Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
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Mercury Levels in Svalbard Reindeer Tissue and Faeces in Relation to Diet and Season

Master's thesis in Environmental Toxicology
Supervisor: Bjørn Munro Jenssen
Co-supervisor: Malin Andersson Stavridis & Tomasz Maciej Ciesielski
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Sammendrag

Kvikksølv (Hg) er et allstedsværende giftig grunnstoff som finnes i det arktiske miljøet, inkludert i den arktiske biotaen, til tross for begrensede utslippskilder. Hg kan transporteres til fjerne områder via langt utstrakt transport via vind og hav strømmer. Den metylerte formen for Hg kan bioakkumuleres og biomagnifiseres i næringskjeden. Vi analyserte avføringsprøver fra Svalbardrein (*Rangifer tarandus platyrhynchus*) fra Colesdalen (n = 43) og Hollendarbukta (n = 25), samt nyre-, lever-, avførings-, muskel-, hjerne-, pels-, røde blodceller- og serumprøver fra avlivete (i oktober 2021) Svalbardrein (n = 18) for Hg-konsentrasjoner. Avføringsprøvene viste en økning i Hg-konsentrasjonen mot slutten av juli og begynnelsen av august 2022 (uke 32 for Hollendarbukta, uke 29 og 32 for Colesdalen), sammenlignet med juni 2022. Årsaken til økningen er ukjent, men kan skyldes lokale forurensningskilder eller sesongvariasjon, som muligens er påvirket av endringer i reinens kosthold og økt konsumsbehov i møte med vinteren.

Blant de analyserte reinvevene ble de høyeste Hg-konsentrasjonene funnet i nyrene (662,9 ± 279,6 µg/kg, d.w), etterfulgt av leveren (130,5 ± 50,5 µg/kg, d.w), avføringen (60,8 ± 17,2 µg/kg, d.w), pelsen (12,7 ± 10,9 µg/kg, d.w), muskelen (8,2 ± 6,9 µg/kg, d.w) og hjernen (1,3 ± 1,2 µg/kg, d.w). Hg-konsentrasjonene i de røde blodcellene og serumet var henholdsvis 0,4 ± 0,4 µg/kg (w.w) og 0,09 ± 0,2 µg/kg (w.w). Sammenlignet med andre arktiske pattedyr opplever reinen lave nivåer av Hg forurensning og er under nivået for å observere effekter ved forgiftning. Konsentrasjonene i organene og hvor det akkumuleres påvirkes antageligvis av kostholdets nivåer av uorganisk Hg og metylert Hg.

Det ble estimert signifikante korrelasjoner mellom Hg-konsentrasjonene i avføringen og Hg-konsentrasjonene i nyrene ($r = 0,673$, $p < 0,01$), leveren ($r = 0,735$, $p < 0,001$), hjernen ($r = 0,495$, $p < 0,05$) og muskelen ($r = 0,498$, $p < 0,05$). Disse korrelasjonene er muligens relatert til den dominante Hg arten i de ulike vevene. Vi utviklet lineære modeller for å forutsi Hg-konsentrasjoner i nyrene, leveren, hjernen eller muskelen basert på Hg-konsentrasjonene i avføringen. Dette kan være et nyttig, ikke-invasivt verktøy for overvåking av reinpopulasjoner med tanke på Hg-forurensning. Hg-konsentrasjonene i Svalbardrein i Colesdalen og Hollendarbukta ble forutsagt basert på modellene og avføringsprøver, og viste ingen risiko for forgiftning basert på risiko verdiene beskrevet i the Arctic Monitoring and Assessment Program, AMAP (2021).

Abstract

Mercury (Hg) is a ubiquitous toxic element found in the Arctic environment and biota, despite limited emission sources. Hg is transported to remote areas through long-range atmospheric transport and ocean currents. The methylated form of Hg can bioaccumulate and biomagnify in the food web, posing a health risk to wildlife. We analysed the Hg content in faeces samples from Svalbard reindeer (*Rangifer tarandus platyrhynchus*) from Colesdalen (n = 43) and Hollendarbukta (n = 25), as well as kidney, liver, faeces, muscle, brain, fur, red blood cells, and serum samples from Svalbard reindeer from Colesdalen and Semmeldalen (October 2021) (n = 18). The faeces samples showed an increase in Hg concentrations at the end of July and the start of August 2022 (week 32 for Hollendarbukta, week 29 and 32 for Colesdalen) compared to June 2022. The cause of the increase is unknown but could be attributed to local pollution sources or seasonal variation, possibly influenced by a shift in the diet of the reindeer and increased consumption requirements in preparations for the winter.

Among the reindeer tissues analysed, the highest concentrations of Hg were found in the kidneys (662.9 ± 279.6 µg/kg, d.w), followed by the liver (130.5 ± 50.5 µg/kg, d.w), faeces (60.8 ± 17.2 µg/kg, d.w), fur (12.7 ± 10.9 µg/kg, d.w), muscle (8.2 ± 6.9 µg/kg, d.w), and brain (1.3 ± 1.2 µg/kg, d.w). The Hg concentrations in red blood cells and serum were 0.4 ± 0.4 µg/kg (w.w) and 0.09 ± 0.2 µg/kg (w.w), respectively. Compared to other Arctic mammals, reindeer experience low levels of Hg contamination and remain below the threshold of toxic effects. The dietary levels of inorganic and methylated Hg presumably influence the organ concentrations and final accumulation site.

Significant correlations between Hg concentrations in faeces and Hg concentrations in kidney ($r = 0.673$, $p < 0.01$), liver ($r = 0.735$, $p < 0.001$), brain ($r = 0.495$, $p < 0.05$), and muscle ($r = 0.498$, $p < 0.05$) were estimated. These correlations may be related to the dominant Hg species in the different tissues. These correlations were used to develop linear models to predict Hg concentrations in the kidney, liver, brain, or muscle based on Hg concentrations in faeces. The models could be helpful non-invasive tools for monitoring Hg pollution in Svalbard reindeer. Based on these models and collected faeces samples, we conclude that Hg concentrations in Svalbard reindeer in Colesdalen and Hollendarbukta pose no risk of toxicity, as per the risk level determined by the Arctic Monitoring and Assessment Program, AMAP (2021).

Acknowledgement

I would like to express my sincere gratitude to all those who have contributed to completing this thesis. First and foremost, I am deeply grateful to my supervisors for their invaluable guidance, support, and expertise throughout the research process. Their continuous encouragement and insightful feedback have helped shape the direction and quality of this work. Their contribution was crucial in obtaining the data needed to address the research objectives. A special thanks to my supervisor Malin for making my stay in Svalbard truly wonderful, for being a good friend and for letting me tag along on fieldwork.

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Table of Content

Abbreviations

AIC	Akaike's Information Criterion
BBB	blood-brain barrier
BIC	Bayesian Information Criterion
Cd	cadmium
Co	cobalt
CRM	certified reference material
d.w	dry weight
DNA	deoxyribonucleic acid
HCl	hydrogen chloride
Hg	mercury
Hg(0)	elemental mercury
Hg^{1+}	mercurous
Hg^{2+}	mercuric
HgSe	tiemannite
LRT	long-range transport
MeHg	methyl mercury
MT	metallothionein
NTNU	the Norwegian University of Science and Technology
Pb	lead
PHg	particulate mercury
RBC	red blood cell/erythrocyte
RS^-	thiolate anion
Se	selenium
SH^-	sulfhydryl
THg	total mercury
UNIS	the University Centre in Svalbard
w.w	wet weight
Zn	zinc

1. Introduction

Mercury (Hg) is a natural element in the crust of the Earth (Klaassen & Amdur, 2019). It is released into the atmosphere from erupting volcanoes, forest fires, the weathering of rocks, and evaporation from soil and ocean (Kim et al., 2016; Klaassen & Amdur, 2019). Anthropogenic activities like gold mining and coal combustion contribute to emissions of Hg into the atmosphere (Klaassen & Amdur, 2019; Lavoie et al., 2013). Hg is toxic and found in different chemical forms (Berlin et al., 2015). The elemental form, Hg(0), is the reduced form of Hg and a subject for long-range transport (Tarbier et al., 2021). Hg can bind to other elements and form inorganic mercurous (Hg^{1+}) and mercuric (Hg^{2+}) salts (Klaassen & Amdur, 2019). Hg^{2+} is the primary cation of Hg in environmental settings (Bridges & Zalups, 2005). Inorganic Hg can be methylated into its organic form, methyl Hg (MeHg), by microbial or chemical processes (Celo et al., 2006). Entering the biological systems, MeHg can bioaccumulate in tissue and biomagnify in the food web, posing a risk to human and wildlife health (Klaassen & Amdur, 2019; Lavoie et al., 2013). Further, in this thesis, Hg refers to total Hg (THg; all forms of Hg combined) unless mentioned otherwise.

Hg(0) is inhaled, absorbed in the respiratory tract, and distributed to other organs via the circulatory system (ATSDR, 2022). In blood and other organs, Hg(0) can be oxidized to inorganic Hg by the enzyme catalase. Unmetabolized Hg(0) is excreted through exhalation, while metabolised can be eliminated as Hg^{2+} through urine and faeces. Hg^{2+} is mainly absorbed in the gastrointestinal tract after consumption. 1-16% of the ingested Hg^{2+} is absorbed in human adults, while for rodents, the absorption ranges from 0.4 – 42% (ATSDR, 2022). After absorption, Hg^{2+} is distributed to all tissues. The highest inorganic Hg concentrations in rats (*Rattus*) and monkeys (*Saimiri*) are found in the kidneys, followed by the liver (Berlin et al., 1969). The major elimination routes of absorbed Hg^{2+} are renal and faecal excretion, and in humans, the half-life is estimated to be 49-120 days (ATSDR, 2022). MeHg is close to 100% absorbed in the gastrointestinal tract and distributed throughout the body (Berlin et al., 1975; Clarkson, 1971). MeHg can be demethylated to inorganic Hg, making excretion more efficient (ATSDR, 2022). However, during repeated exposures, the demethylation rate does not exceed the absorption and cannot eliminate the total absorbed MeHg dose. MeHg can be eliminated through faeces, urine, and hair/fur (ATSDR, 2022). Most MeHg is found in the liver, but the highest fraction of MeHg is found in the brain, heart and spleen (Matsuo et al., 1989).

Due to the high affinity of Hg to sulphur and thiol (sulfhydryl, SH^-) groups, Hg easily binds to enzymes, transporters, and other proteins and forms S-conjugates (Berlin et al., 2015). This bond disrupts the structure of the protein and interferes with its activity and function (Berlin et al., 2015). In general, the mechanism of toxicity for Hg is common to all cells by interfering with intracellular calcium homeostasis, cytoskeleton, mitochondrial function, causing oxidative stress and deoxyribonucleic acid (DNA) methylation (ATSDR, 2022). In addition, Hg can interfere with the release of neurotransmitters (ATSDR, 2022).

Thiolate anions (RS^-) are present in almost all tissues (ATSDR, 2022). However, organs experience different toxic effects. Accumulation of Hg in the brain can cause neurodevelopmental effects at pre-natal and post-natal stages (Ekino et al., 2007; Grandjean et al., 1994). While in adults, Hg can cause neurobehavioral effects (Ekino et al., 2007). Renal toxicity includes, for instance, damage to proximal tubules and glomerular membrane and is dose- and duration-dependent (ATSDR, 2022; Pletz et al., 2016). Hg can also increase blood pressure and cause other cardiac alternations (ATSDR, 2022). Also, reproductive effects have been documented, affecting fertility and decreasing sperm number and mobility (ATSDR, 2022).

Mercury in the Arctic

The Arctic is a sink for major pollutants, including Hg, in the Northern Hemisphere (Ariya et al., 2004; Wei et al., 2022). The Arctic (as defined by AMAP (1998)) is a remote region (Yang et al., 2020), yet pollutants, including Hg and other heavy metals, are found in numerous Arctic species (see: Andvik et al., 2023; Chastel et al., 2022; Hilgendag et al., 2022; Lippold et al., 2020). The Arctic lacks significant anthropogenic Hg emissions sources. The Hg concentration in the Arctic has increased after the industrial period (starting from ~ 1850), and 92% of the Hg content in the hard tissue of Arctic animals originates from man-made contributions outside of the Arctic (Dietz et al., 2009). According to Outridge et al. (2018), the global emission of Hg to the atmosphere is 4.4 kt/year from terrestrial sources, where 2.5 ± 0.5 kt/year is from anthropogenic sources, and 3.4 kt/y from marine sources (see Figure 1).

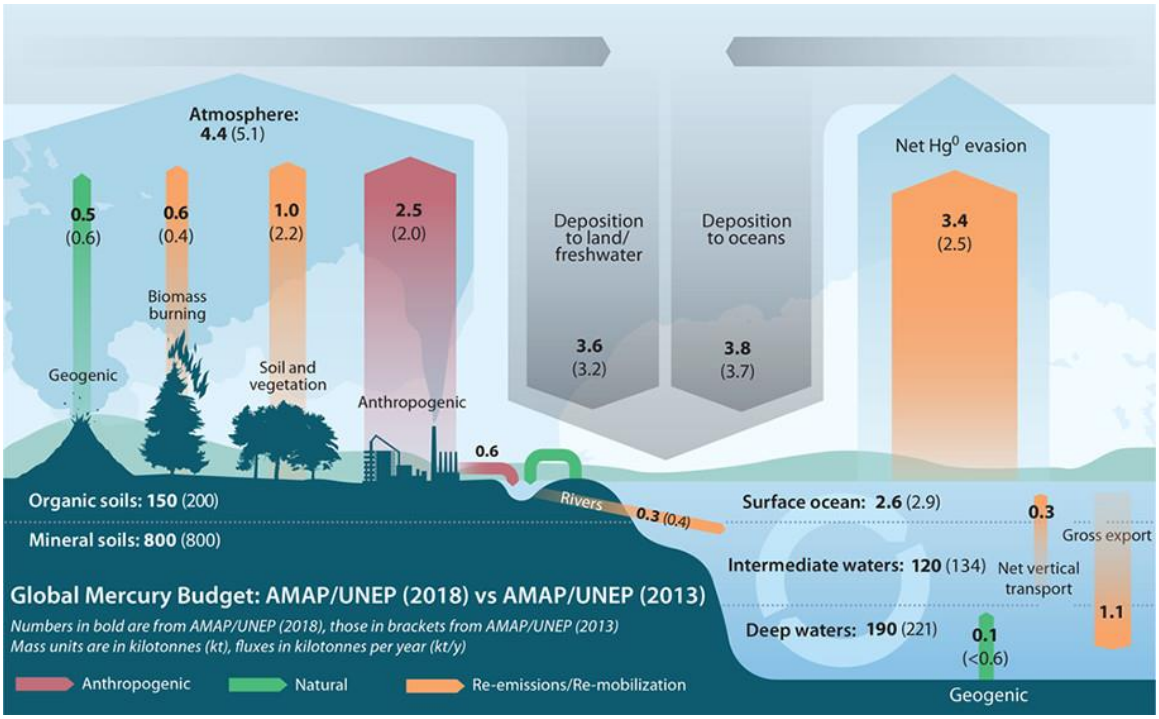

Figure 1. Global Hg budget. Green arrows represent natural Hg sources, yellow represents Hg re-emissions, and red represents the anthropogenic impact since the preanthropogenic period (before 1450 AD). Estimates from AMAP/UN Environment (2019) are in bold numbers, and estimates from AMAP/UNEP (2013) are in brackets. Mass units in kilotonnes (kt), fluxes in kilotonnes per year (kt/y). Illustration gathered from Outridge et al. (2018).

Because of the properties of Hg(0), it can migrate long distances (Tarbier et al., 2021). 98% of the Hg in the Arctic is transported via long-range air and ocean transport from sub-Arctic areas (Dastoor et al., 2022). Atmospheric long-range transport (LRT) to the Arctic during the summer is dominated by transport from Asia and North America, while Russian and European LRT events occur in the winter (Durnford et al., 2010).

