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# Evaluation of the use of reindeer droppings for monitoring essential and non-essential elements in the polar terrestrial environment

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## HIGHLIGHTS

- Reindeer droppings were used to assess bioavailability of 30 elements.
- Significant differences in element concentration between foraging sites
- Seasonal differences occurred for part of the elements.
- Usefulness of older as opposed to freshly collected droppings was assessed.

## GRAPHICAL ABSTRACT


| Method | Elements analysed | Concentration levels (means) |
|---|---|---|
| ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) | Ca, Fe, K, Mg, P, S, Si | 0.44-47.9 mg $g^{-1}$ dw |
| ICP-MS (Inductively Coupled Plasma Mass Spectrometry) | Ag, Al, As, B, Ba, Be, Cd, Co, Cr, Cu, La, Li, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, V, Zn | 0.027-2518 μg $g^{-1}$ dw |
| Thermal vaporization atomic absorption | Hg | 0.082 μg $g^{-1}$ dw |

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## ABSTRACT

Excess or toxic metals, non-metals and metalloids can be eliminated from the organism by deposition in inert tissue (e.g. fur) or excretion with body secretions, urine and faeces. Droppings are one of the main routes for the elimination of multiple elements and they can be collected without direct contact with the animal. Contaminant concentration has been examined in non-lethally collected tissues of several species (especially reptilian, avian and mammalian). However, studies on species residing in polar areas are still limited, especially of mammals from the European Arctic. Reindeers are the only large herbivores living in Svalbard, being an essential part of the Arctic terrestrial ecosystem. Although reindeer presence has a high impact on their surroundings, those huge mammals are rarely part of ecotoxicological studies regarding metal pollution. In this paper, the droppings of Svalbard reindeer were used as a non-invasively collected tissue to examine the excretion pathway of 30 elements. Samples were collected in mesic and moss tundra, representing summer, winter and winter-transitional excretion. For more than a half of the studied elements, significant differences occurred between the samples collected in the two tundra types. The feasibility of older and fresh samples was assessed based on summer droppings, and significant differences were found for K, As, Mn, Na, Ni, and Sb concentrations. No relevant differences in element levels were observed for samples collected from adult females, adult males and calves, except for zinc and potassium. Results show that reindeer droppings are an important vector for the transfer of many metals, non-metals and metalloids including calcium, phosphorus, zinc, aluminium and lead. As a sedentary species, feeding on local food sources, Svalbard reindeer is a valuable indicator of trace element presence in the polar terrestrial ecosystem.



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## 1. Introduction

Tundra is a characteristic biome of the Arctic, covering about one-tenth of the total Earth surface (Poissant et al., 2008). Although local fauna is composed of a limited number of species, low biodiversity is often counterbalanced by high local abundance. The arctic terrestrial ecosystem may be exposed to an enhanced level of metals and metalloids of natural and anthropogenic origin (AMAP, 2005; Ruman et al., 2012; Kozak et al., 2013; Halbach et al., 2017). Thus, studies employing terrestrial fauna and flora are crucial for a comprehensive understanding of ecological consequences to the polar ecosystem. Svalbard Archipelago is inhabited by a number of birds, by polar bears and polar foxes, but Svalbard reindeer (*Rangifer tarandus platyrhynchus)* is the only large mammal herbivore there (Hayashi et al., 2014). In contrast to other *Rangifer* subspecies, Svalbard reindeer is highly stationary and does not migrate long distances under normal circumstances (Tyler and Øritsland, 1989). It eats almost all types of available vegetation with the selection based on plant quantity rather than quality (Van der Wal et al., 2000). Their territory is established in a great part by natural barriers thus genetic differences might occur even at distances <50 $km^2$ (Hansen et al., 2010; Côté et al., 2002). The restricted foraging area, longevity and easiness to spot make the Svalbard reindeer a suitable species to monitor the local pollution level.

A non-lethal sampling includes samples taken with direct contact with an animal (e.g. blood and fur collection) and without direct contact (molten fur, droppings). The non-lethal monitoring of biota is recently gaining a lot of attention worldwide, e.g. in birds (Jerez et al., 2011; Burger et al., 2008; Becker et al., 2016), reptiles (Ikonomopoulou et al., 2011; Schneider et al., 2011; Villa et al., 2015), and mammals (Duffy et al., 2005; Pacyna et al., 2018). Some studies have based their conclusions on completely non-destructive sampling procedures, such as collecting shed skins of cobras (Kaur, 1988), faeces (Xu et al., 2006; Yin et al., 2008), feather (Burger et al., 2008; Jerez et al., 2011), urine (Hart et al., 2018), or fur (Duffy et al., 2005). However, the Arctic has been so far represented to a smaller extent, mostly by bird species (e.g. Burger et al., 2008; Fort et al., 2016).

Faeces generally contain a high concentration of metals and are one of the main routes for the elimination of the majority or excess consumed elements (Yin et al., 2008; Orłowski et al., 2015). Thus, droppings can be used as an indicator of the environmental availability of metals in food items consumed by higher-trophic-level animals (Costa et al., 2012; Yin et al., 2008). This approach was used in previous research, including birds and marine mammals (e.g. Costa et al., 2012; Orłowski et al., 2015; Yin et al., 2008). However, data reporting concentrations of essential and non-essential elements in reindeer living in the Arctic tundra is scarcely available. Previously, reindeer excrements were used to assess exposure close to the station located in Ny Ålesund (Yin et al., 2008). A low level of mercury was found, however, lead levels were unexpectedly high, as compared to the levels found in seabirds and marine mammals. Here, we examined the concentration of 30 elements, including toxic metals of environmental concern (Al, As, Ni, Cd, Pb, Hg) in samples collected from a previously not studied population in the Bellsund region of Svalbard. The key aim of this study is to evaluate the elemental composition of reindeer faeces and to contrast the concentration levels found in: 1) mesic and moss tundra; 2) seasonal excretion (winter, transitional and summer faeces), 3) the droppings of calves, females and males. We also provide information about the usefulness of older samples as opposed to fresh ones.

## 2. Material and methods

### 2.1. Study area

Field sampling was conducted between 6th and 24th of August 2016 on the marine terrace Calypsostranda (south margin of the Bellsund Fjord) and in the valley Chamberlin (south margin of the Recherche Fjord) (Fig. 1). The presence of selected metals in various components of the environment has already been identified in this area (Lehmann et al., 2016; Lehmann-Konera et al., 2018; Szumińska et al., 2018). The meteorological conditions of the study area (the south margin of the Bellsund Fjord, vicinity of the polar station in Calypsobyen) for the years 1986–2011 were as follows: mean air temperature equalled 5.0 °C, mean total precipitation = 32.4 mm and mean wind velocity = 4.3 m $s^{-1}$ (Mędrek et al., 2014).

