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ABSTRACT

The objective of this study was to compare farmed with free-ranging red deer with respect to levels and interactions of 18 selected elements. The study comprised liver samples of 43 free-ranging animals collected from 6 hunting areas, and 52 farmed animals from 9 different herds in Norway. About half of the farmed animals had lower Cu contents than 4 ppm and suffered probably from Cu deficiency. The main structural differences between farmed and free-ranging animals with liver Cu levels 4-40 ppm wm were by partial least square regression analyses related to the higher Al-, Fe- and Mo-concentrations and the lower Cu-concentrations in the farmed animals. This may indicate pronounced element imbalances in the farmed deer since they also in general contained significantly lower levels of trace elements compared to the free-ranging. Comparisons of Cu categories of farmed deer (<2 ppm, 2-3 ppm, 3-4 ppm, 4-5 ppm and > 5 ppm) with free-ranging deer revealed, however, that differences in essential element concentrations diminished with increasing Cu categories of farmed deer. Low Cu concentrations and high Fe concentrations in the farmed deer may have ensued from relatively high absorption of Mo and Al by the farmed deer. We suggest that the farmed deer are influenced by deleterious elements due to a higher rate of ingestion of soil particles than are the free-ranging deer.

INTRODUCTION

Farming of red deer (*Cervus elaphus*) in Norway is a recently established activity based on a Norwegian subspecies of free-ranging deer (*C. e. atlanticus*). Improving trace element nutrition of grazing farmed animals, in a way that is cost efficient and meets consumer perceptions and preferences, is a continuing challenge. Trace element disorders, whether in deficiency or in excess, are generally not characterised by distinct pathological abnormalities (*1*). Especially have the Cu contents of farmed animals been in focus because low Cu levels are found in farmed grazing animals in many countries including Norway. Further, relations between liver Cu levels and deficiency symptoms have been documented in many studies (*2-14*). Dietary Cu requirements vary greatly among species. The recommended levels for one species may be toxic to another. For example, while 10 ppm Cu is the NRC recommended dietary level for dairy cattle, 10 ppm can cause toxicity in sheep under certain conditions (*15*). Low Cu loads in the body may not only be a consequence of low Cu supplies in the feed, but may also result from interactions with other elements. High concentrations of e.g. Mo, Fe, Cd, Pb and Zn are known to induce Cu-deficiency (*16*). The present study was conducted to assess both element levels and interactions in farmed and free-ranging red deer and to evaluate if the element profiles in the farmed individuals reflect a possible satisfactory state of mineral nutrition.

MATERIALS AND METHODS

During the years 1998 - 2000 forty-three livers of free-ranging red deer representing six different hunting areas and fifty two livers of farmed red deer from nine different herds, were collected by hunters and farmers respectively, and kept in freezers (below -18 °C) until chemical analysis. All livers from free-ranging individuals were collected during the hunting season (Sept. - Oct.), and all livers of the farmed individuals except five animals slaughtered in spring, were provided during the autumn. The age, sex and haunt were recorded. From each liver 1.0 - 2.0 g of tissue was sampled, weighed and mixed with 5 mL conc. HNO_3. The samples were coded and then treated in random sequences. After reducing the samples to pulp in a Milestone Mega 1200 microwave oven, they were diluted to standard volume. Each of eighteen elements were analysed by inductively coupled plasma atomic emission spectrometry (ICP-AES). Accuracy and reliability were controlled by use of the international standard Bovine liver 1577b (*17*).

Statistical evaluations were performed by use of MS Excel and Analyse-It version 1.62. Multivariate data analysis, i.e. Principal Components Analysis (PCA), and Partial Least Square Regression (PLS) were computed by use of The Unscrambler ver 7.6 (CAMO ASA, Oslo, Norway). Before performing the multivariate analyses the data variables were scaled to unit variance, which means that all variables had the same numerical range of variations.

The data material was not normally distributed and had unacceptable skewness and kurtosis as shown by Shapiro-Wilk W tests (*18*). Thus, non-parametric tests were chosen to reduce risks of getting incorrect conclusions. Multivariate normalised data were achieved by eliminating those elements that were poorly described by the models. In some cases even individual results had to be defined as outliers to achieve multivariate normalising matrices. PLS is a method for relating the variations in one response variable (Y-variable) to the variations of several predictors (X-variables) with explanatory or predictive purposes. This method performs particularly well when the various X-variables express common information, i.e. when there are large amounts of correlations, or collinearities. The Y-variable contains a column in the data-matrix with information on for example free-ranging or farmed deer, the age of the animal, the sex of the animal or the habitat group. The X-variables contain all individual chemical results from the analyses of 18 elements. The multivariate correlations measure the amount of linear relationships among elements. The correlation is computed as the square root of the covariance between the two elements divided by the product of their variances.

By the comparisons between recorded element concentrations and those found published, results given in dry mass are converted to wet mass by use of the average dry mass % of 73 livers of free-ranging red deer in Norway at 32.2 ± 1.3 (SD) (*19*).

If nothing specified, the significance level is 0.05 %.

RESULTS

Element levels

Farmed Deer vs. Free-ranging Deer

Comparisons of the medians of element concentrations in analysed farmed with free-ranging red deer, revealed significant differences in fourteen of the eighteen elements (Table 1). Farmed deer contained higher concentrations of aluminium (Al) and nickel (Ni), while the free-ranging deer had higher concentrations of twelve elements as shown by Mann-Whitney U-test (Table 1). No significant differences were found regarding iron (Fe), molybdenum (Mo), sodium (Na) and lead (Pb) concentrations.

The samples from both free-ranging and farmed individuals were split into copper (Cu) level categories as seen in Table 2.

Comparisons of Cu categories of Farmed Deer With Free-Ranging Deer

To identify the differences between various Cu categories of farmed deer and the free-ranging deer comparisons by Mann-Whitney U-tests were applied. Exclusively free-ranging deer with less than 40 ppm Cu were included to reduce possible influences such as toxic effects caused by high Cu levels found in some free-ranging deer (Fig. 1).

{Cu} < 2 ppm--Higher levels of Ni (p=0.0445) coincided with lower levels of the 13 elements calcium (Ca) (p<0.0001), cadmium (Cd) (p<0.0001) , cobalt (Co) (p=0.0005), chromium (Cr) (p<0.0001), Cu (p<0.0001), Fe (p<0.0001), potassium (K) (p<0.0001), manganese (Mn) (p<0.0001) , magnesium (Mg) (p=0.0147), Mo (p=0.0001), phosphorus (P) (p<0.0001), sulphur (S) (p<0.0001), and zinc (Zn) (p<0.0001) in farmed deer with Cu levels less than 2 ppm compared with free-ranging deer (Fig. 1).

{Cu} € [2,3> ppm--Higher levels of Al (p=0,0126) and Fe (p=0,0446) coincided with lower levels of the 12 elements Ca (p=0,0005), Cd (p=0,0001), Co (p=0,0109), Cr (p=0,0006), Cu (p<0.0001), K (p<0.0001), Mn (p=0,0155), Mg (p=0,0011), P (p=0,0006), S (p<0.0001), selenium (Se) (p=0,0019), and Zn (p=0,0200) in farmed deer with Cu levels between 2-3 ppm compared with free-ranging deer (Fig. 1). The median Fe level (213 ± 99 ppm) in the farmed individuals of this increased by a factor of 4 compared with that (51± 22 ppm) of the proceeding Cu category (Fig. 1).

{Cu} € [3,4> ppm--Higher levels of Al (p=0,0237) coincided with lower levels of the 7 elements Ca (p=0,0160), Cd (p=0,0359), Co (p=0,0441), Cr (p=0,0114), Cu (p=0,0001), K (p=0,0212), and Mn (p=0,0434) in the farmed deer with Cu levels between 3-4 ppm compared with free-ranging deer (Fig. 1). No other element level differences were revealed (Fig. 1).

{Cu} € [4,5> ppm--Higher levels of Al (p=0,0003), Fe (p=0,0016), Mo (p=0,0553), and Ni (p=0,0597) coincided with lower levels of the 5 elements Ca (p=0,0461), Cd (p=0,0003), Cu (p=0,0001), K (p=0,0060), and Na (p=0,0315) in farmed animals with Cu levels between 4-5 ppm compared with free-ranging deer (Fig. 1).

{Cu} > 5 ppm--Higher levels of Al (p=0,0342), Fe (p=0,0302), Mo (p=0,0078), and Pb (p=0,0571) coincided with lower levels of the 5 elements Ca (p=0,0103), Cd (p=0,0038), Cr (p=0,0359), Cu (p=0,0002), and K (p=0,0100) in farmed deer with Cu levels above 5 ppm compared with free-ranging deer (Fig. 1).

Effects of geographical sites, sex, age and Cu categories

Geographical sites--Comparisons of element levels between free-ranging animals captured from different localities in Norway, revealed site differences in Cd (p=0.0009), Cu (p=0.0038) and Pb (p=0.0011). Equivalent analyses of results from the different enclosures of farmed deer revealed differences concerning Al (p=0.0098), Cd (p=0.0018), Cu (p=0.0095), Fe (p=0.0015), K (p<0.0001), Mo (p=0.0115), and Na (p=0.0032).

Sex--No sex dependent significant differences appeared concerning the element levels from either free-ranging or farmed individuals.

Age--No age dependent differences were found among the free-ranging deer. Farmed deer comprised mainly of the two age categories: 1.5 and 2.5 years. The lowest age category had significantly higher concentrations of Al (p=0.0450), while the oldest category had significantly higher concentrations of Cd (p=0.0254), Co (p=0.0302), Cr (p=0.0027), K (p=0.0254), Na (p=0.0008) and S (p=0.0254).

Cu categories of free-ranging deer--The 43 free-ranging deer were grouped in four Cu categories (Table 2). The Cu categories revealed significant differences regarding Cd (p <0.0001), Pb (p=0.0393), and Se (p=0.0031) as shown by Kruskal-Wallies tests, in addition to the categorising element Cu. Concentrations of 14 elements did not differ significantly among the four Cu categories (Fig. 1).

