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Heavy Metals in Tissues of Hares in Finland, 1980-82 and1992-93

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The anthropogenic load of trace metals from industrial emissions to the atmosphere has significantly increased the aerial deposition of lead, cadmium and zinc and to a lesser extent of copper and nickel during the twentieth century. This is particularly true in southern Finland, where atmospheric deposition, concentrations in water and accumulation in sediments of these metals all exceed those in central and northern Finland. The deposition of trace metals in Finland occurs simultaneously with that of acidic compounds (Verta et al. 1990). Several industrial processes including combustion of fossil fuels, mining, metal processing and as well as refuse incineration contribute to these emissions, which are mainly concentrated in intensively populated industrial areas. Airborne pollutants are transported over long distances and can be detected in remote areas in amounts that greatly exceed the natural background level (Johnson 1987). Long-range transport is the primary source of lead, zinc and cadmium in Finland, but local sources are more important in the deposition of copper and nickel (Verta 1989).

A major investigation of lead, cadmium, copper and zinc was performed at the National Veterinary Institute in 1980-1982. The study included 212 mountain hares (Lepua timidus) and 188 European hares (Lepus europaeus) in 15 game management areas. The results of this investigation have not been published earlier. It was decided in the autumn of 1992 to continue the investigation of hares by taking samples in four game management areas similiar to those of the earlier investigation. The areas were divided so that the game management areas of south-eastern, south-western and southern Finland together comprised the industrial area of Finland. The game management area of Oulu on the other hand represented northern Finland

The aim of the present study was to compare possible area1 differences and to follow the changes in heavy metal concentrations in hares during a period of about ten years.
The environment and diet of mountain hares and European hares often differ clearly from each other, and therefore the investigation provided information about the concentrations of heavy metals both in forests, near cultivated lands and in population centres. The mountain hare can take advantage of different diets at different times. In the summertime it eats mainly grass and the leaves of bushes. In the autumn and in the spring before the beginning of the growing season it eats the twigs of bilberries.Willow and birch bark and twigs are the most important nourishment for mountain hares in the wintertime. The European hare eats considerably less plants with woody stems than the

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mountain hare. It finds nourishment from cultivated land, eating mainly hay and grass. In winter it is able to obtain nourishment by digging under the snowcover. It also resorts to cultivated gardens when other dietary possibilities are exhausted.

MATERIALS AND METHODS

Fifty-one mountain hares and 31 European hares were shot between October 1992 and April 1993 in the game management areas as described in the introduction. Heavy metals were analyzed from muscle, liver and kidney and age was determined from the radius and the ulna. The samples were packed separately, frozen and sent to the laboratory in temperature-controlled containers containing coolant canisters frozen before mailing. The samples were stored at -18°C until analysis.

Samples were dry-ashed and acid-leached as described by Niemi et al. (1993). Determinations of lead, cadmium, chromium and nickel were performed by graphite furnace atomic absorption spectrometry (Perkin-Elmer 5100 PC AAS + Zeeman furnace module + Furnace cooling System + Autosampler AS-70) using pyrolytic THGA-tubes (= transverse heated graphite atomizer tubes). Lead was measured at 283.3nm, cadmium at 228.8nm chromium at 357.9nm and nickel at 232.0nm with hollow cathode lamps. For the determination of lead a matrix-modifier was used (0.2% $Mg(NO_3)_2$ + 4% $NH_4H_2PO_4$/0.1M HNO_3). Copper and zinc were measured by flame absorption spectrometry (Perkin-Elmer 5100 PC AAS) with an air acetylene flame using hollow cathode lamps. Copper was measured at 324.8nm and zinc at 213.9nm. The determinations were carried out using direct comparison with standard solutions in O.1M nitric acid. Standard solutions were made from Titrisol ampoules (Merck, Pb Art 9969, Cd Art. 9960, Cr Art.9948, Ni Art.9989, Cu Art.9987, Zn Art.9953).

The age determinations were performed by investigating the ossification stage of the radius and ulna. The radius and ulna were cleaned and radiographs were taken. The hares were categorized 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).

The methods used have been accredited by the Centre of Metrology and Accreditation of Finland. The laboratory fulfills the requirements of the following standards: SFS-EN 45001 and ISO/IEC Guide 25.

