

Finnish Food Safety Authority Evira
Helsinki, Finland

and

Department of Environmental Science
Faculty of Natural and Environmental Sciences
University of Kuopio
Kuopio, Finland

**THE LEVELS OF HEAVY METALS IN MOOSE, REINDEER AND HARES IN FINLAND-
RESULTS OF TWENTY YEARS' MONITORING**

Eija-Riitta Venäläinen

Academic Dissertation

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Supervised by

Professor Kimmo Peltonen
Unit of Chemistry and Toxicology
Finnish Food Safety Authority Evira
Helsinki, Finland

Professor Pentti Kalliokoski
Department of Environmental Science, University of Kuopio
Kuopio, Finland

Reviewed

Doctor Matti Verta
Finnish Environment Institute
Helsinki, Finland

Docent Mauri Nieminen
Finnish Game and Fisheries Research Institute
Reindeer Research Station
Kaamanen, Finland

Opponent

Docent Georg Alfthan
Department of Health and Functional Capacity
Biomarker Laboratory
National Public Health Institute
Helsinki, Finland

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CONTENTS

ABSTRACT	5
ACKNOWLEDGEMENTS	7
1. LIST OF ABBREVIATIONS	9
2. LIST OF ORIGINAL PUBLICATIONS	11
3. INTRODUCTION	12
3.1. Heavy metals	12
3.2. Heavy metal emission	13
3.3. Atmospheric heavy metal deposition and emission in Finland	15
4. AIMS OF THE STUDY	17
5. REVIEW OF LITERATURE	18
5.1 The occurrence and environmental effects of heavy metals in the environment	18
5.1.1. Cadmium	18
5.1.2. Lead	20
5.1.3. Copper	21
5.1.4. Zinc	23
5.2. Effects of exposure to heavy metals on human health	24
5.2.1. Cadmium	24
5.2.2. Lead	25
5.2.3. Copper	26
5.2.4. Zinc	27
5.3. Indicator species to study the contamination of heavy metals	28
5.3.1. Heavy metal uptake by plants	28
5.3.1.1. Mosses and berries	29
5.3.1.2. Willow and aspen	31
5.3.2. Earthworms	32
5.3.3. Moles	33
5.4. The levels of heavy metals in relevant species in different countries	34
5.4.1. Moose	34
5.4.1.1. Moose in North America	34
5.4.1.2. Moose in Scandinavia and Russia	35
5.4.1.3. Copper deficiency in moose samples	35
5.4.2. Caribou and reindeer	36
5.4.2.1. Caribou in North America	36
5.4.2.2. Caribou/reindeer in Europe and Russia	37
5.4.3. Red deer	37
5.4.4. Hare	38
5.4.4.1. European hare, Mountain Hare and Snowshoe Hare	38
5.5. Analytical technique used to measure heavy metals	39

5.5.1. Atomic absorption	39
6. MATERIALS AND METHODS	41
6.1. Sample collection	41
6.2. Age determination	43
6.3. Analytical methods	44
6.4. Quality control	45
6.5. Statistical analysis	45
7. RESULTS	46
7.1. Heavy metal levels in moose samples	46
7.2. Heavy metal levels in reindeer samples	52
7.3. Heavy metal levels in hare samples	54
8. DISCUSSION	56
8.1. Cadmium levels in moose samples	56
8.2. Lead levels in moose samples	57
8.3. Copper levels in moose samples	57
8.4. Zinc levels in moose samples	58
8.5. Cadmium levels in reindeer samples	58
8.6. Lead levels in reindeer samples	58
8.7. Copper levels in reindeer samples	59
8.8. Chromium and nickel levels in reindeer samples	59
8.9. Cadmium levels in hare samples	60
8.10. Lead levels in hare samples	61
8.11. Copper and zinc levels in hare samples	61
8.12. Chromium and nickel levels in hare samples	62
8.13. Consumption of meat and organs of moose and reindeer	62
9. CONCLUSION AND REMARKS	63
REFERENCES	66
Appendix 1: Tables 2-23	90

ABSTRACT

Lead and cadmium are toxic elements, which are a natural part of earth crust. They are taken up from the soil and transferred upwards in the plant and animal food chain.

Cadmium is widely distributed throughout the natural environment with human activities having an important role in its dispersion into the biosphere. The natural sources of cadmium are volcanic eruptions and old granite rocks, which are an important geochemical source. Anthropogenic sources of cadmium are related mainly to mining, fertilizers and atmospheric deposition.

Leaded gasoline has been most important source of atmospheric lead. However, most countries have now prohibited the use of leaded gasoline. This action has greatly reduced emissions of lead into the atmosphere. Anthropogenic sources of lead other than traffic are typically fossil fuel combustion, non-ferrous metal production and iron and steel production.

Zinc and copper are important constituents in a number of different enzyme functions in man and animals. Zinc and copper reach the environment via industrial releases involving production and refining of metals.

In 1997 emissions of heavy metal particulates were only a third of their values in the early 1990s due to the installation of sulphur removal systems.

In this study lead, cadmium, copper and zinc levels in Finnish game animals (moose, hares) and farmed game animal (reindeer) were studied during twenty years period. About 500 moose were collected between 1980 and 1999 from south western, southern, central and south eastern Finland. About the same number of Mountain and European hare samples were collected during the period 1980 and 1993 from south western, southern, south eastern and northern Finland. About 300 reindeer were collected in 1990–1992 from southern, eastern, western and northern Lapland. After these initial projects, it was decided to collect moose and reindeer samples every year as part of national residue control program; fifteen moose and ten reindeer per year. In this residue control program, also cattle samples have been collected regularly; muscle, liver and kidney from 30 animals in every year.

The lead and cadmium levels in muscle tissue has decreased in all studied animals during the monitoring years being now near to the limit of quantification; 0.01 mg/kg w.w. for lead and 0.001 mg/kg w.w for cadmium. Also the lead levels in liver and kidney samples have decreased during the monitoring period but the moose cadmium levels have increased. The zinc levels had also increased in moose samples but there were no significant

correlation with increasing cadmium and zinc levels. The lead levels in liver and kidney in moose varied in 1999 from 0.04 to 0.07 mg/kg w.w. and 0.05-0.07 mg/kg w.w. The corresponding values for cadmium are 0.71-1.28 mg/kg w.w. and 4.95- 6.18 mg/kg w.w.

The cadmium and lead levels in Mountain hares are higher than in European hares and the kidney cadmium levels in Mountain hares are statistically significantly higher than the kidney cadmium levels in moose.

The lead levels in liver and kidney samples in both adult and calves of reindeer have been invariably below the recommended maximum level in the EU (0.5 mg/kg). However, the kidney cadmium level exceeds the maximum level (1.0 mg/kg) in almost all adult reindeer samples and also in some calves.

The meat of Finnish moose, reindeer and hares does not contain residues of cadmium and lead and therefore consumers can be assured that consumption of the meat is not a health risk. The consumption of the organs of the animals studied may represent a health risk for human.

However, the levels are far from the toxic levels to the animals themselves.

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The data collection for this study was started at the National Veterinary Institute (VELL) and was completed in the Finnish Food Safety Authority Evira. The name and organization of institute have changed from VELL via National Veterinary and Food Research Institute (EELA) to Evira during the time period of this work but this has not disturbed the study and the laboratory facilities have been excellent all the time.

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Helsinki, April

Eija-Riitta Venäläinen

1. LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrometry
AMAP	Arctic Monitoring and Assessment Programme
ATP	Adenosine 5'-triphosphate
BCR	Community Bureau of Reference
Cd	Cadmium
CdCl ₂	Cadmium chloride
Cu	Copper
DNA	Deoxyribonucleic acid
d.w.	Dry weight
EC	European Community
EEC	European Economic Community
EELA	National Veterinary and Food Research Institute
EU	European Union
EVI	National Food Agency
Evira	Finnish Food Safety Authority
FAO	Food and Agriculture Organisation of the United Nations
FAPAS	Food Analysis Performance Assessment
Fe	Iron
GFAA	Graphite Furnace Atomic Absorption
ISS	Istituto Superiore di Sanita
JECFA	Joint Expert Committee on Food Additives
K	Kelvin
LOD	Limit of detection
LOQ	Limit of quantification
mg	Milligram (10 ⁻³ gram)
ML	Maximum level
MMM	Ministry of Agriculture and Forestry
MT	Metallothionein
ng	Nanogram (10 ⁻⁹ gram)
Ni	Nickel
NIST	National Institute of Standard & Technology
NH ₃	Ammonia
NO _x	Oxides of nitrogen
pg	Picogram (10 ⁻¹² gram)
Pb	Lead
PTWI	Provisional Tolerable Weekly Intake
RNA	Ribonucleic acid
SH	Sulphydryl
SO ₂	Sulphide dioxide

t	Ton
VELL	National Veterinary Institute
WHO	World Health Organisation
Zn	Zinc
µg	Microgram (10^{-6} gram)
w.w.	Wet weight

2. LIST OF ORIGINAL PUBLICATIONS

- I Niemi, A., Venäläinen, E.-R., Hirvi, T., Valtonen, M. 1993, Heavy metals in muscle, liver and kidney from Finnish Elk in 1980-81 and 1990. *Bulletin of Environmental Contaminations and Toxicology* 50: 834-841.
- II Rintala, R., Venäläinen, E.-R., Hirvi, T. 1995. Heavy metals in muscle, liver, and kidney from Finnish Reindeer in 1990–91 and 1991–92. *Bulletin of Environmental Contaminations and Toxicology* 54: 158–165.
- III Venäläinen, E.-R., Niemi A., Hirvi, T. 1996. Heavy metals of hares in Finland. 1980-82 and 1992–93. *Bulletin of Environmental Contaminations and Toxicology* 56: 251–258.
- IV Venäläinen, E.-R., Peltonen, K., Anttila, M. 2005. Heavy Metals in Tissue Samples of Finnish moose, *Alces alces*. *Bulletin of Environmental Contaminations and Toxicology* 74: 526–536.

3. INTRODUCTION

3.1. Heavy metals

The definition of heavy metals varies in the scientific literature. However, the most wide definition consider heavy metals as all of those metals whose atomic weight is more than that of hydrogen. According to another definition, a heavy metal is a metal, whose density is above 5 g/cm³ (Anon., 1964; Lapedes, 1974; Sherlock, 1985).

The many heavy metals can be considered as environmental contaminants; however some of them at low concentrations are essential trace elements (zinc, copper and iron). Typical examples of heavy metals are iron, cadmium, nickel, mercury, arsenic, chromium, thallium, cobalt, zinc, copper and lead (Table 2).

Heavy metals are natural components of the Earth's crust and they enter into the biosphere both naturally and as a result of human activities; mostly from industry, traffic and impurities of fertilizers. The metals are predominantly transferred as molecules or particulate matter via the atmosphere, mostly over distances. The amount of anthropogenically derived heavy metals has increased continuously since the beginning of the industrial revolution but public awareness and concern associated with their environmental and health risks have risen sharply in the last decades. One focal point of metal emissions is congested urban areas where there is a high density of industry and traffic (Komarnicki et al., 2005). The soil inherits heavy metals from its parent's materials. Some soils have been found to have a high background of some heavy metals, which are toxic to plants and wildlife, i.e. these soils contain extremely high concentrations of these elements in the parent materials (He et al., 2005).

Heavy metals are dangerous because they tend to bioaccumulate, in other words, there will be an increase in the concentration of the metal in a biological organism over time, compared to its concentration in the environment. Compounds accumulate in living organisms any time that they are taken up and stored faster than they are metabolized or excreted (Pascoe et al., 1994).

The Commission Regulation (EC) No 466/2001 adopted in March 2001 established maximum levels (MLs) of cadmium and lead in certain foodstuffs. The maximum lead levels in the meat of bovine animals and edible offals of cattle are 0.1 mg/kg and 0.5 mg/kg, respectively. The maximum cadmium levels for bovine meat, liver and kidney are 0.05, 0.5 and 1.0 mg/kg w.w., respectively. For copper and zinc no ML values have been established.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established a Provisional Tolerable Weekly Intake (PTWI) for cadmium and lead. The PTWI for cadmium is 0.007 mg/kg bodyweight. This is equivalent to 0.49 mg/week for an individual weighing 70 kg. For lead, the PTWI is set to 0.025 mg/kg bodyweight, equivalent to 1.75 mg/week for a person weighing 70 kg.

Cadmium and lead have been included in monitoring programs because they are toxic and not at all essential for animals or human health. Copper and zinc are essential trace elements but also toxic elements and therefore they too have been chosen for this study.

3.2. Heavy metal emissions

The data of world-wide emissions of trace metals from major anthropogenic sources to the atmosphere in the mid-1990s indicate that the stationary fossil fuel combustions continue to be the major source of chromium (69 %), mercury (66 %), manganese (85 %), antimony (47 %), selenium (89 %), tin (89 %) and thallium (almost 100 %) these mostly coming from coal combustion with the major source of nickel (90 %) and vanadium (100 %) with respect to oil combustion. Combustion of leaded, low-leaded, and unleaded gasoline continues to be the major source of atmospheric lead emissions, accounting for about 74 % to the total anthropogenic emissions of this metal in 1995. The third source of trace metals is non-ferrous metal production, which is the largest source of atmospheric arsenic (69 %), cadmium (73 %), copper (70 %), indium (100 %) and zinc (72 %) (Pacyna et al., 1995; Pirrone et al., 1996; Pacyna & Pacyna, 2001).

There are many high-temperature processes, such as coal and oil combustion in electric power stations and heat and industrial plants, gasoline combustion, roasting and smelting of ores in non-ferrous metal smelters, melting operations in ferrous foundries, refuse incineration, as well as kiln operations in cement plants which emit various trace metals. These metals enter the atmosphere and from there, gain access to the aquatic and terrestrial ecosystems. Practically every industry discharges at least one trace metal into these ecosystems.

Emissions from the incineration of municipal wastes and sewage sludge are the most difficult to estimate because of the lack of information on the actual amounts and composition of wastes incinerated in various countries and their composition. Once more reliable data on the magnitude of wastes incinerated and the level of trace metals in these wastes are obtained, additional focus can be placed in making an inventory of future emissions.

On a global scale, the largest metal emissions come from sources in Asia. This is related to the growing demands for energy in the region and their increasing industrialization. As a

result, the Asian emissions are not only larger than the emissions from other countries, but they also show an increasing trend. In Europe and North America, emissions of trace metals have shown a decreasing tendency over the last decades (Pacyna & Pacyna, 2001).

There are several reasons for the decrease in emissions in Western and Northern Europe; the reduction in coal consumption, the increase in the use of unleaded fuels, the development of industrial manufacturing processes and the tightening of environmental legislation, and in Eastern Europe by the closure of outdated industrial plants and coal-fired power plants, and by the reduction in industrial output.

It is generally accepted that the principal natural sources of trace metals in the atmosphere are wind-borne soil particles, volcanoes, seasalt spray and wild forest fires. It has also been shown that particulate organic matter is the dominant component of atmosphere aerosols in non-urban areas and that 30–60 % of airborne trace metals in forested regions can be attributed to aerosols of biogenic origin (Nriagu, 1989).

The comparison of global anthropogenic emissions with global natural emissions suggests that anthropogenic emission of lead and vanadium are one order of magnitude higher than the natural emissions of these particular metals. Anthropogenic emissions are a factor of two to three times higher than the natural emissions for cadmium and nickel, and the two sources are comparable for copper, mercury, molybdenum, antimony and zinc. The natural emissions of arsenic, selenium and chromium are higher than the anthropogenic emission by a factor of 2 to 3. For manganese the natural sources are by far more significant than the anthropogenic sources (Pacyna & Pacyna, 2001).

For the most toxic metals, the natural fluxes are low compared with emissions from industrial activities, implying that mankind has become the key factor in the global atmospheric cycle of trace metals and metalloids (Nriagu, 1989).

3.3. Atmospheric heavy metal deposition and emission in Finland

Heavy metals possess a strong affinity to be adsorbed onto organic surfaces. The humus layer in coniferous forest ecosystem is also effective in retaining heavy metals, particularly lead and copper, through adsorption and these metals form complexes with organic matter. The mobility and toxicity of heavy metals in soils is strongly related to the acidity and organic matter content. The dependence of metal mobility on acidity has been experimentally demonstrated in a number of simulated acidification studies. The mobility of cadmium, zinc and nickel in soil, lake and stream water has been shown to be strongly related to pH, while lead and copper fluxes tend to be more determined by organic matter considerations (Tyler, 1981; Bergkvist et al., 1989).

Most anthropogenic sources emit air pollutants in the form of gases (SO_2 , NO_x , NH_3) and only a minor part is in a particulate form. Sulphate, nitrate and ammonium ions are formed during long-range transport, the residence time of which is several days, enabling transport over long distance. Some metals, e.g. copper, lead and nickel are often associated with SO_2 emissions from metal smelters and are deposited locally. However, long-range transport of heavy metals via atmosphere can occur with the metals in soluble forms. As a result, many regions of the world are exposed to air pollutants, even areas that are hundreds of kilometres away from the source of the emissions (Lutterman and Freedman, 2000).

Emission of SO_2 in Finland have decreased by more than 80 % since 1981, whereas there has been little change in the emissions of nitrogen compounds. Most of the SO_2 emissions come from energy production and industrial processes, nearly half of the NO_x emissions from traffic and most of the NH_3 emissions from agriculture (EMEP, 2000).

From the Finnish perspective, the emissions of SO_2 are largest in the countries to the south as well as those in the southwest and west of Finland. The change in the amount transported from these sectors together with the lower SO_2 emission reductions, especially in the southern sector, have contributed to the lower reductions in the sulphate concentrations. The highest sulphur dioxide and particulate sulphate concentrations in southern and central Finland originate from the sectors between south and east, while the transport is most frequent from the western sectors. Thus, the atmospheric sulphur exposure is greatly affected by transport via the cleaner westerly winds, although the highest concentrations also have a remarkable effect on the mean concentration level. In the north, the transport from north eastern and northern sectors is also important for the exposure. From the Finnish perspective, the nitrogen oxide emissions are highest in the countries in the south western and western sectors. In these regions, the reduction has been lower than the average in the area contributing to the deposition in Finland. The significant transport from these areas has highly affected the Finnish concentrations and exposure (Ruoho-Airola, 2004).

The total annual emissions of cadmium, lead, copper and zinc into air from various sources fell by 80 %, 95 %, 70 % and 85 %, respectively in the time period 1990 -1997 (Melanen et al., 1999).