According to Elvebakk (2005), the vegetation of the study area belongs to mesic tundra and is dominated by dwarf shrubs, flowering plants and graminoids, mainly *Luzula nivalis*. However, also bryophytes and lichens are common components of vegetation. The following species were dominant in Chamberlindalen in the dryer part of the valley: *Saxifraga oppositifolia* L., *S.axifraga cespitosa* L., *Salix polaris* Wahlenb., *Cetrariella delisei* (Bory ex Schaer.) Kärnefelt & A. Thell, *Collema ceraniscum* Nyl., *Lecidea ramulosa* Th. Fr., *Ochrolechia frigida* (Sw.) Lynge, *Stereocaulon alpinum* Laurer., *Thamnolia vermicularis* (Sw.) Schaer. Near the river flowing through the valley, in places with higher humidity, moss tundra was predominant, consisting mainly of bryophyte species, such as *Aulacomnium turgidum* (Wahlenb.) Schwägr., *Campylium polygamum* (Schimp.) C.E.O. Jensen, *Dicranum fuscescens* Turner, *Dicranum groenlandicum* Brid., *Distichium capillaceum* (Hedw.) Bruch & Schimp., *Drepanocladus fluitans* (Hedw.) Warnst., *Hylocomium splendens* (Hedw.) Schimp., *Pohlia cruda* (Hedw.) Lindb., *Polytrichum alpinum* (Hedw.) G.L.S, *Ptilidium ciliare* (L.) Hampe, *Racomitrium canescens* (Hedw.) Brid., *Racomitrium lanuginosum* (Hedw.) Brid., *Sanionia uncinata* (Hedw.) Loeske, and *Sarmentypnum sarmentosum* (Wahlenb.) Tuom. & T.J. Kop.

### 2.2. Sample collection

54 samples were collected manually to polyethylene zip bags, previously washed with deionized water. To avoid contamination, personnel taking the samples wore polyethylene gloves. After collection, samples were initially dried in the vicinity of the polar station in Calypsobyen. Subsequently, the dried samples were packed into their original zip bags, secured from potential contamination by two more zip bags and transported to the laboratory in Poland. Although all samples were collected within one month, the differences in the sample structure allow for the approximate evaluation of their origin (for further references, see Węgrzyn et al., 2018b). Samples were assessed by their appearance and divided into three groups, representing summer, winter and winter-transitional excretion. Winter faeces were characteristic pellet-shaped droppings and their age was determined (to be within the 2015/2016 season) on the basis of their compact structures, namely no clear cavities as a result of ageing, no cover of cyanobacteria layer on their surface, and their black or black-grey surfaces being shiny with no clear matt coating, which would occur on faeces older than last winter. Summer samples were compact, homogeneous pieces (Fig. 2 presents a schematic drawing with major differences included). The winter-transitional samples had features of both winter and summer faeces (pellet-shaped droppings, but irregular and with the pieces connected to one another).

Opportunistically, fresh samples were collected from individuals with the separation of male adult, female adult and calf. Herein, fresh means that samples were collected immediately after the excretion happened.

### 2.3. Laboratory analysis and QA/QC

Samples (in total 54) were freeze-dried to remove all water residues, then ground with mortar and pestle. Approximately 0.5 g was weighted to the nearest 0.1 mg, and placed in a clean Teflon vessel with 65% $HNO_3$ (Merck, Suprapur, Germany). Digestion was carried out using a high-pressure microwave emitter (Microwave Digestion System, Anton Paar). The temperature was increased from room


**Fig. 1.** Study site A - mesic tundra, B - moss tundra (map source: toposvalbard.npolar.no); fot. (Sara Lehmann-Konera): moss tundra.

temperature to 180 °C (at the rate of approximately 8 °C/min). Such conditions were maintained for 10 min. After that, the temperature was gradually reduced. Subsequently, the mineralized samples were diluted with deionized water (Millipore Milli-Q, France) to 25 mL in clean plastic flasks. To ensure quality control, blank samples were run with every batch.

A subset of the samples was analyzed with a mercury analyser by thermal vaporization atomic absorption method (NIC MA-2000). Freeze-dried samples were weighted to the nearest 0.1 mg and heat decomposed in a ceramic boat with the addition of activated $Al_2O_3$, $Na_2CO_3$ and $Ca(OH)_2$, used to eliminate substances possibly interfering with the measurement. Purified air was used as a carrier gas. The mercury concentration was expressed in μg per 1 g dry weight of droppings. Quality control included blank samples inserted between every batch of 8–9 samples, and sample runs performed in duplicate (and in triplicate when necessary). Measurement precision, presented as the coefficient of variation of the concentrations found in the duplicates or triplicates of a single sample was 3.83 (min-max 0.00–9.61). All samples tested for Hg concentrations were well above the detection limit of 0.54 ppb.

Ca, Fe, K, Mg, P, S, and Si were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES 9820 Shimadzu, Japan) and the following 22 elements: Ag, Al, As, B, Ba, Be, Cd, Co, Cr,

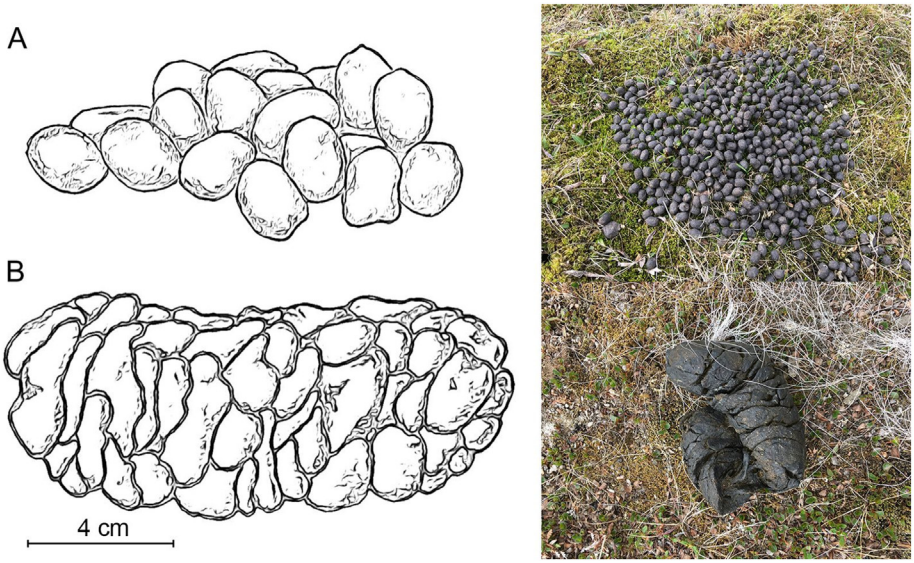


**Fig. 2.** Winter (A) and summer (B) droppings of the Svalbard reindeer.

Cu, La, Li, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, V, and Zn, were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS 2030 Shimadzu, Japan). Analyses were performed in three replicates (mean RSD 3.82% and 6.43% for ICP-OES and ICP-MS measurements, respectively). All measurement conditions and parameters are listed in Tables 1a and 1b. The results were corrected for mean value from 6 procedural blanks in the case of K, Be, Cr, La, Pb and Sb. In the rest of the samples, blank correction was not required, as background contamination was negligibly low. Both the method limit of detection (LOD) and its limit of quantification (LOQ) are listed in Tables 2a and 2b. For statistical analyses, results below the LOD were assigned a half of the LOD value. Results of the ICP-OES analysis of six samples from mesic tundra were excluded from interpretation due to detected technical problems with the instrument.

Calibration of the ICP-MS was performed with the ICP IV multi element standard (Merck, USA) and with single-element standards for As, Sb, Se, Mo and V (Sigma-Aldrich, USA). Additionally, for ICP-MS Sc, Rh, Tb and Ge in supra pure 1% HNO3 (Merck, USA) were used as internal standards. Deionized water was obtained from the Milli-Q Direct 8 Water Purification System (Merck Millipore) and applied for sample (pre)treatment and dilution.

