

1 Svalbard reindeer as an indicator of ecosystem changes in the Arctic terrestrial 2 ecosystem

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14

15 Abstract

16 Over the years, noticeable effort has been directed towards contaminant determination in
17 multiple biotic samples collected from the inhabitants of the Arctic. Little consideration has
18 been given to polar herbivores, however, especially those from the European parts of the
19 Arctic. To provide a broader perspective, we aimed to decipher trace element concentration
20 in hairs of the key species in the Arctic, namely the Svalbard reindeer (*Rangifer tarandus*
21 *platyrhynchus*), and to recognise whether diet variations could correspond with forward
22 exposure. The effect of habitat and diet was investigated using the ratios of stable isotopes of

23 carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$), and previous literature studies on vegetation from the areas
24 of interest. Analysis was performed for eighteen elements in total, both toxic and essential.
25 Metals were present in a decreasing order Fe>Zn>Ba>Cu>Pb>Cr>Ni>V>Ga
26 =La>Rb>As>Li>Co>Hg>Cd>Cs>Be. Similarity in trends in the studied subpopulations was
27 observed for many metals. A significant log-linear correlation was observed for most of the
28 elements, excluding nitrogen and carbon isotopes signature. Extremely high iron levels were
29 determined in some of the samples, suggesting past iron overload. Zinc, in contrast to the
30 remaining metals, did not correlate well with any other element. Mercury was determined at
31 very low levels, in accordance with previous literature regarding its concentrations in moss
32 and lichen species in Svalbard. The analysis of stable isotopes showed a high variation in
33 nitrogen isotopes signatures. Further research is required to properly evaluate the potential
34 health risks and ecological implications of elevated exposure.

35

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

38

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

41

42 **1. Introduction**

43 Constant pollutant emission is undeniably a serious problem and it is considered a huge threat
44 to ecosystem stability. Anthropogenic activities undoubtedly have significant ecological
45 consequences worldwide. The Arctic is an invaluable source of information on the global-scale

46 impact due to long-range contaminant transport (Davis, 1996; Halbach et al. 2017). The
47 accumulation of trace elements, particularly heavy metals, and the resulting enrichment in
48 higher trophic levels, raise questions about its impact on native fauna. Due to its unique
49 geographical location, the Svalbard Archipelago has become a significant recipient of
50 pollutants emitted in the Northern Hemisphere. Natural sources of heavy metal emissions
51 include volcanic activities, biogenic sources, soil-derived dusts, and sea salt aerosols. It is
52 anthropogenic emissions, however, that are assumed to account for the observed heavy metal
53 levels in the Arctic to the greatest degree (AMAP, 2005; Halbach et al. 2017). With only several
54 local sources of pollution (such as mining activities, airport, ship traffic), most contaminants
55 including heavy metals are atmospherically transported long-range from mid- and low-
56 latitudes (Bard, 1999).

57 A growing amount of evidence arose in the recent years concerning the deposition of
58 pollutants in polar, particularly marine biota (e.g. Burger et al. 2007). Physiological and
59 ecological factors affecting the bioaccumulation process vary between terrestrial and aquatic
60 ecosystems (van den Brink et al. 2015). Terrestrial species are often weakly investigated and
61 yet crucial parts of any polar ecosystem. Reindeers are a key component of the Arctic
62 terrestrial ecosystem (Duffy et al. 2005). Because they are a part of a simple food chain, the
63 species is ideal for monitoring changes in the terrestrial trophic network (Elkin and Bethke,
64 1995).

65 In this paper, we investigate the usefulness of molten fur collected from a broadly distributed
66 resident of the European part of the Arctic, namely - the smallest reindeer subspecies
67 (*Rangifer tarandus platyrhynchus*). This large herbivore, endemic to Svalbard, can be found in
68 the majority of non-glaciated areas of the island. The Svalbard reindeer has certain

69 adaptations to the polar environment, including relatively short legs and thick fur with
70 colouring and thickness varying between the seasons (Cuyler and Øritsland, 2002). Its total
71 population size is estimated for 10,000 animals (npolar.no). Monitoring studies conducted in
72 Brøggerhalvøya, Reindalen, Adventdalen, and Edgeøya suggest high annual fluctuations
73 (mosj.no; Reimers, 2012) primarily caused by variations in climate condition (such as snow
74 depth and rain-on-snow events), and partially by competition for food resources.

