Metal concentrations in feathers and blood of Northern goshawk (*Accipiter gentilis*) nestlings from Norway and Spain

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Abstract

Information on metal pollution in the terrestrial environment and its biota is limited compared to the marine environment. In the present study, we collected body feathers and blood of 37 Northern goshawk (*Accipiter gentilis*) nestlings from the regions of Troms (northern Norway), Trøndelag (central Norway), and Murcia (southern Spain) to study both internal and external regional exposure to metals. Blood and body feathers were analyzed for aluminum (Al), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), cadmium (Cd), mercury (Hg) and lead (Pb), and the influence of latitude, urbanization and land usage in proximity to the nesting Northern goshawks was investigated. Most metals were detected below literature toxicity thresholds, except for Zn, Cd, and Hg. Multiple linear models indicated latitude was a significant factor influencing Zn, Cd and Hg feather concentrations, but only for Zn this was reflected in a significant relationship to latitude in the univariate regional comparisons. Trondheim nest sites were located closest to urbanization and agricultural land use. However, neither urbanization nor land use had a significant impact on metal concentrations in feathers. Blood sample metal concentrations were not significantly influenced by any of the investigated factors. The present study's nestlings exposure to metal pollution was minimal, terrestrial raptors should be considered alongside marine species when assessing metal pollution scenarios.

Keywords: Metal pollution, external contamination, terrestrial raptors, biomonitoring, wildlife.

Research highlights:

- Metal exposure was investigated in Northern goshawks over a latitudinal gradient
- Concentrations were investigated in nestling's body feathers and blood
- The influence of latitude, urbanization and agriculture on exposure was investigated
- Only latitude influenced metal levels in body feathers
- Overall metal levels do not indicate toxic effects
- Zn, Cd and Hg levels were however above toxic threshold levels in some locations

1.0 Introduction

Anthropogenic activities are releasing metals into the environment causing a global increase of metal concentrations, particularly in the atmosphere of industrialized and developing countries (Pacyna and Pacyna, 2001). Metals are introduced into the environment from many anthropogenic sources such as fossil fuel (Lough et al. 2005, Wang et al. 2003) and coal combustion (Pacyna and Pacyna 2001, Reddy et al. 2005), waste water irrigation (Xue et al. 2012), and mining and smelting processes (Koptsik et al. 2003, Pacyna et. al. 1991). Above a certain threshold, metals cause damage on the cellular level (Ercal et al. 2001), leading to negative effects at higher organizational levels. For example, metal toxicity has been related to reduced avian clutch sizes and reduced reproductive success, which ultimately threaten wildlife populations (Eeva et al. 2009a), while growth inhibition and metal accumulation in internal organs reduce individual fitness and lower the chances of survival (Eeva et al. 2009b).

Biomonitoring efforts can give information on wildlife exposure to metals (Burger and Gochfeld 1993, Bustnes et al. 2013). Metal concentrations found in certain sentinel species estimate environmental metal pollution as well as could be used to indicate wildlife and human health risks. Top predators, such as birds of prey, can give information on bioaccumulating substances (Lodenius and Solonen 2013). Northern goshawks (*Accipiter gentilis*) are an especially suitable sentinel species because they are opportunistic hunters, seeking out a variety of available prey in their environment.

Goshawks prey on small birds and mammals, mainly several species of grouse (*Tetrao tetrix*), pigeon (*Columba palumnbus*), small birds (e.g. *Garrulous glandarius*, *Corvus corone*), and squirrels (*Sciurus vulgaris*) (Linden and Wikman 1983, Petty 2003, Widen 1997). They have been used as an indicator species to study regional contamination and pollution patterns of an ecosystem (Kenntner et al. 2003). In addition, pre-fledged nestlings are conveniently accessible compared to their parents and provide newly grown feathers, which are still connected to the blood stream. In addition, they provide information on the local environment around the nest. Both agricultural land use and urbanization can influence the Northern goshawk diet by reducing the diversity of prey species available, as well as be a potential source for metal pollutants from the application of agricultural products, aerosols from fuel combustion, and tire dust (Adachi and Tainosho 2004, Besnard et al. 2001, Lighty et al. 2000, Roca et al. 2007, Wauchope 1978). By reducing suitable habitat, both of these factors can also cause Northern goshawks to reduce their territories, influencing diet by the resulting higher competition for prey with neighboring raptors.

Feathers have been used for monitoring of heavy metal pollution for over 50 years (Burger 1993, Weyers and Glück 1988). Feathers are connected to the bloodstream upon growth. Feathers contain keratin, a protein with many disulfide bonding sites. These disulfide sites can chelate metals, depositing them into the feather's structure as it is being formed. After the feather is formed the blood connection is severed leaving the feather with information of circulating blood metal concentrations integrated over the period of feather growth. The most important problem to consider when using feathers is the susceptibility of feathers to external contamination and the variation among feathers at different stages of the molting process (Borghesi et al. 2016, Dauwe et al. 2003, Jaspers et al 2004). Lodenius and Solonen (2006) recommend the use of whole, smaller body feathers from nestlings when monitoring Hg because they contain less variation compared to wing feathers.

