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Life-history Effects of Metal contamination in Arctic snow buntings *(Plectrophenax nivalis)* breeding in Adventdalen, Svalbard.

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Abstract

Factors such as species, sex, body size, season, migration, habitat and feeding ecology may affect accumulation of metals and other elements in birds. Several studies have examined how age and sex may affect accumulation of metals in birds, with varying results. Birds have also been widely used for evaluation of pollutant levels in their environment. It is argued that the most serious effects of metals in the Arctic environment of Svalbard, are due to local pollution. Based on reports from previous studies, the coal industry that characterizes settlements on Svalbard is believed to contribute with significant emissions of metals in this area. Adventdalen is located 0-8 km east of Longyearbyen, and the area is characterized by coal mines, piles of mine waste and potential emissions from the coal-fired power plant located in Longyearbyen. Artificial nest boxes have been installed along the cableway that runs through the valley, and these are utilized by the snow bunting (*Plectrophenax nivalis*). The main objective of this study was to investigate how age and sex may affect accumulation of metals and other elements in snow buntings, and to what degree the level of coal-associated metals reflect expected emissions from the coal industry. During the field season of 2015, feather samples were collected of chicks from 34 nests and from 22 adults. Element analysis was performed at NTNU in Trondheim, and the samples were analysed for 58 chemical elements.

It was a clear distinction in concentrations of elements in feather samples from nestlings and adults. P, K, Na, Mg, Zn and Se, as well as the non-essential elements Hg and Rb, were present in highest concentrations in nestling samples. The remaining analysed elements were highest concentrated in adult samples. Significant difference in concentrations between nestlings and adults were demonstrated for Al, Ba, Cd, Cu, Hg, Mn, Na, Ni, Pb, Sb, Sn, Tl, V and Zn. It was not concluded with a clear age-dependent accumulation, due to absence of significant differences between 1 year old- and 2 years or older birds. No significant impact on accumulation was observed with sex. The role of Rare Earth Elements in PCA could indicate external contamination of adult feathers. Comparison with baseline levels in passerine birds from relatively uncontaminated areas, revealed higher concentrations of Al, Co, Se and Zn in feathers of snow buntings from Adventdalen. These levels may be of toxicological significance for snow buntings, and follow-up studies examining concentrations in blood or other tissues and/or organs are recommended, as well as studies investigating potential toxicological effects.

Sammendrag

Akkumulering av metaller og andre elementer i fugl, påvirkes av faktorer som art, kjønn, kroppsstørrelse, sesong, migrasjon, habitat og foringsøkologi. Flere studier har tatt for seg betydning av alder og kjønn for akkumulering av metaller i fugler, med noe varierende resultater. Fugler har også vært mye brukt for evaluering av forurensningsnivå i miljøet. Det hevdes at de alvorligste effektene av metaller i det Arktiske miljøet på Svalbard skyldes lokal forurensning. Basert på rapporter fra tidligere studier, antas det at kullindustri som karakteriserer bosetninger på Svalbard, bidrar med signifikante utslipp av metaller i nærmiljøet. Adventdalen ligger 0-8 km øst for Longyearbyen, og er et område preget av kullgruver, hauger med gruveavfall og potensielle utslipp fra kullkraftverket lokalisert i Longyearbyen. Kunstige reirkasser har blitt montert langs taubanen som går gjennom dalen, og disse benyttes av snøspurv (*Plectrophenax nivalis*). Hensikten med dette studiet var å kartlegge hvordan alder og kjønn kan påvirke akkumulering av metaller og andre elementer i snøspurv, samt i hvilken grad nivå av kull-assosierte metaller reflekterer forventede utslipp fra kull industrien. I løpet av felt sesongen 2015 ble det samlet inn fjærprøver av unger fra 34 reir, samt 22 fjærprøver fra voksne. Elementanalysene ble utført ved NTNU i Trondheim, og prøvene ble analysert for 58 kjemiske elementer.

Det var et klart skille i konsentrasjon av elementer i fjærprøver fra unger og voksne. P, K, Na, Mg, Zn og Se, samt de ikke-essensielle elementene Hg og Rb, var tilstede i høyest konsentrasjoner i fjærprøver fra unger. De resterende analyserte elementene var i all hovedsak høyest konsentrert i fjærprøver fra voksne. Signifikant forskjell i konsentrasjoner mellom unger og voksne ble demonstrert for Al, Ba, Cd, Cu, Hg, Mn, Na, Ni, Pb, Sb, Sn, Tl, V og Zn. Basert på fravær av signifikante forskjeller mellom 1 år gamle fugler og 2 år eller eldre fugler, ble det ikke konkludert med en tydelig akkumulering med alder. Ingen signifikant forskjell på akkumulering ble observert i forhold til kjønn. Sjeldne jordmetallers rolle i PCA ga indikasjoner om ekstern forurensning av voksne fjærprøver. Sammenliknet med referanse konsentrasjoner i spurvefugler fra relativt ikke-forurensede områder, var Al, Co, Se og Zn til stede i høyere konsentrasjoner i snøspurv fra Adventdalen. Disse nivåene kan være av toksikologisk betydning for snøspurven, og oppfølgingsstudier som undersøker konsentrasjoner i blod eller annet vev og/eller organer anbefales, samt studier som undersøker eventuelle toksikologisk effekter.

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1. Introduction

Metals have been a natural part of the Earth since its formation (Walker et al., 2012), and they occur in all ecosystems with concentrations that reflect the local geology (Marcy, 2005). They are redistributed naturally in the environment through both geological and biological cycles, including weathering of rocks and ores by rainwater and incorporation into food cycles by plants and animals (Klaassen, 2013). Nevertheless, some of these metals pose a serious threat to our environment due to anthropogenic activities at local, regional and global scales (Dauwe et al., 2000). Human activity intentionally shortens the residence time of metals in ore deposits, resulting in an altered concentration in the environment in relation to naturally occurring levels. The chemical form or speciation of metals may also be altered, which are of great importance for their toxic potential (Klaassen, 2013; Marcy, 2005).

Improved national and regional emission inventories have made it possible to acquire an understanding of anthropogenic emissions of metals and other elements to the atmosphere, but it is still difficult to obtain information on such emission from natural sources. A comparison of the global natural emissions (Nriagu, 1989) and the global anthropogenic emissions (Pacyna & Pacyna, 2001) estimates the contribution of a selection of metals from each of the sources (Figure 1). It is however important to emphasize that these relationships may be valid for evaluation at the global scale, but they can be entirely different at the local (e.g., around a waste incinerator) and regional (e.g., industrial area) scale. In addition, point and non-point source discharges of metals to terrestrial, freshwater and marine environments must also be included in the evaluations (Pacyna, 2005).

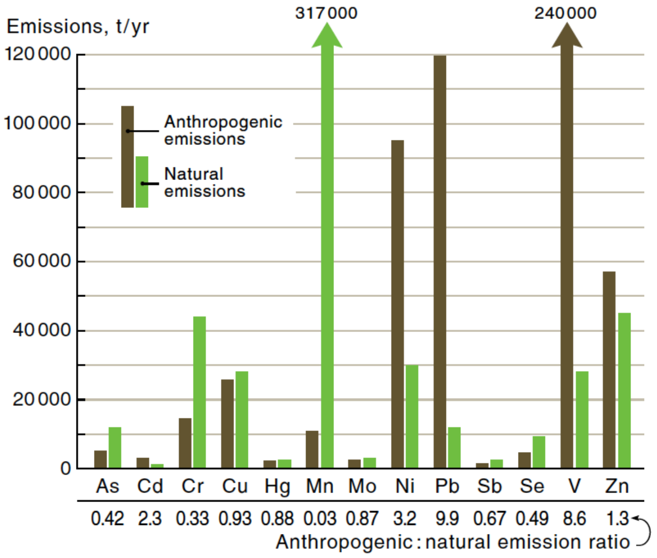


Figure 1: Estimated global emissions of elements (tons/year) in the mid 1990s from anthropogenic sources and natural sources (Pacyna, 2005).

1.1 Metals in the Arctic environment of Svalbard

Metals of both anthropogenic and natural origin, from both distant and local sources, influence the Arctic environment at Svalbard (Rose et al., 2004; Jaworowski, 1989). However, the natural sources of metals that influence the Arctic area are still relatively little studied, but are presumably caused by phenomena such as resuspension of metals from weathered rock surfaces and atmospheric transport from volcanic activity and forest fires. Most of these natural emissions of metals are unlikely to have any adverse effects on the Arctic ecosystem, as they have been present in sufficient time for biota to adapt and develop a stable system (Jaworowski, 1989). However, large emissions related to volcanic eruptions have the potential to overwhelm the biotic system, and affect living organisms negatively (Ragnarsdottir et al., 1994; Jaworowski, 1989).

Research has shown that Svalbard is exposed to higher atmospheric deposition of anthropogenic pollutants than other arctic areas (Maenhaut et al., 1989; Rahn, 1984; Rahn & Show, 1982), and it appears that a large contribution is airborne pollution from eastern Asia, followed by Europe, central and southern Asia, and North America. Some of these areas have large emissions of certain toxic elements. Asia for instance, accounts for 40-60% of total global emissions depending on the metal, and some of this will be deposited in the high Arctic (Pacyna, 2005). However, it is claimed that the most severe effects of certain metals on ecosystems in the Arctic stems from local pollution (Nilsson, 1997). As in other parts of the world, the local environment of Svalbard is largely influenced by fossil fuel combustion associated with transportation. This includes land-based transportation (snow scooters, private cars and heavy duty vehicles), shipping (goods, cruise, coal and research vessels) and aviation (local, domestic and international) (Vestreng et al., 2010). The local environment of Svalbard is also affected by emissions from the coal- and diesel-fired power stations in the settlements (Nilsen, 2013). Much focus has been directed towards the coal industry in recent years, as coal-mining operations encroach on the natural environment and may emit significant pollution, including metals (Store Norske, undated).

1.1.1 Coal industry

The first reports of extraction of coal on Svalbard are dated back to the early 17th century, but the implementation of regular coal mining occurred first in 1906. Until now, it has at peaks of exploitation been as much as ten different operative mines. However, today the only active mines are the Russian-owned operations in Barentsburg, and the Norwegian mines in Svea and Adventdalen (Store norske, undated; Stange, 2013, Orheim, 1982). The two latter ones are operated by Store Norske Spitsbergen Kullkompani AS (SNSK). The Svea Nord mine was put into operation by SNSK in 2001, and this involved a significant increase of coal production for the company. However, SNSKs annual production of coal has declined after the production peak in 2007, in which more than 4 million tons of coal was produced. In 2013, 1,778,325 and 64,687 tons of coal was produced from the Svea Nord mine and mine no. 7 (Gruve 7) in Adventdalen, respectively (Store Norske, 2013). The coal deposits in these mines originate from the older tertiary period (60-65 million years old), have high carbon content and are considered cleaner in use than carboniferous coal. This is due to a high energy content with a corresponding lower content of water and other substances (Store Norske, undated).

Coal mining may cause some emission of pollution through coal dust that escapes during the extraction, storing, loading and transport of the coal. It has been estimated that approximately 100 grams of coal dust is discharged per tonne of coal each year (Store norske, undated). The coal itself is not classified as toxic, but it contains substances that may be harmful to organisms if present in excessive amounts and under given conditions (Store Norske, undated). During extraction of coal, surrounding rocks with low coal content are removed and give rise to mine waste rocks, traditionally deposited in piles above the ground. These rocks often contain iron sulphide minerals, which oxidize in contact with air and water to produce sulphuric acid (H_2SO_4) and heat. The following reduction in pH may lead to leaching of trace elements such as nickel (Ni), zinc (Zn) and lead (Pb) from the sulphide oxidation, in addition to aluminium (Al) and manganese (Mn) from weathering processes nearby. The resulting acidic metal-enriched runoff is referred to as acid mine drainage (AMD) (Askaer et al., 2008). According to the State Pollution Control Authority (Statens forurensningstilsyn) (1998) there exist about 40 mine waste piles at Svalbard, with at least half of them requiring further investigation due to potential environmental hazard (Askaer et al., 2008).

Emissions from coal mining activities are believed to be considerably lower than those from combustion of coal in power plants (Store Norske, undated). The coal power plants in Longyearbyen and Barentsburg supply both municipal and industrial installations with electric power in this area, and emissions from these plants have been confirmed as major local pollution sources on Svalbard (Weinbruch et al., 2015). Combustion of coal produces large amounts of particulate matter (soot, fly ash), gaseous species (trace elements, organic compounds) and slag (bottom ash) (Klein et al., 1975). During the last decades, much research regarding particular matter from coal power plants have focused on the emission of secondary sulphates and nitrates, due to their important contribution to acid rain. In the recent years however, fly ash has gained more attention, as it constitutes a larger proportion of particulate emission to the atmosphere (Weinbruch et al., 2015). The fly ash is the remaining non-combustible parts of the coal associated with the flue gas, and it consists largely of aluminosilicates. The ash may also contain enhanced concentrations of metals, reflecting the metals embedded in the combusted coal (Mandal & Sengupta, 2006). According to a study performed by the US National Research Council (NRC) in 2012, elements of major concern because of known adverse health effects or due to their abundance in coal, include Pb, arsenic (As), boron (B), cadmium (Cd), mercury (Hg), molybdenum (Mo) and selenium (Se). In addition, coal also contains elements of moderate and minor concern, including Mn, Ni, Zn, barium (Ba), bromine (Br), chlorine (Cl), cobalt (Co), chromium (Cr), copper (Cu), fluorine (F), germanium (Ge), lithium (Li), sodium (Na), antimony (Sb), strontium (Sr) and vanadium (V). Elements with negligible concentrations, but of concern are silver (Ag), beryllium (Be), tellurium (Te) and thallium (Tl). Most of these are concentrated in fly and bottom ash in various proportions, while Hg and Se are primarily emitted to the atmosphere in the gas phase (Nalbandian, 2012). At the global scale in the 1990s, it was estimated that coal combustion contributed with Cr (69%), Hg (66%), Mn (85%), Sb (47%), Se (89%), Sn (89%) and Tl (almost 100%) to atmospheric emissions (Pacyna, 2005).

