}$, $ST^{ab}$, $CO^{abc}$, $AL^{a}$, $AP^{abc}$, $BL^{abc}$, $AT^{bc}$, $BA^{c}$ | -0.0175 (0.0271) | 0.0000810 (0.000206) | n.d. | varExp(Days into season) |
| **[α]Mo** | **M** | $AT^{a}$, $ST^{a}$, $BL^{ab}$, $AP^{ab}$, $AL^{ab}$, $BA^{b}$, $AR^{b}$, $CO^{b}$, $NA^{ab}$ | -0.000538 (0.0135) | -0.000208 (0.0000882) | 13-14 > 12-13 | varIdent(Estates) |
| | **F** | n.d. | -0.00581 (0.0678) | - | n.d. | varExp(Days into season) |
| **Se** | **M** | $BA^{a}$, $AR^{ab}$, $BL^{abc}$, $ST^{bcde}$, $AT^{bcd}$, $AL^{cde}$, $AP^{de}$, $CO^{e}$, $NA^{de}$ | -0.000202 (0.00143) | - | n.d. | - |
| | **F** | $BL^{a}$, $BA^{ab}$, $AL^{ab}$, $AT^{abc}$, $AR^{abcd}$, $ST^{abcd}$, $AP^{bcd}$, $NA^{cd}$, $CO^{d}$ | 0.00748 (0.00382) | 0.000000900 (0.0000316) | 12-13 > 13-14 | varIdent(Estates) + varPower(Days into season) |
| **Zn** | **M** | $AT^{a}$, $ST^{a}$, $AP^{ab}$, $CO^{ab}$, $BA^{ab}$, $BL^{ab}$, $AL^{ab}$, $NA^{ab}$, $AR^{b}$ | -0.00774 (0.00519) | -0.0000130 (0.0000352) | n.d. | varIdent(Estates) + varConstPower(Days into season) |
| | **F** | n.d. | -0.00164 (0.00153) | 0.0000120 (0.0000120) | n.d. | - |

[α] Female and male Mn and Mo data were not log transformed.

[ε] Estate abbreviations: Alladale (AL), Altnaharra (AT), Applecross Trust (AP), Ardnamurchan (AR), Badanloch (BA), Ben Loyal (BL), Conaglen (CO), Strathconon (ST), and two neighbouring estates North Harris Trust and Aline (NA). Within the sexes, significant differences between estate Least-Square means (L-S means) (at the 5% level) are denoted by compact letter descriptors; estates that share a common letter did not have significantly different element content. L-S means were used to estimate differences between sampling estate means (log transformed unless stated), while accounting for temporal variation modelled by the GLM or GLS. Similarly, L-S means were used to estimate differences between sampling season means, while accounting for geographic and within season temporal variation.

[β] Parameter estimates show the direction of temporal associations, but are of the log transformed scale unless stated. Dashed lines (-) indicate that temporal parameters were not included in the model, as AIC increased with their inclusion.

[γ] n.s indicates differences in concentrations between seasons of sampling that included zero within 95% confidence intervals (but season was retained in the model because AIC increased with their exclusion).

[ε] Error variance structures: Structures involving days into the season (i.e., varExp(), varConstPower() and varPower()) show error variance increases as the stalking season progresses. Structures involving Estates (i.e., varIdent()) show that error variance varied between estates.