4. AIMS OF THE STUDY

The aims of the study were

- to setup a monitoring program to measure heavy metal levels in some game/farmed game animals.
- to discover locally important emission sources and to identify their role in wild animal exposure.
- to monitor moose, reindeer and hare exposure by using various heavy metals in various organs.
- to speculate on the reasons for the increasing levels of cadmium in selected animal samples.
- to identify the possible toxicity of observed levels to humans and to these particular animal species.

5. REVIEW OF THE LITERATURE

5.1. The occurrence and environmental effects of heavy metals in the environment

5.1.1. Cadmium

Cadmium is a natural element which accounts for about 5×10^{-5} % of the Earth's crust, being one of the rare metals. It is usually found as a mineral combined with other elements such as oxygen (cadmium oxide), chlorine (cadmium chloride), or sulphur (cadmium sulphate, cadmium sulphide). Natural sources of cadmium into the the biosphere include volcanoes, and the weathering of rocks and minerals (UNEP, 2006).

Cadmium is mainly released into atmosphere by domestic and industrial combustion processes; this accounts from 33 % to 72 % of the local cadmium emissions (Yaaqub et al., 1991; Melanen et al., 1999).

Atmospheric transport is probably the most important mechanism of cadmium dispersion in the environment. Once emitted to the atmosphere, cadmium becomes dispersed and carried by the winds and eventually becomes deposited to land or water bodies. The deposition can occur locally (close to source), regionally, or in locations far from emission sources. Some cadmium emissions can be transported by airflows over hundreds or even thousands of kilometres and impact on human health and ecosystems far away from the emission source. The degree of atmospheric dispersion and distances of deposition depend on various factors, including, particle size, stack height, and meteorology (Harrison and Williams, 1982; UNEP, 2006).

Cadmium is and has been used for many different purposes. Cadmium and its compounds are widely used in electroplating metals and alloys because of their anticorrosive properties. The major fields of applications are NiCd batteries, pigments, plating and stabilizers (Alloway, 1995; AMAP, 2005; UNEP, 2006).

Council Directive 91/157/EEC of 18 March 1991 on batteries and accumulators containing certain dangerous substances sets restrictions and a ban on the marketing of certain batteries and accumulators containing dangerous substances such as cadmium, lead and mercury.

Directive 91/338/EEC (Council Directive 91/338/EEC of 18 June 1991 amending for the 10th time Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing

and use of certain dangerous substances and preparations) prohibits the use of cadmium and its compounds in three areas: pigments, stabilisers and surface treatment. Furthermore, it provides a general exemption clause where justification exists on the grounds of safety or reliability and where the use of cadmium is unavoidable.

Table 1. The trend of cadmium production in Finland in tons/year (GSF, 2003)

Metal	Time period				
	1970	1980	1990	2000	2001
Cadmium	258	582	563	682	604

Cadmium is produced mainly as a by-product from mining, smelting and refining of zinc (from sulphide ore) and to a lesser degree, lead and copper (Table 1; UNEP, 2006).

Other major sources include fossil fuel combustion and waste incineration. Some of the cadmium ends up to cultivated soil in fertilizers or is released into surface waters from agrochemical production plants. The cadmium content of the phosphorous fertilizers depends on the cadmium concentration of the raw material used to make the fertilizer. The cadmium content of phosphate rock of igneous origin is relatively low, generally below 5 mg/kg, but these rocks account for only 15 % of world phosphate production. Sedimentary phosphate rocks contain cadmium from 3 to 120 mg/kg. The Finnish phosphate mine at Siilinjärvi, produces phosphate rock of igneous origin. The cadmium content of Siilinjärvi rock phosphate is exceptionally low. In the 1990's, the cadmium concentration in phosphate fertilizers on the Finnish market varied from 1 to 5 mg/kg, whereas the average cadmium level of fertilizers in European markets was 138 mg/kg (Davister et al., 1996; Louekari et al., 2000).

The mean cadmium level of cultivated soil in Europe is 0.5 mg/kg (Davister, 1996) while it is 0.21 mg/kg in Finland though there are regional differences (Sipola and Mäkelä-Kurtto, 1986; Mäkelä-Kurtto and Sippola, 2002). For example, the levels in southern Finland are nearly two times higher than the average level because of more intensive industrial activities and agriculture practices in that part of Finland. In addition, long-range transport from Central Europe can not be ruled out as a significant factor. The cadmium level of cultivated Finnish soils is close to the values reported in other northern European countries, 0.22 mg/kg in Sweden and 0.25 mg/kg in Denmark (Andersson, 1977; Tjell and Hovmand, 1978). Relatively high cadmium levels in cultivated soils have been reported in Germany and France, 0.52 mg/kg and 0.74 mg/kg, respectively (Angelo and Bini, 1992). In Austria, the mean cadmium level is 0.40 mg/kg d.w. (Komarnicki, 2005). In the U.K., the mean cadmium level was 1.0 mg/kg, which indicates that there has been anthropogenic pollution (Jackson and Alloway, 1992). Phosphate fertilisers are believed to be the main source of cadmium in Finnish cultivated soils (Mäkelä-Kurtto and Sippola, 2002). Since the early 1980s, when a phosphate mine was opened at Siilinjärvi, cadmium inputs from

fertilisers have decreased gradually. In 1999, a mean annual cadmium input from the mineral fertilisers was 0.025g / ha (Mäkelä-Kurtto and Sippola, 2002).

The highest cadmium levels in Finnish soil are found in acidic sulphate soils in south western Finland and in conjunction with zinc ore e.g in Pyhäsalmi in Oulu province. High cadmium levels occur in ground water in south western Finland and in the area of Kymijoki in south eastern Finland (Heikkinen, 2000). The major part of soil cadmium is from anthropogenic sources including from the Cu-Ni smelter in Harjavalta in south western Finland, where a logarithmically decreasing gradient, studied at the distance of 0.5, 2, 4, and 8 km from the smelter, was found in the soil cadmium levels (Derome and Lindroos, 1998a and b).

High levels of cadmium in soil may cause many toxic effects in plants, such as a reduction of growth, especially root growth (Weigel and Jäger, 1980; Padjamaja et al., 1990), disturbances in mineral nutrition and carbohydrate metabolism (Moya et al., 1993), inhibition in net photosynthesis (Baszynski et al., 1980; Sheoran et al., 1990a and 1990b; Dong et al., 2005).

In contrast, at lower levels, some potentially positive impacts of cadmium on plant height and photosynthesis have been reported (Wu et al., 2003).

In general, the bioavailability of heavy metals in soil is best estimated by the metal concentrations in soil solutions (Janssen et al., 1997). Cadmium in plants is probably derived from water soluble forms of cadmium and to a lesser extent from atmospheric input (Harrison et al., 1989; Vassilev et al., 1998; Taylor et al., 2001; Ramos et al., 2002; Komarnicki, 2005).

5.1.2. Lead

Lead makes only up to 0.0018 % of the Earth's crust and is rarer than for example cerium, tungsten, vanadium or yttrium. Nevertheless, it was already known by the ancient Egyptians 5000 years ago because it was concentrated in a few large deposits in the form of easily reduced galena. Lead pipes bearing the insignia of Roman emperors, used as drains from the baths, are still in service (Desphane, 2002).

The major natural sources for mobilisation of lead from the Earth's lithosphere to the biosphere are volcanoes and weathering of rocks. Lead-rich minerals most often occur together with other metals, particularly silver, zinc, copper and sometimes gold (UNEP, 2006).

Lead is one of the best examples for anthropogenic environmental metal pollution. The emission of lead was associated for several decades with the use of lead compounds

which were incorporated as anti-knock additives into gasoline. (Ewers and Schlipkötter, 1984). In Finland, lead was banned in gasoline in 1993 and in European Union in 2000. However, tetraethyl lead is still used in some gasoline types though it too is being phased out on environmental grounds. Other anthropogenic sources of lead include the metallurgic industries, ammunition, and trash incineration (Melanen et al., 1999; AMAP, 2005).

Lead is used for a large number of applications. Chemically, lead is acid resistant, corrosion resistant, it has low melting point and is easy to cast. Because of its softness, pure lead is only used for a few applications. In metallic applications, lead is most often alloyed with small amounts of antimony (e.g. in batteries and cable sheathing), copper (e.g. in lead sheets and lead pipes), calcium (e.g. in batteries) or silver (e.g. in solders). In addition, lead is used as an alloying element in alloys of copper (some brass and bronze alloys) and tin (pewter, solders). Lead is present in batteries in both the metallic form and combined with other chemicals. Lead is used in different types of ammunition. One major application is lead shot for shotguns. The most significant changes in the overall use pattern are the increased consumption for batteries, and a decrease in the areas of cable sheathing and gasoline additives (UNEP, 2006).

Lead in the environment is strongly absorbed by sediments and soil particles, and is therefore largely unavailable to plants and animals. Many of the inorganic salts of lead (lead oxides and sulphides) are not readily soluble in water and are sequestered in sediment. In aquatic systems, lead uptake is influenced by great many environmental factors such as temperature, salinity, pH, and the presence of organic matter (AMAP, 2005). Soil contamination is particularly harmful to roots, while the upper plant parts are more influenced by the absorption of airborne lead (Scheffer and Schachtsschabel, 1992).

5.1.3. Copper

Copper makes about up to 0.007 % of the Earth's crust, so it is the 25th most abundant element. Copper sulphides, especially chalcopyrite (CuFeS), are the most important copper ore mineral. Under the oxidizing conditions prevailing in the Earth's crust, copper forms hydroxides and carbonates and even occurs in native states (Rose et al., 1979; Baker and Senft, 1995).

About 40 % of annual production of copper is used for the manufacture of alloys. Due to its excellent heat conductivity, it is used for brewing vats, vacuum pans, soldering irons, heating and cooling coils, etc. Because of their fungicidal properties, copper salts have been used since the ancient times for crop protection and as wood preservatives (Desphande, 2002).

The sources of copper into the soil and atmosphere are weathering of copper ores, fossil fuel combustion, waste incineration, the emissions of metal processing and mining industries, and the use of copper-containing fertilizer (Sillanpää, 1988; Kabata-Pendias and Pendias, 1992; Baker and Senft, 1995). During weathering, copper is almost immobile; it precipitates as water-bearing carbonate or hydroxide under oxidizing conditions and as sulphide under reducing conditions (Kabata-Pendias and Pendias, 1992).

The concentrations of copper are higher in alkaline and neutral soils than in acidic soil, from which copper can be easily extracted. The average abundance of copper in the crust is 15–40 mg/kg. The concentrations are low in sandstone and limestones, higher in argillaceous schists, and very high in graphite schists containing organic matter. However, copper concentrations may increase to 3500 mg/kg near to industrial sources (Kabata-Pendias and Pendias, 1992).

In Finland, the overall copper level is 24 ± 14 mg/kg but in the clays in south-western Finland it can be as high as 59 mg/kg (Salminen et al., 1997). About 88 % of the total copper present in fine fraction of till is acid-extractable. This indicates that copper occurs as a sulphide, which dissolves easily in acids, and therefore in soil, copper is readily available as a nutrient (Koljonen, 1992).

Copper is an essential nutrient in plants and animals and the levels in soil need to be 15-60 mg/kg to meet the requirements of plants and plant-eating animals. Copper deficiency is often observed in plants grown in soil inherently low in copper (coarse textured and calcareous soils) and in soil high in organic matter, where copper is readily chelated (Alloway et al., 1984). A deficiency of copper affects the photosynthesis of plants, impairs protein metabolism, inhibits respiration in plants, as well as the production of DNA and RNA leading to a decline in the growth of the plants (Kabata-Pendias and Pendias, 1992). Excessive exposure to copper inhibits root growth more than shoot growth. Toxic levels of soluble copper cause chlorosis and the deformation of roots (Lexmond and van der Vorm, 1981; Kabata-Pendias and Pendias, 1992). The toxic response increases as a function of the soil acidity. Copper from anthropogenic sources are more soluble than the naturally occurring copper ores and thus are more harmful to plants (Kabata-Pendias and Pendias, 1992). The use of copper containing fungicides and antihelminthic compounds (insecticides) in agriculture has resulted in copper toxicity in some plants, but toxic effects due to naturally occurring copper are relatively rare (Welch et al., 1991).

5.1.4. Zinc

Making up about 0.012 % of the Earth's crust, zinc is close to copper, strontium and vanadium in its abundance. As a very non-noble metal, it only occurs in nature in the form of its compounds, mostly in the divalent oxidation state, in the company of lead and cadmium.

The major use of the zinc produced is its incorporation into galvanized steel. Large quantities are also used in production of the brass, bronze, solders, and other zinc alloys. Zinc oxide is used in glass, ceramics, and dyes, and further uses of zinc compounds are found in the soap, cosmetics, pharmaceutical, and rubber and plastics industries (Desphane, 2002).

In the bedrock, zinc is mostly present in the crystal lattices of silicates where it replaces iron and magnesium, but it also found in sulphides (Rose et al., 1979; Kabata-Pendias and Pendias, 1992). The average abundance of zinc in the earth's crust is 50–100 mg/kg, but the levels may locally rise as high as 900 mg/kg. In Finland, the highest zinc concentrations are found in metamorphic volcanogenic-sedimentary areas in southern Finland, along the Lake Ladoga-Bothnian Bay zone and in central Lapland (Heikkinen, 2000).

The major anthropogenic sources of zinc are fossil fuel combustion and metal smelters. In addition, zinc gains access to the atmosphere and soil via waste incineration and the use of fertilizer (Kabata-Pendias and Pendias, 1992; Kiekens, 1995). The amount of zinc from anthropogenic sources could be often greater than the zinc obtained from the weathering of zinc ores (Kabata-Pendias and Pendias, 1992). However, in some places the weathering of zinc sulphides is believed to be an important source of zinc (Sipilä and Salminen, 1994).

Zinc is an essential nutrient for plants and is toxic only in a large excess (Rose et al., 1979; Kabata-Pendias and Pendias, 1992). It is a component of many essential enzyme functions in the metabolism of plants and it is involved in the formation of ribosomes and RNA. Zinc is believed to strengthen the resistance of plants to mycosis and bacteria (Kabata-Pendias and Pendias, 1992; Kiekens, 1995).

A high pH of soil reduces the availability of zinc by plants and a high clay and phosphorous content and low soil temperature can further promote zinc deficiency. With decreasing soil pH, zinc solubility and uptake is improved and the potential for phytotoxicity correspondingly increases. Zinc phytotoxicity is more severe in plants grown in light-textured than in heavy-textured soils. This is mainly because of differences in specific zinc adsorption capacities of the soil. For example excess exposure to zinc may cause chlorosis, it may weaken the growth of the plants and intake of important other nutrients

(Kabata-Pendias and Pendias, 1992; Kiekens, 1995). However, the toxic limits for zinc are much higher than for other essential elements such as copper (Kabata-Pendias and Pendias, 1992).

5.2. Effect of exposure to heavy metals on human health

5.2.1. Cadmium

Food is the main cadmium source in human and foodstuffs that are rich in cadmium can greatly increase the cadmium exposure (EU-Reports on tasks for scientific cooperation, 2004).

It is believed that from 10-50 % of cadmium is absorbed from the lung, whereas its absorption is usually only a few percent from the gastrointestinal tract. The major route of excretion is the faeces.

After uptake, cadmium is transported in blood plasma, initially bound to protein albumin. Cadmium-albumin is preferentially taken up by the liver where it is bound to a protein known as metallothionein (MT) to form a complex that is then transported to the kidneys. Speciation of the ingested cadmium is a significant factor affecting the toxicokinetics. After ingestion of CdCl_2 , cadmium is more likely to accumulate in the liver, while ingestion of Cd-MT results in an increased cadmium accumulation in kidneys (Brune et al., 1980; Travis et al., 1980; Nordberg et al., 1985; Bremner 1987; Dunn et al., 1987).

Absorbed cadmium is mainly accumulated in liver and kidneys, which account for more than half of the cadmium body burden. Renal tubular damage is probably the most sensitive health effect of cadmium exposure (Nordberg et al., 1993; Järup et al., 1998). Cadmium is excreted very slowly, having a biological half-time of 10–30 years (Nordberg et al., 1985; Chmielnicka et al., 1986).

Another effect of cadmium accumulation in the organism is to evoke a defect in calcium metabolism leading to the appearance of kidney calculi, which in combination with nutritional deficiency of calcium leads to the development of osteomalacia and osteoporosis (Nogawa et al., 1987; Kido et al.; 1991; Järup et al., 1998).

There is also increasing evidence that cadmium may act independently on bone tissue and that also individuals without previous renal lesions may be affected (Roels et al., 1993; Nordberg, 2004).

Data from animal experiments have indicated that cadmium may play a causative role in cardiovascular disease, but the evidence for an association in humans has so far been weak and inconclusive (Friberg et al., 1986; Järup et al., 1998).

Smoking is an important source of cadmium exposure and smoking increases the cadmium exposure by potentiating the uptake of cadmium levels that are present in cadmium-rich food. (Elinder et al., 1976; Ellis et al., 1979; Nilsson et al., 1995; Berglund and Vahter, 1998; Mortada et al., 2004; Ikeda et al., 2005).

Similarly, the blood cadmium levels can be elevated in persons who live near areas with hazardous waste sites or factories that release cadmium into the air (Roels et al., 1983; Carvalho et al., 1986; Maravelias et al., 1989; Zielonka et al., 1993).

In 1993 the International Agency for Research on Cancer (IARC, 1993) classified cadmium as a human carcinogen (category 1). A recent re-evaluation has indicated; however, that a classification of cadmium as a “probable human carcinogen group 2A” would be more appropriate (IARC, 1992; Lamm et al., 1992; Sorahan et al., 1995 and 1997; Järup, 2002).

5.2.2. Lead

The diet is considered as a major source of the total body burden of lead. It has been estimated that dietary sources account for 90 % of the total body burden of lead (EU-Reports on tasks for scientific cooperation, 2004).

Due to of its persistence and high occurrence in the environment, exposure to lead is a major public health concern. Occupational exposure may also result in high lead levels in blood (Pirkle et al., 1998; Ahmed, 1999; Nielsen et al., 2000; Vaglenov et al., 2001).

Lead binds to the sulphhydryl (SH) groups of proteins, but the basic mechanism of the lead toxicity is not yet established (Skerfving et al., 1998).