Cu categories of farmed deer--The 52 individuals were grouped in five Cu categories as seen in Table 2. Due to the considerably lower Cu levels in farmed deer, the Cu ranges of the categories had to be different from the categories of free-ranging deer (Table 2).The Cu categories revealed significant differences with respect to 12 elements: Al (p= 0.0002), Ca (p= 0.0007), Cd (p= 0.0062), Cr (p= 0.0162), Fe (p= 0.0004), K (p= 0. 0002), Mn (p= 0.0019), Mo (p< 0.0001), Na (p= 0.0382), P (p= 0.0004), S (p= 0.0050), and Zn (p<0.0001) as shown by Kruskal-Wallies test, in addition to the categorising element Cu. None of the other element concentrations varied significantly among the 5 Cu categories (Fig. 1).

Element profiles

Free-ranging deer

All individuals--Three clusters of mutually positively interacting elements were formed by the principal component analysis (PCA) of all free-ranging deer, comprised by: 1) the essential macro trace elements S, P, and Mg, 2) Cd, Cu, Pb and Se and 3) Al, Ca, and Zn. The two former clusters were also recognised from some of the PCAs of different Cu-categories of free-ranging deer (Fig. 2,3).

Cu < 40 ppm--The PCAs of free-ranging deer with less than 40 ppm Cu revealed only positive element interactions, except the negative relations between Cu and Mn and between Mo and Na (Fig. 3 A,B). The main structural variations in the liver chemicals coincided with individual differences regarding total element contents (Fig. 3 A,B). Cu had low influences in these models.

Cu > 40 ppm--Many negative element interactions appeared as shown by the PCAs of free-ranging deer with higher Cu than 40 ppm (Fig. 3 C,D). In both of the models, Cu was localised in opposed positions to the essential elements, indicating antagonistic relations. But the interactions among Cd, Cu, Pb and Se differed evidently between the two PCAs (Fig 3 C,D). By the PCA of animals with Cu levels 40-70 ppm all the positively interacting elements Cd, Cu, Pb and Se were jointly in opposed positions to essential elements. In the PCA of animals with higher Cu levels than 70 ppm, solely Cu, Na and Fe held these positions, concomitant as Cu by this model interacted negatively with Cd, Pb and Se, while Fe appeared as an antagonist to Mn, and Na as an antagonist to K (Fig. 3D).

Farmed deer

All individuals--The analyses of all the farmed animals pointed out two discrete subgroups of individuals (FSG 1 and FSG 2). FSG 1 comprised individuals with the lowest concentrations of most of the analysed elements, except Na and Ni, while FSG 2 comprised individuals with the higher element levels (Table 3). The formation of two discrete subgroups and the structural variations of the elements Ca, Cu, Fe, Mn, Mo, S and Zn appeared along the first principal component (PC1). Thus, these elements reflected best the differences between the subgroups (Fig. 4).

By median tests there were no significant differences between the subgroups with respect to the sex ratio, but animals of FSG 2 were significantly older (median=2.0 year) than individuals of FSG 1 (median=1.5 years)(p=0,0362).

Farmed subgroup 1 (FSG 1)--None of the elements in FSG 1 were well explained by the PCA (Fig. 5), but all of them, except Ni, were grouped together, which indicates positive interactions.

Farmed subgroup 2 (FSG 2)--The variations of the elements Mg, Mo, S, and P were best explained by PC1 (Fig. 5). The model revealed three clusters of elements: Cluster 1 comprised Cr, K and Na, Cluster 2 comprised Fe and Al, and Cluster 3 included the remaining elements, but with the essential elements Mn, Mg, Mo, P, S, and Zn as most influential. Elements within same clusters interacted positively, while elements from different clusters were independent or negatively interacting. Element interactions between Cluster 1 and Cluster 2 were either weak or absent. However, both interacted negatively with the essential elements within Cluster 3 (Fig. 5).

FSG 1 vs. FSG 2--Apparently the most biologically relevant differences between FSG 1 and FSG 2 refer to the Fe and Cu contents. The Fe levels (median ±S.D.) of animals in FSG 1 (54 ± 19 ppm) were significantly lower than the Fe levels (124 ± 108 ppm Fe) of free-ranging deer ($p < 0.0001$) while the Fe levels of animals from FSG 2 (212 ± 121 ppm Fe) were significantly higher than those from free-ranging deer ($p < 0.0001$). Simultaneously, the PCAs of FSG 1 and FSG 2 described Cu quite differently (Fig. 5). By the PCA of FSG 1 Cu was localised close to the most important model component (PC1) (Fig. 5) and hence Cu was an important element in describing the structural variations from all elements in this subgroup. Cu from animals comprising FSG 2, however, was by the PCA localised far from the most important model component axis (PC 1-axis), emphasising the minor influence from Cu on the model (Fig. 5).

Effects of Cu-categories on element profiles in farmed deer

{Cu} < 2 ppm--The elements Ni, Al, and Pb had no significant interactions with any other elements by the bivariate analyses (Fig. 6 A). The remaining elements interacted more or less positively. Comparing can recognise many similarities between PCA of FSG 1 and PCA of farmed deer with Cu-levels lower than 2 ppm (Fig. 5 and 6A). The two models had, however, different capacities to explain structural variations in the elements. PCA of FSG 1 explained less than 50 % of the structural variations in the elements by the two first principal components, while the PCA of the present Cu category of farmed deer described more than 50 % of the structural variations of all elements except Pb, Co, and Ni. Accordingly this model produced more evident element relations.

{Cu} € [2,3> ppm--Bivariate analyses revealed significant and negative relations between Cu - Na, and Ni - K (Fig. 6 B). The remaining elements comprised networks of more or less positively interacting elements, except the independent elements Pb and Se.

{Cu} € [3,4 > ppm--In the PCA of this group of farmed deer 6 significant negative element interactions appeared in addition to 10 positive interactions (Fig. 6 C). The main negative interactions appeared close to the first principal axis (PC 1), which included 40 % of the total structural variations in the data from this category. Hence, the negative interactions including Al and Fe appeared as most important and both of them had opposed positions to essential elements as Co, Cu, Na, Mg, P and S.

{Cu} € [4,5> ppm--The two first principal components (PC 1 + PC 2) expressed 39% and 31% of the total structural variations in the data (Fig. 6 D). Hence, both the components must be considered by evaluating the element interactions. The results of the PCA indicated the existence of four interactive element clusters. The cluster one which comprised Al, Cu, Fe, Na, and Ni correlated negatively with the cluster two of Co, Mg, P, and S. In addition negative interactions appeared between a cluster of Ca, Cd, Cr, K and Zn and the element Mo.

{Cu} > 5 ppm--The two first principal components (PC 1 and PC 2) described 39% and 33% respectively of the total structural element variations in this PCA. Hence, both the components had to be considered by evaluating the element interactions (Fig. 6 E). The PCA indicated the existence of four element clusters, which in couples interacted negatively. The main negative interactions appeared along the first principal component axis, comprised Mo, Mg, P, Ni and S at positive side and Na and Cd at the negative side of PC 1. The other couples of clusters included Al, Fe, Mn, Pb and Zn along positive side of PC 2 and Cu, K, Cr, and Co along the negative side of both PC 1 and PC 2.

Effects of different Cu levels on element interactions--An overall comparison of the different PCAs of Cu categories of farmed deer, revealed the tendency: increased Cu levels in the farmed deer coincide with decreased numbers of positive element interactions concomitant with increased numbers of negative element interactions (Fig. 6). The changes in the total element interactions from farmed deer revealed most evidently between individuals with less than 3 ppm Cu and individuals with more than 3 ppm Cu because of the dissimilar numbers of total negative interactions. Regarding the Cu interactions the greatest changes appeared in farmed deer between individuals with less than 4 ppm Cu and individuals with more than 4 ppm Cu because of the dissimilar numbers of negative interactions with Cu.

Comparisons between farmed and free-ranging deer

Elements that best describe differences between farmed and free-ranging deer--To find the elements that most significantly expressed the structural differences between free-ranging and farmed individuals with Cu levels between 4 ppm and 40 ppm a PLS was applied. The Y-variable contained information of the type of animal where "+1" identified the free-ranging individuals and "-1" identified the farmed individuals. The X-variables contained the results of the chemical analyses of 18 elements from each of the animals. Positive regression coefficients indicate higher values for that element in the free-ranging than in the farmed deer, and vice versa as regards negative regression coefficients. The resulting PLS model assigned significantly positive regression coefficient for Cu, and significantly negative regression coefficients for Al, Fe, and Mo (Fig. 7). The other elements displayed no significant influences on the structural differences between farmed and free-ranging deer.

Element levels in selected group--To smooth out some possible effects of the different Cu levels between farmed and free-ranging animals, selective comparisons were performed by exclusively including individuals of both groups with Cu concentrations between 5 and 25 ppm. This was performed by use of the Mann-Whitney U-tests. Despite the performed narrow selections of animals from both groups, significantly higher levels were revealed in the selected farmed deer concerning Al ($p= 0.0145$), Fe ($p= 0.0309$), Mo ($p= 0.0180$), and Pb ($p= 0.0025$), and significantly lower levels of Ca ($p=0.0394$), Cd ($p= 0.0146$), and Cu ($p= 0.0009$) compared with the free-ranging individuals. None of the other element concentrations differed significantly.

Farmed subgroup FSG 1 vs. free-ranging deer--Comparisons of the element medians obtained from animals in FSG 1 with element medians obtained from free-ranging deer by the Mann-Whitney U-tests revealed higher concentrations of Ni ($p=0{,}0099$) in the FSG 1 group. No differences between FSG 1 and free-ranging deer appeared in Al ($p= 0{,}8777$), Na ($p=0{,}9014$), and Pb ($p=0{,}4793$). All of the other elements were lower from FSG 1 compared with the free-ranging deer ($p<0.0001$).