The objectives of the present study were to assess exposure to metal pollution in Northern goshawk nestlings using body feathers and blood. Variations in metal concentrations were investigated according to latitude, land use and urbanization. The present study also reports concentrations in the blood and feathers against literature threshold concentrations of toxicity.

2.0 Methods

Northern goshawk nestlings were sampled from three regions: Murcia in southern Spain, Trøndelag in central Norway and Troms in northern Norway (Figure 1). In each region we sampled the oldest chick from 8-20 nest sites for body feather and a blood sample. From each selected nestling one body feather from the back and 200 µL of whole blood were used for metal analysis. One Murcia blood sample was not available, thus 36 sites were included in the blood analysis and 37 sites were included in the feather analysis. Because of the small sample size in Murcia, all available data was used.

Blood samples were unfrozen in the lab. They were diluted with 0.5 mL of double deionized water (Milli-Q) and subsequently 0.5 mL of concentrated nitric acid. Samples were then digested in a high-pressure microwave system (Milestone UltraClave, EMLS, Leutkirch Germany). Digestion heated samples up to a maximum temperature of 245ºC at approximately 120 bar for 2.5 hours (Supplementary Information Figure SI-1). After digestion the samples were diluted to a volume of 12 mL with Milli-Q water and transferred into 15 mL polypropylene tubes.

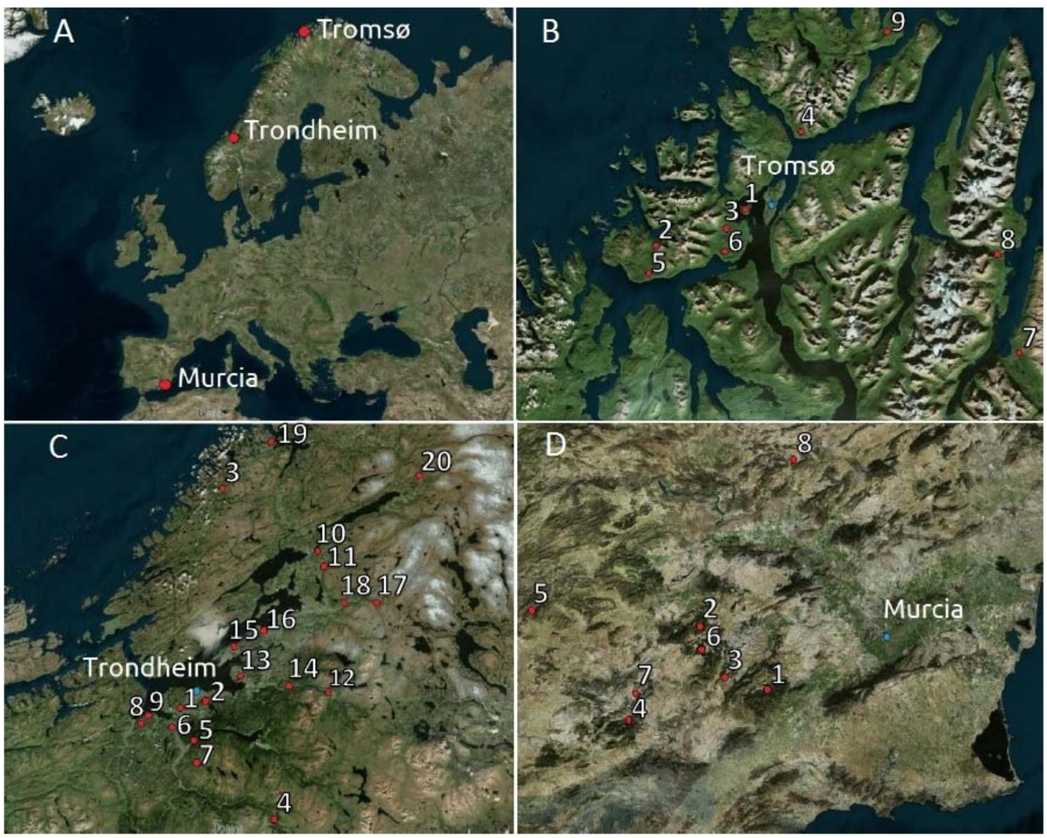

Figure 1 shows A) the three study regions, B) the 9 nests sampled in Tromsø, northern Norway, C) the 20 nests sampled in Trøndelag, Norway, and D) the 8 nests sampled in Murcia, southern Spain.

After each nestling’s body feather mass and length were recorded, it was washed sequentially with Milli-Q 1M nitric acid and acetone (Sigma-Aldrich, ≥99.5%, GC). Feathers were left to dry in the fume hood overnight covered with clean filter paper. The feathers were then diluted with 3 mL of 50% *v/v* nitric acid and digested in a high-pressure microwave system (Milestone UltraClave, EMLS, Leutkirch Germany). Digestion heated samples up to a maximum temperature of 245ºC at approximately 120 bar for 2.5 hours (Figure SI-1). Samples were diluted with Milli-Q to a final volume of 30 mL, and a portion of each was transferred into 15 mL propylene tubes.

A high resolution ICP-MS using argon gas analyzed all diluted and digested samples for 56 elements, using magnet settings of low, medium or high resolution to detect each element. Each element was scanned three times, giving an individual relative standard deviation (RSD) for each number. Rhenium was used as an internal standard. In addition, results of each element were manually adjusted for drift in baseline and sensitivity versus multi-element calibration solutions. Interfering oxides (e.g. molybdenum oxide for Cd and tungsten oxide for Hg) not resolved by resolution, were manually corrected. The detected amounts in individual feather and blood samples were expressed on their specific mass or volume respectively.