Most of the intake via ingestion (90 %) in vertebrates, for example, is transferred to bones, which are the main target organs for lead accumulation. The retention of lead in soft tissues is highest in the liver, followed by kidney, aorta, muscle, and brain in decreasing order (Rabinowitz et al., 1973; Barry, 1975; Whanger, 1982; Andrews et al., 1989). Lead in the hard tissues is tightly bound but an equilibrium state may exist between bone, blood, and soft tissue lead levels, so that hard tissue lead may be an important source of blood and soft tissue lead (Goyer and Chisolm, 1972). Lead has a comparatively long biological half-life (5–20 years for humans) in hard tissues, which results in increased body burdens with age. In contrast, the half-life in body fluids and soft tissues is only a few days (Tsuchia, 1990).

The data on genotoxic and carcinogenic properties of lead are contradictory (Gerber et al., 1980; Fu et al., 1995; Todd et al., 1996; Hagmar et al., 1998; Vaglenov et al., 2001).

In bacterial tests, lead seems to be generally nonmutagenic (Dunkel et al., 1984) but in eukaryotic cells it is genotoxic (Zelikoff et al., 1988; Winder et al., 1993). However, the mechanism has not been well characterized though it is believed to involve indirect damage of DNA, affecting the stabilization of chromatin (Johansson and Pellicciari, 1988) or by an interaction with the repair processes (Hartwig et al., 1990).

The most susceptible populations to lead toxicity are children, particularly toddlers, infants in the neonatal period, and fetuses. There is a body of evidence indicating that low-level lead exposure can cause delayed mental development in children (Ahmed, 1999; Nielsen et al., 2000).

Children living in lead polluted areas have elevated blood lead levels and the critical blood lead level of 100 µg/l can be exceeded (Maravelias et al., 1989; Zielonka et al., 1993; Paoliello et al., 2002). Epidemiological studies show consistently that effects in children are associated with lead levels in blood about 100–150 µg/l. There are indications that lead is harmful even at blood lead concentrations considerably below 100 µg/l and there may be no threshold for these effects (UNEP, 2006).

The blood lead level of Finnish children living in the neighbourhood of a former lead smelter was slightly but statistically significantly higher than the corresponding values in children in the control areas. However, the critical blood level 100 µg/l was not exceeded in any of the children examined. Over the years, the blood lead level of children living in this area has decreased (Taskinen et al., 1981; Louekari et al., 2004). Lead levels in Finnish children in a day-care home in Helsinki located near to urban traffic street have fallen from 48 µg/l in 1983 to 30 µg/l in 1988, and down to 26 µg/l when measured in 1996 (Pönkä, 1997).

The International Agency for Research on Cancer (IARC, 1987) has classified lead and inorganic lead compounds as possible human carcinogens (Group 2B) on the basis that though there is sufficient evidence for carcinogenicity in experimental animals there is inadequate evidence for carcinogenicity in humans.

5.2.3. Copper

Copper is an essential mineral for cell survival. It is present in red blood cells, and it keeps the blood vessels, nerves, immune system and bones healthy. Copper is an integral part of many important enzymes involved in a number of vital biological processes. Muscle and bones contain approximately 25 % and 42 % of the total body copper. Liver and brain

account for about 9 % and blood for about 5 % of body copper with 60 % of blood copper being in the plasma. In general, copper cannot be considered as a metal that is stored in the body. In human and animals, absorption occurs primarily in the duodenum. It is usually readily absorbed from the intestine and is extensively excreted. Copper homeostasis is maintained mainly through excretion (Despande, 2002).

Dietary excess copper intake is not very common, but there is a hereditary disorder, Wilson's disease, that leads to deposits of copper in the liver, brain, and other organs. The increased copper accumulation in these tissues causes hepatitis, kidney diseases, neurological disorders and other detrimental health consequences (Tapiero et al., 2003).

Copper is relatively non-toxic to most mammals and birds but excess of copper may selectively damage neuronal and hepatic systems. The acute symptoms of copper poisoning include nausea, vomiting, diarrhea, headache, dizziness, and weakness. In more severe cases, tachycardia, hypertension, and coma may occur and these may be followed by jaundice, haemolytic anaemia, hemoglobinuria, uremia, and even death (Cai et al., 2005).

The most toxic effects of copper probably result from the production of oxygen radicals by copper chelates, e.g., when Cu^{2+} is reduced by ascorbate. In the liver, which is the first recipient of most of the incoming dietary copper, damage from the oxygen radicals causes scar tissue formation, leading to changes in tissue architecture and a reduction in liver function. Scarring of other tissues and damage to cell membranes (e.g. in kidney tubules and erythrocytes) lead to cell lysis and connective tissue deposition (Shah et al., 1992; Kadiiska et al., 1993; Lind et al., 1993).

Dietary deficiency of copper is not very common in humans. Genetic defects in copper metabolism include Menke's disease (kinky hair syndrome), a very rare congenital disorder of copper metabolism that occurs in male infants. This disease highlights the importance of adequate copper intake during embryogenesis and early development, especially for the central nervous system (Tapiero et al., 2003).

5.2.4. Zinc

Zinc is an important component of neural function and it is essential for human growth, development, and the maintenance of the immune system (Kordas et al., 2003). Zinc is an integral component of a large variety of proteins and enzymes, and it participates in a wide variety of metabolic processes including carbohydrate, lipid, protein and nucleic acid synthesis or degradation. At the molecular level, zinc plays an important role in DNA replication and transcription and thus in protein synthesis, and ultimately it can influence cell division and differentiation (Prasad, 1995; Cai et al., 2005).

The highest amounts of zinc are present in the brain, especially in the hippocampus and cerebral cortex. Zinc deprivation influences brain zinc homeostasis and leads to alteration in behaviour, learning capabilities, mental function and susceptibility to epileptic convulsions (Frederickson, 1989; Takeda, 2000).

Recent data have been emphasized the important role of zinc homeostasis in psychopathology and depression and it has been claimed that zinc possesses potential clinical antidepressant activity (Nowak et al., 2003 and 2005).

Although zinc is essential, it can be toxic in large doses, especially in certain genetic disorders. Zinc toxicity can occur in both acute and chronic forms. Intakes of 150 to 450 mg per day have been associated with low copper status, altered iron metabolism, reduced immune function, and reduced levels of high-density lipoproteins; the so-called good cholesterol (Prasad, 2003; Cai et al., 2005).

A deficiency of zinc remains a global problem, especially among women and children in the developing countries (Aggett, 1989; Bahl, 1998; Brown et al., 1998; Bhutta et al., 1999; Black, 2003). Zinc deficiency poses an increased risk of illness and death from infectious diseases such as bacterial-induced diarrhea, pneumonia infection or malaria (Black, 2003). Although defects in the immune system are known to occur with even mild zinc deficiency, its importance for the risk of childhood infectious diseases have been only recently recognized (Bahl et al., 1998; Shankar and Prasad, 1998; Black, 2001; Baqui et al., 2003).

Meat products are the best source of zinc. Consequently, zinc deficiency may coexist in populations that consume diets with insufficient amounts of animal-based foods (Baqui et al., 2002; Black et al., 2004; Thurlow et al., 2005).

Iron and zinc utilize chemically similar absorption and transport mechanisms (Sandström, 2001; Bagui et al., 2003). New evidence based on cell culture studies has shown that iron may inhibit zinc absorption in some cells at very high ratios of iron to zinc, but not vice versa i.e. zinc does not prevent iron absorption (Kordas et al., 2004).

5.3. Indicator species to study the contamination of heavy metals

5.3.1. Heavy metal uptake by plants

A range of morphological, anatomical, and physiological properties affect the capacity of individual plants to filter, bind, and accumulate elements on their surfaces, and to take them up intracellularly. The greatest difference in the uptake mechanisms for different elements is between vascular plants and cryptogams (bryophytes and lichens). Vascular

plants mainly take up elements via their roots from the soil, although the foliar uptake of gases (e.g. NO₂, NH₃ and SO₂) and soluble elements may also occur. The foliar uptake of heavy metals has been demonstrated in many crop plants (Haslett et al., 2001) but, in evergreen species, the thick epidermis and the waxy cuticle of the leaves provide external protection against toxic elements. Large amounts of metal-containing dust can become attached to the leaf surfaces of trees growing near the emission sources and particles may also become embedded in the cuticular waxes (Rautio and Huttunen, 2003). Acidic deposition causes erosion of the cuticle (Manninen and Huttunen, 1995), which may increase the leaching of elements and the penetration of heavy metals into the foliar tissues.

The bioavailability of heavy metals in the soil is regulated by many physical, chemical and biological properties and processes (Ernst, 1996). The mobility and toxicity of heavy metals are strongly related to the acidity and organic matter content of the soil (Alloway, 1995). Heavy metal ions accumulate first in the cortex of the roots, from where a small proportion passes through the endodermis and is subsequently distributed into the different plant organs. Vascular plants have many species-specific mechanisms to restrict the cellular uptake of heavy metals and also able to detoxify them internally. The role of mycorrhizal fungi in retaining heavy metals in the root system is important in providing resistance for the host plants (Salemaa et al., 2004).

Cryptogams, however, have no real roots, epidermis or continuous cuticle layer, and they absorb water and dissolved elements directly across their surface. Most of the bryophyte and lichen species obtain the majority of their water and nutrients from atmospheric deposition; some species also obtain nutrients from water that has been in contact with the substrate (Salemaa et al., 2004).

5.3.1.1. Mosses and berries

The most widely used bioindicators of air pollution are mosses and lichens. Mosses and lichens generally lack vascular systems, which minimizes the possibility for uptake from substrates and internal translocation. Nutrients and pollutants are accumulated primarily from the atmosphere. The lack of a thick cuticle promotes the migration of heavy metals and other elements to the free cation exchange sites located on the walls of the cells in mosses. The common forest mosses that are most suitable for element surveys are the glittering feather moss (*Hylocomium splendens*) and the red-stemmed feather moss (*Pleurozium schreberi*) (Poikolainen et al., 2004). Together with their broad geographical range and ecological amplitude, these factors make mosses and lichens useful biomonitors of atmospheric deposition (Steinnes, 1995). Mosses have been used in large-scale heavy metals deposition surveys in Europe, and to some extent also in North

America (Rühling, 1994; Ford et al., 1995; Halleraker et al., 1998; Rühling and Steinnes, 1998; Grodzinska et al., 1999).

According to the moss survey conducted during the period of 1985–2000, the heavy metal concentrations in Finland were generally relatively low, apart from those in southern Finland and close to a number of major emission sources. The mean concentrations of all heavy metals decreased during the period covered by the surveys. The metals which have undergone the strongest decrease in concentrations since 1985 are lead (78 %), vanadium (70 %) and cadmium (67 %). The concentrations of other heavy metals decreased by 16–34 %. The concentrations of chromium, copper and nickel were clearly associated with local emission point sources and changes in emissions levels (Poikolainen et al., 2004). The levels of lead, cadmium and vanadium decreased by the most and there was also a steady geographical reduction from south to north of the country. Traffic used to be the principal emission source of lead in Finland.

The survey identified some major emission sources which have a high impact on the deposition of copper, nickel and chromium. Elevated copper and nickel concentrations were found in the Harjavalta-Pori area, which has copper and nickel smelters, and also in north eastern Lapland, which is affected by copper and nickel emissions from the large smelters situated on the Kola Peninsula. Elevated chromium concentrations were found in the Kemi-Tornio area, which has a ferrochrome and stainless steel plants and an opencast chromium mine. The copper and chromium level decreased significantly between 1985 and 2000, but the nickel concentrations did not decrease. The concentration of zinc decreased significantly between 1985 and 2000 but a significant decrease did not occur until between 1995 and 2000. No significant emissions sources for zinc were found on the basis of the concentrations in mosses (Poikolainen et al., 2004). Locally elevated zinc levels were observed around the Imatra paper factory in the south eastern part of Finland and in the Kokkola in western part of Finland around zinc industrial plants in a survey in 1990-1996. However, no significant change has occurred during the survey period (Rühling and Steinnes, 1998).

The decrease in the heavy metal levels in mosses in Finland is mainly due to the decrease in domestic emissions, as well as to the decrease in the long-range transport of heavy metals into Finland. Arsenic, cadmium, copper, lead, nickel and zinc emissions from sources monitored by the environmental authorities in Finland have decreased by over 90 % during the past 20 years and chromium, mercury, iron and vanadium emissions by 60-85 % (Poikolainen et al., 2004). Similar trends in the emissions levels and in the metal concentrations have also taken place in other parts of Europe. Emissions of lead especially have decreased throughout Europe (Rühling and Steinnes, 1998; Sucharova and Suchara, 1998; Grodzinska et al., 1999; Steinnes et al., 2001; Pacyna and Pacyna, 1999 and 2001; Ilyin et al., 2002). However, the long-range transport of some heavy metals (lead, cadmium, mercury) is significant (Ilyin et al., 2002). In the Scandinavian

countries, the atmospheric background deposition levels decreases as one moves toward the north. This pattern is especially pronounced in the case of lead, zinc, vanadium and cadmium and implied that this at least partly had to be associated with long-range transport to southern Scandinavia from the heavily industrialized areas elsewhere in Europe (Rühling and Tyler, 1973; Reimann et al., 1997; Steinnes, 1997; Rühling and Steinnes, 1998; Reimann, 2001).

Elevated heavy metal concentrations were measured in lingonberries (*Vaccinium vitis-idaea* L.) sampled around the same ferrochrome and stainless steel plants and an open-cast chromium mine in the Kemi-Tornio region where elevated heavy metal concentrations in mosses were detected. The maximum concentrations of chromium, nickel, vanadium and cadmium in the immediate vicinity of the point sources were 33; 6; 4 and 8 times higher than the background levels (Pöykiö et al., 2005)

5.3.1.2. Willow (*Salix*) and aspen (*Populus*)

Several studies have shown that many species or clones of *Salix* have the capacity to accumulate high levels of cadmium in the aboveground biomass compartments (Landberg and Greger, 1996; Rosselli et al., 2003; Vandecasteele et al., 2004). Willow foliar cadmium concentrations are strongly correlated with soil and soilwater cadmium concentrations. *Salix* trees on calcareous metal contaminated sites can thus mobilize cadmium from the subsoil and recycle it to the stand surface with leaf fall. This raises concerns of increased cadmium mobility in natural ecosystems and the risk of food chain contamination. Conversely, this also opens the prospect of cleaning cadmium contaminated soils through repeated harvesting of the produced wood and leaf biomass in carefully managed and monitored short rotation forestry schemes (Beyer et al., 1990; Östman, 1994; Landberg, 1996).

High cadmium concentrations were observed in the two tree species *Salix aurita* (eared willow) and *Populus tremula* (aspen) (average of 1.5 mg/kg in leaves and 2.25 mg/kg in twigs). Both *Salix* and *Populus* belong to the *Salicaceae* family. They are generally known to be able to accumulate some heavy metals more than other trees; however, there is considerable variation between species and also between clones (Brekken and Steinnes, 2005). Cadmium concentrations up to 4 mg/kg in foliage have previously been reported in some *Salicaceae* species from some catchments in southern Scandinavia (Gjengedal, 1992; Alriksson, 1998). No reference data about toxic foliar cadmium concentrations for willow species were found, but most authors refer to general toxic plant concentration ranges of 5-30 mg/kg (Vandecasteele et al., 2004). However, willow has been reported to contain elevated concentrations of cadmium within its leaves, even when growing on unpolluted substrates (Vandecasteele et al., 2004).

5.3.2. Earthworms

Earthworms play an important role in soil ecosystems, since they contribute to organic matter incorporation and decomposition, excavation of burrows and production of casts. A decrease in earthworm abundance caused by soil heavy metal contamination may diminish these ecological functions (Lee, 1985; Edwards and Bohlen, 1996). Earthworms are relatively localized animals and therefore in field studies their limited mobility makes them very suitable for monitoring the impact of contaminants (Pankakoski et al., 1994). Generally, earthworms can be used as an indicator of soil impairment because their abundance and biomass decrease with soil contamination (Belotti, 1998; Spurgeon et al., 1999; Lukkari et al., 2004).

In the context of biomonitoring, the earthworms are good biomarkers to predict and to monitor the total-soil metal concentration and provide early warning signals of potential danger to the biota (Morgan et al., 1988; Heikens et al., 2001; Burgos et al., 2005). However, there is also evidence that the accumulated levels in earthworms do not consistently reflect the metal contamination level of the soil. In acidic sandy soils, cadmium can accumulate in earthworms to a considerable extent, even though the soil contamination level may be rather low. Earthworms accumulate much more lead from contaminated acidic sandy soils than from soils which have been limed (Ma, 1987 and 1989).

In a study carried out in the U.K. (Morgan et al., 1988) earthworms (*Lumbricus rebellus* and *Dendrodrilus rubidus*) were sampled from uncontaminated and known metal-contaminated sites. Significant positive correlations were found between the earthworms and 'total' (conc. nitric acid-extractable) soil cadmium, copper, lead and zinc concentrations. The relationships were linear, and the accumulation patterns for both species were similar as far as a single metal was concerned, even though there was a species difference in the mean metal concentrations. Earthworm cadmium concentration exceeded that of the soil, home to the worms; in contrast, the earthworm lead levels were lower than the soil lead concentration in all but one (an acidic, low soil calcium) site. Soil pH coupled with a cation-exchange capacity and soil calcium had a major influence on lead accumulation and cadmium accumulation may be suppressed in extremely organic soils (Morgan et al., 1988).

Heavy metal concentrations in organic rich soils close to the emission sources (1, 2, and 4 km) were high enough to have harmful effects on earthworms and their environments. In general, diversity, total numbers, and biomass of earthworms increased with increasing distance from the emission sources. Positive correlations between metal concentrations in the earthworms and those in the soils were observed (Heikens et al., 2001; Lukkari et al., 2004).

5.3.3. Moles

Mammals used as a source of biomarkers should have a high abundance in urban areas, a low rate of migration and be limited to a small space to ensure the detection of local phenomena according to the variety of local sources in cities (Wren, 1986; Fliclinger and Nichols, 1990). This is the case of the mole (*Talpa europaea L.*) which lives to an age of up to 6 years (Lodal and Grue, 1985). In general, insectivores accumulate more potentially harmful metals like cadmium and lead than other investigated, predominantly herbivorous, small mammal species (Wren, 1986; Ma et al., 1991). One disadvantage, however, lies in the mole's restricted occurrence to particular soil types (Oppermann, 1968; Milner and Ball, 1970; Funmilayo, 1977 and 1979).

