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Svalbard reindeer as an indicator of ecosystem changes in the Arctic terrestrial ecosystem

Aneta Dorota Pacyna[1], Katarzyna Koziorowska[2], Stanisław Chmiel[3], Jan Mazerski[4], Żaneta Polkowska[1*]

[1]Gdansk University of Technology, Faculty of Chemistry, Department of Analytical Chemistry, 11/12 Narutowicza st, Gdansk 80-233, Poland

[2]Institute of Oceanology Polish Academy of Sciences, ul. Powstańców Warszawy 55, Sopot, Poland

[3]Department of Hydrology, Faculty of Earth Sciences and Spatial Management, Maria Curie-Skłodowska University, Kraśnicka Ave. 2 cd, 20-718 Lublin, Poland

[4]Gdańsk University of Technology, Faculty of Chemistry, Department of Pharmaceutical Technology and Biochemistry, 11/12 Narutowicza st, Gdańsk 80-233, Poland

*corresponding author zanpolko@pg.edu.pl

Abstract

Over the years, noticeable effort has been directed towards contaminant determination in multiple biotic samples collected from the inhabitants of the Arctic. Little consideration has been given to polar herbivores, however, especially those from the European parts of the Arctic. To provide a broader perspective, we aimed to decipher trace element concentration in hairs of the key species in the Arctic, namely the Svalbard reindeer (*Rangifer tarandus platyrhynchus*), and to recognise whether diet variations could correspond with forward exposure. The effect of habitat and diet was investigated using the ratios of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), and previous literature studies on vegetation from the areas of interest. Analysis was performed for eighteen elements in total, both toxic and essential. Metals were present in a decreasing order Fe>Zn>Ba>Cu>Pb>Cr>Ni>V>Ga =La>Rb>As>Li>Co>Hg>Cd>Cs>Be. Similarity in trends in the studied subpopulations was observed for many metals. A significant log-linear correlation was observed for most of the elements, excluding nitrogen and carbon isotopes signature. Extremely high iron levels were determined in some of the samples, suggesting past iron overload. Zinc, in contrast to the remaining metals, did not correlate well with any other element. Mercury was determined at very low levels, in accordance with previous literature regarding its concentrations in moss and lichen species in Svalbard. The analysis of stable isotopes showed a high variation in nitrogen isotopes signatures. Further research is required to properly evaluate the potential health risks and ecological implications of elevated exposure.

Capsule: *Keratinised tissues can be a valuable source of information in ecotoxicological studies in the case of polar herbivores.*

Key Words: *Rangifer tarandus platyrhynchus,* hair, essential elements, toxic metals, stable isotopes, tundra

1. Introduction

Constant pollutant emission is undeniably a serious problem and it is considered a huge threat to ecosystem stability. Anthropogenic activities undoubtedly have significant ecological consequences worldwide. The Arctic is an invaluable source of information on the global-scale impact due to long-range contaminant transport (Davis, 1996; Halbach et al. 2017). The accumulation of trace elements, particularly heavy metals, and the resulting enrichment in higher trophic levels, raise questions about its impact on native fauna. Due to its unique geographical location, the Svalbard Archipelago has become a significant recipient of pollutants emitted in the Northern Hemisphere. Natural sources of heavy metal emissions include volcanic activities, biogenic sources, soil-derived dusts, and sea salt aerosols. It is anthropogenic emissions, however, that are assumed to account for the observed heavy metal levels in the Arctic to the greatest degree (AMAP, 2005; Halbach et al. 2017). With only several local sources of pollution (such as mining activities, airport, ship traffic), most contaminants including heavy metals are atmospherically transported long-range from mid- and low-latitudes (Bard, 1999).

A growing amount of evidence arose in the recent years concerning the deposition of pollutants in polar, particularly marine biota (e.g. Burger et al. 2007). Physiological and ecological factors affecting the bioaccumulation process vary between terrestrial and aquatic ecosystems (van den Brink et al. 2015). Terrestrial species are often weakly investigated and yet crucial parts of any polar ecosystem. Reindeers are a key component of the Arctic terrestrial ecosystem (Duffy et al. 2005). Because they are a part of a simple food chain, the species is ideal for monitoring changes in the terrestrial trophic network (Elkin and Bethke, 1995).

In this paper, we investigate the usefulness of molten fur collected from a broadly distributed resident of the European part of the Arctic, namely - the smallest reindeer subspecies (*Rangifer tarandus platyrhynchus*). This large herbivore, endemic to Svalbard, can be found in the majority of non-glaciated areas of the island. The Svalbard reindeer has certain adaptations to the polar environment, including relatively short legs and thick fur with colouring and thickness varying between the seasons (Cuyler and Øritsland, 2002). Its total population size is estimated for 10,000 animals (npolar.no). Monitoring studies conducted in Brøggerhalvøya, Reindalen, Adventdalen, and Edgeøya suggest high annual fluctuations (mosj.no; Reimers, 2012) primarily caused by variations in climate condition (such as snow depth and rain-on-snow events), and partially by competition for food resources.

The primary function of the fur of the Svalbard reindeer is body insulation from cold and wind (Cuyler and Øritsland, 2002). In cervids, the coat is replaced annually. New fur developes from late spring/early summer to late fall. The trace element composition of fully grown hairs largely reflects summer and fall deposition (Drucker et al. 2010). Reindeer hairs develop a hollow, air-filled, stiff, close-packed structure with a primary heat transfer function. It also undergoes seasonal changes. Summer and winter fur of adults and calves is characterised by different properties such as hair length, density, and colour (Cuyler and Øritsland, 2002).

The Svalbard reindeer is the only large grazing mammal in the European High Arctic (Hayashi et al. 2014). It is exposed to contaminants particularly through its diet, composed of different types of vegetation, including lichen and moss (Robillard et al. 2002). Terrestrial plants receive metals sprayed from seawater (if they grow within the distance of sea spray influence), by dry and wet deposition, and from melting glaciers as trapped particles are released from ice (Xie et al. 2006; Samecka-Cymerman et al. 2011). Birds can also be an additional vector for contaminant transport (Savinov et al. 2003), as well as reindeer guano (van der Val et al. 2004). The Svalbard subpopulation eats almost all types of vegetation available. During the growing season, selection for plant quantity rather than quality is observed (Van der Wal et al. 2000).

Plants show variable stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) depending on their physiology and environmental conditions, e.g. temperature, light intensity, air humidity, or precipitation (Drucker et al. 2010). Stable isotopes are incorporated into growing hair from diet, and can be used to assess spatial and temporal variation in diet components, to characterise the trophic niche (Boecklen et al. 2011), unravel the migration path (Hobson and Wassenaar, 2008), or determine habitat selection (Newsome et al. 2009). The ecology of the animal can be therefore investigated based on stable isotope analysis, as their abundance in tissues reflects that in the diet (Drucker et al. 2010).