Farmed subgroup FSG 2 vs. free-ranging deer--Comparisons of this subgroup with free-ranging deer revealed higher concentrations of Al ($p<0.0001$) and Fe ($p<0.0001$) in the FSG 2 subgroup. The concentrations of Ca ($p<0.0001$), Cd ($p<0.0001$), Co ($p=0{,}0399$), Cr (*p*<0.0001), Cu (*p*<0.0001), K (*p*<0.0001), Mn (*p*=0,0212), Mg (*p*=0,0007), S (*p*=0,0076), and Se (*p*=0,0029) were all lower in FSG 2. None of the other element levels differed.

DISCUSSION

Element levels

Free-ranging deer

The Cd, Mo, Pb and Fe levels measured in this study were similar to those that have been reported in other studies in red deer, while the levels of Cu, Se, and Zn were somewhat higher (*19-26*). The levels of Co, Mn, Mg, and Ni were apparently in accordance with results obtained from other ruminants (*1, 19, 21, 23, 26-32*). The levels of Al, Ca, Cr, K, and P measured in the present study were somewhat higher than those recorded in moose (*Alces alces*), reindeer (*Rangifer tarandus*), goat (*Capra hircus*), and sheep (*Ovis ovis* and *Ovis anios*) (*1, 28, 30, 31, 33-36*). No comparisons could be performed on Na and S due to lack of relevant data.

Of the forty-three free-ranging deer from the present study 93 % had adequate Cu levels in accordance with the classification by Wilson and Grace (*11*). Whether the recorded Cu levels may cause toxic effect is a difficult decision because each of the dietary elements Zn, Fe, Cd, and Mo may interact with Cu metabolically, and thus complicate the establishment of a definite maximum tolerable Cu level (*16*). The liver may accumulate large amounts of Cu before signs of toxicity appear (*37*). The median Cu level of animals (n=11) with Cu levels above 70 ppm was 82.7 ppm wm (Fig. 1). In most adult mammals the Cu concentrations in the liver are normally < 30 ppm wm (*38*). In cattle (*Bos taurus*), the hepatic concentration of Cu can be high, and especially high concentrations have been found in livers of sheep (*39*). According to Frøslie (*40*) 50 - 150 ppm of Cu in fresh sheep's liver indicate moderate overloading, and > 150 ppm Cu can induce haemolytic crisis. No Cu levels toxic to sheep were found in free-ranging red deer in the present study. But there are species differences in toxicity and it has been suggested that accumulation of Cu in the liver of moose from Finland is caused by poor ability to regulate Cu metabolism (*38*).

No individuals from the present study had liver Se concentrations below the assumed deficiency level of 0.05 - 0.1 ppm Se wm (*26*). Cd, Cu and Pb demonstrated consistent site-related trends in the livers of the free-ranging red deer. Unequal Cd levels in animals from different geographical locations are previously found (*41*) and in reindeer for Cd, Cu and Pb (*42*).

Farmed deer

The median levels of Cd, Fe, Mo, Pb and Se were within the range expected from previous work in red deer (*19-21, 25, 26*), but Pb and Cd concentrations were lower than those found in red deer in Spain (*24*). P concentrations were at same levels as found from livers of goat (*35*). The levels of Ni and Pb were within levels previously found in moose, goat and reindeer (*30*), while Cu and Zn were lower (*1, 28, 30, 35, 43*). The levels of Mg were also lower than those found in moose (*1*) and goat (*35*).

The levels of Al, Ca, and Cr were higher and Mn lower than previously found in other species such as moose (*1, 28, 30, 43*), reindeer (*30, 33*), goat (*35*) and sheep (*30*)

Cobalt does not normally accumulate in the foetal liver of ruminants, and McNaught (*44*) suggested that 0.013 - 0.0192 ppm wm or less in the livers of sheep and cattle indicate Co deficiency (converted to wet from dry mass), and that 0.026 - 0.039 ppm Co wm indicate a satisfactory Co status (*44, 45*). Only one of the farmed red deer had lower Co concentrations than the suggested threshold of Co deficiency.

The two studies by van Koetsveld (*46*) and Egan (*47*) revealed that ruminant liver Mn values below 10 ppm and 6 ppm dm, respectively, indicate deficiency, which on wet mass basis is equal to about 3.2 and 1.9 ppm wm respectively. Only 7 farmed deer of the analysed 52 had liver Mn concentrations exceeding 3.2 ppm and 37 % of the livers had lower Mn concentrations than 1.9 ppm. Underwood (*48*) believed that Mn concentration in the liver is a useful but not an entirely reliable indicator of deficiency, unless the deficiency is severe. The storage capacity of the liver for Mn is limited compared with its capacity to retain Cu, Fe, and Se, and the reproductive processes are particularly susceptible to lack of Mn (*49*). The low Cu levels found in the present study are reported in Rosef et al. (*14*). No comparisons could be performed on Na and S due to lack of relevant data from literature. Element interactions

Free-ranging deer

All individuals--By the multivariate analysis two apparently independent clusters of elements were formed (Fig. 2). One of them comprised elements known to be stored in livers and thereby promote harmful effects when percent abundantly such as Al, Cd, Cu, Pb and

Se (*13, 50-55*). The other cluster comprised essential elements not known to be extensively stored in livers, such as Co, Cr, K, Mg, P and S (*49, 56, 57*) (Fig. 2).

Due to the recorded dissimilarity in element profiles between animals with less than 40 ppm Cu and animals with more than 40 ppm Cu, the individuals were split in two groups:

{Cu} < 40 ppm--The relatively low influences by Cu in the PCAs of individuals with lower Cu concentration than 40 ppm, may indicate that the individuals had adequate Cu supplies. The potentially toxic elements Cd, Pb, Se, Ni and Al did not interact negatively with nutritional elements, which may indicate that they did not suppress the metabolism of these elements. The lower levels of Cd, Cu, Pb, and Se found in these individuals compared to individuals with higher liver Cu levels may elucidate their low influences (Fig. 1,3A,B).

{Cu} > 40 ppm--Concomitant with higher Cu levels, the animals with Cu levels 40-70 ppm also had higher levels of Cd, Pb and Se compared with animals from the proceeding group. The storage of Cu are primarily in metallothioneins (MT) and superoxide dismutase (SOD) (*58*). Normally, concentrations of MT are low in tissues; however, Cu, Zn, and Cd can cause increases in tissue concentrations of MT (*59*). The sulfhydryl-rich structure of MT has led to experiments designed to investigate its potential as a binding moiety and perhaps detoxifier of hepatotoxins (*60*). In the literature many interactions including Cu, Cd, Pb and Se are demonstrated, but the underlying mechanism is not fully understood. It has been shown that Se has strong tendencies to complex with other metals, such as Cd and Cu, and thereby exert protective effects against toxic influences (*49, 61-66*). From a study of rats it was concluded that the amount of Se in the diet determined whether or not an increase in dietary levels of Cu affected the metabolism of Se (*67*). The presumed protective effect of Se against Cd toxicity is from analyses of humans found to be the result of the diversion in their binding from low to high molecular weight proteins (*66*). In general, the liver is thought to be the first storage site for Cd where it may be bound to a MT, then transported to the kidney which is the final and main storage site for Cd (*68*). Cd and, to a lesser extent Pb, have been shown to affect both the metabolism and absorption of minerals and trace elements like Fe, Cu, and Zn (*69-75*). It is assumed that MT induction is part of a defence mechanism in which cellular metals are conserved during reduction in the dietary supply, and estimates suggest that MT is a fairly rapidly turned over protein, but the exact functions of MT in livers remain to be defined (*76*). Decreased food consumption might also be expected to concomitantly induce liver MT (*76*). The appeared antagonisms between the potentially toxic elements Cd, Cu, Pb

and Se and the essential elements P, S, Mn, Mo and Co seen from the multivariate analyses, may have arisen as a consequence of the higher levels of the mentioned potentially toxic elements compared to animals with less than 40 ppm Cu. Since turn over of MT is supposed to happen rapidly and also dependent on the nutritional status of the animals, there may also exist a homeostatic mechanism that reduce the MT content including the metals bound to it when animals ingest adequate essential elements combined with reduced absorption of potentially toxic elements. If so, the negative interactions may have appeared due to different nutritional status of animals with more than 40 ppm Cu.

When dietary supply of Zn or Cu is sufficiently elevated, induction of MT is found to occur, primarily in the liver and intestine with a succeeding accumulation or redistribution of these metals in both tissues (*76*). Intracellular Zn and Cu are presumed partitioned in two general ways, i.e. as components of metalloenzyme systems and, at least in the case of Zn, with macromolecules, particularly membranes (*76*). Hence, this may explain the independence between Cu and Zn recognised from animals with higher Cu levels than 40 ppm.

While all of Cd, Cu, Pb and Se interacted negatively with essential elements in animals with Cu levels 40-70 ppm, the negative interactions of Cu with Cd and Se prevailed the structural variations of animals with more Cu than 70 ppm. Because the two Cu categories solely had different Cu levels, the concurrently changed Cu interactions may have arisen from this difference. When Cu exceeds certain levels in the body, the MTs are thought to prevent the development of Cu toxicity by binding Cu and slowing the release of Cu into the blood stream (*58*). When MT and other ligands that normally sequester Cu are saturated, additional Cu will complex with and damage microsomes and other proteins (*58*). The results indicate that Cu levels above 70 ppm exert toxic influences, and the elucidation to this may be that the mechanism of sequestering of Cu in these animals was saturated or inadequate. In these animals both Cu and Fe also exerted negative interactions with essential elements (Fig. 3 D). Negative interactions between Fe and Mn, and between Na and K, as recorded from animals in this Cu category, are also described in previous studies (*77-79*).

The significant and positive relations among Pb, Cd and Se, may be associated with published findings of several Pb-binding fractions in the cytosol of livers, where most of the Pb was associated with high molecular weight protein fractions (*55*). From animals with higher Cu than 40 ppm, Pb was negatively correlated with Mo. It has been found that Pb affects absorption or distribution of Se, and that dietary Pb influences the level of Mo in tissues (*80-*

82). As dietary Se given to Pb-exposed rats was increased from 0.015 to 0.5 µg/g, tissue Pb and urinary ALA concentrations decreased, and blood and liver ALAD activity increased (*55*). With Se at 1.0 µg/g diet, tissue Pb concentrations increased, as did urinary ALA and the inhibition of ALAD (*55*). These effects of high Se have been reported in other work with rats *(80)*. The described influences between Se and Pb may elucidate the revealed positive interactions concurrently as the animals with more than 40 ppm Cu also had relatively high levels of Se.