**Table S7**. **Summary of GLM model parameter estimates for three principal components calculated using concentrations of eight elements (cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn)) measured in the livers of male (M; n =) and female (F; n = 352) red deer (*Cervus elaphus*) collected on nine hunting estates in the Scottish Highlands during August to February 2012-13 and 2013-14**.

| PC | Sex | Associated elements | Variance explained (%) | [ε] Element concentrations on estates (lowest to highest) and significant differences (L-S means) in relation to increasing principal components | Days into the stalking season (±SE) | Season culled (L-S mean) |
|---|---|---|---|---|---|---|
| **1** | M | Mn, Mo, Zn | 36.1 | (**High Mn, Mo, Zn**) CO$^{a}$, NA$^{abc}$, AL$^{ab}$, AR$^{abc}$, AP$^{abc}$, BA$^{abc}$, ST$^{abc}$, BL$^{bc}$, AT$^{c}$ (**Low Mn, Mo, Zn**) | 0.0418 (0.00387) | n.d |
| **1** | F | Cd, Co, Cu, Se | 23.6 | (**Low Cd, Co, Cu, Se**) AT$^{a}$, AR$^{ab}$, BL$^{ab}$, AL$^{abc}$, BA$^{abc}$, NA$^{abc}$,AP$^{abc}$, ST$^{bc}$, CO$^{c}$ (**High Cd, Co, Cu, Se**) | 0.0121 (0.00336) | n.d. |
| **2** | M | Cd, Cu, Se | 21.4 | (**Low Cd, Co, Cu, Se**) AR$^{a}$, BA$^{ab}$, BL$^{bc}$, AT$^{c}$, ST$^{cde}$, AL$^{cd}$, NA$^{cde}$, CO$^{de}$, AP$^{e}$ (**High Cd, Co, Cu, Se**) | 0.0152 (0.00291) | 13-14 > 12-13 |
| **2** | F | Mn, Mo, Zn | 20.9 | (**Low Mn, Mo, Zn**) NA$^{ab}$, ST$^{ab}$, CO$^{ab}$, AL$^{a}$, AR$^{ab}$, AP$^{ab}$, BL$^{ab}$, AT$^{ab}$, BA$^{b}$ (**High Mn, Mo, Zn**) | -0.0136 (0.00319) | n.d. |
| **3** | M | Fe, Co | 12.7 | (**Low Fe, Co**) BL$^{a}$, AP$^{ab}$, BA$^{ab}$, AT$^{ab}$, AL$^{ab}$, CO$^{ab}$, ST$^{bc}$, NA$^{abc}$, AR$^{c}$ (**High Fe, Co**) | 0.0119 (0.00246) | 12-13 > 13-14 |
| [α]**3** | F | Fe | 15.0 | (**Low Fe**) AP$^{ab}$, AR$^{a}$, BL$^{ab}$, CO$^{ab}$, NA$^{ab}$, AT$^{ab}$, ST$^{ab}$, AL$^{ab}$, BA$^{b}$ (**High Fe**) | 0.00291 (0.000965) | 12-13 > 13-14 |

[α]Female PC3 data were [log (PC3+3)] transformed to coerce normality and homoscedasticity in residuals of a simple linear regression.

[ε]Estate abbreviations: Alladale (AL), Altnaharra (AT), Applecross Trust (AP), Ardnamurchan (AR), Badanloch (BA), Ben Loyal (BL), Conaglen (CO), Strathconon (ST), and two neighbouring estates North Harris Trust and Aline (NA). Within the sexes, significant differences between estate Least-Square means (L-S means) (at the 5% level) are denoted by compact letter descriptors; estates that share a common letter did not have significantly different element content. L-S means were used to estimate differences between sampling estate means (log transformed unless stated), while accounting for temporal variation modelled by the GLM or GLS. Similarly, L-S means were used to estimate differences between sampling season means, while accounting for geographic and within season temporal variation.