Standard reference materials were analyzed in duplicate together with each sample series. The analysis results of standard reference materials are shown in Table 1. Blank samples were also analyzed with each sample series to check the contamination. Calibration of all the metal analyses was continuously checked using standard samples in different concentrations. The recoveries of metals were studied by adding known amounts of standard solution to samples. The amounts added were selected so that they would be close to the amounts normally found in meat, liver and kidney. The recoveries in different sample materials were 82-90% in liver and 99-114% in kidney for lead; 80-100% in muscle, 84-85% in liver and 94% in kidney for cadmium; 92-94% in muscle, 89-98% in liver and 81-99% in kidney for chromium; 89-92% in muscle, 79-86% in liver and 82-104% in kidney for nickel; 109-112% in muscle, 105-106% in liver and 103-106% in kidney for copper; 104-105% in muscle, 102-106% in liver and 104-107%

in kidney for zinc. The limits of determination (mean of 20 blanks ± three times the standard deviation of blanks) were as follows: Pb 0.01, Cd 0.001, Cr 0.01, Ni 0.02, Cu 0.2 and Zn 0.2mg/kg wet weight.

Table 1. Results of the analyses of standard reference materials. Metal concentrations are expressed as mg/kg wet weight (No. = number of samples).

Metal	Standard Reference Material	No.	Our results mean ± SD	Certified value ± uncertainty
Lead	BCR 184 bovine muscle	19	0.245 ± 0.025	0.239 ± 0.011
	BCR 185 bovine liver	22	0.497 ± 0.039	0.501 ± 0.027
	BCR 186 pig kidney	10	0.337 ± 0.054	0.306 ± 0.011
Cadmium	BCR 184 bovine muscle	26	0.019 ± 0.003	0.013 ± 0.002
	BCR 185 bovine liver	37	0.291 ± 0.026	0.298 ± 0.025
	BCR 186 pig kidney	20	2.72 ± 0.17	2.71 ± 0.15
Copper	BCR 184 bovine muscle	14	2.39 ± 0.18	2.36 ± 0.06
	BCR 185 bovine liver	18	192 ± 11.0	189 ± 4
	BCR 186 pig kidney	10	32.1 ± 0.86	31.9 ± 0.40
Zinc	BCR 184 bovine muscle	10	151 ±13.5	166 ± 3
	BCR 185 bovine liver	20	142 ± 8	142 ± 8
	BCR 186 pig kidney	8	123 ± 11	123 ± 3

RESULTS AND DISCUSSION

The lead concentrations in muscle, liver and kidney are shown in Table 2.
The lead contents of hares were clearly lower in 1992-1993 than in 1980-1982. This can be at least partly a consequence of the fact that the consumption of unleaded petrol has increased during the intervening period. When the lead contents of mountain hares and European hares in 1980-1982 were compared no differences were observed. However in the present study the lead contents of European hares were lower than those of mountain hares.

Lead contents in muscle were higher than those in muscle samples of Finnish elks and reindeers (Niemi et al. 1993 and Rintala et al. 1995), which were near the limit of detection, 0.01mg/kg. Some muscle samples of hares contained very high lead concentrations, 8-10mg/kg, probably due to the fact that the samples were taken from near the shooting wound. These samples were not included in this study.

The average lead contents in liver and kidney of hares were below 0.5mg/kg, which is the recommended maximum for liver and kidney of pigs and cattle (The Contaminant Group subordinated to the Nordic Council of Ministers 1991). An exception was formed by the kidneys of mountain hares from industrial Finland, which had an average lead content of 0.63mg/kg. The lead contents in liver and kidney of hares were at same level as or higher than those in elks. The lead contents of mountain hares were at same level as those in adult reindeer but in European hares the contents were at the same level as reindeer calves.

Table 2. The lead concentrations (mg/kg wet wt) of muscle, liver and kidney in mountain hares and European hares in industrial Finland and northern Finland (n = number of samples, x = mean, SD = standard deviation).

Lead	Industrial Finland			Northern Finland		
	n	x	SD	n	x	SD
Mountain hares						
1980-1982:						
muscle	28	0.13	0.12	7	0.09	0.05
liver	65	0.59	0.47	35	0.59	0.34
kidney	65	0.91	0.74	35	0.71	0.60
1992-1993:						
muscle	32	0.07	0.08	4	0.04	0.01
liver	43	0.29	0.22	8	0.23	0.29
kidney	43	0.63	0.40	7	0.21	0.06
European hares						
1980-1982:						
muscle	31	0.13	0.12	2	0.08	0.05
liver	86	1.01	0.86	14	0.47	0.14
kidney	88	0.90	0.73	15	0.76	0.73
1992-1993:						
muscle	26	0.05	0.04	3	0.01	0.01
liver	28	0.17	0.13	3	0.06	0.01
kidney	28	0.19	0.21	3	0.05	0.01