Development of the coal industry has led to emission control technologies for most targeted pollutants, including sulphur dioxide (SO_2), nitrogen oxides (NO_x), particulate matter and some other pollutants such as Hg and other trace elements. Technologies such as selective catalytic reduction, electrostatic precipitators, fabric filters, flue gas desulfurization and mercury control methods has led to decreased emissions in many plants (Moretti & Jones, 2012). In December 2013, Longyearbyen Community Council (Longyearbyen Lokalstyre Bydrift KF) signed a contract with French LAB (www.lab.fr, Lyon) regarding the establishment of a new treatment plant at Longyearbyen Powerplant (Longyear Energiverk). The agreement entailed the process of establishing 3 purification steps of the flue gas for reduction of SO2, NOx and other particulate matter (Longyearbyen Lokalstyre, 2013). The running period of the treatment plant started September 10, 2015, and for the first time in Svalbard, the flue gas was sent through a purification process before it was emitted through the chimney (Longyearbyen Lokalstyre, 2015). Longyearbyen Energiverk deposits the produced and collected slag and ash at the landfill in Adventdalen (Appendix 1). A total amount of 13000 m^3 slag and ash was deposited there during the years 2008-2010, and the annual production is expected to be stable and remain at about 5000 m^3 (Lyche & Nedland, 2011). This landfill can be an important point source of metal contamination to surrounding media, through leaching of combustion waste once it has been deposited (Løtveit, 2012; Gulec et al., 2001).

1.2 Potential effects of metal contamination

Metals are nonbiodegradable, which means that unlike many other pollutants, they cannot be broken into less harmful components. This means that organisms exposed to metals have to excrete or detoxify them by other means, for instance by hiding active metal ions within proteins like metallothionein, depositing them for long-term storage or excrete them with feces. However, even though metals are nonbiodegradable, they do exist in various species with different bioavailability. Hence, some metal species are more easily absorbed than other species, such as the organic compound methyl Hg (MeHg) compared to inorganic Hg^0 (Klaassen, 2013; Walker et al., 2012). When an organism absorbs a metal from the abiotic environment and/or diet, the circulatory system distributes the metal throughout the body and a fraction may be taken up in specific organs. If the organism's ability to absorb the metal exceeds the ability to excrete it, the metal will accumulate. The extent of long-term accumulation therefore depends on excretion rate (Walker et al., 2012, Nilsson, 1997). Factors such as species, age, sex, body size, seasonality, migration, habitat use and feeding ecology may also affect accumulation (Klaassen, 2013).

Some elements are essential for an organism to ensure normal growth, development and maintenance. In addition to the main essential elements (carbon (C), hydrogen (H), nitrogen (N) and oxygen (O)) and the major minerals required for ionic balance and as integral parts of amino acids, nucleic acids and structural compounds (Cl, Na, calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P) and sulphur (S)), a selection of metals are essential to various biological processes. These include As, Co, Cr, Cu, Mn, Mo, Ni, Se, V, Zn, iron (Fe), iodine (I), and silicon (Si), and are referred to as trace elements (Kronborg, 2013; Walker et al., 2012). These trace elements are intentionally bioacumulated in organisms, a process that involves active transport and storage. However, excessive exposure can still make these elements toxic to organisms, because they overwhelm biological systems and bind to unwanted sites. Nonessential toxic metals, such as Pb and Cd, may also exploit this intentional bioaccumulation, by mimicking trace elements and thus gain access to, and disrupt key cellular functions. This may explain the accumulation of metals without any known biological functions (Klaassen, 2013).

In addition to disturbance of essential metal homeostasis, metals may also bind to biomolecules or promote formation of reactive oxygen species (ROS). In particular nonessential metals interact with biomolecules such as enzymes, which can cause fatal consequences by inhibiting their biological function. Some metals, such as nickel and chromium, promote oxidative damage to biomolecules (e.g. proteins, DNA) such as proteins by generating ROS or other reactive intermediates. This illustrates the large variation in chemical properties and toxic endpoints among metals, which implies that metals can interact with biological systems in a large variety of ways. It is also important to remember that organisms in the environment are exposed to a complex cocktail of inorganic pollutants, which together can result in a detrimental effect on behaviour, physiology, biochemistry or histology of organisms (Klaassen, 2013).

1.3 Birds as sentinels of metal contamination

Birds have often been used to evaluate pollutant levels in the environment. This is partly due to their presence in ecosystems worldwide, an easy identification, an established classification and systematic, and a good background knowledge of their biology (Martinez, 2012). Many birds are also long-lived species that constitute a relatively high trophic level in the food chain, which makes them particularly suitable as monitors (Martinez, 2012; Burger, 1993). However, several passerine species have also successfully been used to monitor polluted environments (Berglund et al., 2011).

The birds' exposure to metals occurs through the surrounding environment and/or diet. When the metal has entered the bird, it may excrete it via feces or by depositing them in the uropygial gland, salt gland (Burger and Gochfeld, 1985) and/or feathers (Burger 1993). Bird feathers have thus been widely used to evaluate metal exposure in birds (Costa et al., 2013; Berglund et al., 2011; Costa et al., 2011; Dauwe et al., 2000; Denneman et al, 1993; Goede et al., 1975). The levels of metals in feathers can reflect levels in blood during the short period of feather growth. During this period the feathers are connected with the blood vessels, and the heavy metals will be incorporated into the keratin structure. This incorporation makes the metals inert and stable (Burger 1993), as the blood vessels atrophy and the feathers becomes physiologically separated from the birds circulation after they are fully formed (Burger, 1993; Denneman & Douben, 1993). A significant proportion of metals in their body may in this way be eliminated via the plumage during the molting period (Hughes et al., 1997; Burger, 1993), and result in lower concentration of certain metals in internal tissue than in feathers (Grue et al., 1986). In the case of female birds, metals may also be excreted through their eggs (Dauwe et al., 1999; Burger & Gochfeld, 1993). An obvious advantage of using feathers for biomonitoring, is the fact that they are easy to obtain and can be collected repeatedly – without affecting the condition and health of the studied individuals (Markowski et al., 2013). External contamination should however be cautiously discussed, as feathers are subjected to contamination through atmospheric deposition, contact with soil, dust or water, or from deposition of contaminants on feathers during preening. Several studies have shown that trace elements in feather analysis were of external origin (Borghesi et al, 2016; Dauwe et al, 2003; Ek et al, 2004), and according to Borghesi et al. (2016), the presence of rare earth elements (REE) are considered good indicators of such contamination.

1.4 Snow bunting

The snow bunting (*Plectrophenax nivalis*) is the northernmost nesting passerine species, and the only one that regularly breed in the high Arctic environment of Svalbard (Hoset et al, 2014). The male individuals usually arrive at the breeding site in this area, 3-4 weeks before the females, usually in late March – early April (Cramp & Perrins, 1994). The males that arrive first secure the best territories, making them more attractive to the females (Hindrum, 1994). As the snow buntings and their eggs are vulnerable to predation by the Arctic fox (*Vulpes lagopus),* glaucous gulls (*Larus hyperboreus*) and skuas (*Stercoraiidae*) at Svalbard, is it important to ensure a good breeding site (Strøm, undated). The female snow bunting prepares their nests in natural breeding sites such as crevices, cavities in screes and under boulders. Artificial sites such as cavities and nooks on buildings and other man-made constructions (e.g. nest boxes) are also used (Hoset et al., 2014). The nest is bowl-shaped, and at Svalbard it consists mainly of straw, moss, feathers and reindeer (*Rangifer tarandus platyrhynchus*) hair. The female lays 5-6 eggs, and some birds may breed twice during a good season. The eggs are incubated for 12-13 days, followed by a nestling period where both parents supply the nestlings with insects and spiders. This period includes both the 10-12 days until the nestlings leave the nest, but also a corresponding time after fledging (Skjøstad, 2008; Hindrum, 1994). Unlike nestlings, adult snow buntings primarily feed on seeds. They may however supply their diet with insects during the nestling period (Strøm, undated). The snow buntings leave Svalbard in August – September, and moult before the autumn migration (Green & Summers, 1975). Hence, the concentration of elements in snow bunting feathers are likely to reflect the background levels at Svalbard. The detailed migratory route for snow buntings breeding at Svalbard is uncertain.

1.5 Aim of this study

The main objective of this study was to investigate how life-history traits such as age and sex may affect accumulation of metals and other elements in snow buntings in Adventdalen, Svalbard, by examining feathers of nestlings and adults. The Arctic environment of Adventdalen may be influenced by pollution from coal industry and coal combustion, an important anthropogenic source to metal emissions. The aim was therefore to assess how accumulation of elements varies with sex and age in snow buntings, and to which extent the levels of coal-associated elements in the feathers reflect expected emissions from the coal industry.

Based on the fact that accumulation of some elements occurs over time, is it hypothesized that the concentrations of metals will increase with age. Sex is also expected to influence accumulation, with a significantly lower accumulation in females due to sequestering of metals to their eggs. Additionally, it is hypothesized that accumulation of metals in snow buntings in Adventdalen will reflect levels in their ambient environment. As coal is a major contributor to local pollution, it is assumed that levels in the birds to some extent reflect emissions associated with the coal industry and coal combustion.

2. Materials and Methods

2.1 Study area and sampling

The fieldwork was carried out in Adventdalen valley, Svalbard, from 0 to 8 km east of Longyearbyen (Appendix 1). The project is linked to Research in Svalbard (RIS) ID 2272, and permission to carry out the capture of adult birds was provided by the Norwegian Animal Research Authority (NARA) (FOTS ID 4701). The collection area contains a cableway, which was earlier used to transport coal from the mines into the city (Appendix 1). In this area, snow buntings breed in both artificial nest boxes placed on the cableway (Hoset et al. 2014; Espmark et al., 2004), and in natural breeding sites such as crevices and under boulders. Feather samples from a selection of breeding birds (adults; 1 year, ≥2 years) were sampled in this area during the summer of 2015 (Appendix 1, Appendix 2). All birds were captured in their nest boxes. The feathers were plucked from the chest. Age (nestling, 1 year old, 2 years or older) and sex were determined based on feather colouration following Svensson (1992). In addition, feathers samples from three dead adults from previous years were included in the study. The sex of these was registered, but not age. The feather samples from the nestlings were collected in both artificial and natural nests along the cableway, when the nestlings were about eight days old. The feathers from the nestlings were also plucked from the chest. Regarding the adult birds, each feather sample corresponds to an individual bird, while samples of the nestlings correspond to a pool of feathers collected from several chicks in the same nest. In total, 22 feather samples from adults and 34 feather samples from clutches were included in this study (Appendix 2). The samples were placed in paper envelopes marked with nest number, clutch size, age, sex and date of collection.

2.2. Preparation and analysis of feathers

Feathers were washed alternately (2x) with double deionized water (MiliQ) and acetone (Sigma-Aldrich) in the laboratory, and dried beneath clean filter paper in room temperature (24°C). The dry weight was calculated and the feathers were subsequently digested with nitric acid (HNO_3, 50%, 3 mL), in a high-pressure microwave system (Milestone UltraClave, EMLS, Leutkirch, Germany) (Appendix 3). After digestion, the samples were diluted by adding deionized water to a total volume of 30 ml in teflon-bottles, and subsequently transferred to 15 ml PP-vials. Final determination of 58 chemical elements was carried out by high-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) using a Thermo Finnigan model Element 2 instrument (Bremen, Germany) at the Department of Chemistry, NTNU.

Limit of detection (LOD) were calculated based on known instrument detection limits (IDLs) for different elements, or based on three times standard deviation of the blanks. The highest value was used (Appendix 4). Elements with 50% of the element concentrations below LODs were excluded from the statistical analysis, and includes Ag, Cr, Mo, dysprosium (Dy), hafnium (Hf), iridium (Ir), lutetium (Lu), niobium (Nb), platinum (Pt), scandium (Sc), thorium (Th), thulium (Tm) and zirconium (Zr). The concentrations of Al, As, Ba, Ca, Cd, Co, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, P, Pb, S, Sb, Se, Si, Sn, Sr, Tl, V, Zn, gold (Au), bismuth (Bi), cerium (Ce), caesium (Cs), erbium (Er), gallium (Ga), lanthanum (La), neodymium (Nd), praseodymium (Pr), rubidium (Rb), samarium (Sm), terbium (Tb), titanium (Ti), uranium (U), tungsten (W), yttrium (Y), ytterbium (Yb) were determinable in at least 50% of the samples, and their results were thus subjected to statistical analysis. In the case of results lying below LOD in these elements, the concentration of the sample in question was excluded from all statistical analysis. All concentrations are presented in µg/g dry weight (dw).

2.3 Reference material

The analytic procedure was verified with the use of INCT-OBTL-5 (Oriental Basma Tobacco Leaves), certified reference material from the Institute of Nuclear Chemistry and Technology Warszawa – Poland. The recoveries ranged from 85 to 115%, and the precision of the analytic procedure was expressed by the relative standard deviation (RSD) from 0.6 to 14.7% (Appendix 5).

2.4 Statistical analysis

2.4.1 Principal component analysis

SIMCA (Version 13, Umetrics, Umeå, Sweden) was used to perform a principal component analysis (PCA) on nestling and adult (1 year and ≥2 years) samples. PCA is a multivariate technique that extracts the most important information and patterns from a dataset, while it retains maximum level of variation. The simplified dataset is expressed by a set of new, orthogonal variables referred to as principle components. The highest level of variance is expressed by the first principle component (PC1), and the second highest level of variance by the second principle component (PC2). The second principle component is however computed under the constraint of being orthogonal to the first principle component (Kronborg, 2013; Abdi & Williams, 2010).

Two PCA plots were created, one of the samples (score plot) (Figure 2) and one of the variables (loading plot)(Figure 3). Each of the feather samples in the analysis is characterized by two values, one for each of the two principle components. This score locates the samples in the model, and can be utilized to detect patterns, groupings, similarities or differences (Gergen & Harmanescu, 2012). Samples that are closely associated in the plot are expected to have similar variable composition (Eriksson et al., 2006). In the loading plot, each variable receive a loading that reflects their contribution to the variation in the dataset, in addition to an interpretation of the relationship between the variables. The size of the variables residual variance indicates their importance in the PCA model. This can be utilized for variable selection, as it reveals which variables are most significant for the variation contained in the data (Kronborg, 2013; Gergen & Harmanescu, 2012).

2.5.2 Statistical tests and presentation

The Kruskal-Wallis tests were performed to test the effect of age and sex on individual metal concentrations. I selected 18 elements *a priori* known to be associated with coal mining.

Differences between groups were presented in bargraphs. Bonferroni correction was used to counteract the problem of multiple comparisons. The significance level (0.05) was divided by the number of metals tested in this analysis (18), giving a new significance level of 0.00278. A post hoc test, Dunn's multiple comparison, was performed on the significant results from the Kruskal-Wallis test to examine which age groups differed significantly.

