Biogeochemistry and metal biology

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Abstract: Environmental monitoring of metals on two trophic levels is presented: a biogeochemical technique developed at the Geological Survey of Sweden, using aquatic mosses and roots of aquatic plants, and a bioanalytical–chemical technique developed at the National Veterinary Institute based on organ tissues from the moose (*Alces alces* L.), a wild ruminant living in the Swedish forests.

The usefulness of the techniques is exemplified by monitoring of Cd in southern Sweden. The results of both monitoring systems are in close agreement. Together with analysis of crops and drinking water the results indicate a region with elevated Cd burden. Also the changing environment is monitored by the moose. Decreasing concentrations of essential and toxic metals (cations) and increasing molybdenum concentration (anion) were found in a strongly acidified region of Sweden by comparison with a reference material from 1982. pH increase of the environment of the moose is indicated, probably by liming. It resulted in severe copper and chromium deficiency of the moose and was suggested as the cause of a 'mysterious' disease in the moose.

Comparison of the techniques confirms the advantage of using metal-monitoring maps in interpreting biological data and also the predictive value of the monitoring maps in biological contexts.

The Centre for Metal Biology was established in 1993 on the initiative of Swedish politicians. The members of the Centre are Uppsala University, the Swedish University of Agricultural Sciences, the Geological Survey of Sweden, the National Veterinary Institute, the University Hospital at Uppsala and the municipality of Uppsala. The Centre was created as a scientific platform with the important tasks of performing research and informing various target groups such as politicians, physicians, veterinarians, teachers and students about the present state of knowledge in the field of heavy metals and diseases related to them in humans and animals.

In order to solve intricate biological/environmental problems in modern society, and to work towards explaining the interactions between metals, scientists working in different fields of biology, toxicology, nutrition and health, were brought together to facilitate interdisciplinary co-operation. The broad spectrum of participants, representing different scientific fields, enables the Centre to cover knowledge and research in many areas with impact on human and animal health. The Centre is expected to provide guidelines and to propose studies with environmental impact, including suggestions on solving, eliminating or counteracting problems identified. Such measures could be exemplified by detoxifying agents and agents for large-scale metal elimination from, for example, industrial waste water.

A model problem which was identified at an early stage was the study of the possible adverse effects of mercury from dental amalgams. Approaches to shed light upon this problem have been proposed and tried by physicians at the Amalgam Unit at the University Hospital. Knowledge emanating from the toxicological research within the centre proved to be of great value in this case and is an example of interdisciplinary co-operation.

Through the Centre for Metal Biology opportunities for co-operation between disciplines are thus greatly increased. The present paper is a result of such an interdisciplinary co-operation between the Geological Survey of Sweden and the National Veterinary Institute.

Environmental monitoring

Introduction

Contamination of the environment with hazardous compounds and elements of anthropogenic origin is of increasing concern because of the

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effect on the whole biosphere, i.e. the micro-flora and -fauna of soils, plants and higher life, including humans and animals. Use of different monitors at various trophic levels has been suggested to collect relevant information. Biological monitoring should be designed to obtain and make use of the optimum amount of available information by complementing existing environmental studies, or through the simultaneous collection of other environmental data (Wren 1983). Monitors have recently been defined as organisms in which changes in known characteristics can be measured to assess the extent of environmental contamination so that conclusions on the health implication for other species of the environment as a whole can be drawn (O'Brien *et al.* 1993). Monitors may provide information about the environmental concentration of essential as well as toxic metals of importance for life, displaying deficiency and toxicity, respectively.

Some elements may derive from environmental pollution while some enrichments may be natural. The metals may affect life on different trophic levels. Essential and toxic elements in bedrock or soils may become a direct risk for human and animal health and may be the underlying cause of both deficiency and toxicity (Crounce *et al.* 1983; Låg 1990, 1991). Aerial deposition of sulphuric and nitric oxides (acid rain) may influence weathering of bedrock and soils. The concentration of metals in upper soil layers may change as a result of mobilization and the metals become more available via plants to grazing animals. Elements that are easily mobilized are Ca, Mg, Mn, Al, Ni, Zn and Cd, and to a lesser extent Hg, Pb and Cu. When the buffering capacity of the soil is insufficient, acid rain may cause deficiencies in plants and via plants in herbivorous animals as a result of leaching and eluting of metals essential to plants and animals. Certain essential trace elements, such as Se and Mo, become less soluble in acidic environment and their availability to plants decreases. Changes in the uptake via plants may result in changed metal concentrations as well as changed relationships between metal concentrations in organ tissues, with severe consequences for grazing animals.