Hg(0) has an atmospheric residence time of 6 to 12 months (Ren et al., 2020). Hg can deposit from the air to the surface of the Earth via either wet or dry deposition (AMAP, 2021). Wet deposition occurs as rain, snow, fog or ice and is defined as an air-to-surface flux in the presence of precipitation (AMAP, 2021). Dry deposition is air-to-surface Hg flux in the absence of precipitation (AMAP, 2021). Hg(0) can be oxidized to the ionic species, which can bind to particles or aerosols (referred to as PHg) (AMAP, 2021). Oxidized Hg species and PHg undergo wet and dry deposition, while Hg(0) is believed to dry deposit (Lindberg et al., 2007). Once deposited to the surface, Hg becomes available for methylation by bacteria in the soil and waters (AMAP, 2021). MeHg can bioaccumulate and biomagnify in food webs or be re-emitted to the atmosphere (AMAP, 2021). Biomagnification through food webs is highest in cold systems

with low productivity (Lavoie et al., 2013). Aquatic organisms in cold climates experience suppressed growth rates, restraining the effect of biodilution (Lavoie et al., 2013).

Mercury in Terrestrial Arctic Ecosystems and Biomonitoring

The atmospheric deposition of Hg to Arctic terrestrial surfaces (including aquatic areas) is calculated to be 118 ± 20 Mg/year (AMAP, 2021). Hg is stored in reservoirs such as glaciers, snowpacks, and soil. Estimates show storage of 2415 ± 22 Mg Hg in Artic glaciers and 39 Mg in the snowpack (AMAP, 2021). The Arctic soil is estimated to contain in total ~ 858 000 Mg Hg, where ~ 49 000 Mg are found in the surface soil, ~ 212 000 Mg in the active layer and ~ 597 000 in the permafrost (AMAP, 2021). This Hg can be available for vegetation or runoff into the freshwater and marine systems (AMAP, 2021; Wojtuń et al., 2013).

Atmospheric Hg has three pathways to enter the terrestrial biosphere. Hg(0) deposits over terrestrial areas and can (1) bind to soil organic matter, (2) be taken up in stomata in leaves of vascular plants/be absorbed on the surface of lichen and moss, and (3) Hg (inorganic/organic) in the soil can enter vascular plants by root absorption (Bargagli, 2016; Liu et al., 2020; Schaefer et al., 2020). Hg in the Arctic terrestrial environment can be monitored by measuring Hg levels in soil and vegetation (Wojtuń et al., 2013). In addition, biological matters, e.g., faeces samples, can be used to monitor metals in the environment (Yin et al., 2008). A relationship between Hg levels in fungi and faeces has been established for ruminant animals (Pokorny et al., 2004).

Correlation or extrapolation models are a tool to monitor the metal concentration in animals and are non-invasive if it uses tissues that are available by non-lethal collection (Treu et al., 2018). Models can be used to predict internal pollution levels, and based on established relationships, pollution levels can be extrapolated between biological matrixes (Bechshoft et al., 2019; Treu et al., 2018). Extrapolation models using fur have been created to predict Hg levels in the kidneys and liver tissue of Arctic foxes (*Vulpes lagopus*) and muscle tissue of polar bears (*Ursus maritimus*) (Bechshoft et al., 2019; Treu et al., 2018).

Reindeer (*Rangifer tarandus*) are the most abundant large ruminant herbivore in the Arctic (McKinney et al., 2022). Because of their high abundance and distribution, in addition, to be easily spotted in the terrain because of their size, reindeer are a valuable bioindicator for contaminants in the terrestrial environment (Pacyna et al., 2019). During the summer, Svalbard

reindeer (*Rangifer tarandus platyrhynchus*) consume food based on plant availability and quality, while during the winter, they are less selective (Bjørkvoll et al., 2009). In addition, Svalbard reindeer are relatively non-migratory (Tyler & Øritsland, 1989). The average home range size during the summer is 24.4 km^2 and 29.2 km^2 during the winter (Kinck, 2014), and Svalbard reindeer populations can, therefore, represent specific terrestrial regions.

This thesis aims to investigate (I) if the Hg levels in Svalbard reindeer faeces vary in different weeks during the summer season (June – August) and at different locations at Nordenskiöld Land, Spitsbergen, Norway, and (II) how Hg is distributed in reindeer tissues, based on samples from culled animals in October. The tissue Hg concentrations will be compared to other Arctic mammals in the discussion to put the results into a more holistic perspective and to better elaborate the observed distribution pattern in reindeer. This thesis lastly aims to investigate (III) if there is a correlation between faeces and other internal tissues. If any correlations are estimated, correlation models will be created, and internal Hg levels of Svalbard reindeer in the field will be predicted based on faecal Hg levels.

2. Material and Method

'Field Sampling of Faeces' was carried out in June, July, and August of 2022, where samples of Svalbard reindeer faeces were collected. 'Tissue Collection' was conducted in October 2021 and included sampling of Svalbard reindeer organs (brain, muscle, kidney, liver), tissue (red blood cells, serum), fur and faeces from euthanised reindeer. For simplification, all sample types in 'Tissue Collection' will be referred to as tissue when mentioned collectively further in this thesis.

2.1 'Field Sampling of Faeces'

Sampling Site

R. tarandus faeces samples were collected from Colesdalen (*n* = 43; 26 males and 17 females) and Hollendarbukta (*n* = 25; 21 males and 4 females) in weeks 25, 27, 29 and 32 in 2022. The sampling sites for the faeces samples are shown in Figure 2. The coordinates are listed in Table A1 in Appendix A. Fresh faeces samples were collected a few minutes after the faeces was dropped by Svalbard reindeer that were previously identified by observation with binoculars. The samples were collected using plastic gloves and transferred to plastic polyethylene zip-lock bags. The age of the reindeer (juvenile/adult) and sex were determined by binocular observation. Adult and sub-adult reindeer, excluding calves, were selected. In addition, some Svalbard reindeer were already marked or tagged as part of another research program (Research in Svalbard project; RIS-ID 2909), and the code was noted.

Faeces samples (*n* = 10) were also collected from Bjørndalen (coordinates listed in Table A1 in Appendix A). These results are not a part of this thesis but are provided in Table A10 in Appendix D.

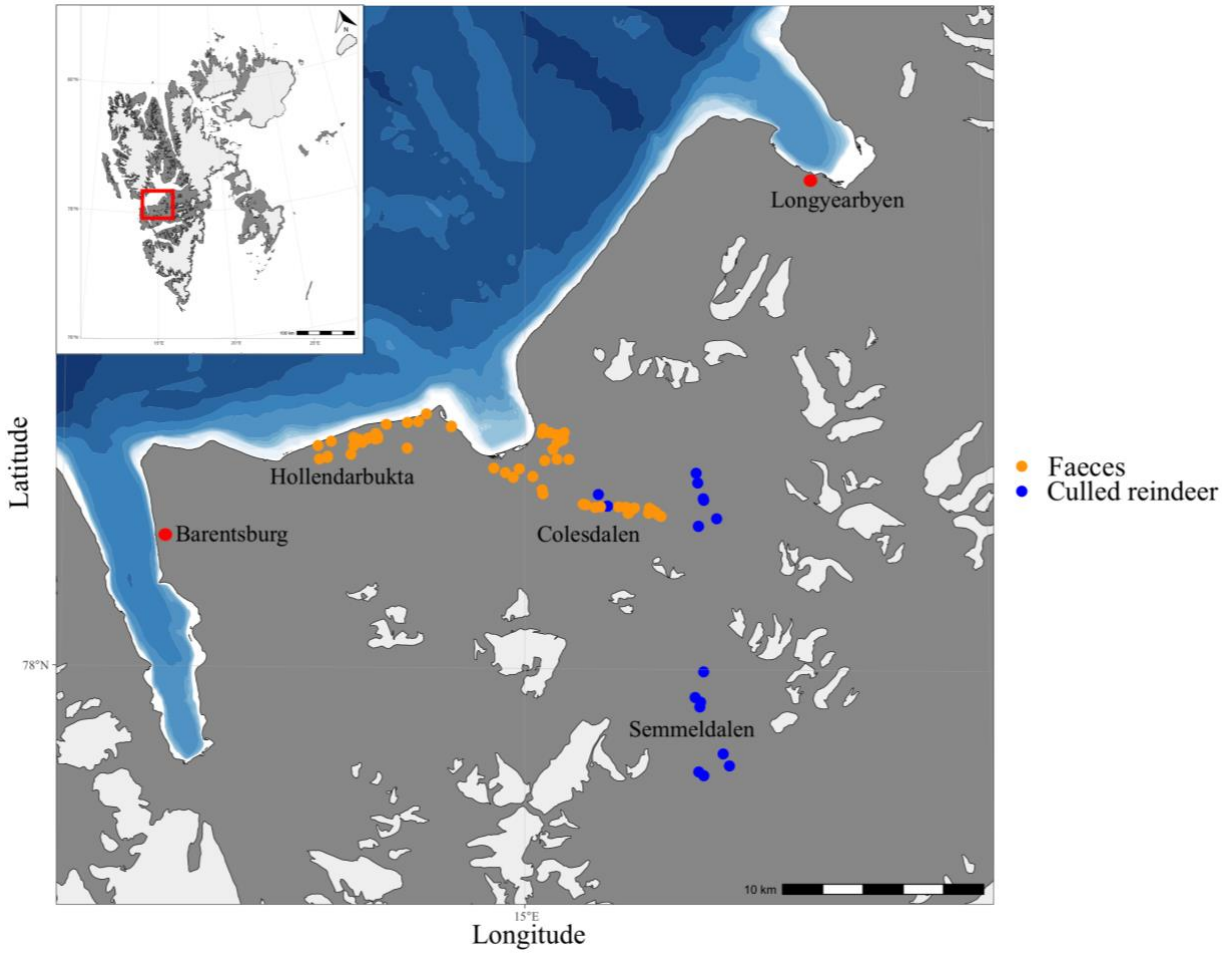

Figure 2. Sampling site in decimal degree for *R. tarandus* individuals in October 2021 (blue) and *R. tarandus* faeces (orange) in the summer of 2022. A map of Svalbard, Norway, is shown in the left corner, with a red square illustrating the fieldwork area on the island. The locations Colesdalen, Hollendarbukta and Semmeldalen, in addition to the two cities (red) Longyearbyen and Barentsburg, are given by name. Map created in RStudio using the package *ggOceanMap*.

Sample Preparation

The wet weight (w.w) of the faeces samples was recorded (Mettler Toledo, ± 0.01 g). The samples were freeze-dried for 48 hours at 0.004 mbar at -50 °C (FreeZone Benchtop Freeze Dryer, Labconco) at the University Centre of Svalbard (UNIS), and the dry weight (d.w) was recorded (Mettler Toledo, ± 0.01 g). The samples were homogenised in plastic bags using a plastic hammer.

2.2 'Tissue Collection'

Sampling Site

Collaborating Research in Svalbard projects, RIS-ID 2909 and RIS-ID 11828, were responsible for collecting the samples. RIS-ID 2909 received the permits for euthanising the reindeer from Sysselmesteren (ref: 16/01632-38).

Female Svalbard reindeer (n = 18) were euthanised in Colesdalen and Semmeldalen in October 2021. The sampling sites of the reindeer are shown in Figure 2, and the coordinates are listed in Table A2 in Appendix A. The carcasses were transported by helicopter to UNIS for dissection.

Sample Collection

Whole blood samples were collected in the field by a cut in the neck (*sternocleidomastoideus*) immediately after euthanasia. The blood was collected in paper cups, transferred to tubes (9 ml Sodium Heparin tubes, VenoSafe and 10 ml Serum tubes, Vacuette), and kept at body temperature (about 37°C). Once back at UNIS, the blood was separated into serum and red blood cells (RBCs) by centrifugation at 1000g for 10 minutes. RBCs were transferred from the Sodium Heparin tubes to Eppendorf tubes (1 ml, Eppendorf), and serum was transferred from the Serum tubes to Eppendorf tubes (1 ml, Eppendorf). All the samples were frozen at -80°C.

Back at UNIS, faeces, brain, fur, muscle, liver, and kidney samples were collected, 6-12 hours after euthanasia. Fur samples were collected from the abdomen. The animals were skinned, and the organs were cut out with stainless-steel scalpels. The outer layer of the organ was removed to avoid contamination. Samples from the medial thigh muscle (*semimembranosus*) were taken. The liver and one kidney, excluding the ureter, were sampled. The skull was opened with a saw, and the brain was removed. Brain samples from the brainstem area were collected for individuals 1-7 and 15-18. One whole half of the brain was sampled for individuals 8-14. Faecal samples were collected from the large intestine.

Sample Preparation

The wet weight of all organs, fur and faeces was recorded (Mettler Toledo, ± 0.01 g). The fur was washed with acetone and purified water (Milli-Q) in cycles and left to dry at room temperature. The samples of the organs, fur and faeces were freeze-dried for 48 hours at 0.004 mbar at -50 °C (FreeZone Benchtop Freeze Dryer, Labconco) at UNIS. The dry weight of all

organs was recorded (Mettler Toledo, ± 0.01 g). The samples were homogenised by pulverising them using a plastic hammer and further subsampled.

Prior to the analysis of Hg, the serum samples were thawed and thoroughly mixed (Vortex MS2, IKA Works, Inc) at the Norwegian University of Science and Technology (NTNU, Trondheim). The RBC samples were thawed and homogenised by ultrasonicate (Cole Parmer Ultrasonic Processor GEX 400) at NTNU. The serum and RBC were subsampled (1.8 ml CryoPure tubes, Sarstedt) for Hg analysis. The number of samples from each tissue is shown in Table 1.

Table 1. The number of samples for each tissue from *R. tarandus* from Colesdalen/Semmelsdalen in October 2021.

Tissue	**Number of samples**
Faeces	18
Brain	18
Fur	18
Muscle	18
Liver	18
Kidney	18
Red blood cells	18
Serum	14

2.3 Analysis

The samples were analysed for THg using a Direct Mercury Analyser (DMA-80, Milestone, Sorisole, Italy) at NTNU in Trondheim in October and November 2022, except from liver and kidney, which were analysed in May 2022.

The calibration curve was created by making standard Hg solutions with 2% hydrochloric acid (HCl, aq.), MilliQ water and Hg with known concentrations (see Appendix B.1 for a detailed method description). More information about DMA-80, the calibration points and equations used in the calibration curve is shown in Appendix B.1. Technical issues which occurred during the analysis are further explained in Appendix B.2.

Approximately 100 mg of a sample was used for the analysis. The producer estimated detection limit of DMA-80 is 0.0003 ng or 0.003 μg/kg, if 100 mg of the sample is used in the analysis (Milestone, 2021). MODAS–3 Herring Tissue (M–3 HerTis) was used as certified reference material (CRM) to control the accuracy of the analysis. The temperature protocol used in the analysis was according to the instructions from the manufacturer (shown in Table A8 in Appendix B.1).

One tissue type was analysed in the same run to avoid contamination across samples. All samples were run in singletons, but a triplicate of every 18 samples was run to check the variance within the samples. In addition, six blanks were analysed for each analysis, three at the start and end of each run, to control for instrumental noise and signal drift. The average of the Hg absorbance of the blank values was subtracted from the tissue/faeces samples to correct for the background Hg concentrations. This was done within each analysis, so the blanks in one analysis were subtracted from the samples that had been analysed in the same analysis run. The same procedure was done for all sample types.

2.4 Statistical Analysis

An average of the sample triplicates was used for the statistical analysis, giving one value for each sample. The normality and distribution of the residuals for the data and models were checked using scatterplots, Q-Q plots, and histograms in RStudio (R Core Team, 2021). The impact of potential outliers was reviewed with Cook's Distance, but no data points were removed. The data from 'Field Sampling of Faeces' and 'Tissue collection' were $\log_e$ - transformed (natural logarithm) to obtain normally distributed residuals. The significant level used was $p\text{-}value < 0.05$.