Hg:Se molar ratios were calculated for each blood sample. Se being an important cofactor for antioxidant enzymes, where Se acts as a binding site for demethylated Hg, reducing the potential for secondary toxicity from methyl Hg (Eagles-smith et al. 2009).

2.1 Calculation of geographic factors

In the present study there were no metal pollution sources identified within 50 km of the sampled nests, with exception from urban encroachment and agricultural land use. In the absence of obvious pollution sources, we chose to evaluate the effect of three variables, i.e. latitude of the nest location, degree of urbanization, and anthropogenic land usage, on metal concentrations in the feathers.

Latitude identifies broad regional differences in metal contaminations, allowing for a north-south comparison of abiotic transport. The three separate regions were at 37.79-38.46°, 62.89-63.° and 69.40-69.99° northern latitude.

Degree of urbanization was calculated by quantifying and summing the area vector layers of all man-made structures defined as “developments” within a 5km radius around each nest site (Figure SI-2) using quantum geographic information systems (QGIS) software program (QGIS Development Team 2015). Austen (1993) found a nesting home range for Northern goshawks in the southern Cascade mountains to be an area equivalent to 3.46 km radius around each nest, while Rutz (2006) found home ranges in Germany to be an area equivalent to 1.7 km radius around the nest. Tornberg et al. (2006) reported home range radii for Northern goshawks of 4.4 km in southern Sweden, and 4.8 km in Finland. In the present study, we calculated based on a conservative 5 km radius to account for adults possibly traveling further in one study region compared to another. In the present study, developments were defined as roads (Aasetre 2005), railroads, and buildings. Land usage was similarly calculated using QGIS, quantifying the vector layer areas of man-made agricultural fields and pastures within a 5 km radius of each nest.

2.2 Statistics

In order to analyze differences in metal concentrations, as well as Hg:Se ratios, in feathers and blood among regions, parametric ANOVA or non-parametric Kruskall-Wallis models were used. RStudio software, version 0.98.507 for Linux (RStudio Team 2015) was used to assess normality and homoscedasticity. Normality was visually assessed with quantile-quantile plots, as well as Shapiro-Wilk’s hypothesis tests. Homoscedasticity was determined by visually assessing the residuals and fitted values of the ANOVA models, looking for uniform distribution patterns. Tukey and Dunnett models were used for post-hoc clarification when needed. The H_o was rejected and groups were assumed significant if they were outside of the 95% confidence interval ($\alpha = 0.05$). No metal concentrations were transformed and no outliers were removed, because pollution exposure can include rare high outlying values (Zuur et al. 2007). Potential outliers were evaluated looking for unusually high value patterns observed in the same sample for multiple metals (suggesting contamination) and if the ICP MS's RSD values were high (>10%) for that specific sample. High values were determined visually using Cook's distance graphs and influential point charts.

Adonis, a function of Rstudio software in the vegan package, was used to look for significant causal relationships between the three explanatory factors and the metal concentrations as a group using distance matrices (Oksanen 2015). Multiple linear regressions, using the same three factors (latitude, urbanization, land use) to explain individual metal concentrations were performed for those metals exceeding avian toxicity thresholds.

3.0 Results

3.1 *Variation in metal concentrations by region*

The full list of metals along with their median and range in body feathers and blood are represented in the supplementary information (Table SI 1 and SI 2). Of all metals investigated, nine metals (Al, Ni, Cu, Zn, As, Se, Cd, Hg, Pb) are further compared between regions. These nine were chosen either because of their toxicity (Cd, Hg, and Pb), because they are frequently found in other metal pollutant studies (As, Cu, Ni, and Zn), or they offer information on the behavior of other metals (Al, Se).

Three metals, As, Se and Hg, were found to differ geographically in blood samples. The trend for these three metals generally followed the same trend in the feathers. Furthermore, there were geographical variations in Al and Zn in feather samples that were not detected in blood samples.

3.1.1 Blood metal concentrations

For most of the metals analyzed, we did not find significant variation among study regions (Figure 2). Blood Se ($F_{2,33} = 15.19$, $p < 0.01$) and Hg ($F_{2,33} = 6.06$, $p < 0.01$) concentrations were significantly different among regions, with significantly higher Se and Hg in Trondheim compared to Tromsø (Se: $t_{33} = 3.83$, $p < 0.01$; Hg: $t_{33} = 2.73$, $p = 0.03$) and Murcia (Se: $t_{33} = -4.89$, $p < 0.01$; Hg: $t_{33} = -2.85$, $p = 0.02$). Nestling from nest 18 from Trondheim had the highest blood Hg concentration: 0.13 µg mL^{-1} (Figure 1C). The molar ratio of Hg to Se ranged from 0.007 to 0.080 in nestling blood samples. The Hg:Se ratio did not vary significantly between locations ($F_{2,33} = 3.41$, $p = 0.05$). Blood As concentrations were significantly different among regions ($H_2 = 15.26$, $p < 0.01$) with lowest values found in Murcia ($W_{TOS, MUR} = 112.00$, $p < 0.01$; $W_{TRD,MUR} = 136.00$, $p < 0.01$). Blood As concentrations were below the limit of quantification (LOQ) in six sites, feather As concentrations were below the LOQ in 24 of the sites, both were below the LOQ, particularly in Murcia.