Cadmium

Cadmium found in soils, waters, plants and other environmental matrices not affected by pollution, can be regarded as natural. There is little difference among the igneous rocks in Cd content while among sedimentary rocks, the carbonaceous shales, formed under reducing conditions, contain the most Cd. The Zn/Cd ratio in terrestrial rocks is about 250 (Thornton 1986). In non-contaminated, non-cultivated soils, Cd concentration is largely governed by the amount of Cd in the parent material (Purves 1985; Adriano 1986). In an extensive survey of Swedish soils Andersson (1977) found an average of 0.22 mg Cd $\mathrm{kg^{-1}}$ (0.03–2.3 mg/kg range) for both cultivated and non-cultivated Swedish soils. The soil geochemical mapping carried out by the Geological Survey of Sweden so far has analysed 10 000 samples from all parts of Sweden. The median value of these samples is 0.1 mg Cd $\mathrm{kg^{-1}}$ in natural unweathered podzolic soils, while the 90th percentile is *c.* 0.4 mg $\mathrm{kg^{-1}}$, and the maximum value is 6.4 mg $\mathrm{kg^{-1}}$ (M. Andersson, pers. comm.).

Cd is readily taken up by roots and is distributed throughout the plants. The uptake in plants can be both active and passive (Kabata-Pendias & Pendias 1992), however, these aspects will not be discussed in the present context. The amount of uptake is influenced by soil factors such as pH, cation exchange capacity, redox potential, phosphatic fertilization, organic matter, other metals and other factors. In general, there is a positive, almost linear correlation between the different Cd concentrations in the substrate and the resulting Cd concentration in the plant tissues (Adriano 1986).

In a *Filipendula ulmaria* meadow ecosystem Balsberg (1982) found that water solutions of different Cd concentrations added to the soil, $< 10\%$ of the total Cd in the ecosystem was retained in the plant biomass. Root concentrations exceeded those in the soil and were several times the concentration in above ground organs. The Cd concentration in various plant parts decreased in the order: new roots > old roots > rhizomes > stem leaf-stalks > stem leaf-blades > reproductive organs.

In the present paper two examples are given of monitoring metals, in this case especially cadmium, in the environment by use of (i) aquatic mosses and the roots of aquatic higher plants, a technique developed at the Geological Survey of Sweden; and (ii) organ tissues of the moose (*Alces alces* L.), a wild ruminant used for monitoring by the Chemistry Department of the National Veterinary Institute since 1980.

Aquatic roots and mosses as environmental monitors

In order to delineate the geochemical distribution of metals, the Geological Survey of Sweden started a nationwide mapping programme in 1980. The purpose of the programme is to compile a geochemical atlas of the entire country. The maps give a general outline of the distribution for heavy metals in the surficial environment. A new method is used, whereby metal concentrations are determined in organic material consisting of aquatic mosses and roots of aquatic higher plants. These are barrier-free with respect to trace metal uptake and reflect the metal concentrations in stream water (Brundin *et al.* 1987).

Aerial parts of many plant species do not generally respond to increasing metal concentrations in the growth medium because of physiological barriers between roots and above ground parts of plants. These barriers protect them from uptake of toxic levels of metals into the vital reproductive organs (Kabata-Pendias & Pendias 1992; Kovalevsky 1987). The roots and mosses respond closely to chemical variations in background levels related to different bedrock types in addition to effects of pollution (Brundin & Nairis 1972; Brundin *et al.* 1988; Selinus 1988, 1989; Nilsson & Selinus 1991). Variation of uptake with growing season and between plant species is of concern only for above earth parts of plants but not for the roots (Brundin *et al.* 1988).