The recorded dependencies among element levels and interactions shown in present and former works, render evident explanations on the element interactions difficult, because transfer of knowledge on element interactions between studies presuppose equivalence in element levels.

In animals with more than 40 ppm Cu most of the elements Cu, Cd, Se and Pb are probably sequestered in binding fractions in the liver to reduce deleterious effects. By the comparisons of farmed deer results with results obtained from free-ranging deer with less than 40 ppm Cu, the strong influences of high levels of Cu, Cd, Se and Pb are avoided.

Farmed deer

All individuals--The findings of two discrete subgroups by the analyses of farmed deer, resulted in separations of the individuals in subgroups FSG 1 and FSG 2 (Fig. 4).

Farmed subgroup 1 (FSG 1)--The animals in this subgroup had consistently lower levels of all analysed essential elements both compared to free-ranging deer with less than 40 ppm Cu and farmed individuals in FSG 2. The Cu levels of 1.5 ± 0.7 ppm (mean ± SD) found in animals in FSG 1 are similar to those associated with Cu deficiency with extensive health effects in red deer (*3, 6-8, 10-13*). Common clinical signs of Cu deficiency in domestic and wild ruminants are loss of appetite and weight, leading to emaciation (*83*). Low Cu in the body may affect the metabolism of many known Cu proteins, as: 1) the ferroxidase activity of ceruloplasmin, 2) the monoamine oxidase enzymes, 3) lysyl oxidase and 4) the enzymes cytochrome c oxidase and 5) SOD (*16*). The effects by low Cu levels on element profiles may therefore be influenced by many biochemical mechanisms. A study by Wolkers et al. (*84*) of the effect of long-term dietary restriction on the composition of the liver in red deer, revealed that malnutrition was associated with a reduction in the total body mass and a relatively large reduction in protein and liver mass expressed by cell size as well as cell number. The most structured variations that appeared in FSG 1 coincided with the individual variations in the total contents of elements, except Ni. This subgroup of farmed deer was probably underfed caused by low Cu loads.

Farmed subgroup 2 (FSG 2)--The comparisons of FSG 2 with free-ranging deer revealed concomitant findings of the:

- higher levels of Al, and Fe,
- negative interactions exerted by both Fe and Al on the essential elements, especially S, Mo, Mg and P,
- lower levels of essential elements, as Ca, Co, Cr, Cu, K, Mn, Mg, S, and Se,
- relatively modest influence on the model by Cu, and
- negative interactions exerted by Na on essential elements (Fig. 5).

This group of farmed deer had mean Cu levels of 5.0 ± 3.9 ppm (mean ± SD), which indicates that most of the animals had marginal but not deficient Cu levels (Table 3) (*11*). However, some individuals within the FSG 2 group also had Cu levels that could cause Cu deficiency (Table 3). But as a group the Cu supplies did not appear as a critical factor in the multivariate screening of the element profiles (Fig. 5).

Fe is an essential element required for the synthesis of haemoglobin and myoglobin and the many Fe-containing enzymes, which are necessary for normal cellular function (*85*). Nevertheless, when there is excessive accumulation in the body (Fe overload), Fe displays toxic properties, producing widespread cellular damage owing mainly to the production of toxic free radicals, which can cause lipid peroxidation and other oxidative damage to cellular constituents (*86, 87*). Increased accumulation of tissue Fe has been associated with pathogenesis in a variety of diseases although the extent of any toxicity will, in part, be dictated by the localisation of the Fe complex within the cell, i.e., ferritin or haemosiderin, as well as the ability of the cell to prevent the generation and propagation of free radical species by the wide range of antioxidants and cytoprotective enzymes present in that cell (*88, 89*). Because liver cells have high antioxidant protection they normally will be less susceptible to Fe induced oxidative stress than cells with less protection (*90*). The animals in FSG 2 had higher levels of Fe (239 ± 121 ppm, mean ± S.D.) than the free-ranging deer (145 ± 108 ppm, mean ± S.D.). This in addition to the recorded negative interactions with Fe involved as shown by both the multivariate and the bivariate analyses, may indicate that Fe exerted restrained influences on the metabolism of essential elements such as Mg, S and P. It is assumed that Fe is regulated in the body by a mechanism where relatively high loads of liver Fe stimulate synthesis of ferritin, at the same time as the ability of transferrin to bind Fe is degraded (*90*). The Cu containing enzyme ceruloplasmin oxidises Fe^{2+}, which is first liberated in this form from the depot tissue, to the Fe^{3+} state and thereby makes possible its transfer to transferrin (*91*). Hence the mobilisation of Fe from storage sites in mucosa and liver does not occur at a normal rate during Cu deficiency with resulting anaemia as a consequence (*92*). It has been found increased Fe storage in various organs of animals with Cu deficiency, while the haematological parameters such as haemoglobin concentration, haematocrit, and erythrocyte count were greatly decreased (*93*). Since ceruloplasmin responds fast to changes in Cu supply, it is understandable that the deposition and mobilisation of Fe in the liver is affected with similar speed (*94-97*). Fe accumulation resulting either from dietary Cu depletion or from Zn-induced Cu-deficiency has been shown to normalise again with dietary Cu supplementation (*98, 99*). Hence antagonistic interactions between Fe and Cu could be expected from the multivariate models of farmed deer. In absence of such evident interactions, however, other elements in addition might affect the interactions.

The relatively high Al loads found in some livers of farmed deer, at the same time as the multivariate models revealed positive correlations between Al and Fe, makes it reasonable to focus on Al. Coincidental increases in liver levels of Al and Fe have been reported in sick moose from Sweden (*1*), in sheep (*100*), and in rats (*101*). The metabolism of Fe has been shown to interact with that of Al in relation to intestinal absorption, transport in the blood plasma, and the induction of lipid peroxidation and cellular damage, but Morgan (*85*) found little evidence for interaction between Fe and Al metabolism in rats. Although Al has been implicated in various neuropathological states with ageing due to its involvement in neurotoxicity, the exact role of the metal ion is still unclear, but Al is not considered as an essential dietary compound (*102, 103*). In a study of growing rats Boudey (*104*) found that side effects of small variations of Al intake can be enhanced when they are combined with other mineral imbalances. One known consequence of Al toxicity is anaemia, due to the inhibition of enzymes in the heme biosynthetic pathway (*105*). Even in absence of signs of anaemia, ingested Al may depress haematopoiesis by affecting red blood cell production and cell destruction (*106*). Both Al and Fe can bind to the protein transferrin, although transferrin has a lower affinity for Al than for Fe, it is believed to be the major Al transport protein (*101,* *107-113*). Al has been shown to inhibit the ferroxidase (ceruloplasmin) activity and it has been suggested that Al might prevent the unloading of transferrin Fe onto a specific tissue or receptor or else be unloaded instead of Fe^{3+} (*114, 115*). Al is also suspected to promote stimulatory effect on Fe^{2+}- initiated lipid oxidation by inhibition of the autooxidation of the ferrous ion (Fe^{2+}) (*116, 117*). The combined effects of relatively high liver Al concentrations in some of the farmed deer, which may block the ferritin synthesis and relatively low levels of available Cu, which may block the synthesis of ceruloplasmin, may exert disorders in the metabolism of Fe. The higher levels of Al and Fe and their corresponding negative interactions on essential elements e.g. Mg, Mo and S, as found from FSG 2 compared with free-ranging animals, have not been reported previously as structurally differences between farmed and free-ranging red deer.

Effects of different Cu levels of farmed deer

Comparisons of different Cu categories of farmed deer with free-ranging deer (Cu < 40 ppm) revealed four tendencies:

- essential element level differences decline when categories of farmed deer with increasing Cu levels up to 5 ppm are compared with free-ranging deer,
- higher levels of the potentially toxic elements Al, Fe, Mo, Ni, and Pb in farmed deer with Cu levels above 4 ppm compared to free-ranging deer (Fig. 1),
- lower levels of Ca, Cd, Cu, and K in farmed individuals with more than 4 ppm Cu than in free-ranging deer, and
- the significantly negative influences by Na on Mg, Mo and P in animals with more Cu than 4 ppm.

The approximations in levels of essential elements between farmed and free-ranging individuals with increasing Cu concentrations in the farmed deer may concurrently indicate approximations between the two groups regarding the metabolism of the essential elements (Fig. 6). However, in both the PCAs of farmed deer with Cu more than 4 ppm, also Na was designated as an evident antagonist to the essential elements P, Mg and Mo which appeared along the first principal component. Generally, Na and P are considered the minerals most limiting to growth and reproduction of mammalian herbivores world-wide and earlier studies from the Netherlands, suggested that red deer, as a consequence of poor mineral availability, were in Ca, P, and Na stress (*21, 118, 119*).