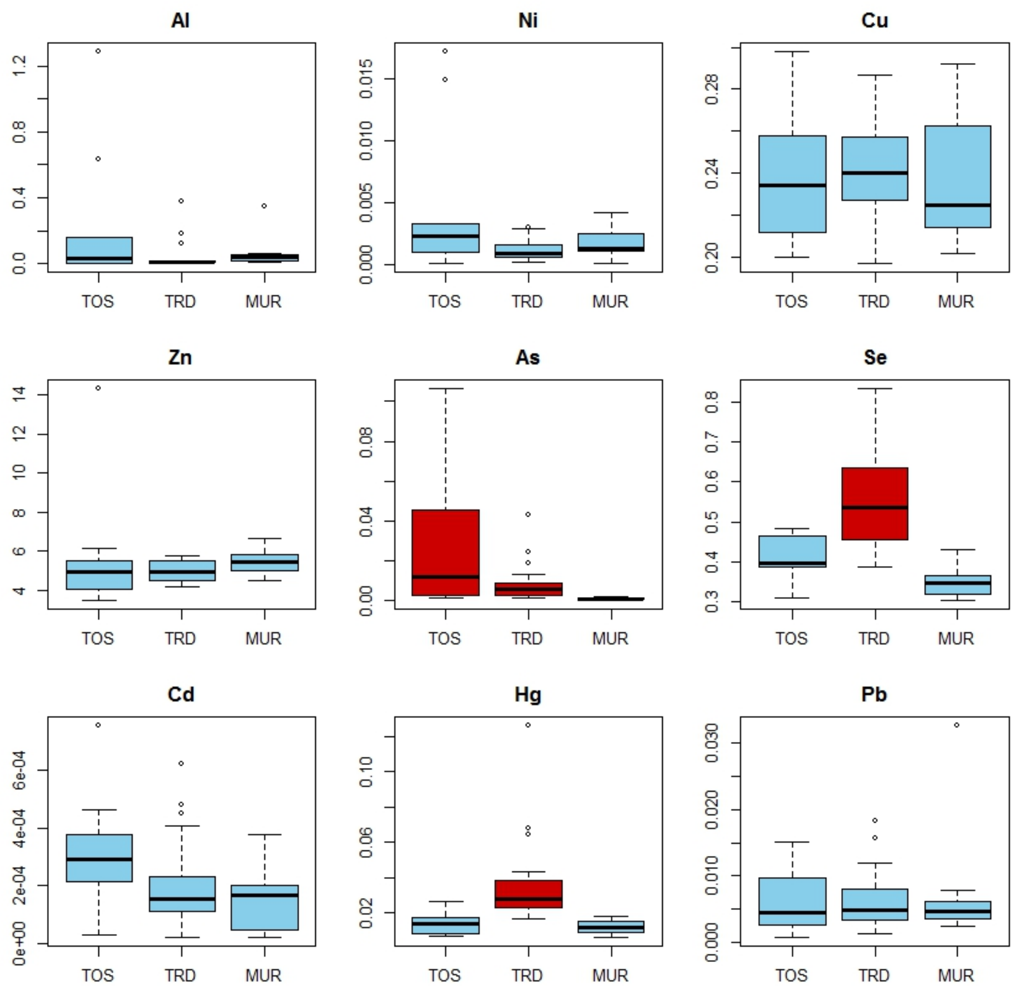

Figure 2 Blood concentrations (µg mL^{-1}) of nine elements grouped by region: Tromsø (TOS), Trondheim (TRD) and Murcia (MUR). Red colors indicate statistically significant differences. The box shows the inter-quartile range (25-75th percentile), the bold line is the median, the whiskers show adjacent values (upper adjacent value is the highest value within the upper quartile range plus 1.5 the length of the inter-quartile range), and dots indicate high/low values outside of both the inter-quartile range and the whiskers.

3.1.2 Body feather metal concentrations

Nestling body feather Al concentrations (Figure 3) were significantly different among regions ($F_{2,34}$ = 8.91, p < 0.01). Feathers sampled in Murcia were significantly higher compared to Trondheim (t_{34} = 3.87, p < 0.01) and Tromsø (t_{34} = 3.71, p < 0.01). There was one high concentration of 23.7 µg g^{-1} dw in nest 8 of Murcia (Figure 1D).

Feather Zn concentrations were significantly different among regions ($F_{2,34}$ = 8.02, p < 0.01). Zn levels were significantly higher in nestlings from Trondheim (t_{34} = 2.73, p = 0.03) and Murcia (t_{34} = 3.97, p < 0.01) compared to those from Tromsø.

Concentration of As in body feathers varied significantly among locations as well ($F_{2,34}$ = 3.91, p = 0.03). As levels in feathers from Tromsø nestlings were significantly higher than those in Murcia (t_{34} = -2.66, p = 0.03). There was one high concentration in Tromsø of 0.08 µg g^{-1} dw in nest 9

(Figure 1B).

