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Heavy Metals in Roe-Deer Liver and Alimentary Tract and their Content in Soil and Plants in Central Poland

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Babińska-Werka J. & Czarnowska K., 1988: Heavy metals in roe-deer liver and alimentary tract and their content in soil and plants in Central Poland. Acta theriol., 33, 15 : 219—230 [With 6 Tables & 1 Fig].

The flow of heavy metals (Pb, Zn, Cu, Mn and Fe) in the system: plant — herbivorous animal was studied on roe-deer *Capreolus capreolus* (Linnaeus, 1758) living in a small woodland (about 200 ha) surrounded by farmlands. The heavy metals content in the soil, litter and in plants which the roe-deer consumed was on an intermediate level between that of unpolluted and heavily polluted regions. The content of Mn and Fe was very high, due to the strongly acid reaction of the soil. Heavy metals pass through the organism of a roe-deer and accumulate in small amounts in the liver.

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1. INTRODUCTION

The roe-deer, *Capreolus capreolus* (Linnaeus, 1758), has often been studied for heavy metal accumulation (Sawicka-Kapusta, 1978, 1979; Sawicka-Kapusta, Perzanowski & Bobek, 1981; Kryński *et al.*, 1982; Tataruch, 1984). Since the species is sensitive to changes in its environment it may be used as a bioindicator of environmental contamination (Sawicka-Kapusta, 1978). Furthermore, in Poland, 33 thousand roe deer are shot annually (Grodziński, 1975).

Until recently, studies on the heavy-metal content in roe-deer have mainly concentrated on animals living in large forest complexes. The present study aimed at assessing heavy-metal accumulation in the food, alimentary tract, and in the liver of roe-deer living in a small, moderately contaminated woodland surrounded with farmlands.

2. MATERIALS AND METHODS

The studies were performed on the Forest Experimental Station of the Warsaw Agricultural University in Rogów, Central Poland (51°48'N, 19°53'E). Materials were collected from 200 ha of forest, where three forest types were distinguished, *i.e.* (1) fresh mixed-coniferous forest — FMC (*Pino-Quercetum typicum* Kozł. 1925) — 45%; (2) fresh mixed-deciduous forest — FMD (*Tilio-Carpinetum calamagrostietosum* Tracz. 1962) — 50%, and (3) fresh deciduous forest — FD (*Tilio-*

Carpinetum typicum Tracz. 1962) — 5% of area. The forest was surrounded by small private farms.

In order to trace the flow of heavy metals in the system: plant-herbivorous animal, the content of heavy-metals in the soil, litter and in the vegetation which constitutes potential food for the roe-deer were studied. In addition, the respective portions of the alimentary tract as well as the feces of the animals were studied.

Since soils in the study area were differentiated, the chemical properties of soil from two forest sites, varying in type and soil, *i.e.* forest compartment no. 119 * — fresh mixed forest with gray-brown soils, and forest compartment no. 106 — (FMC) with podsolized gray-brown soils were analyzed. In both cases the micromonolit was cut down to the depth of 30 cm, from which 300 g of litter (A_0) and 500 g of mineral soil (from the A_1 level) were taken. The soils in the study area were developed from a shallow fine-silt formation deposited upon the moraine material — clay and sand (Konecka-Betley & Czępińska-Kamińska, 1979).

Samples of plants composing the roe-deer diet were taken at the end of November 1983, just before the first snow fall. The plants were selected after the roe-deer diet was determined in the Rogów Forest (Babińska-Werka, in prep.). Tree and shrub branches with leaves or buds were cut 30—150 cm from the ground level, *i.e.* at the typical browsing height of the roe-deer (Bobek & Dzięciołowski, 1972). In order to characterize the respective species of plants, the plant material obtained from 10 trees or shrubs was treated as one sample. Likewise, one sample of herbaceous vegetation contained specimens collected from 10 different spots in the study area.

