Mätningar i älg

Utfört av
Naturhistoriska Riksmuséet
Gruppen för Miljögiftsforskning
och
Statens Veterinärmedicinska Anstalt

Programområde
Skog
Kontrakt nr 219 0110, 221 0130, 221 0038
Time trends of metals in organs of moose (Alces alces) from Sweden, 1980-2000

Swedish monitoring programme in terrestrial biota

2002-06-07

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Miljögifter i biota - skogsmark

Avtal nr 219 0110
Metaller i älg - Grimsö, 1980-1990

Avtal nr 221 0130
Metaller i älg - Grimsö

Avtal nr 221 0038
Metaller i älg

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2002-06-07
TIME TRENDS OF METALS IN ORGANS OF MOOSE (*Alces alces*) FROM SWEDEN, 1980-2000

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Introduction
The long-term monitoring of persistent and bio-accumulating chemicals in the Swedish environment is part of the Swedish National Environmental Monitoring Programme. It is based on chemical analyses of tissues and organs from species collected in selected reference areas of the Swedish mainland, lakes and coastal areas (Odsjö & Olsson 1979a,b, Bernes 1985). As part of the terrestrial contaminant monitoring programme, specimens of muscle, liver and kidney of moose (*Alces alces*) have been collected since 1980 from Grimsö, a reference area in the monitoring programme and a coherent hunting district in the Örebro county (T) in south-central Sweden. In 1996, the monitoring was extended by collection and chemical analysis of organs of moose from six further counties and districts in Sweden. These districts are situated in the Norrbotten county (BD), Jämtland county (Z), Västmanland county (U), Älvsborg county (P), Jönköping county (F) and Kronoberg county (G) (Figure 1).

Moose, with a diet dominated by twigs and leaves of trees and shrubs (Cederlund *et al.* 1980), was chosen in the monitoring programme as a representative of biota in the Swedish forest areas. Since the moose is distributed almost all over the country, it was considered as an ideal material also for studies of spatial distribution of environmental pollution and bio-accumulation, which is the reason for the extended collection of samples in 1996 onwards.

This report presents levels and time trends of Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, V and Zn in liver and kidney and Hg and Se in liver and muscle from the period 1996-2000. For Grimsö data from an extended period, 1980-2000 is presented.

Material and methods

Grimsö area
From the start of the collection in the Grimsö area (Figure 1), samples of liver, kidney and muscle have been collected from approximately 45-50 individuals annually during the hunting season in the autumn and, with special permit also in the winter and spring. Samples were taken from all individuals shot in the area during hunting despite age and sex. This was done to make it possible to select the most appropriate and homogeneous material for contaminant monitoring according to influence of biological variables (e.g. age, sex, etc.) on the concentrations. The samples were extracted at the slaughter, prepared in laboratory and stored in a temperature of -25°C until analysis. Individual age was determined by tooth sectioning after slaughter. Calves and, certain seasons also males were initially well represented in the material. However, the age structure of the material has changed considerably during the
period, which may have consequence in future for the choice of material from a smaller and spatial concentrated population like that in the Grimsö area. According to the extended hunting period and date of collection, selection of individual calves for analyses was restricted to the period October 1 - April 30 each hunting season. The selection of specimens started with the earliest shot animals each season. No significant variation in levels of Cd according to date of collection during the hunting season was revealed (Odsjö 2001).

From the Grimsö area, tissue samples of male calves were selected for analyses with some few exceptions that were from female calves. Completion of male samples with samples of females was acceptable after studies of the relation level/sex for cadmium in kidney, which showed no difference according to sex. Further tests and discussion of selection criteria of matrices of moose are reported elsewhere (Odsjö 2001). The time series from Grimsö includes 21 years of analyses and 219 individual samples from calves. For calcium, chromium, lead, nickel and vanadium some samples were either missing or below detection limit (see below).

Other districts

From the other six districts (Figure 1), samples of blood serum, muscle, liver, kidney, spleen, hair and half the lower jaw (mandible) were collected from approximately 40 calves and 50 adult moose per year. The samples were collected from animals shot during the ordinary hunt in the autumn; mid-October to the end of December in southern Sweden, and September to the end of October in northern Sweden except for three weeks interruption during mating season. These samples were also extracted at the slaughter, prepared in laboratory and stored in a temperature of -25°C until analysis. Specimens of varying ages and sex were selected for analysis. The ages vary from calves (approximately six months) up to animals 2.5 and 3.5 years old.

The outer layer of the tissue specimens was cut off and samples were taken from the inner part to avoid any surface contamination.

In order to achieve information on the within-year variation in concentration of the studied populations, 10 or more individuals (of different ages) were analysed per year and district. However, in some years it has not been able to achieve the required number of requested individuals. From the individual analyses a geometric mean value was calculated and used as a value of the year in the time trend study.
Figure 1 Sampling sites in the various counties indicated with dots. The star indicates the Grimsö field station where the moose for the 21-year time series are collected.
Analytical methods

Pre-treatment of samples

The outer layer of the tissue specimens was cut off and samples were taken from the inner part to avoid surface contamination.