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

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

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

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

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

113 **2. Materials and Methods**

114 2.1 Study area and sampling

115 Fur samples were collected in two consecutive summer seasons: in August 2015 from
116 Longyearbyen region (N78° E015°, n=11) and in September 2016 from the Fuglebekken
117 catchment in the vicinity of the Polish Polar Station in Hornsund (N77° E015°, n=16) (Fig.1).
118 Samples were collected from the ground, after a herd moved to a new place. To avoid
119 pseudoreplication, only freshly molten fur was collected (one sample per at least 4 m²
120 distance). We assumed that samples were from separate individuals. All samples were
121 individually packed in clean zip bags, and stored at a temperature of 4°C prior to analysis. Long,
122 straight, white on entire length (except darker tip) guard hairs were collected. Mean
123 temperature during the period of sample collection amounted to 2.9°C in August 2015
124 (Longyearbyen) and 3.9°C in September 2016 (Hornsund) (yr.no). Sample weight varied from
125 16 to 80 mg for samples collected from Longyearbyen, and from 9 to 100 mg for samples
126 collected from the Hornsund area.

127

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

130 The Svalbard reindeer, unlike other reindeer subspecies, is highly stationary. It is reluctant to
131 migrate beyond its territory range mostly established by natural barriers (thin sea ice, glaciers,
132 steep mountains) (Hansen et al. 2010b). Genetic differences between populations might occur
133 even at distances <50 km² (Côté et al. 2002). Therefore, the studied herds are most likely from
134 completely separate populations. Predation is almost non-existing, with the exception of local
135 hunting and occasional evidence of polar bear hunting attempts (Hansen et al. 2011).

136 2.2 Analytical methods

137 18 trace elements and nitrogen and carbon stable isotopes composition were analysed. The
138 basic course of the analytical procedure involves removal of external contamination and then
139 elemental analysis preceded by acid mineralization in microwave emitter (trace elements
140 except for mercury), thermal vaporization (mercury) and high temperature oxidation ($\delta^{13}\text{C}$ and
141 $\delta^{15}\text{N}$).

142 **2.2.1. Trace elements (except for mercury)**

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



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

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

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

166 **2.2.2. Mercury analysis**

167 External contamination was washed out using the same procedure as for other trace
168 elements. The pooled dry sample was cut into smaller pieces using sterilised stainless scissors,
169 weighed (to the nearest 0.01 mg), and analysed by the thermal vaporisation atomic absorption
170 method (MA-3000 Nippon Instruments Corporation). The samples were heat decomposed in
171 a ceramic boat, first heated to 180°C for 120 s, and then to 850°C also for 120 s. The mercury
172 collector collects the atomised mercury gas in a form of gold amalgam, condensing and
173 purifying the mercury. After heat decomposition, the mercury collection tube was heated to
174 650°C to liberate the mercury gas. Absorbance at a wavelength of 253.7 nm was then
175 measured. Oxygen flow amounted to 0.4 L/min. Total mercury concentration was determined
176 in triplicates, and based on them the variation coefficient was calculated. Quality control
177 included blank samples every 5-6 subsamples run. The median of the coefficient of variation
178 between replicates was equal to 10.0 (7.91-13.95) in samples collected from Longyearbyen,
179 and 3.65 (1.64-8.98) in samples from Hornsund. Reference materials MODAS-4 Cormorant
180 Tissue (M-3 CornTis), MODAS-3 Herring Tissue (M-3 HerTis), MODAS-5 Cod Tissue (M-5
181 CodTis) were used to determine analytical accuracy, and to perform method and quality

182 control. Recovery of reference materials measured on three replicates of each RM varied from
183 94 to 100%.

184 **2.2.3. Stable isotopes**

185 The analyses of carbon and nitrogen stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were done in an Elemental
186 Analyser Flash EA 1112 Series combined with an Isotopic Ratio Mass Spectrometer IRMS Delta
187 V Advantage (Thermo Electron Corp., Germany). Details of these measurements are described
188 by Kuliński et al. (2014). In short, the samples were dried, homogenised, and weighed into
189 silver capsules (about 1 mg). This sample weight guarantees C and N loads significantly higher
190 than those given by the limit of quantification (C = 20 μg , N = 20 μg). Next, samples were
191 oxidised in 1020°C in presence of Cr_2O_3 and Co_3O_4 . After catalytic oxidation, gases including
192 CO_2 , NO_x and H_2O , were transported to the second reactor, where NO_x was reduced to N_2 on
193 the metallic Cu (650°C). Subsequently, the analysis products were dried with $\text{Mg}(\text{ClO}_4)_2$ and
194 separated on GC (45°C). The separated gases (CO_2 and N_2) were transported to the IRMS. The
195 isotopic composition of carbon and nitrogen was calculated using laboratory working pure
196 reference gases (CO_2 and N_2) calibrated against IAEA standards: CO-8 and USGS40 for $\delta^{13}\text{C}$ and
197 N-1 and USGS40 for $\delta^{15}\text{N}$. Results of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were given in the conventional delta
198 notation, i.e., versus PDB for $\delta^{13}\text{C}$ and versus air for $\delta^{15}\text{N}$ as parts per thousand (‰) according
199 to the following equation:

$$200 \quad \delta X (\text{‰}) = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000$$

201
202 where: X is the stable isotope ratio of $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$; R is the ratio of $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$.
203 The measurement precision was better than 0.20‰ for $\delta^{13}\text{C}$ and 0.18‰ for $\delta^{15}\text{N}$
204 (n = 5).