Due to chemical weathering processes, the metal concentrations in the stream water reflect the chemical composition of the surrounding bedrock and soils. When the groundwater reaches surface waters some of the metals may precipitate. Enrichment takes place both in the roots and in iron- and manganese hydroxides. The exchange of metals between the water and the roots is a slow process whereby the influence from seasonal variations is of minor importance. One great advantage of using biogeochemical samples instead of water samples is that the biogeochemical samples provide integrated information of the metal contents in the water for a period of some years. Water samples suffer from seasonal and annual variations depending on, for example, precipitation. One great advantage of using biogeochemical samples instead of water samples is that the biogeochemical samples provide integrated information of the metal contents in the water for a period of some years. The biogeochemical samples also provide information on the time-related *bioavailable* metal contents in aquatic plants.

All sampling points are chosen in such a way that they each represent a drainage area (one sample every 6 km^2). All samples are analysed after ashing by X-ray fluorescence (XRF) for Al, As, Ba, Ca, Co, Cr, Cu, K, Mg, Mo, Nb, Na, Ni, P, Pb, Rb, S, Si, Sr, Ti, U, V, W, Y, Zn, Zr. Every fifth sample is also analysed by Atomic Absorption (AAS) for Hg, Se and Cd. After analysing the samples, all analytical results are normalized with respect to organic content and limonite content (Fe and Mn) using stepwise regression analysis (Selinus 1983).

The biogeochemical mapping programme now (1996) covers about 50% of the land area of Sweden (30 000 sample points), where about 75% of the population of Sweden lives. This means that the Geological Survey now has an extensive database of analyses for use in environmental and geomedical research. The samples are related to the periods in which they are sampled, which means that resampling and follow-up research will render invaluable monitoring information in the future. An existing sample bank with all biogeochemical samples could also be used for future environmental research. The geochemical mapping programme also includes geochemical soil surveys and bedrock surveys. The soil surveys provide information on the regional variation of major, minor and trace element distributions in the unaffected part of the till cover of the country. The bedrock surveys are used for separating the natural background of the metals from anthropogenic sources by means of multivariate statistical methods (Selinus & Esbensen 1995).

Cadmium levels in biogeochemical samples (roots and aquatic mosses) from southern Sweden are shown in Fig. 1 (Ressar & Ohlsson 1985). The contents are enhanced in the southernmost counties and along the west coast of Sweden. The latter distribution is derived mainly from transboundary atmospheric transport and deposition of anthropogenic origin. The contents of Cd in the southernmost part are, however, much higher, and the highest levels so far detected by the Geological Survey since 1982 are located in this region. This region is a densely populated farming region from which growing crops are distributed to the rest of Sweden. The important source of uptake of Cd for the non-smoking Swedish population is cereals. Therefore, a sampling programme of wheat in the affected areas was performed by the farmers' organizations after contact with the Geological Survey.

In 1989 samples of autumn wheat were taken in certain geographical regions, and in the Skåne region 54 samples were taken. The results showed that samples from Skåne had an average of 73 μg Cd kg^{-1} d.w., with several areas exceeding 100 μg Cd kg^{-1} d.w. In comparison, an area in central Sweden yielded on analysis only 29 μg Cd kg^{-1} d.w. on average in autumn wheat