The analyses of farmed deer with Cu levels above 4 ppm indicated negative interactions between Cu and S, Mo and Zn (Fig.1, 6 D,E). Earlier work has also documented that high levels of Mo may exert negative influences on the metabolism of Cu (*13*). Mo tends to accumulate in plants grown in poorly drained soils, and in the rumen, S and Mo combine to form a thiomolybdate complex, which is able to irreversibly bind Cu, rendering it unavailable for absorption (*120*). While it has been shown that thiomolybdate or derivatives reduce the absorption of dietary Cu from the gut, there is also evidence that the compounds can be absorbed and do affect Cu metabolism systematically (*81, 121-125*). In addition a number of Cu enzymes, including ceruloplasmin, cytochrome oxidase, superoxide dismutase and tyrosine oxidase have been shown to be inhibited (*126, 127*). The formation of trithiomolybdate by ruminants is likely to be the key event in the biochemical pathogenesis of the widespread Mo-induced syndromes (*128*). While the changes which occur are complex they can be best understood in terms of an alteration in the affinities for Cu of some of the competing ligands which leads to a change in distribution of Cu between the different systemic pools and an overall depletion (*128, 129*). The binding of Cu to thiomolybdates is even stronger than that to metallothioneins, which are the principal Cu-binding protein in tissue (*1, 130, 131*). When thiomolybdate forms, total Cu concentrations may overestimate the functional Cu status in tissues, thus further complicating the diagnosis of Cu deficiency (*132, 133*). It has also been shown in lambs that Mo enhances the Cu excretion (*134*). The significant negative interactions Cu-Mo-S seen from farmed deer with Cu above 5 ppm (Fig. 6) are probably related to influences of thiomolybdates, and hence the Cu concentrations may overestimate the functional Cu status in tissues (*132, 133, 135*). In the free-ranging deer, however, no bivariate correlations between Cu and Mo revealed from the present analyses of different Cu categories, probably as a consequence of low influences by thiomolybdates.

Ni occurs in low concentrations in all animal tissues and fluids without remarkable concentration in any tissue or organ, but without any firmly established biological functions (*136, 137*). However, it has been shown that Ni deficient animals had impaired utilisation of Fe, concurrently with changed trace element profiles in livers (*136*). Ni interacts synergistically with Fe to affect haematopoiesis in rats fed dietary Fe^{3+}-sulfate only, but not as a mixture of Fe^{3+}- and Fe^{2+}-sulfates (*138, 139*). On the other hand, when a ferric-ferrous mixture was supplemented to the diet, Ni deprivation elevated the liver content of Fe (*140*). In farmed deer Ni is positively correlated with Cu in animals with Cu levels 4-5 ppm, and both elements correlated positively with Fe by the multivariate model of these animals. But these interactions were not recorded from the other farmed animals and the mechanism underlying these influences by Ni remains unknown.

Differences between farmed and free-ranging deer

The farmed animals with lower Cu contents than 4 ppm which comprised about half of the analysed farmed animals in this study, suffered probably mainly of Cu deficiency. A PLS of individuals with Cu concentrations between 4 and 40 ppm, revealed that the structural differences between farmed and free-ranging deer were best expressed by higher Al-, Fe- and Mo- levels and lower Cu-levels in the farmed deer (Fig. 7). This result in addition to the recorded strong influences by Na on the essential elements in these animals may indicate that the farmed animals have unsatisfactory composition of dietary minerals. Restrained effects on the metabolism due to some of the described element interactions are probably not prevented only by giving the farmed deer artificial Cu supplies. But some of the negative influences on the essential elements seemed to be diminished by an increase of Cu levels in farmed individuals with lower Cu contents than 4-5 ppm. In animals with higher Cu contents, however, the screening of element profiles revealed much more complex relations, caused by many negative element interactions including those apparently important influences exerted by Al, Fe, Mo and Na. The differences between free-ranging and farmed red deer from the present study share signs of equality with changes regarding Cu, Fe and Mo found in moose from Sweden where Frank et al. suggested that the changes were mainly caused by increase in the formation of thiomolybdates which bind Cu (*1, 141*). However, the higher levels of Al found in the present study of farmed deer are not exclusively elucidated by the incidence of thiomolybdate alone.

Management implications

The present comparisons of farmed with free-ranging red deer revealed pronounced differences in the concentrations of most elements. In farmed animals with presumed adequate levels of liver Cu (> 4 ppm) the levels of the potentially toxic elements Al, Fe, Pb, Mo and Ni were considerably higher and the levels of the essential elements Ca, Cu, K, and Na correspondingly lower than those in free-ranging deer. Why these differences appeared, is not clear. One possible explanation is ingestion of mineral particles from soil and water. Wild ruminants may compensate for soil deficiency of particular micronutrients due to a more varied diet than confined ruminants restricted largely to grass forage and thereby prevent element deficiencies and imbalances (*142*). Skipworth (*143*) suggested that animals may consume soils as a source of trace elements when vegetation lacks these elements. But from many studies deliberate ingestion of soil by animals is related to Na hunger (*144, 145*). A study on moose from areas where Na concentrations in terrestrial plants were significantly below the dietary requirement, while levels were sufficient in aquatic plants but coincidental with high concentrations of toxic heavy metals, the physiological need for Na was the anticipated cause of overintake of heavy metals (*146*). Consumption of submerging aquatic plants by North American moose has also been linked to Na hunger (*147*). Salt intake of grazing animals is often influenced by environmental conditions (temperature, rainfall), pasture management (grazing intensity, fertiliser application), and biological factors (maturity stage, selective grazing, species or breed etc.) (*148*). The strong negative relations that Na and K exerted on the nutritional elements P, Mg, Mo and Zn in farmed animals (FSG 2) may be elucidated by disturbances in Na-K relationsships leading to Na hunger in some of the animals.

Animals may also ingest soil inadvertently as by soil particles adhering to the ingested plant material (*144, 149*). Stark (*150*) suggested that the mean intake of soil by grazing animals was likely to be about 10 % of total diet dry matter intake. In a study performed by Arthur and Gates (*151*) the soil ingestion by pronghorns (*Antilocarpa americana*) represented more than 50 % of the daily dry mass intake of Fe and Na, and more than 30 % of the daily intake of Cr and Mn, while vegetation ingestion resulted in greater than 90 % of the daily intake of Ca, Cu and P. Estimated rates of soil ingested by free-ranging elk (*C. elaphus*), moose and mule deer (*Odocoileus hemionus*) were less than 2 % of diet in dry mass in all three species (*144*). This may indicate that browsers like red deer in Norway, normally have low rate of soil ingestion. Concurrently the evolution of these species may have resulted in less tolerance than species normally exposed to higher rates of soil ingestion.

Soil ingestion depends on many factors including soil type, weather conditions, type of sward, pasture availability, stocking rate, type of animal and grazing behaviour (*152*). Higher exposure to Al and Fe by ingestion of soil particles may occur at sites where heavy grazing and trampling pressure concurrently have reduced the plant layers so the animals ingest increased mass of soil particles while grazing (*92*). In the domesticated species sheep, cattle and swine soil was found to be the main source of exposure to environmental contaminants, e.g. Pb (*153-157*). Ingestion of soil particles may also contribute to high Al concentrations in the body as Al occurs abundantly in soil (*115*). Al -hydroxide and -oxides are 100 - 1000 times more soluble at pH 4.2 than at pH 6.2 - 8.1. It has been suggested that Al might be more readily absorbed in the stomach or proximal duodenum, and gastric pH might have a major effect on Al absorption (*115, 158*). Direct soil consumption by grazing animals can result in adverse effects due to high intakes of both Fe and Al (*159*). Lambs exposed to high dietary levels of Fe and Al showed reduced food consumption (*159*). Rosa also found that added dietary P reduced hepatic Fe while high dietary Al increased liver Fe concentrations. These interrelations among Al, Fe and P from lambs coincide with findings in the present study of farmed deer, but not of free-ranging deer. The animals may also ingest Al and Fe by drinking water with suspended soil particles or ingestion of aquatic plants from puddles in the enclosures.

The relatively lower concentrations of Cd in farmed deer is probably related to nutritional differences. Contaminated dust may accumulate on the plant surface over years (*160, 161*). Free-ranging animals ingest to a larger extent older plants which may accumulate Cd in stems and leaves, in contrast to farmed deer that feed mainly on young plants and hey with a growth time of less than 2 months, resulting in lower ingestion of Cd (*20*).

The Cu-Fe interaction is of practical significance because in ruminants exposed to high Fe intakes from the two sources, ingested soil or mineral supplements, the Fe will inhibit Cu absorption (*132*). In both cases the Fe is present largely in insoluble forms such as Fe oxides and it has been assumed that compounds such as Fe_2O_3 were inert (*162*). However, Fe_2O_3 inhibits Cu absorption when added to the diet of sheep, and Fe-rich soils have a similar effect (*163, 164*). The message is clear: in areas where hypocuprosis is likely to occur, the risk will be reduced by avoiding the use of mineral supplements of high Fe content, minimising the use of bare winter pasture and avoiding the excessive contamination of silage with soil during harvesting (*165*). The latter point is confirmed by the observation that 30 per cent reduction in the availability of Cu from silage to which soil was added raised its Fe concentration by 2.35 g/kg dm (*162*). The mechanisms for the Cu-Fe-antagonism is not fully understood, and Suttle et al. (*164*) showed the soil Fe effect to be S-dependent, like the Cu-Mo antagonism, and associated with enhanced rumen S^{2-} concentrations; they postulated formation of insoluble CuS following the trapping of S^{2-} as FeS in the rumen (*132*). Surprisingly, wild ruminants appear to be better adapted to low-Cu diets than most domesticated ruminants, as the required level of Cu in the liver tissue of sheep and cows (35 mg/kg dm) is higher than that for deer (10-20 mg/kg dm) (*3*). This may in general be related to a higher intake of soil particles by farmed animals combined with a less diversity of diet qualities compared with free-ranging animals. Several studies on cattle and sheep have shown that under lush vegetational conditions 1-2 % of their diet is soil, but when forage is sparse the value may be as high as 18 % (*166*). Because free-ranging red deer ingest low rates of soil particles they also may have developed low tolerances to such particles. If this is correct farmed red deer may be considerably more vulnerable to sparse vegetational conditions than domestic animals.

