The accumulation of metal runoff from acidic sulphate soils in two avian predators, white-tailed eagle (*Haliaeetus albicilla*) and great cormorant (*Phalacrocorax carbo*)

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Acidic sulphate soils are one of the largest sources of metal contamination in Finland, exceeding in some cases industrial emissions. Sulphate soils occur naturally in the Finnish coastal areas, but anthropogenic land modification and land rising causes the metals and metalloids in the soil mobilize and end up in water bodies with runoff. Metal leaches from sulphate soils are harmful to aquatic organisms, but the concentrations and accumulation of metals from sulphate soils in apex avian species has not been studied. Using inductively coupled plasma -mass spectrometry, concentrations and spatial trends of 17 metals and metalloids associated with sulphate soils were analysed from the blood of nestlings of white-tailed eagles and great cormorants collected from sulphate soil and control areas in the western Finnish coast. For most metals, there were no differences in blood concentrations between control and sulphate soil areas, except for the arsenic concentrations in white-tailed eagles, which were higher in the sulphate soil areas, and lithium concentrations of cormorants, which were higher in the control areas. The concentrations of most metals were within background levels, not known to cause toxic effects. Concentrations of mercury, cadmium and lead were at the level of mild sublethal toxicity. Concentrations of several elements correlated between species. Latitudinal trends and intraspecies correlations of metals were also observed. The results indicate, that the white-tailed eagles and great cormorants do not get their metal burden from the sulphate soils, and that metal contamination and their accumulation into apex species varies along the Finnish coast.

Happamat sulfaattimaat ovat yksi Suomen suurimmista metallien ympäristölähteistä, ja monen metallin päästöt sulfaattimaista ylittävät teollisuuden päästöt. Sulfaattimaita esiintyy luonnostaan Suomen rannikolla, mutta maaperän altistuminen hapettaville olosuhteille maanmuokkauksen ja maankohoamisen takia saa aikaan maaperän happamoitumisen. Maaperän happamoitumisen seurauksena siinä olevat metallit liukenevat, ja joutuvat valuman mukana vesistöihin. Sulfaattimaiden metallipäästöt ovat haitallisia vesieliöille, mutta sulfaattimaista peräisin olevien metallien pitoisuuksia tai kertymistä ravintoketjun huipulla oleviin lintuihin ei ole tutkittu. Tutkimuksen tavoite on selvittää, onko sulfaattimaiden läheisyydessä pesivien merikotkien ja merimetsojen metallikuorma suurempi kuin kauempana sulfaattimaista pesivien lintujen, ja onko metallien pitoisuuksissa alueellista vaihtelua. Käyttäen ICP-massaspektrometriaa, 17 sulfaattimaihin yhdistetyn metallin ja puolimetallin pitoisuudet analysoitiin Suomen rannikolta sulfaattimaa- ja kontrollialueilta kerätyistä merikotkan ja merimetson poikasten verinäytteistä. Suurimassa osassa metalleja ei ollut pitoisuuseroja sulfaatti- ja kontrollialueiden välillä. Merikotkien arseenipitoisuus oli suurempi sulfaattimailla kuin kontrollialueilla, ja merimetsojen litiumpitoisuus oli suurempi kontrollialueilla. Useimman metallin pitoisuudet olivat tasolla, jonka ei tiedetä aiheuttavan toksisia vaikutuksia. Elohopean, lyijyn ja kadmiumin pitoisuudet olivat tasolla, jonka tiedetään aiheuttavan lieviä myrkytysvaikutuksia. Monen aineen pitoisuudet korreloivat alueellisesti lajien välillä. Lisäksi metallien pitoisuuksissa oli pohjoiseteläsuuntaista vaihtelua, ja lajin sisäisiä korrelaatioita metallien välillä. Tulosten perusteella happamat sulfiittimaat eivät ole merikotkien ja merimetsojen metallikuorman lähde, ja metallien pitoisuudet vaihtelevat alueellisesti Suomen rannikolla.

Key words: white-tailed eagle, great cormorant, acidic sulphate soils, metals

Contents

1. Introduction

Acidic sulphate soils, cited as “the nastiest soils in the world” by Dent and Pons (1995), and found globally on multiple continents, are one of the largest environmental sources of metals and metalloids in Finland. The emissions of many metals, e.g. manganese, aluminium, nickel and zinc, from sulphite soils are multiple times higher than the Finnish industrial emissions (Sundström et al. 2002). Finland has largest area of acidic sulphate soils in Europe, and the sulphate soil area has been estimated to cover 1600-3000 km^2, mostly in the western and south-western coastal areas (Fältmarsch et al. 2008). Sulphate soils in coastal areas of Finland were formed in the anoxic conditions in Littorina Sea, and they are characterised by the high sulphur concentrations. The sulphate soils become acidic when the soils are exposed to atmospheric oxygen due to land rising of the coastal areas and anthropogenic land modification for e.g. agricultural purposes. The exposure to oxidising conditions causes the sulphur in the soils to form sulphuric acid (H_2SO_4), which in turn lowers the pH of the soils (Dent and Pons 1995). Acidic sulphate soils do not have higher concentrations of metals and metalloids in them compared to other soil types, but the low pH conditions (pH <4) increase the dissolving and mobilisation of metals in the sulphate soils, thus increasing the leaching compared to other soil types (Sohlenius and Öborn 2004). The acidity and the composition of metals and metalloids leaching from sulphate soils varies spatially (Fältmarsch et al. 2008; Wallin et al. 2015). The sulphur acid is formed when the soil is dry, and metals are washed away by water, and along with the runoff, the metals are carried to near-by water systems, such as rivers and lakes (Sohlenius and Öborn 2004; Fältmarsch et al. 2008).

The acidic metal effluents from sulphate soils can be harmful to fresh water invertebrates and fish. Metal emissions from sulphate soils can affect the development of aquatic insect larvae, causing morphological abnormalities (Vuori 1996). Sulphate soil emissions are also harmful to fish eggs and larvae, and timing of the breeding of the fish at the same time with the large effluents from the sulphate soils, e.g. in during spring and autumn, has been observed to affect whole fish populations (e.g. Hudd and Kjellman 2002). Large metal leaches from sulphate soils have even caused mass kills of fish in the rivers of Western Finland (Fältmarsch et al. 2008).

Carried by the river water, the metals mobilised from sulphate soils end up in the brackish estuaries in the coast of Baltic. The leaching of metals from sulphate soils is highest and most wide spread during seasons of high flow, such as autumn and spring, when there is heavy rainfall and melting water that increase the flushing of the soils, and spread the metals in a wider area in the river estuaries (Nystrand and Österholm 2013; Nystrand et al. 2016). In the estuaries the metals can end up in the sediments, or remain in the water column, where they can potentially end up in aquatic organisms (Nystrand et al. 2016). Even though the metals leaching from the sulphate soils are known to harmful to fresh water organism, there haven’t been studies focusing on the effects of sulphate soil effluents to the brackish Baltic species. Risk assessment of 14 river estuaries affected acidic sulphate soils in Western Finnish coast revealed elevated metal concentrations in both the water column and sediments, and deteriorated benthic invertebrate communities in many of the study sites (Wallin et al. 2015). Wallin et al. (2015) assessed the ecological risk caused by acidic sulphate soils to be high or moderate in several of the studied estuaries, demonstrating the significance of the ecological impacts caused by the acidic sulphate soils.

Although effects of metal leached from sulphate soils on organism have been studied on aquatic plants, invertebrates and fish, there are no studies on the effect or concentrations of metals from acidic sulphate soils in aquatic birds. While some metals are essential for proper function of metabolism, excessive metal contamination is known to have negative effects on birds. Large concentrations or long exposure of metals can cause both acute and chronic toxic effects in organisms, depending on their mode of toxicity. The toxicity of metal can be based on actual cytotoxicity of the metal, or the metal can have a disruptive effect on the normal metabolism by resembling and behaving in a similar way in the organism as the essential trace metals needed in the normal metabolism (Scheuhammer 1987). Even very low concentrations of non-essential metals have been found to be harmful to organisms by causing increased oxidative stress (e.g. Espín et al. 2014b, a). Some metals and metalloids are known to accumulate into different tissues and organs of an organism over time if the metals are not excreted, or the excretion is slow (e.g. Lebedeva 1997; Nam et al. 2005; Berglund 2018). Some metals, especially mercury, can also biomagnify in the aquatic food webs (e.g. Barwick and Maher 2003; Nfon et al. 2009; Cui et al. 2011; Guo et al. 2016). Despite the high availability of metals in coastal sulphate soil estuaries and their bioaccumulation and biomagnification capability, metal concentrations in high trophic level organisms has not been studied. Biomagnification and accumulation of metals poses threat to especially to apex species of the food web, as they can ingest and accumulate toxic levels of metals in their tissues over time.

The objective of this master's thesis is to find out, do apex avian species of the Baltic Sea coastal food web nesting in the proximity of acidic sulphate soils have higher metal burden than their counterparts living further away from the effect of the sulphate soils, and whether there are spatial trend and covariation in the metal concentrations. I will study two avian apex predator species, the white-tailed eagle (*Haliaeetus albicilla*) and great cormorant (*Phalacrocorax carbo*). As top predators, the contaminant concentrations in white-tailed eagles and cormorants reflect also the contamination in their prey species. The Finnish white-tailed eagles are known to suffer from metal contamination (e.g. Cd, Hg and Pb in Krone et al. 2006 and Pb in Isomursu et al. 2018), but it has not been studied whether white-tailed eagles suffer from metal contamination from sulphate soils. Great cormorant populations elsewhere in the world have been found to have accumulated metal burden (e.g. Nam et al. 2005; Hribsek et al. 2017), but the metal contamination or its origin in the great cormorant population in the Baltic Sea has not been studied. I will study the metal burden in the white-tailed eagles and cormorants by using blood samples taken from nestlings of both species collected from both sulphate soil and control areas along the western Finnish coast. My hypotheses are that individuals from the proximity of sulphate soils have higher concentrations of metals in their blood than the individuals from further away from sulphate soils, and that due to spatial covariation in metal contamination and availability for food web magnification there is spatial correlation in the metal concentrations found in white-tailed eagles and cormorants.

2. Materials and methods

2.1. Study species

2.1.1. White-tailed eagle (Haliaeetus albicilla)

White-tailed eagle (here after WTE) is the largest predatory bird in Finland. WTEs' range from the temporal areas from Norway to East Asia (Birdlife International 2004). In Finland the WTE is a year-round resident mainly in the coastal areas of the Baltic. In the past the Baltic WTE population declined due to environmental toxins, such as mercury and POPs, but the population has recovered since (Helander et al. 2008, Saurola et al. 2013). In 2018, the growth of the WTE population appeared to have stopped in most areas along the Finnish coast excluding the Quark and Gulf of Finland (WWF 2018). The Finnish WTEs are not migratory, but Finnish WTE individuals are known to move in a large area around the Baltic, Central Europe, and Russia (Saurola et al. 2013).

Based on food remnants collected around the WTE nests during breeding season, the diet of Finnish WTE consist mostly of birds and fish, mammals being only a small proportion (Sulkava et al. 1997; Ekblad et al. 2016). Amount of different prey items vary spatially, as there is a positive correlation between the proportion of fish and the land cover in the territory, and negative correlation between the proportion of birds and the land cover (Ekblad et al. 2016). In Germany a seasonal WTE dietary shift has been observed as the proportion of mammal carcasses increases in the winter time (Nadjafzadeh et al. 2013, 2015).

There are multiple possible food web pathways of exposure to metals for WTEs. The most consumed bird species by WTEs in Finland is the common eider (*Somateria mollissima*) (Ekblad et al. 2016), for which the Finnish population is known to have metal burden (Fenstad et al. 2017), and the eiders feed on benthic invertebrates, which may accumulate metals from the sediments. Also, during nesting time, the WTE diet includes littoral fish species, such as pike (*Esox lucius*) and common bream (*Abramis brama*) (Ekblad et al. 2016), which can accumulate metals either though diet, including other fish and aquatic invertebrates, or through their membranes (Pagenkopf 1983).

2.1.2. Great cormorant (Phalacrocorax carbo)

The great cormorant (*Phalacrocorax carbo sinensis*) (here after cormorant) is an avian species with globally wide distribution. Cormorants retuned to Finland's list of breeding species in 1996 after disappearing from the Finnish coastal areas for few hundred years (Lehikoinen 2006). The Finnish cormorant population grew fast after returning to its old nesting areas in Finland, growth being almost exponential in the beginning, but since the growth has stabilized, and in 2018 26 700 cormorant nests were counted in Finland (Finnish Environmental Institute 2018). Cormorants are migratory, and the main wintering areas for cormorants nesting in Finland are in Central Europe and Mediterranean (Saurola et al. 2013).