Body feather metal concentrations showed trends for Hg and Se similar to those found for blood. Concentrations of Se ($F_{2,34}$ = 7.22, p < 0.01) and Hg ($F_{2,34}$ = 4.04, p = 0.03) in feathers were significantly different among regions, with higher concentrations of Se in Trondheim compared to Tromsø (t_{34} = 2.49, p = 0.04) and both Se (t_{34} = -3.47, p < 0.01) and Hg (t_{34} = -2.66, p = 0.03) were significantly higher in Trondheim compared to Murcia. One exceptionally high Hg concentration (0.68 µg g-1 dw) was found in the nestling from nest 9 (Figure 1B).

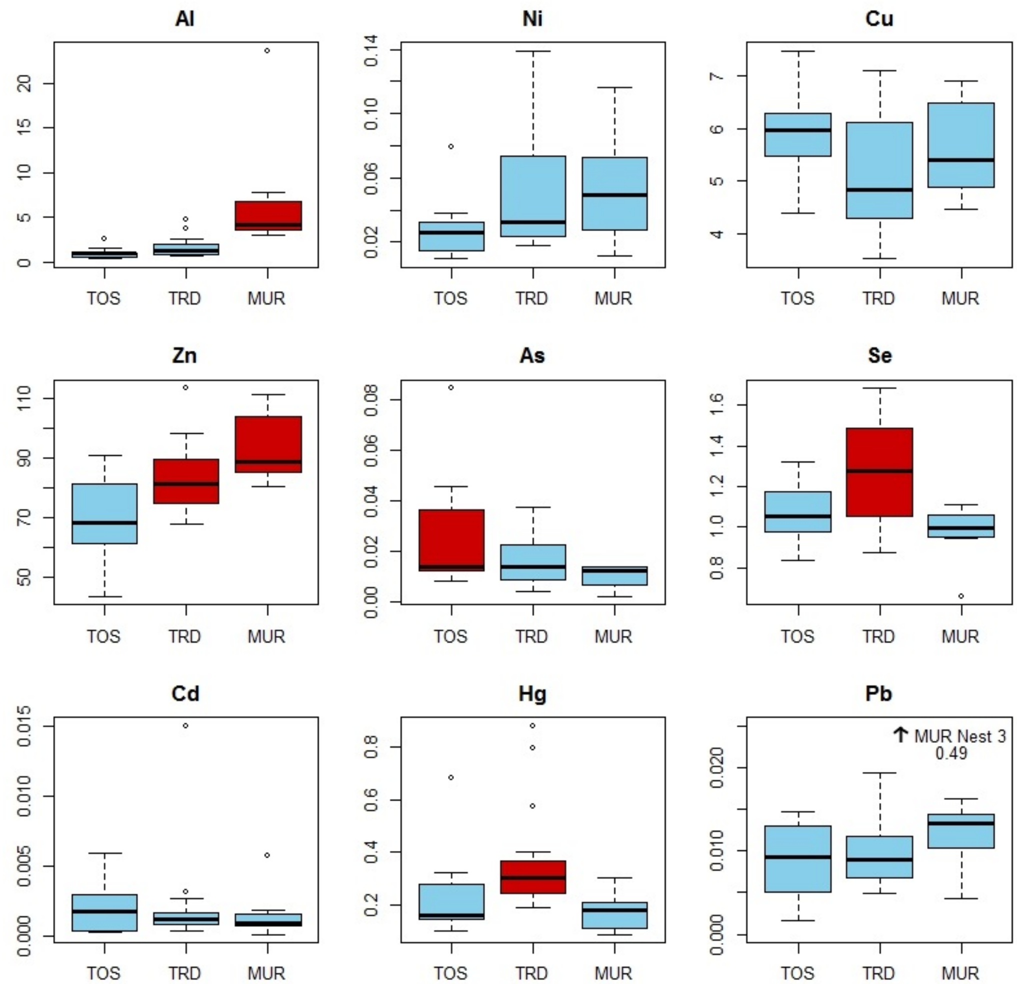

Figure 3 Nestling body feather concentrations ($\mu g\ g^{-1}$ dw) of nine elements of interest grouped by region: Tromsø (TOS), Trondheim (TRD) and Murcia (MUR). Red colors indicate statistically significant differences. Nest 3 in MUR, with a Pb concentration of 0.49 $\mu g\ g^{-1}$dw, is indicated but not to scale with the Pb boxplot, in order to aid visualization. The box shows the inter-quartile range (25-75th percentile), the bold line is the median, the whiskers show adjacent values (upper adjacent value is the highest value within the upper quartile range plus 1.5 the length of the inter-quartile range), and dots indicate high/low values outside of both the inter-quartile range and the whiskers.

Blood and feather concentrations correlated for As, Se and Hg and Pb (R^2 = 0.28-0.46, F =12.92-66.21, p < 0.01), but not for Al, Ni, Cu, Zn, or Cd (R^2 = 0.01-0.07, F = 0.12-2.62, p = 0.1-0.7).

3.1.3 Explanatory variables

The degree of urbanization around the nests ranged between 4 and 461 ha, with the majority of sites having less than 10 ha of development. There was an average of 13, 76, and 24 hectares of roads and buildings per 5 km nest radii in Tromsø, Trondheim, and Murcia respectively.