The ICP-OES 9820 (Shimadzu, Japan) calibration was completed with single-element standards (Sigma Aldrich, USA) containing 1000 mg $L^{-1}$ of Ca, Mg, K, Fe, P, S and Si, of which calibration standard solutions were made. The accuracy of the analyses was verified by means of two certified reference materials (CRMs): Trace metals ICP - sample 1 and Trace metals ICP - sample 2. The recovery of the selected elements in both CRMs ranged from 96% to 104%.

### 2.4. Statistical methods

Samples were separated according to their location and time of origin. Ag was below LOD in 70% of all samples, thus it was rejected from statistical analysis. The analysis was performed separately for samples collected in mesic and moss tundra with the season differentiation (MeS – mesic tundra summer, MeW – mesic tundra winter, MoS – moss tundra summer, MoW – moss tundra winter). Statistical differences between groups were tested using the Analysis of Variance (ANOVA) and then by the non-parametric U Mann-Whitney test, as the normal distribution and homogeneity of variance were not achieved for all groups of samples. Statistical differences were also tested for fresh and older samples from the summer season, collected in the mesic tundra.

We employed principal component analysis (PCA) for the whole dataset as an exploratory technique to detect the closely correlated variables and to search for seasonal characteristics of the studied samples. The variables were log-transformed to bring their distribution closer to normality, although in the case of Fe, Al, Ba, Co, Cr, La, Li, Ni, Sb, V and Zn, the log-transformed dataset was still showing deviations from normality in a Q-Q plot. Due to the missing values in the dataset, the variables were divided into two groups with a different number of cases (corresponding to different laboratory analysis methods: ICP-OES and ICP-MS). The PCA was conducted separately for each of these variable sets, with the exception of aluminium being grouped together with the ICP-OES results, since its concentrations were much higher than for all other variables analyzed by ICP-MS.

The statistical analysis was performed using two software: Statistica 13.1 for ANOVA and Mann-Whitney test, and R version 3.4.4 for the PCA (*prcomp* function).

**Table 1a**
ICP-MS 2030 (Shimadzu, Japan) measurement conditions and parameters.

| Parameter and accessories | Value |
|---|---|
| Radio frequency power generator | 1.2 kW |
| Gas type | argon |
| Plasma gas flow rate | 8.0 L $min^{-1}$ |
| Auxiliary gas flow rate | 1.1 L $min^{-1}$ |
| Nebulization gas flow rate | 0.6 L $min^{-1}$ |
| Torch | Mini-torch (quartz) |
| Nebulizer | Coaxial |
| Spray chamber temperature | 3 °C |
| Drain | Gravity fed |
| Internal standard | Automatic addition |
| Sampling depth | 5 mm |
| Collision cell gas flow (He) | 6 mL $min^{-1}$ |
| Cell Voltage | −21 V |
| Energy Filter | 7.0 V |
| Number of replicates | 3 |

**Table 1b**
ICP-OES 9820 measurement parameters (Shimadzu, Japan).

| Parameter and accessories | Value |
|---|---|
| Radio frequency power generator | 1.2 kW |
| Gas type | Argon |
| Plasma gas flow rate | 10.0 L $min^{-1}$ |
| Auxiliary gas flow rate | 0.6 L $min^{-1}$ |
| Nebulization gas flow rate | 0.7 L $min^{-1}$ |
| Plasma view | Vertical torch; axial/radial view |
| Torch | Mini-torch (quartz) |
| Nebulizer | Coaxial |
| Chamber | Cyclone (glass) |
| Drain | Gravity fed |
| Injector tube | Quartz (1.2 mm i.d.) |
| Background correction | 2-points |
| Number of replicates | 3 |
| Exposure time | 15 s |

## 3. Results

Significant differences in element concentration occurred between samples derived from mesic and moss tundra (without season differentiation). Statistically significant differences (non-parametric U Mann-Whitney test $p < 0.05$) occurred for: Ca ($p < 0.0002$), Fe($p < 0.0001$), S ($p < 0.0380$), Al ($p < 0.0001$), Ba ($p < 0.0000$), Be ($p < 0.0001$), Co ($p < 0.0000$), Cr ($p < 0.0000$), Cu ($p < 0.0004$), La ($p < 0.0005$), Li ($p < 0.0001$), Mn ($p < 0.0063$), Mo ($p < 0.0036$), Ni ($p < 0.0000$), Pb ($p < 0.0239$), Sb ($p < 0.0133$), Sr ($p < 0.0000$), and V ($p < 0.0001$). Thus it suggests that differences in vegetation type growing in mesic and moss tundra and the meteorological conditions affecting the soil surface have a prevalent role in reindeer exposure assessment.

With the data divided only by season, statistically significant differences (non-parametric U Mann-Whitney test; $p < 0.05$) occurred for K ($p < 0.0024$), P ($p < 0.0001$), S ($p < 0.0016$), Cd ($p < 0.0017$), Cu ($p < 0.0044$), Mn ($p < 0.0167$), Se ($p < 0.0341$), and Zn ($p < 0.0010$).

Analyzing simultaneously by tundra type and season enables a clearer view of the whole data set. Several significant differences occurred in samples representing summer deposition from mesic and moss tundra and, in parallel, winter deposition from mesic and moss tundra (Tables 3a and 3b), with more dissimilarities occurring during the summer season. Between older and freshly collected summer faeces, statistically significant differences (Tables 4a and 4b) were found for K ($Z = -2.3372$, $p < 0.0195$), As ($Z = -2.3176$, $p < 0.0205$), Mn

**Table 2a**
Limit of detection and limit of quantification from ICP-OES [mg $L^{-1}$].

| Element | WL (nm) | LOD (3 s) | LOQ (10 s) |
|---|---|---|---|
| Ca | 396.8 | 0.005 | 0.016 |
| Fe | 259.9 | 0.001 | 0.003 |
| K | 766.4 | 0.008 | 0.026 |
| Mg | 285.2 | 0.0004 | 0.001 |
| P | 177.4 | 0.010 | 0.035 |
| S | 180.7 | 0.031 | 0.104 |
| Si | 251.6 | 0.001 | 0.004 |

**Table 2b**
Limit of detection and limit of quantification from ICP-MS [μg $L^{-1}$].

| Element | Quantified isotope | LOD (3 s) | LOQ (10s) |
|---|---|---|---|
| Ag | 107 | 0.0006 | 0.0019 |
| Al | 27 | 0.1554 | 0.5183 |
| As | 75 | 0.0128 | 0.0427 |
| B | 11[a] | 0.6734 | 2.244 |
| Ba | 138 | 0.0089 | 0.0296 |
| Be | 9[a] | 0.0003 | 0.0009 |
| Cd | 111 | 0.0016 | 0.0055 |
| Co | 59 | 0.0024 | 0.0082 |
| Cr | 52 | 0.0113 | 0.0377 |
| Cu | 63 | 0.0836 | 0.2787 |
| La | 139 | 0.0004 | 0.0014 |
| Li | 7[a] | 0.0023 | 0.0078 |
| Mn | 55 | 0.0137 | 0.0457 |
| Mo | 98 | 0.0007 | 0.0023 |
| Na | 23[a] | 0.6569 | 2.189 |
| Ni | 60 | 0.0043 | 0.0144 |
| Pb | 208 | 0.0031 | 0.0102 |
| Sb | 121 | 0.0008 | 0.0025 |
| Se | 78 | 0.0399 | 0.1330 |
| Sr | 88 | 0.0033 | 0.0111 |
| V | 51 | 0.0015 | 0.0050 |
| Zn | 66 | 0.0389 | 0.1299 |

[a] Analysis was performed without gas in the collision cell.

