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Lead, Cadmium, and Zinc in Tissues of Roe Deer *(Capreolus capreolus)* near the Lead Smelter in the Koroška Region (Northern Slovenia)

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Keith (1996) used the term "hot spots" for areas where wildlife is the most threatened by toxic substances. These include areas near industrial and electricity generating centres. Koroška, an industrial area in the northern part of Slovenia with a centuries-old tradition of mining and manufacturing of lead and zinc, represents an extreme example of a "hot spot" environment. Lead excavation started in Koroška in 1424, while the start of well-organised metallurgical activity dates from 1893. Lead production reached its peak in the seventies when approximately 28,000 tons of lead were produced per annum. The concentration of Pb in the air was reduced by about 90 % after a filter system had been built in 1979 (Prpic-Majic 1996). Nevertheless, the persistence of heavy metals in the soil has remained a major problem in the Koroška region. The concentrations of Pb, Zn and Cd in the upper 10-20 cm of the soil reach up to 5898 µg/g, 1244 µg/g and 37 µg/g, respectively (Kugonic and Zupan 1999).

In the year 1997 ERICo Velenje started a research project entitled *The accumulation of heavy metals in tissues of wildlife - with emphasis on roe deer - in the polluted areas of Slovenia.* The main aim of the study was to monitor levels of heavy metals in game tissues and to make a risk assessment for wildlife as well as for human health. In the present work, a comparison between the Koroška region and two other contaminated areas in Slovenia is made. One of them is the Šalek valley where the major Slovene power plant of Šoštanj is located, and the second one is Zasavje where again a large power plant as well as a cement factory are operated. Comparison is also made with the Triglav National Park (TNP), which represents the area without any local pollution sources *(Figure 1).* A comparison with similar research performed in 1986 (Osrajnik 1990) is also made. The lead smelter at Zerjav stopped primary manufacture of lead ore in 1990. It is expected that a reduction in levels of heavy metals would follow in all constituent segments of the environment.

MATERIALS AND METHODS

Samples from 188 roe deer, including 43 animals shot in the Koroška region were collected in the period from 1st June to 31st December 1997 by local hunting authorities in the above mentioned areas. All animals were shot with legal permission. Each animal was assigned basic data such as sex, estimated age, weight, weight of antlers, the date and shooting location with the altitude. Each sample was composed of the following tissues: kidney, liver, spleen and muscular tissue from the neck. After dissection, tissues were packed separately, frozen and sent to the laboratory at ERICo Velenje where they were stored at - 18 °C until analysis.

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Figure 1. Map of Slovenia showing areas where the study was carried out.

Tissues were homogenised with Büchi-Mixer B-400 equipped with a ceramic knife. A CEM MSP 1000 microwave digestion system was used for wet digestion of samples. Tissue samples (1.4 to 1.5 g, wet weight) were weighed to ± 0.1 mg in microwave digestion vessels to which 7 ml of conc. HNO_3 and 1 ml of H_2O_2 were added. The heavy metal content was determined using a Perkin Elmer SIMAA 6000 atomic absorption spectrometer. The electrothermial technique was used for the determination of lead and cadmium while the flame technique was used for determination of zinc. Pb, Cd and Zn were measured with hollow cathode lamps at 283.3 nm, 228.8 nm and 213.9 nm, respectively. Standard reference material (Bovine liver BCR 185) was used for analytical quality assurance. The agreement for Pb (BCR 185: 501 ± 27 ng/g) was 98-103 %, for Cd (BCR 185: 298 ± 25 ng/g)) 94-108 % and for Zn (BCR 185: 142 ± 3 ng/g)) 97-105 %. Analysis of variance and the t-test were used for statistical evaluation of the sample data. Statistical analyses were made for the organs where the major accumulation of a particular element takes place (kidney for Cd and Zn; liver for Pb). All results are given as mg/kg on a wet weight basis.

RESULTS AND DISCUSSION

The levels of Pb and Zn in roe deer tissues from the Koroška region are presented in *Table 1* and Cd levels are presented in *Table 2.* The arithmetic means (a) with the confidence limits, relative standard errors (SE %), minimum and maximum values as well as the percentage of samples exceeding the permitted values defined by Slovene regulations (Ur. list SFRJ 79/1987) are given.

Cadmium is the only element of interest for which a positive correlation between the age of the animal and the concentration in the viscera is significant ($p<0.01$) *(Table 2).* The extremely long retention period, reaching 10 to 30 years in kidney and liver of mammals (Cooke and Johnson 1996), is the main cause for this correlation.

Table 1. Pb and Zn in tissues of roe deer shot in the Koroška region in 1997 (mg/kg wet weight).