The available data on exposure assessment in polar herbivores is still limited, particularly to the Alaskan and Canadian populations. Also studies concerning stable isotope analysis in reindeer tissues are scarce. To fill this gap in knowledge, the present study focused on the investigation on 18 trace elements (Fe, Zn, Ba, Cu, Pb, Cr, Ni, V, Ga, La, Rb, As, Li, Co, Hg, Cd, Cs, Be), and nitrogen and carbon stable isotopic composition in hairs collected in the summer season from reindeer herds. The Svalbard reindeer is a sedentary species, migrating only in the case of significantly reduced food resources (Hansen et al. 2010b). It is therefore vulnerable to any changes in local foraging conditions. Hairs can be used as a long-range record of contaminants deposition as they accumulate elements continuously by bounding them to sulphur-rich hair proteins during the hair growth period (Duffy et al. 2005).

The primary objective of this paper is to provide new background data on the levels of metals in reindeer fur, and a comparison between two subpopulations living in distant areas in order to establish the pollution level and determine variations in nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotope composition.

2. Materials and Methods

2.1 Study area and sampling

Fur samples were collected in two consecutive summer seasons: in August 2015 from Longyearbyen region (N78° E015°, n=11) and in September 2016 from the Fuglebekken catchment in the vicinity of the Polish Polar Station in Hornsund (N77° E015°, n=16) (Fig.1). Samples were collected from the ground, after a herd moved to a new place. To avoid pseudoreplication, only freshly molten fur was collected (one sample per at least 4 m^2 distance). We assumed that samples were from separate individuals. All samples were individually packed in clean zip bags, and stored at a temperature of 4°C prior to analysis. Long, straight, white on entire length (except darker tip) guard hairs were collected. Mean temperature during the period of sample collection amounted to 2.9°C in August 2015 (Longyearbyen) and 3.9°C in September 2016 (Hornsund) (yr.no). Sample weight varied from 16 to 80 mg for samples collected from Longyearbyen, and from 9 to 100 mg for samples collected from the Hornsund area.

Fig. 1 Study area with main coordinates, A – Longyearbyen area, B – Hornsund area [map source: toposvalbard.npolar.no]; Svalbard reindeer (*Rangifer tarandus platyrhynchus)*

The Svalbard reindeer, unlike other reindeer subspecies, is highly stationary. It is reluctant to migrate beyond its territory range mostly established by natural barriers (thin sea ice, glaciers, steep mountains) (Hansen et al. 2010b). Genetic differences between populations might occur even at distances <50 km^2 (Côté et al. 2002). Therefore, the studied herds are most likely from completely separate populations. Predation is almost non-existing, with the exception of local hunting and occasional evidence of polar bear hunting attempts (Hansen et al. 2011).

2.2 Analytical methods

18 trace elements and nitrogen and carbon stable isotopes composition were analysed. The basic course of the analytical procedure involves removal of external contamination and then elemental analysis preceded by acid mineralization in microwave emitter (trace elements except for mercury), thermal vaporization (mercury) and high temperature oxidation ($\delta^{13}C$ and $\delta^{15}N$).

2.2.1. Trace elements (except for mercury)

First, each hair strand was separated manually from the collected sample with clean tweezers to separate from any parts of moss collected with the fur ball. To remove the adherent external contamination such as dust and loosely bound particulate matter, each pooled sample from one individual was cleaned by vigorous shaking at least 2 times in double deionised water for 15 min in an automatic shaker, and then air-dried for 24 hours. Only white hairs were used, and all visible dust particles were washed out. Next, dry hairs were homogenised by cutting into small parts, weighed to the nearest 0.1 mg, and placed in a clean teflon vessel with 65% HNO_3 (Merck, 99% purity). Digestion was carried out using a high-pressure microwave emitter (Microwave Digestion System, Anton Paar). The temperature was increased from room temperature to 90°C (app. 6-8°C/min). Such conditions were maintained for 25 min. After that, temperature was gradually cooled down. Subsequently mineralised samples were diluted with deionised water into 25 ml in clean plastic flasks. To ensure quality control, blank samples were run with every batch. The metals were determined by means of a quadrupole spectrometer ICP-MS Xseries2 by Thermo with inductively-coupled plasma. For the purpose of reduction of isobaric and polyatomic interferences, a collision/reaction cell was used with the application of a mix of helium and hydrogen gases, and the kinetic energy discrimination function (KED).

The accuracy of the analyses was verified by means of certified material Standard Reference Material NIST 1643e Trace Elements in Water and Analytical Reference Material EnviroMAT ES-H-2 CRM SCP SCIENCE. The retrieval of the elements water ranged from 87% to 109%.

The determination was performed at the Department of Hydrology, Faculty of Earth Sciences and Spatial Management, Marie Curie-Skłodowska University in Lublin.

Tab.1 Detail information about analytical instrumentation (Supplementary Material)

2.2.2. Mercury analysis

External contamination was washed out using the same procedure as for other trace elements. The pooled dry sample was cut into smaller pieces using sterilised stainless scissors, weighed (to the nearest 0.01 mg), and analysed by the thermal vaporisation atomic absorption method (MA-3000 Nippon Instruments Corporation). The samples were heat decomposed in a ceramic boat, first heated to 180°C for 120 s, and then to 850°C also for 120 s. The mercury collector collects the atomised mercury gas in a form of gold amalgam, condensing and purifying the mercury. After heat decomposition, the mercury collection tube was heated to 650°C to liberate the mercury gas. Absorbance at a wavelength of 253.7 nm was then measured. Oxygen flow amounted to 0.4 L/min. Total mercury concentration was determined in triplicates, and based on them the variation coefficient was calculated. Quality control included blank samples every 5-6 subsamples run. The median of the coefficient of variation between replicates was equal to 10.0 (7.91-13.95) in samples collected from Longyearbyen, and 3.65 (1.64-8.98) in samples from Hornsund. Reference materials MODAS-4 Cormorant Tissue (M-3 CornTis), MODAS-3 Herring Tissue (M-3 HerTis), MODAS-5 Cod Tissue (M-5 CodTis) were used to determine analytical accuracy, and to perform method and quality control. Recovery of reference materials measured on three replicates of each RM varied from 94 to 100%.