The cadmium concentrations in muscle, liver and kidney are shown in Table 3. The cadmium contents of mountain hares showed no clear differences during a period of over ten years, whereas the cadmium contents of European hares decreased. The cadmium concentrations of European hares were lower than those of mountain hares. The concentrations in northern Finland were lower than those in industrial southern Finland. Cadmium reaches the environment by industrial release from concentration and refining processes of metals and from fertilizers and wastes. Half of the total Finnish emissions of cadmium and copper originate from south-western Finland in the vicinity of non-ferrous metal industry in Harjavalta and Kokkola (Jussila et al. 1991, Jokinen et al. 1991). Cadmium deposition coming from foreign sources represents the major part of the total fallout in Finland (Louekari et al. 1991).

The cadmium contents in muscle in both mountain hares and European hares are low and similar to the cadmium concentrations in Finnish pigs and cattle (Niemi et al. 1991) and elks and reindeers (Niemi et al.1993 and Rintala et al. 1995). The cadmium content in muscle was near the limit of detection, 0.001mg/kg wet weight.

The cadmium contents in liver were below 0.5mg/kg, which is the proposed for maximum cadmium concentration in liver of pigs and cattle (The Contaminant Group subordinated to the Nordic Council of Ministers 1991). Concentrations were lower than in the livers of Finnish elks and were at the same level as in the livers of Finnish reindeer calves. The cadmium contents in kidney were rather high, over the proposed limit of 1mg/kg. In southern Finland the cadmium contents of mountain hares were higher than in the kidneys of Finnish elks and reindeers. In northern Finland the

Table 3. The cadmium concentrations (mg/kg wet wt) of muscle, liver and kidney in mountain hares and European hares in industrial Finland and northern Finland (n = number of samples, x = mean, SD = standard deviation).

Cadmium	Industrial Finland			Northern Finland		
	n	x	SD	n	x	SD
Mountain hares						
1980-1982:						
muscle	36	0.014	0.010	7	0.010	0.007
liver	65	0.485	0.341	36	0.390	0.300
kidney	65	11.1	12.8	35	4.55	4.04
1992-1993:						
muscle	39	0.006	0.004	8	0.005	0.004
	1	<0.001				
liver	43	0.445	0.273	8	0.185	0.107
kidney	43	10.7	9.34	7	3.73	3.16
European hares						
1980-1982:						
muscle	38	0.008	0.008	2	0.003	0
liver	88	0.330	0.330	15	0.171	0.153
kidney	88	3.83	4.23	13	1.46	1.07
1992-1993:						
muscle	26	0.003	0.002	3	0.001	0
	2	<0.001				
liver	28	0.160	0.152	3	0.057	0.029
kidney	28	1.91	2.1	3	0.627	0.439

contents in the kidneys were at the same level as in the kidneys of adult reindeers. Cadmium accumulates in the kidneys with age, as shown in Figure 1.

It was surprising that the lead and cadmium contents in liver and kidney of mountain hares were higher than in the same organs of European hares. The difference probably results from the different diets: 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, during which they accumulate heavy metals.

The chromium and nickel contents of muscle, liver and kidney were low, practically at the same levels as in pigs and cattle.

The copper and zinc concentrations are shown in Tables 4 and 5.
The copper contents increased slightly during the intervening ten years but the zinc contents did not change. There were no major differences in copper and zinc contents between areas and also no differences between mountain hares and European hares. The copper contents in livers of hares were much lower than in elks and reindeers and zinc concentrations in muscle were only half those in muscle of elk. Kubin (1990) used