Combustion of organs (5 g liver and kidney, respectively, for multi-metal determination; about 5 g liver and muscle, respectively, for analysis of Hg and Se) was performed by automatic wet digestion according to a standard program (Frank 1976, Frank, and Petersson 1983, Frank 1988, Frank et al. 1992). An electrically heated block of aluminium was used (Foss Tecator Digestion System, Model 40, Foss Tecator AB, Höganäs, Sweden). For digestion conditions see Table 1.

Table 1. Conditions for wet digestion of organ tissues.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca, Cd, Co, Cu, Fe, Mg, Mn, Mo, Ni, Pb, V, Zn</td>
<td>65% HNO₃, 70% HClO₄</td>
<td>65% HNO₃, 70% HClO₄, 95% H₂SO₄</td>
<td>quartz glass</td>
<td>240</td>
</tr>
<tr>
<td>Hg</td>
<td>65% HNO₃, 70% HClO₄</td>
<td>65% HNO₃, 70% HClO₄</td>
<td>boro-silicate glass</td>
<td>180</td>
</tr>
<tr>
<td>Se</td>
<td>65% HNO₃, 70% HClO₄</td>
<td>65% HNO₃, 70% HClO₄</td>
<td>boro-silicate glass</td>
<td>225</td>
</tr>
</tbody>
</table>

Analysis

Analysis of 13 elements (Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, V and Zn) in material from 1996 was performed using direct-current plasma atomic emission spectrometry, DCP-AES (SpectraSpan IIIA, Applied Research Laboratories Inc., Valencia, CA, USA) and inductively coupled plasma atomic emission spectrometry, ICP-AES (JY 50P, Instruments S.A., division Jobin Yvon, Longjumeau, France) (Frank and Petersson 1983) The limits of detection for each element are presented in Table 2. For material from 1997-2000 the analysis was performed using only inductively coupled plasma atomic emission spectrometry, ICP-AES, (JY 238, Instruments S.A., division Jobin Yvon, Longjumeau, France). The limits of detection for the elements are presented in Table 2. (The method is accredited since June 1998).

For the determination of Hg in material from 1996-98, flow injection cold vapour atomic absorption spectrometry was used, FI-CV-AAS. The limit of detection (blank + 3s) for Hg was ~ 0.3 ng/g, wet weight (Table 3). For the determination of Se in material from 1996-98, flow injection hydride generation atomic absorption spectrometry was used, FI-HG-AAS (Galgan and Frank 1988, Galgan and Frank 1993). The limit of detection (blank + 3s) was 1.0 ng/g, wet weight. (The methods are accredited since June 1998).

The determination of both Hg and Se in material from 1999 and 2000 was performed by hydride generation inductively coupled plasma atomic emission spectrometry, HG-ICP-AES. The limit of detection (blank + 3s) was for Hg and Se, 0.46 and 2.1 ng/g, wet weight, respectively (Table 3). (The method is accredited since Oct. 2000)

Quality control was performed using appropriate reference materials like NIST (National Institute of Standards and Technology) SRM 1577b bovine liver.

The analysis of the thirteen elements in moose tissues from Grimsö, 1980-2000, was performed using ICP-AES and the analysis of Se and Hg by use of HG-ICP-AES. (Detection limits, see Table 2 and 3). The number of individual samples from Grimsö below detection limit of the elements is given in Table 4.
The chemical analyses were carried out by the Department of Chemistry, National Veterinary Institute, Uppsala.

Table 2.
Detection (DL) limits for the various analytical techniques and years estimated from samples of 5 g, µg/g wet weight.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>0.146</td>
<td>0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.020</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>0.002</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>0.002</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>0.018</td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>0.056</td>
<td>0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>0.024</td>
<td>0.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>0.010</td>
<td>0.014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mo</td>
<td>0.020</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>0.006</td>
<td>0.022</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>0.020</td>
<td>0.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>0.002</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>0.112</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.
Detection limits for the various analytical techniques and years, (blank + 3s) estimated from samples of 5 g, ng/g, wet weight.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Se</td>
<td>Se FI-HG-AAS</td>
<td>Se FI-HG-AAS</td>
<td>Se FI-HG-AAS</td>
<td>HG-ICP-AES</td>
</tr>
<tr>
<td>Hg</td>
<td>Hg FI-CV-AAS</td>
<td>Hg FI-CV-AAS</td>
<td>Hg FI-CV-AAS</td>
<td>HG-ICP-AES</td>
</tr>
<tr>
<td>Se</td>
<td>0.26</td>
<td>0.29</td>
<td>0.30</td>
<td>0.46</td>
</tr>
<tr>
<td>Hg</td>
<td>0.29</td>
<td>0.30</td>
<td>0.46</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.
Number (n) of individual samples of moose from Grimsö out of a total of 219, found with concentrations below detection limit (D.L.). (l)=liver, (k)=kidney.