205 **2.3 Quality assurance/quality control (QA/QC)**

206 To ensure high quality of results, the obtained data were subject to strict quality control
207 procedures. All the analytical equipment was carefully washed before analysis. Based on
208 duplicate and triplicate samples, the variance coefficient of metal concentration was
209 calculated. If the coefficient >15%, samples were excluded from the analyses, assuming
210 unreliable estimation of metal concentration. Background contamination was present in
211 metal method blanks prepared after mineralisation, therefore blank correction was
212 performed for all elements. Blank correction involved subtracting the total amount of analyte
213 detected in the method blank from the total amount of analyte detected in the hair samples.
214 Negative numbers and numbers below the limit of detection were reported as half of the limit
215 of detection for statistical analysis. The obtained results were also corrected for sample
216 weights and method dilution factor, and are reported as $\mu\text{g/g dw}$. All reagents were of the
217 highest purity. Ultrapure water was produced from a Mili-Q Gradient A10 (Milipore, France).
218 ICP-MS equipment calibration employed the multi-element standard by Inorganic Ventures
219 ANALITYK - CCS-1, CCS-4, CCS-6. The optimised and validated methods showed good linearity
220 ($R^2 > 0.999$) over a wide range with low limits of detection. Both the method limit of detection
221 (LOD) and the limit of quantitation (LOQ) were calculated based on the standard deviation of
222 the response (s), and the slope of the calibration curve (b) according to the following formulas:
223 $\text{LOD} = 3.3(s/b)$, $\text{LOQ} = 10(s/b)$ (LOD/LOQ - Li, Fe, V, Cr, Ni, As, Rb, Ba, Pb 0.1/0.3 ppb; Be, Co,
224 Ga, Cs, Cd, La 0.01/0.03 ppb; Cu, Zn 0.5/1.5 ppb). For mercury the method limit of detection
225 and quantification was equal to 0.54 and 1.62 ppb, respectively.

226 Due to the fact that metals are bound to the keratin structure with variable affinity, removal
227 efficiencies differ significantly among compounds when stronger solvents such as acetone are

228 used. Therefore, only double deionised water was used as a washing agent. Some part of
229 surface contamination might not have been removed. Because it is difficult to distinguish
230 between internal and external exposure, it can be assumed that hairs provide information of
231 integral exposure.

232

233 **2.4 Statistical methods**

234

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

245

246 **3. Results**

247 Median, mean, and standard error, log transformed mean, and t-difference test of means are
248 presented in Table 2. For compiled samples, correlation coefficients are mostly high, many are
249 close to one (Table 4). The correlation of variables with regard to the sampling site was also
250 tested. In the majority of cases, stronger correlations between metals were observed in
251 samples from the Longyearbyen area, compared to the Hornsund samples. For zinc,

252 correlations with other metals were notably lower (the highest occurs with gallium content:
253 $R^2_{\text{tot}} = 0,54$). Those coefficients were used to measure similarity of variables by data clustering
254 (Fig.2). As a result, two groups were obtained: zinc as an isolated element, and other elements
255 forming a single cluster. After further division, we obtained a five-elemental cluster
256 (containing V, Fe, Li, Cs, and La), a three-elemental cluster (As, Ga and Ba), and the remaining
257 elements as isolated items. High variation was observed for nitrogen isotope composition. T-
258 difference test of means ($p < 0.05$) for nitrogen isotopes ($\delta^{15}\text{N}$) was equal to -5.16, and for
259 carbon ($\delta^{13}\text{C}$) to -3.12. Three individuals from the Longyearbyen area showed elevated
260 contents of all the measured elements, with extremely high levels of iron, chromium, nickel,
261 and lead. The average value of nitrogen isotope $\delta^{15}\text{N}$ for those outliers was equal to 6.95 [‰].
262 Outliers were not excluded from statistical analysis.