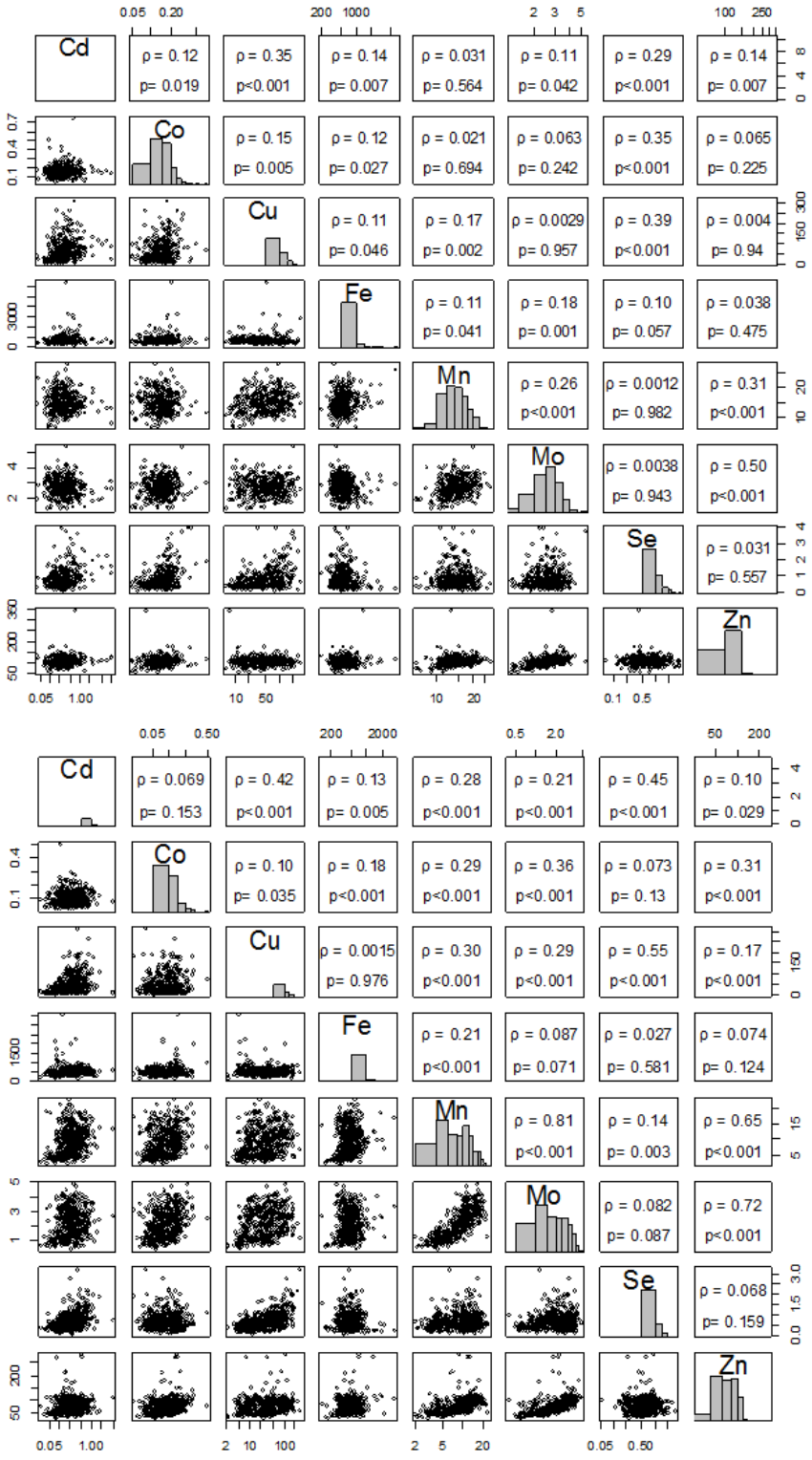


**Figure S1. Pairwise Spearman's ρ correlations between element (cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn)) concentrations in red deer (*Cervus elaphus*) liver samples (n = 770) collected on nine hunting estates in the Scottish Highlands during August to February 2012-13 and 2013-14**. The top panel illustrates data for females, and the bottom panel for males. Note that none of the liver element data were log-transformed prior to this analysis, but axis scales are logarithmic to aid visual clarity.