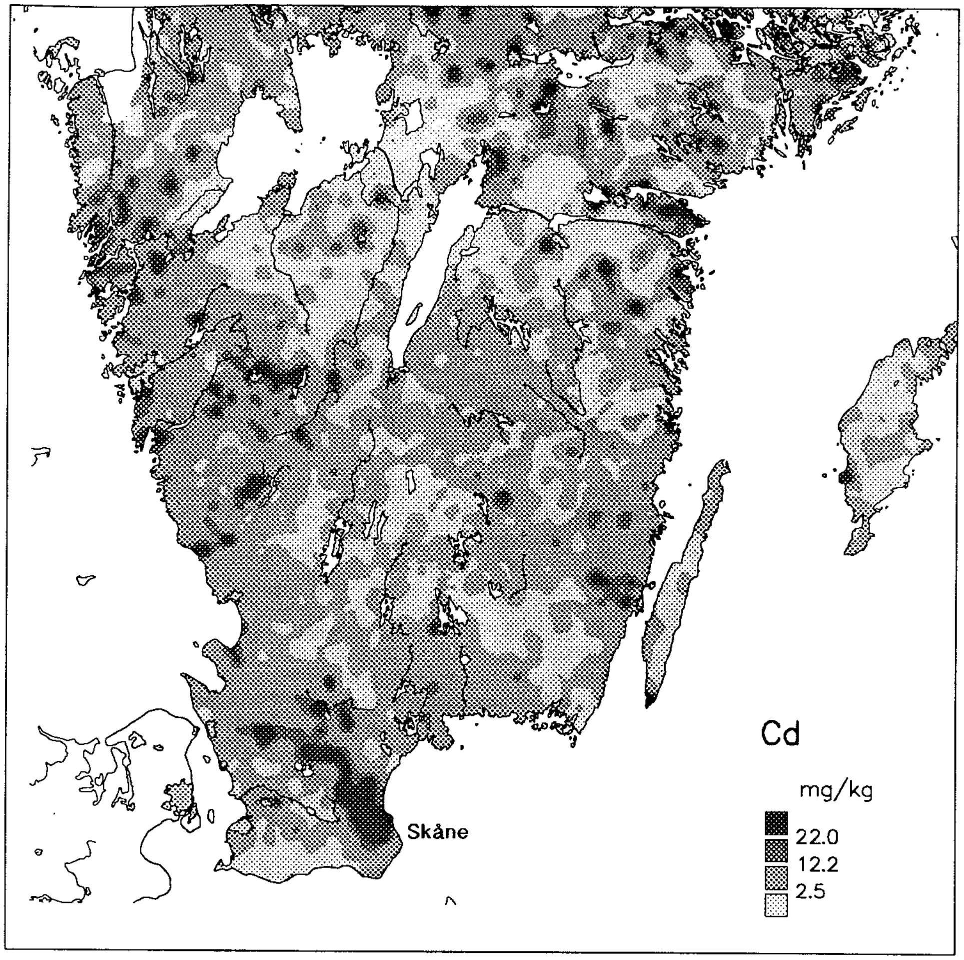
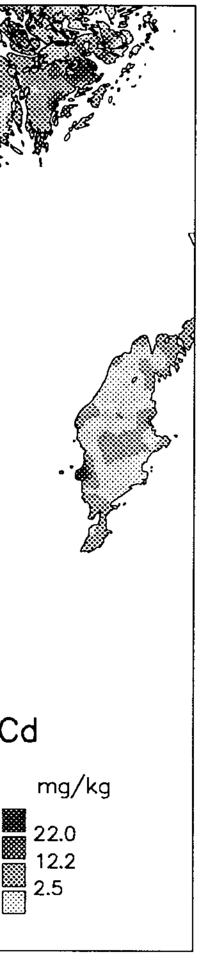


Fig. 1. Cadmium in biogeochemical samples (roots and mosses) from southern Sweden.

(A. Jonsson, pers. comm.). Japan is known to have the highest cadmium burden in the world, especially in home produced rice. It is noteworthy that the concentration of cadmium in polished rice was found to be 30–300 $\mu g\,kg^{-1}$, and an average value from 200 samples of polished rice was 66 $\mu g\,kg^{-1}$ w.w. (Friberg *et al.* 1974). The Cd contents in wheat from Skåne could therefore be a matter for concern. In this region drinking water is taken from many wells. In those that have been analysed for Cd, the results show an almost identical distribution of high Cd contents as depicted in the biogeochemical map. For drinking water, the WHO has set a limit of 5 $\mu g\,Cd\,l^{-1}$ (WHO 1984). In comparison, the wells in Skåne, within the region with high Cd burden, have average levels of about 400 $\mu g\,Cd\,l^{-1}$.

Several factors may interact with each other and contribute to the high Cd concentrations found in this region, for example, deposition of airborne Cd as well as acid rain from Eastern, Western and Central Europe. Cadmium may also originate from local sources, for example, from phosphate fertilizers used in agriculture or sewage sludge, or high contents of natural Cd derived from the sedimentary bedrock in Skåne. Research is now being carried out in order to investigate combinations of these factors.

The concurring results from biogeochemistry (Fig. 1) and analyses of crops and drinking water indicate, thus, a region of pronounced Cd contents. Similar results were found when monitoring was performed by analysing samples from a wild herbivorous ruminant, the moose.