3. Results

The PCA resulted in a significant model with two principal components, which explained 81.1 % of the variation. PC1 explained 69% of the variation, followed by PC2 (12%). The score plot showed a clear separation of nestling and adult samples ("1 year" and "≤2 years") along PC1, while no difference between female and male samples could be explained by neither PC1 nor PC2 (Figure 2). This was verified by Kruskal-Wallis test (Appendix 7). Neither age nor sex explained the variance along PC2, with p-values from the Kruskal-Wallis test of 0.2482 and 0.6761, respectively (Appendix 7). The loading plot (Figure 3) revealed which elements were responsible for the age grouping observed in Figure 2. P, K, Na, Mg, Zn, Se, Hg and Rb were associated with nestling samples and elements such as Al, As, Ba, Cd, Co, Cu, Mn, Ni, Pb, Sb, Sn, Sr, Tl and V were associated with adults.

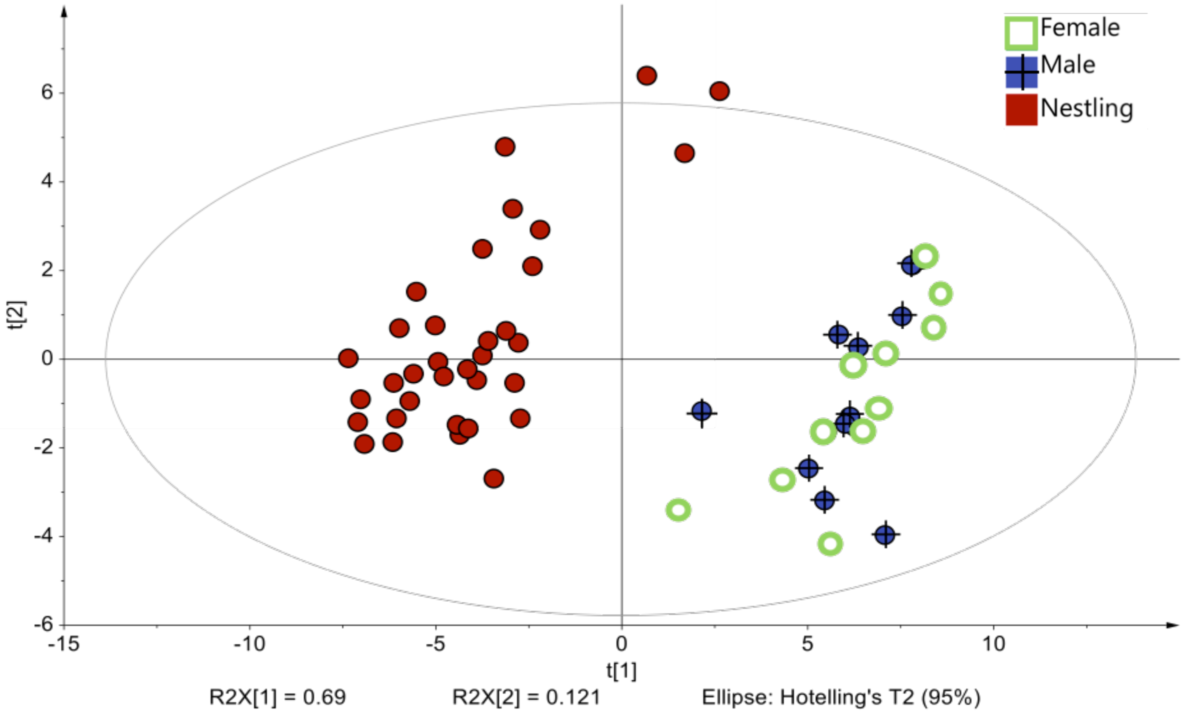


Figure 2: PCA Score plot for feather samples of snow buntings (*Plectrophenax nivalis*) collected in Adventdalen, grouped into nestlings (red, n=34), adult males (blue, n=10) and adult females (green, n=12). PC1 was explained by age, while neither age nor sex explains PC2. Scores attached in Appendix 9.

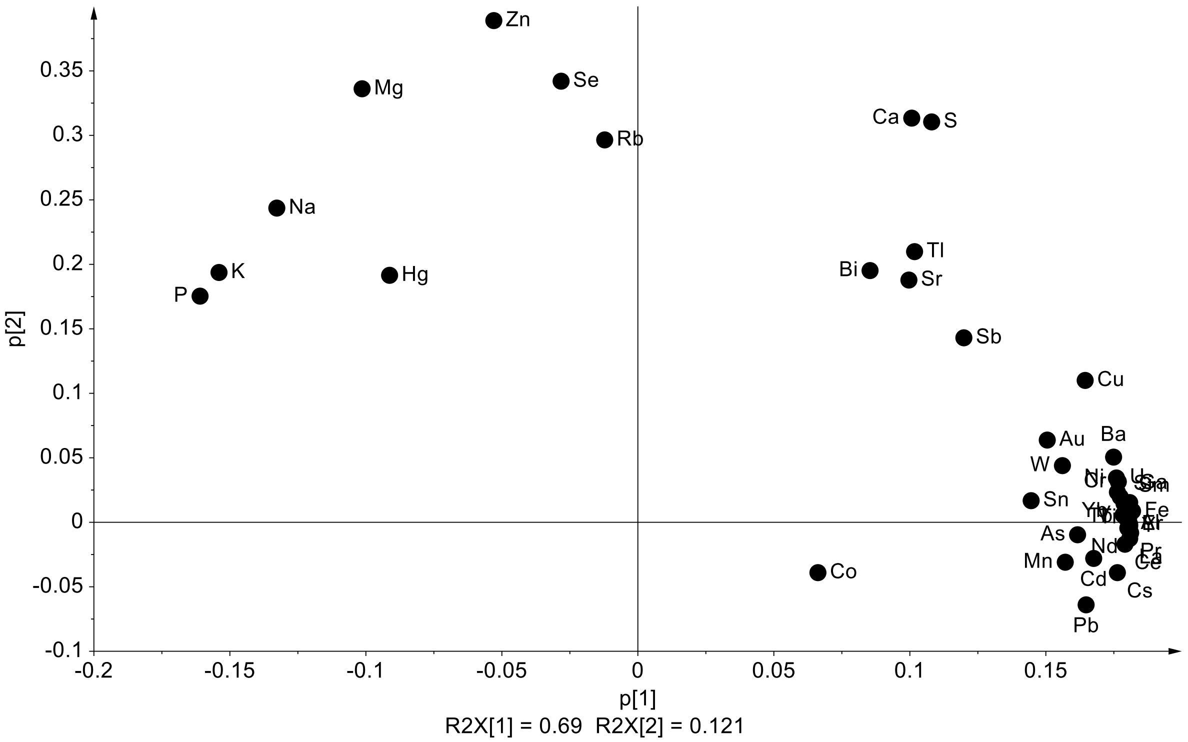
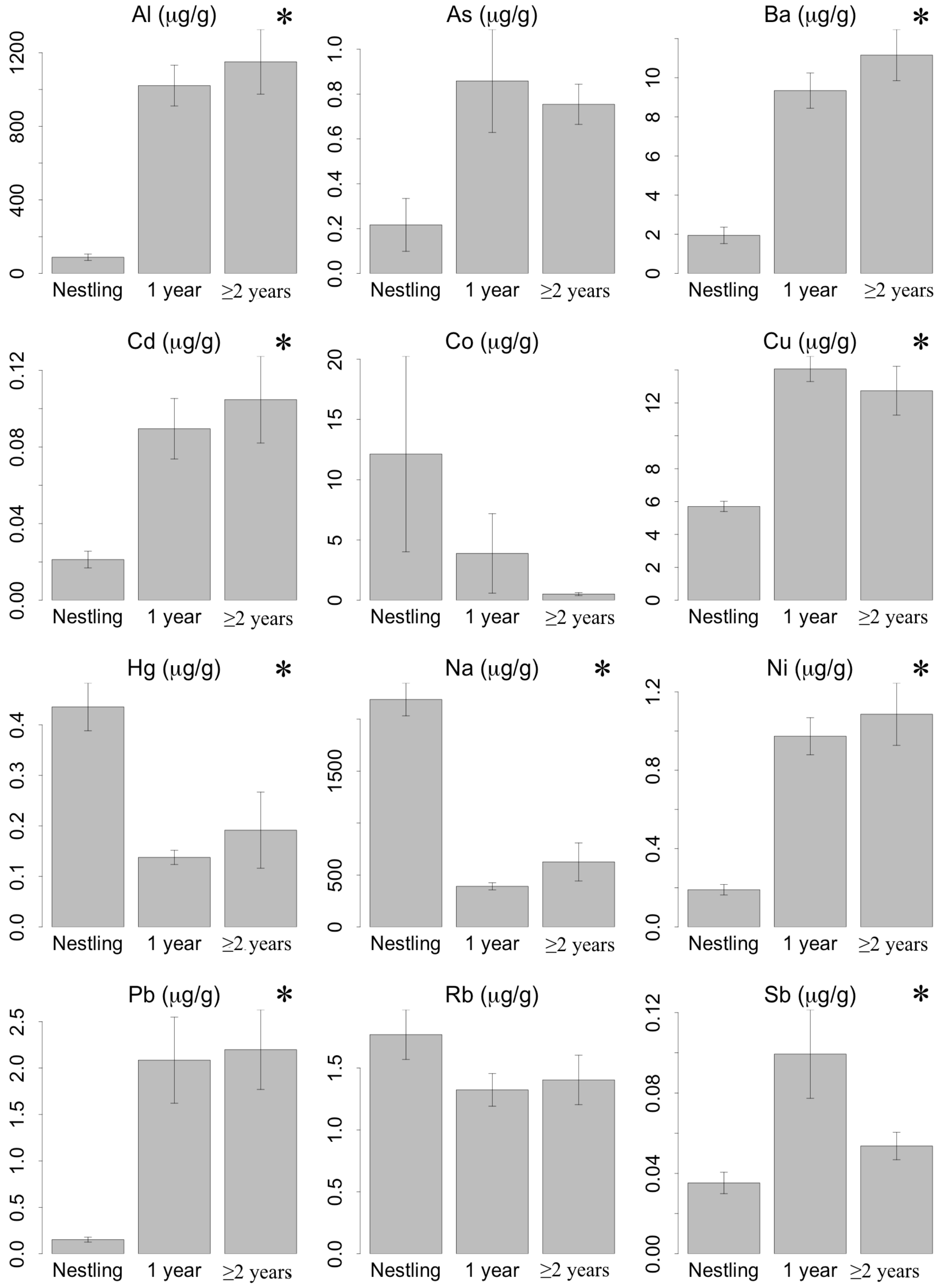


Figure 3: PCA loading plot for principal component 1 and 2. Loading for each metal attached in Appendix 10.

A complete overview of the concentrations of elements in analysed feather samples is presented in Table 6 (Appendix 6). The bargraphs illustrate how the concentrations for a variety of elements varied within and between the age groups in snow buntings in Adventdalen (Figure 4). Bargraphs with an asterisk mark significant differences (Kruskal-Wallis test) between the nestling group and the adult group (1 years and ≥2 years) (Appendix 7). Dunn's test reported no significant differences between 1 year old and 2 years or older birds for any selected element (Appendix 8). Figure 4 illustrates a significant biodillution from nestling to 1- and 2 years or older birds for Hg, Na and Zn, while an accumulation from nestling to 1- and 2 years or older birds are demonstrated for Al, Ba, Cd, Cu, Ni, Pb, Sb, Sn, Tl and V. None significant differences among the groups were evident for As, Co, Se and Sr.


Al (µg/g) *
0
400
800
1200
Nestling
1 year
≥2 years
As (µg/g)
0.0
0.2
0.4
0.6
0.8
1.0
Nestling
1 year
≥2 years
Ba (µg/g) *
0
2
4
6
8
10
Nestling
1 year
≥2 years
Cd (µg/g) *
0.00
0.04
0.08
0.12
Nestling
1 year
≥2 years
Co (µg/g)
0
5
10
15
20
Nestling
1 year
≥2 years
Cu (µg/g) *
0
2
4
6
8
12
Nestling
1 year
≥2 years
Hg (µg/g) *
0.0
0.1
0.2
0.3
0.4
Nestling
1 year
≥2 years
Na (µg/g) *
0
500
1500
Nestling
1 year
≥2 years
Ni (µg/g) *
0.0
0.4
0.8
1.2
Nestling
1 year
≥2 years
Pb (µg/g) *
0.0
0.5
1.0
1.5
2.0
2.5
Nestling
1 year
≥2 years
Rb (µg/g)
0.0
0.5
1.0
1.5
Nestling
1 year
≥2 years
Sb (µg/g) *
0.00
0.04
0.08
0.12
Nestling
1 year
≥2 years

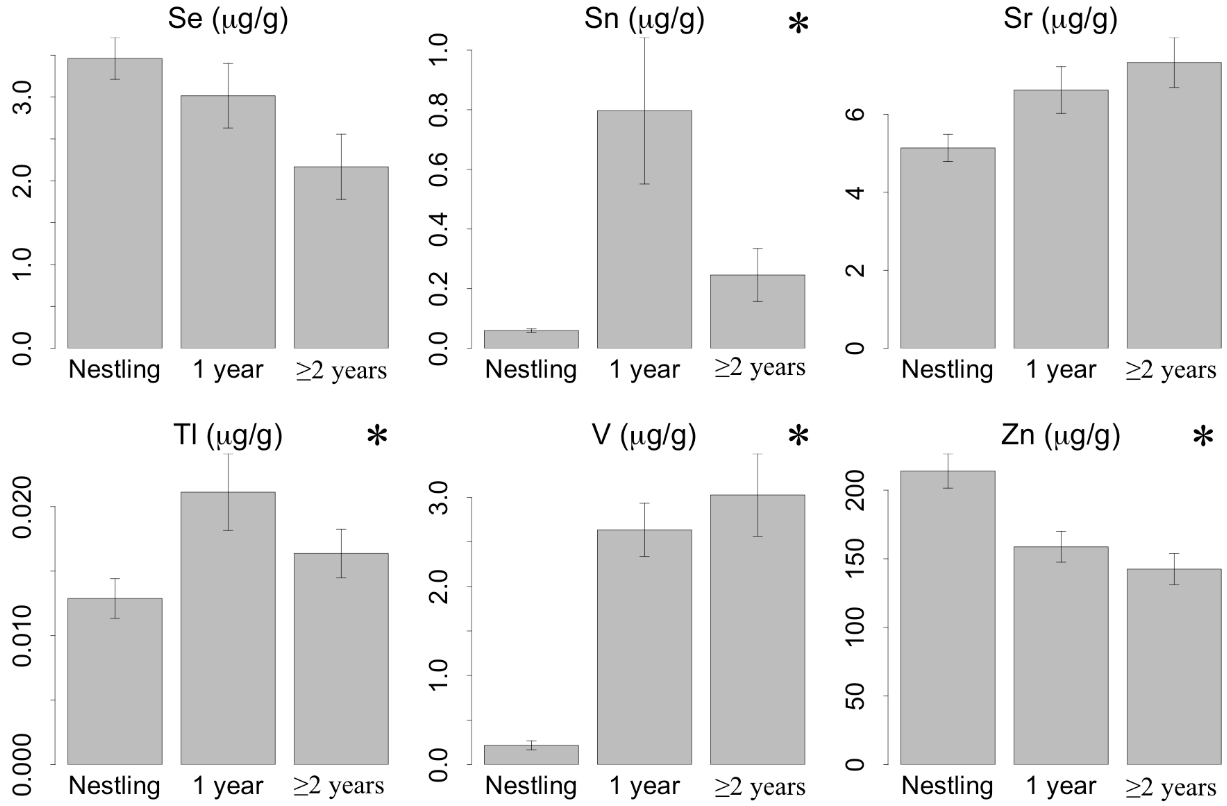


Figure 4. A selection of element concentrations in feather samples of snow buntings (*Plectrophenax nivalis*) collected in Adventdalen, grouped into "nestlings" (n=34), "1 year" (n=11) and "≥2 years" (n=). Asterisk marks significant differences between nestlings and adults. There were no significant differences between the two groups: "1 year" and "≥ 2 years".