In Finnish rural areas, the concentrations of cadmium, copper, zinc, molybdenum and lead in the liver and kidneys of moles, *Talpa europaea L.* (Insectivora) were lower in juveniles except for zinc in the liver, which was lower in adults. If the animals were divided according to their age (0–6 years), cadmium and molybdenum concentrations in the liver increased significantly with age, while concentrations of copper, zinc and chromium tended to decrease. Female moles had higher lead concentrations than males, especially adult females, which also had lower levels of copper in their liver than the adult males. Moles in the metropolitan area of Helsinki clearly differed from those in rural areas in that the concentrations of heavy metals in these moles were higher (especially for the most toxic metals: cadmium, lead and mercury), and their body weight was lower (Pankakoski et al., 1993).

The accumulation of heavy metals in moles reflects the bioavailability of these metals to earthworms (Ma et al., 1989; Pankakoski et al., 1993; Komarnicki et al., 2000). However, accumulated levels in earthworms and moles do not consistently reflect the metal contamination level of the soil. In acidic sandy soils cadmium can accumulate in earthworms to a considerable extent, and critical levels of cadmium toxicity in moles which eat the worms can be exceeded even though the soil contamination level may be rather low. Earthworms and moles also accumulate more lead from contaminated acidic sandy soils than from soils which have been limed. At the same level of soil contamination, lead can exceed critical levels of toxicity in moles living in acidic sandy soils, while the animals reveal no elevated tissue levels if they live around limed sites (Ma, 1987).

5.4. The levels of heavy metals in relevant species in different countries

5.4.1. Moose (*Alces alces*)

Moose are ruminants that feed selectively on the available shrub and herb species during the course of the year. The important food plants of moose are aspen, birch, willow, Scots pine and fireweed (Nygren, 1990; Robbins, 2001). Of the the plant species, willow and aspen are by far the most important cadmium contributors. In areas where high cadmium accumulators grow widely, the daily intake by moose could be as high as 7 mg or more (Vandecasteele et al., 2002; Brekken and Steinnes, 2004).

The cadmium, lead, copper and zinc levels of moose are summarized in Tables 3-7.

5.4.1.1. Moose in North America

The Canadian Arctic is richly endowed with mineral resources, including cadmium. The physical and chemical weathering of bedrock can be a natural source of cadmium exposure (Wotton et al., 1986; Glooschenko et al., 1988; Ford, 1995; Kim et al., 1998; Jin et al., 2003). Toxic pollutants are transported to the Canadian Arctic and sub arctic areas from industrially active mid-latitudinal areas through ocean currents and air flows and also from natural processes and local discharges into the environment (Crete, 1987; Lockhart et al., 1992; Gamberg and Scheuhammer, 1994; Kim et al., 1998; AMAP, 2005; Gamberg et al., 2005; Arnold et al., 2006).

Although moose from the Canadian Arctic generally carry relatively low contaminant burdens, moose in the Yukon (in the northwest part of Canada) have high renal selenium levels, and high renal cadmium levels, which may put some animals at risk of suffering toxicological effects. The mean cadmium levels in muscle, liver and kidney of Yukon moose were 0.03 mg/kg, 4.94 mg/kg and 28.1 mg/kg in w.w., respectively (Gamberg et al., 2005b). Some of the moose in the Yukon had renal cadmium levels that fell within or exceeded the threshold range of 100–300 mg/kg w.w. at which renal tubule dysfunction has been shown to occur (Kjelstrom et al., 1986; Beyer et al., 1996). Sublethal effects would be expected at a much lower level, 30 mg/kg w.w. (Outridge et al., 1994).

In Alaska, the individual levels of cadmium in liver of moose ranged from 0.06 mg/kg to 9.0 mg/kg w.w. and in the kidney cortex the levels were from 0.10 mg/kg to 65.7 mg/kg in w.w. depending on the geographic location of the sample. The data from kidney cortex represents the upper limit of cadmium concentration present in kidney and levels were significantly associated with location and age. The arithmetic mean in liver and kidney cortex ranged from 0.44 to 2.51 mg/kg and from 1.68 to 22.8 mg/kg in w.w., respectively. The highest means of cadmium in liver (2.51 mg/kg) and kidney cortex (22.8 mg/kg) were

detected in moose sampled near Galena, Alaska. The Galena area is known to contain mineral deposits generally associated with cadmium, such as galena (lead sulphide) and sphalerite (zinc sulphide) (Arnold et al., 2006). The cadmium levels in liver and kidneys in various areas of Alaska exhibited some variability (O'Hara et al., 2001). In the Colville River area (the north slope of Alaska) the reported values were 3.13 and 21.6 mg/kg w.w. in liver and kidneys. In the areas outside of the north slope (Nome, Nowitna and Fairbanks) the corresponding values were 11.9 mg/kg and 73.1 mg/kg w.w. In the same geographic regions, the levels of copper and other minerals were low- close to a level of deficiency reported in some farm animals (Puls, 1988).

In general, samples from agricultural areas had higher levels of cadmium, copper, molybdenum and selenium compared samples from bog and forest areas (Table 7) (Scanlon et al., 1986).

5.4.1.2. Moose in Scandinavia and Russia

In Russia, the cadmium and lead levels in muscle samples were relatively high, 0.11 and 1.43 mg/kg w.w., respectively. Liver and kidney levels of cadmium (1.18 and 4.78 mg/kg w.w.) and lead (1.69 and 0.54 mg/kg w.w.) were lower than the corresponding values from North America (Medvedev, 1999).

Tables 3-6 show the levels of heavy metals of moose in Sweden during the time period of 1973-1976 (Frank, 1986), as well as the time periods of 1980-2000 and 1996-2000 (Odsjö et al., 2002). The longer time series of 1980–2000 included one hunting district (Grimsö), as a reference area. No significant linear time trends were detected for cadmium and copper concentrations. A significant positive trend was detected in liver samples and a significant negative trend was detected in kidney samples from Grimsö for zinc. However, a significant negative trend was detected for lead concentration both in liver and kidney.

In general, higher levels of cadmium were detected in Norwegian moose compared with those in Sweden (Frosli et al., 1986; Scanlon et al., 1986).

5.4.1.3. Copper deficiency in moose samples

During 1990-95, the moose population in north western Alaska (Colville River Drainage) declined and it was hypothesized that low level of copper could have predisposed the animals to poor reproductive performance, immunosuppression, anemia, and other ailments ultimately compromising the entire population (O'Hara et al., 2001 and 2003; Custer et al, 2004). The low hepatic copper levels (40.3 mg/kg w.w.) in some moose herds in the Yukon (in the northwest part of Canada) may be evidence of supply of copper

insufficient to meet metabolic demands, particularly in females (Gamberg et al., 2005a). The hepatic copper concentrations in the Yukon moose, although lower than the levels (103.9 mg/kg w.w.), found in a “healthy” moose population in Alaska (O’Hara et al., 2001 and 2003), were higher than the levels found in moose exhibiting signs of a moose wasting disease associated with a copper deficiency in Alaska (O’Hara et al., 2001 and 2003, Frank et al., 2004) and Sweden (Frank et al., 1994 and 2000; Frank, 1998). The copper concentrations were 9.80 mg/kg and 11.4 mg/kg w.w., respectively. However, Yukon moose exhibited no symptoms of the moose wasting disease (Gamberg et al., 2005b).

5.4.2. Caribou and reindeer (*Rangifer tarandus*)

When the Earth’s surface is unfrozen, reindeer and caribou mainly feed on grass and sedge like horsetail, willow-herb and shoots of deciduous trees. The nutritional value of summer food is high. At the end of summer and in early autumn reindeer eat mushrooms. Mushrooms contain much protein, sugar, fat and vitamins. The winter diet of these animals consists primarily of lichen (Elkin et al., 1995).

The cadmium, lead, copper and zinc levels of reindeers/caribous are summarized in Tables 8-11.

5.4.2.1. Caribou in North America

The reported heavy metal levels of North America caribou are summarized in Tables 8-11 (Crete et al., 1989; Gamberg et al., 1994; Elkin et al., 1995; Kim et al., 1998; MacDonald et al., 2000; Robillard et al., 2002).

The mean cadmium levels in kidney in North America varied from 2.13- 7.92 mg/kg w.w. but in some individuals the level of 30 mg/kg were exceeded. This is the level at which sublethal effects may to be expected to occur (Outridge et al., 1994). The cadmium levels in kidney samples of caribou in the Yukon (in the northwest part of Canada) was 9.08 mg/kg w.w. with standard deviation 27.8 which means that some individuals carried rather high levels of cadmium (Gamberg et al., 2005a). A seasonal variation in the cadmium concentrations has been reported, with levels being higher in winter than in autumn (Crete et al., 1989).

The high lead levels (7.76 mg/kg w.w.) measured in caribou liver samples (Macdonald et al., 2000; Falandysz et al., 2005) exceed the level (6 mg/kg w.w.) where physiological changes have been documented (Johnson et al., 1982; Hutton 1983; Grue et al., 1984; DeMent et al., 1987; Friend, 1987; Scheuhammer, 1989; Schilderman, 1997). The high

lead levels in liver were found from caribou hunted in mineral-rich area in Alaska, but the levels were not at levels of concern in terms of toxicoses.

Copper levels were in some areas under the level (10 mg/kg w.w.) where copper deficiency has been reported in farm animals (Flynn et al., 1977; Frank et al., 1984; Puls, 1988). The relatively low hepatic copper levels in some caribou herds may indicate a shortage of copper to meet metabolic demands, particularly for female animals (Macdonald et al., 2000). Adequate dietary levels for cattle and sheep are considered to be 25–100 mg/kg w.w. (Puls, 1988).

The concentration of zinc in these caribou appears to be of no concern (O'Hara et al., 2003).

5.4.2.2. Caribou/reindeer in Europe and Russia

The cadmium and lead levels in caribou and reindeer in Greenland and Russia were rather similar with the exception of higher muscle cadmium and lead levels in Russian samples (Tables 8-11). Liver and kidney levels of cadmium and lead were lower than those found in North America (Aastrup et al., 2000; Espelien et al., 1999; Melnikov et al., 200; AMAP, 2005).

Higher levels of cadmium were detected in Norwegian reindeer (Froslic et al., 1986) than in Sweden (Frank et al., 1986; Jorhem et al., 1998; Odsjö et al., 2005). A similar trend had been seen with cadmium in moose. In Sweden, reindeer samples were analyzed during the time period 1989–2003. Cadmium concentrations in muscle and liver samples and lead concentrations in muscle showed no significant change while the concentrations in liver showed a decreasing change during the time period. Copper concentrations in liver samples have decreased while the concentrations in muscle showed no significant change during the same time period. The zinc concentrations in liver showed no change or a significant increasing change depending from which district the samples were collected (Odsjö et al., 2005).

5.4.3. Red deer (*Cervus elaphus*)

The red deer is one of the largest species of deer in the world. The red deer is widespread in Eastern and Middle Europe. In Finland, red deer were introduced in the 16th century. In many parts of the world, the meat of red deer is widely used as food.

The heavy metal levels of red deer are summarized in Tables 12–15 (Froslic et al., 1984; Michalska et al., 1992; Wolkers et al., 1994; Falandysz, 1994; Drozd et al., 1997;

Kottferová et al., 1998; Santiago et al., 1998; Szkoda et al., 2001; Szymczyk, 2001; Falandysz et al., 2005; Lazarus et al., 2005; Kramárová et al., 2005; Vikoren et al., 2005). The mean lead and cadmium levels in red deer liver samples were lower than the recommended maximum limit set by EU (0.5 mg/kg), but cadmium levels in kidney in many cases were above the limit (1.0mg/kg). However, cadmium and lead levels in these particular organs are far from being so high as to be toxic to the animals; at the concentrations of 100 to 300 mg/kg w.w renal tubule dysfunction has been reported to occur (Beyer et al., 1996) and at a level of 30 mg/kg w.w. sublethal effects might be expected (Outridge et al., 1994). Acute lead poisoning usually combined with serious physiological lesions is seen at an average lead concentration of 6 mg/kg w.w. (20 mg/kg d.w.) (Falandysz et al., 2005).

The levels of lead in the muscle of many carcasses exceeded the legal limit of EU (0.05 mg/kg), the average values being 0.09-0.39 mg/kg w.w. Cadmium levels were also detected in muscle, but these (0.005-0.030 mg/kg w.w.) levels were below the EU limit with the exception of the levels (0.046-0.12 mg/kg w.w.) in Polish red deer.

Copper and zinc levels are reported to be at physiological levels and therefore do not pose any threat to animals or consumers.

5.4.4. Hare

5.4.4.1. European hare (*Lepus europaeus*), Mountain Hare (*Lepus timidus*), Snowshoe Hare (*Lepus Americanus*)

The European Hare or Brown Hare is a species of hare native to northern, central, and western Europe and western Asia. In northern parts of Finland and Sweden, the other native hare species, the Mountain Hare competes for habitat with the European Hare. The snowshoe hare also called the varying hare is a species of hare found in North America. It has the name "snowshoe hare" because its back feet are so big, it looks as though if it is wearing large shoes to walk on the snow. The animal's large feet prevent it from sinking into the snow when it hops and walks. Aspen and birch are most important winter food supply of hares. Mountain hare eat plants with wood stems (willow, aspen, birch) but European hare more grass (Robillard, 2001).

Tables 16-20 summarized data of cadmium, lead, copper and zinc levels in tissues of hares in various countries (Bukovjan et al., 1991; Kottferova et al., 1998; Massányi et al., 2003; Olsen et al., 2003; AMAP, 2005; Myslek et al., 2006).

Cadmium and lead levels in Mountain hares were relatively low according to a Russian study and the spatial pattern was similar for cadmium and lead, with the highest and the

lowest values being at the same level (Melnikov et al., 2002; AMAP, 2005). The Norwegian data indicate that lead levels tend to increase with age in Mountain hares (Kålås et al., 2000; AMAP, 2005).

Cadmium and lead levels in the liver and the kidneys of European hares were studied as a function of season, age and sex. This study showed that the levels tended to be highest during the winter period. As far as gender was concerned, the liver levels of lead were significantly higher ($p < 0.05$) in male hare (0.216 vs 0.127 mg/kg w.w.). However, the liver cadmium levels were significantly higher ($p < 0.001$) in females (1.46 vs 1.38 mg/kg w.w.). No difference in the lead levels was seen if age was the determinant. However cadmium levels were significantly higher ($p < 0.001$) in liver and in kidneys in adult animals (Massányi et al., 2003).

Cadmium levels in liver and kidney samples from the snowshoe hare were reported to decrease but interestingly the levels of lead in liver seem to have increased over a fifteen years time span (Table 16). However, the number of samples was rather low, 2-5 samples, (Champoux et al., 1999; AMAP, 2005).

5.5. Analytical technique used to measure heavy metals

5.5.1. Atomic absorption

Atomic absorption is the process that occurs when a ground state atom absorbs energy in the form of light of a specific wavelength and the atom becomes elevated to an excited state. The amount of light absorbed at this wavelength will increase as the number of atoms of the selected element in the light path increases. The relationship between the amount of light absorbed and the concentration of analyte present in known standards can be used to determine unknown concentrations by measuring the amount of light they absorb and extrapolating from the standard curve. Instruments readouts can be calibrated to display concentrations directly.

The basic instrumentation for atomic absorption consists of a primary light source, an atom source, a monochromator to isolate the specific wavelength of light to be used, a detector to measure the light accurately, and electronics to treat the signal, and a data display or logging device to display the results. The light source normally used is either a hollow cathode lamp or an electrode-less discharge lamp (Price, 1974; Welz and Sperling, 1999).

The atom source used must produce free analyte atoms from the sample. The source of energy for the free atom production is heat, in flame technique this heat is most commonly in the form of an air-acetylene flame or nitrous oxide-acetylene flame. The sample is

introduced as an aerosol into the flame. The flame burner head is aligned so that the light beam passes through the flame, where the light is absorbed (Beaty and Kerber, 1993).

The major limitation of atomic absorption using flame sampling (flame AAS) is that the burner-nebulizer system is a relatively inefficient sampling device. Only a small fraction of the sample reaches the flame, and the atomized sample passes quickly through the light path. With graphite furnace atomic absorption (GFAA), the flame is replaced by an electrically heated graphite tube. The sample is introduced directly into the tube, which is then heated in a programmed series of steps to remove the solvent and major matrix components and then to atomize the remaining sample. All of the analyte is atomized, and the atoms are retained within the tube (and the light path, which passes through the tube) for an extended period. As a result, sensitivity and detection limits are significantly improved.

GFAA analysis times are longer than those for flame sampling, and fewer elements can be determined using GFAA. However, the enhanced sensitivity of GFAA and the ability of GFAA to analyze very small samples and to directly analyze certain types of solid samples significantly expand the capabilities of AAS (Schlemmer and Radziu, 1989).

Graphite furnace atomic absorption allows the determination of over 40 elements in microliter sample volumes with detection limits typically 100 to 1000 times better than those than can be achieved with flame atomic absorption systems (Schlemmer and Radziuk, 1989).

Spectral interferences are those in which the measured light absorption is erroneously high due to absorption by a species other than the analyte element. The most common type of a spectral interference in atomic absorption is "background absorption" (Beaty, 1993). Background absorption arises from the fact that not all of the matrix materials in a sample are necessarily completely atomised. Since atoms have extremely narrow absorption lines, there are a few problems involving interferences where one element absorbs at the same wavelength as another.

Even when an absorbing wavelength of another elements fall within the spectral bandwidth in use, no absorption can occur unless the light source produces light at that wavelength, i.e., that element is also present in the light source. However, undissociated molecular forms of matrix materials may have broadband absorption spectra, and tiny solid particles in the flame may scatter light over a wide wavelength region. When this type of nonspecific absorption overlaps with the atomic absorption wavelength of the analyte, the background absorption will be the result. To compensate for this problem, the background absorption must be measured and subtracted from the total measured absorption to determine the true atomic absorption component (Price, 1974; Welz and Sperling, 1998).

6. MATERIALS AND METHODS

6.1. Sample collection

Moose and hare samples were collected after receiving permission from the Hunters Central Organisation. The hunters were informed on how to conduct the sampling, and they were informed on how to pack the samples. All the materials needed for shipping the samples to the laboratory were provided. Muscle, liver, kidney and moose tooth samples were packed separately, frozen and sent to the laboratory in temperature controlled chambers. In the laboratory, the samples were stored at -18°C until analyzed.