In the study of 'Field Sampling of Faeces', some marked animals were sampled two or three times. One/two of the duplicates/triplicates were randomly selected and removed from the data to avoid pseudoreplication. We assumed that no unmarked animals were sampled multiple times. The analysis for 'Field Sampling of Faeces' was carried out with linear regression between Hg levels (response variable) and the interaction of week and location (week*location; explanatory variables). Sex was excluded from the model since Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC) tests gave better results without sex as a variable (See Table A9 in Appendix C for the AIC and BIC results).

The distribution of Hg (in dry weight) in faeces, brain, fur, kidney, liver and muscle from 'Tissue Collection' was estimated using linear regression and illustrated with a boxplot using the *ggplot2* package in RStudio (R Core Team, 2021). All data is presented as dry weight as the water content varies among organs. Dry weight removes the influence of different water content and makes the Hg levels in the organs comparable.

The correlations between the Hg levels in the different tissues (response variable) and faeces (predictor/explanatory variable) were first tested with Pearson's product-moment correlation. Only faeces were in focus in this study, and only correlations between faeces and the other tissues were further investigated. For the tissues, which showed a correlation to Hg concentrations in faeces, linear regression models with the tissue as the response variable and faeces as the predictor variable was created using the *ggplot2* package in RStudio (R Core Team, 2021). The equation for the regression models resulted in the extrapolation model. Based on the Hg concentration reported in the faeces samples from the field ('Field Sampling of Faeces'), the internal Hg concentrations in the tissues of Svalbard reindeer were predicted. The

faecal Hg concentrations were added to the equations and solved by the natural logarithm (See Appendix E for calculations).

In multiple RBC and serum samples, the Hg concentrations were below the detection limit, and in these samples, Hg levels were set to half of the detection limit: 0.0015 μg/kg. Blood and serum were not freeze-dried, and concentrations are thus presented on a wet weight basis. The correlation between the Hg levels in faeces and the Hg levels in RBCs and serum was tested by transforming concentrations in faeces to wet weight (formula provided in Appendix E). However, no correlation was found when serum, RBCs and faeces were tested with Pearson's product-moment correlation on wet weight. Because RBCs and serum concentrations were reported on wet weight and were under the detection limit, it was removed from the results representing the Hg concentration and distribution in the other organs. However, the Hg levels are presented in Appendix D (Table A11) and discussed separately.

Because RBC and serum constitute whole blood, it was further investigated whether their distribution differed. A separate test to check the difference in the Hg concentration between RBCs and serum was conducted using Wilcoxon signed-rank test. The Wilcoxon signed-rank test is a non-parametric test between two dependent samples (here: RBCs and serum). The test was chosen because the residuals were not normally distributed, the sample size was small, and the $\log_e$ transformation did not improve the residuals of the data.

3. Results

3.1 Hg Levels in Faeces from Colesdalen and Hollendarbukta

In Colesdalen, the Hg concentrations in Svalbard reindeer faeces was stable between week 25 and 27. In weeks 29 and 32, it was a significant elevation in Hg concentrations compared to week 25 (see Figure 3 and Table A10 in Appendix D). In Hollendarbukta, the Hg levels in weeks 25, 27 and 29 were similar (about 3.6 µg/kg, $\log_e$ scale). However, a significant increase in Hg concentrations in week 32 in Hollendarbukta was observed (see Figure 3 and Table A10 in Appendix D).

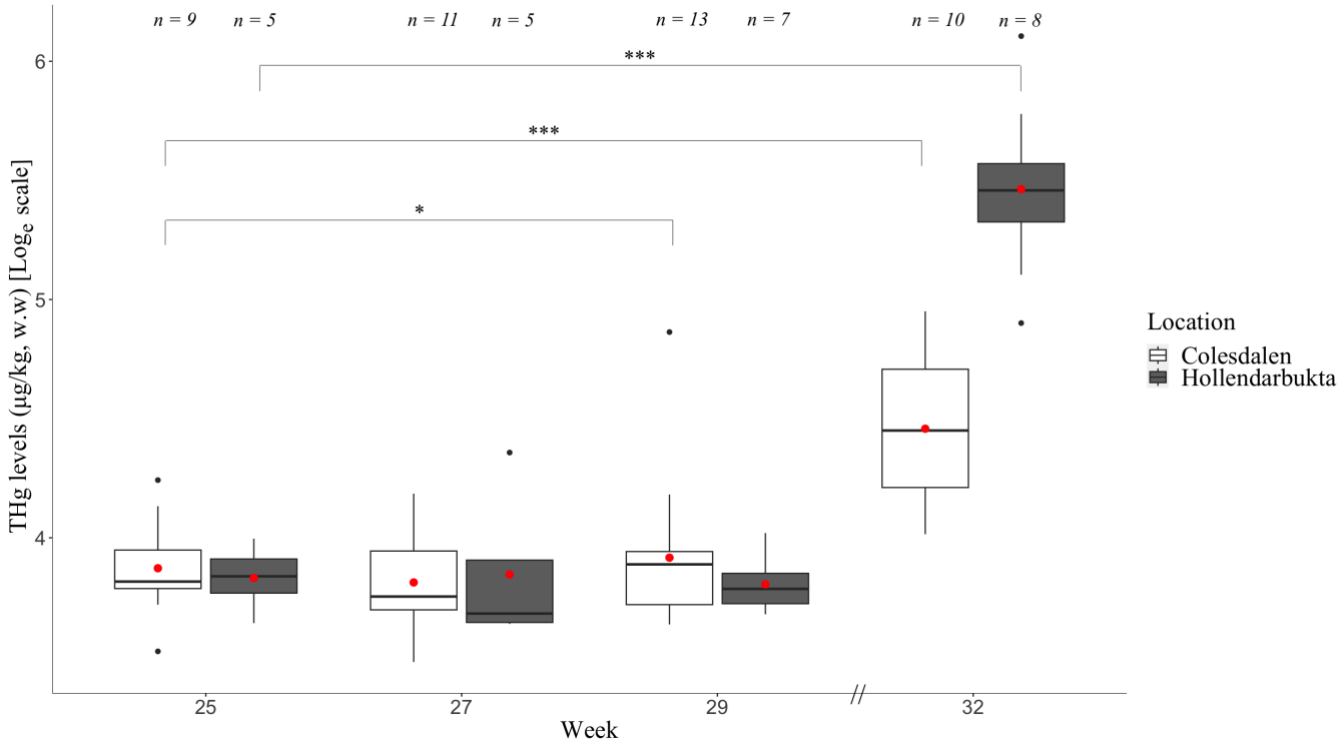

Figure 3. Hg levels (µg/kg, d.w) in *R.tarandus* faeces on $\log_e$ scale in Colesdalen (white) and Hollendarbukta (grey) in weeks 25, 27, 29 and 32 2022. The red point represents the average Hg level. *** represents the significant level $p < 0.001$, and * represents the significant level $p < 0.05$. *n* indicates the sample size of each boxplot. Note that the time difference between 29 and 32 is greater than the time difference between the other weeks.

3.2 Tissue Distribution of THg

The Hg distribution pattern in Svalbard reindeer tissues (from high to low) was kidney > liver > faeces > fur > muscle > brain and is shown on $\log_e$ scale in Figure 4. The average Hg concentration for each tissue is shown in Table A11 in Appendix D. Although Hg concentrations were somewhat higher in RBC than in serum (0.43 ± 0.43 vs 0.092 ± 0.195 µg/kg w.w: Table A11, Appendix D), there was no significant difference between the Hg concentrations in these two matrices (Wilcoxon signed-rank, $p = 0.065$).

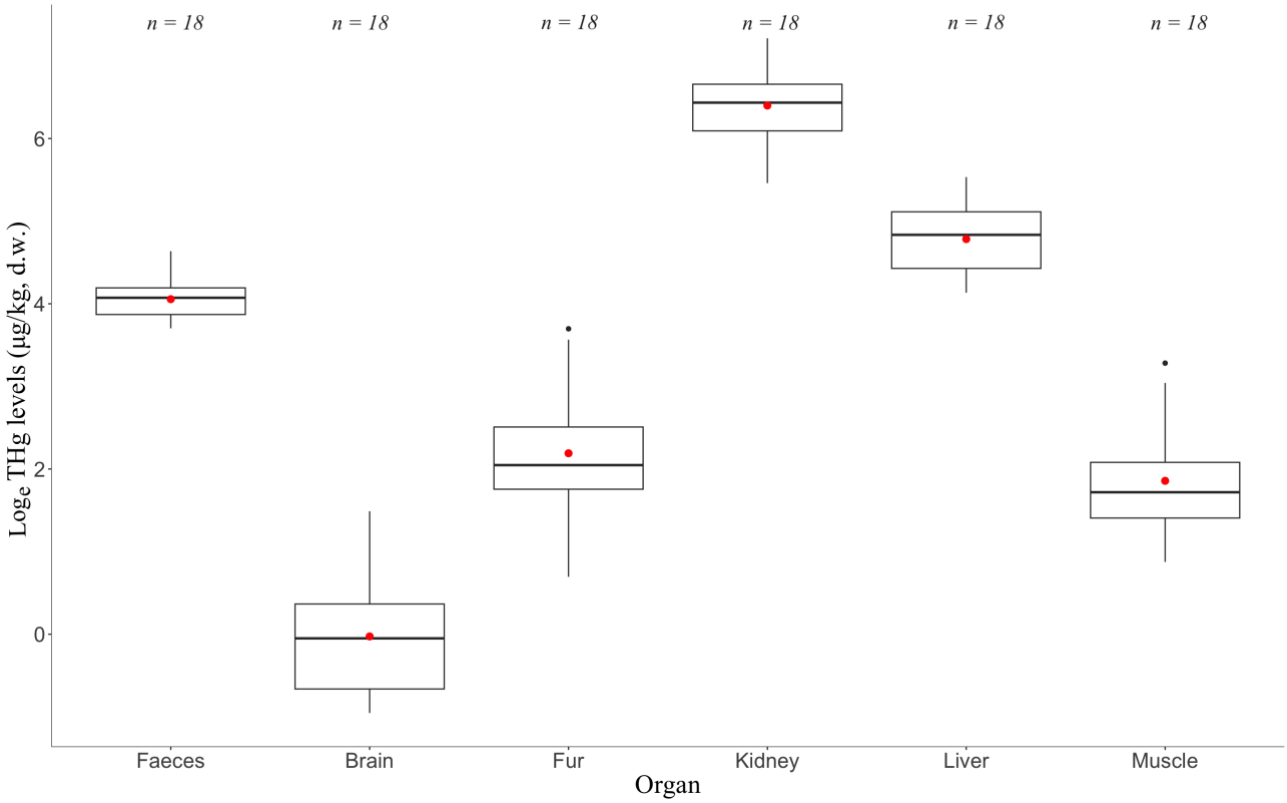

Figure 4. Total Hg levels (µg/kg, d.w) on $\log_e$ scale in tissue for *R. tarandus*. *n* indicates the sample size for each boxplot. The red points represent the average $\log_e$ THg level for each tissue; 4.073 ± 0.268 for faeces, -0.0517 ± 0.788 for brain, 2.252 ± 0.770 for fur, 6.413 ± 0.428 for kidney, 4.799 ± 0.393 for liver and 1.848 ± 0.694 for muscle.

3.3 Correlation Models

In the culled reindeer ('Tissue Collection'), the Pearson's product-moment correlations showed correlations between faeces and kidney (r = 0.673, $p < 0.01$), liver (r = 0.735, $p < 0.001$), brain (r = 0.495, $p < 0.05$) and muscle (r = 0.498, $p < 0.05$) and this data was further used to create correlation models. Linear regression for relationships between Hg concentrations in faeces and concentrations in kidney, liver, brain, and muscle are shown in Figures 5, 6, 7 and 8, respectively, with their linear equation.

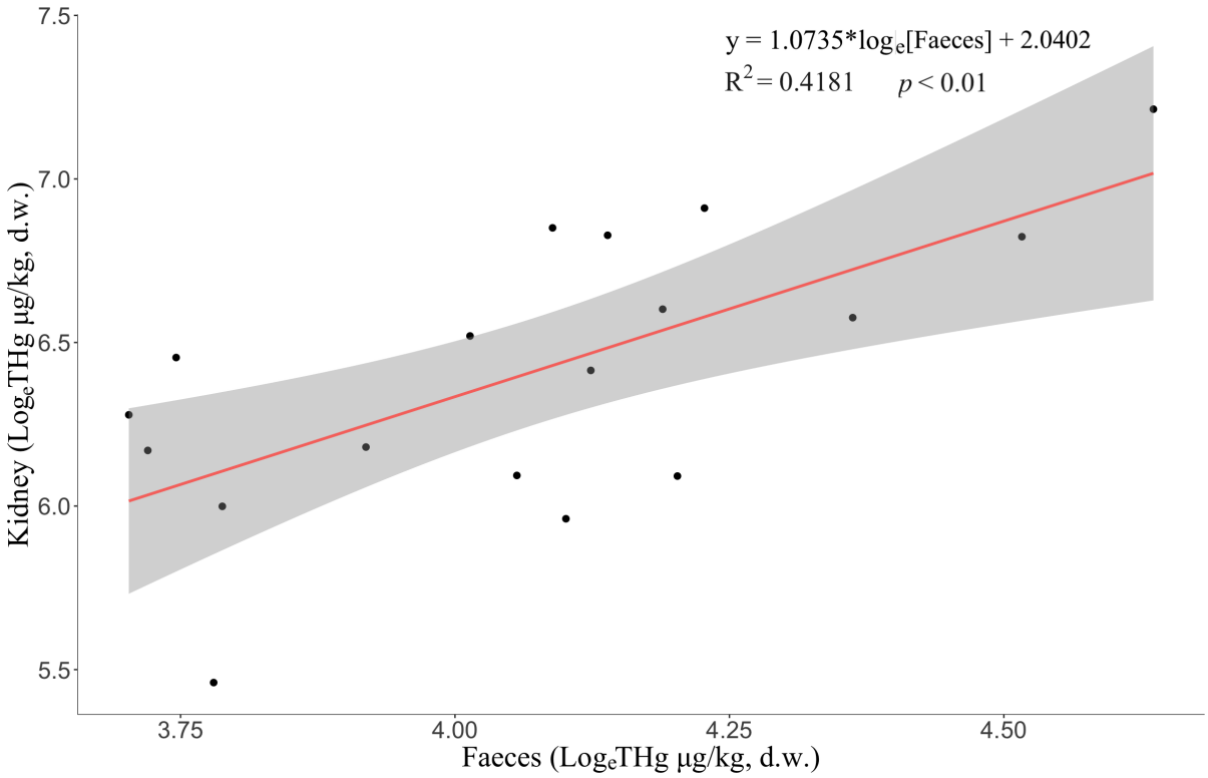

Figure 5. The linear regression (red) of Hg levels (µg/kg, d.w) between *R. tarandus* kidney and faeces on $\log_e$ scale. The grey area represents the 95% confidence interval. The equation, R^2 value and p-value for the regression are shown in the upper corner of the figure.