4. Discussion

To my knowledge, there are no published studies on metal contamination in snow buntings. However, similar studies have been conducted with a number of other passerine species such as great tit (*Parus major*), rock bunting (*Emberiza cia*), Eurasian blackcap (*Sylvia atricapilla*), European robin (*Erithacus rubecula*) and blackbird (*Turdues merula*) (Costa et al., 2013; Costa et al., 2011; Dauwe et al., 2001; Llacuna et al., 1995). As far as I know, this is the first study that examines variations in element concentrations in relation to age and sex of Arctic snow bunting.

4.1 External contamination

Many of the elements that explained the variance in PC1 (69%) (Figure 2) and hence, the age grouping, are classified as REE (Appendix 10). These elements have many similar properties, correlate well with each other and are litophilic, which means that they are enriched in the earth's crust and comprise significant amounts of some minerals (Borghesi et al, 2016; Castor & Hedrick, 2006). As mentioned in the introduction, Borghesi et al. (2016) consider these metals as good indicators of external contamination of feathers. Based on their high values for PC1 coinciding with the feather samples of the adult birds, this could indicate a significant external contamination of adult feathers. This contrasts the results from the nestling samples, which were mainly associated with elements characterized by low loadings in PC1, and thus not REEs. This could imply that feathers from the nestlings are less affected by external contamination, and to a greater extent represent internal contamination, possibly from maternal transfer, diet and/or their ambient environment. Considering the fact that the environmental exposure of the nestlings are limited to the nest box, it is not unlikely that the adults, which are moving freely in the environment, are more susceptible to external contamination. This also reinforces the idea that nestlings are more suitable for studies on feathers than adult birds (Borghesi et al., 2016). If the results on adults from this study originate from exogenous rather than endogenous contamination, this involves a certain probability of misleading results due to masking of the actual internal accumulation of adult snow buntings. A follow-up study that includes samples from several organs of snow buntings is therefore strongly recommended in order to verify the results of this study. However, many studies have used feathers to assess accumulation of metals in birds (Lester & Van Riper, 2014; Costa et al., 2013; Berglund et al., 2011; Llacuna et al., 1995).

4.2 Accumulation

As previously mentioned, an organism will accumulate metals and other elements if it absorbs more from the abiotic environment and/or diet than it eliminates (Walker et al., 2012; Nilsson, 1997). In addition to excretion rate, factors such as age and sex may also affect the extent of accumulation in organisms (Klaassen, 2013).

4.2.1 Significance of age

The significance of age on concentrations of metals and other elements in snow buntings became very clear based on the results from the PCA (Figure 2), and this was verified by the Kruskal-Wallis test (Appendix 7). The expectations prior to this study entailed an accumulation of metals with age, meaning that older birds will have a greater body burden of metals than younger ones. Several studies support this hypothesis for some metals. Cul et al. (2013) showed significantly greater concentrations of both Cd in all tissue and Pb in lung tissue of older pigeons (*Columba livia domestica*) compared to younger ones. Berglund et al. (2011) also conducted a study in which they observed a clear accumulation of both Cd and Se with age, in two passerine species, pied flycatcher (*Ficedula hypoleuca*) and great tits. Accumulation of elements with age is a natural consequence, if the absorption of the bird exceeds its ability to excrete them. However, several factors should be considered when evaluating accumulation. Firstly, different bird species might differ with respect to toxicokinetics. This implies that there may exist inter-species differences regarded absorption, distribution and elimination of toxicants. Observed accumulation of a metal in one bird species does not imply that it also accumulates, or accumulates to the same extent, in other species. This was highlighted in the study of Berglund et al. (2011), in which higher concentrations of metals were observed in the pied flycatchers than in the great tits, under the same exposure conditions. Secondly, different organs may also contain different concentrations of metals. Some metals may be excreted to a large extent in feathers, while others are retained in the body (Borghesi et al, 2016). Thirdly, the environment and/or diet of the birds must contain metal concentrations sufficient for absorption to exceed elimination. Which concentrations the snow buntings must be subjected to over time to accumulate metals, will vary with each specific metal, as they have different properties and thus toxicokinetics. Some metals are more easily absorbed and/or eliminated, while others require more time. This is highly dependent on the specific form the element is available in, which to a large extent determines its bioavailability (Klaassen, 2013). A metal characterized by a low rate of absorption and a high rate of elimination for instance, would not be expected to accumulate. In the opposite case, an accumulation would be conceivable. The significance of external contamination must further be evaluated if the feather analysis is utilized to assess accumulation, as it has the potential to mask the extent of accumulation in the birds (Borghesi et al., 2016; Dauwe et al., 2003). To exclusively rely on concentrations in feathers may thus lead to misinterpretation of the results, as mentioned earlier.

The PCA loading plot clearly indicates which elements were responsible for the cluster of samples corresponding to each of the age groups (nestlings, adults), and how the examined metals correlate with each other (Figure 3). Variables located close to each other tend to be positively correlated, while those located on opposite sides of the plot origin tend to be negatively correlated (Nilsson et al., 2006). This implies that adult associated elements grouped at the lower, right corner in the loading plot for instance, could be positively correlated. Their trends of accumulation in snow buntings will thus most likely be similar. This is clearly evident in Figure 4, which shows how a selection of these positively correlated elements all had a significant accumulation from the nestling to the adult group (e.g. Al, Ba and Cd). This contrasts the observed trends of elements negatively correlated to the adult associated elements. In general, this applies to elements associated with the nestlings, such as

Hg, Na and Zn. Negatively correlated elements act in the opposite way, with a rise in one concentration resulting in a decrease of the other and vice versa (Nilsson et al., 2006). The importance of this relationship becomes apparent when studying the accumulative trends of negatively correlated elements in Figure 4. While most of the elements that were high in the adults showed the expected accumulation from the nestling to the adult group, concentrations of elements that were high in the nestlings dropped significantly in the adults. This is not surprising for the essential elements P, K, Na, Mg, Zn and Se measured in this study, as nestlings are developing and have diversifying tissues, and thus could have a high demand for these elements. Ciesielski et al. (2004) for instance, reported similar trends for Cu and Zn in Baikal seal (*Phoca sibirica*) pups. The high levels of Hg in nestlings in relation to adults, is however very surprising and particularly interesting. Since several studies have shown that external contamination of Hg in feathers is negligible (Borghesi et al, 2016), is it believed that concentrations of Hg almost exclusively represent levels in the blood during formation of the feathers. This indicates a higher absorption of Hg by the nestlings than by the adults, most probably due to a higher exposure. This could be due to the different diet of the nestling- and adult birds, with nestlings feeding on insects and spiders, while adults mainly consume seeds (Kristoffersen, 2012). This means that the nestlings are at a higher trophic level than the adults, with the possibility of accumulation and trophic transfer of Hg with each successive step in the food chain (Zhang et al, 2009). This is particularly applicable to Hg, if it is present as the organic compound methyl mercury (MeHg). MeHg has very good biomagnifying properties. However, the inorganic form Hg^0 does not biomagnify to the same extent (Klaassen, 2013). Varying results regarding the extent and significance of such a biomagnification through a food chain have been reported, and it is believed that this process is highly species dependent. However, as plants may accumulate elements through surface deposition of particles, uptake of vapours and/or directly from the soil (Kristoffersen, 2012), significant quantities of Hg may also be present in seeds. The contribution of diet to the significant higher concentration of Hg in nestlings is thus uncertain. Another possible source to a potential high exposure of nestlings could be the environment of their nest, which is made up of straw, moss, feathers and reindeer hair (Hindrum, 1994). Contamination of these constituents could involve an increased absorption through the respiratory- and gastro intestinal tract of the nestlings. This could result in an elevated accumulation compared to adults. The study of Beitveit (2015) indicated relatively high levels of mercury in moss from the collection area, compared to reference levels (G. Beitveit, pers.com.). Some of the elements included in this study did not vary significantly between the age groups at all, such as As, Co, Se and Sr (Fig. 4).

4.2.2 Significance of sex

As discussed in the section on principal component analysis, the results from this study confirmed the importance of age in relation to accumulation, but disproved differences between females and males (Figure 2). The latter finding is in contrary to my expectations, as I expected a greater body burden of metals in males than in females. This hypothesis was largely based on the fact that females may deposit a part of their body burden of metals through their eggs (Dauwe et al., 1999; Burger & Gochfeld, 1993). Such a transfer would involve an increased elimination of metals in the females during oviposition, most likely with a significant impact on the extent of accumulation. As the males do not have this additional opportunity of elimination, elevated metal concentrations in comparison to female levels would be a natural consequence. Several studies indicate that such a sequestering of metals occurs under certain conditions in the nature, and elevated levels of mercury (Fimreite et al., 1982; Haseltine et al., 1980; Fimreite et al., 1974), lead, cadmium (Maedgen et al., 1982) and certain other metals (Gochfeld & Burger, 1987) have been reported in eggs. Based on the results of the present study, with no significant difference of metal accumulation in females and males, maternal transfer of elements to eggs seem to be of little importance in female snow buntings. This was also suggested by Eastin and O'Shea (1981), in which no detectable levels of heavy metals were discovered in eggs from experimentally dosed female mallards (*Anas platyrhynchus*) with elevated tissue levels. It is however important to emphasize that the present study does not provide any basis to conclude whether the female snow buntings excrete metals to their eggs or not, and if so, which metals they transfer to the greatest extent. Nevertheless, the results imply that such transfer is of minor significance when it comes to accumulation of metals by the females. This conclusion is based on an assumption that the female and male birds had approximately the same body burden of metals at the time of egg laying. It also excludes the possibility that external contamination of feathers could mask a potential difference in accumulation between the sexes. A study including concentrations in internal organs/tissues could validate whether sex has any significance for the accumulation of metals, by eliminating the uncertainty associated with external contamination. Another follow-up study on metal concentrations in females and associated eggs could also provide a better understanding of maternal transfer in snow buntings, and valuable information regarding which metals, and to which extent these metals are transferred to eggs. Although a potential transfer does not necessarily result in a significant reduction in the metal accumulation of females, it may still be of great importance when it comes to nestling development and survival.

4.3 Local pollution

Although there is some uncertainty regarding the use of feather analysis for assessment of metal accumulation in birds (Borghesi et al., 2016; Dauwe et al., 2003), several studies have confirmed the reliability of bird feathers as non-destructive, suitable bioindicators of metal contamination (Markowski et al., 2013, Salah-Eldein et al., 2012; Goede & Bruin, 1986). This may be due to the fact that both the internal and any external contamination of the feathers interact to reflect pollution levels in the ambient environment. In particular, nestling feathers have been identified as the most credible bioindicators of local metal pollution (Borghesi et al., 2016), due to the clearly defined time period of metal contamination obtained from a limited parental foraging area (Dauwe et al., 2000). However, it is important to keep in mind that each metal may be excreted to a varying degree in the feathers, meaning that feather analysis can provide inaccurate information regarding internal metal concentrations in the birds (Borghesi et al., 2016).

It is assumed that the local environment in the vicinity of Longyearbyen is influenced by coal industry and coal combustion. Accordingly, one would expect emissions of metals from both mining, waste stockpiles, land fills and the coal power plant located in Longyearbyen. The latter is predicted to be the largest source of emissions, especially given the absence of flue gas purification until September 2015 (Longyearbyen lokalstyre, 2015). Since this study was completed during the summer of 2015, it was expected elevated levels of coal-associated elements present in the local environment at the time of sampling. As mentioned in the introduction, coal contains various amounts of most elements. Elements discussed in this section have been chosen based on their abundance in coal and/or their toxic potential, and in light of available reference material (Table 1). A complete overview of concentrations of all elements examined in the feathers of snow buntings is attached in Appendix 3.

Table 1: Average metal concentrations (µg/g dw) for selected elements in feathers of nestling and adult snow buntings, and in a selection passerine species from other publications (Llacuna et al., 1995, Dauwe et al., 2001, Costa et al., 2011 & Costa et al., 2013). Detailed information regarding number of samples for each element in adult and nestling snow buntings attached in Appendix 6.