($Z = 2.1559$, $p < 0.0311$), Na ($Z = -3.2877$, $p < 0.0011$), Ni ($Z = 2.4793$, $p < 0.0132$), and Sb ($Z = 2.6948$, $p < 0.0071$). Finally, concentrations in calf, female and male reindeer excrements were at a similar level, except zinc and potassium (Fig. 3), although due to small sample sizes, no further analysis was performed.

To reveal a synthetic picture of the connections between variables, principal component analysis (PCA) was performed. In the variable set with the higher concentrations and with a few missing records, the two main PCs explained 67.5% of the total variability in the dataset. In the space defined by those two components, clusters related to seasons could be found (Fig. 4a), as well as clusters of similar tundra types (Fig. 4b). The further PCs (3 and 4, explaining 20.6% of the total variability), despite their potential importance suggested by the scree plot, did not exhibit any clear division with respect to season or tundra type.

Based on a scree plot, we analyzed 3 PCs for the second variable set (with lower concentrations and no missing data records; the total variability explained by the three factors was 74.9%) and marked the points on the graph according to the collection season and tundra type. The first PC, explaining 39.2% of the dataset variability, was connected to the type of environment in which the sample was collected (mesic or moss tundra, Fig. 5b). None of the principal components, however, has divided the values clearly by season (Fig. 5a).

## 4. Discussion

Svalbard Archipelago is affected by long-range-transported pollution, including multiple heavy metals (Pacyna, 1995). Contaminants may be delivered into land by wet and dry deposition, redeposited from melting glaciers, sea aerosol and biota (Wojtuń et al., 2013). Through droppings, metals excreted by fauna reach the Arctic soil. Animal droppings can serve as a biomonitoring tool, as they exhibit significant spatial differences in trace element accumulation (Yin et al., 2008). Thus, it is necessary to understand the place of reindeer droppings in the elemental cycles of this environment.

A direct source of trace metal intake for reindeer is their diet which consists of mosses, lichens and vascular plants, all of which may accumulate significant amounts of metals and metalloids (Wojtuń et al., 2013; Węgrzyn et al., 2016, 2018a). Moss is a dominant form of vegetation in the Arctic tundra ecosystems, and due to its physiology, it may easily intercept, retain, and accumulate metals from dry and wet deposition (Wojtuń et al., 2013, 2018). Some elements in the faeces, including Cu, may also correspond to the geochemical background levels in the area of interest (Yin et al., 2008). It was suggested that through their long-term interaction with lichens and mosses, reindeer have adapted evolutionarily to tolerate compounds occurring at high levels in lichens and moss, e.g. phenolic compounds or iron (Borch-Iohnsen and Thorstensen, 2009; Sundset et al., 2010). However, the interactions and effects of excess exposure to many compounds in not well known, thus it cannot be concluded yet that the high levels of elements are not harmful to their health.

### 4.1. Differences by location: mesic and moss tundra

Differences in the plant types growing in mesic and moss tundra may imply differences in metal exposure. High levels of Co, Cr, Cu, Fe, Hg, Mn, Ni, and Pb were found in moss *Racomitrium lanuginosum, Sanionia uncinata, and Straminergon stramineum* from variable wet tundra environments (Wojtuń et al., 2013). The moss tundra receives water from spring runoff and melting ice or snow, which increases the load of available metals and metalloids (Wojtuń et al., 2013). Sea aerosol is an additional source of elements including sodium, lead, mercury and cesium (Kłos et al., 2017; Wojtuń et al., 2018). Thus we suspected higher element levels in droppings collected from the moss tundra type. Since some of the elements accumulate very efficiently in mosses (e.g., Cd, Co, Cr, Cu, Fe, Mn, and Zn) (Wojtuń et al., 2013), the Zn-Cd-Cu-Mn and Mo element correlation in our studies (Fig. 5) may be explained by their dietary intake from moss tundra. Al-Fe could be correlated (Fig. 4) due to a common geological source, and be introduced to reindeer diet through rock weathering and subsequent absorption by vegetation.

Mercury contamination in polar regions has raised substantial concerns, as it may easily enter the food chain (Poissant et al., 2008). Primary sources of mercury in the Arctic are transport via air, ocean

**Table 3a**
Levels of the analyzed macro-elements [mg $g^{-1}$ dw] in reindeer droppings. Values reported are: mean ± SD (median) for groups of samples referring to their location and season. The significance level of the statistical difference between seasons (based on the U Mann-Whitney test) with p-values <0.05 is highlighted. Abbreviations: MeS – mesic tundra summer, MeW – mesic tundra winter, MoS – moss tundra summer, MoW – moss tundra winter.

| Element | Mean ± SD (median) [mg $g^{-1}$ dw] [droppings grouped by tundra type and season] | | | | | Statistical difference between mesic and moss tundra [U Mann-Whitney test; p-values] | |
|---|---|---|---|---|---|---|---|
| | Mesic tundra summer (n = 22) | Mesic tundra winter (n = 9) | Mesic tundra winter transitional (n = 3) | Moss tundra summer (n = 7) | Moss tundra winter (n = 7) | p (MeS-MoS) | p (MeW-MoW) |
| Ca | 54.9 ± 12.9 (57.3) | 58.3 ± 16.0 (61.9) | 36.1 ± 6.05 (33.3) | 23.4 ± 5.32 (22.8) | 41.8 ± 17.9 (37.8) | **0.0001** | 0.112 |
| Fe | 2.85 ± 0.76 (2.71) | 3.36 ± 0.72 (3.36) | 2.37 ± 0.61 (2.01) | 9.23 ± 3.48 (10.7) | 8.41 ± 6.50 (4.93) | **0.0002** | 0.112 |
| K | 2.55 ± 1.58 (2.18) | 0.80 ± 0.85 (0.32) | 1.57 ± 0.24 (1.45) | 1.41 ± 0.97 (0.88) | 1.18 ± 0.73 (1.37) | 0.120 | 0.397 |
| Mg | 6.37 ± 2.91 (5.33) | 3.37 ± 1.25 (2.81) | 6.15 ± 3.23 (4.88) | 6.48 ± 2.07 (6.53) | 6.33 ± 2.47 (5.76) | 0.899 | **0.015** |
| P | 3.32 ± 1.03 (3.05) | 1.88 ± 0.52 (1.55) | 2.84 ± 0.53 (2.59) | 3.10 ± 1.12 (2.62) | 2.21 ± 0.87 (1.91) | 0.558 | 0.341 |
| S | 4.63 ± 0.44 (4.65) | 4.04 ± 0.55 (3.88) | 4.29 ± 0.36 (4.14) | 4.26 ± 0.72 (4.06) | 3.67 ± 0.63 (3.58) | 0.252 | 0.397 |
| Si | 0.46 ± 0.04 (0.45) | 0.44 ± 0.06 (0.44) | 0.48 ± 0.02 (0.47) | 0.39 ± 0.08 (0.44) | 0.44 ± 0.08 (0.46) | 0.161 | 0.916 |

**Table 3b**
Levels of analyzed elements (μg $g^{-1}$ dw) in reindeer droppings: mean ± SD (median). Columns represent division by sampling location or season. The level of statistical difference between seasons (based on the U Mann-Whitney test) with p-values < 0.05 was highlighted. Abbreviations: MeS – mesic tundra summer, MeW – mesic tundra winter, MoS – moss tundra summer, MoW – moss tundra winter. Sample size listed in the table heading, except for Hg (see footnote).