Cormorants nest in colonies in the Baltic sea. Cormorants are piscivorous, and there is seasonal and spatial variation in the diet species composition (Lehikoinen 2005, Boström et al. 2012). Cormorants feed on smaller fish species, the most consumed species of fish in Finland being roach (*Rutilus rutilus*), perch (*Perca fluviatilis*), and eelpout (*Zoarces viviparus*) (Lehikoinen 2005; Lehikoinen et al. 2011). With diet including bottom-dwelling species such as eelpouts, cormorants may be exposed to metals already precipitated into the sediments. Cormorants feed also on pelagic species of fish, such as herring (*Clupea harengus*) (Lehikoinen et al. 2011), thus one possible route of metal exposure for cormorants being through the pelagic food chain in addition to the littoral one. Cormorants switch their diet during the breeding season, as the proportion of smaller fish species, such as viviparous blennies (*Zoarcidae*), is increase when the chicks are small (Lehikoinen 2005, Boström et al. 2012).

2.2. Metals of study

The metals and metalloids chosen for the analysis were silver (Ag), aluminium (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), lithium (Li), manganese (Mn), nickel (Ni), lead (Pb), rubidium (Rb), selenium (Se), thallium (Tl), uranium (U) and zinc (Zn). All of the chosen elements are known to be leached from the sulphate soils near the Finnish coastal areas, amounts of different metals varying spatially (e.g. Roos and Åström 2005; Fältmarsch et al. 2008; Nyberg et al. 2012; Nystrand and Österholm 2013).

Mercury (Hg) was also chosen for the analysis despite not being associated with the acidic sulphate soils in literature, as it is a well-known environmental toxin especially in aquatic environments (Scheuhammer 1987), with high potential to biomagnify in aquatic food chains, thus being most harmful to apex species such as WTEs and cormorants (Nfon et al. 2009).

2.3. *Data collection and laboratory analyses*

The data was collected from sulphite soil and control areas in the Finnish coast. Cormorant colonies and WTE territories in sulphate soil group were in the proximity of sulphate soil areas or estuaries, where sulphate soil contaminated rivers carry metals. Control group included colonies and territories in areas which did not have sulphate soils or estuaries of rivers with sulphate soils along them. Possible river estuaries in proximity of control sites were of rivers with no sulphate soils close by. Information of the sulphate soil locations was obtained from the map of acidic sulphate soils produced by the Finnish Institute of Geology. The map is based on multivariate analysis done using spatial data software, which uses soil data and airborne geophysical data, and catchment area-specific field surveys collected and done by the Finnish Institute of Geology, and elevation data and base maps of Land Survey of Finland (Finnish Institute of Geology 2018). The used map can be found on the website of Finnish Institute of Geology (http://gtkdata.gtk.fi/Hasu/index.html, 3.10.2018).

The samples of WTEs' and cormorants' nestlings were collected during May and June 2017 and 2016. In 2017, nine samples from control areas and seven samples of WTEs and total of 15 control samples from four colonies and total of 15 sulphite soil area samples from six colonies of cormorants were collected. One nestling per WTE territory and three randomly chosen nestlings per cormorant colony were sampled, except of one cormorant colony in Oulu, where only one individual was sampled. Chicks were captured from the nest, and blood sample was taken from the ulnar vein using needle and syringe. The samples were frozen in -18 °C.

In addition to the WTE samples collected in 2017, eight WTE blood samples collected in 2016 were added to the analyses. I assigned the samples to control and sulphate soil groups using same criteria that was used for the samples collected 2017. Out of the samples collected in 2016, three were sulphate soil area samples, and five from control areas. I received the data from my supervisors and I did not participate in the collection or laboratory analyses of the samples. I was, however, taking an active role in planning and facilitating the analysis of the samples in the lab since spring 2018 when the topic of my thesis was decided.

The metal analyses for the samples were done in Norwegian University of Science and Technology, Trondheim, Norway. The laboratory analysis was done for 17 metals and metalloids to find out their concentration in the WTE and cormorant blood. The analysis was done using high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS). All concentrations reported by the laboratory were given in µg/l, the concentrations were within ±20 % of the reported value. Full blood was used as a matrix for the analyses.

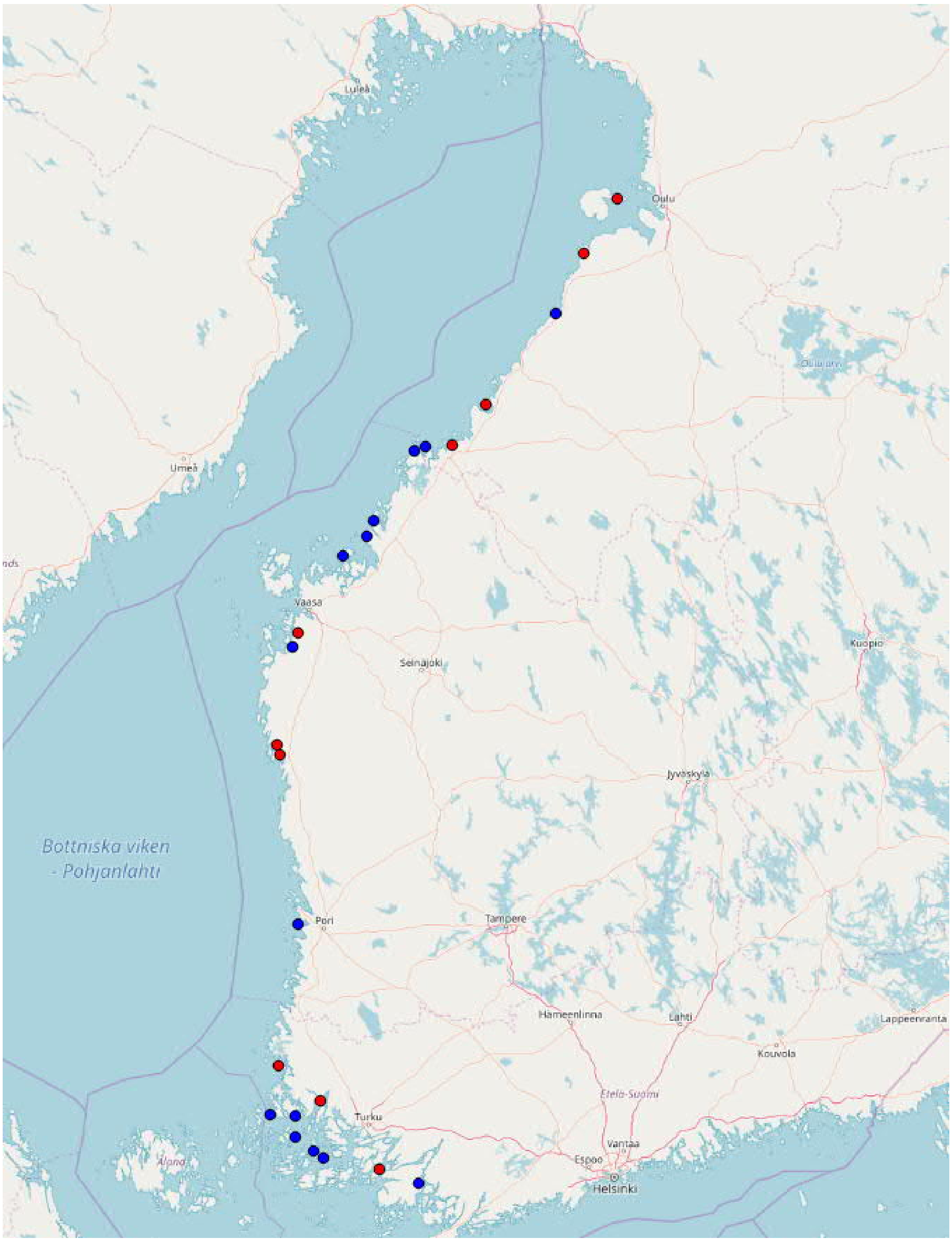

Figure 1. Map of locations of WTE sampling sites. Blue dots (•) are control sites, and red dots (•) are sulphate soil sites.

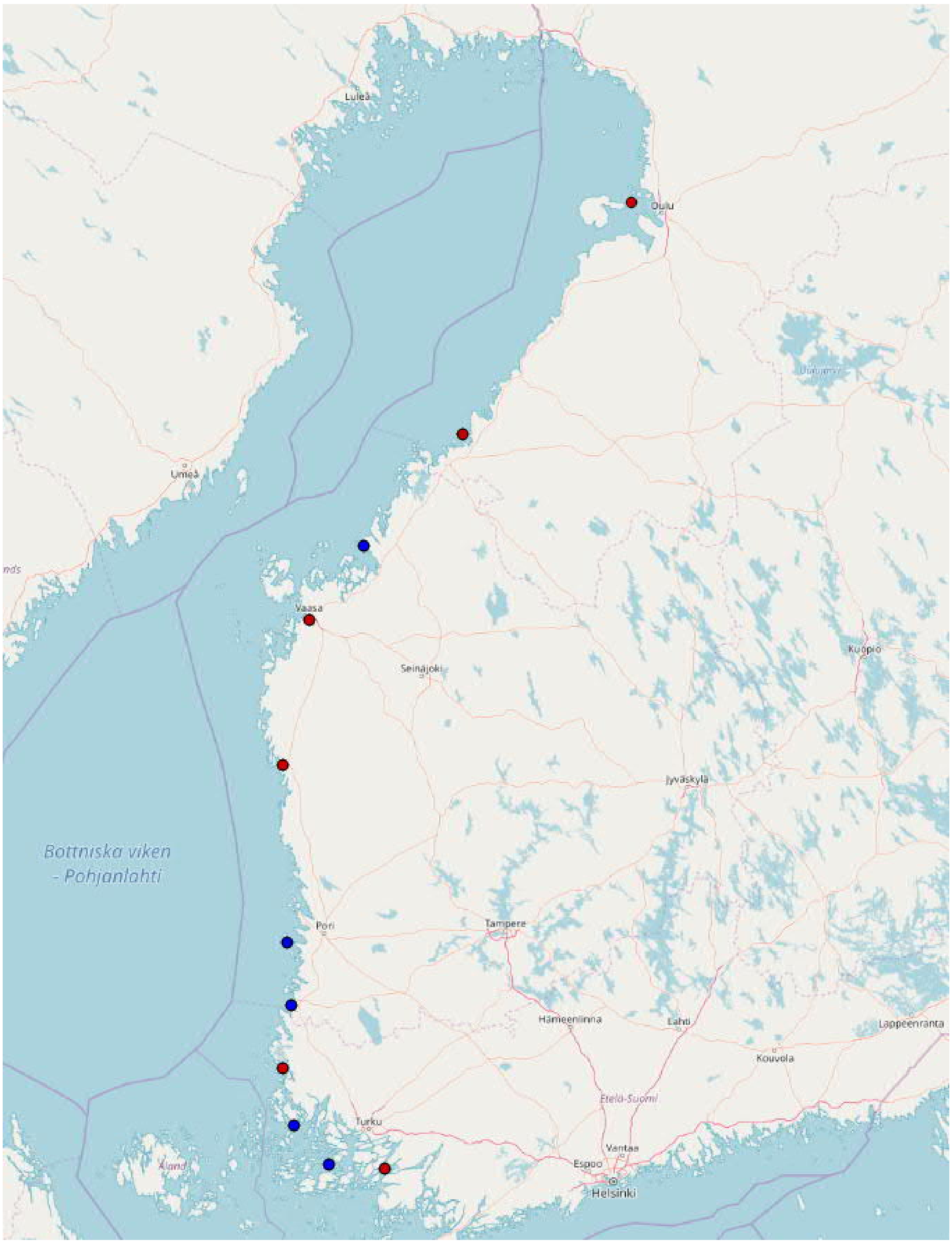

Figure 2. Map of locations of cormorant sampling sites. Blue dots (•) are control sites, and red dots (•) are sulphate soil sites.

2.4. Statistical analyses

After receiving the data from the laboratory, I did the statistical testing for it. For statistical analyses, I used Microsoft Office Excel (v. 16.0.) and SAS (v. 7.12.), and R (v. 3.5.1.) for producing the figures. Before the analyses, I did exploratory data analysis for the data. I removed one negative value of uranium concentration of WTE from the data, as blood concentration of metal cannot be negative.

2.4.1 Correlations

To see if there were geographical correlations in the concentrations of elements in the cormorants and WTEs, I calculated Spearman's correlation coefficients (r_s) with their corresponding p-values. For calculating the correlations, the samples were divided into smaller areas, each being a sulphate or a control area. For each area, the mean concentration of each element was calculated for both species individually. Means of the element separately for the species for each area formed one data point. Some areas did not have both species, and those points were excluded from the interspecies correlation analyses. They were however included in the intraspecific correlations, in which the concentrations of the different elements in the same sites were tested for both species separately.