<table>
<thead>
<tr>
<th>Element</th>
<th>n below D.L.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr (l)</td>
<td>13</td>
</tr>
<tr>
<td>Cr (k)</td>
<td>16</td>
</tr>
<tr>
<td>Ni (l)</td>
<td>178</td>
</tr>
<tr>
<td>Ni (k)</td>
<td>91</td>
</tr>
<tr>
<td>Pb (l)</td>
<td>27</td>
</tr>
<tr>
<td>Pb (k)</td>
<td>3</td>
</tr>
<tr>
<td>V (l)</td>
<td>48</td>
</tr>
<tr>
<td>V (k)</td>
<td>37</td>
</tr>
</tbody>
</table>
**Statistical treatment and graphical presentation**

**Trend detection**

One of the main purposes of the monitoring programme is to detect trends. Since only four years time-series are yet available, except for Grimsö (10 years), only log-linear regression analyses is carried out.

The slope of the line describes the annual change. A slope of 5% implies that the concentration is halved in 14 years whereas 10% corresponds to a similar reduction in 7 years and 2% in 35 years. See table 5 below.

<table>
<thead>
<tr>
<th></th>
<th>1%</th>
<th>2%</th>
<th>3%</th>
<th>4%</th>
<th>5%</th>
<th>7%</th>
<th>10%</th>
<th>12%</th>
<th>15%</th>
<th>20%</th>
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</thead>
<tbody>
<tr>
<td><strong>Increase</strong></td>
<td>70</td>
<td>35</td>
<td>24</td>
<td>18</td>
<td>14</td>
<td>10</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td><strong>Decrease</strong></td>
<td>69</td>
<td>35</td>
<td>23</td>
<td>17</td>
<td>14</td>
<td>10</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

**Legend to the plots**

The analytical results from each of the investigated elements are displayed in figures. Each site/tissue is represented by a separate plot except for time series shorter than 4 years.

The plot displays the geometric mean concentration of each year (circles) together with the individual analyses (small dots) and the 95% confidence intervals of the geometric means.

The overall geometric mean value for the time series is depicted as a horizontal, thin, dashed line.

The trend is presented by one or two regression lines (plotted if $p < 0.05$, two-sided regression analysis); one for the whole time period and one for the last ten years (if the time series is longer than ten years). Ten years is often too short a period to statistically detect a trend unless it is of considerable magnitude. Nevertheless the ten-year regression line will indicate a possible change in the direction of a trend. Furthermore, the residual variance around the line compared to the residual variance for the entire period will indicate if the sensitivity have increased as a result of e.g. an improved sampling technique or that problems in the chemical analysis have disappeared.

The log-linear regression lines fitted through the geometric mean concentrations follow smooth exponential functions.

Each plot has a header with a letter for the investigated county, BD = Norrbotten, Z = Jämtland, U = Västmanland, P = Älvsborg, F = Jönköping and G = Kronoberg county. Below the header of each plot the results from several statistical calculations are reported:

$n(tot)$= The first line reports the total number of analyses included together with the number of years ($n(yrs)$). Note that values below the detection limit are included in this number.

$m$ = The overall geometric mean value together with its 95% confidence interval is reported on the second line of the plot (N.B. d.f. = $n$ of years - 1).
**slope** = reports the slope, expressed as the annual change together with its 95% confidence interval.

**SD(lr)** = reports the square root of the residual variance around the regression line, as a measure of between-year variation, together with the *lowest detectable change* in the current time series with a power of 80%, one-sided test, \( \alpha = 0.05 \). The last figure on this line is the estimated *number of years* required to detect an annual change of 5% with a power of 80%, one-sided test, \( \alpha = 0.05 \).

**power** = reports the power to detect a log-linear trend in the time series (Nicholson & Fryer, 1991). The first figure represents the power to detect an annual change of 5% with the number of years in the current time series. The second figure is the power estimated as if the slope where 5% a year and the number of years were ten. The third figure is the *lowest detectable change* for a ten-year period with the current between year variation at a power of 80%.

**y(00)** = reports the concentration estimated from the regression line for the last year together with a 95% confidence interval, e.g. \( y(00)=2.51(1.92,3.27) \) is the estimated concentration of year 2000 where the residual variance around the regression line is used to calculate the confidence interval. Provided that the regression line is relevant to describe the trend, the residual variance might be more appropriate than the within-year variance in this respect.

**r²** = reports the coefficient of determination (\( r^2 \)) together with a p-value for a two-sided test (\( H_0: \text{slope} = 0 \)) i.e. a significant value is interpreted as a true change, provided that the assumptions of the regression analysis is fulfilled.