263

264 **Table 2. Trace element concentration in reindeer fur samples collected from two separate**
265 **populations ($\mu\text{g/g dw}$)**

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

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

269 **Fig. 2. Hierarchical dendrogram for clustering chemical elements. Lines indicate distance**
270 **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,**
271 **11,5-Be, 13-Ni, 14,5-Cr, 16-Co, 17,5-Pb, 19-Cu, 20,5-Cd, 22,5-Zn**

272

273 **4. Discussion**

274 This study reports the levels of essential and toxic elements and stable isotope composition in
275 Svalbard reindeer hair samples collected from herds living in distant parts of the island.
276 Keratinised tissues such as hairs, fur, or feathers can be collected non-lethally, and have been
277 successfully used for stable isotopes and heavy metal analysis for many years (Duffy et al.
278 2005; Burger et al. 2007; Sergiel et al. 2017). Hair tissue has several advantages in practical
279 use. Owing to its stability, samples can be stored for a long time, they are relatively
280 metabolically inactive (Duffy et al. 2005), and elements are accumulated over extended
281 periods of time. Therefore, the exposure assessment covers several weeks or months. Molten
282 hairs can be collected without direct contact, avoiding difficulties related to capturing a free-
283 living individual. However, because factors such as specimen age and gender are often
284 unknown, this mode of sampling also limits the possibility of result interpretation.

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

292 Spatial and temporal heterogeneity in diet components might be responsible for significant
293 seasonal differences in contaminant distribution across studies (Robillard et al. 2002). In our
294 study, the majority of elements showed a strong positive correlation with multi-element
295 totals, excluding zinc. High variability in trace element composition was observed even above
296 an order of magnitude within samples of reindeer from one location. This is probably related
297 to differences in age (herds were composed from both young and older individuals), gender,

298 and food preference. Due to lack of previous studies regarding trace elements in reindeer
299 hairs, our data can be used as a reference for future investigations in the Svalbard Archipelago
300 concerning reindeer and closely related species.

301 **4.1 Accumulation route**

302 Vegetation covers only 6-7% of the area of Svalbard. The growing season lasts approximately
303 90 days (Kłos et al. 2015). Because of the short grazing season, the Svalbard reindeer must
304 restore its body reserves after winter, and accumulate fat at this time (Staaland, 1984). The
305 plant species-specific physiology, age, and sampling location will correspond with forward
306 exposure. Lower trace element levels are observed in vascular plants as compared to mosses
307 and lichens (Wojtuń et al. 2013). This may be related to their higher morphological similarity,
308 and more selective accumulation process (Chiarenzelli et al. 2001). Due to the lack of root
309 system, slow growth rate, longevity, vast surface area, and lack of well-developed cuticle,
310 plants such as lichens and bryophytes are prone to accumulating a varied cocktail of toxic
311 compounds from the atmosphere (Robillard et al. 2002; Gamberg et al. 2005, Samecka-
312 Cymerman et al. 2011). Essential elements such as copper and zinc, necessary for plant
313 growth, can also be accumulated beyond physiological demands (Samecka-Cymerman et al.
314 2011, Józwik, 1990). For instance, for zinc, enhanced exposure in lichens is above 500 µg/g,
315 cadmium can be tolerated between 1 and 30 µg/g, and copper between 1 and 50 µg/g
316 (Nieboer et al. 1978).

317 The accumulation route can be passive by water transpiration passage (e.g. Cu in lichens),
318 active (e.g. zinc), and metabolic (e.g. manganese), or a mix of those factors (Józwik 1990).
319 Mosses are evidenced to accumulate notably high levels of Cd, Co, Cr, Cu, Fe, Mn, and Zn,
320 even higher than lichens (Wojtuń et al. 2013). Particularly moss species such as *Aulacomium*
321 *palustre*, *A. turgidum*, *Hylocomium splendens*, *Sanionia uncinata*, and *Tortula ruralis* are
322 suspected to be very good heavy metal accumulators in Svalbard (Grodzińska and Grodzik,
323 1991).

324

325 **4.2 Toxic elements**

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

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

343 Mercury and cadmium previously showed a clear pattern of accumulation towards higher
344 trophic levels in the terrestrial ecosystem (Dietz et al. 2000). Cadmium binds to the low
345 molecular weight sulphur-rich proteins, and accumulates mostly in kidneys (Chan et al. 2001).
346 It also may significantly increase with age (Danielsson and Frank, 2009). In our study, however,
347 age differences were not analysed, and hair bounding capacities are different than in internal
348 tissues. Literature studies on both areas showed low cadmium exposure in vegetation (Wojtuń

349 et al. 2013; Samecka-Cymerman et al. 2011; Węgrzyn et al. 2013; Kłos et al. 2015), and as
350 expected we found low levels in reindeer hair. To our best knowledge, no study has been
351 published concerning cadmium accumulation in Svalbard mammal herbivores, therefore no
352 comparison is possible.

353 On the other hand, high lead levels were found in the majority of samples, suggesting an
354 accumulation path by vegetation. High levels of lead were also previously found in Greenland
355 soils. However, it does not tend to accumulate towards higher trophic levels, as reindeers had
356 lower lead levels than lichens (summarized in Dietz et al. 2000 based on Greenlandic studies
357 of the AMAP programme). Notice that only reindeer internal tissues were used. In Svalbard
358 area, levels of lead in vegetation is highly variable. Threshold values for lead in lichens are
359 from 5 to 100 $\mu\text{g/g}$ and 15 $\mu\text{g/g}$ is a boundary for enhanced exposure (Nieboer and Richardson,
360 1981). In hairs, lead is accumulated both externally and internally over a long period of time,
361 until molting. It is possible that apart from internal contamination accumulated by foraging on
362 high-lead level food sources, part of external contamination was not washed out during the
363 cleaning procedure.