Element	n	ā	SE %	Min	Max	Excess [1]
Pb						
kidney	40	0.03 ± 0.01	14 %	<0.05	0.20	0
liver	29	0.71 ± 0.65	44 %	<0.05	9.3	14 %
muscle	38	0.05 ± 0.03	31 %	<0.05	0.55	3 %
Zn						
kidney	39	47.1 ± 8.3	9 %	8.3	144.2	/
liver	40	32.6 ± 6.6	10 %	3.3	105.6	/
muscle	36	51.6 ± 6.0	6 %	19.1	90.5	/

[1] The percentage of samples exceeding the permitted values defined by Slovene regulations (Ur. list SFRJ 79/1987) given as:
Cd: viscera (0.5 mg/kg) except kidney (1.0 mg/kg); meat (0.1 mg/kg);
Pb: viscera (1.0 mg/kg), meat (0.5 mg/kg);
Zn: permitted levels are not specified.

Table 2. Cd in tissues of roe deer shot in the Koroška region in 1997 (mg/kg wet weight).

Age class	n	ā	SE %	Min	Max	Excess [1]
Cubs						
kidney	3	2.91 ± 2.92	51 %	0.45	5.6	66 %
liver	3	1.38 ± 1.99	73 %	0.15	3.4	66 %
Yearlings						
kidney	16	7.13 ± 4.43	29 %	1.0	30.7	100 %
liver	16	1.06 ± 0.77	34 %	0.07	6.2	63 %
muscle	17	0.03 ± 0.02	38 %	0.01	0.19	6 %
2+ animals [2]						
kidney	22	22.73 ± 8.92	19 %	2.3	76.0	100 %
liver	23	3.92 ± 0.88	22 %	0.26	13.5	87 %
muscle	21	0.04 ± 0.01	14 %	0.01	0.12	5 %

[1] The percentage of samples exceeding the permitted values defined by Slovene regulations (Ur. list SFRJ 79/1987) given as:
Cd: viscera (0.5 mg/kg) except kidney (1.0 mg/kg); meat (0.1 mg/kg);
Pb: viscera (1.0 mg/kg), meat (0.5 mg/kg);
Zn: permitted levels are not specified.

[2] 2+ animals: two or more year old animals (the remark hold true till the end of the paper).

The Cd concentrations in kidneys (22.73 ± 8.92 mg/kg; max: 76.0 mg/kg) and liver (3.92 ± 1.82 mg/kg; max: 13.5 mg/kg) of 2+ animals are very high. In comparison with other areas of interest the average as well as the maximum Cd concentrations in both organs are threefold to fivefold higher *(Figure* 2). Furthermore, they exceed the majority of the levels ever measured in viscera of European free-living ruminants. Nevertheless, the levels are well below the concentrations dangerous for the animals' health (100-300 mg/kg wet weight) as defined by Beyer et al. (1996).

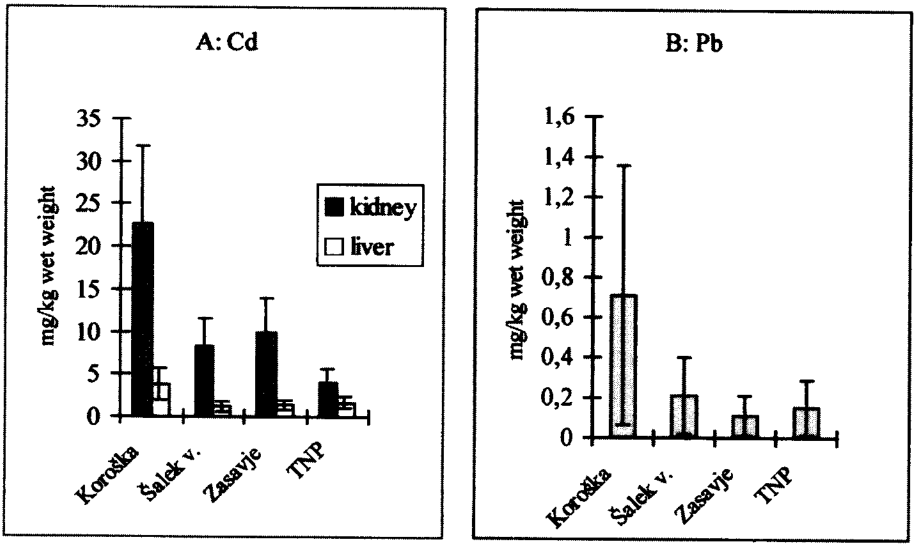

Figure 2. The Cd concentrations in kidneys and liver of 2+ animals (A) and the Pb concentrations in liver of roe deer (B) in four areas of Slovenia (Koroška: n=40; Šalek valley: n=75; Zasavje: n=28; TNP: n= 20).