2.2.3. Stable isotopes

The analyses of carbon and nitrogen stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) were done in an Elemental Analyser Flash EA 1112 Series combined with an Isotopic Ratio Mass Spectrometer IRMS Delta V Advantage (Thermo Electron Corp., Germany). Details of these measurements are described by Kuliński et al. (2014). In short, the samples were dried, homogenised, and weighed into silver capsules (about 1 mg). This sample weight guarantees C and N loads significantly higher than those given by the limit of quantification (C = 20 μg, N = 20 μg). Next, samples were oxidised in 1020°C in presence of Cr_2O_3 and Co_3O_4. After catalytic oxidation, gases including CO_2, NO_x and H_2O, were transported to the second reactor, where NO_x was reduced to N_2 on the metallic Cu (650°C). Subsequently, the analysis products were dried with $Mg(ClO_4)_2$ and separated on GC (45°C). The separated gases (CO_2 and N_2) were transported to the IRMS. The isotopic composition of carbon and nitrogen was calculated using laboratory working pure reference gases (CO_2 and N_2) calibrated against IAEA standards: CO-8 and USGS40 for $\delta^{13}C$ and N-1 and USGS40 for $\delta^{15}N$. Results of $\delta^{13}C$ and $\delta^{15}N$ were given in the conventional delta notation, i.e., versus PDB for $\delta^{13}C$ and versus air for $\delta^{15}N$ as parts per thousand (‰) according to the following equation:

$$\delta X\ (‰) = [\frac{R_{sample}}{R_{standard}} - 1] \times 1000$$

where: X is the stable isotope ratio of $\delta^{13}C$ or $\delta^{15}N$; R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The measurement precision was better than 0.20‰ for $\delta^{13}C$ and 0.18‰ for $\delta^{15}N$ (n = 5).

2.3 Quality assurance/quality control (QA/QC)

To ensure high quality of results, the obtained data were subject to strict quality control procedures. All the analytical equipment was carefully washed before analysis. Based on duplicate and triplicate samples, the variance coefficient of metal concentration was calculated. If the coefficient >15%, samples were excluded from the analyses, assuming unreliable estimation of metal concentration. Background contamination was present in metal method blanks prepared after mineralisation, therefore blank correction was performed for all elements. Blank correction involved subtracting the total amount of analyte detected in the method blank from the total amount of analyte detected in the hair samples. Negative numbers and numbers below the limit of detection were reported as half of the limit of detection for statistical analysis. The obtained results were also corrected for sample weights and method dilution factor, and are reported as µg/g dw. All reagents were of the highest purity. Ultrapure water was produced from a Mili-Q Gradient A10 (Milipore, France).
ICP-MS equipment calibration employed the multi-element standard by Inorganic Ventures ANALITYK - CCS-1, CCS-4, CCS-6. The optimised and validated methods showed good linearity (R2>0.999) over a wide range with low limits of detection. Both the method limit of detection (LOD) and the limit of quantitation (LOQ) were calculated based on the standard deviation of the response (s), and the slope of the calibration curve (b) according to the following formulas: LOD = 3.3(s/b), LOQ = 10(s/b) (LOD/LOQ - Li, Fe, V, Cr, Ni , As, Rb, Ba, Pb 0.1/0.3 ppb; Be, Co, Ga, Cs, Cd, La 0.01/0.03 ppb; Cu, Zn 0.5/1.5 ppb). For mercury the method limit of detection and quantification was equal to 0.54 and 1.62 ppb, respectively.

Due to the fact that metals are bound to the keratin structure with variable affinity, removal efficiencies differ significantly among compounds when stronger solvents such as acetone are used. Therefore, only double deionised water was used as a washing agent. Some part of surface contamination might not have been removed. Because it is difficult to distinguish between internal and external exposure, it can be assumed that hairs provide information of integral exposure.

2.4 Statistical methods

Data were log-transformed to meet the assumptions of normality, and consequently parametric tests were performed. A T–difference test of means was performed for trace metals and stable isotopes. A Pearson's correlation test was performed to investigate the relationships between metals and continuous explanatory variables (hair $\delta^{13}C$ and $\delta^{15}N$ values). High correlation values between the primary values of the metals in the analysed samples justify the principal component analysis. Two main components have been designated for interpretation, accounting for 81.79% of the cases. However, the analysis provides no meaningful information for the interpretation of data analysis. Therefore, data clustering was performed to provide an insight into the data structure. Clustering was done by the nearest neighbour's method, adopting tangent distance as a measure of distance.

3. Results

Median, mean, and standard error, log transformed mean, and t-difference test of means are presented in Table 2. For compiled samples, correlation coefficients are mostly high, many are close to one (Table 4). The correlation of variables with regard to the sampling site was also tested. In the majority of cases, stronger correlations between metals were observed in samples from the Longyearbyen area, compared to the Hornsund samples. For zinc, correlations with other metals were notably lower (the highest occurs with gallium content: R^2_{tot} = 0,54). Those coefficients were used to measure similarity of variables by data clustering (Fig.2). As a result, two groups were obtained: zinc as an isolated element, and other elements forming a single cluster. After further division, we obtained a five-elemental cluster (containing V, Fe, Li, Cs, and La), a three-elemental cluster (As, Ga and Ba), and the remaining elements as isolated items. High variation was observed for nitrogen isotope composition. T–difference test of means ($p<0.05$) for nitrogen isotopes ($\delta^{15}N$) was equal to -5.16, and for carbon ($\delta^{13}C$) to -3.12. Three individuals from the Longyearbyen area showed elevated contents of all the measured elements, with extremely high levels of iron, chromium, nickel, and lead. The average value of nitrogen isotope $\delta^{15}N$ for those outliers was equal to 6.95 [‰]. Outliers were not excluded from statistical analysis.

Table 2. Trace element concentration in reindeer fur samples collected from two separate populations (µg/g dw)

Table 3. Nitrogen and carbon stable isotopes concentration in Svalbard reindeer hairs

Table 4. Pearson correlation values indicating correlation between the various trace elements measured (n=26)

Fig. 2. Hierarchical dendrogram for clustering chemical elements. Lines indicate distance 0.27 and 0.5, respectively. From the left: 1-V, 2-Fe, 3-Li, 4-Cs, 5-La, 6,5-Rb, 8-As, 9-Ga, 10-Ba, 11,5-Be, 13-Ni, 14,5-Cr, 16-Co, 17,5-Pb, 19-Cu, 20,5-Cd, 22,5-Zn

4. Discussion

This study reports the levels of essential and toxic elements and stable isotope composition in Svalbard reindeer hair samples collected from herds living in distant parts of the island. Keratinised tissues such as hairs, fur, or feathers can be collected non-lethally, and have been successfully used for stable isotopes and heavy metal analysis for many years (Duffy et al. 2005; Burger et al. 2007; Sergiel et al. 2017). Hair tissue has several advantages in practical use. Owing to its stability, samples can be stored for a long time, they are relatively metabolically inactive (Duffy et al. 2005), and elements are accumulated over extended periods of time. Therefore, the exposure assessment covers several weeks or months. Molten hairs can be collected without direct contact, avoiding difficulties related to capturing a free-living individual. However, because factors such as specimen age and gender are often unknown, this mode of sampling also limits the possibility of result interpretation.