Spearman's correlation (r_s) was used for all correlation analyses, as it is less sensitive to non-normally distributed data. I calculated the correlations using SAS's CORR-procedure using the mean concentrations of each area. The interspecies correlations were calculated between the same metal's concentration for WTEs and cormorants. Scatterplots were drawn using R v. 3.5.1. for the statistically significant ($p < 0.05$) and near significant ($p < 0.10$) correlations.
Also, intraspecific correlations were calculated for both species. The same means for each area as calculated for interspecies correlations were used, and points with only one of the two species were also included in the analysis.

2.4.2 General linear mixed model (GLMM)

To test if there was a difference in nestling metal concentrations between control and sulphate soil areas, I did used a general linear mixed model (GLMM) for each metal and separately for both the species. I did not use multivariate analysis of variance (MANOVA) to the data due to the small number of observations in relations to dependent variables. I did the GLMMs using the SAS MIXED-procedure. In each model, the element of interest was the dependent variable, and treatment (sulphate soil or control) was the independent variable. Also, to test the effect of latitudinal location of the sampling area in the metal concentration, I standardized the latitudinal coordinates of the sampling locations by subtracting the arithmetic mean of the coordinates from each latitude coordinate, and added the derived standardized location to the GLMM as an independent variable. The interaction of the treatment and latitude as independent variable was also tested for each model. If the interaction of treatment and location was non-significant ($p > 0.05$), the interaction was removed from the model, and the main effects of treatment and location were tested. For cormorants, the colony was used in the model as a random effect to control for the similarity of metal concentrations between individuals in the same colonies.

I also checked if the data met the GLMM assumptions, normality and heteroscedasticity of the residuals. To test for normality, I visually examined the graphs describing the data. I also used Shapiro-Wilk -test, which tests if the sample came from a normally distributed population. I did the Shapiro-Wilk -test using SAS UNIVARIATE -procedure. If the data looked visually close to normal and the p of Shapiro-Wilk -test was > 0.1, data was considered normally distributed. I tested for homoscedasticity by doing a Levene's test, which test for the equality of variances for groups. Residuals were also checked visually from plots.

One of the most common problems with the data was the skewness of the residuals to the right, thus making the data non-normally distributed. To make the data fit the model better, I did a log-normal transformation for the concentrations of the elements which did not fill the assumptions of GLMM. For cormorants, I did the log-normal transformation to silver, aluminium, cadmium, copper, chromium, manganese, nickel, lead, selenium and thallium, and for WTEs to silver, aluminium, arsenic, cadmium, cobalt, chromium, mercury, lithium, nickel and lead. To the log-normal transformed data, I applied the same model that was used previously, with metal concentration as dependent variable and treatment, latitude, and their interaction as independent variables. I checked the statistical significance of the interaction again, and if p was > 0.05, I omitted the interaction from the model. The interaction of treatment and latitude was non-significant in all models, so it was removed in all cases.

From the results of both models, I took the estimated marginal means of the metal concentrations for each group with their 95% confidence limits (LS means statement in SAS). I transformed the means and their 95% confidence limits of the log-normal scale back to normal scale. I plotted the means and their 95% confidence limits using R v. 3.5.1.

3. Results

The marginal means with lower and upper 95 % confidence limits, median, and minimum and maximum concentrations for each element for control and sulphate soil areas are given for WTEs in Table 2. and for cormorants in Table 1. The range of the concentrations varied between different elements 10^7-fold. Uranium had the lowest concentrations in both species, and zinc had the highest concentrations.

Table 1. Estimated marginal means, lower and upper 95 % confidence limits (CL), minimum and maximum value and median of the metal element concentrations (μg/l) in cormorant nestlings in control and sulphate soil areas in the Finnish Baltic Sea coast. N is the number of nestlings.

	Control						
Element	**Mean**	**Lower 95% CL**	**Upper 95% CL**	**Min**	**Max**	**Median**	**n**
Ag	0.082	0.035	0.19	0.012	0.5	0.089	15
Al	22.3	11.8	42.0	4.86	101	20.2	15
As	74.5	51.5	97.5	18.5	175	77.4	15
Cd	0.74	0.35	1.56	0.074	2.35	0.92	15
Co	2.85	1.72	4.73	1.15	6.27	2.35	15
Cr	0.29	0.19	0.43	0.052	1.07	0.34	15
Cu	355	320	390	319	395	363	15
Hg	272	91.0	452	94.7	386	257	15
Li	3.26	3.04	3.48	2.59	3.75	3.21	15
Mn	96.7	75.7	124	48.7	151	91.4	15
Ni	0.44	0.18	1.09	0.029	3.04	0.39	15
Pb	0.83	0.62	1.11	0.26	3.37	1.03	15
Rb	2111	1867	2355	1200	2680	1930	15
Se	657	527	820	490	1060	626	15
Tl	0.15	0.095	0.22	0.048	0.26	0.13	15
U	0.0066	0.0046	0.0087	0.0012	0.014	0.0061	15
Zn	10727	9954	11500	9630	11800	10700	15

	Sulphate soil						
Element	**Mean**	**Lower 95% CL**	**Upper 95% CL**	**Min**	**Max**	**Median**	**n**
Ag	0.082	0.036	0.18	0.022	0.23	0.083	16
Al	16.7	9.09	30.7	5.49	72.1	17.6	16
As	76.1	54.0	98.3	24.7	179	40.8	16
Cd	0.38	0.18	0.77	0.21	0.83	0.41	16
Co	2.30	1.42	3.73	0.76	9.73	3.05	16
Cr	0.25	0.17	0.37	0.076	1.16	0.24	16
Cu	386	353	419	308	477	386	16
Hg	350	178	522	214	871	295.5	16
Li	2.77	2.56	2.99	2.12	3.8	2.75	16
Mn	103	81.5	131	69.5	229	98.4	16
Ni	0.50	0.21	1.19	0.018	2.31	0.5	16
Pb	1.02	0.77	1.35	0.42	2.28	0.89	16
Rb	2208	1973	2443	1500	3070	2285	16
Se	660	534	815	491	1110	632.5	16
Tl	0.082	0.055	0.12	0.04	0.36	0.08	16
U	0.0048	0.0027	0.0068	0.0012	0.011	0.0048	16
Zn	10345	9606	11085	8890	12200	10400	16

Table 2. Estimated marginal means, lower and upper 95 % confidence limits (CL), minimum and maximum value and median of the metal element concentrations (µg/l) in white-tailed eagle nestlings in control and sulphate soil areas in the Finnish Baltic Sea coast. N is the number of nestlings.

Control

Element	Mean	Lower 95% CL	Upper 95% CL	Min	Max	Median	n
Ag	0.085	0.055	0.13	0.035	0.44	0.085	14
Al	18.5	12.5	27.4	7.8	46.9	18	14
As	23.2	19.1	28.2	13.1	71.3	25.2	14
Cd	0.13	0.071	0.24	0.039	1.36	0.1	14
Co	1.06	0.80	1.40	0.43	3.76	1.01	14
Cr	0.32	0.17	0.60	0.13	1.17	0.25	14
Cu	580	508	652	382	863	611	14
Hg	219	147	325	96.5	481	222	14
Li	4.85	4.22	5.56	3.28	8.39	4.61	14
Mn	51.5	37.0	66.1	19.6	85.9	55.6	14
Ni	0.47	0.20	1.10	0.036	59.5	0.47	14
Pb	3.85	1.89	7.83	1.09	14.9	4.64	14
Rb	2002	1580	2424	506	2420	935	14
Se	1018	804	1232	860	3110	2360	14
Tl	0.039	0.032	0.046	0.015	0.071	0.038	14
U	0.0032	0.0016	0.0048	0.00038	0.0089	0.0027	13
Zn	9723	8115	11332	5530	12600	11250	14

Sulphate soil

Element	Mean	Lower 95 % CL	Upper 95% CL	Min	Max	Median	n
Ag	0.080	0.047	0.13	0.031	0.47	0.063	10
Al	17.9	11.2	28.5	6.02	84.7	13.8	10
As	36.0	28.6	45.3	11.8	84.2	37.1	10
Cd	0.12	0.058	0.24	0.0068	0.48	0.15	10
Co	1.12	0.81	1.56	0.67	3.01	1.04	10
Cr	0.56	0.26	1.18	0.13	16.3	0.33	10
Cu	545	460	631	363	719	540	10
Hg	241	151	385	67.3	1260	241	10
Li	4.09	3.47	4.81	2.38	6.97	4.02	10
Mn	62.5	45.2	79.8	17.8	144	55.2	10
Ni	0.65	0.23	1.80	0.19	5.57	0.58	10
Pb	4.73	2.03	11.0	0.49	75.4	3.27	10
Rb	2082	1582	2583	1130	2810	2255	10
Se	967	713	1220	509	1400	937	10
Tl	0.032	0.024	0.040	0.015	0.057	0.034	10
U	0.0041	0.0023	0.0060	0.001	0.011	0.0033	10
Zn	10934	9025	12842	5870	14600	11550	10

3.1. Correlation in metal concentrations between species

The Spearman correlations and their p-values were calculated for each metal between WTEs and cormorants using the means of each element calculated for each area. There were statistically significant ($p < 0.05$) and moderately strong ($r_s > 0.6$) positive Spearman's correlations in the concentrations of mercury, lead, and thallium (Table 3). In addition to these, the silver concentrations between WTEs and cormorants has a statistically marginally non-significant ($p = 0.07$) moderately strong correlation (Table 3). No obvious correlations were found in other elements the ($p \geq 0.2$). All the interspecies Spearman's correlation coefficients with their corresponding statistical significances are given in Table 3. Scatter plots were drawn to illustrate correlations of mercury, lead, thallium, and silver (Figure 3). For both mercury and lead, one area had highest metal concentrations in both WTEs and cormorants. For mercury, the highest concentrations were measured from the northernmost sulphate soil sampling point, while highest concentrations of lead were measured from the southernmost sulphate soil sampling point.

Table 3. Interspecific Spearman correlation coefficients (r_s) and their p-values. P-values < 0.05 are marked with *. For each correlation n = 10 areas.

	Ag	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Hg**	**Li**
r_s	0.60	-0.33	0.30	-0.35	0.24	-0.03	0.04	0.66	-0.21
p-value	0.07	0.347	0.405	0.328	0.511	0.934	0.907	*0.038	0.556

	Mn	**Ni**	**Pb**	**Rb**	**Se**	**Tl**	**U**	**Zn**
r_s	0.18	0.02	0.71	-0.44	0.02	0.66	-0.39	-0.02
p-value	0.627	0.960	*0.022	0.200	0.960	*0.038	0.260	0.967

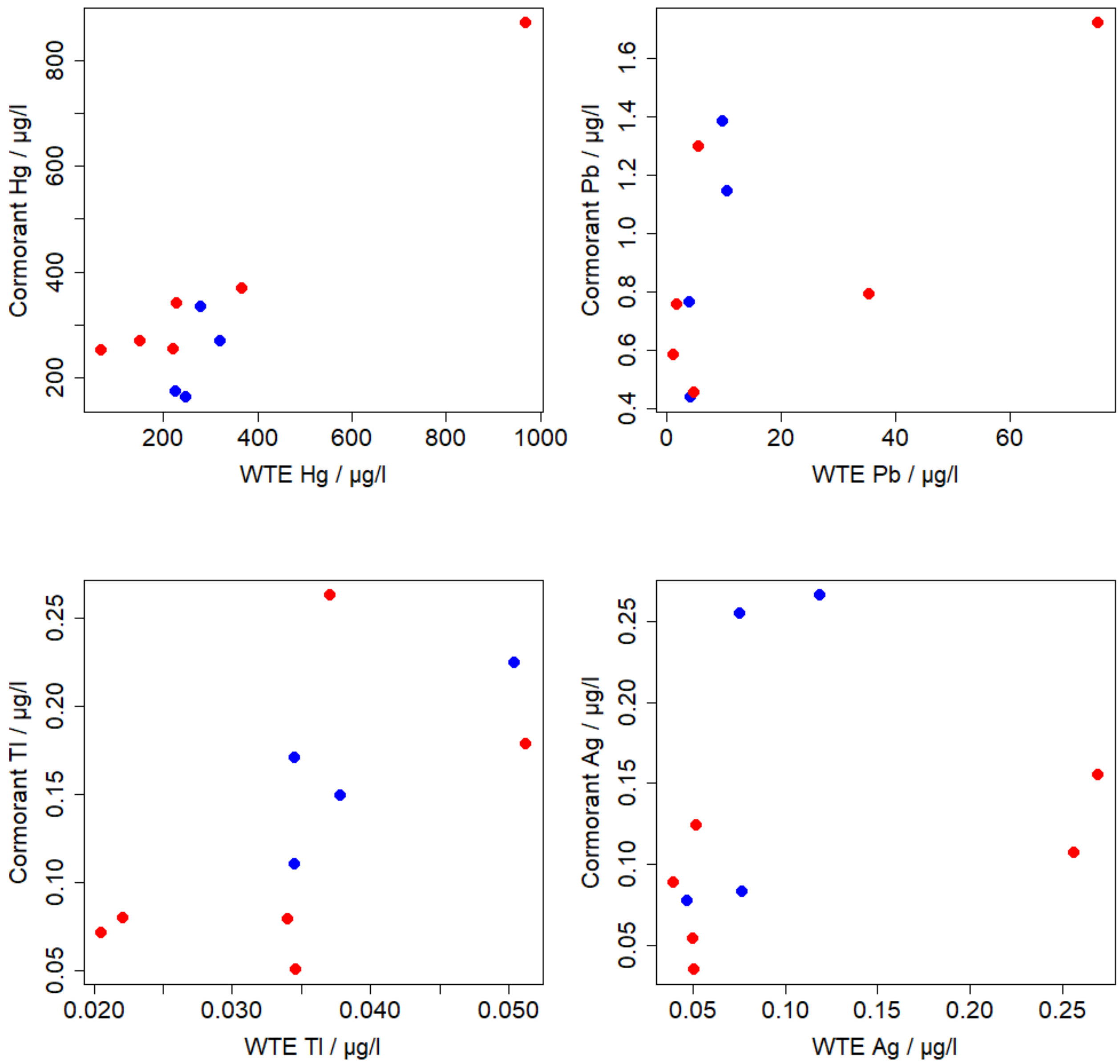

Figure 3. Scatter plots of statistically significant or near significant interspecific correlations presented in Table 3. Blue dots are the control areas, red dots are sulphate soil areas.

