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Master thesis

Blood concentrations of lead (Pb), mercury (Hg) and cadmium (Cd) in Scandinavian and Alaskan brown bears (Ursus arctos)

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Abstract

Lead (Pb), mercury (Hg) and cadmium (Cd) are among the most serious environmental heavy metal pollutants with long half-life times that will make them present in both the environment and living organisms over a long period of time. They are found naturally in all ecosystems but the exposure is often due to human activities. Both humans and animals are exposed to these through ingestion or inhalation. With this bakground I will in this study test differences in the bear blood concentrations among brown bears (*Ursus arctos*) in Scandinavia and Alaskan National Parks. The measurements of the bears in Alaska are taken in Lake Clark, Gates of the Arctic and Katmai. In general, the results shows a trend in differences depending on the heavy metal in relation to the study area. The results revealed elevated blood Pb concentrations among the sampled brown bears from Scandinavia compared to Alaska. On the other hand, they also revealed significantly low blood Hg concentrations among Scandinavian brown bears compared to Alaskan brown bears. For blood Cd concentrations there was less clear differences among the study areas. Bears in Gates of the Arctic and Scandinavia had higher blood Cd concentrations than in Katmai and Lake Clark. Overall, we see that the differences in the heavy metal concentrations of the brown bears in this study are due to location. Studied locations differed in primary food resources and aerial heavy metal depositions mainly human caused, but also natural occurences. The exposure of heavy metals can also be due to both new and old emissions as they have accumulation capacity both in the environment and in animal body tissues. A study like this can provide opportunities for further research where the actual results can be seen in the context of other analyzes of blood concentrations of heavy metals.

Content

1.Introduction

Heavy metals are defined as metals with a density higher than $5g/cm^3$ (University of Oslo (UiO), 2018). Some heavy metals work as micronutrients and are easily absorbed by for example plants (UiO, 2018), while others have no known biological function such as lead (Pb), mercury (Hg) and cadmium (Cd) (Norwegian Food Safety Authority, 2013). These metals are found naturally in all ecosystems but the exposure is often due to human activities like mining, agriculture, incineration, road traffic, industry and wastewater discharges (UiO, 2018, Jaishankar et al., 2014). UiO (2018) classifies heavy metals as a very serious environmental problem that gives harmful effects on agriculture and health of both humans and animals. Pb, Hg and Cd are among the most serious environmental heavy metal pollutants with long half-life times that will make them present in both the environment and living organisms over a long period of time (Matportalen.no, 2019). Both humans and animals are exposed to these through ingestion of contaminated food and water, or inhalation of air, dust and vapor. On ATSDR's (Agency for Toxic Substances and Disease Registry) substance priority list that considers the level of toxicity, frequency and potential for human exposure, Pb, Hg and Cd are placed in second, third and seventh place, respectively (ATSDR, 2020).

Given the toxicity of these metals, the degree of exposure and the health hazards, it is therefore important to be able to map the amount in the environment and organisms, enabling to monitor change and reduction of exposure risk. Being able to combine the focus on animal welfare at the same time as human health, will be crucial in creating a general understanding of the severity of heavy metals and their destructions.

In this study, samples of brown bears (*Ursus arctos*) from both Scandinavia and Alaska have been measured to map the amount of Pb, Hg and Cd. The study areas differ in landscape as well as resource composition and availability, with effects on body size and population density (Mangipane et al., 2017, Swenson et al., 2007). In general, bears are omnivores and generalists with a holarctic distribution (Cook & MacDonald, 2009). They eat what is available in their habitat and they are highly adaptable to both spatial and temporal variations in resources (Bojarska & Selva, 2012, Mowat & Heard, 2006, Van Daele et al., 2012). Most of the bears in the Scandinavian population live predominantly in the boreal forest and the diet composites of berries, ungulates, plants and insects (Stenset et al., 2016). In Alaska, on the other hand, there is also a larger network of salmon *(Salmo salar)* rivers and the bears makes extensive use of these (Mangipane et al., 2017).

Although home ranges are set by dietary needs and competition among individuals (Dahle & Swenson, 2003), there will also be variations between the different areas when it comes to external environmental factors. Heavy metals are one of these external factors that can have large variations in location and may affect individuals differently from region to region due to differences in geology and human activities (Amap, 2002). Estimates of heavy metal emissions show that there are three main sources of human activity that lead to pollution of the atmosphere. These three are waste combustion, fossil fuel combustion and non-ferrous metal production (Amap, 2002). Studies shows that in 1995, emissions of both Pb and Hg to the atmosphere after stationary fossil fuel combustion are considerably higher in Europe than in North America, while emissions of Cd are over twice as high in North America than in Europe (Pacyna & Pacyna, 2001). Combustion of Pb through the use of leaded gasoline has also been an environmental problem for many decades, but due to the ban of its use, aerial depositions was higher earlier than it is today (von Storch et al., 2003, Nriagu, 1990).

According to Amap (2002), aerial deposition, natural background levels and heavy metal measured in different species vary greatly between Alaska and Scandinavia. In reindeer/caribou *(Rangifer tarandus)*, Pb-, Hg- and Cd-concentrations in liver varied between Scandinavia and Alaska, but also within Scandinavia and within Alaska (Amap, 2002). With this background, I will in this study test whether these variations on landscape scale are represented in the bear blood concentrations among brown bears in Scandinavia and Alaskan National Parks. The measurements of the bears are taken in Lake Clark, Gates of the Arctic and Katmai National Parks in Alaska and I have used them in this study to primarily see if there are any differences in heavy metal content compared to Scandinavia.

With a lower human population density in close proximity to the national parks in Alaska, in combination with higher aerial deposition of some of the heavy metals in Northern Europe I have formulated following predictions; 1) Pb, Hg and Cd are higher in blood of Scandinavian brown bears compared to Alaskan brown bears and 2) Heavy metal concentrations will also differ between the three Alaskan national parks.

In addition to examining the actual values of the heavy metals and looking at possible differences, I will also discuss the differences in light of published literature about possible sources.