Svalbard reindeers consume various plants, including vascular plants, bryophytes, and lichens, all determined to accumulate high levels of essential and heavy elements (Jóźwik, 1990, Samecka-Cymerman et al. 2011, Garty, 2001). Their levels found in polar plant species can be elevated due to natural processes (such as volcano eruptions, rock weathering) or atmospheric deposition, mainly from long distance transboundary transport from lower latitudes (Grodzińska and Grodzik, 1991). Sea aerosol can be an additional source of elements such as lead, mercury, and cesium (Kłos et al. 2017).

Spatial and temporal heterogeneity in diet components might be responsible for significant seasonal differences in contaminant distribution across studies (Robillard et al. 2002). In our study, the majority of elements showed a strong positive correlation with multi-element totals, excluding zinc. High variability in trace element composition was observed even above an order of magnitude within samples of reindeer from one location. This is probably related to differences in age (herds were composed from both young and older individuals), gender, and food preference. Due to lack of previous studies regarding trace elements in reindeer hairs, our data can be used as a reference for future investigations in the Svalbard Archipelago concerning reindeer and closely related species.

4.1 Accumulation route

Vegetation covers only 6-7% of the area of Svalbard. The growing season lasts approximately 90 days (Kłos et al. 2015). Because of the short grazing season, the Svalbard reindeer must restore its body reserves after winter, and accumulate fat at this time (Staaland, 1984). The plant species-specific physiology, age, and sampling location will correspond with forward exposure. Lower trace element levels are observed in vascular plants as compared to mosses and lichens (Wojtuń et al. 2013). This may be related to their higher morphological similarity, and more selective accumulation process (Chiarenzelli et al. 2001). Due to the lack of root system, slow growth rate, longevity, vast surface area, and lack of well-developed cuticle, plants such as lichens and bryophytes are prone to accumulating a varied cocktail of toxic compounds from the atmosphere (Robillard et al. 2002; Gamberg et al. 2005, Samecka-Cymerman et al. 2011). Essential elements such as copper and zinc, necessary for plant growth, can also be accumulated beyond physiological demands (Samecka-Cymerman et al. 2011, Jóźwik, 1990). For instance, for zinc, enhanced exposure in lichens is above 500 µg/g, cadmium can be tolerated between 1 and 30 µg/g, and copper between 1 and 50 µg/g (Nieboer et al. 1978).

The accumulation route can be passive by water transpiration passage (e.g. Cu in lichens), active (e.g. zinc), and metabolic (e.g. manganese), or a mix of those factors (Jóźwik 1990). Mosses are evidenced to accumulate notably high levels of Cd, Co, Cr, Cu, Fe, Mn, and Zn, even higher than lichens (Wojtuń et al. 2013). Particularly moss species such as *Aulacomium palustre, A. turgidum, Hylocomium splendens, Sanionia uncinata*, and *Tortula ruralis* are suspected to be very good heavy metal accumulators in Svalbard (Grodzińska and Grodzik, 1991).

4.2 Toxic elements

Mercury is a global pollutant that enters the Arctic terrestrial ecosystem mainly through rock weathering and long-range atmospheric deposition (Gamberg et al. 2015). During spring, atmospheric Hg(0) is oxidised into Hg(II), and deposited in the snow, ice, or ocean surfaces from where can be partly reemitted or further retained, transformed, and transported (Schroeder et al. 1998; Halbach et al. 2017). In addition to snow and ice, soil is believed to be a major land mercury reservoir in the Arctic (Gamberg et al. 2015). Our study shows low mercury contents in both studied subpopulations. Elevated mercury level is indeed usually found in marine biota, in contrast to terrestrial mammals, especially herbivores with a short food chain.

To the best of our knowledge, no studies are available regarding contaminant deposition in the hair of the Svalbard reindeer subspecies. Duffy et al. (2005) conducted a study on mercury levels in the hair of the Alaskan reindeer population, indicating low exposure (mean total mercury for free ranging individuals was equal to 0.055 µg/g). Mercury was also a major research interest in Lokken et al. (2009) pilot study performed on lichen and the Alaskan caribou population (mean hair levels varied from 0.0146 to 0.0834 µg/g). In the present study, the highest level was found in the Longyearbyen population. It does not exceed 0.160 µg/g (median equal to 0.112 and 0.060 µg/g).

Mercury and cadmium previously showed a clear pattern of accumulation towards higher trophic levels in the terrestrial ecosystem (Dietz et al. 2000). Cadmium binds to the low molecular weight sulphur-rich proteins, and accumulates mostly in kidneys (Chan et al. 2001). It also may significantly increase with age (Danielsson and Frank, 2009). In our study, however, age differences were not analysed, and hair bounding capacities are different than in internal tissues. Literature studies on both areas showed low cadmium exposure in vegetation (Wojtuń et al. 2013; Samecka-Cymerman et al. 2011; Węgrzyn et al. 2013; Kłos et al. 2015), and as expected we found low levels in reindeer hair. To our best knowledge, no study has been published concerning cadmium accumulation in Svalbard mammal herbivores, therefore no comparison is possible.

On the other hand, high lead levels were found in the majority of samples, suggesting an accumulation path by vegetation. High levels of lead were also previously found in Greenland soils. However, it does not tend to accumulate towards higher trophic levels, as reindeers had lower lead levels than lichens (summarized in Dietz et al. 2000 based on Greenlandic studies of the AMAP programme). Notice that only reindeer internal tissues were used. In Svalbard area, levels of lead in vegetation is highly variable. Threshold values for lead in lichens are from 5 to 100 µg/g and 15 µg/g is a boundary for enhanced exposure (Nieboer and Richardson, 1981). In hairs, lead is accumulated both externally and internally over a long period of time, until molting. It is possible that apart from internal contamination accumulated by foraging on high-lead level food sources, part of external contamination was not washed out during the cleaning procedure.