The average Cd levels in kidneys of yearlings have increased 3.5-fold since 1986 (from 2.05 mg/kg in 1986 to 7.13 ± 4.43 mg/kg in 1997; $p<0.05$) and in kidneys of 2+ animals by more than tenfold (from 2.21 mg/kg to 22.73 ± 8.92 mg/kg; $p<0.001$) *(Figure 3).* Such enormous differences cannot be explained only by the increased emissions of Cd into the environment (industry, agriculture etc.), or by the increasing acidification of soil caused by acid rain (influence on the bioavaibility of Cd). Some discrepancies in the methodology between our research and the research made in 1986 (Osrajnik 1990) should also be taken into account. Among them the higher average altitude of places where the animals were shot should be mentioned (820 m and 730 m above sea level in 1997 and 1986, respectively; $p<0.1$). Rather than annual plants a higher portion of perennials (containing higher levels of Cd due to accumulation over a longer period) occurs in the nutrition of wildlife with increasing altitude (Venäläinen et al. 1996). Another difference in the methodology was the time of culling the animals. The majority of animals were shot up to July in 1986, while almost 70 % of animals were shot after the rut in 1997 (approximately 40 % of them were shot after September). It is well known (Crete et al. 1989) that in the process of catabolism the weight of soft organs decreases during the winter, resulting in an increase of the elemental concentrations expressed on a wet weight basis. Accordingly, comparison of the two studies should be made with great caution.

In contrast to Cd the average Pb levels in roe deer tissues from the Koroška region have significantly decreased since 1986 *(Figure 3):* from 1.72 to 0.04 ± 0.01 mg/kg ($p<0.00l$) in kidneys; from 1.15 to 0.71 ± 0.65 mg/kg ($p>0.05$) in liver and from 0.60 to 0.05 ± 0.03 mg/kg ($p<0.01$) in muscular tissue. Moreover, the percentage of samples exceeding the permitted values defined by Slovene regulations (Ur. list SFRJ 790987) has also declined in the same period from 32 % to 0 %, from 17 % to 14 % and from 23 % to 3 % for kidneys, liver and muscular tissue, respectively. Niemi et al. (1993) found the same trends - a decrease of Pb levels and an increase of Cd levels - in elk tissues from Finland.

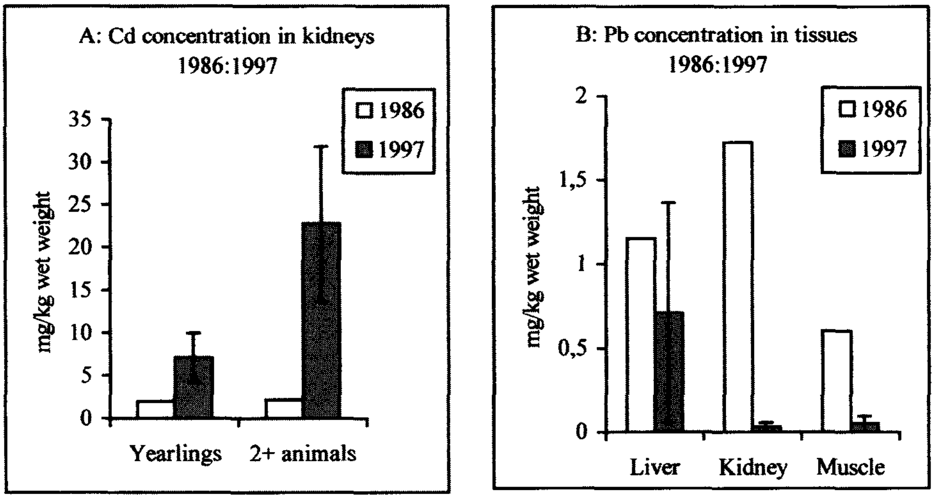

Figure 3. Comparison between the years 1986 and 1997: Cd concentrations in kidneys (A) and Pb levels in tissues (B) of roe deer shot in the Koroška region.

The reduction of Pb emissions is doubtless the main cause for the decline of Pb levels in roe deer tissues. Since traffic is one of the most important anthropogenic sources of Pb, the introduction of unleaded petrol in the nineties is probably the most significant contribution to the decreasing levels of Pb in the environment. Many authors (e.g. Niemi et al. 1993; Tataruch 1995) report decreasing levels of Pb in game tissues after the introduction of unleaded petrol. Nevertheless, the lead smelter at Zerjav stopped primary manufacture of lead ore in 1990. The stoppage could certainly be the main reason for the marked decline of Pb levels in roe deer tissues from the Koroška region. Although recent research (Kugonic and Zupan 1999) shows that soils are still highly polluted with Pb, its accumulation in wildlife tissues is reduced due to its limited bioavaibility along the food chain. In contrast to Cd, which has a retention time over 10 years in animal tissues, the half-time for Pb in soft organs of mammals is measured in weeks (Ma 1996). Accordingly, Pb levels in viscera and muscles are indicators of the present exposure of animals. Since the uptake of Pb into plant tissues is very low (Arndt et al. 1987), wildlife primarily consume Pb, which is deposited as dust on the surfaces of leaves. After the cessation of primary smelting of lead ore, a significant decrease in lead emission and dust deposition occurred. The decrease of Pb levels in game tissues is therefore an expected response. Consequently, the value of roe deer as a bioindicator of environmental pollution with lead as well as with some other heavy metals is clearly confirmed.