2. Material and Methods

2.1 Study area

2.1.1 Scandinavia

Norway's land area consists of just under 400 000 km^2 (Kartverket, 2020), of which 37% is covered by forest (NIBIO, 2017). Unlike Sweden's approximately 450 000km^2 (United Nations Association of Norway, 2017), the total land area is covered by 69% of forest (Skogssverige, 2017). Most of the Scandinavian bears are found in the boreal forest where the dominating tree species are Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) (Nordisk ministerråd, 2008). The bears in this study are found in south-central Sweden and south-eastern Norway [61°N, 15°E] where the size of the area is approximately 100 km in radius. Most of the area is covered by production forests with mainly pine and spruce and in some places the elevation rises above 850 m.a.s.l. (Fuchs et al., 2020). During the spring and summer, plant food is an important food source together with insects and vertebrates for these bears. In the autumn on the other hand berries are the main energy supplement during hyperphagia preparing for five to six months of hibernation (Evans et al., 2016, Stenset et al., 2016).

The total brown bear population in Scandinavia is concentrated in different areas, with the majority found in Sweden (Figure 1) (Naturvårdsverket, 2019). In Norway, 138 individuals were registered in 2018 (Figure 2) with an estimate of 7,7 reproductions (Fløystad et al., 2019), while Sweden estimated their population in 2017 to 2877 (Kindberg & Swenson, 2017).

2.1.2 Lake Clark National Park, Alaska

Lake Clark National Park, located southwest of Alaska (Figure 3), extends over 16 000 km^2, and is the most remote national park in the country (National Park Service, Lake Clark, 2019). The National Park houses four out of five biotic communities in Alaska. Everything from coasts, rivers, wetlands, volcanoes and glaciers, to mountains, boreal forest and tundra exists in the park and it is a wilderness that provides habitats for countless fish and animal species (National Park Service, Lake Clark, 2018).

The bear population of Lake Clark is divided into two groups; coastal bears and inland bears. Both brown bears and grizzly bears *(Ursus arctos lasiotus)* are common names for the same species, but are apart in geographically location which influences its behavior, diet and size. Generally, coastal bears are called brown bears and inland bears are grizzly, while in Lake Clark the definition is brown bears for all bears. The food availability controls the bears, and in Lake Clark the density of bears is greater along the coast where there is plenty of food. Recently, biologists have counted a number of 219 bears within an area of 140 km^2, and this is one of the highest densities of bears in the world (National Park Service, Lake Clark, 2018). While coastal bears feed on salmon, whales and other marine carcasses, clams, berries and plants, the inland bears feed on roots, insects, ground squirrels *(Ictidomys tridecemlineatus)*, berries, salmon, moose calves and reindeer carcasses. The diet is much the same, but inland they lack the salt marshes which contains protein-rich vegetation. In addition, there are larger distances and the bears have to travel over larger areas to obtain the same amount of food as the coastal bears receive in a small area. Due to the difference in food access, competition is also much higher for the inland bear and they are more solitary here than they are on the coast. Thus, the density of inland bears is automatically lower (National Park Service, Lake Clark, 2018).

2.1.3 Gates of the Arctic National Park, Alaska

Gates of the Arctic, located north-central of Alaska (Figure 3), comprises almost 34 000 km^2 and is known as the premier wilderness park of all Alaska national parks. The park has an arctic mountainous ecosystem that also consists of a large network of rivers and houses habitat for several populations of world-important plants and animals (National Park Service, Gates of the Arctic, 2018). Brown bears are found in all habitat types throughout the Gates of the Arctic, but are often seen in open tundra or alpine habitats. Like Lake Clark, the population is also larger along the largest rivers and lakes. The food is also about the same as in Lake Clark, but due to their vicinity to the larger rivers that allow free access to huge quantities of salmon, this is a very important food source. Yet the food in the park is spread over large areas and there is estimated an average of one brown bear per 259 km^2 (National Park Service, Gates of the Arctic, 2019).

2.1.4 Katmai National Park, Alaska

Located south-west in Alaska (Figure 3), Katmai National Park totals just over 16 000 km^2 (National Geographic, 2009) and was established as a monument in 1912 to preserve the areas after the huge volcanic eruption that year (National Park Service, Katmai, 2018). In recent times, it has become equally important to preserve the brown bears and their widely varying habitats (National Park Service, Katmai, 2018). The national park as it is today was established in 1980 and has North America's largest population of protected brown bears (National Geographic, 2009). It is estimated that Katmai national park hosts about 2 200 brown bears. With a decline in bear populations around the world, however, Katmai has some of the few remaining unaltered bear habitats that exist (National Park Service, Katmai, 2018). Brown bears in Katmai are found along the entire coastal and lake regions where the food is very similar to the coastal bears in Lake Clark National Park (National Park Service, Katmai, 2019).

Figure 1. Distribution of brown bears (Ursus arctos) in Scandinavia. Darker color indicates higher bear density. (Kindberg, J. & Swenson, J.E., 2017).

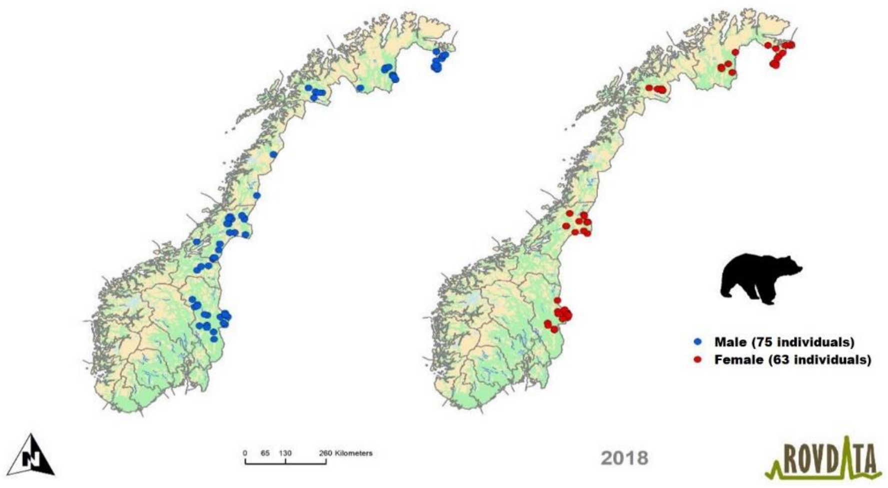

Figure 2. Distribution of 138 brown bears (Ursus arctos) registered in Norway 2018. Blue dots shows registered males and red dots shows registered females. (Rovdata, 2019).