Fig. 3a,b. Plot of average Cd, Pb and Hg and Fe, Zn, Ba and Cu concentration in Longyearbyen (dark colors) and Hornsund (light colors) reindeer hair samples. Values are log transformed. The horizontal lines represent medians, the boxes – upper and lower (25-75% quartiles) and whiskers – minimum and maximum values

4.3 Other elements

The studied samples showed particular patterns such as high intra-individual variations in the level of several compounds (iron, chromium, zinc etc.). All the analysed elements occur in broad concentration ranges. Relatively high levels of mean nickel in the Longyearbyen subpopulation, before also observed in the population of moss *Hylocomium splendens*, could be associated with past mining activities in the area (Kłos et al. 2015). The main source of nickel in Longyearbyen is most likely rock waste derived from mining activities and aviation emissions, although discharges transported long-range from the Kola Peninsula are also suspected (Kłos et al. 2017). Iron was significantly elevated in some of the samples from the Longyearbyen area, with the highest level at 14640 µg/g dw. Other two samples were also above 5000 µg/g dw of iron. The effect of spontaneous iron overload was previously described in liver tissues of Svalbard reindeer (Borch-Iohnsen and Nilssen, 1987; Borch-Iohnsen and Thorstensen, 2009). It was caused by high uptake of dietary iron consumed with iron-rich forage plants (Borch-Iohnsen and Thorstensen, 2009). In Svalbard reindeers, spontaneous seasonal iron overload with massive siderosis is considered natural, and occurs mostly in winter when available vegetation is of poorer quality (Borch-Iohnsen and Thorstensen, 2009). It is possible that when reindeers' nutritional conditions improved after winter (Borch-Iohnsen and Thorstensen, 2009), accessory iron was redistributed from the liver to hairs. If that is the case, hairs can be used to reveal past iron overload. All other elements were also significantly elevated in those individuals, suggesting some health implications (with examples presented in Table 5, Supplementary material.). Mercury was not analysed in those samples. Levels of iron in samples from the Hornsund area were lower, not exceeding 5000 µg/g. In two cases, more than 1100 µg/g of iron was detected.

Table 5. Outliers with significantly elevated element levels µg/g dw (Supplementary Material)

Because reindeer subspecies *Rangifer tarandus platyrhynchus* lives exclusively in the Svalbard Archipelago, the nominative species was expected to receive more attention. Studies on Canada and Greenland caribou and reindeer populations mostly concerned internal tissues (Elkin and Bethke, 1995, Robillard et al. 2002, Larter and Nagy, 2000, Aastrup et al. 2000). Medvedev (1995) reported cadmium and lead levels in the bone, teeth, and antlers of forest reindeer (*Ragnifer tarandus fennica*) from north-west Russia. The highest mean levels of cadmium and lead were found in the bone tissue (2.1±1.1 and 41.6±23.7 μg/g dw, respectively). The levels did not depend on sex or age of individuals. Heavy metal levels were also reported for North Norway population in samples collected from semi-domesticated reindeer. Cadmium, lead, arsenic, nickel, and vanadium were determined in the muscle, liver, tallow, and bone marrow tissues, with the highest level of all the elements in the liver (except nickel) (Ali Hassan et al. 2012). A reliable comparison between those studies is not possible, however, because the relationship between deposition of compounds in hairs and internal tissues is not always clear. Svalbard is an Arctic semi-desert compared to other places inhabited by reindeers, with low precipitation and humidity, cold winter temperatures, and high wind speed, resulting in different feeding behaviour and patch choice (Lindner, 2002). The Svalbard reindeer also differs from other reindeer subspecies in its anatomy and physiology (Lindner, 2002).

4.4 Stable isotopes of carbon and nitrogen

Stable isotopes (SI) of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) are increasingly employed as an indispensable tool in ecological studies (Sergiel et al. 2017). The main sources of nitrogen in the Arctic include atmospheric discharge of NO_x, NH_x, primary N2-fixation from the atmosphere, and bird guano (Skrzypek et al. 2015). In nitrogen-limited terrestrial ecosystems such as Arctic tundra, soil microbes are recognised to function as main nitrogen pools, competing for nitrogen with plants (Bardgett et al. 2007). Plant growth is limited by nitrogen availability. Consequently, the capacity for carbon sequestration is also restricted (Skrzypek et al. 2015). Arctic tundra contains a significant percent of the global soil carbon reserve. Its storage is controlled by factors such as e.g. temperature, vegetation type, soil hydrology, or shifts in vegetation state. The latter can be induced by herbivores (Van der Val, 2006; Speed et al. 2010).

Forage patch choice by reindeers and nitrogen content in plants are largely influenced by the timing of snowmelt (van der Wal et al. 2000). In Svalbard, seasonal variability of plant and soil nitrogen pools are mostly controlled by changes in temperature and soil moisture over the growing season. Such changes, however, are markedly lower than in the other seasonally cold ecosystems (Bardgett et al. 2007). Also Arctic tundra has a high capacity to retain nitrogen transported after extreme events, with non-vascular plants acting as a short-term sink, and vascular plants as a long-term reservoir (Choudhary et al. 2016). Our results indicate high variability in the ^{15}N:^{14}N ratio, suggesting that reindeers consume vegetation with different ^{15}N values. In the Fuglebekken catchment (Hornsund), high loads of nutrients are deposited by large bird colonies such as little auk (*Alle alle*). This influx impacts soil fertility and subsequently plant productivity and structure (Skrzypek et al. 2015). As a result, the available vegetation differs in protein, sugar composition, and digestibility (Staaland, 1984). Bird guano and additional N-sources from colonies, such as carcasses, dead chicks, and eggshells, constitute a huge N-load compared to other sources (Skrzypek et al. 2015). It could account for significant differences between the two subpopulations.

Moss tundra serves as an important sink for carbon sequestration (Nakatsubo et al. 2015). Here, relatively low variability was observed for stable carbon isotopes. No significant correlation was observed between C and N values and metal concentration, apart from zinc.

No previous studies are available concerning stable isotope analysis in the keratinised tissues of the Svalbard reindeer. Mosbacher (et al. 2016) showed high inter- and intra-annual seasonality in the diet of the Greenland muskox (*Ovibos moschatus*) by the application of sequential data on nitrogen stable isotopes derived from guard hairs. Drucker (et al. 2010) studied the dietary references and habitat use of moose (*Alces alces*) and caribou (*Rangifer tarandus*) in plucked hair samples from Canada populations. The dietary strategies of those species differ in spite of the same habitat range. Differences in stable isotope abundance were significantly linked to the species' dietary specialisation (Drucker et al. 2010).

Fig. 4. Plot of average nitrogen (blue) and carbon (green) stable isotope composition in Longyearbyen (L) and Hornsund (H) reindeer hair samples. The horizontal lines represent medians, the boxes – upper and lower (25-75% quartiles) and whiskers – minimum and maximum values excluding outliers

The long-term variation in weather conditions may impact vegetation quality, consequently affecting the ungulates' nutritional profile and foraging conditions. Lower snow layer hardening in winter leads to changes in snow-pack properties, including ground icing, resulting in snowpack with impenetrable vegetation underneath (Hansen et al. 2011, Loe et al. 2016). Food availability can also be restricted by overgrazing (Węgrzyn et al. 2016). Therefore, some populations are more likely to expand their foraging area, or alternatively use less preferred food sources such as goose droppings (van der Wal and Loonen, 1998) or marine algae (Hansen and Aanes, 2012). Because many factors are responsible for seasonal availability of various food sources, and Svalbard reindeers tend to forage for plant quantity rather than quality (Van der Wal et al. 2000), a complex study program concerning trace element levels in vegetation may help assess their future potential exposure.