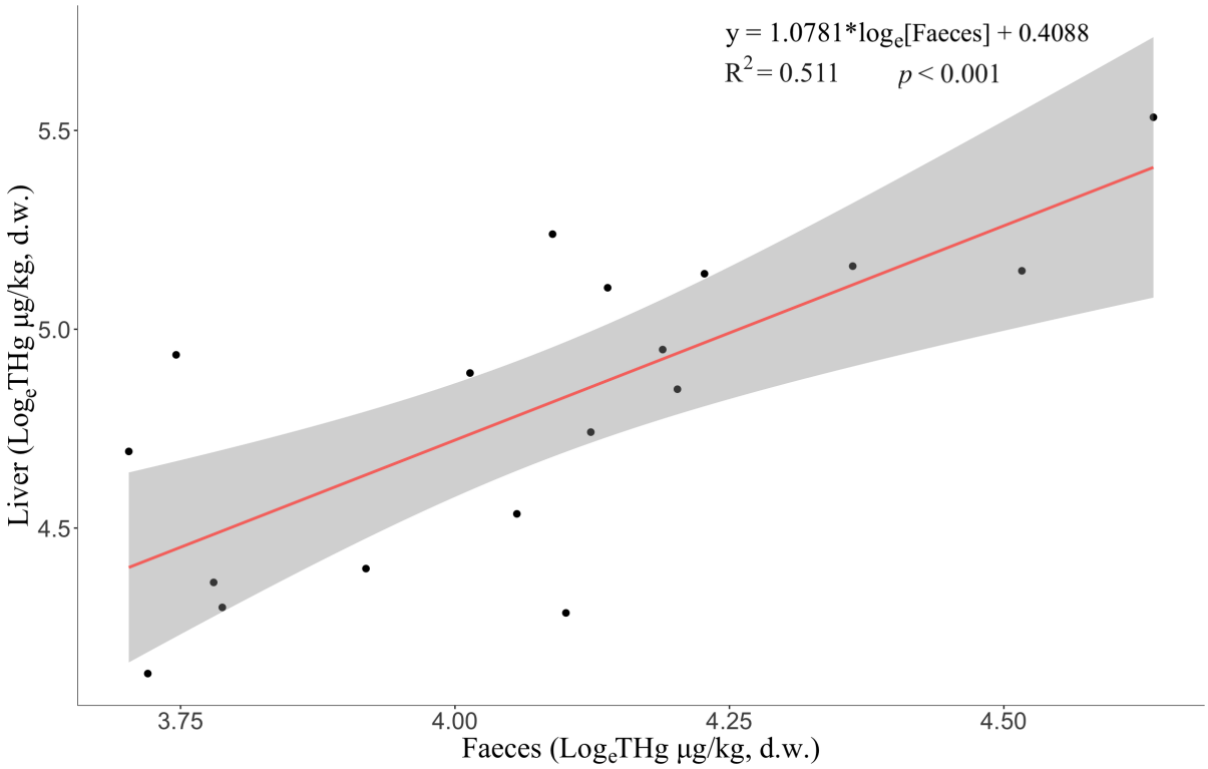

Figure 6. The linear regression (red) of Hg levels (µg/kg, d.w) between *R. tarandus* liver and faeces on $\log_e$ scale. The grey area represents the 95% confidence interval. The equation, R^2 value and p-value for the regression are shown in the upper corner of the figure.

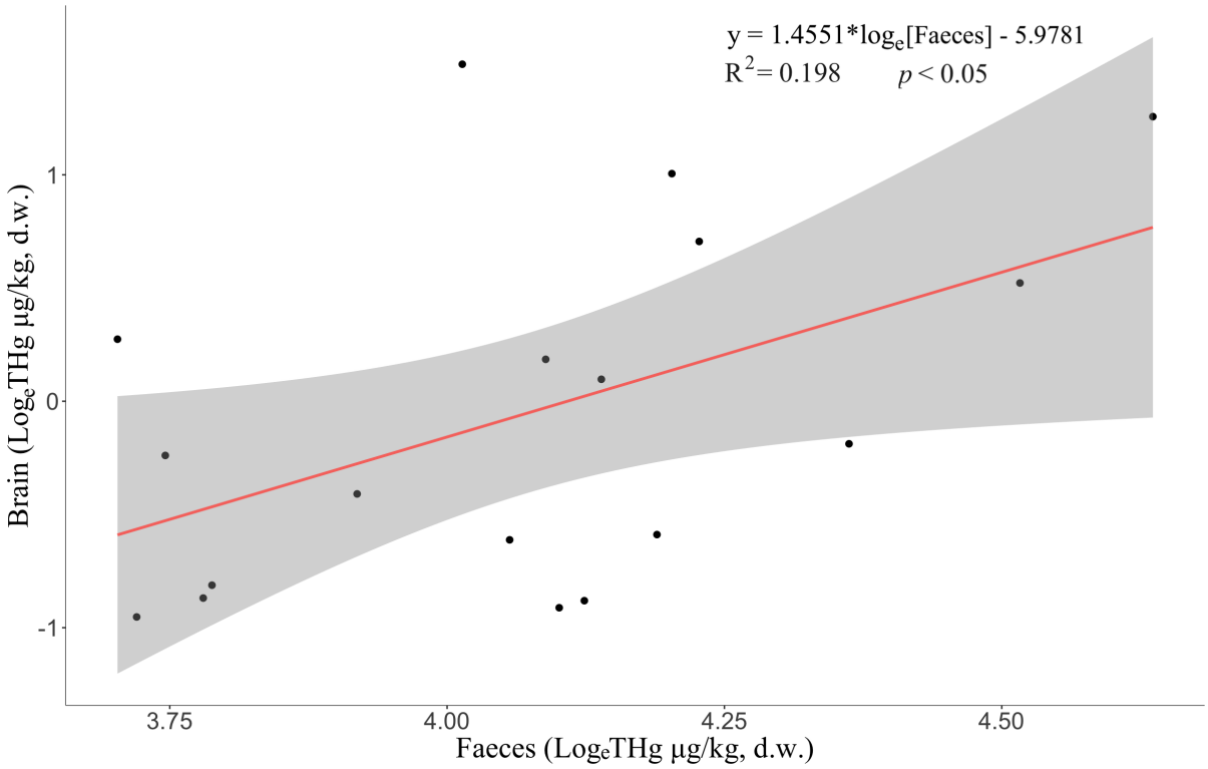

Figure 7. The linear regression (red) of Hg levels (µg/kg, d.w) between *R. tarandus* brain and faeces on $\log_e$ scale. The grey area represents the 95% confidence interval. The equation, R^2 value and p-value for the regression are shown in the upper corner of the figure.

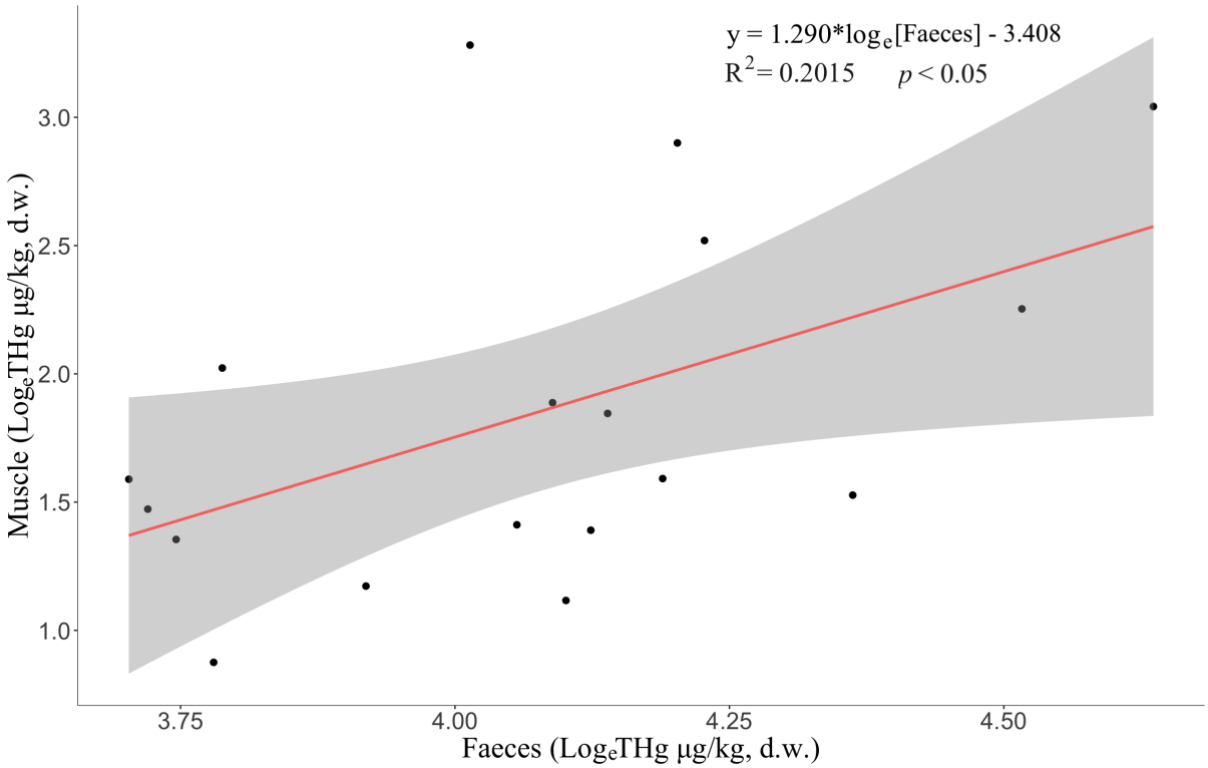

Figure 8. The linear regression (red) of Hg levels (µg/kg, d.w) between *R. tarandus* muscle and faeces on $\log_e$ scale. The grey area represents the 95% confidence interval. The equation, R^2 value and p-value for the regression are shown in the upper corner of the figure.

3.4 Predicted Internal Hg Concentrations Based on Faeces Samples from the Field

Using the equations in Figure 5 – 8, the Hg concentrations in the kidney, liver, brain, and muscle were predicted in Svalbard reindeer in Colesdalen and Hollendarbukta based on the faecal samples collected in the field ('Field Sampling of Faeces'). The results (shown in Figure 9 and Table A13 in Appendix D) showed elevated Hg concentrations in week 32 for all tissue samples in Colesdalen and Hollendarbukta.

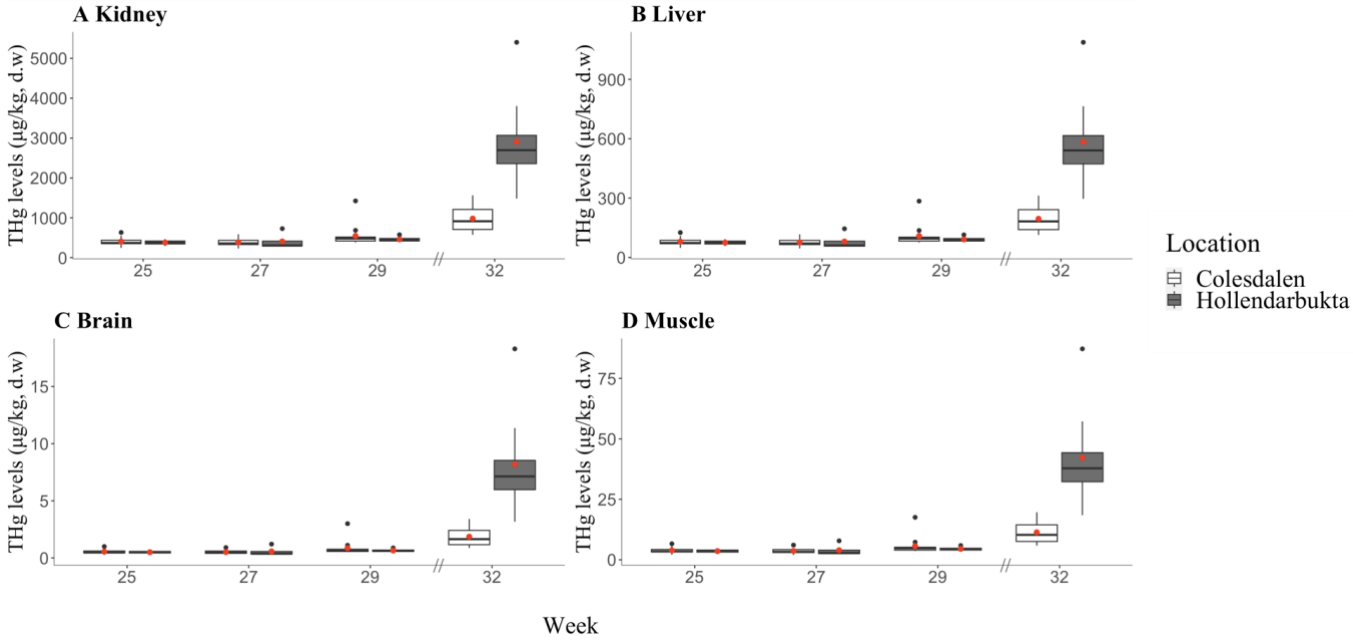

Figure 9. Predicted Hg concentrations (µg/kg, d.w) in the kidney (A), liver (B), brain (C) and muscle (D) tissue for Svalbard reindeer in Colesdalen and Hollendarbukta, based on faecal Hg concentrations. The red point represents the average Hg concentration.

4. Discussion

Hg levels in Faeces from Colesdalen and Hollendarbukta

Local Pollution Site

Based on the results from the faeces samples from Colesdalen and Hollendarbukta (Section 3.1), elevated Hg concentrations occurred in week 32. Comparing Hollendarbukta in week 25 and week 32, week 32 had significantly elevated Hg levels. Samples from Colesdalen in week 32 also had significantly elevated levels compared to Colesdalen in week 25. However, it seems to be local differences between Colesdalen and Hollendarbukta due to the high levels observed in Hollendarbukta in week 32. In the study of Pacyna et al. (2019), Hg levels in the faeces of Svalbard reindeer were 67 μg/kg d.w (0.067 μg/g d.w) and 100 μg/kg d.w (0.1 μg/g d.w) in mesic and moss tundra, respectively. The sampling in that study occurred in the areas of Calypsostranda and Chamberlin in August 2016. The levels resemble the Hg levels detected herein in Colesdalen and Hollendarbukta in weeks 25, 27 and 29. The fact that these regions in Svalbard, about 60 km in airline apart, detected almost the same Hg levels in reindeer faeces suggests that the increased Hg levels in week 32 may be due to local pollution. Since elevated Hg concentrations were observed in both Hollendarbukta and Colesdalen in week 32, it could potentially be a common source of pollution. The faecal Hg levels in Colesdalen week 32 were lower than in Hollendarbukta week 32, and the source of pollution might be closer to Hollendarbukta but still affect Colesdalen.

Hollendarbukta is closer to Barentsburg, a Russian town in Svalbard, than Colesdalen is (see Figure 2). It is known that mining occurs in the area (Duda et al., 2022), and the heat power plant operates year around (Lebedeva et al., 2018). The average Hg concentration in the snow cover in the area around Barentsburg was 0.003 μg/L in the period of 2003-2010, while the reference background sites were 0.001 μg/L (Demin et al. (2011); Russian reference in Lebedeva et al. (2018)). Hg can be deposited from the atmosphere to the environment by deposition, and wet deposition of Hg is most dominant during the summer months (AMAP, 2021). Thus, Hg could potentially be transported from Barentsburg by air and further dry or wet deposit to the study sites. Unfortunately, no precipitation or wind direction data is available for Barentsburg.

One should be aware that if a deposition event would occur, it would be a delayed timeframe from the exposure event to the detection of Hg in the faeces, when interpreting the data. Hg can

deposit into freshwater sources or exchange with vegetation (Dastoor et al., 2022). 7 days after a one-time intraruminal injection dose of 500 μCi ^{203}Hg, 25.32% of the Hg dose was removed through faeces in Guernsey cow (*Bos primigenius taurus*) (Sell & Davison, 1975). Thus, Hg levels observed in the faeces samples in Colesdalen and Hollendarbukta could thus reflect approximately 25% of a pollution event, potentially from Barentsburg, that occurred one week prior.

Seasonal Variation

The Hg levels in Colesdalen were not only significantly elevated in week 32, but also in week 29 compared to Colesdalen in week 25. However, the Hg concentrations in Hollendarbukta in week 29 were not significantly elevated compared to Hollendarbukta in week 25. The increase in both weeks in Colesdalen indicates that the elevated Hg concentrations might not be due to local pollution but rather an increase of Hg at the end of the summer months. A dietary shift, i.e., consumption of other plant species due to seasonal differences in growth and presence, can potentially explain the elevated Hg levels. As the introduction describes, atmospheric Hg has three pathways to enter the terrestrial biosphere, including absorption through the roots and stomata in the leaves (Liu et al., 2020; Schaefer et al., 2020). Once absorbed, Hg is translocated to different parts of the plant; leaves and wood (Schaefer et al., 2020). The Hg accumulation potential varies among plant species (Liu et al., 2017), resulting in some species containing more Hg.