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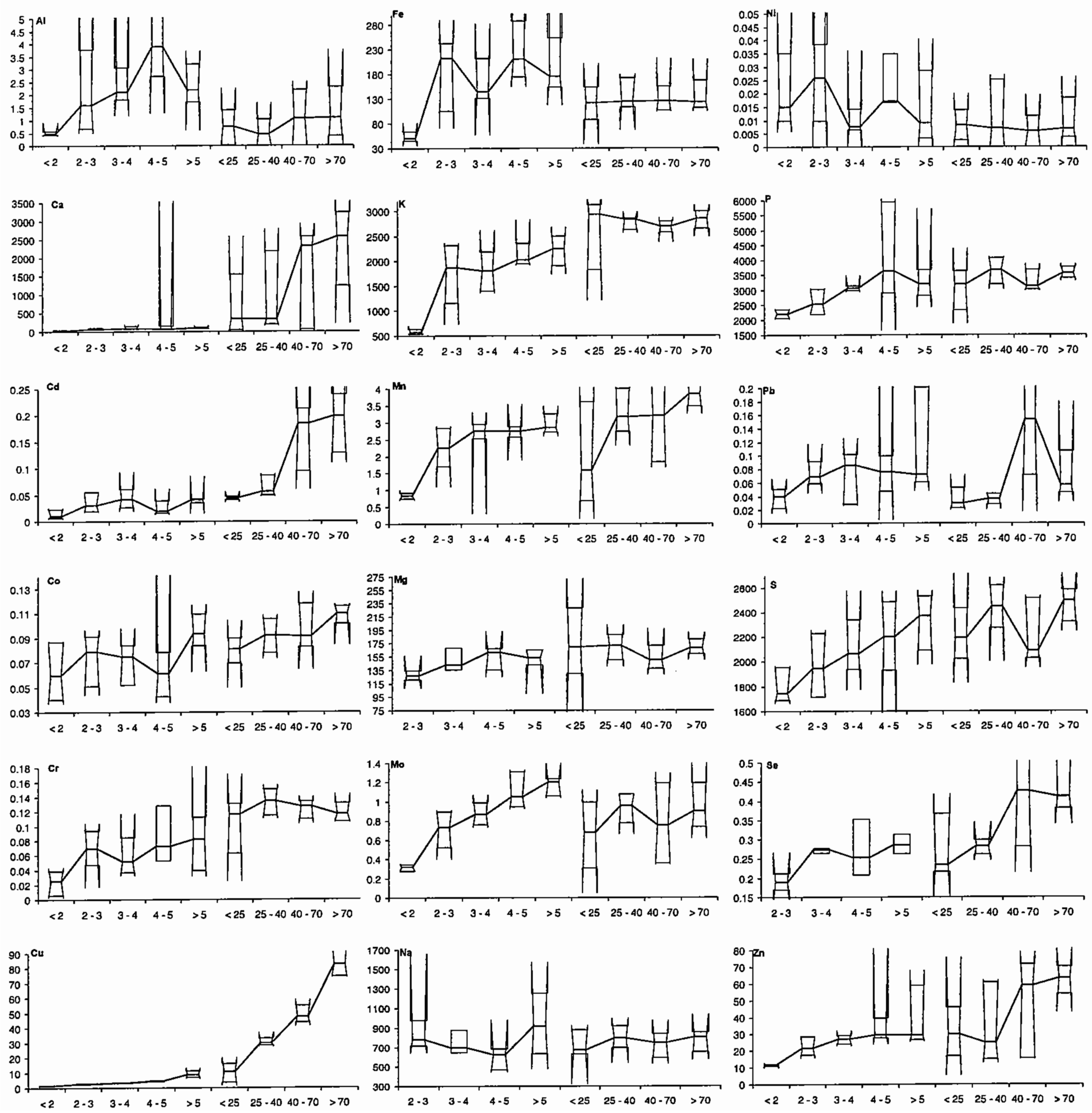

Fig. 1 Box plots of Cu-categories of farmed and free-ranging deer from each liver element

The liver elements are indicated at top of the Y-axis. The box-plots graphically show the median value of liver elements indicated by horizontal lines. The medians of farmed and free-ranging deer, respectively, are connected. The X-axis show the Cu category of farmed deer (< 2, 2-3, 3-4,4-5 and > 5) and of free-ranging deer (<25, 25-40, 40-70 and >70). The Y-axis show the liver element concentrations in ppm wm. The notched box shows the median, lower and upper quartiles, and the confidence interval around the median.

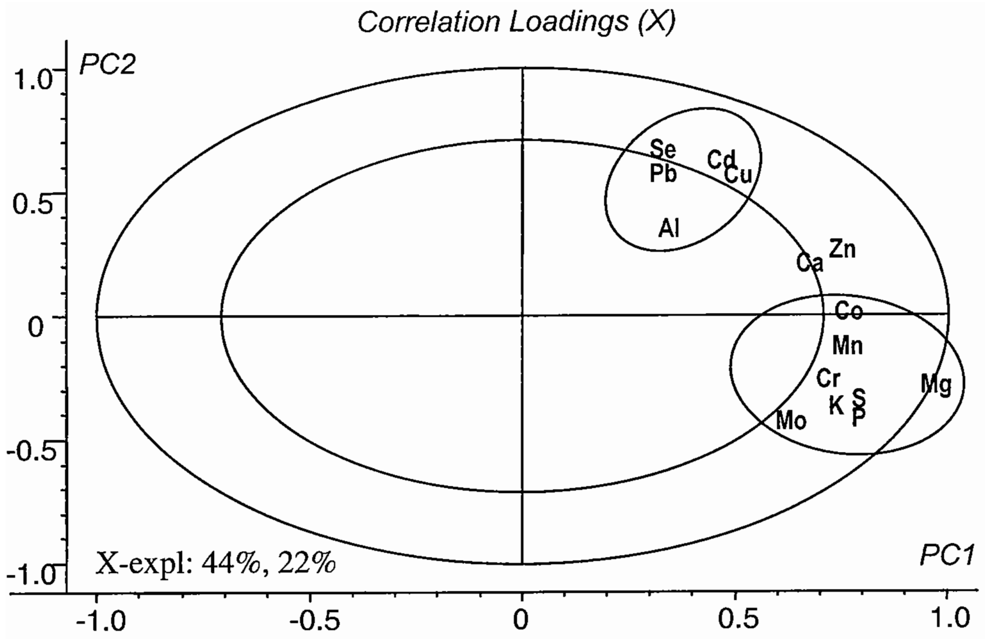

Fig. 2 Liver element loadings by a PCA of free-ranging individuals. Elements close to each other will have a high positive correlation if the two components explain a large portion of the variance of the elements. The same is true for elements in the same quadrant lying close to a straight line through the origin. Elements in diagonally-opposed quadrants will have a tendency to be negatively correlated. There seems to be some degree of independence between the cluster comprised of Mg, Mo, K, S, P and Cr, and the cluster comprised of Se, Pb, Cd and Cu, because the two clusters have independent variations and were localized orthogonally in proportion to origin. The two concentric circles show the correlation loadings by the 50% (inner) and 100% (outer) of explained variance limits. Elements inside inner circle are explained with less than 50 % of their structural variations, and elements close to origin are poorly explained by the two principal components. PC 1 and PC 2 explain 66 % (44% + 22%) of the total structural variations in the data.

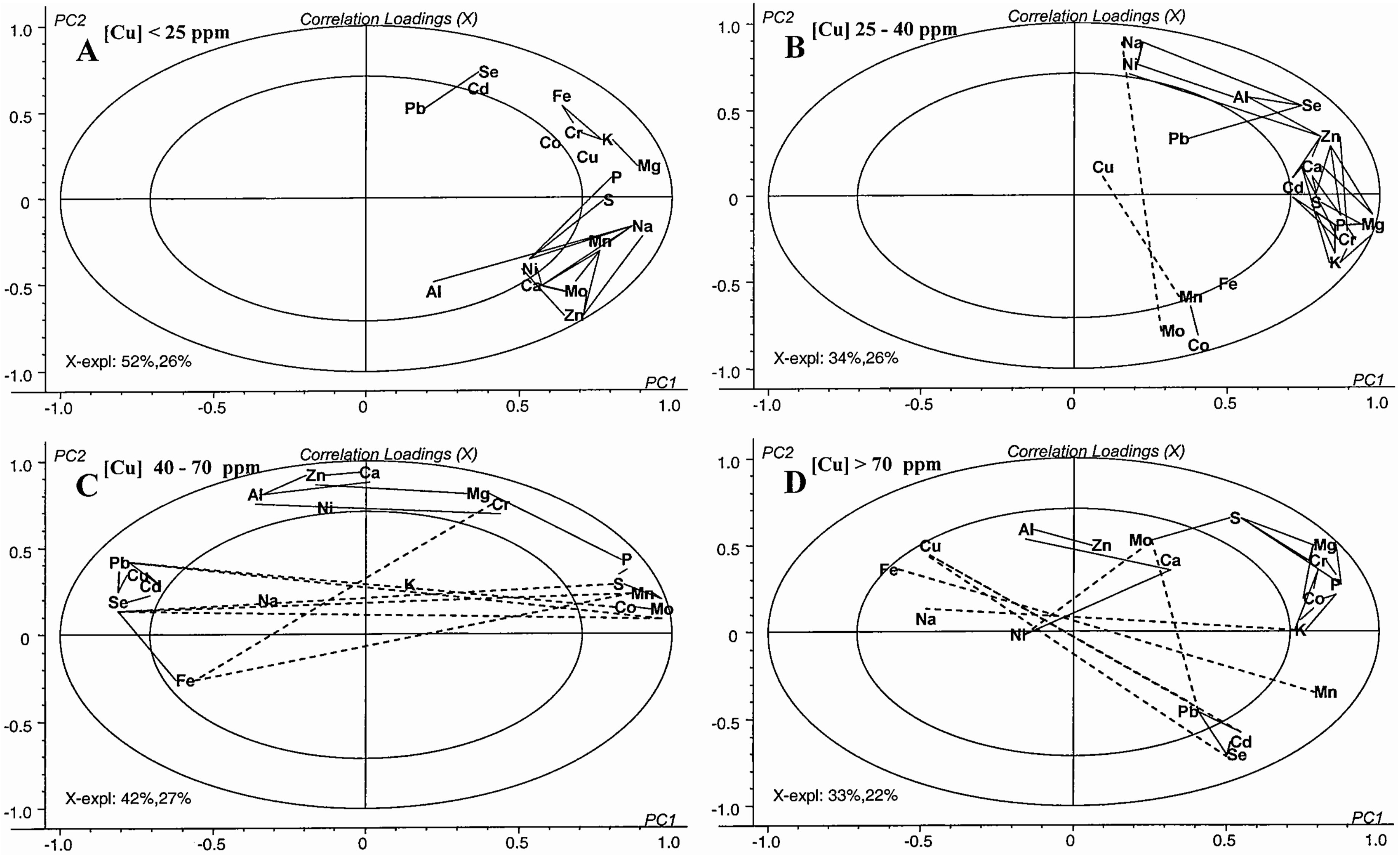

Fig. 3 Combined results of PCA and bivariate correlation analyses of free-ranging deer. For explanations see Fig. 2