3.2. Intraspecific correlations

For WTEs, the Spearman's correlation matrix for all metals is given in Appendix 4. There were both negative and positive correlations between metals. Very strong correlations ($r_s > 0.80$) occurred between copper and selenium ($p < 0.0001$), copper and zinc ($p = 0.0002$), mercury and rubidium ($p < 0.0001$), manganese and selenium ($p < 0.0001$), and rubidium and zinc ($p = 0.0001$). All other correlations were between r_s = 0.4-0.8 and r_s = -0.4- -0.8, indicating moderate to strong correlations between metals.

The correlation matrix for all metals in cormorants is given in Appendix 3. As in WTEs, both negative and positive correlations occurred between metals. Very strong correlations ($r_s > 0.80$) occurred between chromium and aluminium ($p = 0.002$) and arsenic and lead ($p = 0.002$). All other correlations were between $r_s = 0.5\text{-}0.8$ and $r_s = -0.5\text{-} -0.8$, meaning strong correlations. The species had some similarities in their correlations. In both species, there were significant negative correlations between silver and mercury and lithium and lead. Positive correlations occurred between aluminium and chromium, aluminium and uranium, cobalt and lithium, lithium and lead, lead and selenium and rubidium and zinc.

3.3. Results of GLMM

In cormorants, there was a significant difference between control and sulphate soil group in lithium concentrations, control group being 0.5 µg/l larger than sulphate soil group (Table 4, Figure 4). For WTEs, there was a significant difference in blood arsenic concentrations between the two study groups, concentration of sulphate soil group being 12.8 µg/l larger than control group (Table 5, Figure 5). For rest of the elements in either species, there were no statistical difference in blood metal concentrations between the control and sulphate soil group. The estimated marginal means for all elements with 95 % confidence limits are shown for cormorants in Figure 4, and for WTEs in Figure 5. The F-values, degrees of freedom and p-values for cormorants are shown in Table 4, and for WTEs in Table 5.

The assumptions of GLMM, normal distribution and heteroscedasticity, were checked visually from plots and tested using Levene's test and Shapiro-Wilk -test. For the metals which did not fill the assumptions one or both assumptions, problems were mainly the skewness of the data to the right, outliers, and heteroscedasticity of the residuals. For those metals a log-normal transformation was done to make the data normally distributed. Tables 4 and 5 show for which elements log-normal transformation was done to. For cormorants, all elements met the assumptions of GLMM after the transformation. For WTEs, aluminium, chromium, mercury and nickel did not fill the GLMM assumption even after the log normal transformation due to non-normal distribution and heteroscedasticity. The reason for chromium and nickel not fitting the assumptions of GLMM was one outlier in the WTE data of both metals. For chromium, the maximum without outlier was 1.17 µg/l (outlier 16.3 µg/l), and for nickel the maximum without outlier was 5.57 (outlier 59.5 µg/l). The outliers were either natural outliers, or possibly due to contaminations by stainless steel sometime during the laboratory analyses. To see if the outlier affected the results, the two outliers were dropped from the chromium and nickel data, and GLMM was repeated for the data without outliers. After the outlier removal, nickel and chromium with log-normal transformation fit the GLMM assumptions. There was no statistically significant difference between the sulphate soil and control group in either metal (chromium: $F_{1, 21} = 0.42$, $p = 0.5$, nickel: $F_{1, 21} = 2.31$, $p = 0.14$) (Figure 6).

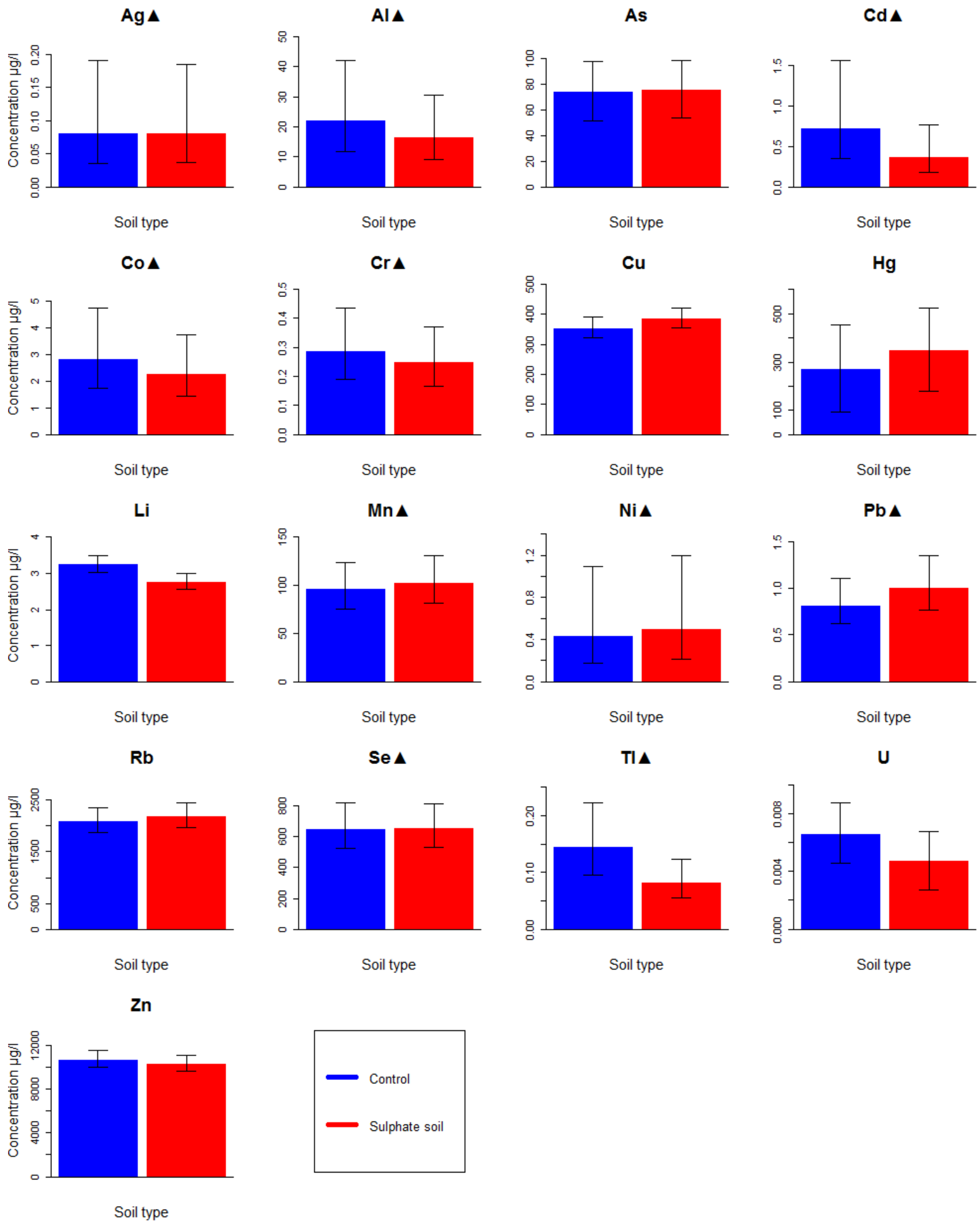

Figure 4. The estimated marginal means and their 95 % confidence limits of blood metal concentrations in control and sulphate soil areas in cormorant nestlings. Marginal mean values and confidence limits gotten by back-transformation of the log-normal values given by the GLMM in Table 4 are marked with ▲-symbol next to the name of the metal.

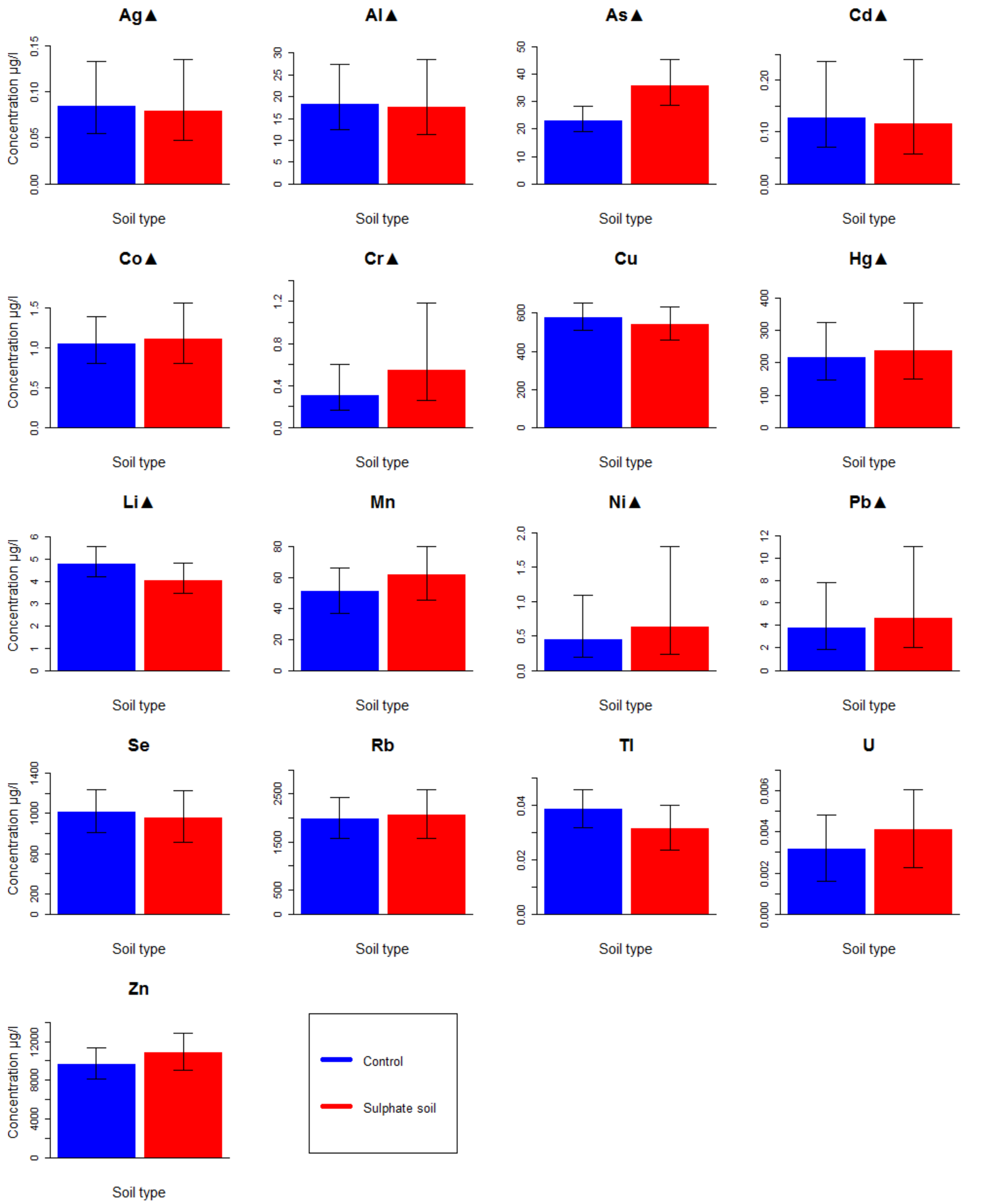

Figure 5. The estimated marginal means and their 95 % confidence limits of blood metal concentrations in control and sulphate soil areas for white-tailed eagle nestlings. Marginal mean values and confidence limits gotten by back-transformation of the log-normal values given by the GLMM in Table 5 are marked with ▲-symbol next to the name of the metal.