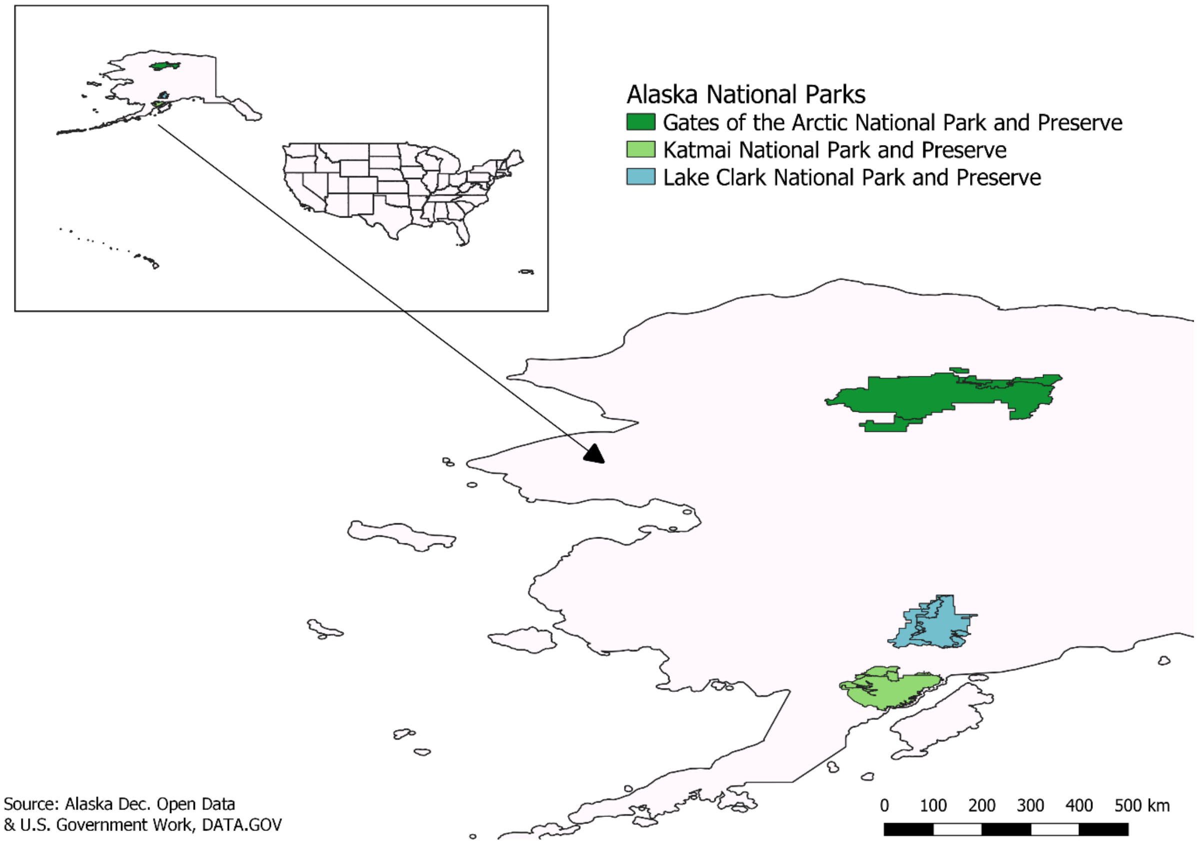

Figure 3. Gates of the Arctic (dark green), Katmai (light green) and Lake Clark (blue) National Parks in Alaska, United States.

2.2 Sample size

A total of 153 bears are blood sampled in this study. Some bears are sampled over several years in both Scandinavia and Katmai National Park, Alaska, and the total sample size are 245 blood samples (N=245). From the Scandinavian bear population there are 109 bears sampled and with a total of 189 blood samples. Of these bears, 62 are females, 43 are males and 4 unknown. Collection of blood samples of Scandinavian brown bears were conducted between 2010 and 2019.

In 2017, 13 brown bears from Katmai National Park in Alaska were sampled and in this study there are 25 blood samples. Of these there are 11 females and two males. In Lake Clark National Park, Alaska, 12 bears were blood sampled in 2016. Six of these are females and six are males. From Gates of the Arctic National Park, Alaska, there are 19 bears used in this study which are blood sampled in 2016. Of these are 12 of them females and seven males.

2.3 Bear captures and data collection

During the ongoing Scandinavian Brown Bear Research Project, the brown bears from Scandinavia in this study were anesthetized and blood sampled in the spring (April/May). The bears were darted from a helicopter and GPS coordinates were registered by the helicopter crew. All bears were weighed with a digital spring scale and age-determined if they were captured for the first time as adults. To determine the age, the cementum layer of a premolar tooth was measured. The bears that were captured as one-year-olds were microchipped and tattooed for later individual recognition (Fuchs et al., 2020).

The blood samples were taken from the jugular vein and stored in either 4 ml evacuated K3EDTA tubes or in 8 ml evacuated heparine trace element tubes (Vacuette, Greiner Bio-One International GmbH, Kremsmünster, Austria). These were frozen the same day at – 20 °C and kept frozen while shipping to the laboratory (ALS Scandinavia AB, Luleå, Sweden) (Fuchs et al., 2020).

Blood from Alaskan bears were sampled in the same way as the Scandinavian through aerial darting where they determined sex, estimated age through tooth measuring and weighed each individual. In Gates of the Arctic National Park, blood samples was collected during spring (May/June). Bears from Lake Clark National Park, were blood sampled in May while bears from Katmai National Park mostly were blood sampled in May and July, but also some were measured in October.

All captures and sampling of Scandinavian brown bears were approved by the Swedish Ethical Committee on Animal Research (Uppsala, Sweden; #C18/15). Captures and sampling of Alaskan brown bears were approved by Institutional Animal Use and Care Committees of the National Park Service (AKR_GAAR_Gustine_GrizzlyBear_2014) and the U.S. Geological Survey, Alaska Science Center (2014-01, 2015-04, 2015-06) (Mangipane et al., 2020).

2.4 Data analysis

To find the statistical difference between heavy metal concentration in blood between the different study areas, I conducted a general linear model (GLM). The four different study areas were used as explanatory variables (x) in each analyses, and the metals were used as response variable (y), one for each analyses.

To avoid fitting estimates below 0 and to meet visual inspection distribution of the data, I used gamma distribution with an identity link function. With a gamma distribution the estimated values also do not fall below 0, which in turn is important since Pb-, Hg- or Cd-concentrations cannot be measured in minus.

For these models, I removed yearlings (n=47) in the Scandinavian dataset because milk dependent cubs are correlated with their mothers (Fuchs et al., 2020). Statistical significance was considered at the 0,05% level.