For agricultural land usage, there was an average of 359, 1735 and 1710 ha within the 5 km nest radii in Tromsø, Trondheim, and Murcia respectively.

Adonis multivariate comparisons for the nestling blood sample data found that none of the three variables, i.e. latitude ($F_{1,32}$ = 1.37, p = 0.25, R^2 = 0.04), degree of urbanization ($F_{1,32}$ = 0.17, p = 0.75, R^2 = 0.01), or land usage ($F_{1,32}$ = 0.89, p = 0.27, R^2 = 0.03), significantly explained the multivariate dataset of nine metals. Multiple linear models performed on each element separately, each containing three variables (latitude, degree of urbanization, and land use) were composed to explain Zn, As, Cd, and Hg concentrations in nestling blood, as concentrations of these metals either varied among regions (Figure 2) or were exceeding avian blood toxicity threshold values reported in the literature. Variation in concentrations was for none of the three metals significantly explained by latitude ($F_{1,32}$ = 0.01-3.18, p = 0.08-0.92), degree of urbanization ($F_{1,32}$ = 0.01-1.06, p = 0.31-0.99).

However, adonis multivariate comparisons for the nestling feather sample data found that latitude (representing study region) significantly influenced the group of nine metals ($F_{1,32}$ = 13.24, p = <0.01, R^2 = 0.29). Multiple linear models, similar to those described above for blood, were composed to explain separate metal concentrations in nestling feathers for metals Al, Zn, As, Cd, and Hg because concentrations of these metals varied by region or were above reported toxic threshold values. Latitude significantly explained Al ($F_{1,33}$ = 12.5, p < 0.01), Zn ($F_{1,33}$ = 9.82, p < 0.01), As ($F_{1,33}$ = 3.99, p = 0.05), Cd ($F_{1,33}$ = 4.04, p = 0.05), and Hg ($F_{1,33}$ = 5.45, p = 0.03) concentrations in the feather samples.

4.0 Discussion

4.1 Blood concentrations

The nestling blood metal concentrations were generally at background levels, with some exceptions (Zn, Cd, Hg). Northern goshawk nestlings from all three regions had blood Zn levels of 5.0-5.6 µg mL^{-1} ww, which were above avian reference levels for the Psittacines genus (1.25-2.29 µg mL^{-1}, Osofsky et al. 2001, Puschner et al. 1999). In wild adult Northern goshawks from the Pennsylvania Appalachian Mountains, background levels of Zn in the blood plasma were low compared to the blood concentrations in the present study (1.29-2.30 µg mL^{-1} ww, Stout et al. 2010). The only Zn point source in Norway is the Zn smelter at Odda, in southern Norway. Generally, Zn is produced by metal industry, and found atmospherically deposited at higher levels in industrially developed areas of central Europe, with moss samples showing concentrations of 60-80 µg g^{-1}, dw, Zn close to Murcia in Spain (Rühling and Steinnes 1998). However, Honda et al. (1986) observed that high levels of Zn are common in growing Great eastern white egret chicks (*Egretta alba modesta*), as Zn is involved in the keratinization of feathers and in tissue proliferation. Adult blood Zn levels of White-tailed eagles (*Haliaeetus albicilla*) from contaminated areas of the Baltic Sea showed levels of 7.5 mg kg^{-1} ww (Falandysz et al. 1988). From these above avian studies we conclude that Northern goshawk nestlings have a higher background blood level of Zn compared to adult birds, and that toxicity thresholds are therefore also assumed to be higher. Since the nestlings from this study did not surpass the 7.5 mg kg^{-1} ww Zn levels from contaminated adult birds of prey we conclude the nestlings in this study are not harmfully contaminated with Zn.

Because of the low concentrations of blood Cd in the present study, we did not find significant variation among regions. This also suggests that there are no critical point sources. Cd was found at the highest blood concentration, 0.00076 µg mL^{-1}, in nest 2 from Tromsø. Notably high values in Tromsø nests 2, 3, 5 and 8 suggest that Tromsø Northern goshawks are exposed to higher levels of Cd compared to the other study regions. Eleven of the sampled nests, mainly from Trondheim and Murcia, were below the instrument's quantification limits (estimated at 0.00012 µg mL^{-1}), creating an ambiguity in the quantification of Cd in this study's blood samples. This ambiguity could be clarified by sampling more nests, particularly in Tromsø. Cd is a very potent toxin and the levels found in the nestling's blood approach sub-lethal oxidative stress toxicity thresholds cited in the literature for Griffon vultures (*Gyps fulvus*) (>0.0005 µg mL^{-1} ww, Espin et al. 2014b) and Eagle owls (*Bubo bubo*) (>0.0002 µg mL^{-1} ww, Espin et al. 2014). Blood samples from all three regions in the present study were below reference Cd concentrations established for wild Spanish birds (0.001 µg mL^{-1}, Garcia-Fernandez et al. 1996, Donazar et al. 2002).