The first extensive moose study of lead, cadmium, copper and zinc was performed in 1980–1981 in which over 300 moose were collected from 15 game management areas all around the Finland. In the autumn of 1990 a second study of heavy metals was started by collecting samples (93 moose) in three areas where previously samples had been collected; south western Finland, southern Finland and central Finland. In the third phase of the project in 1999 moose samples (83 moose) were again collected from the same three game management areas as in 1990 and also from south eastern Finland (Figure 1).

The first investigations of lead, cadmium, copper and zinc levels in hare were performed in 1980–1982. A total 212 Mountain hares (*Lepus timidus*) and 188 European hares (*Lepus europaeus*) were collected from 15 game management areas. In 1992, the investigations were continued by taking samples from the four same game management areas as previously. This was arranged such that the game management areas of south eastern, south western and southern Finland together represented the industrialized area of Finland. The game management area of Oulu on the other hand represented northern rural Finland. Mountain hares ($n = 51$) and European hares ($n=31$) were sampled between October 1992 and April 1993 in the game management areas as described above. Heavy metals were analyzed from muscle, liver and kidney and age was determined from the radius and the ulna (Figure 1).

Samples for heavy metal determinations in reindeer were collected in the autumn slaughtering seasons 1990–1991 and in 1991–1992 from western, southern, northern and eastern Lapland. A total of 325 muscle, 210 liver and 325 kidney samples of adult reindeer (over 2 years) and calves (under one year) were studied. During the first slaughtering season in 1990–1991, samples were taken from both adult reindeer and calves and during the 1991–1992 seasons mainly from calves (Figure 2).

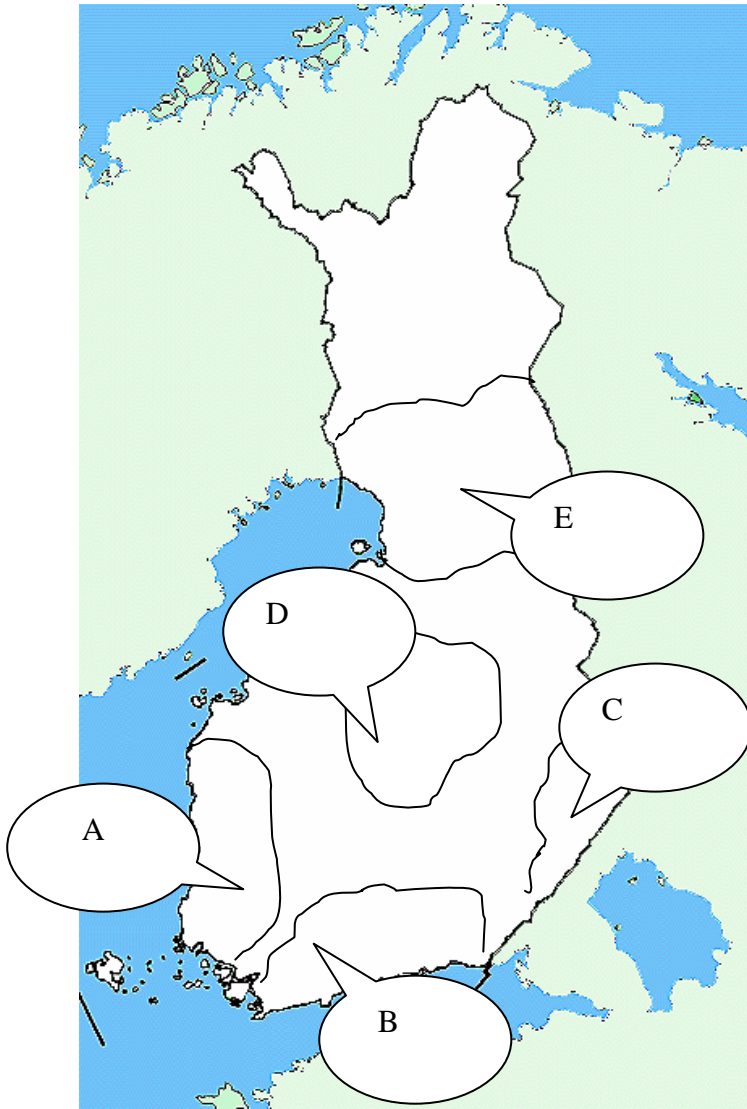


Figure 1. Map of the sample areas of moose and hares. A = South western Finland, B = Southern Finland, C = South eastern Finland, D = Central Finland, D= Northern Finland

After these initial projects, moose and reindeer samples were analyzed in the national residue control program, which is carried out annually in accordance with both national and European Union legislation. Annually fifteen moose and ten reindeer muscle, liver and kidney samples are collected by veterinarians in various slaughterhouses. The area for moose samples could vary each year but reindeer samples were collected from northern, western and southern Lapland. No age determination was done for these animals.

Samples from cattle (1987-2004) were obtained from 8-15 Finnish slaughterhouses. The animals are selected randomly from the slaughter line and samples of muscle, liver and kidney are taken from the same animal. The kidney samples are taken from the cortex part of the organ, which is the primary site of heavy metal accumulation. The samples are packaged separately, immediately frozen and sent to the laboratory in temperature-

controlled containers containing coolant so that they were frozen before mailing. The packaged samples are marked with the date of slaughter, the name of the slaughterhouse, the carcass number, the animal species and the type of sample. The samples are stored at -18 °C until analysis. Sampling is carried out by official inspectors (veterinarians). Every year 30 muscle, 30 liver and 30 kidney samples are analyzed.

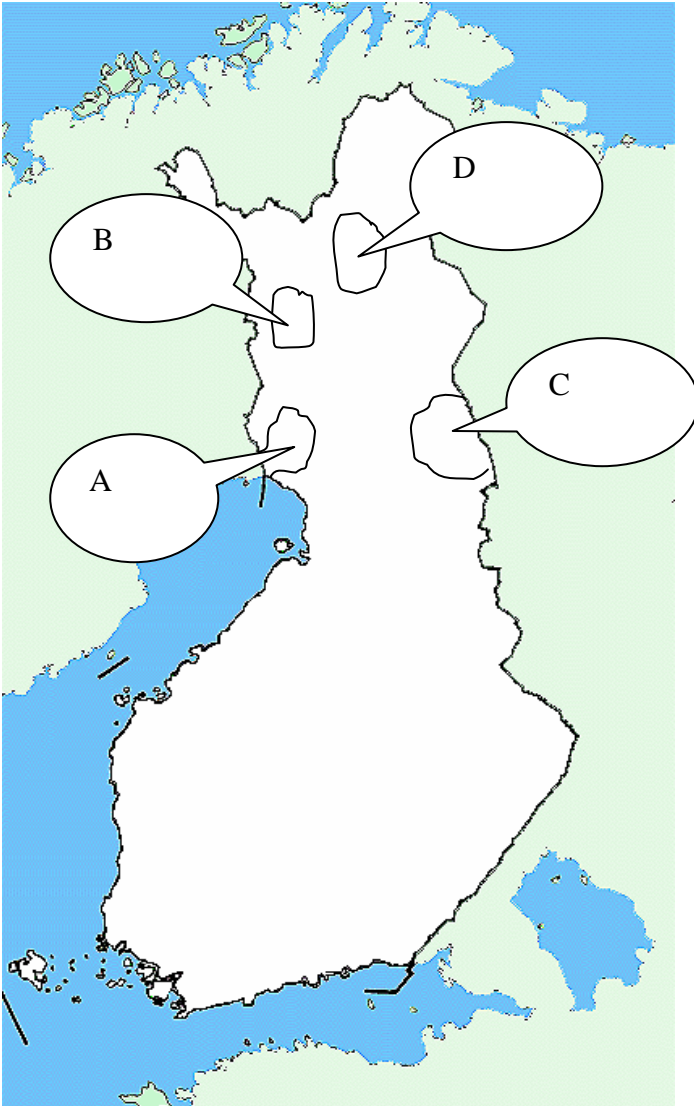


Figure 2. Map of the sample areas of reindeer. A = Southern Lapland, B = Western Lapland, C = Eastern Lapland, D = Northern Lapland

6.2. Age determination

The hunters were asked to send a half of a mandible of the moose without breaking off the root tip. The samples were used to determine the age of each particular moose at the Game and Fisheries Research Institute (samples of 1990) and at Matson's Laboratory

(Matson's laboratory, Milltown, USA) (samples of 1999). Before the teeth were dispatched to Matson's laboratory they were cleaned in the Department of Pathology in National Veterinary and Food Research Institute (EELA) according to the method recommended by Matson's Laboratory. Briefly, the periodontal membrane was softened in hot water (temperature below than 80 °C) and then the teeth were cleaned with a nylon mesh material. Teeth were extracted from the skulls and mandibles and finally they were packed carefully in paper envelopes, placed in a very sturdy cardboard box and mailed to Matson's Laboratory. The teeth were analyzed microscopically and the age was estimated on the basis of the cement layers corresponding to the age of the animal.

The age determination of hare was performed in the Game and Fisheries Research Institute by investigating the ossification stage of the radius and ulna. The radius and ulna were cleaned and radiographs were taken. Hares were sub-divided according to their age into three groups; 1) Juvenile, still growing, epiphyseal cartilage visible, 2) Juvenile, fully grown, epiphyseal cartilage ossified, 3) Adult; Mountain hare older than nine months, European hare 7-9 months (Soveri et al., 1986).

6.3. Analytical methods

Before the chemical analysis muscle (10 g), liver (5 g) and the kidney cortex samples (5 g) were homogenized and subsequently weighed into quartz dishes and dried in a water bath. The dried samples were dry-ashed overnight in a thermostat controlled muffle furnace at 450 °C. The ashed samples were allowed to cool down to room temperature, after which deionised water (2-3 ml) and concentrated nitric acid (1 ml) were added and the samples were evaporated and heated up to 450 °C in a muffle furnace and kept at that temperature overnight. The ash was dissolved in concentrated nitric acid (0.5 ml) and the sample volume was adjusted (liver and kidneys to 20 ml, muscle to 10 ml) with deionised water (Niemi et al., 1991).

Cadmium and lead were measured with a graphite furnace atomic absorption spectrometer applying pyrolytically coated tubes and pyrolytic THGA-tubes (transverse heated graphite atomiser tubes) with a Zeeman furnace module (Perkin-Elmer, Singapore). Cadmium was measured at 228.8 nm and lead at 283.3 nm with hollow cathode lamps. Copper and zinc were measured with the same instrumentation except that the air-acetylene flame technique at 324.8 nm and 213.9 nm wavelength was used. All data was collected using a Perkin Elmer 5000, 5100 PC or with an AAnalyst 800 atomic absorption spectrophotometer. Quantification was carried out by using external standard solutions made up in 0.1 M nitric acid (Merck, Reagecon).

6.4. Quality control

The limit of detection (LOD) and the limit of quantification (LOQ) were determined with reagent blank samples. Blank samples (n =20) were analyzed along with samples and the limit of detection was calculated. The limit of detection (LOD) is the average of 20 samples plus three times the standard deviation. The limit of quantification (LOQ) is two times the LOD. LOQ values for cadmium, lead, copper and zinc were 0.001 mg/kg w.w., 0.01 mg/kg, w.w. 0.2 mg/kg w.w. and 0.2 mg/kg w.w. respectively.

Measurement uncertainty was calculated with random and systematic errors (FINAS S33/1996). The uncertainty depends on the concentration of the analyte. The expanded uncertainty values were 12–40 %, 20-60 %, 10-30 %, 10-30 % for cadmium, lead, copper and zinc, respectively.

The trueness of the method was tested with standard reference materials and with recoveries. Standard reference materials (BCR 184 bovine muscle, BCR 185, BCR 185R, NIST 1577a bovine liver and BCR 186 pig kidney) were used and all samples were analyzed two times. The reference material data was $\pm 10\%$ from the certified mean values. Quantification was performed by using standards with different concentrations and instrument contamination was monitored by analysing blank samples in the sample series. The recoveries of cadmium, lead, copper and zinc were determined by adding a known amount of a particular standard solution into the samples. The quantities of standards used were close to the amounts normally detected in samples. The recovery varied from 70 % to 110 %. Our laboratory participates annually in proficiency tests (Food Analysis Performance Assessment Scheme = FAPAS, Istituto Superiore di Sanita= ISS) in which good results with a z-score of ± 2 are regularly achieved.

The method used to analyze lead, cadmium, copper and zinc was accredited in 1993 by the Centre of Metrology and Accreditation in Finland (EELA 8104 flex: Determination of metals in animal tissues with graphite furnace and flame atomic absorption spectrometry).

6.5. Statistical analysis

The analysis of variance was used to determine whether there were statistically significant differences between various hunting areas in terms of heavy metal levels or in heavy metal levels between tissues (IV). Analysis of variance, t-test and Pearson correlation were used to determine whether there were statistically significant differences between hare and moose cadmium levels and whether there was a correlation between the increased cadmium and zinc levels.

7. RESULTS

7.1. Heavy metal levels in moose samples

The results of moose samples are collected in Table 21.

Cadmium accumulates into kidney and liver as a function of the age of the animals. The mean cadmium levels in 1999 in various hunting areas ranged from 4.95 to 6.18 mg/kg w.w. in kidney and 0.895–1.28 mg/kg w.w. in liver (IV). The level of cadmium in liver and kidney samples seems to have increased during the sampling years (Figures 3 and 4).

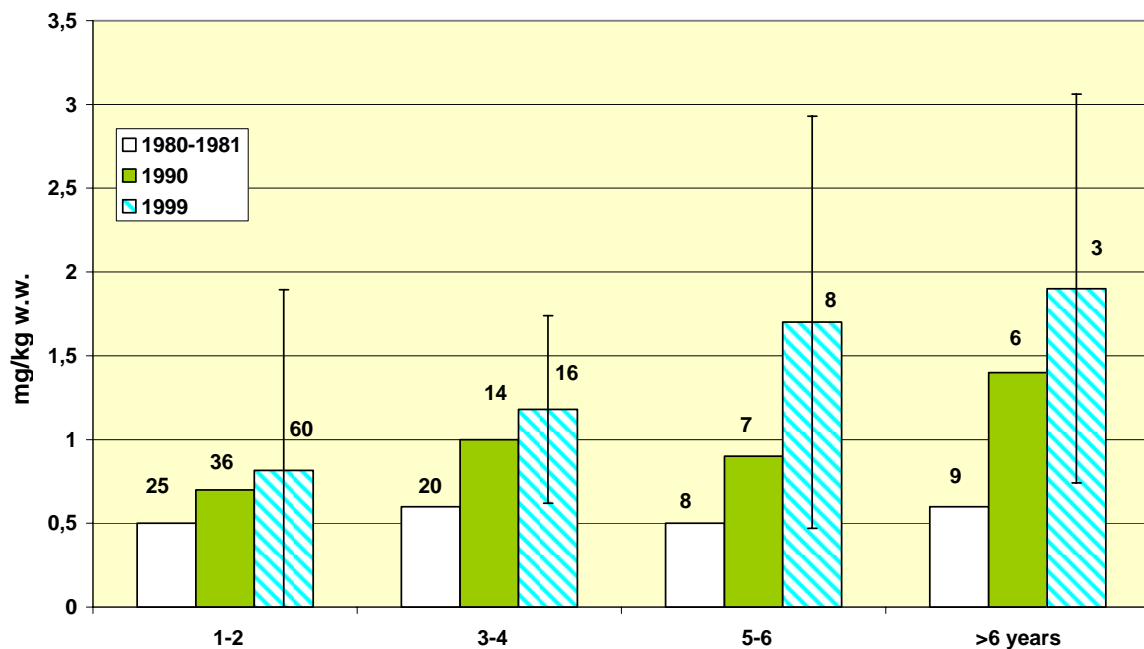


Figure 3. The cadmium levels in liver of moose in different age groups.

The number at the top of a particular column indicates the number of the samples analyzed. The range can only be provided for the 1999 samples.

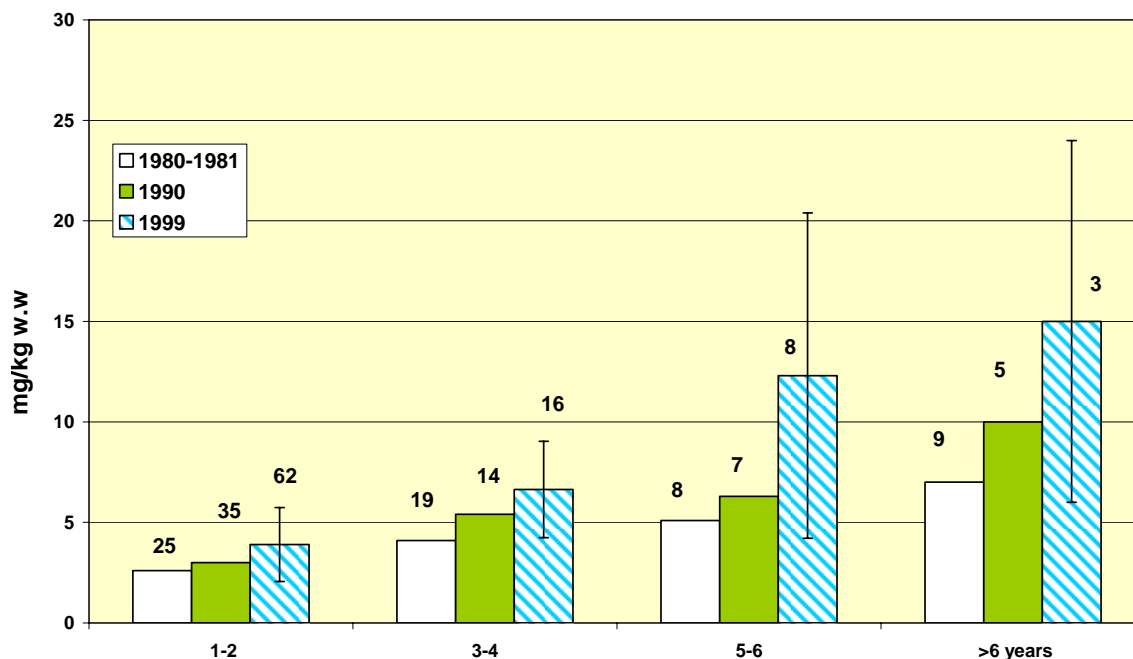


Figure 4. The cadmium levels in kidneys of moose in different age groups. The number on the top of a particular column indicates the number of the samples analyzed. The range can only be provided for the 1999 samples.