All values in this study are reported in µg/L for Pb, Hg and Cd concentrations. I have used R (R Core Team, 2018) for statistical analysis.

3. Results

3.1 Data overview

Of the whole sample size with 245 samples, Scandinavia (n=189) had the highest mean blood lead (Pb) value of 90.95 µg/L. Gates of the Arctic National Park (n=19) had a mean blood Pb value of 38.41 µg/L and Lake Clark National Park (n=12) had its mean blood Pb value of 16.67 µg/L. Katmai National Park (n=25) had the lowest mean blood Pb value of 6.81 µg/L (Table 1). When it comes to Pb, Scandinavia stands out with a maximum value of 220.53 µg/L (Table 1).

Of all mercury (Hg) values in blood, Lake Clark National Park had the highest mean blood Hg value of 40.11 µg/L, Katmai National Park had a mean blood Hg value of 18.70 µg/L, while Gates of the Arctic National Park had a mean blood Hg value of 15.86 µg/L. Scandinavia had the lowest mean value of 1.57 µg/L (Table 1). Of all Hg values, Lake Clark National Park also stands out with a maximum Hg blood value of 119.19 µg/L (Table 1).

When it comes to cadmium (Cd), all study areas had low values and most of them below 1.0 µg/L. Gates of the Arctic National Park had the highest mean value of Cd in blood of 0.40 µg/L and with a maximum value of 1.03 µg/L. Scandinavia had the second highest mean value of Cd in blood of 0.38 µg/L, but with the highest maximum value of 1.20 µg/L (Table 1). Lake Clark National Park had the second lowest value of 0.21 µg/L and Katmai National Park had the lowest mean blood value of Cd of 0.11 µg/L (Table 1).

Table 1. Overview of sample size (N), mean, min (minimum), max (maximum), median and standard deviation (SD) of lead (Pb), mercury (Hg) and cadmium (Cd) blood concentration measured in brown bears from Gates of the Arctic, Katmai and Lake Clark National Parks in Alaska, and Scandinavia. Values are written as µg/L.

Sample Area	**N**	**Mean Pb**	**Min Pb**	**Max Pb**	**Median Pb**	**SD Pb**
Gates of the Arctic National Park, Alaska	19	38.41	4.65	138.58	28.47	32.26
Katmai National Park, Alaska	25	6.81	1.40	37.11	4.95	7.18
Lake Clark National Park, Alaska	12	16.67	2.69	52.54	12.56	13.23
Scandinavia	189	90.95	32.25	220.53	83.80	35.90
Sample Area	**N**	**Mean Hg**	**Min Hg**	**Max Hg**	**Median Hg**	**SD Hg**
Gates of the Arctic National Park, Alaska	19	15.86	1.54	43.52	3.24	16.46
Katmai National Park, Alaska	25	18.70	3.10	39.90	17.54	10.70
Lake Clark National Park, Alaska	12	40.11	5.56	119.19	35.56	32.56
Scandinavia	189	1.57	0.18	21.29	1.38	1.60
Sample Area	**N**	**Mean Cd**	**Min Cd**	**Max Cd**	**Median Cd**	**SD Cd**
Gates of the Arctic National Park, Alaska	19	0.40	0.12	1.03	0.34	0.26
Katmai National Park, Alaska	25	0.11	0.04	0.26	0.10	0.06
Lake Clark National Park, Alaska	12	0.21	0.10	0.40	0.20	0.08
Scandinavia	189	0.38	0.08	1.20	0.35	0.20

3.2 Pb concentration in blood

From 198 samples, estimates from the GLM revealed significant differences in blood Pb concentrations between all study areas ($p < 0.001$). Estimated blood Pb concentration in Scandinavia (89.22µg/L, SE: 6.93) was over twice as high as in the samples from Gates of the Arctic (38.41 µg/L, SE: 5.28), five times higher than in the samples from Lake Clark (16.67 µg/L, SE: 6.02) and 13 times higher than samples from Katmai (6.81 µg/L, SE: 5.34) (Figure 4).

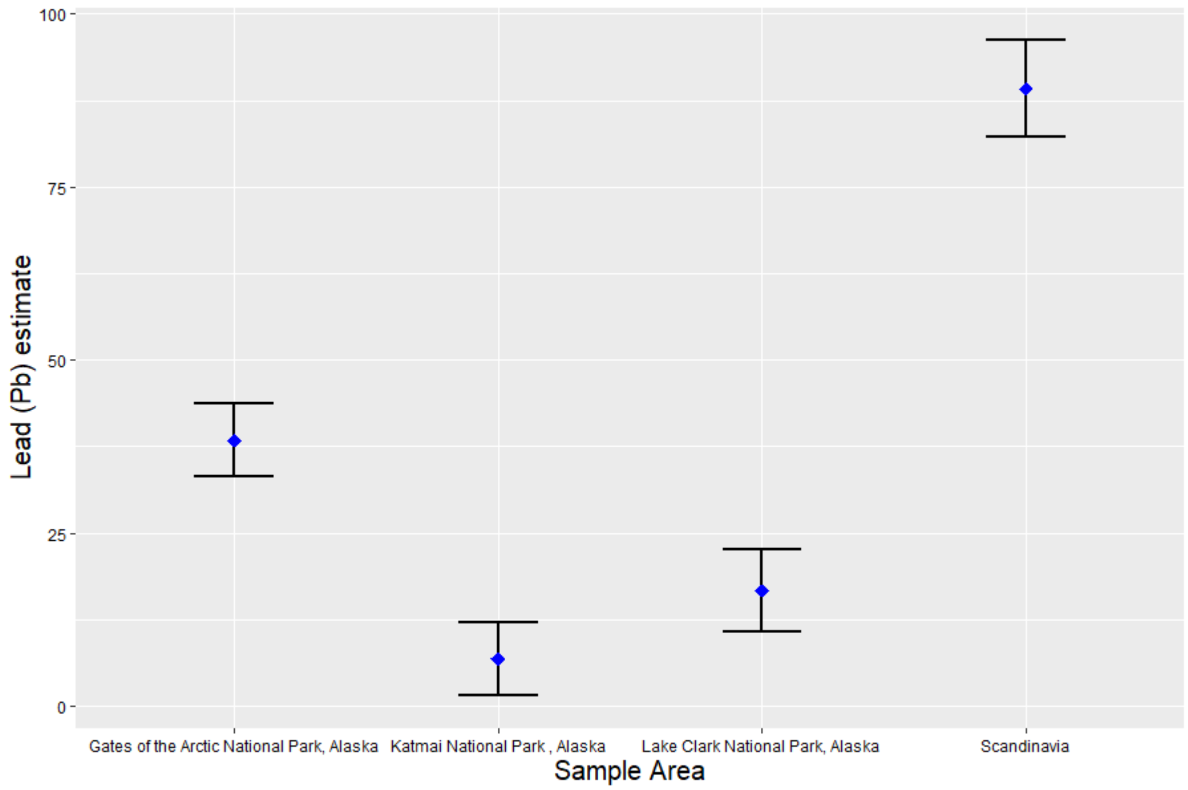

Figure 4. Boxplot with estimates ± SE from a GLM estimating the lead (Pb) concentration in blood of brown bears in Gates of the Arctic, Katmai and Lake Clark National Parks in Alaska, and Scandinavia.