The growth season for Arctic vascular plant species has different lengths and start times (Kelsey et al., 2021). The timing of life-cycle events is called plant phenology (Barbour et al., 1987). The phenological stages are controlled by genetics and depend upon abiotic factors, such as the amount of growing degree days (McMaster, 1997). One degree day is when the daily temperature is at least one degree above the lower developmental threshold (Miller et al., 2001). The phenological state of a plant can impact the body weight of a ruminant, as in the early stages of the phenology, the plant contains higher nutritional qualities in terms of energy and protein (Mysterud et al., 2001). Accordingly, reindeer consume plants based on plant availability and quality in terms of nutritional state (Bjørkvoll et al., 2009). The Hg levels in the plant vary during the different phenology stages (vegetation, reproduction and senescent), where plants in the reproductive stage experience the highest Hg concentrations (Anjum et al., 2013).

In addition to the phenology of the plants, the digestibility of a plant species changes during a season and influences the feeding behaviour of reindeer, where plants that are easily digested are preferred (Staaland, 1985). Polar willow (e.g. *Salix polaris*) is easier digestible for reindeer at the start of the summer compared to the end and is, therefore, more consumed at the beginning of the summer season (Staaland, 1985; Staaland et al., 1983). Svalbard reindeer also consumes moss (Bjørkvoll et al., 2009; Staaland, 1985), which has low digestibility making less nutrition available and is not the preferred feed (Staaland, 1985). However, in early winter (October), Svalbard reindeer consume more moss than in summer (Bjørkvoll et al., 2009). Moss contains more Hg than vascular plants (Wojtuń et al., 2013). It is documented that Svalbard reindeer also eat moss in August (Staaland et al., 1983). A shift from dominantly eating one type of plant to a new one, and potentially moss, might be the reason for the elevated Hg levels observed in week 32.

Svalbard reindeer feeding activity increases during the summer, with the highest activity in August (Loe et al., 2007). Eating during the summer, when more plants are available, is a strategy to increase the fatty layer to tolerate the harsh winter months (Nilsen, 1985; Tyler, 1986). As the plant ages, the protein level decreases (Staaland, 1985). This occurs at the end of July and in August (Staaland, 1985). Therefore, it is possible that reindeer increase their daily feed intake in these months to cover their nutritional needs, which would increase their exposure to Hg, as observed in weeks 29 and 32.

To summarise, the Hg accumulation potential in plants varies (Liu et al., 2017) and might also include Arctic plants. The Hg levels differ depending on the phenological stage of the plant (Anjum et al., 2013), and Arctic plants start their growth season at different times (Kelsey et al., 2021). The elevated levels observed during the late summer might therefore be explained by reindeer eating plants with higher Hg concentrations due to different accumulation potentials and/or eating plants that were in their reproductive stage. If the plants are in their reproductive stage at the end of July/start of August would depend on the phenology of the plant. However, the digestibility of the plant would influence the plant preference of the reindeer (Staaland, 1985). The exposure rate of Svalbard reindeer depends upon the feeding activity, which increase during the end of the summer (Loe et al., 2007).

Ruminant animals, such as Svalbard reindeer, consume soil matter as they graze near the ground (Johnsen & Aaneby, 2019; Staaland, 1985; Thornton & Abrahams, 1983; Tóthová et al., 2006). Hg ground levels have been found to increase during the summer, which could affect Hg levels in plants and soil. Angot et al. (2016) found atmospheric Hg(0) concentrations at ground-based Arctic sites (including Ny-Ålesund, Svalbard) to be highest during the summer (June-August). Based on the data from the study of Angot et al. (2016), Ny-Ålesund had the highest Hg levels in July (2011, 2014, 2015) and August (2012, 2013). Hg stored in vegetation and soil predominately derives from atmospheric Hg(0), and the uptake is enhanced during the summer (Obrist et al., 2017). Increased Hg(0) deposition during the summer, as documented by Angot et al. (2016), can therefore result in increased Hg levels in plants consumed by reindeer.

Specific reasons for elevated levels of Hg at Artic ground sites in the summer months are unknown and environmental processes driving the interannual variations of Hg need more research (Angot et al., 2016). Studies have shown a positive correlation between increased air temperature and Hg levels in polar bears (Morris et al., 2022) and ringed seals (*Pusa hispida*) (Houde et al., 2020) in the Arctic region. Increased temperatures will affect the Hg levels in terrestrial systems by increasing the summer growing season (Tagesson et al., 2012), causing changes in the precipitation patterns (Bintanja & Andry, 2017), including increased summer wet deposition (AMAP, 2021), and by thawing permafrost (Tarbier et al., 2021). This could eventually increase the terrestrial Hg levels in the Arctic environment and exposure to Svalbard reindeer. Further research is required to confirm whether the elevated Hg levels in reindeer faeces are due to seasonal change, a local pollution site, or a combination of both.

Svalbard Reindeer Faeces as a Biomonitoring Tool

Faeces samples collected in the field can pose as a potential tool for biomonitoring. Monitoring faecal samples is a known method for monitoring environmental levels of contaminants (Yin et al., 2008). The study of Roggeman et al. (2013) investigated metal concentration in soil, plant and in fur, blood and faeces in cows (*Bos taurus*) to assess how metals are transferred from soil to plant and further to animals. A relationship between soil and plant was established for cadmium (Cd), cobalt (Co) and zinc (Zn), and a relationship between lead (Pb) levels in plant and cow faeces. Unfortunately, the Roggeman et al. (2013) study did not include Hg.

Moreover, a relationship between Hg concentrations in plants and ruminants has been demonstrated in the study of Pokorny et al. (2004), where Hg concentrations in fungi and faeces of roe deer (*Capreolus capreolus*) correlated. However, one should be aware of species differences concerning their potential to excrete Hg and dietary preferences. Furthermore, a soil-to-plant relationship for Hg has been found between Arctic soil and *Salix polaris* (Wojtuń et al., 2019). However, if a relationship between *Salix polaris* and Svalbard reindeer, representing a plant-to-animal relationship in the Arctic environment, occurs is unknown.

When considering plant-to-animal relationships, one should be aware of the risk of overestimating vegetational metal concentrations. The correlation between Pb levels in plants (d.w) and cow faeces (d.w) showed metal levels 2-3 times higher in faeces (Roggeman et al., 2013). This elevation of Pb in faeces compared to plants should be considered to avoid overestimation when using faeces to monitor metal levels in vegetation (Roggeman et al., 2013). According to Araya Piqué (2023) the Hg level in *Salix polaris* Colesdalen during the summer of 2022 was approximately 12 ug/kg d.w. Compared to the faecal level in Svalbard reindeer, about 40 ug/kg d.w, the Hg levels in faeces are about 3 times higher. Notably, the faecal metal concentrations reflect cumulative Hg concentrations from ingested food, drinking water and consumed soil and should be considered when interpreting the results from large herbivores (Roggeman et al., 2013). More research is needed to confirm if a relationship among Hg concentration in soil, plant and Svalbard reindeer occurs to be further able to use Svalbard reindeer faeces as a bioindicator for Arctic environmental Hg concentrations.

Hg Tissue Distribution

The distribution pattern for Hg in Svalbard reindeer was found to be kidney > liver > faeces > fur > muscle > brain (see Figure 4). No previous studies have assessed the Hg distribution in multiple tissues in reindeer.

A study on the Hg distribution in cow (*Bos primigenius taurus*), another ruminant animal, found a similar pattern with the highest concentrations of radioactive ^{203}Hg in the kidney > liver > muscle > brain > skin and hair (Sell & Davison, 1975) (only relevant tissues are presented, see reference for more tissue types). The pattern between these two ruminant animals is similar, however, reindeer experience higher concentrations in fur/hair than cows. Svalbard reindeer have a thick winter coat to protect themselves against the cold winters (Pedersen et al., 2019), potentially influencing the different Hg distribution between the species (more information in *Fur* in this section).

Tissues

In general, the data has a lot of variability, which is most likely due to individual differences in both dietary exposure and excretion ability. In addition, the exact age of the reindeer has not been accounted for and might influence the variance (Verdouw et al., 2011). The within-sample variance for each tissue type is however low, see Table A11 in Appendix D.

Further in this section, the distribution is discussed, trying to answer why the concentrations vary among organs. The Hg levels are compared to other reindeer if data is available. In addition, the Hg levels in reindeer are compared to the Hg levels in other Arctic mammals to put the concentrations into a holistic aspect in relation to Arctic pollution. Relevant mechanisms involved in the distribution of Hg are brought to attention. Both MeHg and inorganic Hg are discussed, as it is relevant for the Hg pattern distribution and diet composition. For limitation purposes, elemental Hg is excluded from the discussion.

RBC and Serum

Blood and the circulatory system transport Hg from the absorption site to the target site (Carrier et al., 2001; Farris et al., 1993). The results for RBCs and serum in the present study showed low Hg levels, 0.428 ± 0.434 μg/kg w.w and 0.0918 ± 0.194 μg/kg w.w, respectively. Whole blood concentrations of Hg in polar bears from the Southern Beaufort Sea were 52 μg/kg w.w

(0.052 μg/g w.w, median) (Cardona-Marek et al., 2009), which are substantially higher than the levels in Svalbard reindeer. Hg levels in whole blood reflect the diet (Bjermo et al., 2013; Fuchs et al., 2023). Therefore, the difference in Hg levels can be explained by polar bears being carnivores while reindeer are herbivores. Polar bears are in a higher trophic level, feeding on prey with higher Hg concentrations as Hg biomagnifies (Dietz et al., 2000).

The Wilcoxon signed rank test did not show a significant difference between RBCs and serum. However, it was close to significant ($p = 0.065$). The sample size was small, and samples were outside the limit of the detection range of DMA-80. In blood, the concentration of Hg rapidly decreases after exposure, indicating the uptake of Hg by cells (Bridges & Zalups, 2017; Weed et al., 1962). Hg in whole blood is mainly absorbed by erythrocytes (RBCs) (Cember et al., 1968). MeHg is shown to accumulate in erythrocytes (Neathery & Miller, 1975). The levels of Hg found in the reindeer RBCs might, therefore, represent MeHg concentrations. If so, this could explain the low levels of Hg, as reindeer are not feeding on plants with high MeHg levels (Moore et al., 1995). In addition, the rapid absorption of Hg by cells could explain why the serum and blood levels of this study were low.

Kidneys

The highest Hg levels found in the studied reindeer tissues were in the kidneys (see Figure 4). One function of the kidneys is to filter metabolic waste products from the blood into the urine (Klaassen & Amdur, 2019; Marieb & Hoehn, 2016). The blood is transported to the glomerulus, where it gets filtered through the nephrons to remove waste products from the blood and transport it into the urine (Marieb & Hoehn, 2016). During this process, Hg is taken up by the proximal tubular cells (ATSDR, 2022; Zalups, 1993).

The results showed an average renal Hg concentration of 650.93 ± 279.62 μg/kg d.w (140.16 ± 58.73 μg/kg w.w) in Svalbard reindeer. In the study of Gamberg et al. (2020), the renal levels of reindeer in Canada and Greenland were 1400 μg/kg d.w (1.4 μg/g d.w) and 1900 μg/kg d.w (1.9 μg/g d.w) of Hg for male and female, respectively. Svalbard reindeer generally do not eat lichen (Bjørkvoll et al., 2009), which is common for reindeer at other locations (Lokken et al., 2009). Lichen accumulates atmospheric Hg and contains higher Hg levels than vascular plants (Monaci et al., 2022; Steinnes & Krog, 1977; Wojtuń et al., 2013), which may explain the different Hg levels among the reindeer.

The difference in Hg levels between the population at Svalbard and Canada/Greenland can also be explained by the local environmental differences in Hg levels. Hg levels in polar bears are higher in the Canadian High Arctic and North-western Greenland than in other Arctic regions, including Svalbard (Dietz et al., 2022). Svalbard reindeer can, therefore, potentially be used as a baseline for Arctic reindeer that are "free" for Hg pollution, as the site seems to be less polluted. Renal Hg concentrations in polar bears from East Greenland ranged from 1000 – 50 000 μg/kg w.w (1 to 50 μg/g w.w) (Sonne et al., 2007), which is approximately 7 – 350 times higher than the levels observed in Svalbard reindeer.

Inorganic Hg binds to metallothionein (MT) and is found in the kidney and liver of mammals (Das et al., 2000). A MT-Hg complex immobilises Hg and is a detoxification mechanism (Bridges & Zalups, 2017; Das et al., 2000; Zalups & Cherian, 1992). MTs contain sulfhydryl groups, with which Hg has a high affinity (Berlin et al., 2015). In the kidneys, Hg is an efficient inducer of the synthesis of MTs (Piotrowski et al., 1974; Zalups & Cherian, 1992). The MT-Hg complex accumulates in the kidneys, and some of the complexes are excreted through urine (Zalups et al., 1993). However, MT cannot bind to MeHg, and the detoxification potential of MTs may be limited to Hg^{2+} (Das et al., 2000). The renal Hg levels can, therefore, potentially reflect the inorganic Hg in the diet.

Liver

The liver was the second most contaminated tissue in Svalbard reindeer. The function of the liver is to detoxify xenobiotics, and toxicants tend to accumulate in the organ (Klaassen & Amdur, 2019). Selenium (Se) is an element that occurs naturally in rocks and soil and can bioaccumulate and biomagnify in the food web (Davis et al., 1988; Ogle et al., 1988). Se can form bindings with Hg, particularly MeHg (Martoja & Berry, 1980). Once bound, the Se-MeHg complex can crystalise into tiemannite (HgSe) and accumulate as inorganic Hg in the liver (Das et al., 2000; Martoja & Berry, 1980). This detoxicates MeHg to a less toxic form of Hg (Martoja & Berry, 1980).

The Hg levels in Svalbard reindeer were 130.51 ± 50.51 μg/kg d.w (38.14 ± 13.66 μg/kg, w.w). Reindeers in Alaska and Western Hudson Bay had hepatic Hg levels ranging from 190 – 1240 μg/kg w.w (0.19 – 1.24 μg/g w.w) (Dietz et al., 2022). In Northern Québec, the mean Hg

concentrations in the liver were 670 μg/kg w.w (0.67 μg/g w.w) in reindeer (Robillard et al., 2002). The average hepatic Hg levels in Icelandic Arctic foxes, a terrestrial carnivore, was 4690 μg/kg w.w (4.69 μg/g w.w) (Treu et al., 2018).

The level of MeHg in the diet seems to influence the distribution pattern of Hg as the detoxification of MeHg by Se appears to influence the Hg concentration observed in the liver. In general, the diet of reindeer contains low levels of MeHg. Moss is consumed by Svalbard reindeer (Staaland, 1985). However, as discussed, the quantity of consumption depends on the season (Staaland, 1985). Moss from Ontario contained 80.3 ± 49.5 μg/kg d.w Hg, where 38.2% was MeHg (Moore et al., 1995). At Svalbard, the Hg concentration in moss was lower and about 20 μg/kg, d.w (0.02 mg/kg d.w, median), and about 10 μg/kg d.w (0.01 mg/kg d.w, median) in vascular plants (Wojtuń et al., 2013). If MeHg levels in Svalbard moss and vascular plants are 38.2% of the Hg levels, Svalbard reindeer are exposed to low levels of MeHg. In comparison, the diet of Arctic fox includes marine vertebrates (Bocharova et al., 2013), and about 90% of the Hg (THg: ~ 400 ± 300 μg/kg w.w) found in the muscle tissue of ringed seals from the Arctic areas in North America was MeHg (Wagemann et al., 1998). Other Arctic mammals are exposed to higher levels of MeHg than reindeer, and the dietary differences may explain the variation in hepatic Hg levels.

Brain

The brain is protected from exogenous substances by an endothelial cell layer regulating the influx and efflux of compounds called the blood-brain barrier (BBB) (Aschner & Aschner, 1990). However, MeHg can easily cross the BBB by binding to transporter proteins that cross this barrier (Aschner & Aschner, 1990; Bridges & Zalups, 2017). Once crossed, MeHg can demethylate to inorganic Hg and accumulate in the brain (Vahter et al., 1995).