Table 4. The, F-values, degrees of freedom (d.f), p-values and transformation used for GLMM of great cormorant blood metal concentrations as dependent variable, and treatment as independent variable. Statistically significant p-values (< 0.05) are marked with *.

Metal	F-value	d.f.	p-value	Transformation
Ag	0	1, 7.9	0.99	Log normal
Al	0.57	1, 7.04	0.47	Log normal
As	0.01	1, 7.14	0.91	None
Cd	2.08	1, 7.87	0.19	Log normal
Co	0.48	1, 7.5	0.51	Log normal
Cr	0.24	1, 28	0.63	Log normal
Cu	2.12	1, 7.62	0.18	None
Hg	0.5	1, 7.49	0.5	None
Li	9.79	1, 28	*0.004	None
Mn	0.19	1, 7.15	0.67	Log normal
Ni	0.08	1, 3.53	0.79	Log normal
Pb	1.32	1, 7.81	0.29	Log normal
Rb	0.42	1, 7.84	0.54	None
Se	0	1, 7.63	0.98	Log normal
Tl	4.68	1, 7.97	0.063	Log normal
U	2.46	1, 5.77	0.17	None
Zn	0.64	1, 7.7	0.45	None

Table 5. The F-values, degrees of freedom (d.f.), p-values and transformation used for GLMM of WTE metal concentrations as dependent variable, and treatment as independent variable. Statistically significant p-values (< 0.05) are marked with *. Aluminium, chromium, mercury and nickel did not fill assumptions even after log-normal transformation.

Metal	F-value	d.f.	p-value	Transformation
Ag	0.04	1, 21	0.85	Log normal
Al	0.01	1, 21	0.91	Log normal
As	9.06	1, 21	*0.007	Log normal
Cd	0.05	1, 21	0.83	Log normal
Co	0.08	1, 21	0.78	Log normal
Cr	1.38	1, 21	0.25	Log normal
Cu	0.41	1, 21	0.52	None
Hg	0.11	1, 21	0.75	Log normal
Li	2.72	1, 21	0.11	Log normal
Mn	1	1, 21	0.33	None
Ni	0.26	1, 21	0.62	Log normal
Pb	0.15	1, 21	0.7	Log normal
Rb	0.06	1, 21	0.8	None
Se	0.1	1, 21	0.75	None
Tl	1.82	1, 21	0.19	None
U	0.61	1, 21	0.44	None
Zn	1	1, 21	0.33	None

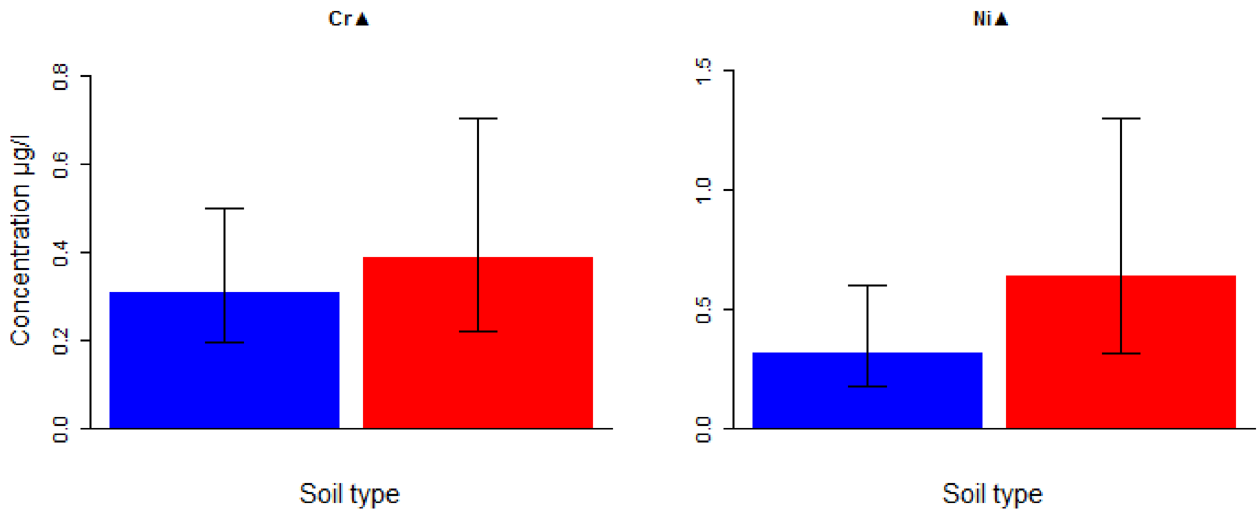

Figure 6. The back-transformed estimated marginal means with their 95 % confidence limits for chromium and nickel of WTEs without outliers (that are included in Fig. 5)

Latitude had a significant effect on the blood metal concentrations of multiple elements in both species. For cormorants, the metal concentrations were higher in south for arsenic ($F_{1,\ 8.68} = 28.75$, $p = 0.0005$) and lead ($F_{1,\ 9.38} = 21.89$, $p = 0.001$). The concentrations were higher in north for cobalt ($F_{1,\ 8.45} = 6.62$, $p = 0.03$), lithium ($F_{1,\ 28} = 13.27$, $p = 0.001$), rubidium ($F_{1,\ 9.45} = 15.72$, $p = 0.003$), and thallium ($F_{1,\ 9.02} = 8.97$, $p = 0.02$) (Figure 7).

For WTEs, concentrations were higher in the south for arsenic ($F_{1,\ 21} = 30.93$, $p = <0.0001$), cadmium ($F_{1,\ 21} = 8.77$, $p=0.0075$), manganese ($F_{1,\ 21} = 6.13$, $p=0.0219$), and selenium ($F_{1,\ 21} = 7.95$, $p = 0.0103$), and higher in north for thallium ($F_{1,\ 21}=5.13$, $p=0.003$) (Figure 8). The parameter estimates with their 95% confidence limits for the latitudinal association of all elements are given in the Appendix 1 for cormorants and in Appendix 2 for WTEs.

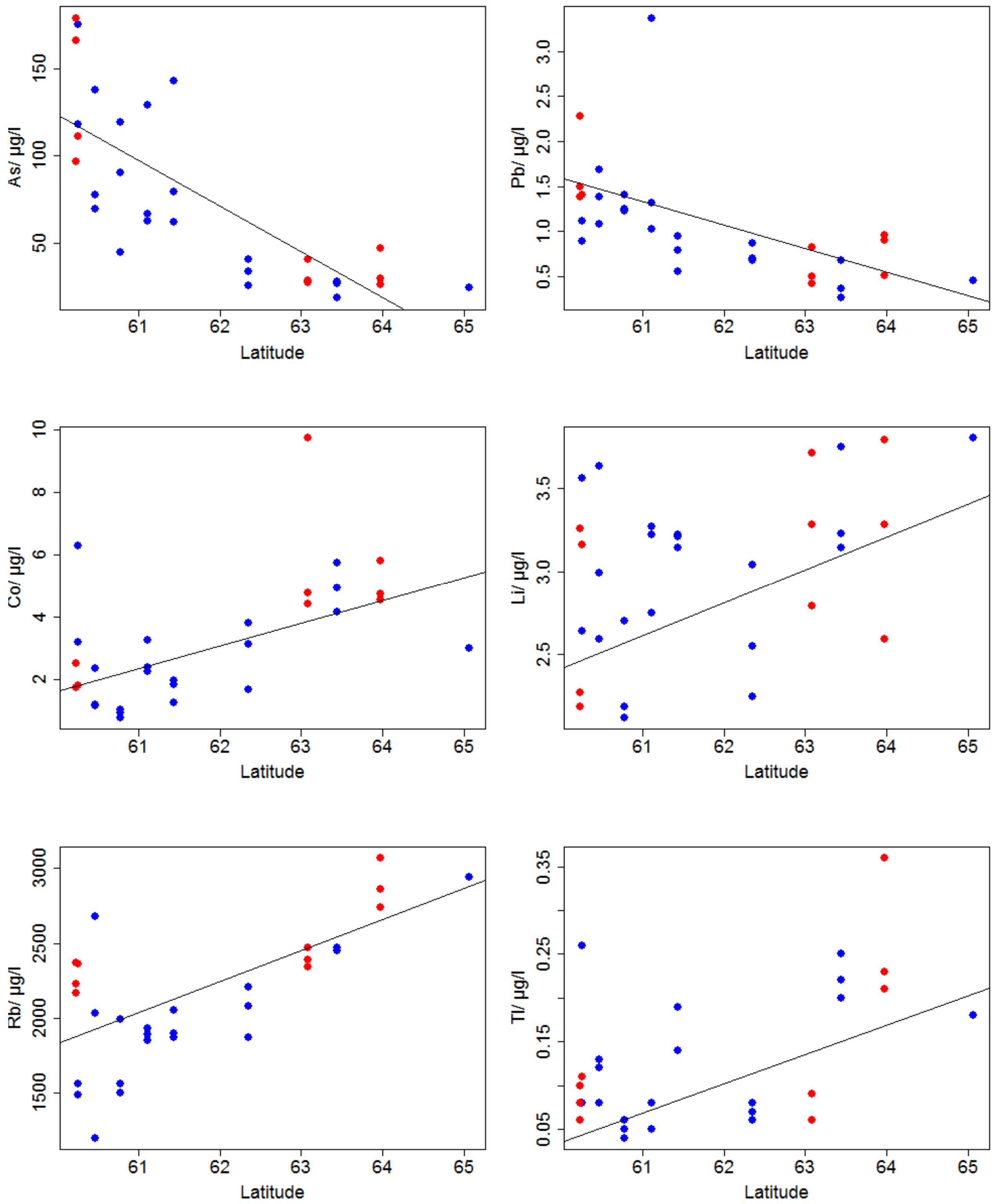

Figure 7. The metal blood concentrations of cormorants with plotted against the latitude. The blue dots are control samples and red dots are sulphate soil samples. All plots are drawn using untransformed data.

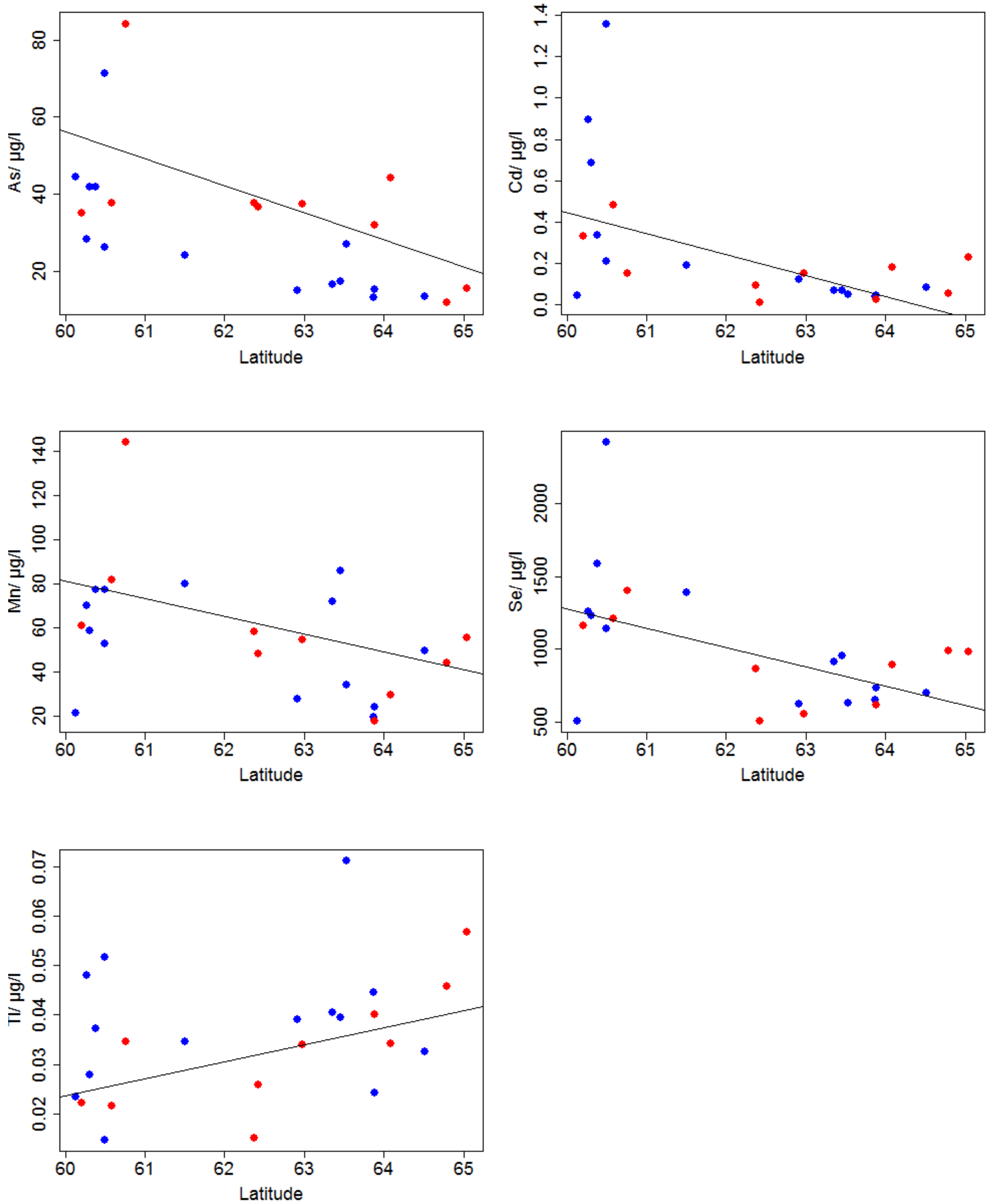

Figure 8. The metal blood concentrations of white-tailed eagles plotted against the latitude. The blue dots are control samples and red dots are sulphate soil samples. All plots are drawn with untransformed data.