3.3 Hg concentration in blood

With the same model of 198 samples, the estimates revealed no significant difference in blood Hg concentration between Gates of the Arctic (15.86 µg/L, SE: 3.59) and Katmai (18.90 µg/L, SE: 5.17) ($p = 0.56$). Statistically, estimates from Lake Clark (40.11 µg/L, SE: 11.96) revealed a difference in blood Hg concentration compared to the other study areas with an estimate twice as high as Katmai ($p < 0.05$, Figure 5). Estimates also shows that Scandinavia (1.72 µg/L, SE: 3.59) with the lowest blood Hg concentration had a significant difference in relation to the three national parks in Alaska with an estimate nine times lower than Gates of the Arctic, and 23 times lower than Lake Clark ($p < 0.001$, Figure 5).

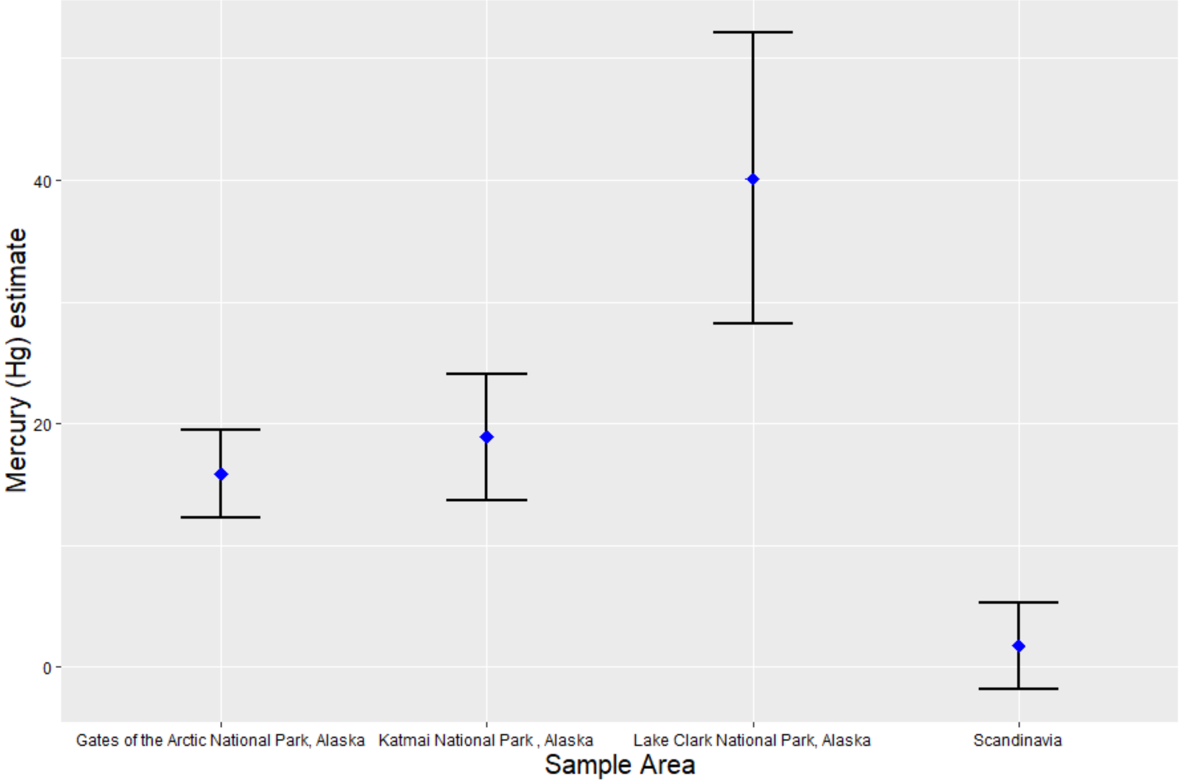

Figure 5. Boxplot with estimates ± SE from a GLM estimating the mercury (Hg) concentration in blood of brown bears in Gates of the Arctic, Katmai and Lake Clark National Parks in Alaska, and Scandinavia.

3.4 Cd concentration in blood

Also from a GLM with 198 samples, the results revealed that Gates of the Arctic (0.40 µg/L, SE: 0.05) and Scandinavia (0.39 µg/L, SE: 0.05) had no significant difference in blood Cd concentration ($p = 0.94$, Figure 6). Estimates revealed that Katmai (0.11 µg/L, SE: 0.05) was significant lower from the other study areas ($p < 0.001$, Figure 6). It had estimates three times lower than both Gates of the Arctic and Scandinavia. Estimates also revealed that Lake Clark (0.21 µg/L, SE: 0.06) had a significant different blood Cd concentration compare to the other study areas ($p < 0.01$, Figure 6). It had estimates nearly twice as high as Katmai, and nearly twice as low as Gates of the Arctic and Scandinavia.