The accumulation in muscle was not related to the age of the animal, higher cadmium levels were observed only in moose which were older than six years. The mean cadmium level in 1999 in muscle was 0.004 mg/kg w.w. A significant difference in the cadmium levels was observed between kidney and liver samples as well as between kidney and muscle samples ($p = 0.0099$ and 0.0051). However, the cadmium concentration in muscle tissue has decreased during the monitoring years, being now close to the limit of quantification (0.001mg/kg) (I, IV). Moose samples in 2001-2004 were also analyzed in the Finnish residue control program (EVI, EELA, MMM, 2005). The cadmium levels in moose liver and kidney are substantially higher than in cattle samples (Figure 5).

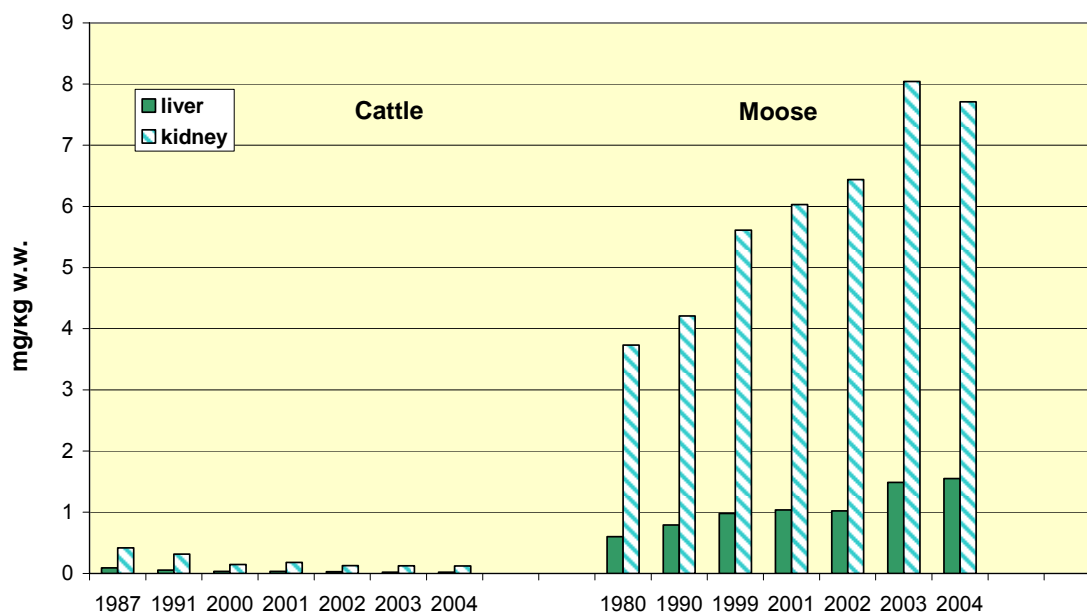


Figure 5. The average cadmium levels in liver and kidney samples. The number of the animals sampled in each case varied from 30 to 90 animals.

During the time period 1980 -1990, lead levels in liver and kidney have decreased clearly. The liver lead level in 1980 -81 varied from 0.21 to 0.25 mg/kg w.w. and in 1990 from 0.05 to 0.07 mg/kg w.w. The kidney lead level in 1980 -81 varied from 0.36 to 0.53 mg/kg w.w. and in 1990 from 0.05 mg/kg w.w. to 0.07 (I). During the time period from 1990 to 1999, no changes occurred in lead levels in liver and kidney samples, with the mean levels varying in 1999 samples from 0.04 - 0.07 mg/kg w.w. and from 0.05-0.07 mg/kg w.w. in liver and kidney samples, respectively with the highest levels of lead measured in samples originating from southern and south western Finland (IV). However, the difference in lead levels between different geographical areas was not statistically significant ($p=0.1529$). In the samples collected in 1999, the lead levels in moose samples were at the same level as those detected in cattle tissues analyzed in Finnish residue control program (Figure 6). The decreasing trend in lead levels of moose can be observed also in the samples of taken in this current century, at least in kidney samples as in cattle samples (Figure 6)

The lead levels in moose muscle have clearly decreased from the levels detected in samples collected in 1980 or 1990 (I), and in 1999 the level was so low as to be close to the limit of quantification 0.01 mg/kg (IV)

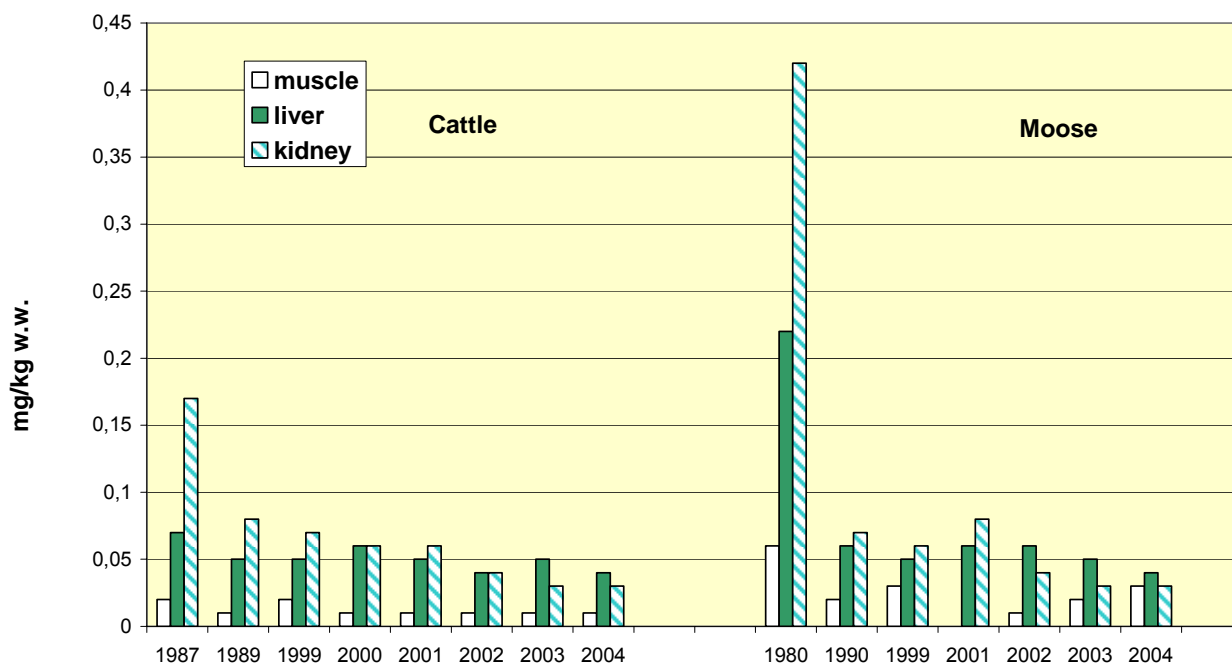


Figure 6. The lead levels in muscle, liver and kidney samples. The number of animals sampled in each case varied from 30 to 90 animals.

In general, copper accumulates in liver. In 1999, the liver copper level was between 33.0 and 50.5 mg/kg w.w. The liver copper levels in 1980–81, 1990 and in 1999 in south western Finland, in southern Finland and in central Finland were 29.7 mg/kg, 46.0 mg/kg, 33.0 mg/kg; 26.2 mg/kg, 46.1 mg/kg, 44.4 mg/kg and 38.9 mg/kg, 54.4 mg/kg, 48.8 mg/kg w.w. respectively. Samples (n= 28) originating from south eastern Finland in 1999 exhibited the highest level of copper in liver tissues (50.5 mg/kg w.w.) and a statistically significant difference was seen when these were compared with samples of south western Finland ($p=0.0191$). Compared to the other sampling areas, the difference in copper levels were not statistically significant (Figure 7).

The level of zinc is highest in muscle tissues followed by kidney and liver samples. In the sampling conducted in 1999, the zinc levels in different hunting areas were in muscle 56.9–62.1 mg/kg, in liver 23.7–32.1 mg/kg and in kidney 30.0–32.4 mg/kg w.w.

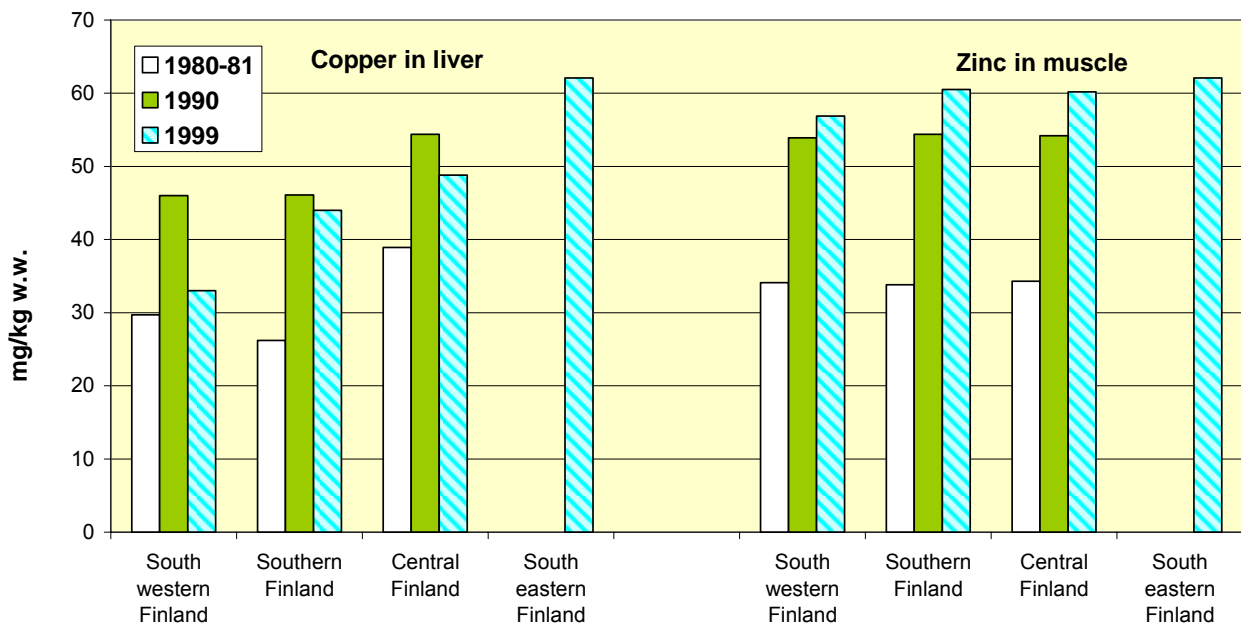


Figure 7 . The copper and zinc levels in Finnish moose. The number of animals sampled in each case varied from 15 to 50 animals.

The zinc concentrations have increased slightly during the past twenty years (Figure 7). The levels of zinc in muscle in 1980–81, 1990 and in 1999 in south western Finland, in southern Finland and in central Finland were 34.1 mg/kg, 53.9 mg/kg, 56.9 mg/kg; 33.8 mg/kg, 54.4 mg/kg, 60.5 mg/kg and 34.3 mg/kg, 54.2 mg/kg, 60.2 mg/kg w.w. There were no significant differences between different game management areas (I, IV) and not were there any correlation between increased zinc and cadmium levels ($p=0.650$).

The level of zinc and copper was not dependent on age of the animals (Figures 8 and 9) (IV).

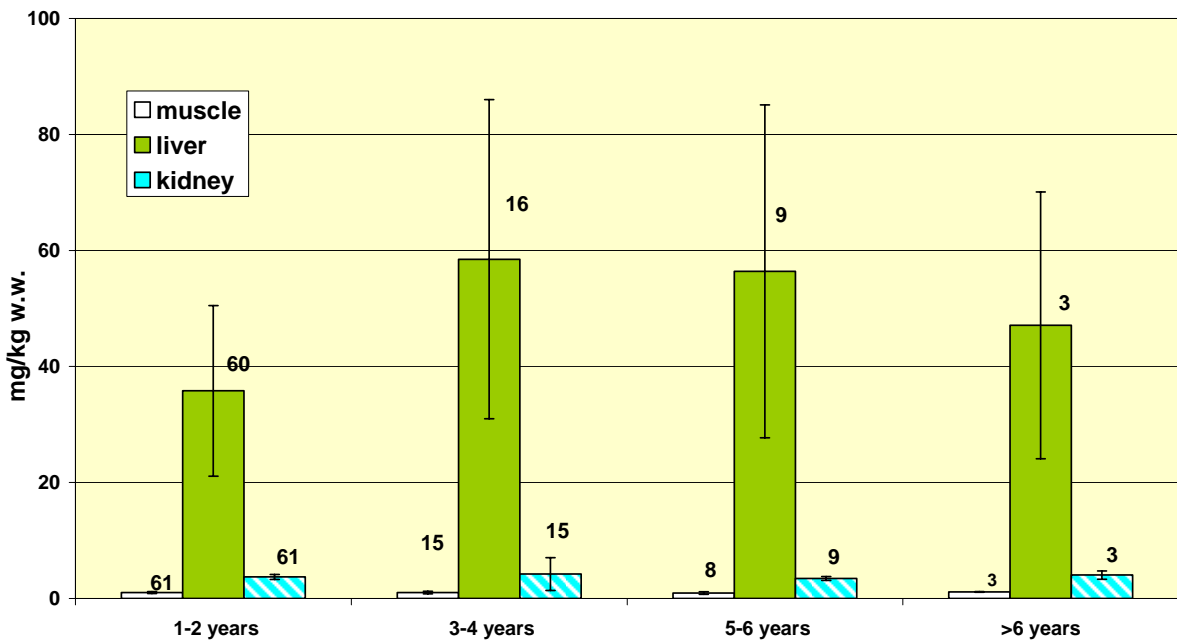


Figure 8. The copper levels in muscle, liver and kidneys of moose in different age groups in 1999. The number at the top of a particular column indicates the number of the samples analyzed. The range is indicated by a bar.

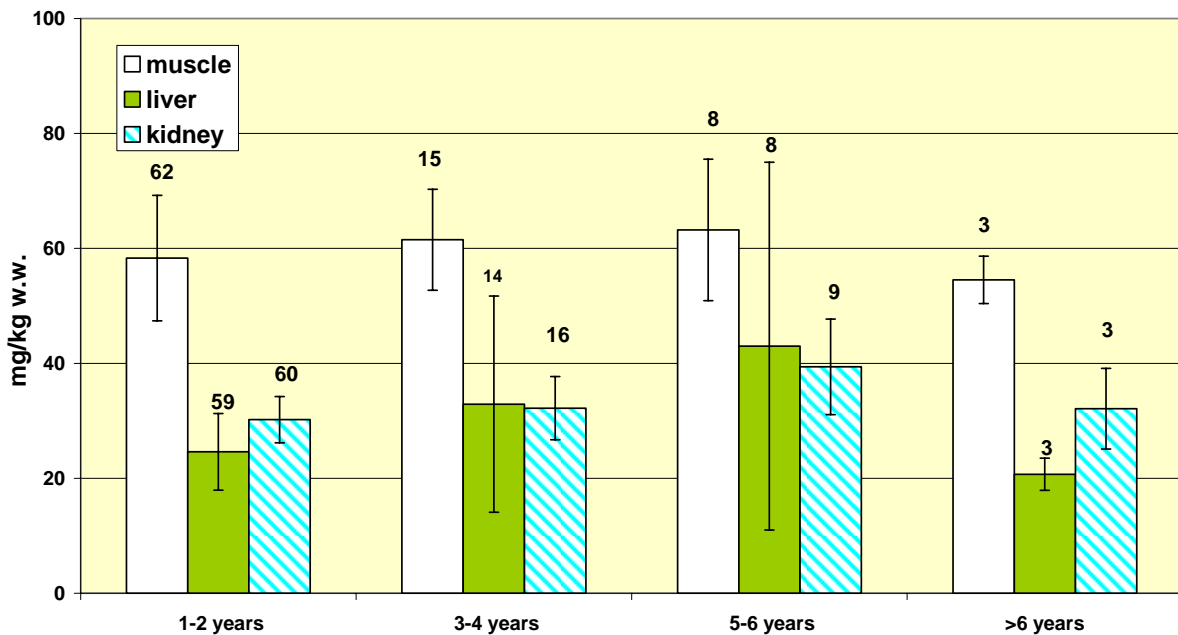


Figure 9. The zinc levels in muscle, liver and kidneys of moose in different age groups in 1999. The number at the top of a particular column indicates the number of the samples analyzed. The range is indicated by a bar.

7.2. Heavy metal levels in reindeer samples

The results of reindeer samples are collected in Table 22.

The arithmetic mean cadmium in muscle for adult reindeer (n= 54) and calves (n = 153) varied between 0.001–0.006 mg/kg w.w. Around 8 % of analyzed calve samples were below the limit of quantification (0.001 mg/kg). The arithmetic means of cadmium in liver in various areas were 0.402–0.958 mg/kg (n =103) and 0.190–0.388 mg/kg w.w. (n = 116) for adult and calves, respectively. The corresponding values for kidney were 1.72–4.62 mg/kg (n =103) and 0.525–1.22 mg/kg w.w. (n= 222) (II).

The arithmetic mean of muscle lead in adult reindeer (n= 61) and calves (n = 151) in Lapland was 0.01 mg/kg w.w. In all, 30 % of the analyzed adult reindeer samples and 40 % of calve samples were below the limit of quantification (0.01 mg/kg w.w.) (II).

The arithmetic means of lead in liver in various areas were between 0.16–0.43 mg/kg (n =103) and 0.13–0.36 mg/kg w.w. (n=116) for adult and calves, respectively. The corresponding values for kidney were 0.27–0.34 mg/kg (n =103) and 0.15–0.33 mg/kg w.w. (n= 192). (II).

The lead and cadmium levels in reindeer are higher than the corresponding values in cattle (Figures 10 and 11).

The arithmetic mean of copper levels in the muscle samples for adult reindeer (n= 103) and calves (n = 206) in Lapland in various areas varied between 1.63–1.91 mg/kg and 1.65–1.92 mg/kg w.w., respectively. The arithmetic means of the copper levels in liver samples in various areas were 29.7-71.0 mg/kg (n =103) and 29.2-82.2 mg/kg w.w. (n = 206) for adult reindeer and calves, respectively. The corresponding values for kidney were 4.14–5.41 mg/kg (n= 103) and 4.02–5.68 mg/kg w.w. (n= 206) (II).