**tao** = reports Kendall's 't', and the corresponding p-value.

**SD(sm)** = reports the square root of the residual variance around the smoothed line. The significance of this line could be tested by means of an Analysis of Variance. The p-value is reported for this test. A significant result will indicate a non-linear trend component.

Below these nine lines are additional lines with information concerning the regression of the last ten years.

**Note. In most cases the y-axis representing the concentrations are different for liver and kidney tissue.**

**Summary**

Significant increasing trends of calcium (0.58% a year, liver), magnesium (0.49% a year, liver), manganese (1.1% a year, liver), molybdenum (both liver; 2.8% a year and kidney; 3.9% a year) and zinc (0.70% a year, liver) were shown for the time series from Grimsö. 21 years of analyses are now available. Significant decreasing trends of iron (-4.1% a year, liver, the last 10-year period), nickel (both liver; -7.9% a year the last 10-year period and kidney; -12% a year the last 10-year period) and lead (both liver; -3.7% a year and kidney; -5.7% a year) were shown for the time series from Grimsö.

Since only 2-5 years of analysis are yet available for the six other areas, it is not likely to find any significant trends in the time series unless relatively large systematic changes have occurred and few significant trends have actually been detected. The same thing is also true for geographical differences where only relatively large regional differences can be detected with the material yet available.
References


Calcium  Ca

Temporal variation
A significant positive time trend was detected in kidney tissue from Grimsö (annual increase 0.64%, p<0.023). Moreover, a significant negative trend in liver tissue from Kronoberg county (G) was detected (annual decrease 11%, p<0.030).

The number of years required to detect an annual change of 5% was 8 years for liver tissue and 7 years for kidney tissue in samples from Grimsö where 21 years of analyses are available. These time series are likely to detect an annual change of about 3 and 2% in liver and kidney respectively, provided that the power is fixed to 80% and the significance level is set to 5%. For the shorter time-series where only five years are yet available, the number of years required detecting an annual change of 5% varied between 4 and 12 years for liver tissue and between 6 and 13 years for kidney tissues. In general, time series of ten years are likely to detect an annual change between 1 and 8%.

Spatial variation
No significant differences in concentrations between the various counties were detected.

Differences between analysed tissues
The calcium concentrations in kidney tissue were significantly higher, about 1.4 times, compared to the concentrations found in liver for the 21 years time series from Grimsö. For the shorter time series the number of years are sometimes not sufficient for a statistical significant difference but there is a general pattern of higher calcium concentrations in kidney tissue (1.64).
Calcium, µg/g fresh w., moose liver (above) / kidney (below)
Cadmium  Cd

Temporal variation
No significant linear time trends were detected for cadmium concentrations. The ANOVA test showed that the smoothed line for concentrations of cadmium in kidney tissue from the Grimsö area indicates a significant non-linear trend component (p<0.036).

The number of years required to detect an annual change of 5% was 13 years for liver tissue and 14 years for kidney tissue in samples from Grimsö where 21 years of analyses are available. These time series are likely to detect an annual change of about 7 and 9% in liver and kidney respectively, provided that the power is fixed to 80% and the significance level is set to 5%. For the shorter time series where only five years are yet available, the number of years required detecting an annual change of 5% varied between 15 to 23 years for liver tissue and between 16 and 26 years for kidney tissue. Time series of ten years are likely to detect an annual change between 10 and 24 %.

Spatial variation
No significant differences in concentrations between the various counties were detected.

Differences between analysed tissues
The cadmium concentrations in kidney tissue were significantly higher, on average 4 to 5 times, compared to the concentrations found in liver.

Cadmium, µg/g fresh w., moose from Grimsö

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Cadmium, µg/g fresh w., moose liver (above) / kidney (below)
**Cobalt Co**

**Temporal variation**
No significant time trends were detected except for a significant negative trend in liver tissue from Norrbotten county (BD) (annual decrease 17%, p<0.041) and Jönköping county (F) (annual decrease 6.8%, p<0.014). The ANOVA test showed that the smoothed lines for concentrations of cadmium in liver and kidney tissues from the Grimsö area indicate a significant non-linear trend component (p<0.007, liver; p<0.012, kidney).

The number of years required to detect an annual change of 5% was 10 years for liver tissue and 8 years for kidney tissue in samples from Grimsö where 21 years of analyses are available. These time series are likely to detect an annual change of about 4 and 3% in liver and kidney respectively, provided that the power is fixed to 80% and the significance level is set to 5%. For the shorter time series where only five years are yet available, the number of years required detecting an annual change of 5% varied between 5 and 21 years for liver tissue and between 8 and 17 years for kidney tissue. Time series of ten years are likely to detect an annual change of between 1 and 16%.

**Spatial variation**
No significant differences in concentrations between the various counties were detected.

**Differences between analysed tissues**
The cobalt concentrations in liver tissue were generally significantly higher, on average 2 to 2.5 times, compared to the concentrations found in kidney tissue.