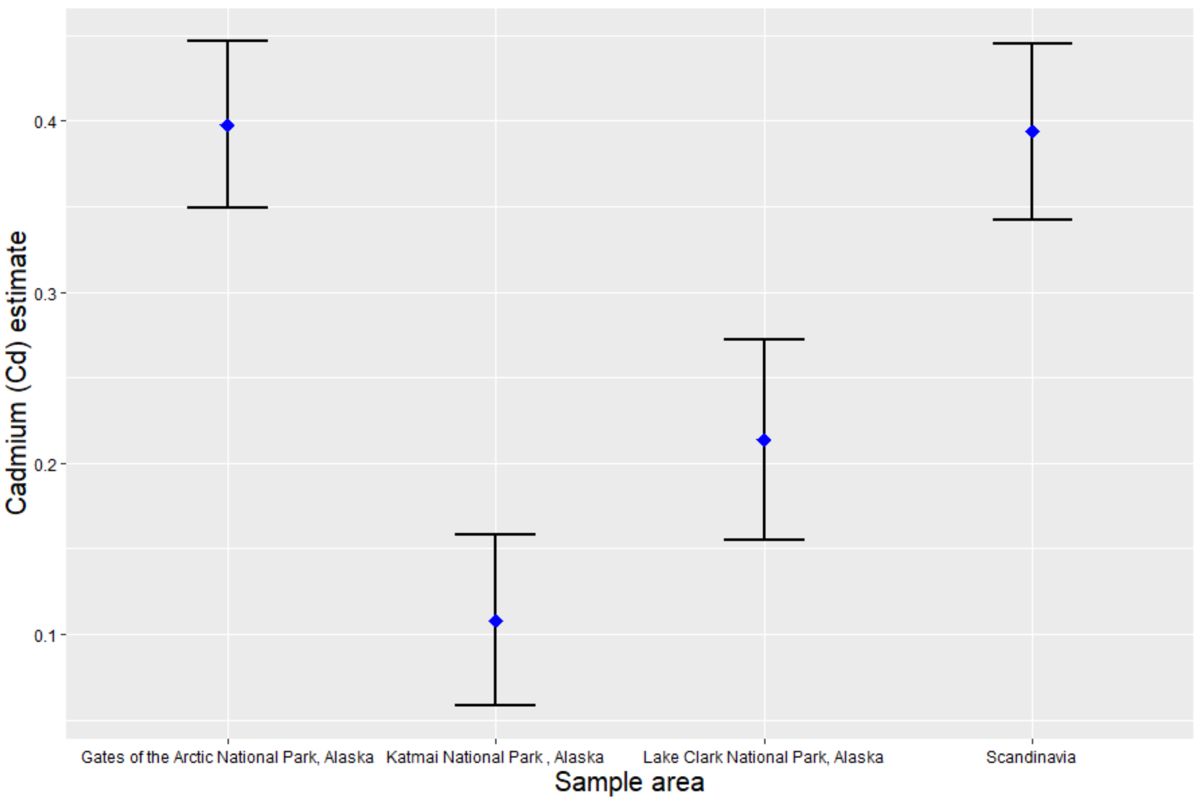

Figure 6. Boxplot with estimates ± SE from a GLM estimating the cadmium (Cd) concentration in blood of brown bears in Gates of the Arctic, Katmai and Lake Clark National Parks in Alaska, and Scandinavia.

4. Discussion

The aim of this study was to investigate the heavy metal blood concentrations in brown bears from Scandinavia and three national parks in Alaska to see if there was any differences.

In general, the results show a trend in differences depending on the heavy metal in relation to the study area. I found that Scandinavia stands out with a very elevated blood Pb concentration compared to Alaska. It was the only study area with a maximum value of Pb of over 200 µg/L and the estimated mean value of the samples was 13 times higher than the samples from Katmai, which had the lowest estimated mean value. For Hg on the other hand, Scandinavia showed an extremely low blood concentration with an estimated value 23 times lower than Lake Clark which had the highest estimated mean value. Lake Clark National Park was also the only study area with a blood Hg concentration measured over 100 µg/L. For blood Cd concentrations there was less clear differences. Still, Gates of the Arctic and Scandinavia had three times higher blood Cd concentrations than Katmai that had the lowest.

4.1 Pb

With these results we see that Pb in Scandinavia is the heavy metal that stands out in differences compared to both Hg and Cd. From other studies (Rogers et al., 2012, Lazarus et al., 2020) we see that an average value of Pb of between 40-60µg/L is twice recorded in bears and that in this study, Scandinavia stands out with extremely elevated Pb values in bear blood. With this result, it is natural to ask whether this is due to natural occurrences of Pb, or whether the exposure is due to differences in human activity.

On a global scale, it has been shown that, on average, biogenic sources account for between 30 and 50% annually Pb emissions to the atmosphere from natural sources (Amap, 2002). Soil-derived dust accounts for 20-30% of Pb emissions to the atmosphere annually, while volcanic releases can annually account for 20-40% of Pb emissions to the atmosphere. (Amap, 2002). Natural sources on a more local scale, have been measured from both soil, moss and lichens. The measurements show a decreasing south-north gradient of Pb concentrations in Scandinavia. Pb concentration in Scandinavian moss was between < 5-30 mg/kg dry weight (dw) in 1995, while in Arctic Alaska measurements taken between 1990 to 1993, had a median value of 0.62 mg/kg dw. The bear population in Scandinavia in this study is within the field of higher values for that time which was between < 5-10 mg/kg dw (Amap, 2002).

When it comes to sources of pollution through human activities, combustion of leaded gasoline was for a long time one of the major contributors. Worldwide, in 1995, an average of 88 700 tonnes were emitted from this combustion. Europe was the continent with the second highest emission of around 19 500 tonnes, while North America was the continent with the third highest emission of between 10- and 15 000 tonnes (Pacyna & Pacyna, 2001). The emission of lead into the atmosphere has dropped sharply following the ban on the use of leaded gasoline. In Norway, for example, lead emissions have decreased by 90% since 1995 from a total of 610 tonnes in a year to 87 tonnes in 2017. This is mainly due to the transition to unleaded gasoline, but this is also due to a decline in the mineral and chemical industries (Norwegian Environment Agency, 2019). Although emissions have dropped sharply in number of tonnes, it will continue to have a significant impact on the environment for a long time since much have been stored in the soil. In addition to this, new emissions will help keep the environment polluted for many years to come. For Norway today, the biggest emission of Pb is leaded ammunition. As much as 66.7 % of the total Pb emission in 2017 was leaded ammunition, and 85 % of the total Pb emission ends up in the soil (Norwegian Environment Agency, 2019). Unlike in Scandinavia, Alaska's national parks do not allow public hunting (Alaska Public Land Information Centers). This can be a very important factor when it comes to differences in blood Pb concentrations in the bears as there will be less to no access to offal after hunting.