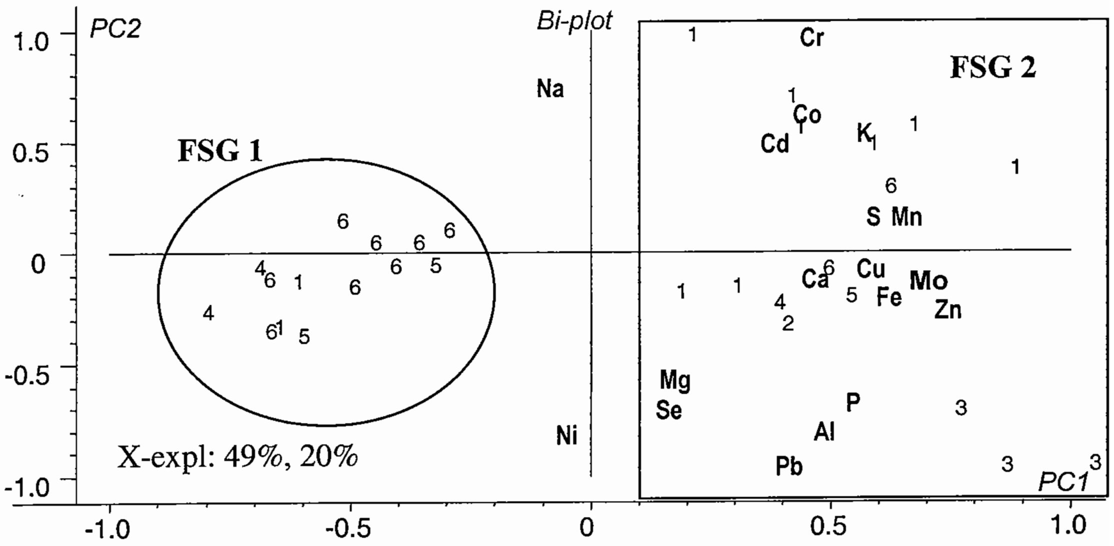

Fig. 4 Loadings of liver elements and scores of individual results showed simultaneously by the PCA of farmed deer. Elements located close to each other were positively correlating, as the the cluster of Cd, Co, Cr and K or the cluster of Cu, Mo, Fe, and Ca show. Because of the opposite positions of Na and Ni, these elements were negative correlated. Because the animals included in this analysis had a bimodal distribution, they had to be treated as subgroups of farmed deer and denoted as FSG 1 and FSG 2. The subgroup of farmed deer comprised of FSG 2 had higher levels of all liver elements located at positive axis of PC1, consequently the subgroup FSG 1 had lower levels of the same elements because of its opposite positions on PC1-axis.

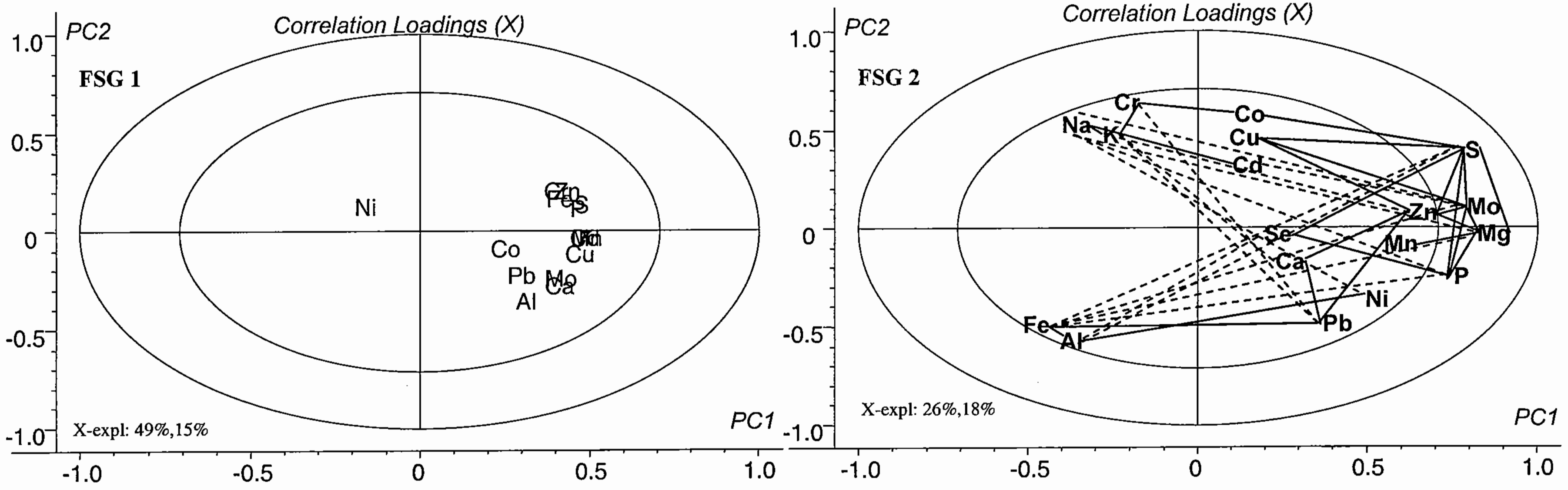

Fig. 5 PCA of the subgroups of farmed deer (FSG 1 and FSG 2) and bivariate correlations of FSG 2. For explanations see Fig 2.

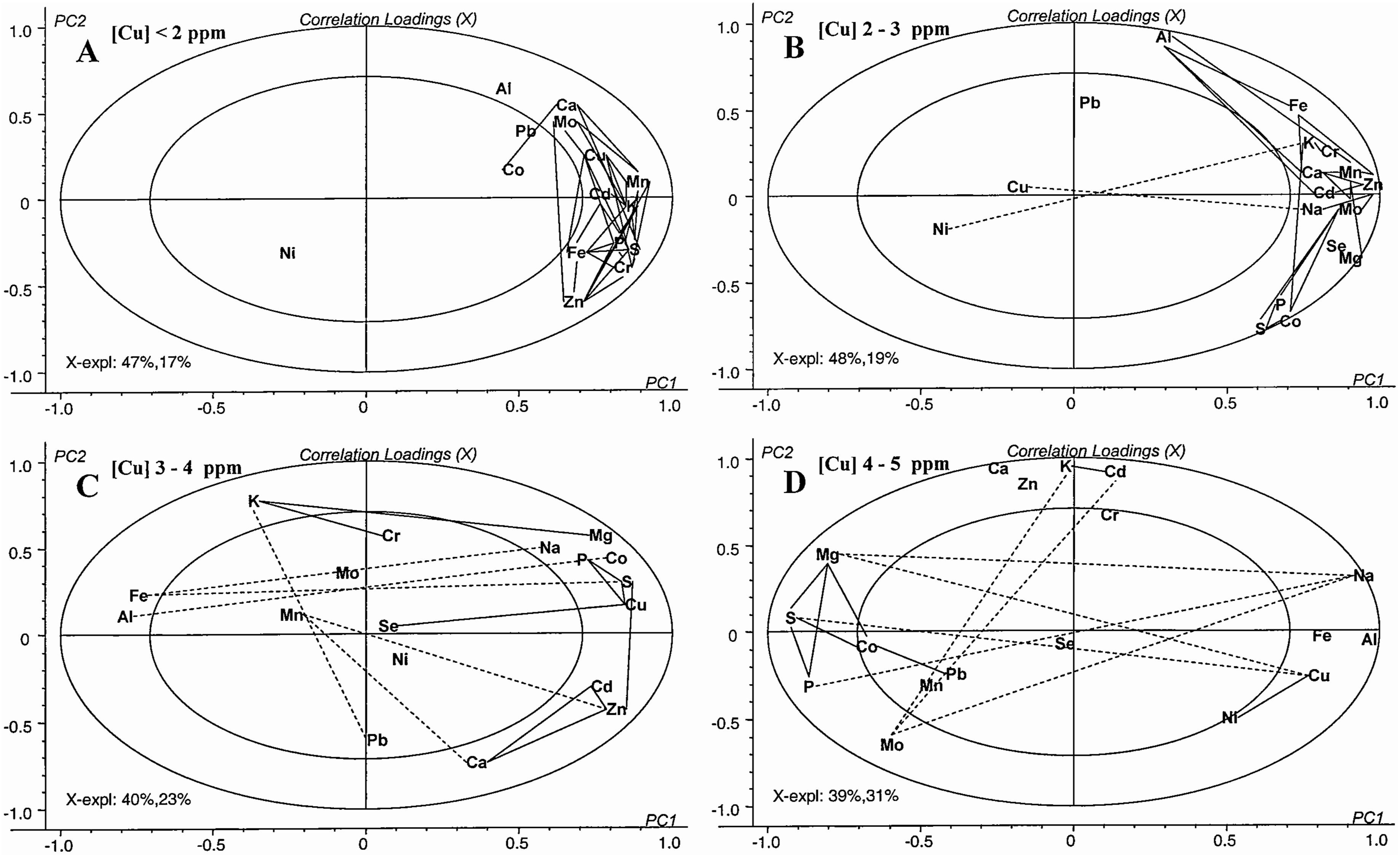

Fig. 6 Combined results of PCA and bivariate correlation analyses of farmed deer. For explanations see Fig. 2.