**Cobalt, µg/g fresh w., moose from Grimsö**
Cobalt, µg/g fresh w., moose liver (above) / kidney (below)

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>n(tot)</th>
<th>n(yrs)</th>
<th>m</th>
<th>slope</th>
<th>SD(lr)</th>
<th>slope</th>
<th>power</th>
<th>r²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD</td>
<td>51</td>
<td>5</td>
<td>.083</td>
<td>.122</td>
<td>.057</td>
<td>4.7%</td>
<td>.20</td>
<td>.81</td>
<td>&lt;.041</td>
</tr>
<tr>
<td>Z</td>
<td>26</td>
<td>3</td>
<td>.073</td>
<td>.187</td>
<td>.029</td>
<td>17%</td>
<td>.24</td>
<td>.96</td>
<td>96</td>
</tr>
<tr>
<td>U</td>
<td>79</td>
<td>5</td>
<td>.107</td>
<td>.250</td>
<td>.092</td>
<td>11%</td>
<td>.28</td>
<td>.98</td>
<td>98</td>
</tr>
<tr>
<td>P</td>
<td>50</td>
<td>5</td>
<td>.118</td>
<td>.125</td>
<td>.071</td>
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<td>.35</td>
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<td>F</td>
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<td>5</td>
<td>.108</td>
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<td>.094</td>
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<td>.49</td>
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<tr>
<td>G</td>
<td>59</td>
<td>5</td>
<td>.132</td>
<td>.153</td>
<td>.114</td>
<td>10%</td>
<td>.52</td>
<td>.95</td>
<td>95</td>
</tr>
</tbody>
</table>

Contaminant Research Group /NRM, Dep.Env.Assess./SLU  01.08.17  14:24, co
Chromium  Cr

Chromium is an element extremely sensitive for contamination that may interfere with natural levels in the samples.

Temporal variation
No significant linear time trends were shown for chromium in liver and kidney, neither for the longer time series from Grimsö, nor for the shorter ones. The ANOVA test showed that the smoothed lines for concentrations of chromium in liver and kidney tissues from the Grimsö area indicate a significant non-linear trend component (p<0.025, liver; p<0.034, kidney).

The number of years required to detect an annual change of 5% was 20 years both for liver and for kidney tissue from Grimsö where 21 years of analyses are available. These time series are likely to detect an annual change of 15 and 16 % respectively for liver and kidney tissues, provided that the power is fixed to 80% and the significance level is set to 5%. For the shorter time series where only five years are yet available, the number of years required detecting an annual change of 5% varied between 16 and 27 years for liver tissue and between 17 and 30 years for kidney tissue. Time series of ten years are likely to detect an annual change between 11 and 31%.

Spatial variation
No significant differences in concentrations between the various counties were detected.

Differences between analysed tissues
No differences in concentrations between liver and kidney were shown.

Chromium, µg/g fresh w., moose from Grimsö
Chromium, µg/g fresh w., moose liver (above) / kidney (below)
Copper Cu

Temporal variation
No significant linear trends were detected for copper concentrations. The ANOVA test showed that the smoothed line for concentrations of copper in kidney tissues from the Grimsö area indicates a significant non-linear trend component (p<0.004).

The number of years required to detect an annual change of 5% was 11 year for liver tissue and 8 years for kidney tissue in samples from Grimsö where 21 years of analyses are available. These time series are likely to detect an annual change of about 6 and 3% respectively for liver and kidney tissue, provided that the power is fixed to 80% and the significance level is set to 5%. For the shorter time series where only five years are yet available, the number of years required detecting an annual change of 5% varied between 6 and 21 years for liver tissue and between 5 and 9 years for kidney tissue. Time series of ten years are likely to detect an annual change of between 1 and 16%.

Spatial variation
No significant differences in concentrations between the various counties were detected.

Differences between analysed tissues
The copper concentration in liver is significantly higher compared to kidney, between 3 – 9 times.

Copper, µg/g fresh w., moose from Grimsö
Copper, µg/g fresh w., moose liver (above) / kidney (below)