A study of Pb exposure in large carnivores from the greater Yellowstone ecosystem shows that grizzly bear was the species with highest concentration of Pb in the blood with a mean value of 55µg/L, and the highest measured value at 186µg/L. The second highest values was from black bear with a mean value at 19µg/L and with the highest measured value at 69 µg/L. Both wolf and cougar had even lower Pb values in the blood (Rogers et al., 2012). These results show that the grizzly bear and, in part, the black bear stand out with higher Pb values than the other species. This could probably be due to a wide variety of diets. Unlike the other species in the aforementioned study, bears are omnivores and can absorb heavy metals from far more sources than the species that are only carnivores. In addition, Yellowstone in the context of the study areas in my study shows major differences when it comes to location in relation to diet availability. The bears in Scandinavia, Yellowstone and Gates of the Arctic in Alaska live far from the ocean and the diet consists more or less of terrestrial food, except for access to salmon. One of the reasons for the differences in heavy metal concentrations between the areas may therefore be due to differences in food resources. Bears with more access to marine food may often equally select this, and this may indicate that elevated values of Pb are due to sources in the terrestrial food chain. These assumptions can be strengthen by looking at my results where Gates of the Arctic is the study area with the second highest mean Pb value and with a maximum value above 130 µg/L. Lake Clark and Katmai which is close located to the sea, had very low values compared to this and with maximum values similar to mean values from other studies of Pb concentrations in bears (Roger et al., 2012, Lazarus et al., 2020).

When I look at the same study of Pb exposure in large carnivores from Yellowstone in the context of my study, there are also big differences when it comes to location in relation to human population density. The Scandinavian bear population, like the bears in Yellowstone National Park, is in closer proximity to larger populated cities and is more exposed to local-transported contaminated air. With higher human population density, there will also be increased human activity. As we have seen, Pb emissions from vehicular traffic have long been one of the major contributors to pollution of the atmosphere. Another big contributor of Pb emissions to the atmosphere in relation to larger cities is waste disposal. Studies of this show that Pb is the metal with the second highest emission when it comes to both municipal waste and sewage sludge waste. On a regional scale, emissions of Pb from waste disposal were higher in Europe than in North America (Pacyna & Pacyna, 2001). The differences in emissions of heavy metals from human activities can thus also be a major cause of the large differences in blood concentration of Pb in the bears between Scandinavia and Alaska. When comparing atmospheric emissions of natural sources of metals to emissions based on human activity on a global scale, it shows that for Pb, emissions from human activity are significantly higher than emissions from natural sources with a ratio of 9.9 (Pacyna & Pacyna, 2001).

4.2 Hg

From the results we also see that Scandinavia stands out with relatively low blood concentrations of Hg compared to the study areas in Alaska. On the other hand, Lake Clark National Park stands out with elevated Hg concentrations in blood. Results from a study of heavy metal concentration in blood of captive and free-ranging brown bears in both Poland and Croatia (Lazarus et al., 2020), shows a mean value lower than 1.31 µg/L. Like my results from Scandinavia, these results also reveals low levels of Hg in the blood of brown bears. Put these results in context, Alaska stands out with elevated Hg values. The inequality may be due to distinct differences in the occurrence of Hg between Europe and North America.

It has been found that on average, biogenic sources account for over 50% of Hg emissions to the atmosphere from natural sources and volcanic releases can account for 40-50% of Hg emissions to the atmosphere annually on a global scale (Amap, 2002). Natural sources on a more local scale have also been measured in moss. When we look at the measurements of Hg concentrations in feather moss from both Scandinavia and Arctic Alaska, Alaska appears to have higher values. Values from Sweden show a decreasing south-north gradient with values from <0.025 – 0.1 mg/kg dw. The study area in Scandinavia in this study is within the field with the lowest values. In Arctic Alaska, concentrations are between 0.02 and 0.112 mg/kg dw (Amap, 2002).

Sources of pollution of Hg through human activities have also been investigated and on a global scale it shows that stationary fossil fuel combustion gave the highest emissions (Pacyna & Pacyna, 2001). Among other sources, both Hg emissions from non-ferrous metal production and Hg emissions from waste disposal was higher in North America than in Europe in 1995. Still, the total amount of Hg emissions was higher in Europe than in North America (Pacyna & Pacyna, 2001). Pacyna & Pacyna (2001) also compared Hg atmospheric emissions from natural sources of metals to Hg emissions based on human activity on a global scale, and it shows that emissions from natural sources are slightly higher than emissions from human activities with a ratio of 0.88.

Food availability differed greatly between the study areas. Since marine mammals often carry substantial concentrations of Hg (Amap, 2002) it is also important to take into account the brown bear's intake of marine mammals. My results shows an increasing Hg concentration between the study areas in connection with access to the marine food chain. Lake Clark National Park had the highest mean value of Hg concentration in blood and are one of two study areas where the bears have direct access to the ocean. Katmai and Gates of the Arctic National Park had no significant difference in Hg concentration, but they still had higher concentration than the Scandinavian bears where the diet consists only of terrestrial food (Stenset et al. 2016). Studies of Hg concentrations in marine mammals in Arctic North America shows in general a decreasing west-to-east pattern (Amap, 2002). Studies of for example beluga whales in Alaska shows Hg mean values of 1.16 mg/kg ww in muscle, 12.4 mg/kg ww in liver and 4.58 mg/kg ww in kidney (Amap, 2002). Since the bears in both Lake Clark and Katmai consumes a lot of carcasses of marine mammals, there is reason to believe that this contributes to the differences between the study areas. The bears in Scandinavia also do not have access to salmon, which, in contrast, is a major food source for the bears in Alaska. In general, fish are seen as an important vector of Hg for both humans and wildlife, as they can accumulate high concentrations in the body (Amap, 2018). A study from 2007-2010 shows how seafood affects the blood concentration of Hg in humans and it shows a high positive relationship between intake of high-mercury fish and the content of Hg in the blood. Among other species, salmon showed a high positive association (Nielsen et al., 2014).

4.3 Cd

From the results of this study, we also see that Scandinavia and Gates of the Arctic National Park have relatively similar mean values of blood Cd concentration, which is twice the mean value of Lake Clark National Park. Katmai National Park has an even lower mean value. When we also compare the results with the study of blood concentrations in brown bears in Croatia and Poland, we see that the Cd concentration in the measured bears had an average value of 0.251 µg/L (Lazarus et al. 2020). These results, together with my results, give a higher sample size, thus it is conceivable that both Scandinavia and Gates of the Arctic have elevated values.