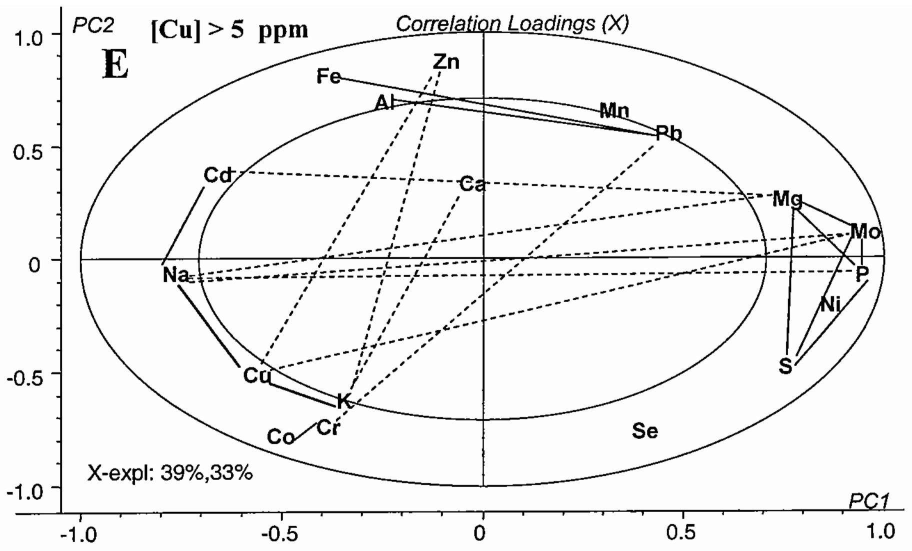

Fig. 6 continued

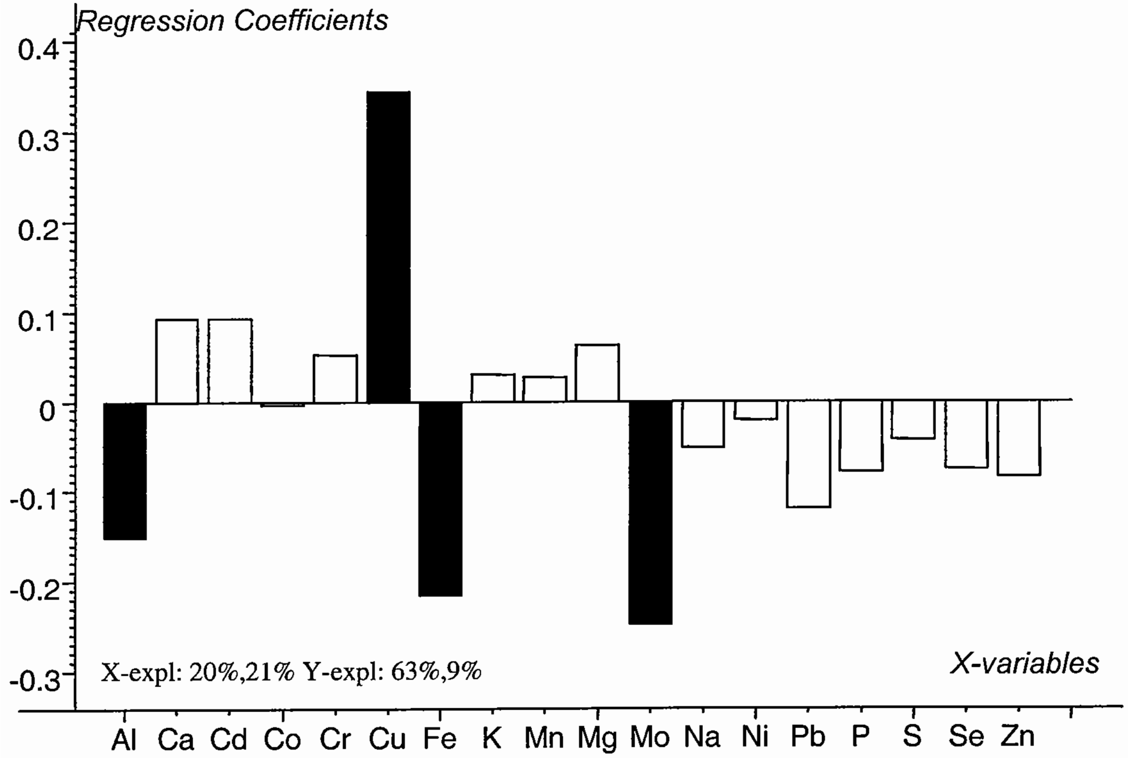

Fig. 7 PLS 1 The power of the elements to describe differences between farmed and free-ranging individuals with [Cu] $\in$ [4,40] ppm. Black columns indicate significant elements, while empty columns indicate not significant elements.

Table1 Liver elements in free-ranging and farmed deer. Mean, Median, SD, Lowest and Highest values in ppm.

	Free Ranging Deer						Farmed Red Deer						*M-W U*
	n	Mean	Median	SD	Min.	Max.	n	Mean	Median	SD	Min.	Max.	*p =*
Al	43	1.3	0.8	1.8	0.0	9.8	49	3.7	1.8	5.2	0.0	23.0	*0.0019*
Ca	43	1472	1274	1360	0	4051	52	148	71	499	2	3657	*< 0,0001*
Cd	43	0.123	0.087	0.104	0.010	0.476	52	0.035	0.027	0.028	0.003	0.137	*< 0,0001*
Co	43	0.094	0.092	0.026	0.028	0.171	44	0.073	0.075	0.031	0.008	0.183	*0.0003*
Cr	43	0.118	0.129	0.040	0.002	0.184	37	0.060	0.044	0.043	0.003	0.183	*< 0,0001*
Cu	43	44.2	39.2	28.7	0.8	111.6	52	4.0	3.0	3.6	1.0	21.8	*< 0,0001*
Fe	43	145	124	108	27	754	45	168	155	129	30	726	*0.3925*
K	43	2665	2798	719	536	4377	52	1669	1902	838	482	4021	*< 0,0001*
Mn	43	3.1	3.6	1.4	0.0	6.1	52	2.1	2.6	1.1	0.0	4.2	*< 0,0001*
Mg	39	167	170	41	56	272	28	150	141	36	100	302	*0.0024*
Mo	43	0.82	0.90	0.38	0.05	1.44	52	0.78	0.84	0.38	0.10	1.50	*0.4637*
Na	39	768	777	192	321	1234	28	831	715	332	446	1661	*0.9797*
Ni	42	0.010	0.007	0.011	0.000	0.045	36	0.024	0.016	0.037	0.000	0.218	*0.0211*
Pb	42	0.090	0.046	0.114	0.005	0.566	47	0.082	0.066	0.071	0.006	0.353	*0.316*
P	43	3462	3495	799	1023	5570	52	3078	2904	1171	1673	6276	*0.0004*
S	43	2358	2375	405	1067	3592	52	2058	1973	359	1379	3037	*< 0,0001*
Se	39	0.40	0.34	0.23	0.15	1.32	22	0.27	0.26	0.13	0.12	0.78	*0.0028*
Zn	43	46.1	47.7	27.9	3.7	112.8	51	26.2	25.0	16.4	6.3	88.1	*0.0027*

Table 2 Liver Copper groups of farmed and free-ranging deer. Due to different liver Cu levels in farmed and free-ranging deer, they were divided in different Cu categories. The third column show numbers of individuals in each category.

[Cu] (ppm, ww)	Type	# of samples
< 1	Farmed deer	13
1 - 2	Farmed deer	12
3 - 4	Farmed deer	10
4 - 5	Farmed deer	8
> 5	Farmed deer	9
< 20	Free-ranging deer	10
20 - 40	Free-ranging deer	13
40 - 70	Free-ranging deer	9
> 70	Free-ranging deer	11

Table 3 Liver elements in the subgroups of farmed deer (FSG 1 and FSG 2). Mean, Median, SD, Lowest and Highest values in ppm.

	FSG 1						FSG 2						*M-W U*
	n	Mean	Median	Std.dev.	Max.	Min.	n	Mean	Median	Std.dev.	Max.	Min.	*p =*
Al	16	0,74	0,51	0,65	3,06	0,38	31	5,41	2,50	5,84	23,00	0,61	*< 0.0001*
Ca	16	41	32	42	187	2	34	202	85	612	3657	38	*< 0,0001*
Cd	16	0,02	0,01	0,02	0,09	0,00	34	0,04	0,04	0,03	0,14	0,01	*0.0019*
Co	15	0,05	0,05	0,03	0,10	0,01	27	0,08	0,08	0,03	0,18	0,04	*0.0093*
Cr	12	0,03	0,02	0,03	0,11	0,00	23	0,07	0,06	0,04	0,18	0,03	*0.0006*
Cu	16	1,7	1,5	0,7	3,3	1,0	34	5,0	3,9	3,9	21,8	2,0	*< 0,0001*
Fe	16	55	54	19	87	30	27	239	212	121	726	125	*< 0,0001*
K	16	811	569	860	4021	482	34	2012	2033	451	2830	1221	*< 0,0001*
Mn	16	0,8	0,8	0,2	1,2	0,3	34	2,7	2,8	0,7	4,2	0,0	*< 0,0001*
Mg	1	302	302		302	302	25	144	139	22	194	100	*n.a.*
Mo	16	0,30	0,30	0,09	0,49	0,10	34	0,99	0,96	0,22	1,50	0,56	*< 0,0001*
Na	1	909	909		909	909	25	808	695	332	1661	446	*n.a.*
Ni	11	0,04	0,03	0,06	0,22	0,00	23	0,02	0,02	0,01	0,05	0,00	*0.2896*
Pb	12	0,04	0,04	0,02	0,08	0,01	33	0,10	0,08	0,08	0,35	0,01	*0.0131*
P	16	2267	2194	294	3048	1853	34	3448	3069	1268	6276	1673	*0.0001*
S	16	1865	1747	361	3037	1379	34	2133	2089	330	2765	1450	*0.0090*
Se	1	0,27	0,27		0,27	0,27	19	0,27	0,27	0,14	0,78	0,12	*n.a.*
Zn	15	11,5	11,0	2,4	16,0	6,3	34	32,9	28,4	16,1	88,1	18,0	*< 0,0001*

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