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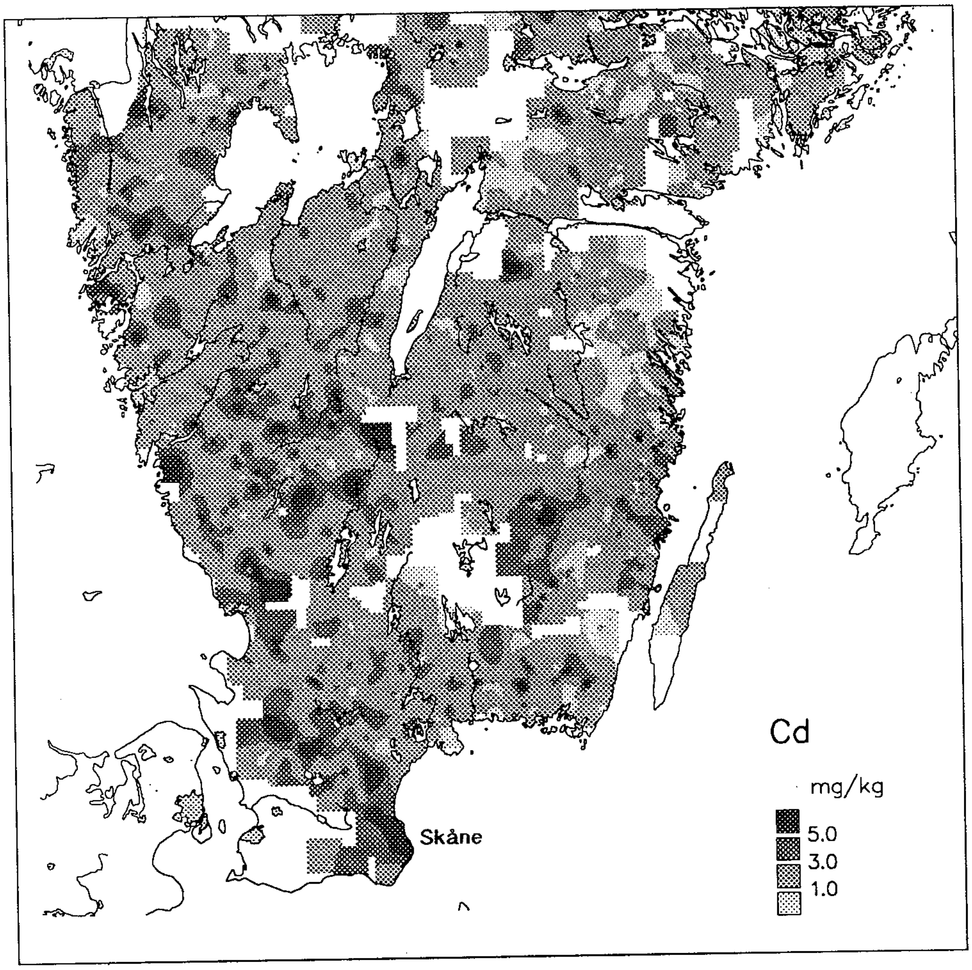

Fig. 2. Average yearly cadmium uptake in moose kidney in southern Sweden expressed in mg Cd kg^{-1} w.w.

The moose (*Alces alces* L.) as an environmental monitor

Cadmium in organ tissues of the moose

In the search for monitors among the wild Swedish fauna during 1973–1976 that could be used for cadmium, the moose (*Alces alces* L.) was found to be useful (Frank 1986). This large wild ruminant is found in most parts of Sweden. The moose is relatively sedentary, the range of migration within the territory seldom exceeds 50–80 km to some extent also depending on the density of the population. Tissues may be obtained through co-operation with the hunters' organization during regular hunting seasons in the autumn. Regional differences in cadmium burden were demonstrated by Frank *et al.* (1981). By far the highest burden was found in the region of Skåne (Mattsson *et al.* 1981; Frank 1982).