When it comes to natural sources on a global scale, it has been shown that on average, biogenic sources provides 30-50% of Cd emissions to the atmosphere annually, and that volcanic releases provides 40-50% of Cd emissions to the atmosphere annually. Studies of Cd concentrations have been done in both soil and moss on a local scale and samples of surface humus taken north of the Arctic Circle estimated the background level to be 0.17 mg/kg dw. In Norway there was a decreasing south-north gradient in the Cd concentration of forest podzol, and in southern Sweden it was found to be five to ten times higher than the background level. Soil cores in Alaska were measured to have a Cd concentration between 0.21 to 0.80 mg/kg dw (Amap, 2002). Feather moss studies also showed a decreasing south-north gradient in Sweden with Cd concentrations between 0.05 and above 0.30 mg/kg dw. The study area in this study is within the field with a Cd concentration of between 0.10 and 0.20 mg/kg dw. Cd concentrations measured in spring moss in Alaska showed concentrations between 0.02 to 0.98 mg/kg dw (Amap, 2002).

When it comes to emissions through human activity, studies have shown that the production of non-ferrous metals has been the major contributor to Cd emissions on a global scale. The second highest emission values were found to come from stationary combustion of fossil fuels. (Pacyna & Pacyna, 2001). On a regional scale, Europe had higher emissions from non-ferrous metal production than North America. From production of pig iron and steel, Europe also had higher Cd emissions than North America. Overall, it still turned out that in total North America had greater emissions of Cd from human activities than Europe (Pacyna

& Pacyna, 2001). Cd emissions from natural sources have also been compared with emissions from human activity, and it has been found that human activity overall has higher emissions with a ratio of 2.3 (Pacyna & Pacyna, 2001).

4.4 Body tissues

As the concentration of heavy metals varies in different species, according to different studies it also varies with different body tissues. In reindeer/caribou, the mean Pb concentration in liver showed 0.1 mg/kg wet weight (ww) for Swedish reindeer and 0,6 mg/kg ww (Hardangervidda) and 1.2 mg/kg ww (Rondane) for Norwegian reindeer. Liver lead concentrations in Arctic Alaskan reindeer varied widely, but had its highest level near a Pb-Zn mine northwest in Alaska with a concentration at 1.7 mg/kg ww (Amap, 2002). Mean Cd concentrations in liver from Alaskan caribou ranged from 0.4 mg/kg ww to 1.9 mg/kg ww, while Swedish reindeer had a mean Cd concentration in liver at 0.4 mg/kg ww and Norwegian reindeer had a concentration at 1.1 mg/kg ww (Amap, 2002). For Hg, Swedish reindeer had a mean concentration in liver at 0.4 mg/kg ww. Norwegian reindeer had a mean concentration in liver at 0.13 and 0.16 mg/kg ww and Alaskan caribou had a mean concentration at 0.6 and 0.4 mg/kg ww (Amap, 2002).

Since blood levels of the metal is an indicator of acute and recent exposure (Lazarus et al., 2020, Rogers et al., 2012), it is also conceivable that the concentrations vary greatly in the different body tissues in the same individual in relation to excretion of waste substances in the body. For Cd it appears that the concentrations in mammals are generally higher in the kidneys than in the liver. Studies of Scandinavian and Russian reindeer show that Cd in the kidneys was five to ten times higher than in the liver. Cd in the liver was again ten to 100 times higher than in the muscles (Amap, 2002). Hg concentrations in reindeer also appear to decrease in the order; kidney, liver, muscle (Amap, 2002).

From a study tracing Pb in human bodies, the distribution of the metal in the body can be understood as a three-compartment model between blood, soft tissues and bones. Pb in the blood as compartment one also moves from the bones back to the blood (Rabinowitz et al., 1976). With increased calcium needs, the chance of reabsorbing Pb from bone to blood will also increase. This is seen during both pregnancy and lactation in humans (Silbergeld, 1991). Exposure of metal to the blood can thus occur from the outside through the environment or from the inside back from the bones. Although blood is used as an indicator of recent exposure, it doesn't necessarily have to be due to environmental exposure. Elevated values from the results of this study may therefore also be due to reabsorption of "old" metal exposure from bone to blood. In this context, it is also important to consider the bear's age, as the Pb accumulates in bones and has a half-life of 10-30 years (Rabinowitz et al., 1976). Older bears may therefore have more Pb in bones than younger ones.

According to a Swedish study, almost half the proportion of the litters remains with the mother for 2.5 years, and the other half remains for 1.5 years (Van de Walle et al., 2018). The period of lactation can thus last for several months. The sample size from Scandinavia in this study are considerably higher than the areas in Alaska. A larger sample size is most likely to provide greater variation and will be more representative of bear populations than small sample sizes. Nevertheless, the Scandinavian bears consist of more female bears than male bears in this study. The Pb values from female bears can thus affect the mean value. With knowledge of reabsorption of Pb from bone to blood, elevated values in my results can thus also be due to the time of data collection in relation to lactation in female bears.

5. Conclusion

From my results, I see that the differences in Pb, Hg and Cd concentrations in brown bears are partly reflected in the recorded differences in natural sources both locally and globally. The differences are also partly reflected in emissions of heavy metals into the environment due to human activity. Similar to findings of different occurrences in both natural sources of Pb and emissions due to human activity, my results shows that the blood concentration of Pb in brown bears was decidedly higher in Scandinavia than Alaska. Although the Pb emissions to the environment have decreased with time, there is still much left in the soil that continues to affect wildlife.

For Hg concentrations overall, we see that different findings on a global scale shows a very small difference between natural sources and emissions due to human activity. The greatest factor for the differences in Hg concentrations in my study is most likely to be the marine nutritional approach, where the study areas with direct access to the coast are found to have higher Hg concentrations in the blood.

I found weak relationships between the study areas in Cd concentrations from natural sources and human activity. With this background, I also find no clear links between the differences in Cd concentrations in the blood of the bears in this study.

Overall, we see that the differences in the heavy metal concentrations of the brown bears in this study are due to location, with differences in food access and air deposition as a result of both natural occurrences, but mostly due to human activity. The exposure of heavy metals can also be due to both new and old emissions as they have accumulation capacity both in the environment and in animal body tissues.

A study like this can provide opportunities for further research where the actual results can be seen in the context of other analyzes of blood concentrations of heavy metals. The results can also be transferable to other species, as we see how much the diet affects animal health. I hope also this will make us more aware of needed assessments and actions to reduce both the use and exposure of the various heavy metals.

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