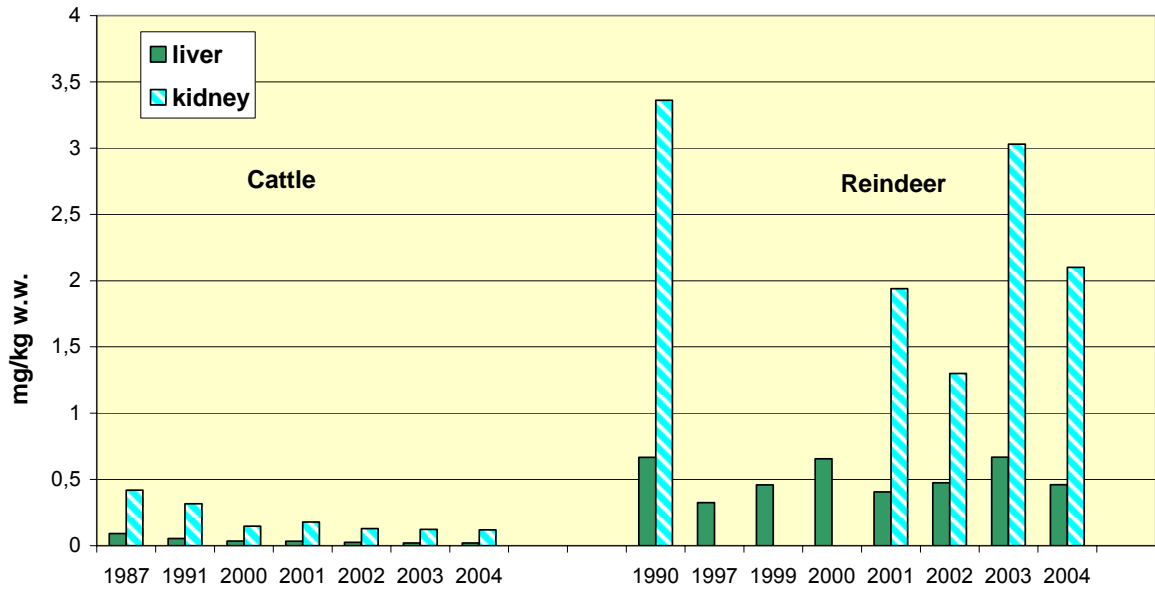


Figure 10. The average cadmium levels in muscle, liver and kidney samples of cattle and adult reindeer. The number of the animals sampled in each case varied from 10 to 100 animals.

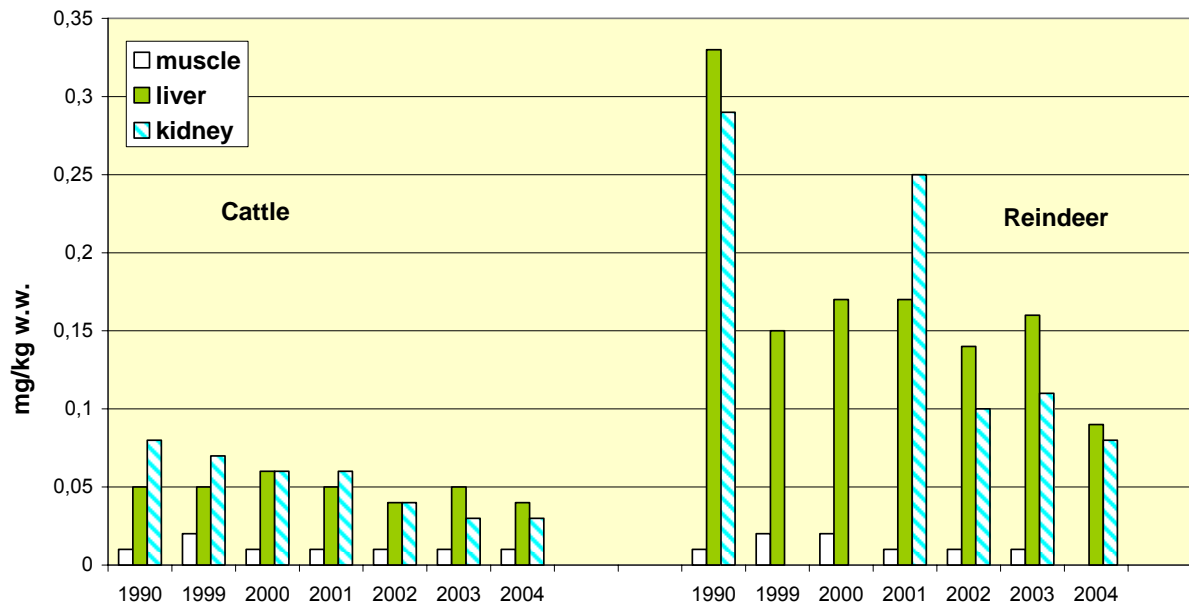


Figure 11. The average lead levels in muscle, liver and kidney samples of cattle and adult reindeer. The number of the animals sampled in each case varied from 10 to 100 animals.

7.3. Heavy metal levels in hare samples

The results of hare samples are collected in Table 23.

The arithmetic means of liver cadmium in Mountain hares in 1992–1993 were 0.445 mg/kg (sd = 0.273) from industrial Finland and 0.185 mg/kg w.w. (sd = 0.107) from the northern part of the country. The corresponding values for European hares were 0.160 (sd = 0.152) and 0.057 mg/kg w.w. (sd = 0.029). The arithmetic means of kidney cadmium in Mountain hares were 10.7 mg/kg w.w. (sd = 9.34) in the industrial area compared to 3.73 mg/kg w.w. (sd = 3.16) northern part of Finland. The corresponding values for European hares were 1.91 mg/kg (sd = 2.19) and 0.627 mg/kg w.w. (sd = 0.439), respectively (Figure 12). The cadmium level in muscle was near to the limit of quantification, (0.001 mg/kg w.w.) The mean cadmium levels in kidney of Mountain hares were significantly higher than those detected in kidney of moose ($p < 0.01$).

The arithmetic means of all of the measured lead levels in liver and kidney samples in 1992–1993 were lower than 0.5 mg/kg w.w. However, kidney samples of Mountain hares hunted in the industrial areas of Finland had an average lead value of 0.63 mg/kg. The lead levels in muscle were much lower, 0.01–0.07 mg/kg w.w.

The copper levels increased slightly during the ten years of the first part of this study (1980–82 and 1992–93). The arithmetic means of liver copper in Mountain hares were 4.71 mg/kg in industrial areas and 4.17 mg/kg w.w. in samples from the northern part of Finland. The corresponding values for European hares were 5.15 mg/kg and 5.32 mg/kg w.w. The arithmetic means of kidney copper in Mountain hares were 4.36 mg/kg and 4.14 mg/kg w.w. in industrial area and northern part of country. The corresponding values for European hares were 4.49 mg/kg and 4.64 mg/kg w.w., respectively.

The arithmetic means of liver zinc in Mountain hares were 31.7 mg/kg and 31.4 mg/kg w.w. in industrial area and the northern part of Finland, respectively. The corresponding values in the European hares were 37.1 mg/kg and 42.8 mg/kg w.w., respectively. The arithmetic means of zinc in kidney samples of Mountain hares were 34.8 mg/kg in industrial areas and 27.5 mg/kg w.w. in the northern regions. The corresponding values for European hares were 28.8 mg/kg and 28.9 mg/kg w.w.



Figure 12. The cadmium levels in livers and kidneys of European hares and Mountain hares in industrial Finland in 1992-1993

8. DISCUSSION

8.1. Cadmium levels in moose samples

Although the atmospheric emissions of cadmium have decreased in Finland by about 80 % from 1990 to 1997, paradoxically the cadmium concentration in moose liver and kidney has increased considerably over the same time period (I, IV). The cadmium level is also higher in older animals than in young animals.

The pH of Finnish cultivated soil is close to 5.8 which is mainly due to the high proportion of organic matter in soil. Plants growing in acidified areas absorb cadmium from the soil more readily than plants growing in soils of alkaline or neutral pH. The slow decomposition rate of organic matter is a result of the cold and humid climate (Louekari et al., 2000). In Central European countries, the pH of the cultivated soil is closer to pH 7 and the organic level of soil is low. The above mentioned factors make it possible for soil in Finland to accumulate high amounts of cadmium in a soluble form; and this in turn makes cadmium biologically available for plants.

The clay soil in southern Finland has a low concentration of zinc which poses a risk by allowing cadmium to accumulate in plants (Louekari et al., 2000). Of all the trace elements, cadmium is the most easily taken up by plants.

The mobility and toxicity of heavy metals in soils is strongly related to the acidity and organic content. In general, increasing acidity tends to increase the mobility of heavy metals. In particular, the mobility of cadmium and zinc is regulated by acidity. And since also the zinc levels of moose have increased during the period of this study, it could be postulated that the forest soils have become more acidic making it possible for cadmium and zinc to translocate more easily to plants and subsequently to the animals grazing on these plants.

One explanation for the increased cadmium levels could be the cultivation of forest soil. The purpose of this process is to improve the productivity of the soil and therefore the humus, which acts as an insulating material layer, is removed away. This means that the temperature conditions of mineral soil are changed; the soil warms and cools more rapidly if it still possessed a humus layer. In addition, the moisture balancing effect of humus is lost. This change in the water economy can increase the movement of cadmium to plants and may encourage the growth of sprout forest e.g. willow trees. It is well known that willow (*Salix*) is a preferred food for moose and this tree has been shown to be a hyperaccumulator of cadmium (Vandecasteele et al., 2002).

One possibility is related to some change in the grazing area and nutritional habits of the moose; perhaps they graze more in areas populated by willows and aspens.

The high cadmium concentrations are a reflection the region's natural mineral levels, acid precipitation, long range atmospheric transport pollutants from industry, waste burning and mining in both central and eastern Europe and transfer of these elements from sediment to moose via the plants growing in the soil which are consumed by the moose.

However, the cadmium concentration in muscle tissue has decreased during the monitoring years, which is partly due to the better and more sensitive analysis techniques. The cadmium concentration in muscle tissue is now close to the limit of quantification (0.001mg/kg) (I, IV),

8.2. Lead levels in moose samples

The moose data from the sampling period of 1980-81 detected a geographical difference in the lead concentration in kidney (I). The kidney samples from southern Finland, where there is highest density of car traffic, had the highest levels of lead. In the samples taken in 1990, the geographical locations of the game management area no longer played any significant role in the lead levels in the detected samples (I, IV). The atmospheric emissions of lead decreased substantially in the 1990s; it has been estimated that emissions have declined by as much as 95 % from 1990 to 1997 even though industrial production of lead has increased over the same time period (Melanen et al., 1999). This has been achieved partly through investments in better purification processes for fuels and gases and advanced combustion technologies together with enhanced process automation. However, the most important reason for the decrease of environmental lead level is the use of unleaded or gasoline with low lead content and this is now mirrored in the lower levels of lead in muscle, liver and kidney samples compared to the samples from the 1980s and 1990s (I, IV). In Finland, the changeover to unleaded gasoline took place in 1993. The decrease is also partly due to the closure down of two secondary lead smelters and a waste incineration plant in the Helsinki area in the beginning of 1980.

8.3. Copper levels in moose samples

The liver has a higher ability to accumulate copper than kidney and muscle tissues. Samples originating from south eastern Finland had the highest level of copper in liver tissues and a statistically significant difference was seen compared to samples collected from south eastern or south western Finland ($p=0.0191$). Compared to the other sampling areas, the difference in copper levels was not statistically significant. The level of copper was not dependent on age (IV).

Copper levels increased during the period of 1980–1990 but since then have declined due to the decrease of copper emission from the beginning of the 1990s (Derome, 2000). Copper levels have decreased slightly in southern and central Finland but the decline has been even more dramatic in south western Finland (I, IV). The highest copper levels are found in south eastern Finland and the lowest levels in south western Finland (IV). Industries using copper now pay much more attention to effective emission control systems and have improved technical processes. The geochemistry is reflected in the copper distribution in moose samples. The copper concentrations in fine fraction of till are higher in eastern Finland than in other parts of Finland (Koljonen, 1992).

However, the copper levels are at the levels which are adequate to the animals themselves. The adequate levels for cattle in muscle, liver and kidney are 1.3–1.5 mg/kg, 25–100 mg/kg and 4.0–6.0 mg/kg w.w., respectively (Puls, 1988).

8.4. Zinc levels in moose samples

The zinc concentrations have increased during the twenty year period but there are no significant differences between the different game management areas. Furthermore there was no correlation with increased zinc and cadmium levels. The acidity is reflected in mobility and levels of zinc; increasing acidity increases the mobility of zinc. However, the levels are at the levels which are not toxic for animal health. The adequate levels for cattle liver and kidney are 25–100 mg/kg and 18–25 mg/kg w.w., respectively (Puls, 1988).

8.5. Cadmium levels in reindeer samples

The average cadmium levels of reindeer liver and kidney samples were quite high. The results point to cadmium accumulation in kidneys with age but also reveal the rather wide variation of cadmium levels between samples. Lower cadmium levels in reindeer muscle, liver and kidney samples were detected in southern Lapland. Cadmium levels of adult reindeer liver and kidney samples from other areas were at about the same level as those found in 1990–91 from Finnish moose liver and kidney samples (I). Slightly higher cadmium levels were measured from reindeer liver and kidney samples in a previous study (Salmi and Hirn, 1981).

8.6. Lead levels in reindeer samples

The lead contents of reindeer muscle, liver and kidney samples have decreased from the first study of Finnish reindeers (VELL, 1980), but the trend is not as clear as with moose (I, IV). However, the lead levels both in reindeer liver and kidney samples (II) are clearly

higher than those found in moose (I, IV). In general, the high lead levels in reindeer liver and kidney samples may be traced back to the lead level of lichen, which is a very important part of the diet of reindeer (Frøslie et al., 1984). Lichens are effective accumulators of heavy metals, but changes in the level of atmospheric lead are reflected only slowly in reindeer organ samples, because of the long lifetime of lichens. The lead levels of reindeer liver samples from southern Lapland were lower than those from other areas but generally no major differences were observed between the various areas. This may be a result of the use of commercial feeds in southern Lapland (i.e. the reindeer were not so dependent on lichen as the mainstay of their diet).

Lead and cadmium levels in reindeer are analysed in the Finnish residue control program annually. The lead level in liver and kidney samples in both adult and calves have been invariably below the recommended maximum level in the EU (0.5 mg/kg). However, the kidney cadmium level exceeds the maximum level (1.0 mg/kg) in almost all adult reindeer samples and also in some calves.

8.7. Copper levels in reindeer samples

Copper accumulates mainly in liver tissue and so liver is a better indicator than other tissues when investigating differences between areas. Higher copper levels were detected in reindeer liver samples from northern and eastern Lapland compared to the samples from other regions. Furthermore, the copper concentrations of reindeer liver samples, excluding samples from western Lapland, appear to be higher than the concentrations in reindeer liver samples in the earlier study of VELL (1980). Kubin (1990) reported a high copper level in the lichen samples from northern and eastern Lapland. Niskavaara and Lehmuspelto (1992) also reported similar results from moss samples collected in northern Lapland. These regions are affected by copper emissions from Kola Peninsula. However, the higher copper concentrations in the reindeer liver samples from northern and eastern Lapland were at the same level as those normally detected in liver samples from Finnish cattle (VELL, 1992). The adequate levels for cattle in muscle, liver and kidney are 1.3–1.5 mg/kg, 25–100 mg/kg and 4.0–6.0 mg/kg w.w., respectively (Puls, 1988).

8.8. Chromium and nickel levels in reindeer samples

The chromium levels of reindeer muscle and kidney samples from eastern Lapland in 1990-1991 were exceptionally high (0.07-0.10 mg/kg w.w.). In other years, the mean levels of chromium were low (0.02 mg/kg w.w.). The reason for the different levels could be normal sample variation in the population or it could also be a sign of regional differences in the environmental chromium burden.

In many samples from southern, western and eastern Lapland the nickel levels were below the limit of quantification. Although no significant differences were found between the studied areas, no samples below the limit of quantification were analyzed from northern Lapland and the mean concentrations of reindeer kidney samples were somewhat higher in the north than those found in kidney samples from other areas.

Kubin (1990) used epiphytic lichen as a bio-indicator of airborne substances and reported higher chromium concentrations in eastern and southern Lapland compared to the north and west of that region. This may be linked to steel manufacture and ore mining in these areas. In the case of reindeer samples from southern Lapland there was no sign of higher chromium concentrations. This may be attributed to the use of commercial feed supplied to the reindeers in that area. Juntto (1992) also measured high chromium and copper concentrations from samples of airborne substances near the research station of Värriö in eastern Lapland.

8.9. Cadmium levels in hare samples

The cadmium levels of Mountain hares (in muscle, liver and kidney) have not changed much over ten year period, whereas the cadmium levels of European hares have declined somewhat (III). The cadmium levels of European hares were lower than those of Mountain hares and levels in northern Finland were lower than those detected in industrial southern Finland.

The cadmium levels in muscle in Mountain hares and European hares are low and similar to the cadmium concentrations in Finnish pigs and cattle (Niemi et al. 1991; EVI, EELA, MMM, 2004) as well as moose and reindeers (I, II, IV). The cadmium level in muscle was near to the limit of detection, 0.001mg/kg w. w. The cadmium levels in liver were below 0.5 mg/kg w.w., which is the proposed maximum cadmium level in the EU in the liver of pigs and cattle. The concentrations in hare's liver were lower than those found in the livers of Finnish moose (I,IV) being at the same level as Finnish reindeer calves (II). The cadmium levels in kidney were rather high, over the proposed safety limit (1 mg/kg). In southern Finland, the cadmium levels of Mountain hares were higher than those detected in the kidneys of Finnish moose and reindeer (III, IV). In northern Finland, the kidney cadmium levels were at the same level as those found in the kidneys of adult reindeers (II).

It was surprising that the lead and cadmium levels in liver and kidney of Mountain hares were higher than in the same organs of European hares. The difference probably can be attributed to the different diets of these two species; Mountain hares eat more plants with wood stems and European hares eat more grass. The grass regenerates yearly, whereas for example willow and birch are exposed to the influence of air pollution for longer periods, and thus have a longer time to accumulate heavy metals.