4. Discussion

4.1. Minor effects of acid sulphate soils on nestling metal concentrations

Against the initial hypothesis that birds in proximity of sulphate soils would have higher concentrations of metals in their blood than birds from further from the sulphate soils areas, most the metal concentrations in cormorant and WTE blood did not differ between individuals from sulphate soil and control areas. Only element with significant differences in concentrations were arsenic of WTEs and lithium in cormorants. The results indicate, that the sulphate soils might not be the source of the metal contamination of WTEs and cormorants in the study areas. It is known that metals and metalloids leach in higher quantities from sulphate soils than from other types soils (Sohlenius and Öborn 2004), but the effect was not reflected in the blood metal concentrations of the birds of this study. This was the first study investigating do the metals and metalloids leaching from the sulphate soils accumulate in the apex avian species.

The result can be due to various reasons, one being that the metals from the sulphate soils do not reach the breeding or feeding areas of the birds. When metals leach from the sulphate soils, they usually end up in the river water or other fresh water systems with the runoff. Compared to fresh water river systems, the metals behave differently in the brackish water. The mobility and bioavailability of different metals in brackish estuarine systems is determined by many factors, such as water properties, including pH and salinity, water discharge and sediment properties and processes, and biological processes, such as microbial activities (de Souza Machado et al. 2016). Different chemical forms of the metals differ in their bioavailability, as hydrated ions and inorganic complexes are bioavailable for organisms, while colloidal metal forms are not considered very bioavailable (Nystrand et al. 2016). Nystrand et al. (2016) studied the forms of metals in the estuarine water of sulphate soil -affected river Vöyri, and found that most of the metals were occurring in the dissolved form, which is considered most toxic to organisms. However, higher salinity increases the buffering capacity of the water, resulting in the pH becoming more alkaline, which decreases the solubility of many metals and metalloids. Already 20-30 % saline estuarine water mixed with fresh river water is enough to halve the concentrations of most dissolved and colloidal metals leached from the sulphate soil, and concentrations of aluminium, cadmium, cobalt, chromium, copper, manganese, nickel, and zinc have been found to decrease with the increasing distance from the river (Nystrand et al. 2016). Metals from the sulphite soils are also subjected to sedimentation in the river estuary, demonstrated by elevated concentrations of metals associated with sulphate soils found in the sediments of sulphate soil affected estuaries (Nordmyr et al. 2008b, a).

For WTEs, the only element showing difference in concentration between control and sulphate soil areas was arsenic, the concentration in sulphate soil areas being 1.5 times the concentrations in control areas. Nystrand et al. (2016) did samplings in two subsequent years in the estuary of sulphate soil affected river Vöyri in Western Finland, and found concentrations of arsenic to increase with the distance from the river. Similar results were found by Wallin et al. (2015) in Kyrönjoki and Maalahdenjoki. Arsenic concentrations not reducing in the brackish estuarine environment could explain, why there was a difference in arsenic concentrations between the treatment groups of WTEs, but not in other elements. Interestingly, the arsenic concentrations of cormorants were higher than those of WTEs in either group (Tables 1 and 2), but there was no difference in arsenic concentrations between cormorants from sulphite and control areas (Figure 4). Arsenic does not have a known biological function, and it has been found to have multiple toxic effects in birds, including decreased reproduction, liver damage, behavioural effects and developmental problems, e.g. decreased growth rate (Sánchez-virosta et al. 2015). I did not find established toxicity levels of arsenic for blood, and most studies related to arsenic toxicity have studied tissues other than blood (Sánchez-virosta et al. 2015). However, the arsenic levels of WTEs were similar to levels reported for common eiders (*Somateria mollissima*) in Baltic region (Fenstad et al. 2017), indicating that WTEs' arsenic levels might be within background levels. The arsenic levels of cormorants of this study were higher than those reported on eiders, but were similar to levels reported in adult and fledgling white storks (*Ciconia ciconia*) (Maia et al. 2017). Predatory and piscivorous birds such as WTEs and cormorants have been found to accumulate higher levels of arsenic compared birds with other diets (Lebedeva 1997). For both species there was a similar decreasing trend in arsenic concentrations from south to north, suggesting that cormorants and eagles get their arsenic burden at least partially from same sources, and larger concentrations of arsenic in cormorants could be due to higher amounts of fish in their diet compared to WTEs' diet. Most studies have not found evidence of biomagnification of arsenic in the aquatic food chains (Cui et al. 2011; Guo et al. 2016; Einoder et al. 2018), but there has also been some evidence for possible biomagnification (Barwick and Maher 2003).

There was also statistically significant difference in cormorant lithium concentrations, but controversial to the hypothesis that the metal concentrations would be higher in the sulphate soil areas, the lithium concentrations of cormorants were higher in the control areas than in sulphate soil areas. In WTEs, there was no difference in lithium concentrations between different groups (Figure 5), and the concentrations in WTEs were slightly higher than in cormorants. Higher lithium concentrations in cormorants from control areas, and similar concentrations of lithium in WTEs, indicate that sulphate soils might not be the primary source of lithium contamination for cormorants or WTEs. For cormorants, there was also a decreasing trend in lithium concentrations from north to south, indicating differences in exposure in different parts of the coast. Lithium does not have a known biological role, but it has not been found to be very toxic. Lithium behaves biologically similarly to sodium, and it can disrupt ion metabolism (Aral and Vecchio-sadus 2008). I did not find established toxicity levels of lithium for blood for birds, but for humans, 10 mg/l of lithium carbonate in blood causes mild lithium poisoning (Aral and Vecchio-sadus 2008). The highest lithium concentration measured was 3.8 µg/l for cormorants, and 8.4 µg/l for WTEs, which is over thousand times lower than the limit of mild lithium poisoning for humans. This combined with the low toxicity for lithium suggests that the birds of this study would not be suffering from toxic effects caused by lithium.

For aquatic birds such as WTEs and cormorants the most probable mode of uptake of metals is through gastro-intestinal track (Szefer 2002), the metals need to get into the prey of the birds before they can be transferred to the birds. Although studies have measured elevated metal concentrations in sulphate soil estuaries (e.g. Wallin et al. 2015; Nystrand et al. 2016), non-bioavailable form and dilution and sedimentation of the metals before they accumulate in the fish and other prey species mean that the metals are not available for the uptake of the birds.

The metals found on the birds could be of natural background metal contamination, or the source of metals could be in other anthropological activities, for example industry or agriculture. At least some of the metal burden of the birds could also be originated from outside the sampling sites and transferred to the chicks maternally through the egg (Burger 1994). Some of the metals could have been accumulated to the females before egg laying possibly from the wintering or feeding areas. Cormorants are migratory with main breeding areas in Central Europe and Mediterranean, and WTEs can move in large areas around the Baltic, Central Europe, and Russia (Saurola et al. 2013). Some of the metal burden originating from outside the sampling sites could explain why there generally was no difference in the metal burden of the birds between the control and sulphate soil areas. Though blood usually reflects recent exposure to contaminants, blood levels of arsenic, cadmium and lead have been also found to correlate with the accumulation in the liver (Berglund 2018). Maternal transfer of metals into chicks could probably explain the results of this study only partially. Also, there are differences in maternal transfer rates between different metals (Agusa et al. 2005), and maternal transfer might thus not be relevant source of contamination for chicks for all metals.

4.2. Spatial variation and covariation in nestling metal concentrations

Concentrations of many metals correlated spatially between species, and there were also latitudinal trends in the concentrations of several elements in both WTEs and cormorants. The spatial correlations and similarities in latitudinal trends in both species, as well as intraspecific correlations in concentrations of metals show that there is spatial variation in the metal contamination, and that concentrations of some metals have similar patterns of variation along the Finnish coast. The similarities in spatial trends of both species also indicate, that some of the metal burden of the WTEs and cormorants is originated from the same contamination sources.

In case of arsenic concentrations in both species, lead of concentrations cormorants, and cadmium, manganese and selenium concentrations of WTEs, there was a latitudinal trend of concentrations being higher in the southern sampling points than in the north. For lead, the highest concentrations of both cormorants and WTEs were from the southernmost sulphate soil sampling point. For these metals, the contamination seems to be higher in the southern parts of the Finnish coast. Airborne emissions from industrial sources are known to relevant source of contamination at least for lead and cadmium (HELCOM 2010). Arsenic can also be transferred in small amount as atmospheric emissions, but generally atmospheric arsenic concentrations are low, and most of arsenic contamination is of natural origin, leaching from soils and sediments (Smedley and Kinniburgh 2002). Manganese is released to the environment with waste water and sewage sludge, and from mining activities, and it also can be spread via air (Howe et al. 2004).

Latitudinal trend of higher concentrations in the northern sampling areas was observed from thallium in both species, and in cobalt, lithium and rubidium of cormorants. For thallium, there was also correlation between WTE and cormorant thallium concentrations. The similarities in the thallium exposure trends in both species indicate that the thallium burden in WTEs and cormorants could be from the same contamination sources. Thallium is released into environment mainly from industrial sources, e.g. smelters, and it can be transferred as atmospheric emissions from long distances (Karbowska 2016). The trends in WTE and cormorant thallium concentrations indicate higher environmental contamination in the northern parts of the Baltic, thallium being also possibly carried by air currents from the north.

4.3. Contents and potential effects of selected metals in WTE and cormorant nestlings

Despite aluminium being one of the most prominent metals in leaches from sulphate soils (Fältmarsch et al. 2008), no differences were found in the aluminium concentrations between sulphate soil and control areas. While aluminium doesn’t have known biological function, it has not been found to be very toxic to birds, as the toxicity of aluminium compared to other metals is low, and it’s poorly absorbed in the intestine and it’s excreted efficiently (Scheuhammer 1987). The chronic toxicity of aluminium is mostly due to its disruptive effect on the calcium and phosphorus metabolism (Scheuhammer 1987), and it can also affect egg shell formation, making the shell quality poorer and thinner (Rosseland 1990). I did not find any studies with established levels of blood toxicity of aluminium for birds, but the aluminium levels were similar to those of northern goshawks (Dolan et al. 2017). However, despite the poor absorption and efficient excretion, the aluminium levels in the blood of the WTEs and cormorants were higher than some metals, which indicates that the environmental exposure to aluminium in WTEs and cormorants in the study areas is high. Wallin et al. (2015) did find very high concentrations of aluminium in both sediments and water column in proximity of sulphate soils, indicating high environmental exposure at least in some parts of the coast.

The chromium and cadmium had similar levels in both species (Table 2 and 3). The chromium concentrations of WTEs and cormorants were similar to the levels of eiders in the Baltic (Fenstad et al. 2017) and to Spanish white storks (Maia et al. 2017), and smaller than reported on Polish mallards (Binkowski and Meissner 2013), suggesting that the blood levels of WTEs and cormorants are within background concentrations. Chromium in its trivalent form (CrIII) is important for function of normal metabolism, but chromium in hexavalent form (CrVI) is a toxin, causing e.g. oxidative stress, DNA damage and dysfunction of multiple organs, such as kidneys and liver (Bagchi et al. 2002). Excess dietary chromium has been found to cause oxidative stress followed by decreased body weight and changes in behaviour in birds (Mashkoor et al. 2016).