364
365 **Fig. 3a,b. Plot of average Cd, Pb and Hg and Fe, Zn, Ba and Cu concentration in Longyearbyen**
366 **(dark colors) and Hornsund (light colors) reindeer hair samples. Values are log transformed.**
367 **The horizontal lines represent medians, the boxes – upper and lower (25-75% quartiles) and**
368 **whiskers – minimum and maximum values**

369
370 **4.3 Other elements**
371 The studied samples showed particular patterns such as high intra-individual variations in the
372 level of several compounds (iron, chromium, zinc etc.). All the analysed elements occur in

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

393

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

396

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

415

416 **4.4 Stable isotopes of carbon and nitrogen**

417

418 Stable isotopes (SI) of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) are increasingly employed as an
419 indispensable tool in ecological studies (Sergiel et al. 2017). The main sources of nitrogen in
420 the Arctic include atmospheric discharge of NO_x , NH_x , primary N_2 -fixation from the

421 atmosphere, and bird guano (Skrzypek et al. 2015). In nitrogen-limited terrestrial ecosystems
422 such as Arctic tundra, soil microbes are recognised to function as main nitrogen pools,
423 competing for nitrogen with plants (Bardgett et al. 2007). Plant growth is limited by nitrogen
424 availability. Consequently, the capacity for carbon sequestration is also restricted (Skrzypek et
425 al. 2015). Arctic tundra contains a significant percent of the global soil carbon reserve. Its
426 storage is controlled by factors such as e.g. temperature, vegetation type, soil hydrology, or
427 shifts in vegetation state. The latter can be induced by herbivores (Van der Val, 2006; Speed
428 et al. 2010).

429 Forage patch choice by reindeers and nitrogen content in plants are largely influenced by the
430 timing of snowmelt (van der Wal et al. 2000). In Svalbard, seasonal variability of plant and soil
431 nitrogen pools are mostly controlled by changes in temperature and soil moisture over the
432 growing season. Such changes, however, are markedly lower than in the other seasonally cold
433 ecosystems (Bardgett et al. 2007). Also Arctic tundra has a high capacity to retain nitrogen
434 transported after extreme events, with non-vascular plants acting as a short-term sink, and
435 vascular plants as a long-term reservoir (Choudhary et al. 2016). Our results indicate high
436 variability in the $^{15}\text{N}:$ ^{14}N ratio, suggesting that reindeers consume vegetation with different
437 ^{15}N values. In the Fuglebekken catchment (Hornsund), high loads of nutrients are deposited
438 by large bird colonies such as little auk (*Alle alle*). This influx impacts soil fertility and
439 subsequently plant productivity and structure (Skrzypek et al. 2015). As a result, the available
440 vegetation differs in protein, sugar composition, and digestibility (Staaland, 1984). Bird guano
441 and additional N-sources from colonies, such as carcasses, dead chicks, and eggshells,
442 constitute a huge N-load compared to other sources (Skrzypek et al. 2015). It could account
443 for significant differences between the two subpopulations.

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

447
448 No previous studies are available concerning stable isotope analysis in the keratinised tissues
449 of the Svalbard reindeer. Mosbacher (et al. 2016) showed high inter- and intra-annual
450 seasonality in the diet of the Greenland muskox (*Ovibos moschatus*) by the application of
451 sequential data on nitrogen stable isotopes derived from guard hairs. Drucker (et al. 2010)
452 studied the dietary references and habitat use of moose (*Alces alces*) and caribou (*Rangifer*
453 *tarandus*) in plucked hair samples from Canada populations. The dietary strategies of those
454 species differ in spite of the same habitat range. Differences in stable isotope abundance were
455 significantly linked to the species' dietary specialisation (Drucker et al. 2010).

456
457

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

462
463 The long-term variation in weather conditions may impact vegetation quality, consequently
464 affecting the ungulates' nutritional profile and foraging conditions. Lower snow layer
465 hardening in winter leads to changes in snow-pack properties, including ground icing, resulting
466 in snowpack with impenetrable vegetation underneath (Hansen et al. 2011, Loe et al. 2016).
467 Food availability can also be restricted by overgrazing (Węgrzyn et al. 2016). Therefore, some

468 populations are more likely to expand their foraging area, or alternatively use less preferred
469 food sources such as goose droppings (van der Wal and Loonen, 1998) or marine algae
470 (Hansen and Aanes, 2012). Because many factors are responsible for seasonal availability of
471 various food sources, and Svalbard reindeers tend to forage for plant quantity rather than
472 quality (Van der Wal et al. 2000), a complex study program concerning trace element levels in
473 vegetation may help assess their future potential exposure.