The Hg levels in the brain of Svalbard reindeer were 1.30 ± 1.17 μg/kg d.w (0.311 ± 0.277 μg/kg w.w). In general, the Hg levels were low, and thus increase in the uncertainty of the measurements has to be accounted for. Overall, the precision of the measurements might be debatable as the with-in variance of the triplicates varied by 30% to the average of the triplicates (see Table A11 in Appendix D). In addition, the standard deviation was large, indicating a variance between the individuals. During the sampling of brain tissue, half brains and brain parts from the brain stem area were collected. The Hg concentration among brain regions

differs, where some regions experience higher Hg levels and are species-dependent (Desforges et al., 2021). However, a student t-test showed no significant difference between the different sampling sites (brainstem area and whole half-brain) in reindeer brain tissue (p = 0.78). Moreover, the aim of this study did not include and was not designed to estimate a Hg distribution pattern in the brain.

In contrast to the low Hg concentrations observed in the brain of Svalbard reindeer, other Arctic mammals experience high and neurotoxic levels. Beluga whales (*Delphinapterus leucas*) from Canada had Hg concentrations in the brain ranging from 2900 – 4700 μg/kg w.w (2.9 – 4.7 μg/g w.w), crossing the threshold for neurochemical effects (Dietz et al., 2013). The observed levels in reindeer are not close to the lower threshold of toxicity (about 0.8 μg/g or 800 μg/kg w.w) (Dietz et al., 2013). Arctic marine mammals have a diet that contains more MeHg than reindeer (Wagemann et al., 1998), which also explains the higher levels of Hg in their brains.

Muscle

The Hg concentration detected in Svalbard reindeer muscle tissue was 8.20 ± 6.92 μg/kg d.w (2.53 ± 2.19 μg/kg, w.w). In Northern Québec, the average Hg concentration in the muscle of reindeer was 30 μg/kg w.w (0.03 μg/g w.w) () (Robillard et al., 2002). The concentrations are higher for the Northern Québec reindeer and are probably due to local differences in Hg concentrations in the Arctic, as discussed in the *Kidney* paragraph.

Hg accumulates in the muscle tissue by binding to the thiol groups of the protein fraction of the muscle (Bosch et al., 2016). Interestingly, Hg tends to bind differently to the different muscle types that are categorised as "white" (fast, for sudden movement) and "dark" (slow, for continuous movement), where dark muscles have higher inorganic Hg concentrations (Bosch et al., 2016). The different distribution among muscle types is due to different protein compositions (Bosch et al., 2016). Only inorganic Hg distribution differs between the muscle types, while MeHg is uniformly distributed in the muscle tissue (Bosch et al., 2016). However, MeHg is the most abundant Hg species in the muscles (Bosch et al., 2016). The muscle *semimembranosus* (the same muscle as used in the current study) in Hanwoo cattle (*Bos taurus coreanae*) is composed of 92% white fibre tissue (measured as fibre area) (Hwang et al., 2010), and presumably close to all of the observed Hg levels in reindeer muscle tissue are MeHg. As

discussed, based on the diet of the reindeer, it is assumed that they are barely exposed to MeHg, which explains the low levels of Hg in the muscle tissue.

In polar bears, the muscle Hg levels were 600 ± 400 μg/kg d.w (0.6 ± 0.4 μg/g d.w) where 400 ± 300 μg/kg d.w (0.4 ± 0.3 μg/g d.w) was MeHg (Boutet et al., 2023). Another study found that 88% of the Hg in polar bear muscle was MeHg (Bechshoft et al., 2019). Compared to polar bears, the muscle Hg levels in Svalbard reindeer are low. The difference in Hg concentrations between these species can potentially be explained by the different MeHg percentages in their diet.

Hg Excretion

Multiple excretion routes can eliminate Hg from the body, e.g., excretion through faeces, fur/hair, urine, and/or antlers (Carrier et al., 2001; Farris et al., 1993; Pokorny et al., 2004; Sell & Davison, 1975). However, only faeces and fur are discussed in this section, as the data is limited to these two sample types.

Faeces

In this study, the average Hg concentrations in faeces for Svalbard reindeer ('Tissue Collection') was 60.82 ± 17.19 μg/kg d.w. Another study on Svalbard reindeer reported concentrations of 67 μg/kg d.w (0.067 μg/g d.w) and 100 μg/kg d.w (0.1 μg/g d.w) in summer faecal samples, for mesic and moss tundra, respectively (Pacyna et al., 2019). The samples from that particular study are from the 6th and 24th of August 2016, while samples from the current study are from October 2021. However, the Hg levels in the samples are similar.

Hg can enter the faeces in two ways; through secretion from the liver into the bile and via the transfer from the blood into the gastrointestinal epithelium (Carrier et al., 2001; Zalups et al., 1999). MeHg can be excreted from the liver through bile and into the gastrointestinal tract (Dutczak et al., 1991). In the gastrointestinal tract, MeHg can be reabsorbed and enter the enterohepatic cycle or be demethylated to inorganic Hg and excreted through the faeces (ATSDR, 2022; Dutczak et al., 1991; Klaassen & Amdur, 2019).

It has been suggested that methylation and demethylation occur in the intestine of organisms by microbes (Abdulla et al., 1973; Farris et al., 1993; Li et al., 2019). One study found that the

faeces of rats fed with MeHg, only contained 15% MeHg of the initial dose, while 65% was inorganic Hg (Farris et al., 1993). Different animals have different microbiomes in the gastrointestinal tract (Ciber et al., 2014), and the composition of the microbial community affect the demethylation and methylation events (Yin et al., 2022). Based on this, one can expect different methylating/demethylation capacities among species, and the ratio of Hg species in faeces should not be assumed to be similar. Demethylation and methylation of Hg might also happen in ruminants (Martín-Doimeadios et al., 2017), but these organisms have not been extensively studied. In addition, the digestive tract structure among ruminants and non-ruminant animals is different (Harfoot, 1978), and how the structural differences affect Hg excretion rates and speciation is unknown.

Other Arctic mammals have higher faecal levels of Hg than reindeer. The average faecal Hg levels in the Pacific walrus (*Odobenus rosmarus divergens*) in the northern Bering and Chukchi Seas in Alaska was 200 μg/kg d.w (200 ng/g d.w) (Rothenberg et al., 2021). The proportion of MeHg was 2.5% (Rothenberg et al., 2021), indicating high levels of inorganic Hg in the samples. Walrus is, however, a marine mammal in a higher trophic position than reindeer (Rothenberg et al., 2021), which may explain the elevated Hg levels. As discussed, Se-Hg accumulation in the livers is a demethylation mechanism that converts MeHg to inorganic Hg (Martoja & Berry, 1980). Most of the Se-MeHg complex crystallizes and does not transfer to the faeces (Martoja & Berry, 1980). Because of demethylation by the microbiome and Se-Hg immobilisation, the Hg species in the faeces might not accurately reflect the ratio between inorganic Hg and MeHg of the diet. Comparing Hg concentrations in faeces, particularly the Hg species, should be done with precaution because of different diet compositions and presumably demethylation and methylation potential in the intestine.

Fur

Fur represents long-term exposure to Hg, in contrast to the blood, which reflects the most recent exposure (Schoeman et al., 2010). Hg is assumed to be transported from the blood into the hair bulb and further into the hair shaft, where it is irreversibly bound, shed, and eliminated (Farris et al., 1993; Yasutake & Hachiya, 2006). In the present study, the Hg levels in fur were 12.69 ± 10.92 μg/kg d.w for Svalbard reindeer. In another study, the Hg levels in Svalbard reindeer fur were measured to 340 ± 230 μg/kg d.w (0.34 ± 0.23 μg/g d.w) in Longyearbyen and 60 ± 10 μg/kg d.w (0.06 ± 0.01 ug/g d.w) in Hornsund (Pacyna et al., 2018). Comparing the Hg

levels in all the fur samples, a spatial difference is observed, where the highest Hg levels are detected in Longyearbyen, followed by Hornsund and Colesdalen/Semmeldalen, respectively (Pacyna et al., 2018; This thesis, 2023). The difference between studies may be explained by the difference in sampling location, where Longyearbyen is a city and is more impacted by humans (Hovelsrud et al., 2020).

However, it should be noted that the sampling months and years are different. The samples in Longyearbyen were collected in August 2015, Hornsund in September 2016, and the current study, including Colesdalen/Semmeldalen, was in October 2021. As the results in Section 3.1 showed, elevated faecal Hg concentrations were found in August, and the unknown reasons for this could potentially also explain why the highest levels in fur also were found in August.

In addition, different times of sampling in the year can also influence the results as reindeer shed their fur in this period (April to September) (Lokken et al., 2009). Fresh fur samples were collected from the ground in the study of Pacyna et al. (2018), while in the current study, it was collected directly from culled reindeer. The fur sampled from the ground would represent fur at the end of its cycle, while fur collected directly from the animal would not. As fur reflect long-term Hg exposure (Schoeman et al., 2010), sampling at different times in the hair cycle might affect the detected Hg concentration.

Hg levels in polar bear fur ranged from 2600 to 13 300 μg/kg, d.w (2.6 to 13.3 mg/kg d.w) (Bechshoft et al., 2019). Fur accumulates MeHg from the blood and is an efficient method of excreting Hg from the body (Dietz et al., 2013). 77% of the Hg in polar bear hair was MeHg (Bechshoft et al., 2019). A diet containing more MeHg is reflected in the Hg levels in human hair (Aleksina & Komov, 2020). The Hg levels in reindeer fur might therefore be MeHg from the diet or a result of methylation. Genetics impacts shedding and will therefore vary among species (Lucas et al., 2021). In addition, an increased frequency of shedding could consequently result in lower Hg levels in fur.

Extrapolation of Faeces to Kidney, Liver, Muscle, and Brain

Based on the Pearson's product-moment correlation, the Hg levels in the kidney and liver well correlated with the Hg levels in faeces (r = 0.673 and 0.735, respectively, see Figures 5 and 6). Both organs are important in the excretion pathway of Hg. The internal distribution pattern of different Hg species may explain the correlations between THg concentrations in these two organs and the faeces.

The kidneys are the target organ of inorganic Hg, which accumulates by binding to MTs located in the kidneys (Bridges & Zalups, 2017; Carrier et al., 2001; Das et al., 2000). In the blood, tissue rapidly absorbs Hg, especially highly perfused tissue like the kidneys (Carrier et al., 2001; Correia, 2019). The Hg entering the kidneys is excreted by urine or re-enters the blood and is excreted through faeces (Carrier et al., 2001). The kidneys do not directly eliminate Hg into the faeces but need to use blood as a vehicle (Carrier et al., 2001). In rats, the efflux from the kidney to the blood is higher for MeHg than inorganic Hg, which declines slower, indicating renal accumulation of inorganic Hg (Farris et al., 1993). After an exposure period, the renal Hg levels decrease (Carrier et al., 2001). However, if the animal is constantly exposed to Hg, the complete elimination of Hg will be minimal.

The main species of Hg in both kidneys and faeces is inorganic Hg in rats (Carrier et al., 2001; Farris et al., 1993). The observed correlation in Figure 5 might be explained by the excretion route of inorganic Hg. Urine is a minor elimination route of Hg (Smith et al., 1994), and the primary excretion route of inorganic Hg from the kidneys is through the blood, which is transferred to the faeces and further excreted (Farris et al., 1993). In Figure 5, when the renal $\log_e$ Hg values increase, so do the faecal $\log_e$ Hg concentrations, suggesting a linear relationship, as demonstrated. In addition, the Hg levels are higher in the kidneys than in the faeces, which most likely confirms accumulation in the kidneys (see Figure 4 and/or the axis in Figure 5).

However, a linear correlation between kidneys and faeces could be true if both the accumulation and the elimination rate in the kidneys are constant and the main renal Hg load is excreted indirectly through faeces. Assuming that the accumulation is constant might be a fair assumption as the MT synthesis in the kidneys is induced by Hg (Piotrowski et al., 1974; Zalups & Cherian, 1992). Increased Hg levels also increase levels of MTs (Piotrowski et al., 1974). We further assume that the only accumulation site of Hg in the kidneys is via MTs. If the

Hg:MTs ratio is constant, the accumulation and excretion ratio will maintain stable. E.g. if the Hg:MT ratio is 1:1 and the kidneys accumulate 50% of a dose of 10 μg/kg Hg, then 5 μg/g would accumulate in the kidneys and 5 μg/kg excreted to the faeces. A higher dose of 20 μg/kg would result in 10 μg/kg accumulating in the kidneys and 10 μg/kg excreted to the faeces, resulting in a linear relationship. So, an increase in Hg exposure and renal accumulation could therefore be reflected in the faeces and explain the observed correlation.

The liver is a part of the gastrointestinal system, and Hg in the liver can be directly transported to the faeces via the bile or indirectly by entering back into the blood, which eventually can be transported to the faeces (Carrier et al., 2001). A significant correlation between MeHg in faeces and the liver has previously been found in the study of Thomas et al. (1987) for male rats. Both MeHg and inorganic Hg are found in the liver and faeces, and as Hg from the liver is directly transferred to the faeces, it could explain the good correlation between the liver and faeces. However, Hg can undergo enterohepatic re-absorption (ATSDR, 2022; Dutczak et al., 1991; Klaassen & Amdur, 2019) and could affect the correlation.

The correlation between Hg levels in faeces and the brain ($r = 0.495$) and muscle ($r = 0.498$) was weaker than in the kidney ($r = 0.673$) and liver ($r = 0.735$). In addition, the R^2 values for the brain ($R^2 = 0.198$) and muscle ($R^2 = 0.2015$) were low, indicating an insufficient explanation of the variance in the models (see Figures 7 and 8). As discussed, the most dominant Hg species in the muscle and brain are MeHg or originate from MeHg as it is oxidised in the brain (Aschner & Aschner, 1990; Bechshoft et al., 2019; Bosch et al., 2016), while most of the Hg species found in faeces are inorganic Hg (Farris et al., 1993). If the overall body burden is dominated by inorganic Hg, as believed to apply for reindeer in this study, comparing the presumed mainly MeHg in the brain and muscle to inorganic Hg levels in faeces might not be a fair method and cause the weak R^2 values.

The extrapolation models for the kidney and liver had a relatively good R^2 value considered using biological field samples (see Figures 5 and 6) (Møller & Jennions, 2002). In the kidney model, 42.81% of the variability of the dependent variables was accounted for. In the liver model, the dependent variables explained 51.10% of the variance. The models are not optimal, as 49-58% of the variance in the models is unexplained and is based on a small sample size ($n = 18$). The distribution of Hg is dynamic, potentially weakening the correlation between Hg

levels in tissues and faeces. E.g., Hg can re-enter the liver with enterohepatic circulation (ATSDR, 2022; Dutczak et al., 1991; Klaassen & Amdur, 2019). Further, it re-enters the blood after being in one compartment (tissue) and is distributed to a new one (Carrier et al., 2001; Farris et al., 1993). In addition, MeHg can be demethylated to inorganic Hg in the gut and other tissues, including the liver (Abdulla et al., 1973; Farris et al., 1993; Li et al., 2019; Martoja & Berry, 1980). The demethylation would change the affinity of Hg to molecules (Beauvais-Flück et al., 2018), and a shift from one species of Hg to another one could change the distribution pattern (Korbas et al., 2012), making the distribution harder to comprehend.

Influencing Factors and Limitations of the Models

Some influencing factors need to be considered when using these extrapolation models. The study of Gamberg et al. (2020) investigated Hg levels in reindeer kidneys and found different levels among 9 reindeer sub-populations in Northern Canada and Greenland. Different sub-populations can be exposed to different Hg levels. As discussed in the previous section, faeces can potentially reflect renal Hg levels, and the different exposure levels would be accounted for. However, we do not know if sub-populations have different excretion capacities due to genetic variations among them (Cronin et al., 2003). This variation should be considered when using the models and comparing Hg levels to other reindeer populations. The models in the current thesis are based on Svalbard reindeer. Svalbard reindeer have some specialised features to tolerate low temperatures. These features include an instant compact body shape, short legs and a small head, which reduce their surface-to-volume ratio (Williamsen et al., 2019). Cross-species extrapolation should be avoided, as inter-species differences occur.