| Element | Mean ± SD (median) [μg $g^{-1}$ dw] [droppings grouped by tundra type and season] | | | | | Statistical difference between mesic and moss tundra [U Mann-Whitney test; p-values] | |
|---|---|---|---|---|---|---|---|
| | Mesic tundra summer (n = 26) | Mesic tundra winter (n = 11) | Mesic tundra winter transitional (n = 3) | Moss tundra summer (n = 7) | Moss tundra winter (n = 7) | p (MeS-MoS) | p (MeW-MoW) |
| Al | 1857 ± 481 (1807) | 2080 ± 470 (2091) | 1526 ± 266 (1457) | 4188 ± 1436 (3993) | 4413 ± 3088 (2610) | **0.0002** | 0.174 |
| As | 0.737 ± 0.328 (0.660) | 0.942 ± 0.384 (0.854) | 0.60 ± 0.08 (0.57) | 1.04 ± 0.27 (1.20) | 1.042 ± 0.533 (0.913) | **0.045** | 0.856 |
| B | 14.82 ± 3.16 (14.40) | 13.9 ± 6.26 (12.2) | 14.8 ± 2.28 (13.8) | 13.1 ± 4.32 (11.9) | 12.3 ± 3.76 (10.8) | 0.523 | 0.717 |
| Ba | 38.5 ± 7.55 (38.2) | 35.4 ± 5.04 (36.9) | 33.4 ± 7.2 (28.5) | 104.8 ± 42.5 (80.0) | 121.6 ± 35.1 (124.8) | **0.0001** | **0.0006** |
| Be | 0.051 ± 0.038 (0.047) | 0.054 ± 0.016 (0.056) | 0.03 ± 0.01 (0.04) | 0.127 ± 0.051 (0.134) | 0.137 ± 0.080 (0.091) | **0.003** | **0.005** |
| Cd | 1.023 ± 0.417 (0.918) | 0.755 ± 0.340 (0.665) | 1.04 ± 0.38 (0.98) | 1.88 ± 1.20 (1.64) | 0.885 ± 0.518 (0.715) | **0.018** | 0.587 |
| Co | 0.898 ± 0.169 (0.898) | 0.699 ± 0.110 (0.717) | 0.94 ± 0.155 (0.84) | 4.79 ± 2.02 (5.03) | 3.54 ± 1.91 (3.67) | **0.00007** | **0.0006** |
| Cr | 1.849 ± 0.469 (1.82) | 2.24 ± 0.544 (2.12) | 1.45 ± 0.22 (1.41) | 17.1 ± 8.67 (23.6) | 14.1 ± 11.8 (10.8) | **0.00007** | **0.0086** |
| Cu | 8.57 ± 3.13 (9.13) | 4.62 ± 2.18 (3.42) | 9.81 ± 2.79 (9.96) | 17.2 ± 3.99 (16.0) | 10.8 ± 5.70 (10.9) | **0.0002** | **0.003** |
| La | 2.82 ± 0.650 (2.72) | 3.24 ± 0.847 (3.33) | 2.24 ± 0.34 (2.15) | 5.43 ± 1.86 (5.68) | 5.70 ± 3.39 (4.47) | **0.0001** | 0.174 |
| Li | 1.22 ± 0.434 (1.18) | 1.36 ± 0.363 (1.37) | 1.03 ± 0.26 (1.00) | 2.97 ± 1.06 (2.56) | 3.50 ± 2.74 (1.76) | **0.0028** | 0.147 |
| Mn | 241 ± 108 (227) | 141 ± 65.6 (123) | 208 ± 120 (135) | 334 ± 104 (278) | 278 ± 109 (227) | 0.050 | **0.002** |
| Mo | 0.517 ± 0.181 (0.503) | 0.548 ± 0.419 (0.354) | 0.65 ± 0.20 (0.53) | 0.896 ± 0.245 (0.834) | 0.671 ± 0.226 (0.662) | **0.0006** | 0.239 |
| Na | 164 ± 89.9 (142) | 105 ± 52.5 (79.4) | 113 ± 49.6 (115) | 112 ± 27.5 (97.7) | 133 ± 39.6 (128) | 0.708 | 0.174 |
| Ni | 2.015 ± 0.339 (1.93) | 1.72 ± 0.243 (1.76) | 2.51 ± 0.55 (2.77) | 19.04 ± 5.94 (20.6) | 13.4 ± 7.10 (12.3) | **0.033** | **0.033** |
| Pb | 4.58 ± 2.43 (3.63) | 7.58 ± 3.75 (7.92) | 2.88 ± 0.54 (2.99) | 2.47 ± 1.26 (1.85) | 3.88 ± 1.84 (4.33) | **0.0007** | 0.070 |
| Sb | 0.028 ± 0.026 (0.022) | 0.036 ± 0.043 (0.022) | 0.05 ± 0.01 (0.05) | 0.009 ± 0.011 (0.00) | 0.022 ± 0.026 (0.013) | **0.0112** | 0.205 |
| Se | 0.988 ± 0.247 (0.972) | 1.25 ± 0.325 (1.25) | 0.73 ± 0.03 (0.72) | 1.30 ± 0.457 (1.24) | 1.45 ± 0.705 (1.31) | 0.226 | 0.717 |
| Sr | 84.9 ± 53.9 (72.2) | 110 ± 80.8 (74.3) | 63.6 ± 12.4 (66.5) | 142.8 ± 36.6 (145) | 211 ± 114 (154) | **0.00007** | **0.0112** |
| V | 2.78 ± 0.709 (2.77) | 3.79 ± 1.36 (3.64) | 2.35 ± 0.28 (2.50) | 10.9 ± 5.36 (13.2) | 9.97 ± 8.37 (6.91) | **0.00007** | 0.147 |
| Zn | 153 ± 72.8 (155) | 81.8 ± 44.6 (61.5) | 165 ± 62.3 (165) | 236 ± 93.3 (246) | 115 ± 66.6 (93.7) | 0.055 | 0.239 |
| Hg[a] | 0.069 ± 0.016 (0.067) | 0.129 | – | 0.100 ± 0.028 (0.100) | 0.082 ± 0.022 (0.085) | – | – |

[a] MeS n = 11, MeW n = 1, MoS n = 6, MoW n = 5.

currents, and rivers, and subsequently dry and wet deposition (Poissant et al., 2008). Here, the highest mercury level was 0.129 μg $g^{-1}$ dw in the winter samples collected in the mesic tundra. Moss tundra dropping levels did not exceed 0.100 μg $g^{-1}$ dw. This suggests low exposure, since also the level in the vegetation described in previous studies did not exceed 0.190 μg $g^{-1}$ dw (Wojtuń et al., 2013). However, most of the mercury in the body is assimilated, and may easily biomagnify (Yin et al., 2008), thus it would not be excreted effectively with droppings.

Previous studies by Yin et al. (2008) showed a high level of lead in Arctic reindeer excrements (>11 μg $g^{-1}$), probably as a consequence of past industrial activities and leaded gasoline use (Sturges and Barrie, 1989). Although since the 1980s the usage of leaded gasoline was significantly reduced, large amounts of Pb emitted into the atmosphere were still deposited in the Arctic (Sturges and Barrie, 1989). The current atmospheric deposition of lead in the Norwegian Arctic originates mostly from Eastern Eurasia (spring input) and Northern America (summer input), with local crustal inputs playing a minor role (Bazzano et al., 2015). Here, Pb levels were significantly higher ($p < 0.0239$) in mesic tundra, with the mean concentrations of 4.58/7.58 μg $g^{-1}$ dw (summer/winter), than in wet moss tundra (2.47/3.88 μg $g^{-1}$ dw).