	Snow bunting Adult (n= ≤ 22)	Snow bunting Nestling (n= ≤ 34)	Great tit Adult (n=10)	Great tit Adult (n=7)	Great tit Nestling (n=30)	Rock Bunting Adult (n=4)	Blackcap Adult (n=22)	Robin Adult (n=14)	Blackbird Adult (n=11)
Al	992.68	88.16	58.34	-	-	-	-	-	-
As	0.76	0.22	0.96	0.98	0.35	-	-	-	-
Cd	0.09	0.02	0.56	0.11	0.03	-	0.2	0.11	0.12
Co	2.33	12.13	0.04	-	-	-	-	-	-
Cu	12.81	5.70	6.47	5.72	8.29	9.85	15.04	17.06	11.0
Hg	0.16	0.44	0.84	0.39	0.41	-	0.32	2.04	0.5
Mn	16.54	2,44	43.8	-	-	-	-	-	-
Ni	0.96	0.19	0.31	1.66	2.38	1.09	-	1.13	0.78
Pb	2.13	0.15	8.07	2.49	1.27	2.37	3.05	2.04	2.35
Se	2.65	3.46	0.83	0.90	0.92	-	-	-	-
Zn	152.74	213.98	119.5	101.6	112.9	177.6	87.83	113.57	109.78

In order to assess whether the bird feathers have elevated element concentrations due to local pollution, a reference level is needed for comparison. Such a reference sample was however difficult to obtain in the vicinity of Longyearbyen, as it was assumed that this entire area is affected by the coal-fired power plant to some degree. As no research on metal concentrations in feather samples from snow buntings has been published, the results are compared to the reported reference levels of five other passerine species (Table 1). These species include rock bunting, Eurasian blackcap, European robin, blackbird and great tit. The reported metal concentrations in Rock buntings was determined in a study by Llacuna et al. (1995), involving an expected non-polluted zone in Spain, at St. Jaume de Frontanya. Dauwe et al. (2001) reported reference metal concentration in adult great tits, from an area 20 km northeast from an industrial plant in Belgium, while reference concentrations reported in Eurasian blackcap, European robin, blackbird and great tit adult and nestlings were determined in the two studies of Costa et al. (2011) and Costa et al. (2013). Both studies included the very limited polluted National Pine Forest of Quiaios, Portugal, as reference area. All of these reference levels have been derived using the same analytic procedure as the present study. However, it is very important to emphasize that these reference values are indicative when it comes to the baseline levels in snow buntings. This is illustrated clearly in Table 1, with varying reference values reported both within and between species. Varying concentrations within the same species may be due to factors such as age, physical condition, metabolic capability and season for sampling, in addition to their actual exposure. Interspecies differences are even more important to take into account, as these also include differences in biochemistry, feeding habits and trophic levels. Accumulation of contaminants might be affected by all of these factors (Kristoffersen, 2012). Direct comparisons of element levels of different species can still be informative due to the limited range of concentrations reported for several different passerine species. Hence, the probability that the basic levels of the snow buntings also are located within this range is high.

When comparing the results from this study with reference concentrations in other passerine species (Table 1), it appears that some metals were present in concentrations significantly above the references. Some metals were also present below reference concentrations, while others were present in equivalent amounts. It is particularly interesting that Cd, Hg, Mn and Cr, metals of which emissions are largely linked to coal industry, were present in relatively low concentrations in feathers of both nestling and adult snow buntings in Svalbard. In fact, most of the feather samples contained concentrations of Cr below the limit of detection (>50%), and were excluded from this study. Estimates from the mid 1990s calculated that coal combustion contributed to total global emissions with 66%, 85% and 69% of Hg, Mn and Cr, respectively (Pacyna, 2005). One would thus expect relatively high levels of these metals in environment adjacent to coal power plants, with a resulting high exposure to organisms inhabiting such areas. As the feather concentrations of both adult and nestling birds in this study were significantly lower than those of reference birds, this could either indicate constrained absorption and accumulation of these elements by snow buntings, limited sequestering to feathers and/or insignificant amounts of these elements in the surrounding environment. There is also a possibility that the baseline level of these elements is significantly lower in snow buntings than the other passerine birds. This study does not provide a good basis for a conclusion regarding the low concentrations of these metals in feathers of snow buntings, as it is solely based on feather analysis of snow buntings from Adventdalen. Some uncertainties in relation to baseline level, toxicokinetics and concentrations of these elements in the environment consists. It would have provided a better basis for a conclusion if the results of this study could be seen in connection with reference values in snow buntings from uncontaminated areas, results of a geochemical study in the environment of Adventdalen and concentrations in different internal organs of the birds. However, based on the results one may note that the levels of Cd, Hg, Mn and Cr were lower than expected in feathers of snow buntings in Adventdalen, considering the proximity of their habitat to the coal power plant. This also applies to the elements As, Ni and Pb, present in concentrations corresponding to the reference levels in the other passerine species (Table 1).

Compared with the baseline levels in the other passerine species, Al, Co, Se and Zn were present in higher concentrations in snow buntings in Adventdalen. These high levels probably reflect the extent of exposure in the ambient environment, as both accumulation and external contamination of the feathers is dependent on substantial quantities of elements in the environment. It is very likely that the elevated concentrations can be traced to the coal industry, especially in terms of Co, Se and Zn (Nalbandian, 2012; Pacyna, 2005)). Most of these elements are associated with the fly ash and/or the bottom ash from the combustion process in the power plant, while selenium is primarily emitted to the atmosphere in the gas phase (Nalbandian, 2012). Based on the absence of flue gas purification until September 2015 (Longyearbyen Lokalstyre, 2015), significant amounts of these elements could have been emitted with the flue gas, spread in air and deposited on soil and plants in the local environment. Disposal of ash in the landfill of Adventdalen could also contribute to significant contamination of the ambient environment, due to leaching of ash-stored elements to the surrounding water and soil. The conspicuous high concentrations of aluminium in the adult feather samples could also be due to such a leaching process, but more indirectly related to coal industry. As mentioned in the introduction, mine waste rocks emerge during excavation of coal. These rocks give rise to mine waste piles, which often can be characterized by an acidic environment due to the presence of iron sulphide minerals. The acidic environment promotes leaching of certain elements, including aluminium from weathering processes nearby (Askaer et al., 2008). However, it is also very likely that the high concentration of Al in the birds may be caused by sources other than coal industry.

4.4 Toxicological considerations

As toxicological implications were not a main focus of this study, the results mainly report a status regarding element concentrations in feathers of nestling and adult snow buntings in Adventdalen. As mentioned earlier, no studies have been performed to determine baseline levels of essential elements or reference levels of non-essential elements, in snow buntings. It is thus difficult to assess whether this study's reported concentrations in feathers are of toxicological significance for the birds or not. However, as discussed in the section on local pollution, a comparison of levels in snow buntings with levels in other bird species could provide valuable information. Nevertheless, it is important to emphasize that such an extrapolation between species is relatively uncertain and rather indicative. It is likely that the majority of birds inhabiting areas relatively unaffected by elemental contamination, have acceptable levels of both essential and non-essential elements. As this study indicates that As, Cd, Cu, Hg, Mn, Ni and Pb were present in equivalent- or lower concentrations compared to reference levels in feathers of other passerine species (Table 1), this could suggest that these elements are of minor toxicological importance for snow buntings in Adventdalen. Despite the fact that all of these elements may be of little toxicological significance due to excessive amounts, inadequate amounts of essential elements also provide a basis for toxicological considerations (Klaassen, 2013). However, based on limited knowledge regarding normal levels of essential elements in snow buntings and uncertainties related to extrapolation between bird species, this is recommended to be investigated in future studies. The same applies to levels of Ba, Na, Sn, Tl and V, for which there was no basis for comparison of the obtained results in this study.

Al, Co, Se and Zn differs from the rest of the coal-associated elements in focus of this study, as they were present in higher concentrations in feathers of snow buntings than in feathers of passerine species utilized for comparison. This could indicate increased levels of these elements in snow buntings from Adventdalen, possibly due to elevated levels in the environment related to the coal industry, as mentioned earlier. These elevated levels could be of toxicological significance for the birds. Even though Co, Se and Zn are essential elements, excessive amounts are associated with negative effects such as poor reproduction, reduced growth, erythropoiesis and polycythaemia, histopathological lesions, paralysis and mortality (Beyer et al., 2004; Hoffman, 2002; Morera et al., 1996; Diaz et al., 1994). The concentration of the non-essential element Al was conspicuous high in feathers of adult snow buntings from Adventdalen, approximately 17 times higher than in feathers of adult great tits from an uncontaminated area (Table 1). A follow-up study is recommended to examine the importance of the Al level in feathers, as toxic effects associated with Al could be consistent with rickets and include reduced growth, lack of appetite, bone deformities, decreased egg laying, impaired eggshell formation and early feather molt (Sparling et al., 1997; Scheuhammer, 1987).

Even though most of the coal-associated elements were present in concentrations of anticipated minor toxicological significance in feathers of snow buntings, is it still important with follow-up studies and further investigations. Studies that examine levels in blood or other tissues and/or organs could provide a better basis for toxicological considerations.

4.5 Global perspective

In recent years, there has been an increasing focus on coal industry and its negative impact on the local and global environment, including the biotic part of ecosystems. Many countries are today aiming at the development of more environmentally friendly energy sources, in conjunction with a phase-out of coal, particularly in the industrialized countries (Tennbakk, 2013). However, the reduction of use of coal occurs slowly. This, in combination with a large consumption of coal in areas such as Asia, means that coal still forms the basis for a large share of the world's energy consumption (Pacyna, 2005). An understanding of the coal industry's effects on the environment and its inhabitants is thus regarded as very important and relevant today. This study is one among many that examines the impact of coal industry in organisms living in the vicinity to coal mines and a coal power plant. From a global perspective is it conceivable that studies dealing with such issues are considered as extremely important in the on-going phase-out of coal fired plants for energy production.

5. Conclusion

The present study was the first to investigate element concentrations in feathers of both nestling and adult snow buntings in the Arctic environment of Adventdalen. Significant differences in concentrations between nestling- and adult feathers were found, but a clear age-dependent accumulation could not be observed among nestlings, 1 year old- and 2 years or older birds. However, external contamination of adult samples could possibly mask internal accumulation. P, K, Na, Mg, Zn, Se, Rb and Hg was present in highest concentrations in nestlings, while the rest of the examined elements were present in highest concentrations in feathers of adults. No significant effect on accumulation was discovered for sex.

As nestlings hatch and develop in Adventdalen, while adults complete their annual molt in the area, feathers of both groups are considered appropriate to assess local pollution. Compared to reference concentrations in other passerine birds, coal-associated elements were mostly present in lower or equal concentrations in feathers of snow buntings from Adventdalen. Al and Co differs from the other elements, as their concentrations for comparison are higher. Hence, element concentrations in feathers of snow buntings from this area, do not reflect emissions associated with coal industry to a large extent. As toxicological implications were not a main focus of this study, the results primarily report a status regarding element concentrations in nestling and adult snow buntings in Adventdalen. However, based on comparison with reference levels in other passerine species, high concentrations of Al in adults and high concentrations of Co, Se and Zn in nestling and adults could give cause for concern.

Follow-up studies using other tissues and/or organs are strongly recommended to verify and further investigate the role of age and sex in accumulation of elements in snow buntings. Such analysis combined with environmental analysis could also provide a better basis for a conclusion regarding environmental exposure, and hence, emissions from the coal power plant located in Longyearbyen. High concentrations of Al in adults, in addition to high concentrations of Co, Se and Zn in nestlings and adults, require further examinations to verify levels and examine if they are of toxicological significance for the birds.

6. References

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Appendix 1: *Map of Adventdalen with collection spots*

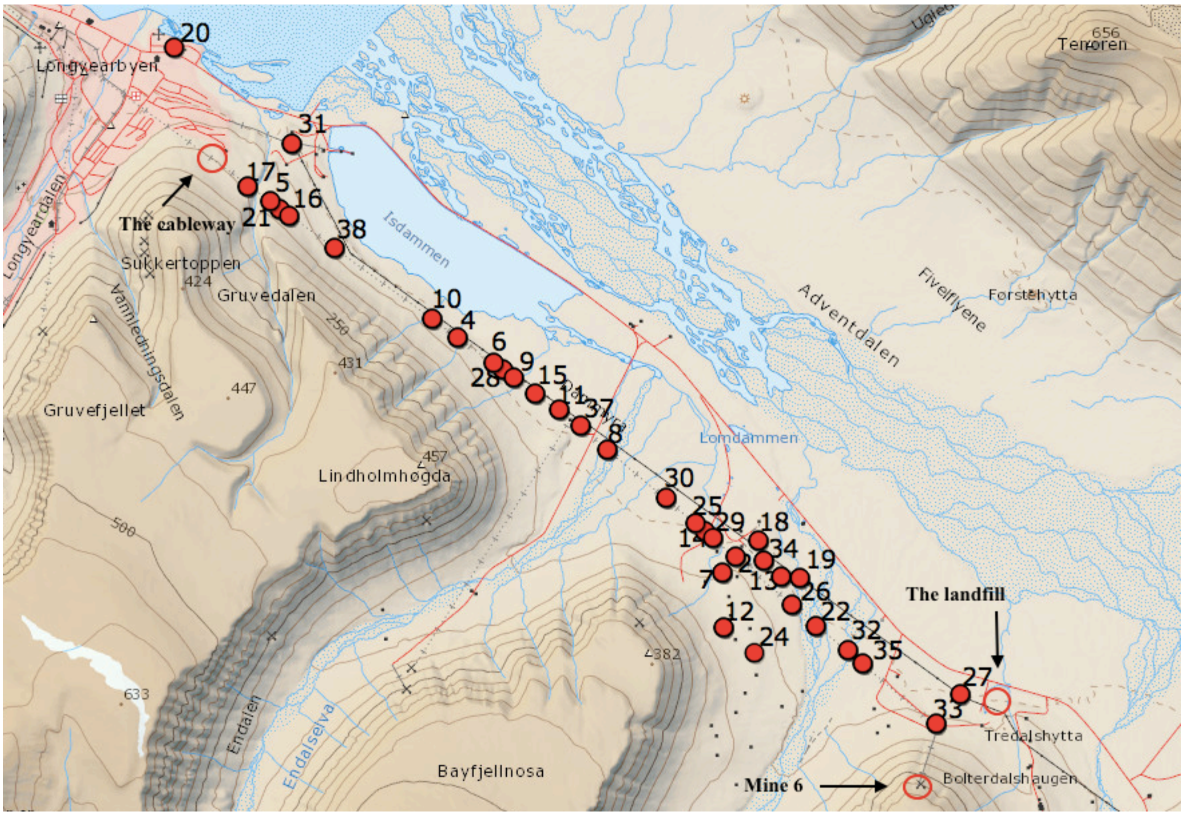

Figure 5. Collection spots for nestling samples along the cableway from mine 6 to Longyearbyen in Adventdalen, Svalbard.