Gamberg et al. (2020) state that renal Hg levels also depend on seasons. The individuals used in the current study were sampled in October 2021. During some months, ruminants consume more plants daily and can thus be exposed to more metals, as shown for roe deer (Pokorny et al., 2004). Increased consumption in specific months also applies to Svalbard reindeer (Loe et al., 2007). In addition, seasonal fluctuation of Hg might occur throughout the season (See section *Hg Levels in Faeces from Colesdalen and Hollendarbukta*).

Moreover, age influences the Hg levels in an animal as it accumulates over time (Verdouw et al., 2011). Age is not accounted for in these models. However, no age relationship was observed between reindeer and Hg (Gamberg et al., 2020), but more research is needed to confirm this.

Sex is an influencing factor (Gamberg et al., 2020). The models in the current thesis are based on female reindeer, which should be kept in mind. In the study of Gamberg et al. (2020), females had higher renal Hg levels than males, 1900 μg/kg d.w (1.9 μg/g d.w) and 1400 μg/kg d.w (1.4 μg/g d.w), respectively. According to the authors, females require more energy to face gestation and lactation relative to their body size. In addition, females can transfer Hg to their foetus by placenta transfer and lactation (Wagemann et al., 1988). The Hg levels in female reindeer might therefore depend on pre-and post-fertilization.

Behavioural and physiological adaptions may also explain the Hg difference between sex. The study of Robillard et al. (2002) found sex differences in Hg levels in reindeer kidneys from Northern Québec, where the Hg levels were lower in males compared to females and were month dependent. This difference might be due to the fluctuation in the mass of the kidney during the stressful rutting season, which ultimately might affect the Hg concentrations (Crête et al., 1989; Robillard et al., 2002). For males, the stress around the mating season causes the kidney to decrease, while the opposite trend occurs for females as a response to gestation and lactation (Robillard et al., 2002).

In addition, Hg was only measured in the kidneys in the study of Gamberg et al. (2020), and it is therefore not possible to know if the faecal Hg levels flux during the season in the same way as the sex-related Hg levels in the kidneys do. However, no significant difference in faecal Hg levels from the field ('Field Sampling of Faeces') was observed between sex during the summer in our study ($p = 0.65$). Using the extrapolation model created in this paper to predict renal Hg levels should be used with precaution.

It is important to know that the models created in the current study are based on faecal Hg levels ranging from 40.56 - 103.15 μg/kg d.w ($\log_e$: 3.70 – 4.63 μg/kg, d.w). The results might be wrong if using faeces levels outside of this range. The assumption of a linear relationship between the Hg levels in faeces and internal organs might not apply to higher or lower levels. And also, the ratio between the Hg levels in faeces and tissues might differ when animals are exposed to higher levels, as detoxification mechanisms might depend on Hg concentrations (Elia et al., 2003).

In addition, when using models to estimate Hg body burden in an animal, one should be aware of all excretion pathways. As mentioned in this thesis, Hg can be excreted through faeces. However, Hg is also eliminated from the body in other pathways; hair/fur, urine, antlers, placental transfer, and through milk (Carrier et al., 2001; Farris et al., 1993; Pokorny et al., 2004; Sell & Davison, 1975). The models could be applied with more confidence if the elimination through these other pathways is constant. However, the pathways are seasonally dependent (Pokorny et al., 2004). Fur, for instance, grows in cycles depending on photoperiods, genetics, and hormones, and placental- and lactating transfer depend on gestation (Mansour et al., 1973; Milner et al., 2002; Yang et al., 1997).

Predictions of Internal Hg Concentrations and Risk Assessment Based on Faeces Samples from the Field

Figure 9 shows the predicted Hg concentrations in different tissues based on the faeces samples collected in the field. Application of the model estimates the Hg concentration in the population as a tool for biomonitoring. Following the risk assessment to AMAP (2021) for terrestrial mammals, the hepatic estimation shows that Svalbard reindeer are not at risk of Hg toxicity. The hepatic Hg levels in Svalbard reindeer from 'Field Sampling of Faeces' ranged from 12.97 – 289.58 μg/kg w.w (see Table A14 in Appendix D for each location and week) and were below 4200 μg/kg w.w (4.2 μg/g w.w), which is the no-risk level reported in AMAP (2021).

As discussed, there is much unexplained variance in the model, and the concentrations are not accurate but might give an indication. Using this model should be done with precaution. The influencing factors and limitations of this model have been discussed in the previous section. The most important argument for these results is that the range of faecal Hg concentrations is outside of the range (especially Hollendarbukta week 32) used to create these models (see Table A10 in Appendix D).

Conclusion and Further Research

In conclusion, this study investigated the variation in Hg levels in different weeks and locations in Nordenskiöld Land, Svalbard, and observed higher levels in weeks 29 and 32 in Colesdalen and week 32 in Hollendarbukta in 2022. The increase in Hg concentrations could be attributed to local pollution sources or seasonal factors, such as increased deposition or changes in the feeding behaviour of Svalbard reindeer, including dietary shifts and feeding frequency. However, further research is needed to determine the reasons for the elevated Hg levels.

Biomonitoring, which assesses pollution by measuring chemical levels in a biological matrix, was employed in this study to monitor environmental Hg levels based on reindeer faeces. The study highlights the potential of using faeces as a monitoring tool for environmental Hg levels. However, it remains uncertain if faeces accurately reflect environmental Hg levels and more research is needed in this area.

The established linear relationships between Hg levels in faeces and kidney, liver, brain, and muscle suggest that it could be used as a non-invasive tool to monitor Hg levels in Svalbard reindeer. Nevertheless, the model used in this study is based on a small sample size and includes uncertainties, necessitating more data and improved estimations.

Compared to other Arctic mammals, the Hg levels in Svalbard reindeer are low, and the internal distribution of Hg in reindeer seems to depend on their diet and the specific Hg species present. The composition of MeHg and inorganic Hg in Svalbard reindeer remains unknown. Further research is recommended to explore the mechanisms behind the observed distribution pattern, including analysing both total Hg and MeHg and examining the relationship between inorganic Hg and MeHg in the tissues and diet of the reindeer. The potential effects of demethylation and methylation in the intestine and Se-Hg interactions in the liver should be investigated to understand the interpretation of faeces as a bioindicator for internal Hg levels. Additionally, future studies should focus on identifying the reasons for the correlations observed in the models and investigate if the Hg species constitutes.

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Appendix

Appendix A – Coordinates

Faecal coordinates

Table A1. Coordinates of sampling location of *R. tarandus* faeces with sex and marked ID (if available) for the individuals.

ID	Latitude	Longitude	Sex	Marked ID
1	78.09507	14.93246	Male	
2	78.09306	14.95732	Male	
3	78.08537	15.03706	Male	
4	78.09090	14.97456	Female	
5	78.08355	15.03896	Male	
6	78.07864	15.13136	Female	
7	78.11380	14.84034	Male	
8	78.11924	14.78588	Male	
9	78.11446	14.69902	Male	
10	78.11037	14.67595	Male	
11	78.10635	14.64689	Female	
12	78.09473	14.98739	Male	
13	78.11579	14.76859	Male	
14	78.11579	14.76859	Male	
15	78.10340	15.06043	Male	
16	78.11255	15.03942	Male	
17	78.11350	14.83982	Male	
18	78.07711	15.23685	Female	
19	78.11132	15.04405	Male	
20	78.09131	15.01681	Female	
21	78.07474	15.26727	Female	187
22	78.07891	15.12598	Male	
23	78.10831	14.66063	Male	
24	78.10801	14.65768	Female	
25	78.10917	14.68035	Male	
26	78.07540	15.22638	Male	
27	78.10387	14.74453	Male	
28	78.10894	14.67176	Male	
29	78.10949	14.67483	Male	
30	78.07771	15.16356	Female	
31	78.07740	15.15185	Male	183
32	78.10949	14.67483	Male	
33	78.07330	15.29433	Female	
34	78.07550	15.27658	Female	
35	78.07637	15.27350	Female	

36	78.07702	15.26910	Female	
37	78.09840	15.04289	Male	268
38	78.09841	15.04290	Female	
39	78.09901	15.06926	Female	
40	78.09892	15.09517	Female	329
41	78.10627	15.06893	Male	
42	78.11080	15.05519	Male	
43	78.11080	15.05519	Male	393
44	78.11537	14.74476	Male	
45	78.10853	14.62816	Male	
46	78.10495	14.62870	Male	
47	78.10101	14.62260	Male	
48	78.10005	14.57199	Male	
49	78.09955	14.57177	Male	
50	78.09880	14.55425	Male	
51	78.11056	15.03697	Male	
52	78.11087	15.08527	Male	
53	78.10995	15.06960	Male	384
54	78.10743	15.07960	Male	181
55	78.10776	15.08303	Female	137
56	78.07767	15.20236	Female	218
57	78.07750	15.21777	Female	208
58	78.07476	15.22331	Female	165
59	78.07536	15.28174	Female	365
60	78.07408	15.28788	Female	247
61	78.10814	14.68026	Male	
62	78.10836	14.67465	Male	
63	78.10817	14.67381	Male	
64	78.10789	14.67539	Male	
65	78.10770	14.63919	Male	
66	78.10723	14.63735	Male	
67	78.10481	14.55109	Male	
68	78.10675	14.57949	Male	

Reindeer coordinates

Table A2. Coordinates for location where female *R. tarandus* were euthanised in October 2021.

ID	Latitude	Longitude
1	78.0037203	15.3842247
2	77.9901811	15.3772542
3	78.0687872	15.3751096
4	78.0881344	15.3741908
5	78.0803753	15.3869191
6	77.9615795	15.4382634
7	77.9669494	15.4250495
8	77.9572693	15.3832372
9	78.0779936	15.1785253
10	77.9880148	15.3756807
11	78.0811026	15.385858
12	77.9589773	15.3724694
13	78.0721104	15.4145244
14	78.0925206	15.3708534
15	78.0687872	15.3751096
16	78.0779936	15.1785253
17	77.9922843	15.3656504
18	78.0832114	15.1588636

Appendix B – Laboratory Procedure

B1. DMA-80 and The Calibration Curve

The technical parts of DMA-80 are shown in Figure A1. The machine is connected to a computer with a Milestone software.

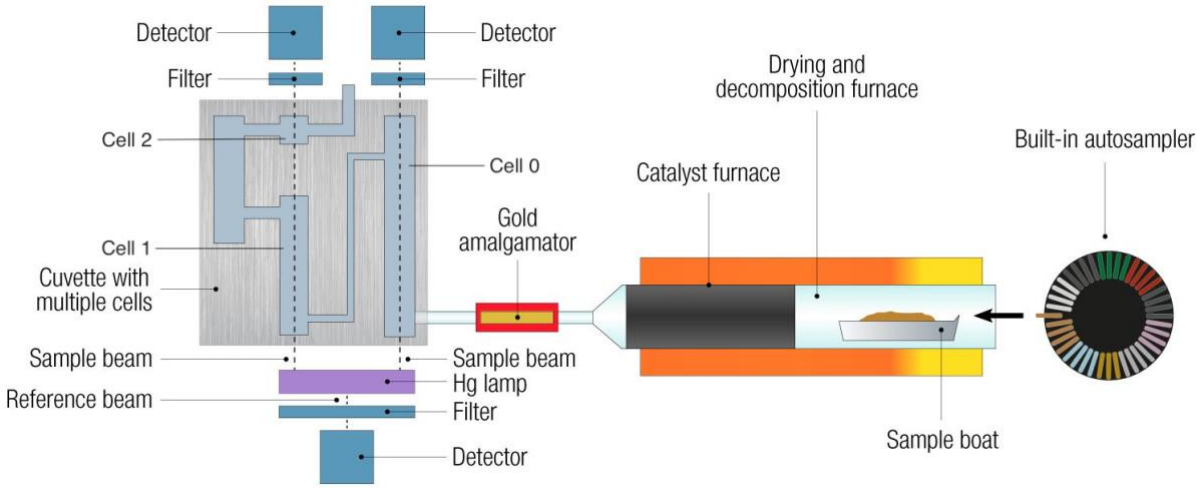


Figure A1. The DMA-80 design from the inside. Figure modified from https://www.milestonesrl.com/products/mercury-determination/dma-80-evo

DMA-80 measures THg in samples. Depending on the concentration in the sample, DMA-80 will measure the Hg levels by absorption in one of three cells shown in Figure A1. Each cell has its own equation (see Table A5, A6 and A7). DMA-80 gives the Hg concentration as ng/g, which is equal to μg/kg, the unit used in this thesis.

UV-protected bottles were cleaved in 450°C for 4 hours a day prior the creation of Hg standard solutions. The calibration solutions were created by mixing HCl, Hg standard/stock solution with known concentration and Milli-Q water, shown in Table A3 into the UV-protected bottles. The solutions were kept cold in a refrigerator a night before creating the calibration curve to stabilise the calibration solution. When the calibration was not in use, they were kept cold in the refrigerator.

Table A3. Method for producing the standard solutions. * This stock solution was not an original fabricated stock solution but created by the laboratory team.

Created Standard Solution (µg Hg/kg)	HCl weight (g)	Stock solution (ng Hg/g)	Hg added from stock solution (g)	Milli-Q Water (g)	Total volume (g)	Hg added in ng	Concentration (ng Hg/g)
1000	0.851	1 000 000	0.028	30.062	30.09	28000	930.5417
100	0.826	1000	3.008	27.013	30.021	3008	100.1965
10	0.854	1000	0.297	29.714	30.011	297	9.8964
1	0.848	1000	0.028	30.031	30.059	28	0.9315
0.1	0.840	10*	0.297	29.764	30.061	2.97	0.0988
0	0.835	0	0	30.07	30.07	0	0.0000

The different calibration points were created by varying the amount (ng) and concentrations of Hg added to DMA-80. The weight of each solution used to create the calibration curve and the total Hg concentration (ng/g) that gave for each calibration point are shown in Table A4.

Table A4. The weight of standard solution used to create calibration points with different concentrations. The standard solution was added to a DMA-80 sample boat.

Calibration point (ng)	Weight of standard solution (g) (in boat, run in DMA-80)	ng/g of standard solution	Total (ng/g) (concentration)
0.005	0.05	0.1	0.05
0.01	0.1	0.1	0.1
0.02	0.02	1	0.2
0.05	0.05	1	0.5
0.1	0.1	1	1
0.2	0.02	10	2
0.5	0.05	10	5
1	0.1	10	10
2	0.02	100	20
5	0.05	100	50
10	0.1	100	100
20	0.2	100	200
40	0.04	1000	400
80	0.08	1000	800
100	0.1	1000	1000

The solutions and samples were measured in both cells, which has different detectors (see Figure A1). Depending on the amount of Hg in the sample and the measured absorption, DMA-80 automatically choose the best fitted cell. Each cell has its own equation (see Table A5, A6 and A7). Because of technical issues (elaborated in Appendix B.2) three calibrations were created, shown in table A5, A6 and A7.

Table A5. Calibration 1 equations, with R^2 values.

Cell	Equation number	Equation	R^2 value
0	I	$-0.0044 \times \mathrm{Hg}^2 + 0.1273 \times \mathrm{Hg} + 0.0193$	0.9983
1	II	$-0.0007 \times \mathrm{Hg}^2 + 0.049 \times Hg + 0.005$	0.9999
2	III	$-2\mathrm{e}^{-7} \times \mathrm{Hg}^2 + 0.0008 \times \mathrm{Hg} + 0.0037$	0.9998

Table A6. Calibration 2 equations, with R^2 values.

Cell	Equation number	Equation	R^2 value
0	IV	$-0.0054 \times Hg^2 + 0.1451 \times Hg + 0.0052$	0.9999
1	V	$-0.0009 \times \mathrm{Hg}^2 + 0.0519 \times Hg + 0.0013$	0.9998
2	VI	$-2\mathrm{e}^{-7} \times \mathrm{Hg}^2 + 0.0008 \times \mathrm{Hg} + 0.0046$	0.9999

Table A7. Calibration 3 equations, with R^2 values.