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>n(tot)</th>
<th>n(yrs)</th>
<th>m (95% CI)</th>
<th>slope (95% CI)</th>
<th>SD (95%)</th>
<th>r²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD</td>
<td>51</td>
<td>5</td>
<td>22.1 (15.5, 31.5)</td>
<td>10% (-17, 38)</td>
<td>27.32%, 14 yr</td>
<td>.32</td>
<td>NS</td>
</tr>
<tr>
<td>Z</td>
<td>26</td>
<td>3</td>
<td>30.9 (4.4, 212)</td>
<td>51% (-87, -15)</td>
<td>6.42%, 6 yr</td>
<td>.09</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>U</td>
<td>57</td>
<td>5</td>
<td>28.2 (21.0, 38.0)</td>
<td>6.8% (-18, 32)</td>
<td>14.50%, 7.6%</td>
<td>.20</td>
<td>NS</td>
</tr>
<tr>
<td>P</td>
<td>50</td>
<td>5</td>
<td>20.8 (15.3, 28.4)</td>
<td>4.4% (-34, 25)</td>
<td>22.46%, 12 yr</td>
<td>.17</td>
<td>NS</td>
</tr>
<tr>
<td>F</td>
<td>59</td>
<td>5</td>
<td>12.4 (5.47, 18.3)</td>
<td>23% (-11.16)</td>
<td>38.48%, 17 yr</td>
<td>.54</td>
<td>NS</td>
</tr>
<tr>
<td>G</td>
<td>51</td>
<td>4</td>
<td>3.88 (3.67, 4.10)</td>
<td>-1.7% (-5.8, 2.4)</td>
<td>.04, 4.4%, 5 yr</td>
<td>.89</td>
<td>NS</td>
</tr>
<tr>
<td>Z</td>
<td>26</td>
<td>3</td>
<td>3.92 (3.26, 4.70)</td>
<td>-3.6% (-45, 38)</td>
<td>.07, 50%, 7 yr</td>
<td>.17</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>U</td>
<td>79</td>
<td>5</td>
<td>3.70 (3.49, 3.93)</td>
<td>.25% (-5.3, 5.8)</td>
<td>.06, 9%, 6 yr</td>
<td>.69</td>
<td>.01</td>
</tr>
<tr>
<td>P</td>
<td>51</td>
<td>5</td>
<td>3.43 (2.94, 3.99)</td>
<td>-1.3% (-17.14)</td>
<td>.06, 1.7%</td>
<td>.06</td>
<td>NS</td>
</tr>
<tr>
<td>F</td>
<td>48</td>
<td>5</td>
<td>3.40 (3.02, 3.83)</td>
<td>2.3% (-8.0, 13)</td>
<td>.10, 11%, 9 yr</td>
<td>.32</td>
<td>.98</td>
</tr>
<tr>
<td>G</td>
<td>59</td>
<td>5</td>
<td>3.68 (3.23, 4.20)</td>
<td>-2.2% (-14.9, 4)</td>
<td>.12, 13%, 9 yr</td>
<td>.28</td>
<td>.11</td>
</tr>
</tbody>
</table>

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Iron  Fe

Temporal variation
A significant negative time trend was detected for iron concentrations in liver from Grimsö during the last ten years (annual decrease 4.1%, p<0.016). A negative trend was also detected for iron concentrations in liver from Norrbotten county (BD) (annual decrease 11%, p<0.007). The ANOVA test showed that the smoothed lines for concentrations of iron in liver and kidney tissues from the Grimsö area indicate a significant non-linear trend component (p<0.025, liver; p<0.022, kidney).

The number of years required to detect an annual change of 5% was 11 years for liver tissue and 8 years for kidney tissue in samples from Grimsö where 21 years of analyses are available. These time series are likely to detect an annual change of about 5 and 3% respectively for liver and kidney tissue, provided that the power is fixed to 80% and the significance level is set to 5%. For the shorter time series where only five years are yet available, the number of years required detecting an annual change of 5% varied between 3 and 12 years for liver tissue and between 7 and 14 years for kidney tissue. Time series of ten years are likely to detect an annual change of between 1 and 8%.

Spatial variation
No significant differences in concentrations between the various counties were detected.

Differences between analysed tissues
The iron concentration in liver is significantly higher compared to kidney, between 1.8 and 2.6 times.
Iron, µg/g fresh w., moose liver (above) / kidney (below)
Magnesium  Mg

Temporal variation
A significant positive time trend was detected for magnesium concentrations in liver from Grimsö (annual increase 0.5%, p<0.035).

The number of years required to detect an annual change of 5% was 6 years for both liver and kidney tissue in samples from Grimsö where 21 years of analyses are available. These time series are likely to detect an annual change of about 2% in both liver and kidney tissue, provided that the power is fixed to 80% and the significance level is set to 5%. For the shorter time series where only five years are yet available, the number of years required detecting an annual change of 5% varied between 5 and 8 years for liver tissue and between 5 and 6 years for kidney tissue. Time series of ten years are likely to detect an annual change approximately between 1 and 3%.

Spatial variation
No significant differences in concentrations between the various counties were detected.

Differences between analysed tissues
No differences in concentrations between liver and kidney were shown.

Magnesium, µg/g fresh w., moose from Grimsö
Magnesium, μg/g fresh w., moose liver (above) / kidney (below)
**Manganese Mn**

**Temporal variation**

A significant positive time trend was detected for manganese concentrations in liver from Grimsö (annual increase 1.1%, p<0.002).