Multiple elements of toxicological concern were found at higher concentrations in the moss tundra in this study. The aluminium concentration in samples derived from moss tundra was more than two times higher compared to mesic tundra ($Z = -3.9181$, $p < 0.0001$). Chromium concentration was the highest during summer in the moss tundra setting, with the mean of 17.1 ± 8.67 μg $g^{-1}$ dw (mesic tundra mean ± SD 1.85 ± 0.47 μg $g^{-1}$ dw; $Z = -5.0037$, $p < 0.0000$). Also nickel

**Table 4a**
Mesic tundra summer samples: comparison of the old and freshly collected droppings (units: μg $g^{-1}$ dw; values reported: mean concentration ± 1 SD and median in brackets). Sample size listed in the table heading, except for Hg (see footnote).

| Element | Mean ± SD (median) [μg $g^{-1}$ dw]/samples grouped by the length of period between excretion and collection/ | |
|---|---|---|
| | Older (n = 17) | Fresh (n = 9) |
| Al | 1828 ± 529 (1720) | 1913 ± 368 (1861) |
| As | 0.694 ± 0.371 (0.650) | 0.819 ± 0.204 (0.782) |
| B | 15.6 ± 3.16 (15.1) | 13.3 ± 2.57 (13.3) |
| Ba | 39.2 ± 8.61 (39.1) | 37.1 ± 4.65 (37.5) |
| Be | 0.059 ± 0.042 (0.056) | 0.036 ± 0.022 (0.028) |
| Cd | 1.11 ± 0.395 (1.15) | 0.866 ± 0.411 (0.677) |
| Co | 0.931 ± 0.184 (0.944) | 0.836 ± 0.213 (0.869) |
| Cr | 1.84 ± 0.49 (1.89) | 1.86 ± 0.427 (1.79) |
| Cu | 9.40 ± 2.92 (9.69) | 7.01 ± 2.91 (5.21) |
| La | 2.69 ± 0.569 (2.56) | 3.05 ± 0.725 (3.40) |
| Li | 1.17 ± 0.484 (1.13) | 1.303 ± 0.301 (1.26) |
| Mn | 274 ± 119 (275) | 179 ± 34.7 (176) |
| Mo | 0.513 ± 0.213 (0.439) | 0.525 ± 0.095 (0.569) |
| Na | 121 ± 67.4 (85.9) | 245 ± 69.9 (247) |
| Ni | 2.13 ± 0.354 (2.06) | 1.79 ± 0.141 (1.81) |
| Pb | 4.24 ± 1.94 (3.43) | 5.23 ± 3.05 (4.56) |
| Sb | 0.037 ± 0.028 (0.027) | 0.012 ± 0.008 (0.014) |
| Se | 0.946 ± 0.206 (0.879) | 1.07 ± 0.296 (1.01) |
| Sr | 89.8 ± 65.1 (68.5) | 75.5 ± 15.6 (77.1) |
| V | 2.69 ± 0.743 (2.74) | 2.93 ± 0.609 (2.80) |
| Zn | 171 ± 69.5 (187) | 120 ± 66.9 (82.1) |
| Hg[a] | 0.068 ± 0.015 (0.075) | 0.069 ± 0.016 (0.067) |

[a] Old n = 3, fresh n = 8.

**Table 4b**
Concentration levels of elements in mg $g^{-1}$ dw. Values reported are means ± 1 SD (median in brackets).

| Element | Mean ± SD (median) [mg $g^{-1}$ dw]/samples grouped by the length of period between excretion and collection/ | |
|---|---|---|
| | Older (n = 13) | Fresh (n = 9) |
| Ca | 50.85 ± 12.7 (51.6) | 60.65 ± 10.8 (63.5) |
| Fe | 2.67 ± 0.76 (2.65) | 3.11 ± 0.68 (2.82) |
| K | 1.85 ± 1.16 (1.67) | 3.56 ± 1.55 (3.82) |
| Mg | 6.05 ± 3.20 (5.10) | 6.83 ± 2.36 (5.57) |
| P | 3.52 ± 1.16 (3.34) | 3.04 ± 0.72 (2.94) |
| S | 4.66 ± 0.53 (4.79) | 4.58 ± 0.26 (4.57) |
| Si | 0.46 ± 0.05 (0.47) | 0.44 ± 0.03 (0.45) |