Cell	Equation number	Equation	R^2 value
0	VII	$-0.0013 \times Hg^2 + 0.1118 \times Hg + 0.0133$	0.9984
1	VIII	$-0.0006 \times \mathrm{Hg}^2 + 0.0474 \times Hg + 0.005$	0.9990
2	IX	$-2\mathrm{e}^{-7} \times \mathrm{Hg}^2 + 0.0008 \times \mathrm{Hg} + 0.0008$	0.9998

The program used for the analysis of the samples is called Feathers program and are specialised for biological tissue. The program is shown in Table A8.

Table A8. Feathers program by Milestone for Hg determination, showing the duration of each step and the temperature the sample was burned at.

Step	Time	Temperature (°C)
1	00:00:01	200
2	00:02:00	650
3	00:01:00	650

B2. The Technical Issues

During the measurements in October and November 2022, DMA-80 was defected for a period, and the amalgamator and catalysator was replaced. This changed the sensitivity of the absorption before and after the replacement and affected the measurements of the samples. Because of the change in sensitivity a new calibration was made. The samples from 'Field Sampling of Faeces' have therefore been measured with two different calibration curves, week 25 and 27 was measured with Calibration 1 (Table A5). The rest of the samples, except liver and kidney (Calibration 3, Table A7), was measured with the Calibration 2 (Table A6). The certified reference material was within the range for all calibrations.

However, some faeces samples measured with Calibration 1 were repeated with Calibration 2 and showed different results. The effect of the Calibration 1 and 2 was statistically estimated by mixed linear regression models (package *lmer4*, in RStudio) accounting for the random effect of individuals and CRM. The estimate showed a significant difference ($p < 0.001$) of 18 µg/kg between Calibration 1 and 2, when considering the repeated individuals and the CRM (before and after replacement of amalgamator and catalysator). However, the estimation of the difference in the faecal samples was based on a small sample size, resulting in a difference of 7.87 µg/kg (elevated levels with Calibration 2). Because the certified reference material was within the range for both calibrations (Calibration 1 and 2), no correction of the data were done. The liver and kidney samples were in addition measured with Calibration 3, however the CRM was also within the range.

Appendix C – Statistics

Two linear models were considered from the study of 'Field Sampling of Faeces'. An Akaike's Information Criterion and a Bayesian Information Criterion test was carried out to choose the best model. The results are shown in Table A9.

Table A9. AIC and BIC results for linear regressions models.

Linear model	AIC value	BIC value
logHg ~ Week*Location	34.71	54.69
logHg ~ Week*Location + Sex	36.57	58.77

Appendix D – Data

The Hg concentration for 'Field Sampling of Faeces' are shown in Table A10. The Hg concentration from 'Tissue Collection' is shown in Table A11. The water content for each organ is shown in Table A12. The formula for converting d.w to w.w is shown in Appendix E. The predicted field values from 'Field Sampling of Faeces' based on the equations in Figures 5 – 8 are shown in Table A13 in w.w for kidney, liver, brain, and muscle and in w.w for liver in Table A14. Calculation for the predicted values are shown in Appendix E.

Table A10. The average, minimum (min) and maximum (max) of the Hg levels, average water content (%), min and max water content in Svalbard reindeer (*Rangifer tarandus platyrhynchus*) faeces samples, in addition to number of samples for each week and location. The dates for each week are presented. * Week 34 is not included in the main results, as the samples were taken in a different location, Bjørndalen.

Week	Dates	Location	Hg concentration (µg/kg, d.w)	Min – Max [Hg]	*n*	Average water content (%)	Min -Max water content (%)
25	20 – 24/06/22	Colesdalen	39.106 ± 10.097	25.156 – 60.814	9	76.98 ± 2.91	73.8 – 81.1
25	20 – 24/06/22	Hollendarbukta	37.709 ± 6.207	29.426 – 45.659	5	75.3 ± 1.53	73.8 – 77.7
27	4 – 7/7/22	Colesdalen	38.219 ± 9.094	23.686 – 56.951	11	71.31 ± 6.86	58.8 – 80.7
27	4 – 7/7/22	Hollendarbukta	40.028 ± 17.084	29.299 – 69.346	5	63.06 ± 9.9	49.2 – 74.4
29	18 – 22/7/22	Colesdalen	52.667 ± 24.281	35.596– 129.473	13	89.04 ± 6.19	72.8 – 95.5
29	18 – 22/7/22	Hollendarbukta	45.192 ± 5.602	39.605 – 55.694	7	94.49 ± 1.69	92 – 96.7
32	8 – 12/8/22	Colesdalen	91.034 ± 31.734	55.419– 141.262	10	90.17 ± 4.78	78.9 – 95.7
32	8 – 12/8/22	Hollendarbukta	250.821 ± 97.619	134.45– 488.407	8	90.71 ± 4.54	81.3 – 94.4
34*	22 – 26/8/22	Bjørndalen	78.334 ± 42. 989	46.344– 209.518	10	89.95 ± 6.13	72.5 – 94.7

Table A11. Mean Hg levels in dry weight (µg/kg, d.w) and wet weight (µg/kg, w.w) for faeces (*n* = 18), brain (*n* = 18), fur (*n* = 18), kidney (*n* = 18), liver (*n* = 18), muscle (*n* = 18), RBC (w.w, *n* = 18) and serum (w.w, *n* = 14) for *R. tarandus.* Minimum (min) and maximum (max) Hg levels for d.w and w.w are shown. With-in variance for each organ are shown, based on the standard deviation of the triplicate for each organ. The percentage of within-variance are shown, based on the proportion of the with-in variance to the average of the triplicates ((standard deviation(triplicates)/average(triplicates))*100%).

Organ	Dry weight (µg/kg, d.w)	Min – Max d.w	Wet weight (µg/kg, w.w)	Min – Max w.w	With-in sample variance; average ± standard deviation of triplicates (µg/kg, d.w)	Percentage of with-in variance
Faeces	60.817 ± 17.187	40.564 – 103.153	13.823 ± 5.434	6.705 – 25.881	61.788 ± 1.532	2.48 %
Brain	1.303 ± 1.174	0.386 – 4.429	0.311 ± 0.277	0.0969 – 1.0201	0.542 ± 0.165	30.38 %
Fur	12.691 ± 10.917	2.004 – 40.332	3.453 ± 3.668	0.647 – 14.299	28.529 ± 2.977	10.43 %
Kidney	662.893 ± 279.615	235.189 – 1357.4	140.158 ± 58.731	51.999 – 279.244	610.76 ± 14.950	3.44 %
Liver	130.51 ± 50.506	62.426 – 252.959	38.135 ± 13.664	17.854 – 71.840	62.426 ± 2.288	3.66 %
Muscle	8.202 ± 6.922	2.399 – 26.619	2.527 ± 2.187	0.809 – 8.182	6.332 ± 0.179	2.82 %
RBC			0.426 ± 0.434	0.0015 – 1.611	0.481 ± 0.0458	9.51 %
Serum			0.0918 ± 0.195	0.0015 – 0.659	0.00515 ± 0.00632	122.74 %

Table A12. Average water content in percentage and minimum (min) and maximum (max) water content for each *R. tarandus* organ.

Organ	Average water content (%)	Mix – Max (%)
Faeces	77.86 ± 2.91	71.72 – 83.75
Brain	75.83 ± 2.26	69.99 – 78.21
Fur	74.55 ± 8.99	52.79 – 85.76
Kidney	78.79 ± 1.58	74.26 – 80.49
Liver	69.98 ± 1,19	67.85 – 72.35
Muscle	69.51 ± 2.30	62.91 – 73.97

Table A13. Predicted average, standard divination and minimum (min) and maximum (max) Hg levels (µg/kg, d.w) for kidney, liver, brain, and muscle in *R. tarandus* from Colesdalen and Hollendarbukta during week 25, 27, 29 and 32.

Organ	Week	Location	Hg level (µg/kg, d.w)	Min – Max (µg/kg, d.w)
Kidney	25	Colesdalen	405.27 ± 101.99	245.26 – 632.67
	25	Hollendarbukta	379.09 ± 66.87	290.22 – 465.10
	27	Colesdalen	385.04 ± 98.42	229.91 – 589.63
	27	Hollendarbukta	405.90 ± 187.57	288.88 – 728.43
	29	Colesdalen	545.42 ± 276.72	381.37 – 1423.87
	29	Hollendarbukta	460.23 ± 61.43	399.24 – 575.68
	32	Colesdalen	979.67 ± 366.59	572.62 – 1563.51
	32	Hollendarbukta	2910.03 ± 1120.76	1482.68 – 5402.78
Liver	25	Colesdalen	80.66 ± 20.39	48.70 – 126.15
	25	Hollendarbukta	75.42 ± 13.36	57.67 – 92.61
	27	Colesdalen	76.62 ± 19.67	45.64 – 117.53
	27	Hollendarbukta	80.80 ± 37.52	57.41 – 145.32
	29	Colesdalen	108.72 ± 55.48	75.88 – 284.89
	29	Hollendarbukta	91.64 ± 12.29	79.45 – 114.73
	32	Colesdalen	195.75 ± 73.56	114.12 – 312.95
	32	Hollendarbukta	584.21 ± 246.19	296.71 – 1087.19
Brain	25	Colesdalen	0.55 ± 0.19	0.28 – 0.99
	25	Hollendarbukta	0.50 ± 0.12	0.35 – 0.66
	27	Colesdalen	0.51 ± 0.18	0.25 – 0.91
	27	Hollendarbukta	0.57 ± 0.37	0.35 – 1.21
	29	Colesdalen	0.86 ± 0.67	0.50 – 3.00
	29	Hollendarbukta	0.65 ± 0.12	0.54 – 0.88
	32	Colesdalen	1.86 ± 0.94	0.87 – 3.41
	32	Hollendarbukta	8.18 ± 4.75	3.17 – 18.29
Muscle	25	Colesdalen	3.91 ± 1.19	2.12 – 6.63
	25	Hollendarbukta	3.59 ± 0.76	2.59 – 4.58
	27	Colesdalen	3.67 ± 1.13	1.96 – 6.09

27	Hollendarbukta	3.96 ± 2.25	2.58 – 7.85
29	Colesdalen	5.67 ± 3.71	3.61 – 17.56
29	Hollendarbukta	4.52 ± 0.73	3.81 – 5.92
32	Colesdalen	11.37 ± 5.11	5.87 – 19.65
32	Hollendarbukta	42.19 ± 21.52	18.44 – 87.21

Table A14. Predicted average, standard divination and minimum (min) and maximum (max) Hg levels (µg/kg, w.w) for liver in *R. tarandus* from Colesdalen and Hollendarbukta during week 25, 27, 29 and 32. The w.w data is calculated based on the average of the water content in the liver (Table.A12).

Week	**Location**	**Hg level (µg/kg, w.w)**	**Min – Max (µg/kg, w.w)**
25	Colesdalen	21.49 ± 5.43	12.97 – 33.60
25	Hollendarbukta	20.09 ± 3.56	15.36 – 24.67
27	Colesdalen	20.41 ± 5.24	12.16 – 31.31
27	Hollendarbukta	21.52 ± 9.99	15.29 – 38.71
29	Colesdalen	28.95 ± 14.78	20.21 – 75.88
29	Hollendarbukta	24.41 ± 3.27	21.16 – 30.56
32	Colesdalen	52.14 ± 19.59	30.40 – 83.36
32	Hollendarbukta	155.61 ± 65.57	79.03 – 289.58

Appendix E – Formulas and Calculations

Calculation of predicted tissue Hg concentrations based on extrapolation equation (an example)

In this thesis 4 extrapolation equations (Figure 5 – 8) predicting internal Hg concentration based on faecal Hg levels are given. Here is an example of how to predict the kidney Hg concentrations in Svalbard reindeer based on the mean Hg concentration in faeces collected in Colesdalen in week 25.

Equation for Hg levels in kidney:

$$y = 1.0735 \times \log_e[Faeces] + 2.0402$$

The average faecal Hg levels from Colesdalen in week 25 was 39.106.

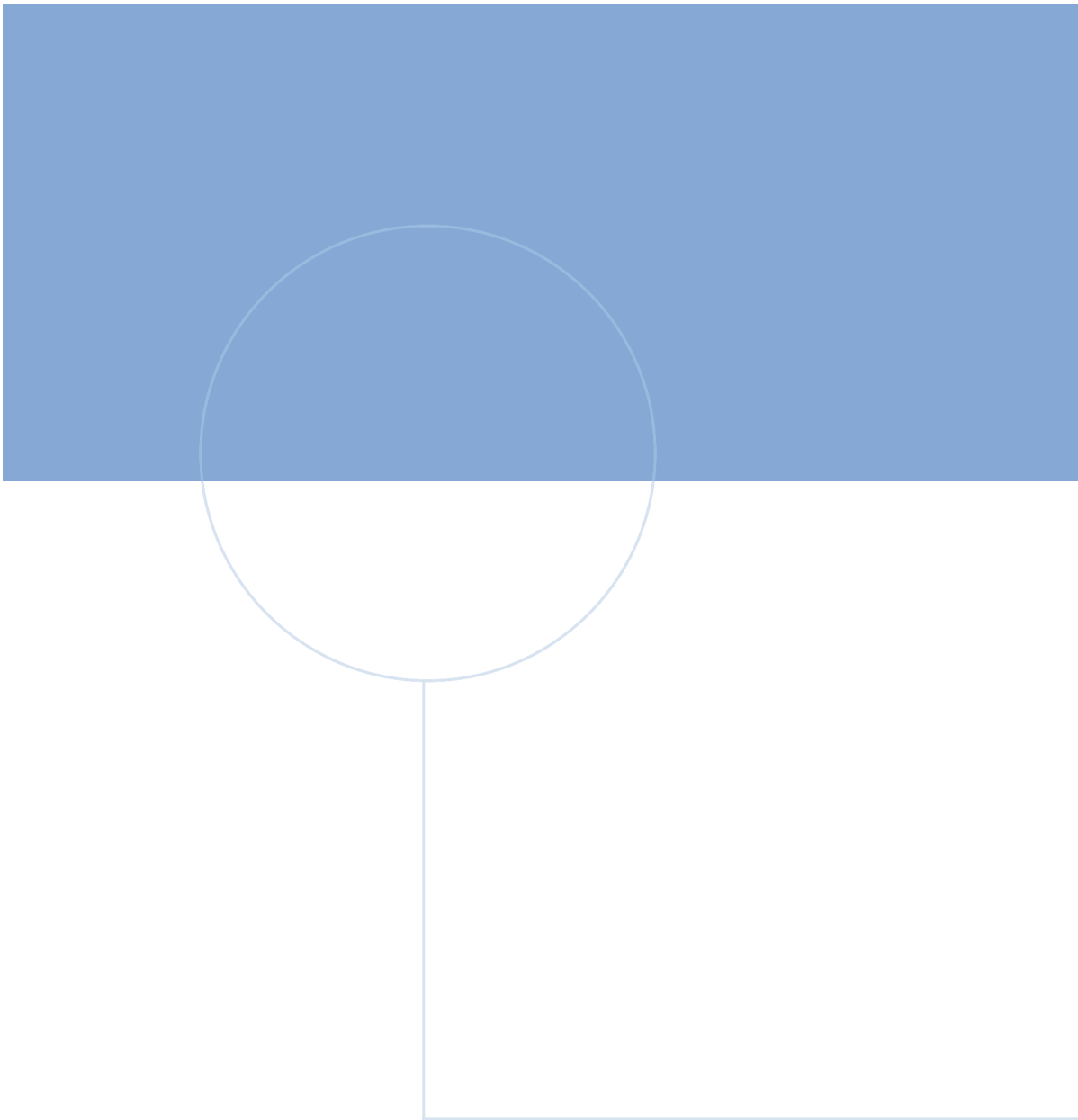
$$y = 1.0735 \times \log_e[39.106] + 2.0402 = 6.079$$

To convert the renal Hg concentration from $\log_e$ scale we solve it by the natural logarithm,

$$e^{6.079} = 436.59 = 436.59\ \mu g/kg$$

Converting dry weight to wet weight

$$\text{Wet weight} = \text{Dry weight} \times \left(1 - \left(\frac{\text{Water content \%}}{100\ \%}\right)\right)$$



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