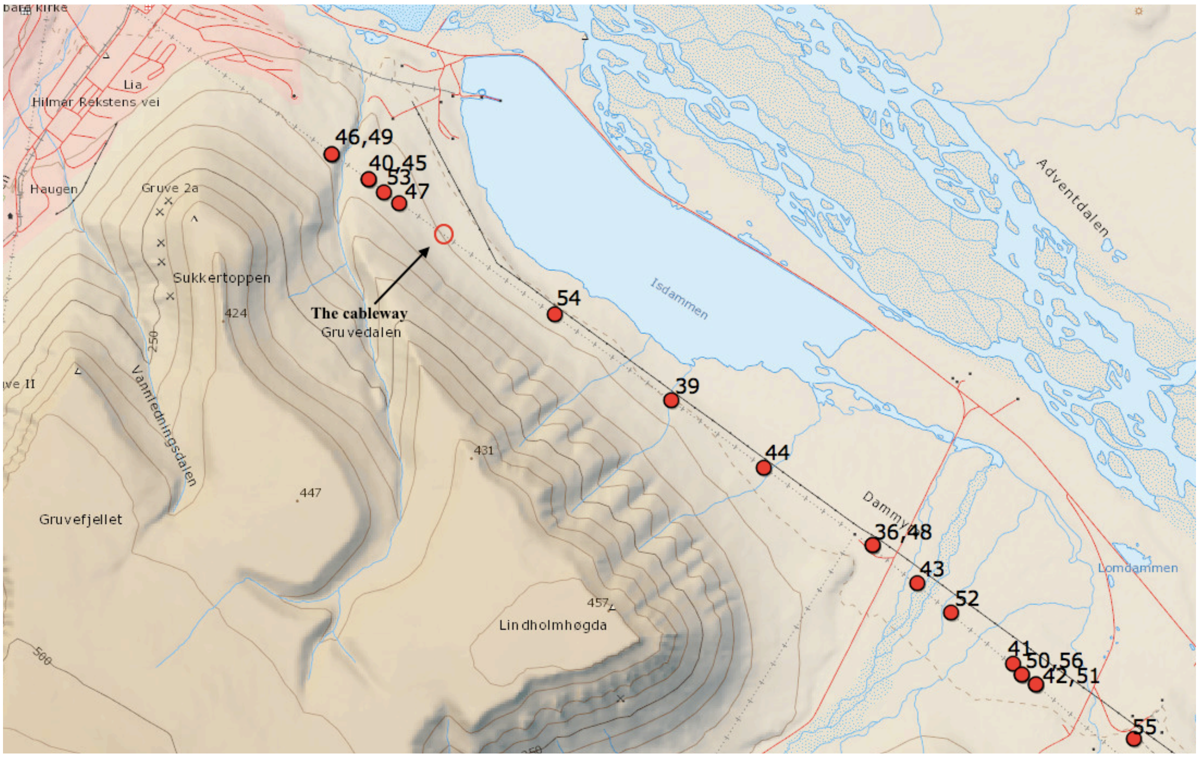

Figure 6. Collection spots for adult samples along the cableway from mine 6 to Longyearbyen in Adventdalen, Svalbard.

Appendix 2: *Information about the samples*

Table 2.1: Raw data with all information about the different samples. The sample number, age, sex, corresponding nest and location, ID, amount of individuals (AoI), initial sample weight (mg), final weight (g) and final volume (ml) are reported.

Nr	Age	Sex	Nest	Location	ID	AoI	Sample weight (mg)	Final weight (g)	Final volume (ml)
4	Nestling	-	-	B36I	-	5	6.8	30.5	30.0
5	Nestling	-	58	B21I	-	5	5.2	30.7	31.3
6	Nestling	-	40	B41I	-	4	2.1	31.3	30.8
7	Nestling	-	51	Ski on wall cabin	-	3	12.5	30.4	29.9
8	Nestling	-	33	B36II	-	6	15.3	30.3	29.8
9	Nestling	-	39	B42II	-	5	6.2	30.3	29.9
10	Nestling	-	**55**	B34I	-	6	5.9	30.7	30.2
11	Nestling	-	36	B46I	-	5	5.4	30.3	29.8
12	Nestling	-	73	New, grey cabin	-	-	1.7	32.0	31.5
13	Nestling	-	20	Wilderness cabin	-	5	20.5	31.0	30.5
14	Nestling	-	25	B25II	-	-	11.9	30.7	30.2
15	Nestling	-	-	B44I	-	-	3.3	32.5	32.0
16	Nestling	-	4	B22I	-	-	3.4	31.2	30.7
17	Nestling	-	-	B17I	-	-	4.9	30.6	30.1
18	Nestling	-	62	Green cabin	-	-	6.8	30.6	30.1
19	Nestling	-	61	Mast 54III	-	-	3.4	30.4	29.9
20	Nestling	-	71	NTNU st.	8N77594	-	1.3	32.5	32.0
21	Nestling	-	-	B19I	-	-	1.2	30.2	29.7
22	Nestling	-	16	Mast 12B	-	-	9.9	30.4	29.9
23	Nestling	-	22	B22II	-	-	2.6	30.3	29.8
24	Nestling	-	68	Bus cabin	-	5	9.8	30.8	30.3
25	Nestling	-	26	B26II	-	5	3.4	30.4	29.9
26	Nestling	-	18	B14II	-	-	2.0	30.4	29.9
27	Nestling	-	-	Mast 68BIII	-	4	8.8	30.5	30.0
28	Nestling	-	41	B40I	-	4	7.0	31.1	30.5
29	Nestling	-	24	B24II	-	-	2.2	30.9	30.4
30	Nestling	-	29	B30II	-	-	8.6	31.5	31.0
31	Nestling	-	83	Wirepit	-	4	3.8	30.2	29.7
32	Nestling	-	17	B10II	-	5	14.1	30.6	30.1
33	Nestling	-	-	Mine 6, lower	-	-	2.0	30.9	30.4
34	Nestling	-	72	Grey cottage	-	4	6.9	30.7	30.1
35	Nestling	-	6	B8II	-	3	3.3	31.9	31.4
36	≥2 years	Male	34	B40II	8N77711	1	5.5	31.0	30.5
37	Nestling	-	34	B40II	-	4	11.4	30.5	30.0
38	Nestling	-	**55**	B26I	-	2	2.5	31.3	30.8
39	≥2 years	Female	42	B36I	8N77723	1	8.6	30.4	29.9
40	1 year	Female	-	B19I	8N77728	1	11.2	31.1	30.6
41	≥2 years	Female	54	B30II	8N77719	1	5.6	30.7	30.2
42	1 year	Male	27	B28II	8N77714	1	9.4	30.9	30.4

Table 2.2: Raw data with all information about the different samples. The sample number, age, sex, corresponding nest and location, ID, amount of individuals (AoI), initial sample weight (mg), final weight (g) and final volume (ml) are reported.

Nr	Age	Sex	Nest	Location	ID	AoI	Sample weight (mg)	Final weight (g)	Final volume (ml)
43	1 year	Female	33	B36II	8N77713	1	9.2	30.2	29.7
44	≥2 years	Male	39	B42I	8N77716	1	4.3	30.5	30.0
45	1 year	Male	59	B19I	8N77721	1	5.3	30.6	30.1
46	1 year	Female	74	B17I	8N77724	1	3.6	30.5	30.0
47	1 year	Male	58	B21I	8N77722	1	3.8	30.6	30.1
48	≥2 years	Female	34	B40II	8N77712	1	6.1	30.5	30.0
49	1 year	Male	74	B17I	8N77720	1	2.8	30.6	30.1
50	1 year	Female	28	B29II	8N77725	1	3.0	30.3	29.8
51	1 year	Female	27	B28II	8N77715	1	4.4	30.5	30.0
52	≥2 years	Male	31	B34II	8N77726	1	2.4	30.5	30.0
53	1 year	Female	-	B21I	8N77727	1	15.9	31.0	30.5
54	1 year	Female	53	B29I	8N77718	1	3.4	30.3	29.8
55	≥2 years	Male	63	Scooter hytta	8N77569	1	40.5	30.8	30.3
56	≥2 years	Male	-	B29II	8N77729	1	3.1	30.7	30.2
57	≥2 years	Female	No. inf.	No inf.	8880924	1	16.7	30.9	30.4
58	-	Female	No inf.	No inf.	Broken wing	1	10.3	30.5	30.0
59	-	Male	No inf.	No inf.	8L48009	1	5.9	30.4	29.9

Appendix 3: *Ultra Clave*

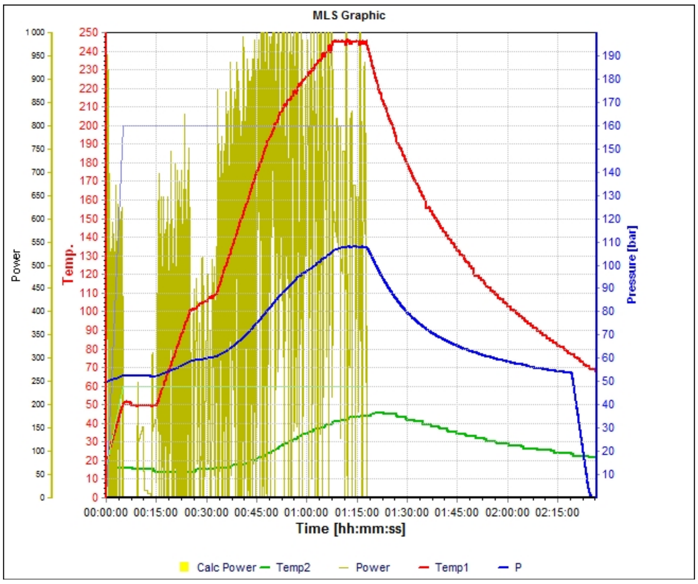

Figure 7: Temperature profile for the digestion with Ultra Clave. HNO_3 (50%, 3 ml) was added to the sample before digestion, and then diluted to 30 mL with ultrapure water.

Table 3: Programme for digestion with Ultra Clave.

Step	**Time (hh:mm:ss)**	**Temp 1 (°C)**	**Temp 2 (°C)**	**Press (bar)**	**Energy (watt)**
1	00:05:00	50	60	160	1000
2	00:10:00	50	60	160	1000
3	00:10:00	100	60	160	1000
4	00:08:00	110	60	160	1000
5	00:15:00	190	60	160	1000
6	00:05:00	210	60	160	1000
7	00:15:00	245	60	160	1000
8	00:10:00	245	60	160	1000

Appendix 4: *Detection limits*

Table 4.1: Calculated detection limits (LODs, µg/g) of detected elements for different weight classes (mg) of feather samples. Each element reported with isotope and resolution.

	Element	Isotope	RES	LOD					
				1-2.5	2.5-5	5-7.5	7.5-10	10-15	15-41
Ag	Silver	109	Mr	0.343	0.160	0.0960	0.0686	0.0480	0.0275
Al	Aluminium	27	Mr	30.7	14.3	8.59	6.14	4.30	2.46
As	Arsenic	75	Hr	0.429	0.200	0.120	0.0857	0.0600	0.0344
Au	Gold	197	Lr	0.0056	0.0026	0.0016	0.0011	0.00078	0.00045
B	Boron	11	Mr	168	78.4	47.1	33.6	23.5	13.5
Ba	Barium	137	Mr	0.427	0.199	0.120	0.085	0.0597	0.0342
Be	Beryllium	9	Mr	0.137	0.0640	0.0384	0.0274	0.0192	0.0110
Bi	Bismuth	209	Lr	0.0573	0.0268	0.0161	0.0115	0.0080	0.0046
Ca	Calcium	44	Mr	67.7	31.6	18.9	13.5	9.47	5.43
Cd	Cadmium	114	Lr	0.0343	0.0160	0.0096	0.0069	0.0048	0.0028
Ce	Cerium	140	Lr	0.0434	0.0203	0.0122	0.0087	0.0061	0.0035
Co	Cobalt	59	Mr	0.340	0.159	0.0952	0.0680	0.0476	0.0273
Cr	Chromium	53	Mr	1.33	0.623	0.374	0.267	0.187	0.107
Cs	Cesium	133	Lr	0.00956	0.0045	0.0027	0.0019	0.0013	0.00077
Cu	Cupper	63	Mr	0.174	0.0814	0.0488	0.0349	0.0244	0.0140
Dy	Dysprosium	163	Mr	0.0343	0.0160	0.0096	0.0069	0.0048	0.0028
Er	Erbium	166	Lr	0.00514	0.0024	0.0014	0.0010	0.00072	0.00041
Fe	Iron	56	Mr	17.5	8.19	4.91	3.51	2.46	1.41
Ga	Gallium	69	Mr	0.120	0.0560	0.0336	0.0240	0.0168	0.0096
Hf	Hafnium	178	Lr	0.0188	0.0088	0.0053	0.0038	0.0026	0.0015
Hg	Mercury	202	Lr	0.0678	0.0317	0.0190	0.0136	0.0095	0.0054
Ir	Iridium	193	Lr	0.00857	0.0040	0.0024	0.0017	0.0012	0.00069
K	Potassium	39	Hr	91.0	42.5	25.5	18.2	12.7	7.30
La	Lantan	139	Mr	0.0343	0.0160	0.0096	0.0069	0.0048	0.0028
Li	Lithium	7	Mr	0.514	0.240	0.144	0.103	0.0720	0.0413
Lu	Lutetium	175	Lr	0.0034	0.0016	0.0010	0.00069	0.00048	0.00028
Mg	Magnesium	25	Mr	20.9	9.74	5.85	4.18	2.92	1.68
Mn	Manganese	55	Mr	0.440	0.205	0.123	0.088	0.0615	0.0353
Mo	Molybdenum	98	Mr	0.343	0.160	0.0960	0.0686	0.0480	0.0275
Na	Sodium	23	Mr	171	80.0	48.0	34.3	24.0	13.8
Nb	Niob	93	Hr	0.429	0.200	0.120	0.0857	0.0600	0.0344
Nd	Neodymium	146	Lr	0.0282	0.0132	0.0079	0.0056	0.0040	0.0023
Ni	Nikkel	60	Mr	0.281	0.131	0.0786	0.0561	0.0393	0.0225
P	Phosphor	31	Mr	26.9	12.6	7.53	5.38	3.77	2.16
Pb	Lead	208	Lr	0.0555	0.0259	0.0155	0.0111	0.0078	0.0045
Pr	Praseodymium	141	Lr	0.00514	0.0024	0.0014	0.0010	0.0007	0.0004
Pt	Platinum	195	Lr	0.0171	0.0080	0.0048	0.0034	0.0024	0.0014
Rb	Rubidium	85	Mr	0.206	0.096	0.0576	0.0411	0.0288	0.0165
S	Sulphur	34	Mr	565	264	158	113	79.2	45.4
Sb	Antimony	121	Mr	0.0417	0.0195	0.0117	0.0083	0.0058	0.0033
Sc	Scandium	45	Mr	0.185	0.0865	0.0519	0.0371	0.0259	0.0149
Se	Selenium	82	Lr	0.857	0.400	0.240	0.171	0.120	0.0688
Si	Silisium	29	Mr	196	91.5	54.9	39.2	27.5	15.7
Sm	Samarium	147	Lr	0.00857	0.0040	0.0024	0.0017	0.0012	0.00069
Sn	Tin	118	Lr	0.0511	0.0238	0.0143	0.0102	0.0072	0.0041
Sr	Strontium	88	Mr	0.429	0.200	0.120	0.0857	0.0600	0.0344
Tb	Terbium	159	Lr	0.0034	0.0016	0.0010	0.00069	0.00048	0.00028
Th	Thorium	232	Lr	0.171	0.0799	0.0479	0.0342	0.0240	0.0137
Tl	Thallium	205	Lr	0.00429	0.0020	0.0012	0.00086	0.00060	0.00034

Table 4.2: Calculated detection limits (LODs, µg/g) of detected elements for different weight classes (mg) of feather samples. Each element reported with isotope and resolution.