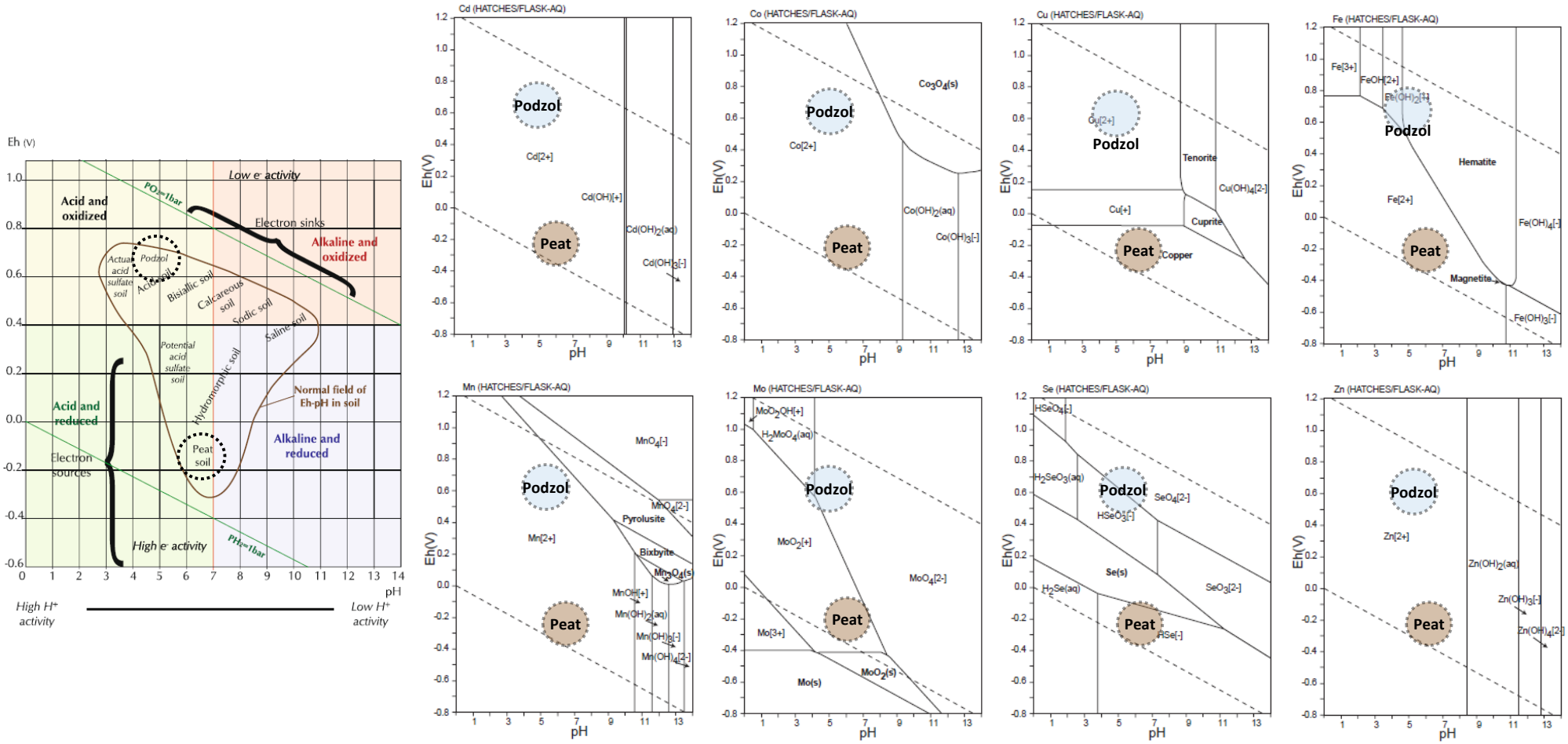


**Figure S2. Eh-pH phase diagrams for eight elements (cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn)) and their relation to soil type (adapted from Husson, 2012; Snakin et al., 2001; Takeno, 2005).**