8.10. Lead levels in hare samples

The lead in hare muscle, liver and kidney were lower in 1992–1993 than those reported in 1980–1982 (III). This can probably be traced to the introduction of low lead gasoline during the same period. In 1980–1982 there were no difference in the the measured lead concentrations of Mountain hares and European hares. However the data from 1992–1993 indicate that the lead levels of European hares were lower than those of Mountain hares.

The lead levels in muscle were higher than those in muscle samples of Finnish moose and reindeers (I ,II, IV), which were near to the limit of detection (0.01 mg/kg w.w.). Some muscle samples of hares contained very high lead concentrations, 8-10 mg/kg, probably due to the fact that the samples were taken from near the shotgun pellet wound. These samples were rejected.

The average lead levels in hare liver and kidney samples were below 0.5 mg/kg, which is the recommended maximum level in EU for liver and kidney of pigs and cattle. One exception was the kidney samples of Mountain hares from industrial Finland, which had an average lead level of 0.63 mg/kg w.w. The average lead levels in liver and kidney of hares were at same level as or even higher than those found in moose (I, IV). The lead levels of Mountain hares were at same level as those in adult reindeer but in European hares, the levels were similar to those found in reindeer calves (II). The lead level in liver and kidney of Mountain hares were higher than the corresponding values in European hares (II).

8.11. Copper and zinc levels in hare samples

There were no major differences in copper and zinc levels between areas and also no differences between Mountain hares and European hares. Copper accumulates mostly in liver and zinc to muscle. However, the copper levels in the livers of hares were much lower than in moose and reindeer and zinc concentrations in muscle were only about half of those in muscle of moose (I, II, IV). Kubin (1990) used lichen to study air pollution in Finnish forests. He reported the highest copper and zinc concentrations in south western Finland, this being apparently attributable to the presence of a copper refinery in that region. High copper concentrations were also observed in eastern Finland in the vicinity of a copper mine. The high copper and zinc concentrations recorded in northern Lapland are evidently the result of long-distance transport from Kola Peninsula, as there are no local sources of these emissions. The hares lived in these areas but the copper and zinc concentrations in hares were at a normal level (Puls, 1988). Copper levels in liver seemed to have increased slightly during the over ten year period (1980–1993) but zinc levels in muscle did not change. Annual emissions of copper increased from 1985 to 1987 from 98 t/year to 140 t/year which could reflect to copper levels (Derome, 2000).

The adequate copper levels for hare's health are 8.0–50.0 mg/kg in liver and 4.0–6.0 mg/kg w.w. in kidney. The corresponding values for zinc are 30–80 mg/kg and 10–30 mg/kg w.w. (Puls, 1988).

8.12. Chromium and nickel levels in hare samples

The chromium and nickel levels of muscle, liver and kidney were low, in practice at the same levels found in pigs and cattle.

8.13. Consumption of meat and organs of moose and reindeer

Cadmium does not pose a health risk as far as moose meat is concerned and the high levels of cadmium in liver and kidney samples are relatively unimportant in terms of dietary intake of the cadmium for the Finnish population. However, liver and kidney may be consumed by the moose hunters themselves and this may have a major impact on the daily cadmium intake of these individuals.

The estimated moose meat consumption is much higher than the consumption of liver and kidneys. Finnish legislation prohibits the use of moose liver and kidneys of animals older than one year for human consumption to avoid excessive exposure to cadmium.

An individual in the highest deciles of consumption of liver and kidney is estimated to have a cadmium intake of 13.9 % of PTWI and 25.5 % of PTWI from liver and kidney respectively (WHO, 2001; Vahteristo et al., 2003).

In Finnish legislation, there are no restrictions placed on the consumption of reindeer meat or organs. The concentrations of heavy metals in the meat are so low that the consumption of reindeer meat does not pose any health risk. However, the amount of lead in liver and kidneys and especially the cadmium levels of those organs are in many cases over the recommended safety limit and it would seem reasonable to recommend that limits should be placed on the consumption of these internal organs. Nevertheless, consumers should be encouraged to eat more reindeer meat since it is known to be a healthy low-fat food containing many vitamins and a high selenium level.

9. Conclusion and remarks

The monitoring program to assess the heavy metal levels in game metal levels in game/farmed game animals was successfully established. Samples were collected in 1980–1999 with the next sampling planned to take place in about 2010. The age determination is an important step in the assessment, and therefore it has been decided to include this aspect also in the future sampling.

The heavy metal emissions from locally sources have been clearly noted to be on the decline. A similar trend is seen also elsewhere in Europe which has led to a decrease in the long-range atmospheric transport of heavy metals to Finland. The overall decrease of lead emissions can be mainly traced to the introduction of low lead gasoline and, since 1993, to the use of totally unleaded gasoline. Locally important emissions sites like lead smelters and a waste incineration plant have made a major contribution to lead emissions. The copper load around the Harjavalta Cu-Ni smelter in south western Finland and chromium load in Kemi-Tornio which has ferrochrome and stainless steel plants in southern Lapland have also declined. The copper and nickel emissions from the Kola Peninsula to north eastern Lapland are also falling.

In Finland, the cadmium levels in moose have increased during the period of 1980–1999 in all age groups. A similar trend has been not found anywhere else in the world. The reason for the increase could not totally be explained with this material, but changes in the acidity of soil, cultivation of forest soil, long-range atmospheric transport, and the underlying geology of the moose's environment and the nutrition of animals could all contribute to the increased cadmium levels found in Finnish moose. The lead levels have decreased during the period of study and the main reason is the use of unleaded gasoline. The copper levels in moose increased from 1980 to 1990 but since then have declined. The copper emissions increased at the end of the 1980s but decreased from the beginning of the 1990s. Copper is often associated with SO₂ emissions and the decrease in SO₂ are also reflected in copper levels in moose. The zinc levels in moose have increased during the period of this study. Possibly, the soil acidity has influenced the mobility of zinc in a similar manner, as this can affect the mobility of cadmium.

In Finland, the lead levels in moose are at same levels as those found in Sweden but cadmium levels are higher than those in Sweden. Cadmium levels are at the same level as in Norwegian samples. In liver and kidney samples of Finnish moose, the cadmium concentrations are higher than those found in red deer in central Europe, but conversely the lead concentrations there are higher there than in Finland.

Cadmium levels in Finnish reindeer are lower than those in moose but lead levels are higher in reindeer samples. The high lead levels are attributed to the lead content of

lichen. In Finland, cadmium and lead levels of reindeer are higher than in Sweden but lower than those in Norway. The copper levels in reindeer liver were higher in northern and eastern Lapland than in samples from other areas probably due to the emissions from Kola Peninsula. But the copper levels were at the same level as in liver samples of Finnish cattle.

No significant changes have occurred in the cadmium levels in Mountain hares, whereas cadmium levels in European hares have decreased. The lead and cadmium levels in liver and kidney of Mountain hares were higher than those detected in the same organs of European hares. The difference probably results from the different diets of these two species. In Finland, the cadmium levels in kidney of Mountain hares were statistically significantly higher than cadmium concentrations in moose kidney. The copper levels in both Mountain and European hares increased slightly during the intervening years (1980-1993) due to the increased copper emission to the atmosphere but the zinc contents have not changed.

In Finland, the cadmium and lead levels in the European hare are lower than those found in central Europe; these regions (southern Poland and the Carpathian part of Romania) appear to be some of the most badly polluted areas of Europe with regard to lead and cadmium.

The levels of lead and cadmium in muscle in moose, reindeer and hares have decreased and today are very low, near to the limit of quantification. In many other studies, the muscle has been contaminated with these heavy metals, for example in Alaska, Russian and Central Europe, where the lead level of the muscle from many carcasses exceeded the legal limit in the EU (0.05mg/kg).

However, in Finland the cadmium levels of moose and reindeer are much lower than those found in north America, (i.e. Canada and Alaska) and are far from toxic levels. The results from North America suggest that some moose there may suffer from sublethal effects of cadmium toxicity. However, it is possible that those moose have evolved a high level of natural cadmium tolerance, either inherited from their parents or by individual induction over their lifetimes. It is possible that moose, as a species, have a high level of cadmium tolerance and therefore they are immune to levels of cadmium which would damage other species.

Because of the increasing trend in cadmium and zinc levels in moose, even though there was no statistically significant correlation between increased zinc and cadmium levels, and because this has not been accompanied by any change in the cadmium levels in Mountain hares, it could be postulated that it is attributable to a change in the acidity of forest soils which is being reflected in the cadmium and zinc levels of these animals.

The meat of Finnish moose, reindeer and hares does not contain residues of cadmium and lead and therefore consumers can be assured that consumption of the meat is not a health risk. However, the consumption of liver and kidneys may represent a health risk because of the elevated lead and especially the high cadmium levels found in these internal organs.

The heavy metal levels are far from the levels which would be toxic to the animals themselves.

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Appendix 1.

Table 2. Physical constants of cadmium, lead, copper and zinc

	Atomic weight	Crystalline form and properties	Density g/cm ³	Melting point °C	Boiling point °C	Atomic number
Cadmium	112.41	Silver-white malleable, metallic, hexagonal,	8.642	320.9	765	48
Lead	207.19	Silver-bluish, white, soft, metallic, cubic	11.344	327.5	1740	82
Copper	63.55	Reddish metallic, cubic	8.92	1083	2567	29
Zinc	65.38	Bluish-white, metallic, hexagonal	7.14	419.6	907	30

Table 3. Cadmium levels (mg/kg w.w.) in moose from various countries

	Muscle	Liver	Kidney
North America	0.03	0.44–11.9	1.68–73.1
Russia	0.11	1.18	4.78
Sweden		0.28–0.583	1.43–2.41
Norway	0.008–0.03	0.3–0.8	0.8–5.13

Table 4. Lead levels (mg/kg w.w.) in moose from various countries

	Muscle	Liver	Kidney
North America		0.002–0.10	0.09
Russia	1.43	1.69	0.54
Sweden		0.03–0.05	0.03–0.06

Table 5. Copper levels (mg/kg w.w.) in moose from various countries

	Muscle	Liver	Kidney
North America		9.80–104	3.47
Russia	3.80	43.5	6.45
Sweden		0.0–30.9	3.43–3.92

Table 6. Zinc levels (mg/kg w.w.) in moose from various countries

	Muscle	Liver	Kidney
North America		30.5–34.9	29.2
Russia	36.8	40.7	28.7
Sweden		26.7–37.0	27.0–31.1
Finland	56.9–62.1	23.7–32.1	30.0–32.4

Table 7. Cadmium, lead, copper and selenium levels (mg/kg w.w.) in livers of moose from Northwest Minnesota in USA (1989–1999) in relation to habitat

	Cadmium	Lead	Copper	Selenium
Agriculture and prairie	0.70	0.01	18.6	0.78
Bog and forest	0.42	0.002	8.64	0.35

Table 8. Cadmium levels (mg/kg w.w.) in caribou/reindeer from various countries

	Muscle	Liver	Kidney
North America		0.50–5.2	1.18–10.8
Greenland caribou reindeer	<0.003	0.121–0.695 0.230–0.426	
Russia (reindeer)	0.014–0.079	0.220–1.73	0.475–3.67
Sweden (reindeer)	0.001–0.003	0.10–0.60	0.45–2.7
Norway (reindeer)		0.7–1.8	2.6–19.8

Table 9. Lead levels (mg/kg w.w.) in caribou/reindeer from various countries

	Muscle	Liver	Kidney
North America	0.03–0.78	0.09–7.76	0.02–10.0
Greenland caribou reindeer	<0.003–0.003	0.027–0.926 0.08–0.67	
Russia (reindeer)	0.09–0.11	0.113–0.9	0.089–0.470
Sweden (reindeer)	0.003–0.013	0.13	0.13

Table 10. Copper levels (mg/kg w.w.) in caribou/reindeer from various countries

	Muscle	Liver	Kidney
North America	2.24–13.8	6.35–100	0.94–28.5
Greenland caribou reindeer	2.69–3.13 2.09–3.60	21.8–57.2 48.8–71.0	
Sweden (reindeer)	1.33	68.2	

Table 11. Zinc levels (mg/kg w.w.) in caribou/reindeer from various countries

	Muscle	Liver	Kidney
Canada (caribou)		62.6–105	2.33–124
Alaska (caribou)	29.2–50.7	22.4–77.3	16.8–44.2
Greenland caribou reindeer	17.5–28.7 25.5–39.6	23.2–29.5 26.2–31.7	
Sweden (reindeer)	80.4	22.6	

Table 12. Cadmium levels (mg/kg w.w.) in red deer from various countries

Region	Muscle	Liver	Kidney
Norway		0.2–0.3	1.0–2.3
Netherlands		0.05–0.260	1.20–6.54
Poland	0.005–0.12	0.039- 0.28	0.98
Slovakia	0.030	0.26- 0.31	2.0–2.39
Spain		0.21	2.2
Croatia			0.099

Table 13. Lead levels (mg/kg w.w.) in red deer from various countries

	Muscle	Liver	Kidney
Netherlands		0.193–0.122	0.449–0.386
Poland	0.09–0.39	0.11–0.70	0.14–0.38
Spain		0.57	0.33
Croatia			0.58
Slovakia	0.35	0.32–1.90	0.48- 0.56

Table 14. Copper levels (mg/kg w.w.) in red deer from various countries

	Muscle	Liver	Kidney
Norway		26	
Netherlands		16.2–25.8	4-4.5
Poland	1.6- 3.5	8.3–15	4.8–7.5
Croatia			5.20

Table 15. Zinc levels (mg/kg w.w.) in red deer from various countries

	Muscle	Liver	Kidney
Netherlands		17.2–32.4	37.3–32.4
Poland	36–40	35–30	26–31
Eastern Croatia			35.1

Table 16. Cadmium and lead levels (mg/kg w.w.) in hares (*Lepus americanus*) in Northern Quebec (Canada)

Cadmium	Muscle	Liver	Kidney
1981–1990	<0.20	0.53	23.8
1991–1997	<0.20	0.37	6.19
Lead			
1981–1990	<0.10	0.22	
1991–1997	<0.10	2.07	

Table 17. Cadmium and lead levels (mg/kg w.w.) in Mountain hares from various countries

Cadmium	Muscle	Liver	Kidney
Russia	0.007–0.018	0.150–0.520	0.830–3.34
Faroe Islands		0.09–0.455	
Lead			
Russia	<0.005–0.059	0.074–0.204	0.039–0.093
Norway		0.14 (2-8 months) 0.29 (>14 months)	

Table 18. Cadmium levels (mg/kg w.w.) in European hares from various countries.

	Muscle	Liver	Kidney
Slovakia	0.03	0.14- 0.31	1.43–2.01
Czech and Slovakia Rebublic		0.26–0.77	3.13–3.61
Poland	0.01	0.45	4.77

Table 19. Lead levels (mg/kg w.w.) in European hares from various countries

	Muscle	Liver	Kidney
Slovakia	0.35	0.184- 0.32	0.083- 0.48
Czech and Slovakia Rebublic		0.09–0.69	0.22–1.43
Poland	0.22	1.06	0.65

Table 20. Copper and zinc levels (mg/kg w.w.) in European hares in Poland

	Muscle	liver	kidney
Copper	1.54	3.97	3.85
Zinc	25.9	31.3	26.5

Table 21. Cadmium, lead, copper and zinc levels (mg/kg w.w.) in muscle, liver and kidney in Finnish moose

Cadmium	Muscle	Liver	Kidney
1980–1981	0.005–0.012	0.45–0.76	3.01–4.43
1990	0.003–0.009	0.69–0.85	3.08–5.25
1999	0.003–0.004	0.71–1.28	4.95–6.18
Lead			
1980–1981	0.05–0.07	0.21–0.25	0.36–0.53
1990	<0.01–0.03	0.05–0.07	0.07–0.08
1999	0.02–0.03	0.04–0.07	0.05–0.07
Copper			
1980–1981	0.92–1.3	26.2–38.9	2.81–3.05
1990	1.03–1.13	46.0–54.4	3.53–3.73
1999	0.97–1.07	33.0–50.5	3.57–3.80
Zinc			
1980–1981	33.8–34.3	17.5–20.1	18.9–22.2
1990	53.9–54.4	22.0–26.4	27.5–28.5
1999	56.9–62.1	23.7–32.1	30.0–32.4

Table 22. Cadmium, lead and copper levels (mg/kg w.w.) in muscle, liver and kidney in Finnish reindeer

Cadmium	Muscle	Liver	Kidney
1990–1992	<0.001–0.003	0.19–0.39 (calves) 0.40–0.96 (adult)	0.525–1.22 1.72–4.62
Lead			
1990–1992	<0.01–0.02	0.13–0.36 (calves) 0.16–0.43 (adult)	0.15–0.33 0.27–0.34
Copper			
1990–1992	1.65–1.92 (calves) 1.63–1.91 (adult)	29.2–82.2 (calves) 29.7–71.0 (adult)	4.02–5.07 (calves) 4.14–5.41 (adult)

Table 23. Cadmium, lead, copper and zinc levels (mg/kg w.w.) in muscle, liver and kidney in Finnish Mountain hares and European hares.

	Muscle		Liver		Kidney	
	Industrial Finland	Northern Finland	Industrial Finland	Northern Finland	Industrial Finland	Northern Finland
Cadmium						
Mountain hares						
1980–1982	0.014	0.010	0.485	0.390	11.1	4.55
1992–1993	0.006	0.005	0.445	0.185	10.7	3.73
European hares						
1980–1982	0.008	0.003	0.330	0.171	3.83	1.46
1992–1993	0.003	0.001	0.160	0.057	1.91	0.627
Lead						
Mountain hares						
1980–1982	0.13	0.09	0.59	0.59	0.91	0.71
1992–1993	0.07	0.04	0.29	0.23	0.63	0.21
European hares						
1980–1982	0.13	0.08	1.01	0.47	0.90	0.76
1992–1993	0.05	0.01	0.17	0.06	0.19	0.05
Copper						
Mountain hares						
1980–1982	1.78	1.68	3.61	4.02	3.86	3.93
1992–1993	2.89	2.70	4.71	4.17	4.36	4.14
European hares						
1980–1982	1.74	1.95	4.61	4.64	4.21	3.76
1992–1993	2.93	2.64	5.15	5.32	4.49	4.64
Zinc						
Mountain hares						
1980–1982	26.8	20.8	34.9	45.0	35.8	34.7
1992–1993	25.5	28.3	31.7	31.4	34.8	27.5
European hares						
1980–1982	22.8	23.7	46.5	38.6	35.6	30.5
1992–1993	23.9	33.5	37.1	42.8	28.8	28.9