Prior to the hunting seasons in 1981 and 1982, standardized packages for sample collection were distributed to the hunters. All but one of the 24 counties of Sweden participated in sample collection. Tissues of liver and kidney, as well as the left mandible (for age determination by cutting the first molar teeth and microscopic examination) were collected from more than 4300 animals (Frank 1989). The samples were pretreated by automatic wet digestion (Frank 1976, 1988). Simultaneous analysis of 13 metals using a direct current plasma-atomic emission

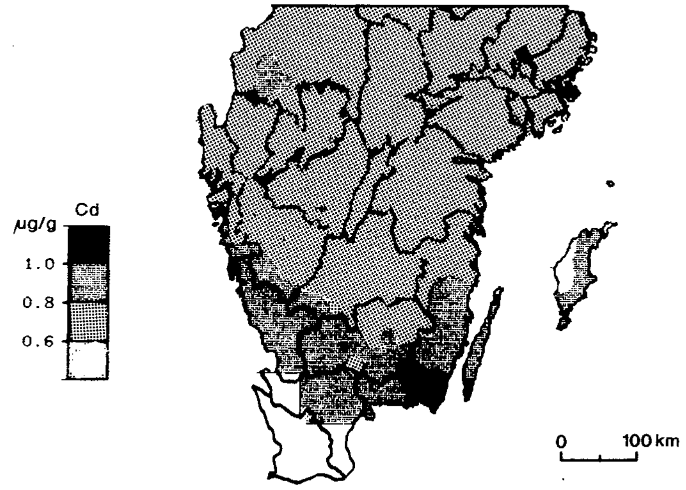

Fig. 3. Cadmium content (µg/g d.w.) in terrestrial moss samples collected in 1975. Adapted from Rühling & Skärby 1979. (The white area in southernmost Sweden (Skåne) is not sampled.)

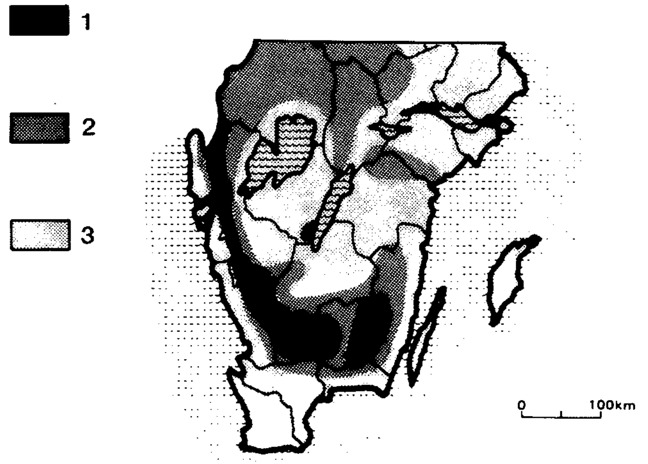

Fig. 4. pH values in the lakes of Sweden in the late 1970s. (1) pH < 5.0 all the year in at least 33% of all lakes; (2) pH < 5.5 some time during the year in at least 50% of all lakes; (3) less than 50% of all lakes acidified. (Adapted from Monitor 1981, Swedish Environmental Protection Agency.)

spectrometer (DCP-AES) was applied in determination of the tissue metal concentrations (Frank & Petersson 1983). The metals determined were Al, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, V, and Zn.

At the time of sample collection, the main interest was focused on determination of the cadmium burden in the different geographical regions of Sweden. In addition, the concentrations of essential elements in liver and kidney gave increased knowledge of normal variations in the different regions, which is important information from a nutritional point of view. All these values represent *time-related reference values for moose in 1982*. Future investigations compared with reference values of the same geographical region would display changes in the environment.

The renal cadmium accumulation is age dependent. To some extent, the rate of accumulation depends on the total amount of cadmium present in the environment. However, influencing factors are the geochemical background, atmospheric transport and deposition of particulate matter, oxides of sulphur and nitrogen, acid rain, anthropogenic activities etc. – all seem to contribute to the flux of cadmium in the biosphere. Acidification mobilizes cadmium, and its availability for uptake via plants in herbivorous animals increases. Early investigations demonstrated very high Cd burdens in the south and southwest of the country. Once again, the highest values were found in the two counties making up the most southern province of Sweden (Frank & Petersson 1984; Frank *et al.* 1984).