There were high Se and Hg blood concentrations observed in Trondheim. The Hg:Se molar ratio was very low and never ranged above 0.08, indicating that Se most likely neutralized toxic effects from Hg. Santolo and Yamamoto (1999) observed no adverse effects from Se in six species of terrestrial raptors that accumulated less than 38 µg mL^{-1} ww blood Se. The present study had concentrations well below this level. Several blood samples though exceeded avian Hg blood toxicity thresholds established in the literature. Ortiz-Santaliestra et al. (2015) found that nestling Bonelli's eagle (*Aquila fasciata*) blood Hg was related to diet, observing higher blood Hg where the preferred prey of rabbits was unavailable, concluding lower quality habitats forced Bonelli's eagles to eat prey containing more Hg. However, in the present study Trondheim nest 18 had minimal impact from urbanization or land usage, thus the low blood level does not appear to be diet or habitat related. Espin et al. (2014b) found that 0.03 µg mL^{-1} ww of Hg in the blood of Griffon vultures induced oxidative stress with elevated concentrations of superoxide dismutase (SOD) enzyme. Eagle owls showed a 102% increase in oxidative stress indicator thiobarbituric acid reactive substances (TBARS) production, with a Hg blood concentration of 0.03 µg mL^{-1} ww (Espin et al. 2014).

Burger and Gochfeld (1997) and Donazar et al. (2002) suggest a reference As blood concentration of 0.02 µg mL^{-1} ww for aquatic avian species, and meadow blackbirds were found to have low levels of <0.005 µg mL^{-1} ww As in their blood (Tsipoura et al. 2008). There were several blood concentrations in Tromsø nestlings from the present study exceeding these thresholds. There was significantly less As in the blood from Murcia nestlings. The multiple linear model for As showed latitude significantly influencing As concentrations, however this simply indicates that the As varies significantly between regions. The sample size of the present study is not big enough in Tromsø and Murcia to make a clear statement on whether or not As is carried north from central Europe and deposited in Norway. Evidence indicates southwest Norwegian bogs get more As surface deposition, decreasing in the far north (Hvatum et al. 1983). There are very few local As sources in Norway and As deposition has been declining steadily in Norway since at least 1986 (Bustnes et al. 2013).

Low Al, Ni, Cu and Pb pollution resulted in no variation among sampling locations in the blood samples of the present study. Background concentrations could be explained by low soil water concentrations in the case of Al (5.4-21.6 mg kg^{-1}, Lange et al. 2006), or atmospheric deposition from industrialized central Europe, in the case of Pb (Rühling and Steinnes 1998). Ni is not found to contaminate environments long distances away from a point source, and there were no Ni point sources near our study regions (Dauwe et al. 2004). Sub lethal toxic effects of Al, Ni, and Pb were only found at blood concentrations much higher than those observed in this study (Al in diet: >1000 mg kg^{-1}, Nybø 1996; blood Ni: 3-62 µg g^{-1} dw, Eeden and Schoonbee 1996; blood Pb: >0.02 µg mL^{-1}, Espin et al. 2014/2014b). Cu data suggests no pollution or toxic effects, with data matching blood Cu reference levels for Hispaniola parrots (*Amazona ventralis*) in the Amazon (0.07-0.19 µg mL^{-1} ww, Osofsky et al. 2001). Stout et al. (2010) found Cu blood concentrations from Northern goshawks in Pennsylvania to be 0.294-0.488 µg mL^{-1} ww, which is slightly higher than in the present study.

4.2 Body feather concentrations

Overall body feathers showed potential as a screening tool in studies focusing on external environmental metal pollutants. However the present study did not include nests close to point sources of metal pollution. Dauwe et al. (2000) measured Cu, Zn, As, Cd, and Pb in nestling feathers of Great (*Parus major*) and Blue (*Cyanistes caeruleus*) tits at both reference and polluted sites, to find only Pb levels to be significantly different between clean and polluted sites. This suggests that nestling feathers may be most useful when screening for specific metals.

In the present study, relatively high feather Al and Zn concentrations in Spain may indicate Al and Zn environmental pollution sources near the Murcia nests. Regional differences in Al and Zn concentrations are supported by the multiple linear models for Al and Zn, which showed latitude as a significant variable. However, the present study's Al and Zn concentrations are not necessarily high relative to other similar studies. Solonen et al. (1999) sampled 21 nestling Northern goshawk feathers with a similar method near Helsinki, southern Finland, to find a median concentration of 24 and 130 µg g^{-1} dw for Al and Zn respectively. The Finland study showed Al values 6x higher and Zn values almost 2x as high as those found in the present study, suggesting Spanish and Norwegian levels to be relatively low.

Nestling feather Ni, Cu, Cd, and Pb concentrations showed similar patterns to blood data, not varying by region. One possible reason may be because the concentrations are very low and close to zero (in the case of Ni, Cd, and Pb), thus variation among regions was minimal. Because all three regions had similar feather concentrations, they are concluded to be either at low homeostatic levels or reflecting external ambient air pollution present at all three study regions. Solonen et al. (1999) found a much higher median Cd feather concentration of 0.19 µg g^{-1} dw, suggesting that the Finland birds were exposed to Cd levels three orders of magnitude higher than the investigated Spanish and Norwegian nestlings. Cu and Pb levels in the Finnish study had medians of 5.8 and 0.1 µg g^{-1} dw, respectively, similar to our reported values, suggesting that both sites were equally unpolluted in Cu, and Pb. Terrestrial avian species from polluted sites can have up to 80 µg g^{-1} dw of Ni in or on their feathers (Outridge and Scheuhammer 1993), which was not seen in our study.