The cadmium levels reported for Baltic eiders (Fenstad et al. 2017), white storks (Maia et al. 2017) and northern goshawks (Dolan et al. 2017) in were similar to WTEs and cormorants of this study, indicating background levels. However, cadmium has been found to start to cause oxidative stress in blood levels of 0.2 µg/l in eagle owls (*Bubo bubo*) (Espín et al. 2014b), suggesting that most individuals in this study might have been suffering from oxidative stress caused by cadmium. Cadmium is most prevalent in liver and kidneys, where it also accumulates, and can cause e.g. renal failure, anaemia and supressed reproductive success (Scheuhammer 1987). Cadmium has not been found to biomagnify in aquatic food chains (Barwick and Maher 2003; Nfon et al. 2009; Guo et al. 2016; Einoder et al. 2018).

Lead affects multiple organs and systems in organism, and symptoms of acute lead poisoning in birds include for example anaemia, weight loss, and dysfunction of nervous system, kidneys and cardiovascular system (Fisher et al. 2006). Lead can also affect reproductive success by reducing egg formation and thinning the egg shells. In birds lead is accumulated mostly in bones, and out of soft tissues to kidneys and females accumulate higher concentrations of lead than males, possibly due to metabolism related to the egg shell formation (Scheuhammer 1987). Lead has not been found to biomagnify higher in the food chain (Barwick and Maher 2003; Nfon et al. 2009; Cui et al. 2011; Guo et al. 2016; Einoder et al. 2018).

The limit of sublethal lead poisoning for bald eagles (*Haliaeetus leucocephalus*) has been proposed to 200 µg/l for blood (Kramer and Redig 1997), while lower limit of toxicity, 25 µg/l, has been proposed for other predatory species, the golden eagle (*Aquila chrysaetos*) (Ecke et al. 2017). Two individuals of WTEs in this study had lead concentrations >70 µg/l, while the concentrations of rest of the individuals ranged from 0.5 to 15 µg/l. Based on the limit of 25 µg/l for sublethal lead poisoning suggested by Ecke et al. (2017), the two WTE individuals with concentrations of >70 µg/l would be suffering from toxic effects caused by lead, while the lead concentrations in rest of the individuals were below the toxicity limit of 25 µg/l, suggesting no lead poisoning. The lead levels in cormorants were lower than those measured from WTEs, with maximum concentration of 3.3 µg/l (Table 1), which is well below of both limits of toxicity suggested by Kramer and Redig (1997) and Ecke et al. (2017). The lead concentrations in blood are highest right after ingestion, before lead is transferred to other tissues (Fisher et al. 2006). This could explain the high concentrations (>70 µg/l) of two WTE individuals, as they could have ingested lead containing prey before sampling. Lead is serious problem for Finnish WTEs, as third of the WTE mortality is caused by lead poisoning (Isomursu et al. 2018). Although the lead concentrations appear to be in the same level as in Baltic eiders (Fenstad et al. 2017), suggesting background levels, it is concerning that even nestlings can have lead concentrations linked with sublethal lead poisoning, as lead accumulates in the tissues of the bird, increasing the risk for chronic lead poisoning (Scheuhammer 1987). High lead concentrations have been linked with higher amounts of mammal carcasses and water fowl in German WTEs' diet (Nadjafzadeh et al. 2013), which could also explain the higher lead concentrations of WTEs in this study compared to the piscivorous cormorants.

Although mercury hasn't been associated to sulphate soils in literature, it is a well-known environmental pollutant, and thus it was taken into the analysis. Mercury can occur in its inorganic form, or as organic methylmercury, which is highly toxic to organisms. Inorganic mercury is mainly distributed to the kidneys, can cause renal toxic effects and affect reproduction (Scheuhammer 1987). Methylmercury affects nervous system and reproduction (Scheuhammer 1987). Mercury is known to biomagnify in aquatic food chains (Nfon et al. 2009; Cui et al. 2011; Guo et al. 2016; Einoder et al. 2018), which is especially problematic for apex species such as cormorants and WTEs, as they can accumulate high concentrations of mercury in their tissues over time. Mercury concentrations in the fish and mussels in the most parts of the Finnish coast are known to be high, exceeding the threshold value of 20 µg/kg ww established in the Environmental quality standards of EU (HELCOM 2018), indicating that cormorants and WTEs could be exposed to high amounts of mercury through their diet. The mercury levels of cormorants varied from 95 to 434 µg/l, except for one individual from the northernmost sampling area, which had mercury level of 871 µg/l. For WTEs, the mercury concentrations varied from 67 to 481 µg/l, except for two individuals from the same northernmost sampling area, which had mercury concentrations of 680 µg/l and 1260 µg/l. Mild toxic effects of mercury have been found to generally start to show in birds in blood levels of total mercury of 200 µg/l, while most effects start to occur between 1000 and 3000 µg/l, and more severe symptoms start from 3000 µg/l (Ackerman et al. 2016), but increased oxidative stress caused by mercury has been associated with mercury blood levels as low as 30 µg/l in Eurasian eagle owl (*Bubo bubo)* (Espín et al. 2014b). Based on these levels of toxicity, the birds of this study, especially those with mercury concentrations higher than 200 µg/l, could be suffering from mild toxic effects of mercury, and all individuals were in the range of increased oxidative stress caused by mercury exposure. The mercury concentrations of WTEs and cormorants were in same range as the mercury in Baltic eiders (Fenstad et al. 2017), but due to their position higher in the food web, WTEs and cormorants may accumulate higher mercury concentrations over time. Also, assessing whether the nestlings were suffering from mercury poisoning can be problematic, as the mercury concentrations in nestlings depend on the age of the chick, concentrations being highest right after hatching due to maternal transfer of mercury into the egg and further to the chick, then declining when chicks start to grow, and concentration increasing again when chicks start to gain mass (Ackerman et al. 2011). Most levels of toxicity have been established on adult birds, for which the concentrations of mercury show less intraindividual variation dependant on the age. Also, assessing toxicity of mercury from total mercury concentration can be misleading, as mercury in its organic form is more toxic than inorganic mercury.

The rubidium concentrations in both species were high compared to most elements, and similar to selenium concentrations (Table 2 and 3). Only concentrations of zinc were higher than those of rubidium. Rubidium does not have known biological role, but it is known to behave physiologically similar to potassium, and it's distributed in large amounts to red cells (Relman 1956), which could explain high concentrations in cormorant and WTE blood samples. Rubidium is not very toxic to organisms, and substitution of potassium by rubidium is tolerated by organisms to some extent (Relman 1956), possibly meaning that though the rubidium concentrations in WTEs and cormorants were high compared to other elements in this study, the birds might not suffer from toxic effects caused by rubidium.

Thallium concentrations in bird blood haven't been studied, and no blood toxicity levels for birds have been established. Thallium concentrations in cormorants and WTEs in this study were among the lowest concentrations compared to the other elements, ranging from 0.01 to 0.3 µg/l. Similar to rubidium, the toxic effect of thallium is based mainly on its disruptive effect on potassium metabolism (Karbowska 2016). For humans, normal blood concentration is 2 µg/l, and concentrations over 100 µg/l are considered toxic (Lansdown 2013). The concentrations in birds of this study were well below the normal thallium blood concentrations of humans, and thousandth part of the toxic concentrations of humans.

Selenium is an essential trace element, that is found e.g. in amino acids and enzymes, and is thus needed for proper function of metabolism (Spallholz and Hoffman 2002). However, high concentrations of selenium are toxic to animals, and the toxicity depends on the form which selenium has ingested. Selenium has multiple mechanism of toxicity, and in birds selenium can cause e.g. teratogenesis and other reproductive disruptions, and dysfunction of liver, kidneys and immune system

(Spallholz and Hoffman 2002). Einoder et al. (2018) did not find selenium to biomagnify, while Barwick and Maher (2003) found evidence for biomagnification of Se in aquatic system. Selenium concentrations in marine birds are often higher than the concentrations in terrestrial birds, for which the blood toxicity level of selenium has been considered to be 100-400 µg/l (Ohlendorf and Heinz 2011), which all the birds of this study exceeded. However, the selenium concentrations of WTEs and cormorants were similar to the selenium levels reported for Baltic eiders (Fenstad et al. 2017), suggesting that they were within background levels. Selenium can function also as mitigating antagonist for mercury by increasing the biotransformation of organic methylmercury into less toxic inorganic mercury (Wang and Wang 2017), thus reducing the toxic effects of mercury.

The zinc levels measured from WTEs and cormorants were highest of all elements included in this study (Table 1 and 2), and slightly higher than reported in Baltic eiders (Fenstad et al. 2017), white-storks (Maia et al. 2017) and northern goshawks (Dolan et al. 2017). The symptoms of zinc poisoning can include pancreatic and intestinal lesions, weight loss, depression and impaired movement (Zdziarski et al. 1994). Blood toxicity levels for birds haven't been established for zinc (Espín et al. 2014a), but high zinc concentrations can partly be explained with the necessity of zinc as a trace metal for the metabolism. However, birds can regulate zinc levels efficiently (Beyer et al. 2004), meaning that even high concentrations of zinc do not necessarily mean poisoning.

For many of the metals in this study, including cobalt, copper, manganese, nickel, silver, and uranium, little or no data on the toxicity for birds exists. While copper and manganese are needed by the metabolism, rest of the elements do not have known biological functions. The blood metal concentrations in this study were similar to those previously reported in birds for manganese (Maia et al. 2017), copper (Fenstad et al. 2017; Maia et al. 2017) and cobalt (Maia et al. 2017). The nickel concentrations in WTEs and cormorants were similar to concentrations reported on Northern goshawks in Norway and Spain (Dolan et al. 2017), and on white-storks (Maia et al. 2017), and lower than reported on Polish mallards (Binkowski and Meissner 2013). The concentrations of silver in cormorants and WTEs were among the lowest of this study, and uranium concentrations were lowest of all elements included. I did not find studies reporting blood concentrations of uranium or silver for birds.

Although most WTE and cormorant individuals did not appear to have very high metal concentrations in their blood compared to blood concentrations reported for other species, some of the sampled chicks had metal levels associated with possible toxic effects. High concentrations of metals in young individuals could be partially explained by maternal transfer, as mechanism of excretion are not yet functioning (Ackerman et al. 2011). Comparing the metal concentrations of young individuals to toxicity levels established in literature can be problematic, as most often toxicity levels have been established for adults. The metal concentrations between chick and adults of same species can differ (e.g. Kim and Oh (2015)) possibly due to bioaccumulation of metals in adults, and also due to difference in metabolic in growing chicks and adults. Young individuals can be susceptible to toxic effects of metals at lower level than older individuals (Scheuhammer 1987), and metal concentrations in chicks can also vary non-linearly in chicks of different ages (Ackerman et al. 2011). Many studies considering toxicity of metals in birds have been done for other tissues than blood, such as liver and kidneys. Although blood levels correlate with metal concentrations from other tissues (Berglund 2018), comparing blood with other tissues' concentrations can be difficult, as blood levels indicate recent exposure, and other tissues long time exposure and accumulation (Fisher et al. 2006; Berglund 2018). However, for young birds, blood levels of contaminants can be more relevant than levels in internal organs, as chicks haven't yet accumulated contaminants in their tissues. Also, as blood indicates recent exposure to contaminants, it is well suited for studying spatial differences in contamination, as the blood levels of contaminants measured from nestlings reflect the contamination in the nesting area.

As I didn't find any studies with blood metal concentrations reported for cormorants and WTEs, I used concentrations from other species as reference for background levels. However, different species can accumulate metals differently due to differences in diet and trophic level, so comparing metal concentrations with other species may be misleading. Also, the birds may be suffering from toxic combination effects of metals. Combination effect of many metals can be hard to predict, as different metals may have interacting effects. While some metals might be more toxic together than alone, thus showing synergistic effects, some metals can function as antagonists, mitigating the toxic effects. For example, arsenic, tellurite, tin and lead have been found to reduce the toxicity of selenium (Howell and Hill 1978). Metals can also be present in many forms, some of which might be more harmful to organism than other. For example, methylmercury is known to be significantly more toxic than inorganic mercury (Ackerman et al. 2016), and chromium is essential for function of metabolism in its trivalent form, but toxin as it's hexavalent form (Bagchi et al. 2002). Thus, the total concentration of the metal is not necessarily descriptive of its potential harmful effects.

Although no difference in concentrations of most metal between birds from sulphate soil and control areas, the metal emissions may have indirect effects on the birds by affecting the populations of their prey species. When carried to the estuaries by the river effluents, the metals often end up in the sediments, where they can end up to the benthic invertebrates and further into species higher in the food chain. Deteriorated benthic communities have indeed been observed in the estuaries affected by the sulphate soils (Wallin et al. 2015). Also, the flushes from the sulphate soils have been found to affect the reproducing of fish, as their eggs and young are susceptible to low pH and high metal concentrations, thus reducing the size of the fish populations (Fältmarsch et al. 2008). Smaller prey populations and lower prey quality in the sulphate soil areas can affect the WTEs and especially piscivorous cormorants.