A number of statistical models were tested to express the cadmium burden of a population of animals. Eventually a statistical model allowing comparison of the cadmium burden of different regions was developed and found most useful (Eriksson *et al.* 1990). It is presented in an abbreviated form as follows. A quotient is formed by dividing the cadmium concentration of the respective organs with the estimated age of the animal. From these quotients, median values are calculated for each age class and region, and the age classes are weighted for age distribution. The resulting constant is characteristic for the region in question and is the average yearly cadmium uptake in liver and kidney respectively at the time of sample collection. Using this model, the cadmium burdens for all the investigated counties were calculated.

A shaded map expressing the cadmium burden of southern Sweden is shown in Fig. 2. When this cadmium map was compared with the map of atmospheric deposition of cadmium, measured by moss analysis (Rühling & Skärby 1979) (Fig. 3), with the bedrock geological map and the acidification map of southern Sweden (Fig. 4), the best agreement was achieved by comparison with the acidification map. Comparison of Fig. 2 with the biogeochemical map based on analysis of roots of water plants (Fig. 1) shows close agreement in the region of Skåne.

Secondary copper deficiency, chromium deficiency and an earlier unknown disease of the moose

In connection with a new type of moose disease of unknown etiology in southwestern Sweden (Steen *et al.* 1989), reexamination of tissue metal concentrations was performed in 1988 and 1992. Decreased hepatic Cu concentrations in 1988 and 1992 (30% and 50% respectively) were observed in comparison with the corresponding value in 1982 (Frank *et al.* 1994). Decrease of other essential and toxic metal concentrations was noted in the liver such as Cr (80%), Fe, Zn, Pb and Cd. In the kidney, decreases of Ca, Mg, Mn indicate severe metabolic disturbances. The Cd concentration in the kidney decreased by about 30%.

Decreasing metal concentrations in organ tissues of the moose, after such a short period of 5–6 years, indicate changes in the concentration of metals via plants in the upper soil surface layer. Thus, the moose appears to be a sensitive monitor of environmental changes. In ruminants, the utilization and availability of Cu in feed is greatly influenced by interactions between Cu, Mo and S (Mills & Davis 1987). Increased Mo concentrations in respect to Cu in the feed causes secondary copper deficiency in ruminants with clinical signs in close agreement with the signs related to the unknown moose disease.

When liver from yearlings, collected in 1982 and 1992, was analyzed for Mo, increased hepatic Mo concentrations were found in northern and southern districts of Älvsborg County (24% and 21%, respectively) during the 10 years between sample collections (Frank *et al.* 1994).

When pH in soils decreases, Mo becomes less available to plants and its uptake diminishes. Nonetheless, the increased Mo uptake by plants in the strongly acidified region is difficult to explain. A small pH increase in the environment causes a marked increase of Mo uptake by plants (Mills & Davis 1987). Thus, the results evidently indicate elevated pH in the soils. The intensive liming of lakes, wetlands, fields and pastures in the western part of Sweden during the later 1980s, and to some extent the liming of forest areas during recent years, are suggested as possible explanations of increased hepatic Mo concentrations in this exceptionally acidified region. This side effect of liming and its harmful consequences for domestic and wild ruminants appear to have been overlooked.

A map showing the ratio of Cu/Mo was recently constructed on the basis of biogeochemical data provided by the Geological Survey of Sweden (to be published). This map demonstrates the risk areas of secondary copper deficiency as well as of copper toxicity in ruminants. The area of the former and the region for the highest prevalance of the unknown moose disease appear to coincide.

Conclusions

Two monitoring techniques were compared. Both techniques map the bioavailabilty of elements in the environment, however, on different trophic levels. The map based on roots of aquatic plants and aquatic mosses, and those of using organ tissues from the moose, a wild ruminant, appear to be remarkably similar, in the vicinity of Skåne, where a region of elevated Cd burden is identified.

The two examples given in the paper elucidate the usefulness of the mapping techniques in monitoring and detecting toxic metal burdens of regions (biospheres) as well as low prevalence of essential elements.

The maps, especially the detailed maps of biogeochemical data comprising about 30 elements, contribute to and facilitate interpretation of biological data and have predictive value in biological contexts. In addition, the moose seems to be a reliable monitor to detect rapid changes in the environment.

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