In spite of the great decrease of Pb concentrations in roe deer tissues since 1986, the average levels of Pb in organs of animals shot in the Koroška region are still higher than in the other areas of interest *(Figure* 2). Nevertheless, the differences are not statistically significant.

For zinc similar conclusions as for lead can be made. The average Zn concentrations in kidneys (47.1 ± 8.2 mg/kg), liver (32.6 ± 6.6 mg/kg), spleen (22.3 ± 3.4 mg/kg) and muscle (51.6 ± 3.0 mg/kg) of roe deer shot in the Koroška region appear higher than in the other areas. Nevertheless, the differences are not statistically significant. The maximum permitted concentrations of Zn in meat and viscera are not defined by Slovene regulations.

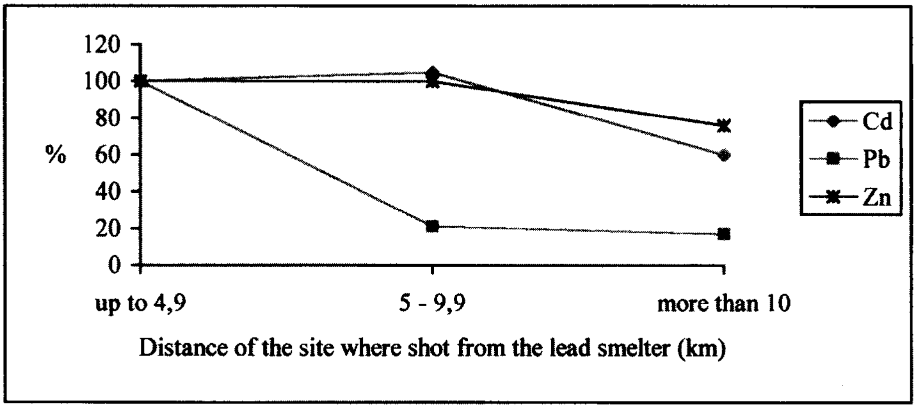

Figure 4. Decline in heavy metal levels in roe deer tissues (kidneys for Cd and Zn, liver for Pb) with respect to the distance of the site where shot from the lead smelter. The levels in the first circle (up to 4.9 km) represent the 100 % value.

Due to the mobility of wildlife, spatial analyses are extremely difficult. Nevertheless, the relation between heavy metal concentrations in roe deer tissues and the distance from the lead smelter at Zerjav (distance) is presented in the following section. Due to the relatively small number of samples (n=40), the correlation between these two variables was not calculated. Instead, all samples were divided into three groups as follows: a) a distance up to 4.9 km, b) a distance between 5 and 9.9 km; c) a distance more than 10 km.

The levels of all metals in roe deer tissues decrease with distance from the lead smelter *(Figure 4).* The reduction is the most evident for Pb, for which the levels in liver decrease by 80 % in the distance between 5 and 9.9 km in comparison with the first spatial group (distance up to 4.9 km). Data demonstrate that lead manufacture is still a very important source of local metal pollution, especially Pb. This finding is consistent with some previous conclusions (Osrajnik 1990; Doganoc and Šinigoj-Gacnik 1995). Besides local sources, remote transport of pollutants should be considered as an additional source of heavy metal contamination in the Koroška region.

The concentrations of all three mentioned heavy metals in roe deer tissues from the Koroška region are much higher than in other Slovene areas. Cd concentrations in roe deer tissues from the Koroška region are the highest ever measured in Europe. Moreover, concentrations are comparable with Cd levels in organs of other species, including brown bears (Zilincar and Zvada 1990), moose (Crete et al. 1987) and small mammals such as shrews (Ma 1996). Our study's data confirms a 1990 Slovenian government directive indicating that kidneys of all game species (irrespective of the animals' age) from all areas of Slovenia are unfit for human consumption due to their high Cd burden. For the same reason we recommend that livers of 2+ animals should also be eliminated as a food source for humans in Slovenia. In contrast, the levels of heavy metals in the muscle (meat) of roe deer do not exceed values permitted by law and are not problematic.

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