4.4. Sources for error

The criteria which was used to decide on the areas considered the proximity of sulphate soil areas and river estuaries, into which the current could carry the sulphate soil material. The division was done using a map showing the probability of occurrence of sulphate soils in each area, based on multivariate analysis model by the Finnish Institute of Geology. However, the actual runoff of contaminants from the sulphate soils is affected largely by the land use in the area, which was not a considered criterium. Also, the spreading patterns of run-off with the coastal water currents were not considered, and the sulphate soil material might thus not be distributed into the colonies and territories of the birds as was thought when planning the experiment. Also, the small patches of sulphate soils were not considered, which might cause error in the control samples. Thus, it might be that the split of sampling areas into the study groups might not reflect the actual impact of the sulphate soils.

5. Conclusions

Based on the results of this study, there is no difference in metal burden in eagles and cormorants in sulphate soil areas compared to individuals outside sulphate soil areas. The result is probably due to sedimentation, dilution and change in the form of the metal when arriving to the saline estuaries, as the water chemistry changes in brackish water compared to fresh water systems. The concentrations of metal varied spatially, and many of the elements had latitudinal trends and spatial correlations. The metal concentrations found in the blood of WTE and cormorant nestlings were generally low, and within background concentrations and below limits of toxicity. For many of the elements often associated with the sulphate soils, e.g. nickel, manganese and zinc, there are no reference levels of bird blood. Although sulphite soils are one of the largest environmental sources of metals in Finland, the metals do not appear to accumulate in food chain.

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8. Appendices

Appendix 1. Solution for fixed effects for cormorants

	Intercept	Lower 95%	Upper 95%	Treatment	Lower 95%	Upper 95%	Latitude	Lower 95%	Upper 95%
Ag	-2.50	-3.31	-1.69	0.00	-1.22	1.21	-0.03	-0.43	0.36
Al	2.82	2.21	3.42	0.29	-0.62	1.20	0.16	-0.14	0.46
As	76.11	53.95	98.27	-1.61	-34.56	31.35	-26.23	-37.35	-15.10
Cd	-0.98	-1.70	-0.26	0.67	-0.40	1.75	0.17	-0.18	0.51
Co	0.83	0.35	1.32	0.22	-0.51	0.94	0.26	0.03	0.49
Cr	-1.39	-1.79	-0.99	0.14	-0.45	0.73	0.00	-0.20	0.21
Cu	386.22	353.16	419.29	-31.27	-80.85	18.31	-7.21	-23.02	8.61
Hg	350.08	177.73	522.43	-78.59	-337.50	180.33	51.04	-30.42	132.51
Li	2.77	2.56	2.99	0.49	0.17	0.81	0.20	0.09	0.31
Mn	4.64	4.40	4.87	-0.07	-0.42	0.29	0.03	-0.08	0.15
Ni	-0.69	-1.56	0.18	-0.13	-1.43	1.17	-0.22	-0.65	0.21
Pb	0.02	-0.26	0.30	-0.21	-0.63	0.21	-0.29	-0.43	-0.15
Rb	2208.21	1973.37	2443.06	-97.40	-447.03	252.23	206.86	89.67	324.04
Se	6.49	6.28	6.70	-0.004	-0.32	0.31	-0.01	-0.11	0.09
Tl	-2.50	-2.90	-2.09	0.57	-0.04	1.18	0.26	0.06	0.45
U	0.0048	0.0027	0.0068	0.0019	-0.0011	0.0049	0.0002	-0.0008	0.0012
Zn	10345.00	9605.50	11085.00	381.58	-726.30	1489.45	278.27	-78.80	635.33

Appendix 2. Solution for fixed effects for WTEs

	Intercept	Lower 95%	Upper 95%	Treatment	Lower 95%	Upper 95%	Latitude	Lower 95%	Upper 95%
Ag	-2.52	-3.04	-2.00	0.06	-0.63	0.76	0.02	-0.18	0.22
Al	2.90	2.44	3.36	0.03	-0.58	0.65	0.04	-0.14	0.22
As	3.48	3.25	3.71	-0.44	-0.74	-0.14	-0.24	-0.33	-0.15
Cd	-2.31	-3.02	-1.61	0.10	-0.84	1.04	-0.39	-0.67	-0.12
Co	0.15	-0.17	0.48	-0.06	-0.50	0.38	0.09	-0.04	0.22
Cr	-0.56	-1.31	0.19	-0.56	-1.56	0.43	0.07	-0.23	0.36
Cu	532.37	447.61	617.13	34.88	-78.06	147.81	-30.07	-63.29	3.16
Hg	5.51	5.05	5.97	-0.10	-0.71	0.52	0.06	-0.12	0.24
Li	1.41	1.25	1.58	0.17	-0.04	0.38	0.01	-0.05	0.08
Mn	59.06	76.15	41.98	-10.96	-33.73	11.81	-7.97	-14.67	-1.28
Ni	-0.44	-1.46	0.57	-0.33	-1.68	1.02	-0.02	-0.42	0.37
Pb	1.45	0.61	2.28	-0.21	-1.32	0.91	-0.25	-0.57	0.08
Rb	2053.98	1558.50	2549.46	-80.27	-740.41	579.88	-65.97	-260.21	128.27
Se	909.37	658.46	1160.28	51.13	-283.16	385.43	-133.37	-231.73	-35.00
Tl	0.033	0.025	0.041	0.007	-0.004	0.018	0.003	0.000	0.007
U	0.0040	0.0021	0.0059	-0.0009	-0.0034	0.0016	-0.0004	-0.0011	0.0004
Zn	10747.00	8856.97	12636.00	-1210.53	-3728.07	1307.01	-434.23	-1174.98	306.53

Appendix 3. Intraspecies Spearman's correlations with their p-values for different metals of cormorants. Correlations with p≤0.05 are marked with green colour.

	Ag	Al	As	Cd	Co	Cr	Cu	Hg	Li	Mn	Ni	Pb	Rb	Se	Tl	U	Zn
Ag	1																
Al	0.22	1															
	0.52																
As	-0.13	-0.22	1														
	0.71	0.52															
Cd	-0.31	-0.24	-0.25	1													
	0.36	0.48	0.45														
Co	0.13	0.25	-0.54	-0.19	1												
	0.71	0.45	0.09	0.57													
Cr	0.26	0.83	0.01	-0.18	-0.26	1											
	0.43	0.002	0.98	0.59	0.43												
Cu	0.72	0.01	-0.14	-0.45	-0.15	0.2	1										
	0.01	0.98	0.69	0.16	0.65	0.56											
Hg	-0.66	-0.09	-0.05	0.19	-0.42	0.05	-0.29	1									
	0.03	0.79	0.87	0.57	0.20	0.87	0.39										
Li	-0.13	0.46	-0.64	0.22	0.72	0.07	-0.45	-0.11	1								
	0.71	0.15	0.04	0.52	0.01	0.83	0.17	0.75									
Mn	0.50	-0.22	-0.41	0.23	0.10	-0.25	0.42	-0.08	-0.06	1							
	0.12	0.52	0.21	0.50	0.77	0.47	0.20	0.81	0.85								
Ni	0.03	-0.05	0.34	-0.09	-0.12	-0.10	0.22	-0.33	-0.43	-0.25	1						
	0.94	0.87	0.31	0.79	0.73	0.77	0.52	0.33	0.19	0.45							
Pb	-0.36	-0.50	0.82	-0.07	-0.58	-0.29	-0.16	0.21	-0.74	-0.39	0.52	1					
	0.27	0.12	0.002	0.83	0.06	0.39	0.63	0.54	0.01	0.23	0.10						
Rb	-0.18	0.36	-0.60	0.08	0.62	0.05	-0.15	0.05	0.68	0.02	-0.16	-0.61	1				
	0.59	0.27	0.05	0.81	0.04	0.87	0.65	0.89	0.02	0.96	0.63	0.05					
Se	-0.26	-0.57	0.17	0.03	0.09	-0.78	-0.18	0.15	-0.26	0.18	0.33	0.54	-0.25	1			
	0.43	0.07	0.61	0.94	0.79	0.005	0.59	0.65	0.43	0.59	0.33	0.09	0.45				
Tl	-0.09	0.44	-0.24	0.32	0.47	0.16	-0.55	-0.06	0.72	0.06	-0.42	-0.50	0.65	-0.28	1		
	0.79	0.18	0.48	0.34	0.14	0.63	0.08	0.85	0.01	0.85	0.20	0.12	0.03	0.40			
U	0.15	0.59	-0.19	-0.06	0.02	0.52	0.01	-0.01	0.33	-0.37	0.13	-0.15	0.09	-0.22	0.07	1	
	0.67	0.06	0.57	0.85	0.96	0.10	0.98	0.98	0.33	0.26	0.71	0.67	0.79	0.52	0.83		
Zn	-0.169	0.620	-0.155	0.023	0.355	0.351	-0.360	0.114	0.565	-0.182	-0.105	-0.314	0.743	-0.228	0.797	0.396	1
	0.62	0.04	0.65	0.95	0.28	0.29	0.28	0.74	0.07	0.59	0.76	0.35	0.01	0.50	0.003	0.23	

Appendix 4. Intraspecies Spearman's correlations with their p-values for different metals of WTEs. Correlations with p≤0.05 are marked with green colour.

	Ag	Al	As	Cd	Co	Cr	Cu	Hg	Li	Mn	Ni	Pb	Rb	Se	Tl	U	Zn
Ag	1																
Al	0.25 0.37	1															
As	0.24 0.40	0.12 0.67	1														
Cd	-0.33 0.23	-0.36 0.19	0.31 0.27	1													
Co	0.40 0.14	0.65 0.01	-0.18 0.53	-0.29 0.29	1												
Cr	0.42 0.12	0.70 0.003	-0.04 0.89	-0.27 0.33	0.71 0.003	1											
Cu	-0.51 0.05	-0.13 0.66	0.10 0.71	0.55 0.03	-0.34 0.22	-0.10 0.73	1										
Hg	-0.63 0.01	-0.02 0.94	-0.11 0.69	0.30 0.27	-0.32 0.24	-0.15 0.59	0.61 0.02	1									
Li	0.39 0.15	0.41 0.12	-0.27 0.33	-0.54 0.04	0.56 0.03	0.38 0.16	-0.29 0.29	-0.49 0.06	1								
Mn	-0.10 0.71	0.10 0.71	0.35 0.21	0.76 0.001	-0.05 0.85	0.09 0.74	0.59 0.02	0.46 0.08	-0.3357 0.22	1							
Ni	0.64 0.01	0.05 0.85	0.10 0.73	-0.21 0.44	0.10 0.71	0.36 0.19	-0.17 0.55	-0.27 0.33	0.11 0.69	-0.22 0.44	1						
Pb	-0.50 0.06	-0.27 0.33	0.11 0.69	0.47 0.08	-0.23 0.42	-0.12 0.68	0.68 0.01	0.40 0.14	-0.45 0.10	0.30 0.28	-0.08 0.78	1					
Rb	-0.62 0.01	0.08 0.77	-0.004 0.99	0.53 0.04	-0.16 0.56	-0.05 0.86	0.75 0.001	0.87 <.0001	-0.40 0.14	0.63 0.01	-0.34 0.22	0.49 0.06	1				
Se	-0.45 0.09	-0.15 0.59	0.25 0.38	0.75 0.001	-0.30 0.28	-0.10 0.71	0.85 <.0001	0.70 0.004	-0.45 0.09	0.81 0.0002	-0.21 0.45	0.69 0.004	0.78 0.001	1			
Tl	-0.25 0.38	0.01 0.96	-0.24 0.40	0.09 0.74	0.22 0.44	0.19 0.51	0.01 0.97	0.26 0.35	0.04 0.88	0.01 0.98	-0.02 0.94	0.32 0.24	0.30 0.27	0.23 0.42	1		
U	-0.07 0.81	0.54 0.05	0.20 0.50	0.10 0.74	0.25 0.39	0.16 0.58	0.12 0.69	0.48 0.08	0.14 0.63	0.55 0.04	-0.41 0.15	-0.17 0.56	0.40 0.15	0.37 0.19	0.02 0.95	1	
Zn	-0.69 0.005	-0.05 0.85	0.09 0.75	0.62 0.01	-0.23 0.42	-0.18 0.53	0.81 0.0002	0.75 0.001	-0.50 0.06	0.61 0.02	-0.41 0.13	0.59 0.02	0.92 <.0001	0.77 0.001	0.05 0.86	0.27 0.35	1