	Element	Isotope	RES	LOD					
				1-2.5	2.5-5	5-7.5	7.5-10	10-15	15-41
Tm	Thulium	169	Lr	0.00857	0.0040	0.0024	0.0017	0.0012	0.00069
U	Uranium	238	Lr	0.00429	0.0020	0.0012	0.00086	0.00060	0.00034
V	Vanadium	51	Mr	0.122	0.0568	0.0341	0.0243	0.0170	0.0098
W	Wolfram	182	Lr	0.0176	0.0082	0.0049	0.0035	0.0025	0.0014
Yb	Ytterbium	172	Lr	0.00686	0.0032	0.0019	0.0014	0.0010	0.00055
Yb	Yttrium	89	Lr	0.0100	0.0047	0.0028	0.0020	0.0014	0.00081
Zn	Zink	66	Mr	7.34	3.42	2.05	1.47	1.03	0.589
Zr	Zirkonium	90	Lr	0.611	0.285	0.171	0.122	0.0855	0.0490

Appendix 5: *Certified reference material INCT-OBTL-5*

Table 5. Results from the certified reference material, reported in µg/g.

Element	Concentrations CRM	Concentrations results	
	Mean ± STD	Mean ± STD	RSD (%)
Ag	0.053 ± 0.011	0.0392 ± 0.0058	14.7
Al	1980 ± 28.0	1786 ± 195	10.9
As	0.668 ± 0.086	0.674 ± 0.024	3.6
Ca	39960 ± 1420	37262 ± 3867	10.4
B	33.6 ± 2.20	33.0 ± 0.6	1.7
Ba	67.4 ± 3.80	60.4 ± 1.2	1.9
Cd	2.64 ± 0.10	2.63 ± 0.031	1.2
Ce	2.99 ± 0.18	3.022 ± 0.072	2.4
Co	0.981 ± 0.067	0.771 ± 0.005	0.6
Cs	0.288 ± 0.02	0.26 ± 0.002	0.7
Cu	10.1 ± 0.40	8.48 ± 0.18	2.1
Er	0.101 ± 0.01	0.1048 ± 0.0028	2.7
Hf	0.291 ± 0.024	0.014 ± 0.0004	2.8
Hg	0.021 ± 0.0013	0.0197 ± 0.0007	3.4
K	22710 ± 760	23034 ± 185	0.8
La	1.69 ± 0.09	1.412 ± 0.022	1.6
Mg	8530 ± 340	8089 ± 721	8.9
Mn	180 ± 8.00	175 ± 16	8.9
Mo	0.414 ± 0.062	0.285 ± 0.004	1.3
Nd	1.33 ± 0.11	1.238 ± 0.076	4.4
Ni	8.5 ± 0.49	6.82 ± 0.08	1.2
P	1700 ± 120	1690 ± 165	9.8
Pb	2.01 ± 0.31	1.725 ± 0.089	5.2
Rb	19.1 ± 1.00	16.95 ± 0.20	1.2
S	4550 ± 910	3891 ± 76	2.0
Sb	0.076 ± 0.0125	0.0305 ± 0.0004	1.2
Sc	0.64 ± 0.027	0.507 ± 0.008	1.6
Sm	0.264 ± 0.01	0.237 ± 0.009	3.6
Sr	105 ± 5.00	98.2 ± 9.5	9.7
Tb	0.035 ± 0.002	0.0341 ± 0.0006	1.9
Th	0.503 ± 0.043	0.4232 ± 0.018	4.2
V	4.12 ± 0.55	4.25 ± 0.07	1.6
Yb	0.115 ± 0.023	0.0884 ± 0.0020	2.3
Zn	52.4 ± 1.80	44.4 ± 0.5	1.1

Appendix 6: *Element concentrations in snow buntings*

Table 6.1: Mean, median, standard deviation (SD), minimum (min) and maximum (max) concentrations for analysed elements reported for nestlings, 1 year old birds, 2 years or older birds, females and males, based on “n” samples. All concentrations are reported in µg/g dw.

Element		**n**	**Mean**	**Median**	**SD**	**Min**	**Max**
Al	*Nestling*	31	88.2	57.5	96.5	20.3	464
	1 year	11	1021	1082	368	533	1780
	≥ 2 years	9	1150	1205	527	336	2170
	Female	12	1091	1094	550	259	2170
	Male	10	894	857	401	188	1566
As	*Nestling*	7	0.217	0.0916	0.312	0.0483	0.912
	1 year	11	0.858	0.570	0.762	0.408	3.02
	≥ 2 years	9	0.754	0.695	0.269	0.446	1.31
	Female	12	0.900	0.595	0.737	0.344	3.02
	Male	10	0.611	0.567	0.212	0.342	1.02
Au	*Nestling*	9	0.00582	0.00232	0.00874	0.000497	0.0267
	1 year	11	0.121	0.0541	0.165	0.0111	0.570
	≥ 2 years	9	0.0426	0.0215	0.0646	0.00157	0.209
	Female	12	0.0538	0.0228	0.0756	0.00312	0.226
	Male	10	0.107	0.0565	0.173	0.00157	0.570
Ba	*Nestling*	34	1.95	1.19	2.45	0.566	14.0
	1 year	11	9.34	9.14	2.99	5.08	15.8
	≥ 2 years	9	11.2	10.8	3.92	5.92	18.0
	Female	12	10.0	10.1	4.14	2.78	18.0
	Male	10	8.89	8.28	3.73	3.34	15.7
Bi	*Nestling*	26	0.0518	0.0351	0.0396	0.0179	0.178
	1 year	9	0.0667	0.0744	0.0266	0.0325	0.113
	≥ 2 years	9	0.0576	0.0513	0.0265	0.0230	0.106
	Female	11	0.0574	0.0650	0.0215	0.0230	0.0804
	Male	9	0.0626	0.0482	0.0319	0.0282	0.113
Ca	*Nestling*	34	1979	1701	770	1267	4408
	1 year	11	2671	2674	641	1780	3694
	≥ 2 years	9	2306	2297	714	997	3245
	Female	12	2667	2602	558	1780	3694
	Male	10	2291	2233	726	997	3245
Cd	*Nestling*	6	0.0212	0.0181	0.0107	0.00760	0.0350
	1 year	11	0.0895	0.0717	0.0524	0.0430	0.220
	≥ 2 years	9	0.105	0.0767	0.0679	0.0454	0.242
	Female	12	0.0941	0.0724	0.0590	0.0379	0.220
	Male	10	0.0929	0.0742	0.0581	0.0430	0.242
Ce	*Nestling*	30	0.0923	0.0604	0.110	0.0157	0.570
	1 year	11	1.26	1.14	0.469	0.5615	2.05
	≥ 2 years	9	1.49	1.43	0.587	0.967	2.74
	Female	12	1.41	1.48	0.640	0.290	2.74
	Male	10	1.09	1.02	0.501	0.284	1.94

Table 6.2: Mean, median, standard deviation (SD), minimum (min) and maximum (max) concentrations for analysed elements reported for nestlings, 1 year old birds, 2 years or older birds, females and males, based on “n” samples. All concentrations are reported in µg/g dw.

Element		**n**	**Mean**	**Median**	**SD**	**Min**	**Max**
Co	*Nestling*	7	12.1	0.721	21.5	0.0361	55.8
	1 year	11	3.90	0.312	10.9	0.163	36.8
	≥ 2 years	9	0.509	0.424	0.368	0.124	1.39
	Female	12	0.487	0.308	0.616	0.0715	2.38
	Male	10	4.18	0.450	11.5	0.210	36.8
Cs	*Nestling*	21	0.00732	0.00536	0.00612	0.00289	0.0285
	1 year	11	0.0764	0.0788	0.0264	0.0327	0.122
	≥ 2 years	9	0.0814	0.0878	0.0365	0.0290	0.149
	Female	12	0.0813	0.0860	0.0367	0.0238	0.149
	Male	10	0.0640	0.0567	0.0286	0.0181	0.110
Cu	*Nestling*	34	5.70	5.00	1.81	3.65	11.8
	1 year	11	14.1	13.6	2.55	9.44	19.5
	≥ 2 years	9	12.7	12.0	4.45	6.11	19.7
	Female	12	13.2	13.2	3.42	5.59	19.5
	Male	10	12.4	11.9	4.57	6.11	19.7
Er	*Nestling*	21	0.00347	0.00256	0.00335	0.000634	0.0157
	1 year	11	0.0317	0.0323	0.0117	0.0157	0.0514
	≥ 2 years	9	0.0367	0.0371	0.0154	0.0136	0.0679
	Female	12	0.0351	0.0337	0.0166	0.00832	0.0679
	Male	10	0.0274	0.0256	0.0117	0.00849	0.0468
Fe	*Nestling*	34	86.7	68.3	81.5	21.7	393
	1 year	11	917	944	317	540	1636
	≥ 2 years	9	1074	1008	431	563	1933
	Female	12	979	963	472	201	1933
	Male	10	838	771	368	176	1444
Ga	*Nestling*	7	0.0470	0.0285	0.0488	0.0108	0.150
	1 year	11	0.273	0.270	0.0925	0.139	0.444
	≥ 2 years	9	0.297	0.302	0.123	0.104	0.526
	Female	12	0.289	0.312	0.132	0.0670	0.526
	Male	10	0.233	0.220	0.100	0.0607	0.385
Hg	*Nestling*	32	0.435	0.369	0.267	0.120	1.42
	1 year	10	0.138	0.125	0.0444	0.0739	0.195
	≥ 2 years	9	0.192	0.106	0.226	0.0516	0.780
	Female	11	0.213	0.175	0.194	0.101	0.780
	Male	10	0.109	0.106	0.0386	0.0516	0.195
K	*Nestling*	34	4216	4030	1084	2130	6799
	1 year	11	471	430	161	264	660
	≥ 2 years	9	475	390	184	258	809
	Female	12	474	410	183	252	809
	Male	10	453	447	152	264	654

Table 6.3: Mean, median, standard deviation (SD), minimum (min) and maximum (max) concentrations for analysed elements reported for nestlings, 1 year old birds, 2 years or older birds, females and males, based on "n" samples. All concentrations are reported in µg/g dw.

Element		**n**	**Mean**	**Median**	**SD**	**Min**	**Max**
La	*Nestling*	28	0.0464	0.0343	0.0482	0.00977	0.243
	1 year	11	0.581	0.547	0.214	0.263	0.963
	≥ 2 years	9	0.671	0.630	0.254	0.440	1.18
	Female	12	0.640	0.642	0.282	0.127	1.18
	Male	10	0.502	0.457	0.229	0.135	0.920
Mg	*Nestling*	34	2065	1894	632	1261	4339
	1 year	11	1168	1034	386	767	2025
	≥ 2 years	9	1060	1065	390	463	1835
	Female	12	1186	1079	342	767	2025
	Male	10	1014	942	389	463	1835
Mn	*Nestling*	34	2.44	1.24	4.12	0.536	21.3
	1 year	11	14.2	11.7	9.60	6.16	39.3
	≥ 2 years	9	18.8	14.1	11.1	8.86	39.0
	Female	12	14.5	11.9	9.13	5.47	39.3
	Male	10	18.6	16.0	11.9	6.16	39.0
Na	*Nestling*	34	2189	1950	920	1083	5254
	1 year	11	392	391	114	204	535
	≥ 2 years	9	626	476	549	181	2006
	Female	12	512	398	486	181	2006
	Male	10	468	444	177	221	860
Nd	*Nestling*	30	0.0479	0.0359	0.0534	0.00892	0.288
	1 year	11	0.570	0.525	0.206	0.265	0.929
	≥ 2 years	9	0.684	0.654	0.261	0.445	1.25
	Female	12	0.643	0.651	0.288	0.145	1.25
	Male	10	0.500	0.483	0.216	0.146	0.862
Ni	*Nestling*	23	0.190	0.155	0.128	0.0531	0.489
	1 year	11	0.974	0.866	0.314	0.456	1.69
	≥ 2 years	9	1.09	0.876	0.477	0.377	1.78
	Female	12	0.949	0.941	0.477	0.254	1.780
	Male	10	0.966	0.835	0.391	0.304	1.578
P	*Nestling*	34	3823	3716	1028	2081	6161
	1 year	11	196	183	44,6	140	267
	≥ 2 years	9	186	169	38,4	155	261
	Female	12	189	171	47,6	139	267
	Male	10	189	180	33,2	151	255
Pb	*Nestling*	25	0.153	0.124	0.132	0.0164	0.592
	1 year	11	2.08	1.44	1.54	0.826	6.22
	≥ 2 years	9	2.20	1.94	1.29	0.964	4.38
	Female	12	2.23	1.89	1.41	0.964	6.22
	Male	10	2.03	1.62	1.29	0.826	4.38

Table 6.4: Mean, median, standard deviation (SD), minimum (min) and maximum (max) concentrations for analysed elements reported for nestlings, 1 year old birds, 2 years or older birds, females and males, based on "n" samples. All concentrations are reported in µg/g dw.