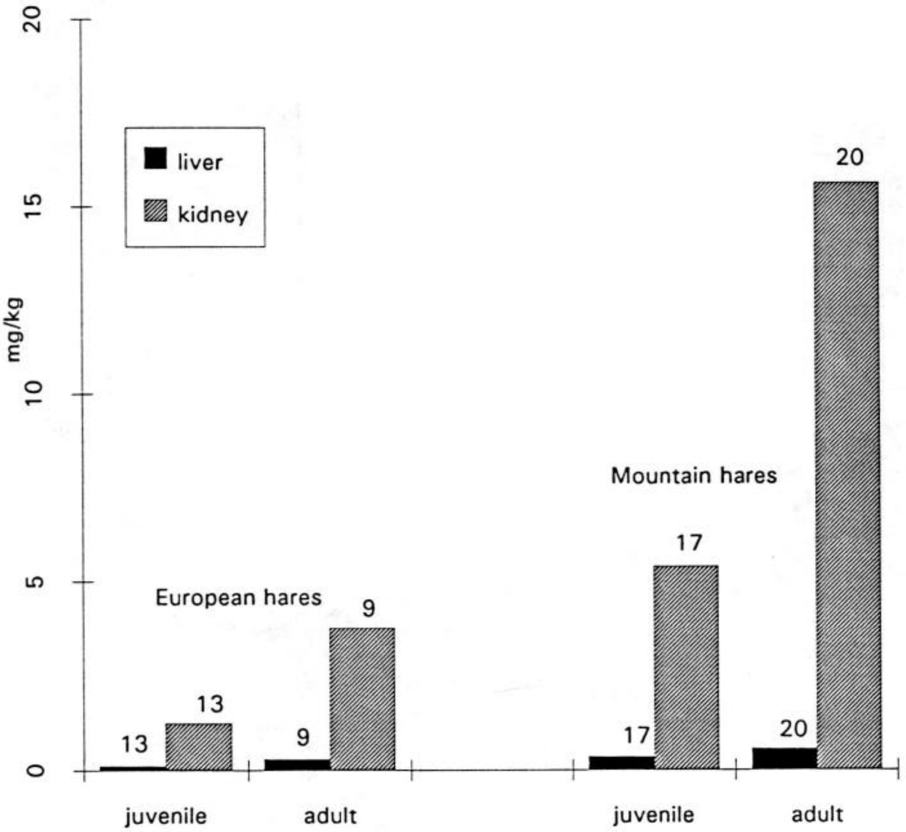

Figure 1. Levels of cadmium (mg/kg wet wt) in livers and kidneys of European hares and mountain hares in Industrial Finland. The number of samples is shown on the top of the bar graph.

lichen to study air pollution in Finnish forests. He reported the highest copper and zinc concentrations in south-western Finland, evidently attributable to the presence of a copper refinery in the 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, as there are no local sources of emissions. The hares were from these areas but the copper and zinc concentrations in hares were at normal level (R.Puls, 1989). Froslie et al. 1987 studied the copper and zinc contents in livers of Norwegian hare. The copper contents were 5.7±7.1 and those of zinc 41±23mg/kg wet weight. At present there are no recommendations concerning the maximum zinc and copper concentrations in foods of animal origin.

Table 4. The copper concentrations (mg/kg wet wt) of muscle, liver and kidney in mountain hares and European hares in industrial Finland and northern Finland (n = number of samples, x = mean, SD = standard deviation).

Copper	Industrial Finland			Northern Finland		
	n	x	SD	n	x	SD
Mountain hares						
1980-1982:						
muscle	36	1.78	0.41	7	1.68	0.82
liver	65	3.61	1.60	36	4.02	2.37
kidney	65	3.86	1.22	35	3.93	2.35
1992-1993:						
muscle	40	2.89	0.82	8	2.70	0.42
liver	43	4.71	0.80	8	4.17	0.56
kidney	43	4.36	0.71	7	4.14	0.34
European hares						
1980-1982:						
muscle	38	1.74	0.49	2	1.95	0.81
liver	88	4.61	1.82	15	4.64	1.76
kidney	88	4.21	1.00	15	3.76	0.50
1992-1993:						
muscle	28	2.93	0.84	3	2.64	0.40
liver	28	5.15	1.08	3	5.32	1.26
kidney	28	4.49	0.47	3	4.64	0.19

Table 5. The zinc concentrations (mg/kg wet wt) of muscle, liver and kidney in mountain hares and European hares in industrial Finland and northern Finland (n = number of samples, x = mean. SD = standard deviation).

Zinc	Industrial Finland			Northern Finland		
	n	x	SD	n	x	SD
Mountain hares						
1980-1982:						
muscle	36	26.8	9.35	7	20.8	6.92
liver	65	34.9	30.6	35	45.0	41.1
kidney	65	35.8	23.6	34	34.7	18.9
1992-1993:						
muscle	40	25.5	7.45	8	28.3	9.35
liver	43	31.7	3.70	8	31.4	2.65
kidney	43	34.8	7.41	7	27.5	4.59
European hares						
1980-1982:						
muscle	38	22.8	10.7	2	23.7	9.50
liver	88	46.5	34.1	14	38.6	26.1
kidney	88	35.6	18.9	14	30.5	21.4
1992-1993:						
muscle	28	23.9	8.21	3	33.5	2.54
liver	28	37.1	12.3	3	42.8	9.99
kidney	28	28.8	4.62	3	28.9	3.07

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