5. Conclusion

The Svalbard reindeer is one of the least studied subspecies amongst family *Rangifer*. In this paper, we present to the best of our knowledge the first communication concerning trace element concentration in hairs of two separate subpopulations. Better knowledge of the potential impacts of metal on the terrestrial ecosystem is needed in polar mammal populations, especially to identify levels related to health dysfunction. In the present study, mercury is indicated as an insignificant thread in terrestrial ecosystem, although levels of lead, chromium, and nickel were noticeably elevated in some of the samples. Because hairs are a dead tissue accumulating elements over long period of time, reindeer may use it in a detoxification process for instance for depositing past iron overload.

Future climate changes will induce higher pressure on all terrestrial species. Rising temperatures, more frequent extreme weather events, heavy rain-on-snow events, and variations in seasonal precipitation patterns may cause negative implications for herbivores (Hansen and Aanes, 2012). In spite of their remarkable abilities to locate food beneath the snow-pack, severe icy conditions may induce changes in reindeer behaviour, including range expansion to mountainous terrain (Hansen et al. 2010), and eating marine algae (Hansen and Aanes, 2012) resulting in potential changes in the foraging profile and contaminant accumulation. The research presented so far provides evidence that keratinised tissues can be a valuable source of information in ecotoxicological studies. Monitoring studies should involve not only marine species, but concurrently more terrestrial key species as an important part of the trophic network.

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Fig. 1 Study area with main coordinates, A-Longyearbyen area, B- Hornsund area [map source: toposvalbard.npolar.no]; Svalbard reindeer (*Rangifer tarandus platyrhynchus)*

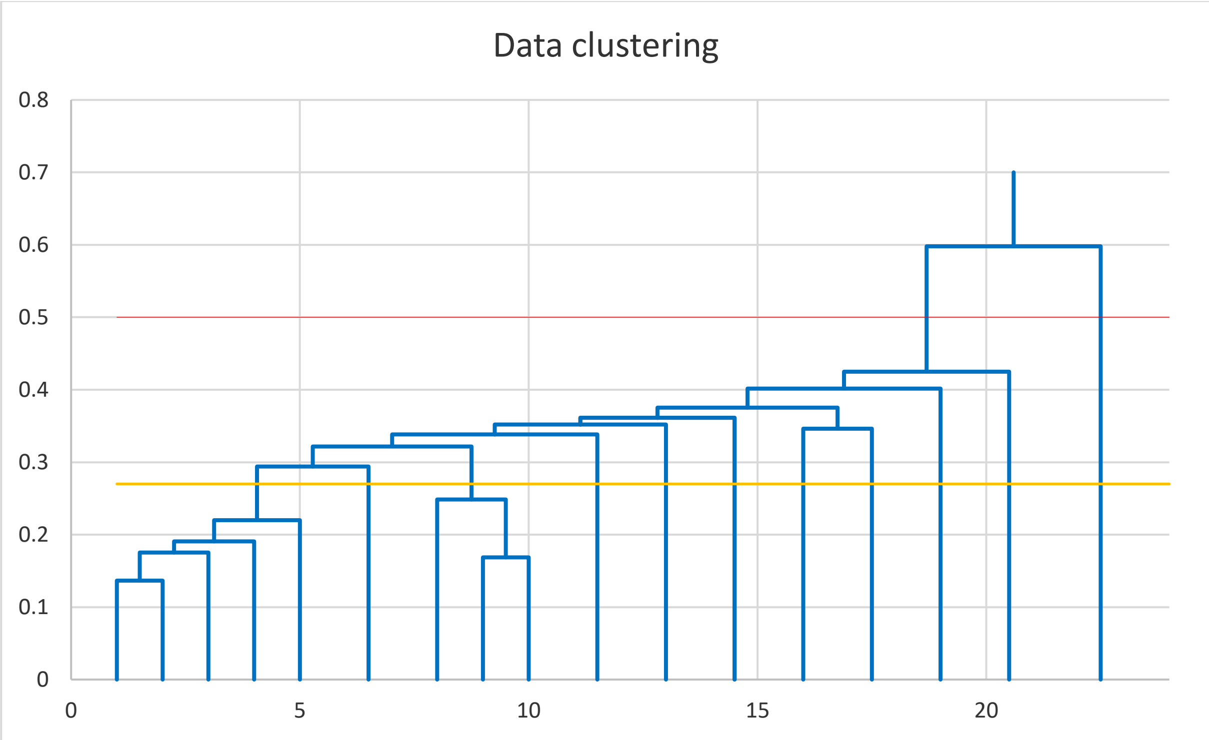

Fig. 2 Hierarchical dendrogram for clustering the chemical elements. Lines indicate distance 0.27 and 0.5, respectively. From the left: 1-V, 2-Fe, 3-Li, 4-Cs, 5-La, 6,5-Rb, 8-As, 9-Ga, 10-Ba, 11,5-Be, 13-Ni, 14,5-Cr, 16-Co, 17,5-Pb, 19-Cu, 20,5-Cd, 22,5-Zn