Element		**n**	**Mean**	**Median**	**SD**	**Min**	**Max**
Pr	*Nestling*	32	0.0110	0.00775	0.0134	0.00240	0.0729
	1 year	11	0.139	0.127	0.0518	0.0654	0.228
	≥ 2 years	9	0.167	0.169	0.0638	0.110	0.302
	Female	12	0.157	0.159	0.0701	0.0338	0.302
	Male	10	0.122	0.112	0.0556	0.0327	0.212
Rb	*Nestling*	34	1.77	1.55	1.17	0.752	7.58
	1 year	11	1.32	1.36	0.438	0.676	2.06
	≥ 2 years	9	1.40	1.42	0.599	0.544	2.50
	Female	12	1.37	1.40	0.612	0.469	2.50
	Male	10	1.18	1.10	0.444	0.585	1.94
S	*Nestling*	34	26588	22815	10489	17903	56812
	1 year	11	33870	33967	5923	25373	43530
	≥ 2 years	9	31277	27963	6826	24218	44213
	Female	12	32008	31821	6108	24681	43387
	Male	10	32765	32585	6708	24218	44213
Sb	*Nestling*	28	0.0352	0.0240	0.0282	0.00872	0.125
	1 year	11	0.0994	0.0717	0.0730	0.0441	0.292
	≥ 2 years	9	0.0536	0.0511	0.0205	0.0304	0.0911
	Female	12	0.0775	0.0576	0.0716	0.0293	0.292
	Male	10	0.0701	0.0680	0.0411	0.0252	0.172
Se	*Nestling*	34	3.46	3.08	1.46	1.60	8.65
	1 year	11	3.02	2.67	1.28	1.16	5.20
	≥ 2 years	9	2.17	1.78	1.17	0.964	4.56
	Female	12	2.46	2.07	1.26	1.16	5.20
	Male	10	2.84	2.89	1.21	0.964	4.56
Si	*Nestling*	23	214	154	218	54,7	845
	1 year	11	1728	1719	719	956	3435
	≥ 2 years	9	1976	1944	913	584	3717
	Female	12	1839	1792	1006	393	3717
	Male	10	1545	1432	706	332	2728
Sm	*Nestling*	28	0.00970	0.00699	0.0104	0.00265	0.0569
	1 year	11	0.102	0.100	0.0377	0.0458	0.177
	≥ 2 years	9	0.118	0.107	0.0471	0.0758	0.216
	Female	12	0.113	0.114	0.0513	0.0287	0.216
	Male	10	0.0892	0.0817	0.0375	0.0319	0.151
Sn	*Nestling*	29	0.0594	0.0515	0.0307	0.0209	0.119
	1 year	11	0.797	0.687	0.815	0.145	3.00
	≥ 2 years	9	0.246	0.143	0.267	0.0830	0.916
	Female	12	0.357	0.191	0.295	0.0644	0.872
	Male	10	0.680	0.177	0.921	0.0474	3.00

Table 6.5: Mean, median, standard deviation (SD), minimum (min) and maximum (max) concentrations for analysed elements reported for nestlings, 1 year old birds, 2 years or older birds, females and males, based on "n" samples. All concentrations are reported in µg/g dw.

Element		**n**	**Mean**	**Median**	**SD**	**Min**	**Max**
Sr	*Nestling*	34	5.14	4.64	2.04	2.67	11.4
	1 year	11	6.62	6.79	1.99	3.84	9.77
	≥ 2 years	9	7.33	7.07	1.92	4.82	10.3
	Female	12	6.91	6.91	2.27	3.25	10.3
	Male	10	6.74	6.93	1.83	4.22	9.51
Tb	*Nestling*	12	0.00177	0.00106	0.00147	0.000560	0.00472
	1 year	11	0.0129	0.0127	0.00478	0.00771	0.0212
	≥ 2 years	9	0.0151	0.0151	0.00663	0.00638	0.0293
	Female	12	0.0143	0.0140	0.00703	0.00332	0.0293
	Male	10	0.0113	0.0106	0.00480	0.00355	0.0192
Tl	*Nestling*	34	0.0129	0.0114	0.00895	0.00423	0.0490
	1 year	11	0.0211	0.0198	0.00986	0.0115	0.0480
	≥ 2 years	9	0.0164	0.0139	0.00565	0.0103	0.0264
	Female	12	0.0192	0.0181	0.0105	0.00860	0.0480
	Male	10	0.0169	0.0161	0.00522	0.0103	0.0247
Ti	*Nestling*	34	6.06	4.41	6.78	1.33	34.1
	1 year	11	66.3	66.0	20.5	33.5	107
	≥ 2 years	9	72.6	69.0	23.8	46.8	116
	Female	12	71.1	74.9	25.0	27.6	116
	Male	10	59.4	60.3	20.8	33.5	103
U	*Nestling*	24	0.00385	0.00245	0.00360	0.00118	0.0134
	1 year	11	0.0274	0.0277	0.00919	0.0127	0.0395
	≥ 2 years	9	0.0310	0.0310	0.0135	0.0106	0.0546
	Female	12	0.0294	0.0314	0.0134	0.00666	0.0546
	Male	10	0.0241	0.0214	0.0113	0.00684	0.0425
V	*Nestling*	26	0.217	0.127	0.257	0.0353	1.12
	1 year	11	2.63	2.79	0.990	1.49	4.82
	≥ 2 years	9	3.03	3.01	1.39	0.862	5.67
	Female	12	2.81	2.85	1.47	0.616	5.67
	Male	10	2.36	2.24	1.08	0.515	4.13
W	*Nestling*	25	0.0221	0.0125	0.0467	0.00321	0.244
	1 year	11	0.0874	0.0869	0.0300	0.0320	0.131
	≥ 2 years	9	0.0768	0.0685	0.0395	0.0314	0.144
	Female	12	0.0832	0.0744	0.0404	0.0152	0.144
	Male	10	0.0692	0.0729	0.0342	0.0221	0.130
Y	*Nestling*	31	0.0249	0.0183	0.0226	0.00681	0.112
	1 year	11	0.278	0.291	0.0986	0.142	0.423
	≥ 2 years	9	0.335	0.341	0.160	0.111	0.683
	Female	12	0.317	0.310	0.160	0.0782	0.683
	Male	10	0.242	0.230	0.106	0.0698	0.426

Table 6.6: Mean, median, standard deviation (SD), minimum (min) and maximum (max) concentrations for analysed elements reported for nestlings, 1 year old birds, 2 years or older birds, females and males, based on "n" samples. All concentrations are reported in µg/g dw.

Element		**n**	**Mean**	**Median**	**SD**	**Min**	**Max**
Yb	*Nestling*	12	0.00354	0.00256	0.00303	0.000768	0.0109
	1 year	11	0.0234	0.0239	0.00815	0.0105	0.0353
	≥ 2 years	9	0.0264	0.0289	0.00962	0.00927	0.0431
	Female	12	0.0253	0.0285	0.0110	0.00606	0.0431
	Male	10	0.0202	0.0198	0.00831	0.00567	0.0319
Zn	*Nestling*	34	214	187	73,0	144	423
	1 year	11	159	148	37,2	107	239
	≥ 2 years	9	142	147	34,0	82,3	201
	Female	12	150	148	25,3	107	191
	Male	10	156	152	45,8	82,3	239

Appendix 7: *PCA scores*

Table 7: Resulting scores for each feather sample in the PCA.

Sample Nr.	PC1	PC2	Sample Nr.	PC1	PC2
4	-4.1566	-0.2206	**32**	-3.4484	-2.6825
5	-2.8893	-0.5256	**33**	-2.9382	3.4050
6	-5.5316	1.5178	**34**	-7.0969	-1.4124
7	-6.9279	-1.9079	**35**	-3.6011	0.4202
8	-4.3694	-1.7031	**36**	5.4406	-3.1820
9	-6.1391	-0.5295	**37**	-4.4280	-1.4783
10	-6.0676	-1.3317	**38**	-7.0256	-0.9084
11	-5.6081	-0.3327	**39**	6.2570	-1.5271
12	-3.1381	4.7943	**40**	5.6121	-4.1252
13	-6.1685	-1.8617	**41**	5.4900	-1.6490
14	-5.7074	-0.9490	**42**	5.0101	-2.4710
15	-7.3672	0.0191	**43**	7.8184	2.1161
16	-2.1884	2.9127	**44**	6.1454	-1.2845
17	-4.7859	-0.3926	**45**	1.5037	-3.3879
18	-3.8964	-0.4669	**46**	8.0481	2.2368
19	-3.7514	0.0826	**47**	6.0091	-1.4481
20	1.6945	4.6505	**48**	8.5431	1.4839
21	2.6191	6.0445	**49**	5.8348	0.5655
22	-4.1398	-1.5685	**50**	6.2827	-0.0764
23	-2.3888	2.1028	**51**	8.4301	0.7128
24	-4.9432	-0.0573	**52**	7.7861	2.1166
25	0.6671	6.4031	**53**	6.9366	-1.0852
26	-3.7479	2.4824	**54**	7.0540	0.1305
27	-2.7932	0.3781	**55**	7.0668	-3.9730
28	-3.1211	0.6383	**56**	7.5300	0.9750
29	-5.0211	0.7684	**57**	4.3047	-2.7333
30	-2.7363	-1.3428	**58**	1.5037	-3.3879
31	-5.9732	0.7011	**59**	2.1499	-1.1634

Appendix 8: *PCA loadings*

Table 8: Resulting loadings for the elements in feather samples.

Element	PC1	PC2	Element	PC1	PC2
Al	0.1803	-0.0012	**Ni**	0.1761	0.0346
As	0.1618	-0.0097	**P**	-0.1609	0.1754
Au	0.1506	0.0639	**Pb**	0.1647	-0.0642
Ba	0.1751	0.0502	**Pr**	0.1811	-0.0080
Bi	0.0853	0.1950	**Rb**	-0.0123	0.2968
Ca	0.1006	0.3137	**S**	0.1079	0.3101
Cd	0.1675	-0.0277	**Sb**	0.1200	0.1429
Ce	0.1792	-0.0167	**Se**	-0.0283	0.3420
Co	0.0661	-0.0389	**Si**	0.1787	0.0143
Cr	0.1768	0.0313	**Sm**	0.1806	0.0129
Cs	0.1762	-0.0392	**Sn**	0.1448	0.0165
Cu	0.1644	0.1102	**Sr**	0.0998	0.1875
Er	0.1808	-0.0016	**Tb**	0.1790	0.0043
Fe	0.1819	0.0084	**Tl**	0.1016	0.2101
Ga	0.1810	0.0151	**Ti**	0.1805	0.0034
Hg	-0.0912	0.1916	**U**	0.1772	0.0199
K	-0.1539	0.1934	**V**	0.1785	0.0059
La	0.1809	-0.0130	**W**	0.1560	0.0440
Mg	-0.1013	0.3358	**Yb**	0.1762	0.0236
Mn	0.1572	-0.0313	**Y**	0.1808	-0.0028
Na	-0.1327	0.2437	**Zn**	-0.0531	0.3889
Nd	0.1802	-0.0044			

Appendix 9: *Kruskal-Wallis test*

Table 9: Results from Kruskal-Wallis test performed on PC1 and PC2.

	X^2	p-value
Score 1/age	37.107	8.754E-09
Score 1/sex	0.90188	0.3423
Score 2/age	2.7868	0.2482
Score 2/sex	0.1746	0.6761

Table 10: Results from Kruskal-Wallis test performed on a selection of metals. Metals marked with an asterisk are significant.

	X^2	p-value	
Al	*35.63*	*1.83E-08*	*
As	9.29	9.59E-03	
Ba	*33.20*	*6.18E-08*	*
Cd	*13.38*	*1.24E-03*	*
Co	0.11	9.46E-01	
Cu	*35.04*	*2.46E-08*	*
Hg	*24.54*	*4.69E-06*	*
Mn	*31.21*	*1.65E-07*	*
Na	*33.80*	*4.58E-08*	*
Ni	*29.77*	*3.43E-07*	*
Pb	*32.64*	*8.16E-08*	*
Sb	*16.80*	*2.25E-04*	*
Se	7.68	2.15E-02	
Sn	*32.94*	*7.05E-08*	*
Sr	11.01	4.06E-03	
Tl	*14.02*	*9.04E-04*	*
V	*33.07*	*6.59E-08*	*
Zn	*15.10*	*5.27E-04*	*

Appendix 10: *Dunn's test*

Table 11: Post hoc test, Dunn's test. Performed on significant results from Kruskal-Wallis test.

	Comparison	Pairwise z-test	Raw p-value
Al	Nestling/≥ 2 years	4.711980	0.0000
	Nestling/1 year	4.698468	0.0000
	1 year/≥ 2 years	-0.300832	0.3818
Ba	Nestling/≥ 2 years	4.568308	0.0000
	Nestling/1 year	4.453836	0.0000
	1 year/≥ 2 years	-0.372838	0.3546
Cd	Nestling/≥ 2 years	3.321942	0.0004
	Nestling/1 year	3.267573	0.0005
	1 year/≥ 2 years	-0.205713	0.4185
Cu	Nestling/≥ 2 years	4.061827	0.0000
	Nestling/1 year	5.113336	0.0000
	1 year/≥ 2 years	0.558542	0.2882
Hg	Nestling/≥ 2 years	-3.653548	0.0001
	Nestling/1 year	-4.091820	0.0000
	1 year/≥ 2 years	-0.226110	0.4106
Mn	Nestling/≥ 2 years	-4.48075	0.0000
	Nestling/1 year	-4.266667	0.0000
	1 year/≥ 2 years	-0.444263	0.3284
Na	Nestling/≥ 2 years	-3.975381	0.0000
	Nestling/1 year	-5.031021	0.0000
	1 year//≥ 2 years	-0.567113	0.2853
Ni	Nestling/≥ 2 years	4.335824	0.0000
	Nestling/1 year	4.465984	0.0000
	1 year/≥ 2 years	-0.150340	0.4402
Pb	Nestling/≥ 2 years	4.526662	0.0000
	Nestling/1 year	4.629597	0.0000
	1 year/≥ 2 years	-0.188219	0.4254
Sb	Nestling/≥ 2 years	1.878164	0.0302
	Nestling/1 year	4.007456	0.0000
	1 year/≥ 2 years	1.571527	0.0580
Sn	Nestling/≥ 2 years	3.430783	0.0003
	Nestling/1 year	5.323865	0.0000
	1 year/≥ 2 years	1.281836	0.1000
Tl	Nestling/≥ 2 years	2.029251	0.0212
	Nestling/1 year	3.523871	0.0002
	1 year/≥ 2 years	1.027090	0.1522
V	Nestling/≥ 2 years	4.647952	0.0000
	Nestling/1 year	4.539498	0.0000
	1 year/≥ 2 years	-0.366669	0.3569
Zn	Nestling/≥ 2 years	-3.299888	0.0005
	Nestling/1 year	-2.744327	0.0030
	1 year/≥ 2 years	0.634253	0.2630