The number of years required to detect an annual change of 5% was 7 years both for liver and kidney tissue in samples from Grimsö where 21 years of analyses are available. These time series are likely to detect an annual change of about 2% for both liver and kidney tissue, provided that the power is fixed to 80% and the significance level is set to 5%. For the shorter time series where only five years are yet available, the number of years required detecting an annual change of 5% varied between 6 and 16 years for liver tissue and between 7 and 13 years for kidney tissue. Time series of ten years are likely to detect an annual change of between 2% and 10%.

**Spatial variation**

No significant differences in concentrations between the various counties were detected.

**Differences between analysed tissues**

There are significant differences between the analysed tissues but the differences are small and not consistent between the regions.

Manganese, µg/g fresh w., moose from Grimsö
Manganese, µg/g fresh w., moose liver (above) / kidney (below)
Molybdenum    Mo

Temporal variation
Significant positive time trends were detected for molybdenum concentrations in liver and kidney tissue from Grimsö (annual increase 2.8%, p<0.001, liver; 3.9%, p<0.001, kidney). The ANOVA test showed that the smoothed line for concentrations of molybdenum in liver tissues from the Grimsö area indicates a significant non-linear trend component (p<0.046).

The number of years required to detect an annual change of 5% was 10 years for liver tissue and 11 years for kidney tissue in samples from Grimsö where 21 years of analyses are available. These time series are likely to detect an annual change of about 5% in both liver and kidney tissue, provided that the power is fixed to 80% and the significance level is set to 5%. For the shorter time series where only five years are yet available the number of years required detecting an annual change of 5% varied between 5 to 20 years for liver tissue and between 8 and 19 years for kidney tissue. These time series are likely to detect an annual change of between approximately 1 and 15%.

Spatial variation
No significant differences in concentrations between the various counties were detected.

Differences between analysed tissues
The molybdenum concentration in liver is significantly higher compared to kidney, between 2.5 and 3 times.
Molybdenum, µg/g fresh w., moose liver (above) / kidney (below)
Nickel  Ni

The nickel concentrations measured in moose, in liver and kidney tissue are close to the detection limit. Hence, the analytical precision plays a larger role for the unexplained between-year variation compared to most of the other investigated trace metals.

Temporal variation

Significant negative trends were detected for nickel concentrations in kidney tissue for the last 10-year period in samples from Grimsö (annual decrease 12%, p<0.039, kidney). A negative trend was also detected for nickel concentrations in liver from Kronoberg county (G) (annual decrease 34%, p<0.028).

The number of years required to detect an annual change of 5% was 18 years for kidney tissue in samples from Grimsö where 21 years of analyses are available. The time series for kidney are likely to detect an annual change of about 13% in kidney tissue, provided that the power is fixed to 80% and the significance level is set to 5%. For the shorter time series where only five years are yet available, the number of years required detecting an annual change of 5% varied between 10 and 19 years for liver tissue and between 8 and 23 years for kidney tissue. Time series of ten years are likely to detect an annual change of between 3 and 20%.

Spatial variation

No significant differences in concentrations between the various counties were detected.

Differences between analysed tissues

The nickel concentration in kidney is significantly higher compared to liver for most of the time series, 1.2 to 3.7 times.

Nickel, µg/g fresh w., moose from Grimsö
Nickel, µg/g fresh w., moose liver (above) / kidney (below)
Lead Pb

Temporal variation
Significant negative trends were detected for lead concentrations in both liver and kidney for the longer time series from Grimsö (annual decrease 3.7%, p<0.001, liver; 5.7%, p<0.001, kidney). The ANOVA test showed that the smoothed line for concentrations of lead in liver tissues from the Grimsö area indicates a significant non-linear trend component (p<0.012).

The number of years required to detect an annual change of 5% was 10 years for liver tissue and 12 years for kidney tissue in samples from Grimsö where 21 years of analyses are available. These time series are likely to detect an annual change of about 5 and 7% respectively in liver and kidney tissue, provided that the power is fixed to 80% and the significance level is set to 5%. For the shorter time series where only five years are yet available, the number of years required detecting an annual change of 5% varied between 11 and 20 years for liver tissue and between 15 and 20 years for kidney tissue. Time series of ten years are likely to detect an annual change of between 5 and 15%.

Spatial variation
No significant differences in concentrations between the various counties were detected.

Differences between analysed tissues
There are no significant differences between the concentrations in liver and kidney.

Lead, µg/g fresh w., moose from Grimsö
Lead, µg/g fresh w., moose liver (above) / kidney (below)
Vanadium  V

The vanadium concentrations measured in moose liver and kidney tissue are close to the detection limit. Hence, the analytical precision plays a larger role for the unexplained between-year variation compared to most of the other investigated trace metals.

Temporal variation
No significant linear trends were detected for vanadium concentrations. However, the ANOVA test showed that the smoothed line for concentrations of vanadium in kidney tissues from the Grimsö area indicates a significant non-linear trend component (p<0.048).