474

475 **5. Conclusion**

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

485 Future climate changes will induce higher pressure on all terrestrial species. Rising
486 temperatures, more frequent extreme weather events, heavy rain-on-snow events, and
487 variations in seasonal precipitation patterns may cause negative implications for herbivores
488 (Hansen and Aanes, 2012). In spite of their remarkable abilities to locate food beneath the
489 snow-pack, severe icy conditions may induce changes in reindeer behaviour, including range
490 expansion to mountainous terrain (Hansen et al. 2010), and eating marine algae (Hansen and
491 Aanes, 2012) resulting in potential changes in the foraging profile and contaminant

492 accumulation. The research presented so far provides evidence that keratinised tissues can be
493 a valuable source of information in ecotoxicological studies. Monitoring studies should involve
494 not only marine species, but concurrently more terrestrial key species as an important part of
495 the trophic network.

496

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

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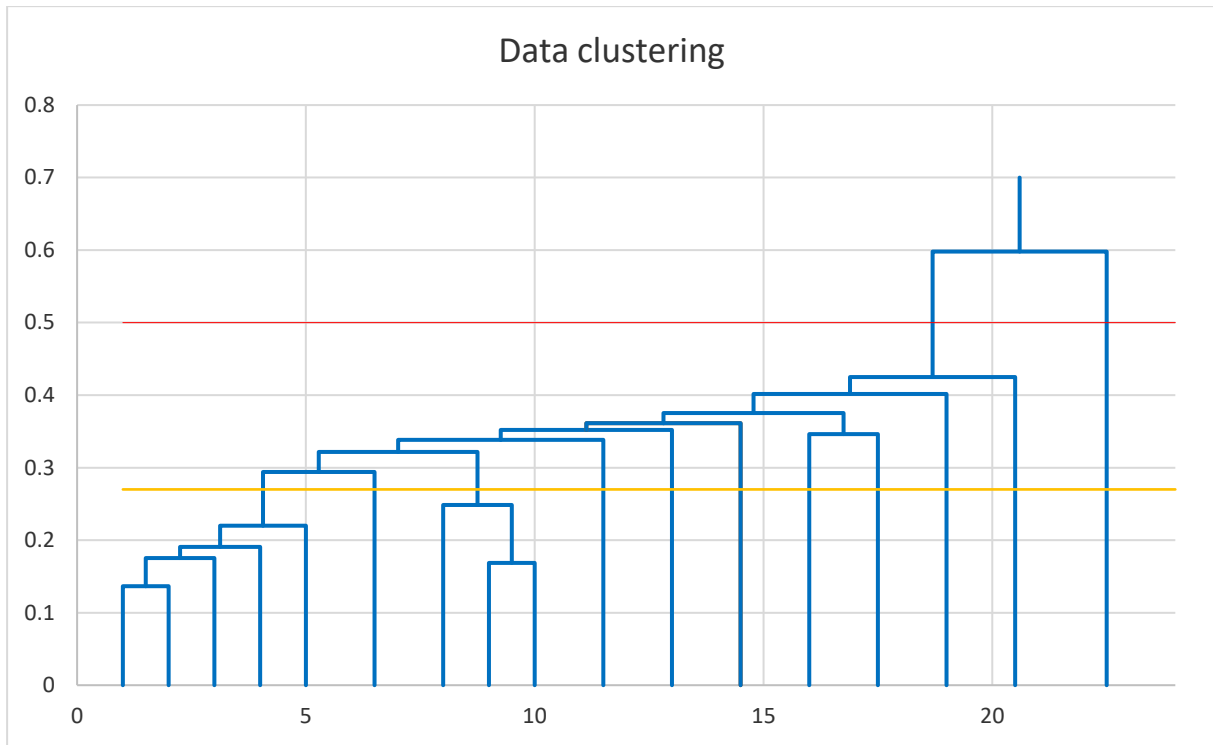
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659 Fig. 2 Hierarchical dendrogram for clustering the chemical elements. Lines indicate distance
 660 0.27 and 0.5, respectively. From the left: 1-V, 2-Fe, 3-Li, 4-Cs, 5-La, 6,5-Rb, 8-As, 9-Ga, 10-Ba,
 661 11,5-Be, 13-Ni, 14,5-Cr, 16-Co, 17,5-Pb, 19-Cu, 20,5-Cd, 22,5-Zn