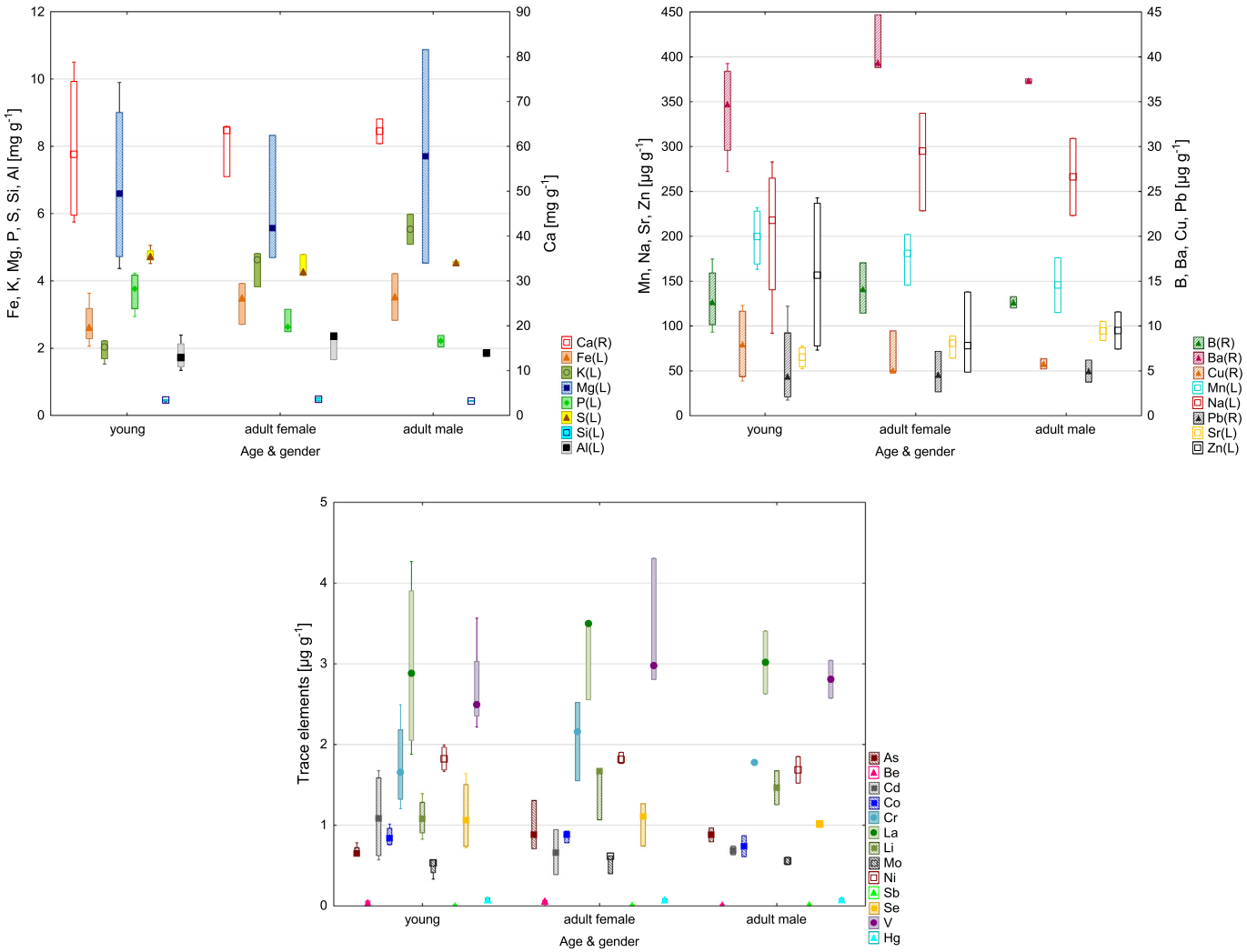


**Fig. 3.** a,b,c. Concentration levels of selected elements in reindeer calves (n = 4), adult females (n = 3) and males (n = 2). The box signifies quartiles (the point marker in the middle is median), whiskers show the full range of values. In the legend, R and L in brackets mean right or left axis of the graph. The variables for each graph were chosen depending on their concentration range, to improve the clarity of presentation.

concentration was significantly lower in the mesic tundra ($Z = -5.5169$, $p < 0.0000$). Cadmium and arsenic levels were also higher in the moss tundra type, although the difference was not statistically significant.

Among all elements, the highest concentration was found for calcium (Table 3a) in the mesic tundra, where it was much higher than in the moss type ($Z = 3.7313$, $p < 0.0002$). Magnesium, potassium and phosphorus concentrations did not differ significantly, in contrast to iron and sulphur (Fe: $Z = -4.0942$, $p < 0.0001$, S: $Z = 2.0755$, $p < 0.0380$).

### 4.2. Feasibility of comparison between the older and fresh samples

Samples collected immediately after excretion and older but excreted during summer (Tables 4a and 4b) were analyzed to compare the concentration levels between them and to assess the utility of the older sample usage. Due to several elemental concentrations departing from the assumptions of ANOVA, such as the normal distribution (in the case of As, B, Be, Na and Sr) and the homogeneity of variance (Mn, Mo, Ni, S, Sb and Sr), we explored the results with a non-parametric technique (the Mann-Whitney test). The statistically significant differences ($p < 0.05$) were found then for K ($Z = -2.3372$, $p < 0.0195$), As ($Z = -2.3176$, $p < 0.0205$), Mn ($Z = 2.1559$, $p < 0.0311$), Na ($Z = -3.2877$, $p < 0.0011$), Ni ($Z = 2.4793$, $p < 0.0132$), and Sb ($Z = 2.6948$, $p < 0.0071$). ANOVA confirmed such statement for potassium, which met its assumptions ($p < 0.0105$, $F = 7.98$), as well as possibly for sodium, manganese, nickel and antimony. Due to the small sample sizes, mercury was not analyzed. For all the other elements, the differences were not statistically significant.

Potassium (and sodium) were more abundant in fresher samples, even up to two times higher than in older droppings. The difference between samples could be caused by atmospheric precipitation, as both elements are readily washed out with water. Apart from arsenic, sodium and potassium, the differences in element concentrations favoured older samples, perhaps due to extra atmospheric deposition over time. The lack of significant differences in lead and cadmium concentrations suggests that older samples are suitable to evaluate exposure to these metals if fresh samples are unavailable.

### 4.3. Season

Within each season, distinct features of element accumulation can be seen, especially in the summer (Tables 3a and 3b). Although Svalbard reindeer are foraging for biomass mostly (Van der Wal et al., 2000), they have certain preferences in choosing foraging areas, dependent on the season (Węgrzyn et al., 2018b). Reindeer prefer open plains and wetlands in winter, whereas after April they mostly choose slopes and

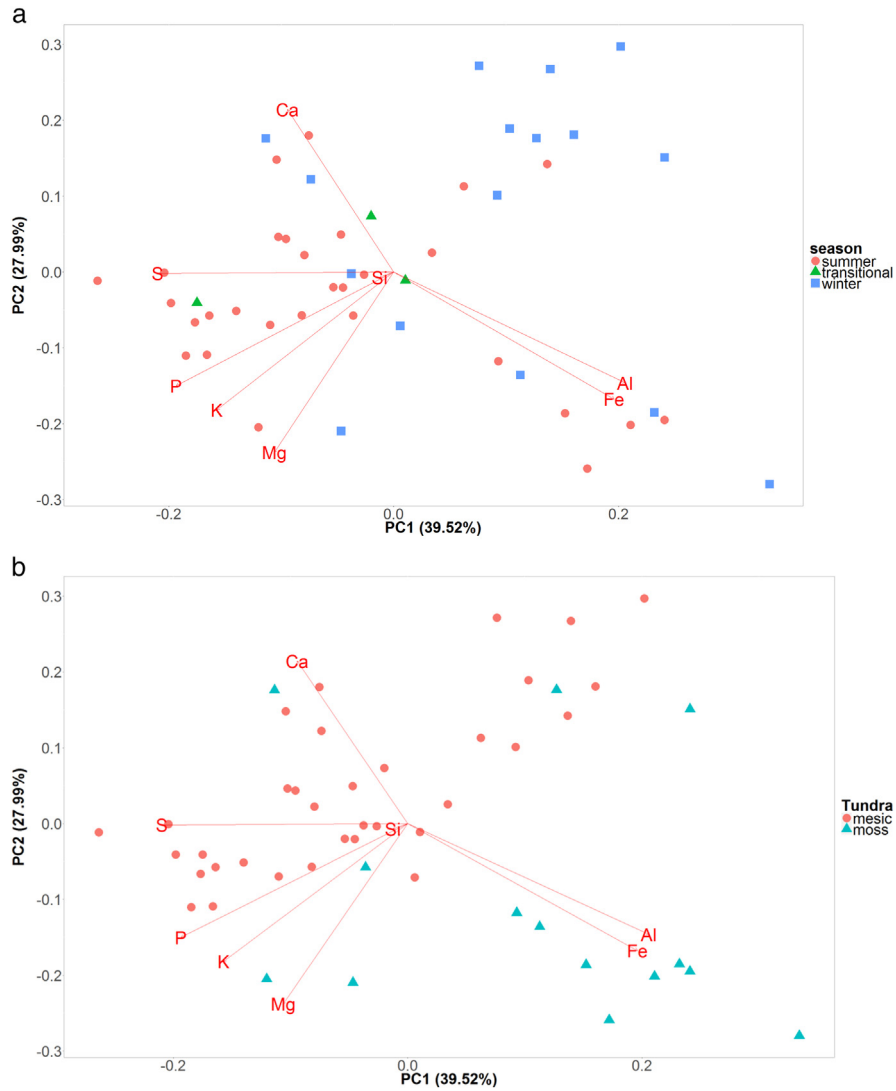


**Fig. 4.** a. PCA of the variable set with higher concentrations. The space defined for PCs 1 and 2 with points marked by season. b. PCA of the variable set with higher concentrations. The space defined for PCs 1 and 2 with points marked by tundra type.

areas with dry vegetation types (Lindner, 2002). Furthermore, food density may be temporarily reduced due to competition or snow accumulation (Lindner, 2002). Thus, the inter-seasonal differences between element levels may result from the restricted food availability and different element concentrations of various vegetation types.