Nestling feather As showed patterns and concentrations similar to the blood As data, with significantly more As present in Tromsø feathers. The multiple linear model for As showed latitude as a significant factor, indicating regional differences. Bustnes et al. (2013) showed that there has been a linear decrease in raptor feather As concentrations in central Norway between 1986 and 2005. Our study's data agrees with Bustnes's linear model for decreasing As, with our feather As values generally below 0.03 µg g^{-1} dw, supporting the claim that As is being atmospherically deposited northward at decreasing levels over the last 30 years.

Body feather Se and Hg concentrations in the present study are considered to be deposited in the feathers internally from the blood, with highest concentrations in Trondheim. Hg is mainly stored in its organic form in the feathers of avian species (Burger 1993). The high correlations between blood and feather seen in this study for both Se and Hg suggest that nestling body feathers are an ideal tissue for monitoring Se or Hg pollution in avian species.

4.3 Effect of latitude and anthropogenic activity

Both degree of urbanization and land usage can influence the choice of prey available to raptors (Donazar et al. 1993). If a site is overly developed with either urban areas or agricultural fields, a raptor has to travel farther from the nest to find its preferred prey and may eat a restricted diet. In restricted urban diets prey is potentially contaminated with metals (Ortiz-Santaliestra et al. 2015). The factors latitude, degree of urbanization and agricultural land usage all did not have an indisputable relationship with observed metal concentrations in either the nestling blood or feather data. Solonen et al. (1999) noted in their study the wide feather metal concentration variability in raptor species depending on their diet (voles > avian prey > fish). As Northern goshawks eat mostly avian prey, this suggests a moderate amount of variation in metal concentration data may be caused by their avian prey diet (see range values in Tables SI-1 and SI-2). This makes it difficult to detect patterns in correlations to factors such as latitude, degree of urbanization and land usage without significantly increasing the number of sampled nests. Together with the fact that most of the metal concentrations observed were below toxic thresholds, we conclude that the birds sampled in this study are not in extreme danger from metal pollution.

Bustnes et al. (2013) found correlations for Zn, As, Cd, Pb between his 19-year study of Tawny owl (*Strix aluco*) feather data and moss sampling by Steinnes et al. (2011), which point clearly to atmospheric deposition onto feather surfaces for these metals. Our study's feather Al, Zn, As, Cd, and Hg data supports Bustnes's conclusion that latitude is a significant regional factor. Since the adonis test detected differences in regions from the latitude factor in the feathers but not the blood of chicks, this indicates that chick feathers may be more suitable than blood sampling for detecting environmental metal contamination.

Although washed, body feathers were still suspected to contain trace metal residues (see Table SI-2). We suggest that future studies should omit feather washing beyond a simple milli Q wash to remove dirt, to focus on the feather as an external environmental pollution screening tool. If dietary metal exposure is a focus of a study, then the rachis alone should be used for this purpose (Bustnes et al. 2013).

Multivariate linear models showed Hg levels to be significantly influenced by latitude. Nestling feathers showed this relationship more clearly than the blood samples. This suggests that either northward aerosol deposition of Hg or inputs from aquatic ecosystems (Cristol et al. 2008, Therrien et al. 2011) are also a threat to terrestrial birds of prey and future studies should consider them alongside marine feeding birds when assessing dietary Hg exposure in northern latitudes. Multiple linear models for Zn and Cd indicated latitude (representing region) to significantly influence Zn and Cd feather concentrations, however univariate results above in figure 3 only reflect this influence of latitude for Zn, with significantly higher values in Murcia.

Since the degree of urbanization and land usage were not correlated with concentrations of one particular metal, our data suggests that urbanization and land use are not significantly influencing metal pollution or prey availability of the nestling birds sampled in the present study. This can be explained by the low human population density in all three regions. Future studies should sample more nests, and use the spatial techniques used (supplementary information) to assess terrestrial raptors inhabiting heavily developed regions, as territory quality is very important for maintaining the health of sedentary raptors (Ortiz-Santaliestra et al. 2015).

5.0 Conclusions

Northern goshawk nestlings were found to be exposed to metal pollution, with Hg and Se present in the blood and deposited in their feathers. Hg accumulates in terrestrial food chains and was found to be significantly influenced by nest latitude. Several nests exceeded Hg toxicity thresholds. However, very low Hg:Se ratios indicate that Hg may not be critically toxic for the investigated birds. Nonetheless, Hg exposure may pose a threat to terrestrial birds of prey and future studies should consider them alongside marine feeding birds when assessing dietary Hg exposure in northern latitudes.

Zn and Cd in blood were also found at levels above reported toxicity thresholds. The Cd in Tromsø and Trondheim (Norway) and the Zn in Murcia (Spain) are potentially approaching sub-lethal toxic levels.

Neither the degree of urbanization nor land use effectively explained metal concentrations. The spatial techniques developed to measure urbanization and land usage could be valuable for studies investigating more polluted regions and are of particular interest in further developing the potential of birds of prey nestlings as sentinels for contamination of their immediate environment. This study hopes to draw attention to the risks of terrestrial birds of prey to metal pollution as terrestrial species are currently under-represented in metal exposure scenarios.

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