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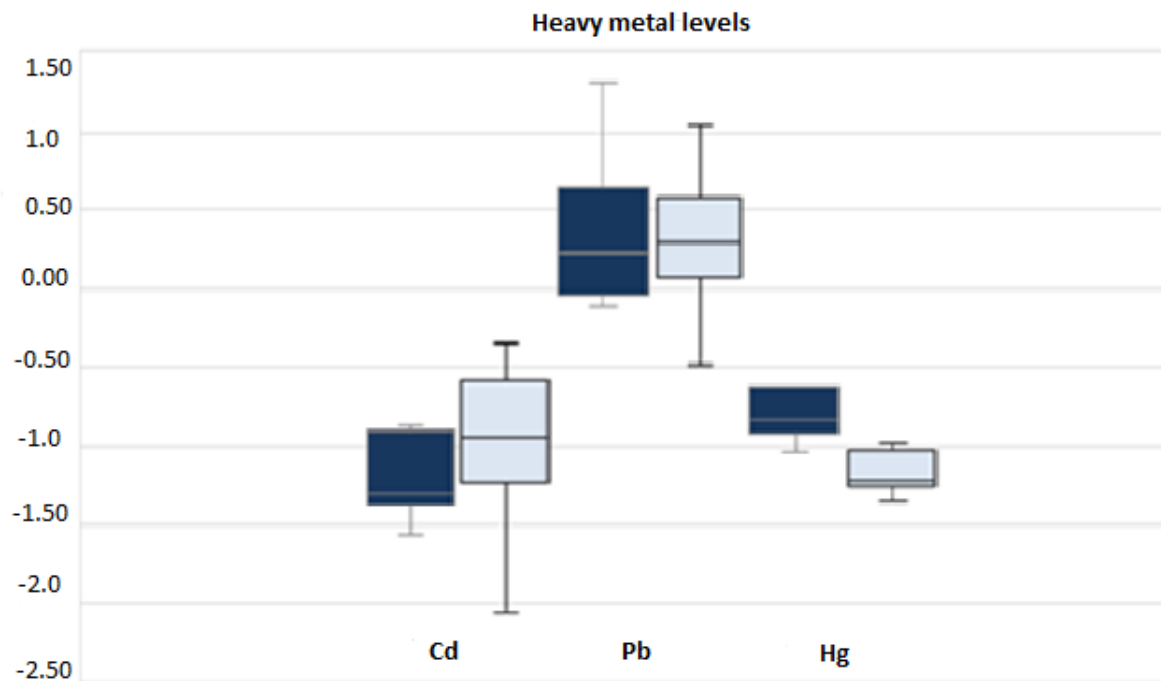
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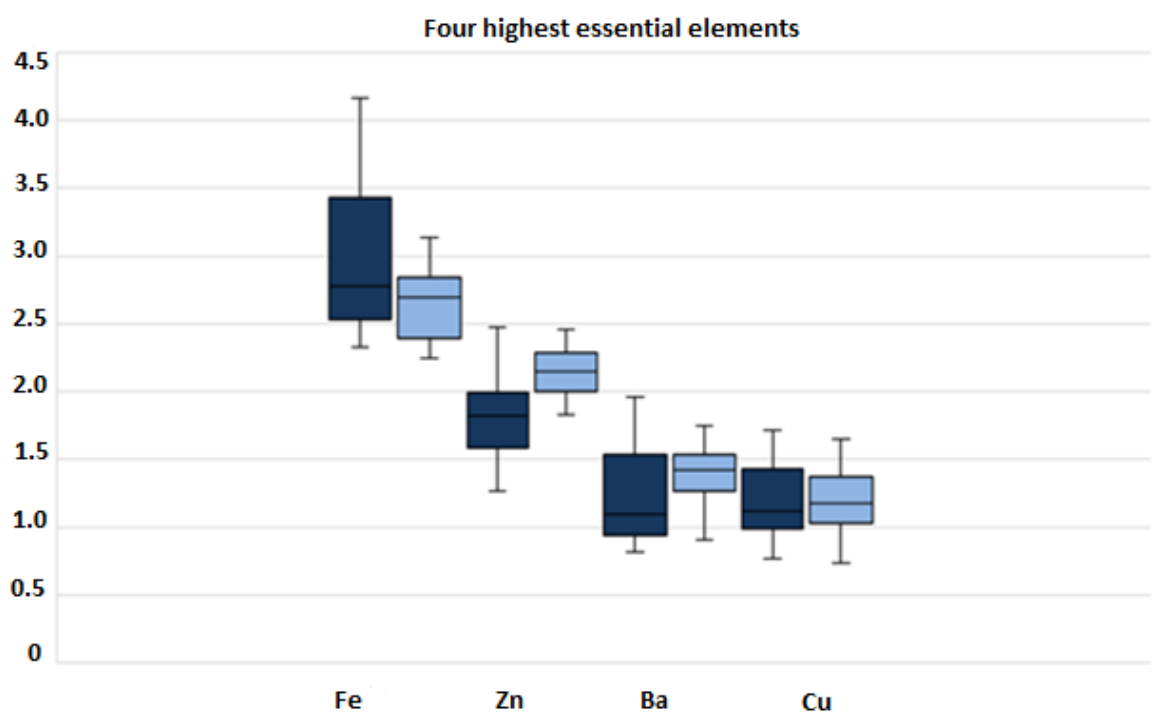
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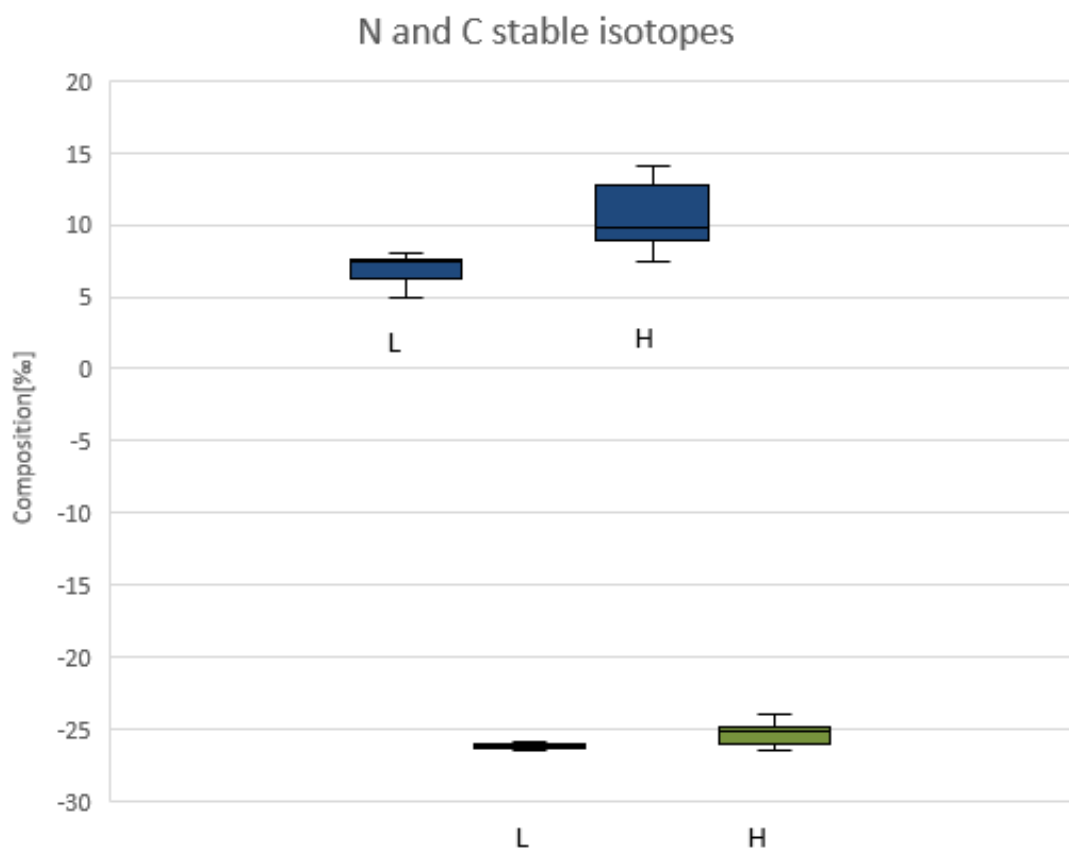
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675 Fig. 3a,b. Plot of average Cd, Pb and Hg and Fe, Zn, Ba and Cu concentration in Longyearbyen
 676 (dark colors) and Hornsund (light colors) reindeer hair samples. Values are log transformed.
 677 The horizontal lines represent medians, the boxes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

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

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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 % H ₂ in He)	5.5 mL/min
Number of replicates	3

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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)

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713 Tab. 2 Trace element concentration in reindeer fur samples collected from two separate
 714 populations ($\mu\text{g/g dw}$)

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

715 *Longyearbyen (n=4), Hornsund (n=5), ^L- low sample size

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

	Longyearbyen (n=10)		Hornsund (n=22)	
	$\delta^{15}\text{N}$ [‰]	$\delta^{13}\text{C}$ [‰]	$\delta^{15}\text{N}$ [‰]	$\delta^{13}\text{C}$ [‰]
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

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742 **Tab. 4 Pearson correlation values indicating correlation between the various trace elements**
 743 **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																		

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755 Tab.5 Outliers with significantly elevated element levels $\mu\text{g/g dw}$ (Supplementary material)

No	$\delta^{15}\text{N}$ [‰]	$\delta^{13}\text{C}$ [‰]	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

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