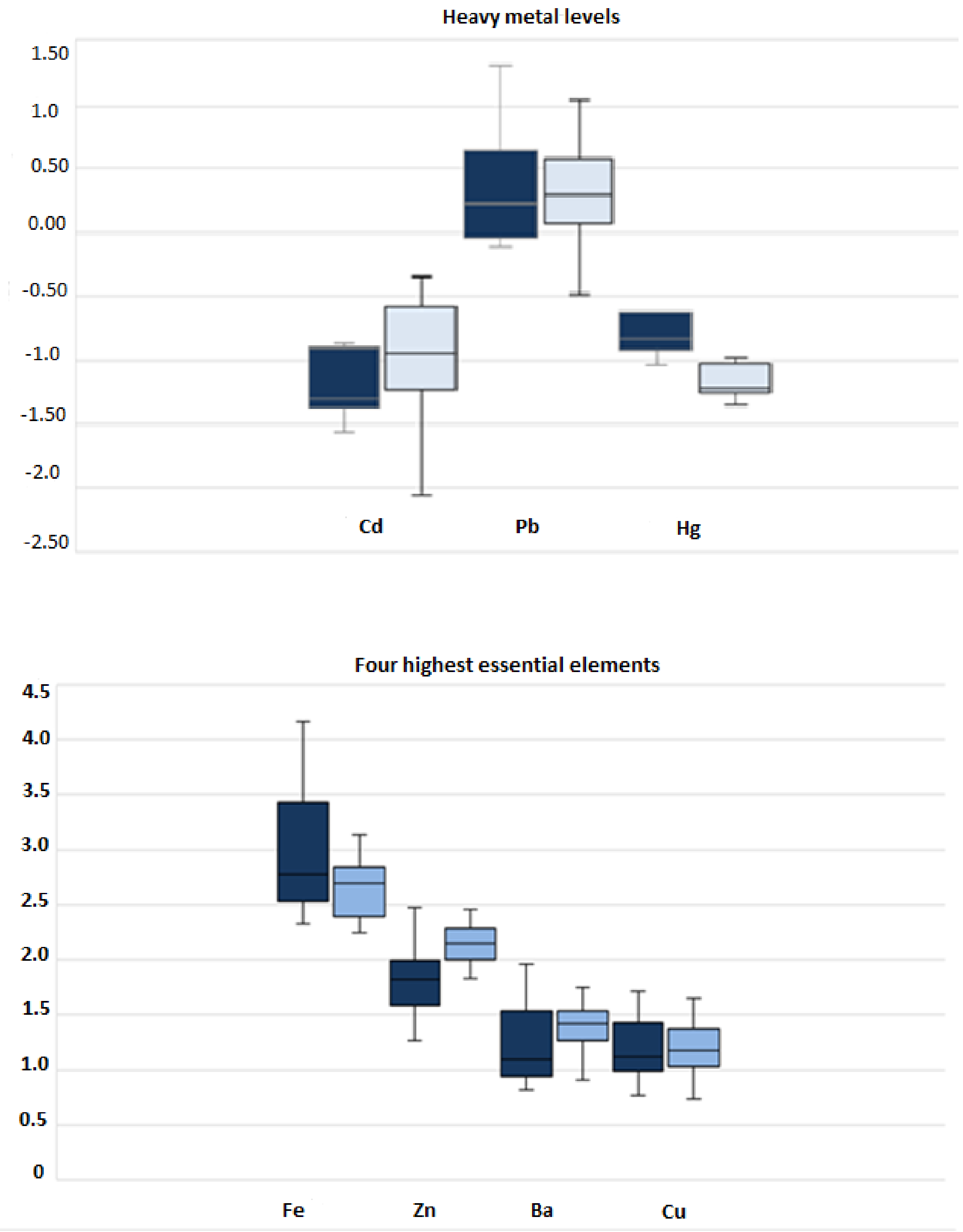

Fig. 3a,b. Plot of average Cd, Pb and Hg and Fe, Zn, Ba and Cu concentration in Longyearbyen (dark colors) and Hornsund (light colors) reindeer hair samples. Values are log transformed. The horizontal lines represent medians, the boxes – upper and lower (25-75% quartiles) and whiskers – minimum and maximum values

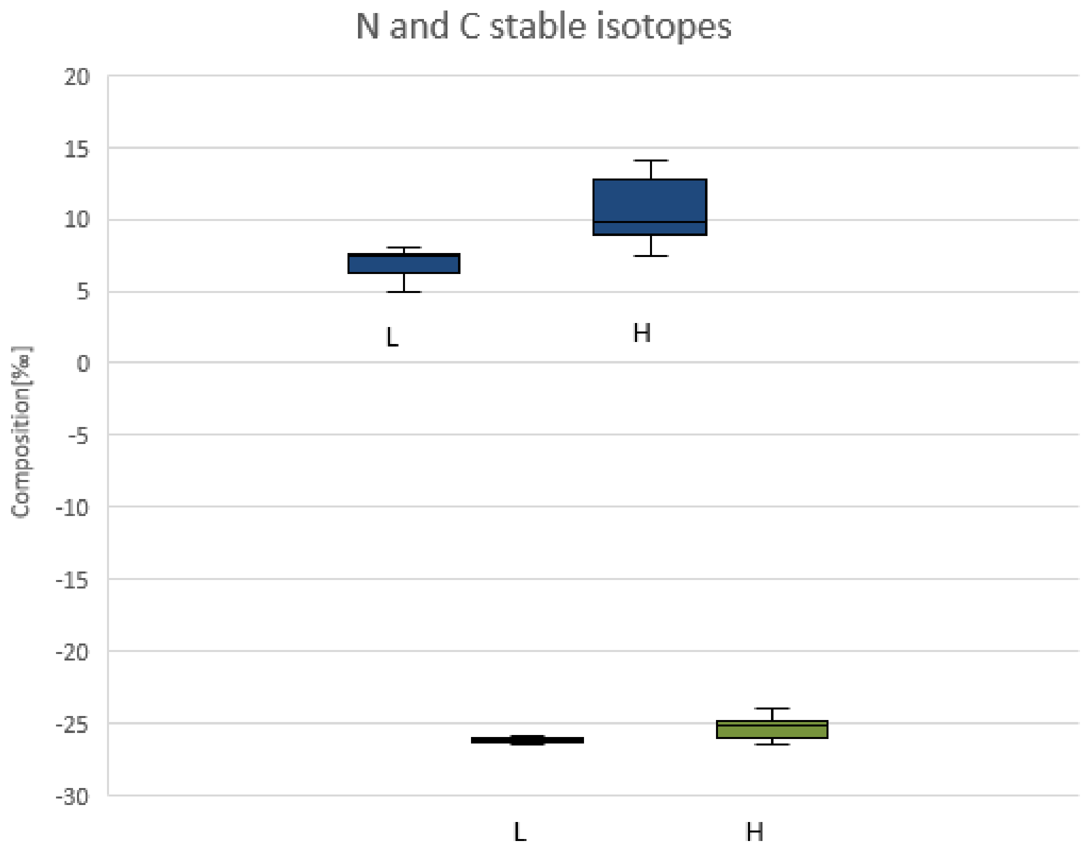

Fig. 4. Plot of average nitrogen (blue) and carbon (green) stable isotope composition in Longyearbyen (L) and Hornsund (H) reindeer hair samples. The horizontal lines represent medians, the boxes – upper and lower (25-75% quartiles) and whiskers – minimum and maximum values excluding outliers

Tab.1 Detail information about analytical instrumentation (Supplementary material)

ICP-MS parameter and accessories	**Value**
Radio frequency power generator	1400 V
Gas type	Argon
Plasma gas flow rate	12 L/min
Auxiliary gas flow rate	0.7 L/min
Nebulization gas flow rate	0.9 L/min
Torch Option	Standard one-piece quartz torch with PlasmaScreenPlus
Nebulizer	Standard glass concentric
Spray chamber	Quartz impact bead
Cones	Xt
Internal Standard	^{6}Li, Sc, Y, In, Tb, Bi
Sample Uptake Rate (mL/min)	0.4 approx.
Sampling depth	98 mm
Collision Cell Gas flow (7 % H2 in He)	5.5 mL/min
Number of replicates	3

Mercury analyzer specification	
Detectors	Photo tubes (Reference-background; Absorption cell 1; Absorption cell 2)
Wave length	253.7nm
Maximum measurement range	70,000ng