Meteorological conditions shape the seasonal differences in element supply through wet and dry deposition, e.g., Bazzano et al. (2015) show the concentrations of atmospheric lead and anthropogenic sulphates were higher in the spring than in the summer. However, very few studies model seasonal deposition sums in the Arctic, so the full atmospheric influence is difficult to discern.

No inter-seasonal difference (summer vs winter) was found for zinc, sodium, boron and selenium. It may be because they are affected by metabolic regulation more than the long-range transport. In the case of sodium and selenium, sea-related emissions may also play a role (Kabata-Pendias and Szteke, 2015; Kłos et al., 2017). Further factors, such as water-soil exchange, precipitation concentration and volume may affect levels in vegetation, and thus the load of metals consumed by reindeer.

### 4.4. Comparison with previous studies

Previous research on fur samples of Svalbard reindeer showed high concentrations of lead, and relatively low mercury and cadmium levels (Pacyna et al., 2018). Yin et al. (2008) studies showed a high level of lead in Arctic reindeer excrements (>11 μg $g^{-1}$). For comparison with other reindeer species, we report data on Alaskan caribou after O'hara et al. (2003). The mean lead level in their faeces (6.18 μg $g^{-1}$ ww), although elevated, was within the normal range for cattle (2.0 to 35.0 μg $g^{-1}$ ww). Cadmium and arsenic were at the level of 0.06 and 0.14 μg $g^{-1}$ ww, respectively. Copper, zinc and iron levels were 11.06, 39.1 and 299 μg $g^{-1}$ ww, respectively.

Samples of liver, teeth and antlers of forest reindeer (*Rangifer tarandus* fennica) (Medvedev, 1995), analyzed for heavy metal and sulphur content, exhibited high lead and cadmium levels in bone tissue (41.6 ± 23.7 and 2.1 ± 1.1 μg $g^{-1}$ dw, respectively). Internal tissues were subject to studies on caribou and reindeer populations from Canada, northern Norway and Greenland (Elkin and Bethke, 1995; Robillard et al., 2002; Larter and Nagy, 2000; Aastrup et al., 2000; Ali Hassan et al., 2012). However, as element excretion is tissue-specific, it is difficult to make a concise comparison between previous research and the current study.

Due to the specific vegetation and climate of Svalbard, which results in a particular feeding behaviour and patch choice by reindeer, and the isolation of the archipelago, the Svalbard reindeer data obtained here should not be directly compared to other reindeer subspecies, such as caribou (Lindner, 2002). However, it can be noted that several elements

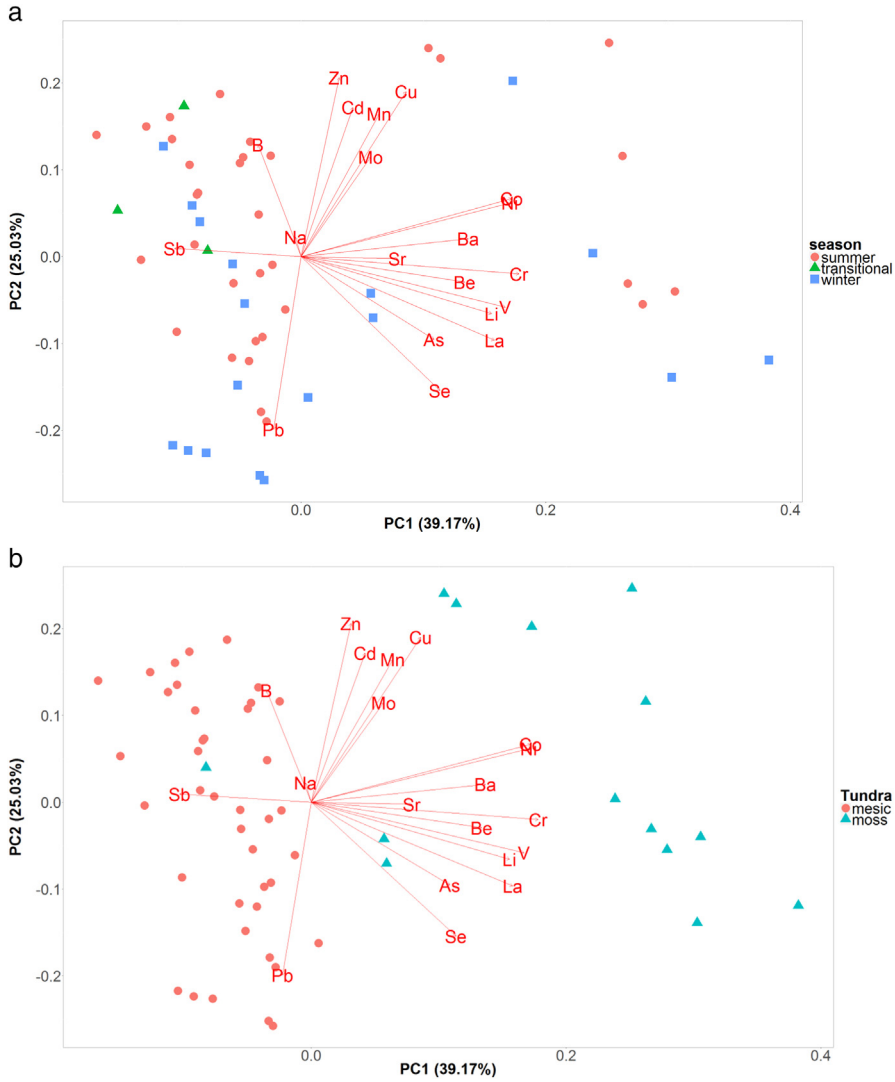


**Fig. 5.** a. PCA of the variable set with lower concentrations, analyzed by ICP-MS. The space defined for PCs 1 and 2, with points marked by season. b. PCA of the variable set with lower concentrations, analyzed by ICP-MS. The space defined for PCs 1 and 2 with points marked by tundra type.

of ecotoxicological interest, including lead and mercury, are found at analogous levels and probably come from similar sources.

### *4.5. Age and gender as an interfering factor*

With few samples assigned to calf, female and male adults, statistical analysis was not feasible, hence we present the data only in a visual form (Fig. 3). The young reindeer were still fed with mother's milk, simultaneously introducing fresh vegetation into their diet. Only for potassium, the level in calves was lower, compared to adult individuals, and in the case of zinc, it was higher. Thus, the age of the excreting reindeer can be a source of uncertainty in the present study, and further studies on more individuals should take place.

## 5. Conclusion

Various biological materials are used as environmental indicators, with special attention given recently to low-cost, noninvasive and easy-to-perform sampling procedures. Droppings are a route of elimination for toxic and excess elements, giving information on the dietary availability of compounds, the level of metal contamination and organism excretion possibilities. In the case of terrestrial species, especially those shy and endangered, it gives a possibility to monitor their population over years, without direct contact with an animal during sample collection. The distribution pattern of metals and metalloids can be affected by the season and the tundra type where the reindeer forage, thus such factors should be taken into account in the study design. Several metals, including cadmium and lead, occurred without significant differences in older and freshly collected samples. Nonetheless, the sample collection should be carried out carefully, with the description of factors affecting reindeer exposure. The presence of metals and metalloids in the terrestrial biota has potential consequences for the local biogeochemical cycle and the quality of the local environment. Since climate change may induce increased exposure from released contaminants, e.g. from melting glaciers and permafrost, we need to understand the ecosystem response to such higher exposure.

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