The number of years required to detect an annual change of 5% was 16 years for liver tissue and 14 years for kidney tissue in samples from Grimsö where 21 years of analyses are available. These time series are likely to detect an annual change of about 10 and 8% respectively in liver and kidney tissue, provided that the power is fixed to 80% and the significance level is set to 5%. For the shorter time series where only five years are yet available, the number of years required detecting an annual change of 5% varied between 8 and 26 (50) years for liver tissue and between 12 and 23 (49) years for kidney tissue. Time series of ten years are likely to detect an annual change of between 3 and 24 (81)%.

Spatial variation
No significant differences in concentrations between the various counties were detected.

Differences between analysed tissues
There are no significant differences between the concentrations in liver and kidney.

Vanadium, µg/g fresh w., moose from Grimsö
Vanadium, µg/g fresh w., moose liver (above) / kidney (below)
Zinc  Zn

Temporal variation
A significant positive trend was detected for zinc concentrations in liver tissue from Grimsö (annual increase 0.7%, p<0.008). A significant negative trend was detected for zinc concentrations in kidney tissue from Grimsö for the ten last years (annual decrease 1.4%, p<0.027).

The number of years required to detect an annual change of 5% was 6 years for both liver and kidney tissue in samples from Grimsö where 21 years of analyses are available. These time series are likely to detect an annual change of about 2% in both liver and kidney tissue, provided that the power is fixed to 80% and the significance level is set to 5%. For the shorter time series where only five years are yet available the number of years required detecting an annual change of 5% varied between 3 and 15 years for liver tissue and between 6 and 10 years for kidney tissue. Time series of ten years are likely to detect an annual change of between 1 to 9%

Spatial variation
No significant differences in concentrations between the various counties were detected.

Differences between analysed tissues
There are no significant differences between concentrations in liver and kidney.

Zinc, µg/g fresh w., moose from Grimsö
Selenium  Se

Temporal variation
No significant trends were detected in the 5-year time series. Only two year’s results of analysis are yet available from Grimsö. Hence, no time trend analysis is available. Note that selenium values are from samples of liver and muscle. From Grimsö are only muscle analysed.

For the short time series where only five years are yet available the number of years required to detect an annual change of 5% varied between 13 to 18 (38) years for liver tissue and between 7 and 19 years for muscle tissue, provided that the power is fixed to 80% and the significance level is set to 5%. Time series of ten years are likely to detect an annual change of between 2 and 14 (47) %.

Spatial variation
No significant differences in concentrations between the various counties were detected.

Differences between analysed tissues
There are significant differences between the concentrations in liver and muscle.

Selenium in muscle, ng/g fresh w., moose from Grimso

Graph showing the concentration distribution with n(tot)=20, n(yrs)=2 and m=40.3 (25.6, 63.3)
Selenium, ng/g fresh w., moose liver (above) / muscle (below)

BD

n(tot)=42, n(yrs)=4
m=215 (101.4, 458)
slope=33% (-15.8, 2)
SD(lr)=.25, 56%, 13 yr
power=.12/ .49/ 7.7%

Z

n(tot)=13, n(yrs)=2
m=117 (.958, 7)
slope=-4.0% (-82, 74)
SD(lr)=.41, 105%, 18 yr
power=.11/.26/13%

U

n(tot)=64, n(yrs)=4
m=230 (22.6, 41)
slope=7.6% (-41, 56)
SD(lr)=.25, 57%, 14 yr
power=.12/.48/7.8%

P

n(tot)=42, n(yrs)=3
m=44.3 (18.5, 106)
slope=-12% (***, 241)
SD(lr)=.43, %, 19 yr
power=.13/.24/14%

F

n(tot)=42, n(yrs)=4
m=42.0 (37.2, 47.4)
slope=3.4% (-11, 18)
SD(lr)=.26/1.0/2.3%

G

n(tot)=52, n(yrs)=4
m=49.2 (34.7, 69.8)
slope=13% (-20, 46)
SD(lr)=.17, 36%, 11 yr
power=.14/.77/5.3%

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Mercury  Hg

The concentrations of mercury in muscle tissue are very close to or below detection limits.

Temporal variation
Significant increasing trends were found for mercury concentrations in muscle tissue from Norrbotten county (BD) and Jönköping county (F) (annual increase 8.3%, p<0.015 (BD) and 20%, p<0.018 (F)). Only two years of analysis of muscle are yet available from Grimsö. Hence no time trend plots are presented.

For the short time series where only five years are yet available the number of years required to detect an annual change of 5% varied between 7 and 26 years for liver tissue and between 6 and 20 years for muscle, provided that the power is fixed to 80% and the significance level is set to 5%. Time series of ten years are likely to detect an annual change of between 2 to 25%.

Spatial variation
No significant differences in concentrations between the various counties were detected.

Differences between analysed tissues
The measured concentrations in liver are significantly higher compared to the concentrations measured in muscle, in general 5 to 6 times.
Mercury, ng/g fresh w., moose liver (above) / muscle (below)