Measuring time	Approx. 5 minutes
Maximum decomposition temp.	Up to 1,000°C
Combustion tube	Quartz (Filled with catalyst)
Gas	Oxygen (>90% purity), 0.1~0.29MPa
Sample boat	Ceramic (standard supply)

Tab. 2 Trace element concentration in reindeer fur samples collected from two separate populations (µg/g dw)

Element	Longyearbyen (n=11)			Hornsund (n=16)			t – difference test of means (p<0.05)
	Median	Mean ±standard error (CI95%)	Log transformed mean	Median	Mean ±standard error (CI95%)	Log transformed mean	
Li	**0.43**	**4.36± 2.18**	**-0.04**	**0.51**	**0.49±0.08**	**-0.49**	**2.15**
Be	**0.01**	**0.09± 0.05**	**-1.11**	**0.02**	**0.025±0.004**	**-1.80**	**1.79**
V	**0.83**	**3.05±1.20**	**0.14**	**0.73**	**0.94±0.21**	**-0.24**	**2.08**
Cr	**0.89**	**2.82±1.17**	**0.08**	**2.24**	**3.28±0.81**	**0.06**	**-0.34**
Co	**0.13**	**1.31±0.65**	**-0.48**	**0.15**	**0.34±0.11**	**-0.97**	**1.76**
Ni	**0.89**	**3.81±1.72**	**0.13**	**1.26**	**1.90±0.54**	**-0.05**	**1.23**
Ga	**0.37**	**0.97±0.38**	**-0.32**	**0.81**	**1.00±0.20**	**-0.12**	**-0.07**
As	**0.54**	**1.06±0.39**	**-0.21**	**0.65**	**0.74±0.13**	**-0.24**	**0.91**
Rb	**0.62**	**3.12±1.42**	**-0.01**	**0.66**	**0.76±0.10**	**-0.19**	**2.02**
Cd	**0.05**	**0.30±0.23**	**-1.08**	**0.11**	**0.17±0.04**	**-1.01**	**0.68**
Cs	**0.09**	**0.73±0.40**	**-0.83**	**0.03**	**0.04±0.01**	**-1.61**	**2.08**
La	**0.32**	**2.22±1.08**	**-0.18**	**0.72**	**0.79±0.14**	**-0.34**	**1.59**
Pb	**1.68**	**5.14±2.19**	**0.37**	**1.96**	**3.20±0.82**	**0.29**	**0.95**
Hg	**0.13***	**0.34±0.23***	**0.29***	**0.06***	**0.06±0.01***	**-1.17***	**-[L]**
Fe	**602**	**3300±1550**	**3.03**	**494**	**530±97**	**2.54**	**2.17**
Zn	**65.9**	**90.6±24.8**	**1.82**	**141**	**154±16**	**2.15**	**-2.23**
Cu	**13.2**	**19.95±4.63**	**1.19**	**15.2**	**18.45±3.04**	**1.18**	**0.28**
Ba	**12.5**	**27.50±8.85**	**1.24**	**26.3**	**26.50±3.73**	**1.33**	**0.11**

***Longyearbyen (n=4), Hornsund (n=5), [L]- low sample size**

Tab. 3 Nitrogen and carbon stable isotopes concentration in Svalbard reindeer hairs

	Longyearbyen (n=10)		Hornsund (n=22)	
	$\delta^{15}N$ [‰]	$\delta^{13}C$ [‰]	$\delta^{15}N$ [‰]	$\delta^{13}C$ [‰]
Arythmetic Mean	**6.73**	**-26.19**	**10.96**	**-25.47**
SD	**1.40**	**0.24**	**2.01**	**0.76**
Median	**7.41**	**-26.22**	**10.66**	**-25.17**
Min	**3.73**	**-26.48**	**7.49**	**-26.67**
Max	**8.00**	**-25.82**	**14.04**	**-24.02**

Tab. 4 Pearson correlation values indicating correlation between the various trace elements measured (n=26)

Variable	15N	13C	Li	Be	V	Cr	Fe	Co	Ni	Cu	Zn	Ga	As	Rb	Cd	Cs	Ba	La	Pb
15N	1.00	-0.06	-0.16	-0.25	-0.21	-0.02	-0.26	-0.16	-0.03	-0.02	0.64	0.24	0.04	-0.10	0.20	-0.36	0.17	-0.04	0.01
13C		1.00	0.08	0.25	0.08	-0.10	0.09	0.07	-0.09	-0.10	-0.32	-0.19	-0.06	-0.07	-0.09	0.12	-0.10	-0.01	-0.07
Li			1.00	0.95	0.97	0.77	0.97	0.77	0.89	0.79	0.44	0.79	0.87	0.92	0.75	0.94	0.81	0.94	0.94
Be				1.00	0.87	0.74	0.86	0.52	0.68	0.59	0.49	0.71	0.79	0.83	0.57	0.90	0.78	0.86	0.56
V					1.00	0.82	0.98	0.76	0.88	0.80	0.39	0.78	0.88	0.87	0.74	0.92	0.82	0.95	0.81
Cr						1.00	0.80	0.48	0.80	0.71	0.52	0.76	0.74	0.65	0.76	0.70	0.82	0.88	0.64
Fe							1.00	0.73	0.88	0.78	0.38	0.75	0.86	0.91	0.71	0.97	0.81	0.95	0.78
Co								1.00	0.73	0.71	0.33	0.65	0.72	0.72	0.66	0.67	0.61	0.64	0.89
Ni									1.00	0.85	0.55	0.80	0.80	0.80	0.85	0.79	0.81	0.89	0.88
Cu										1.00	0.50	0.79	0.80	0.75	0.82	0.67	0.78	0.82	0.86
Zn											1.00	0.74	0.60	0.53	0.57	0.27	0.67	0.55	0.52
Ga												1.00	0.94	0.84	0.81	0.64	0.97	0.84	0.83
As													1.00	0.91	0.74	0.78	0.94	0.87	0.82
Rb														1.00	0.66	0.89	0.85	0.87	0.80
Cd															1.00	0.63	0.83	0.79	0.82
Cs																1.00	0.71	0.87	0.69
Ba																	1.00	0.88	0.79
La																		1.00	0.78
Pb																			1.00

Tab.5 Outliers with significantly elevated element levels µg/g dw (Supplementary material)

No	$\delta^{15}N$ [‰]	$\delta^{13}C$ [‰]	Iron	Chromium	Cobalt	Barium	Nickel	Lead	Arsenic
1	5.88	-25.92	14640	11.1	6.31	69.1	15.5	20.6	4.22
2	7.56	-26.32	11450	8.27	4.36	91.7	13.9	18.0	2.61
3	7.41	-25.82	5810	6.34	2.47	49.4	6.79	7.35	1.61