In order to establish the heavy-metal content in the organism, liver samples were taken from 10 does shot during winter 1983/1984, *i.e.* in December and January. The rumen, duodenum, small intestine, ceacum and colon contents were analyzed separately in order to determine which part of the alimentary tract had the most prominent role in the inhibition of heavy metals. About 50 g of the material were taken from the respective portions of the alimentary tract. On the basis of teeth abrasion the age of the roe-deer was established to be from 2 to 4 years.

At the same time, the heavy-metal content was analyzed in 10 rumens of males shot in June and July 1980, the rumens differing in species composing the animals diet. In addition, the diet of roe-deer was established within the study area (Babińska-Werka, in prep.). In 5 rumens, conifer species were found in addition to the common angiospermous plants.

In order to assess the amount of heavy metals excreted by roe-deer, samples of fresh feces were taken in spring, summer and autumn in the years 1980 and 1981. Ten, 100 g samples were analyzed in each of the seasons. Samples of feces taken from the Białowieża National Park (54°40'N, 23°50'E) were used as a control. According to data obtained by Grodzińska (1971, 1978), Makomaska (1978) and Sawicka-Kapusta (1978) it was assumed that the above area is only somewhat polluted by heavy metals.

The total content of heavy metals in the soil samples was determined in a concentrated extract of acids (NHO_3, H_2SO_4 and HCl) on ashing the organic substance at 480°C. The litter as well as the plant material and feces were dried at 60°C and, subsequently, subjected to grinding and dry mineralization at 450—

* Data obtained from the management plan of the Rogów Forest District in 1979.

—480°C in a muffle furnace for 8 h. Ashes were dissolved under conditions of heating with the use of 6 N HCl (Czarnowska, 1980). Contents of the respective portions of the alimentary tract and the livers were dried at 60°C in order to determine the dry weight. The dry material was subjected to wet mineralization with the aid of concentrated HNO_3 and $HClO_4$ (Czarnowska & Gworek, 1980). In the soil-, plant- and animal solutions, Fe, Mn, Zn, Cu and Pb were determined by atomic absorption spectrophotometry (AAS), 551 model. The analyses were carried out in two replications and the reagent test was made for each series of determinations. Results were calculated for the absolutely dry weight.

3. RESULTS

3.1. Soil and Litter

The soils under study contained relatively large amounts of organic compounds. The organic carbon in the (A_0) litter was 10.5—39.0%, while in the (A_1) mineral, 1.60—5.50%. The organic substance in the soil constituted the raw acid humus of pH ranging from 3.3 to 3.8. A slightly higher pH level was in the A_1 mineral horizon (pH=3.8−4.0).

Table 1

Contents of heavy metals (ppm dry weight) in a forest litter and in a soil in Rogów area.

Layer	Forest section	Fe	Mn	Zn	Cu	Pb
Litter A_0	119	920	960	69	9.0	26
	106	1340	2280	79	10.7	27
Soil A_1 (0—20 cm)	119	3100	107	20	6.2	20
	106	4500	220	20	2.8	16

The samples of soil differed in the amounts of Mn, Fe, and Zn and, to a lesser extent, in the amounts of Cu and Pb (Table 1). The amount of Mn was 9 to 10 times higher in the litter than in the mineral one. In both site types under study the litter had similar amounts of Zn, Pb and Cu. On the other hand, more Fe was found in the (A_1) mineral than in the A_0 organic one.

The total content of heavy metals in the investigated soils was characteristic of an enviroment exposed to slight contamination (Zieliński, 1984).

3.2. Plants

Analyses included herbaceous plants, dwarf shrubs, crop plants, and browse (Table 2). Cu and Pb were present in all samples in almost equivalent amounts. The amount of Fe, however, was twice as great in the ground layer plants and in rye as in the browse. On the other hand, the amount of Mn in the browse plants was triple that in the ground layer plants. All plants from Rogów contained considerable amounts of Pb. Particularly great amounts of Zn were accumulated in *Betula verrucosa* Ehrh. and, among the herbaceous plants, in *Viola silvestris* Rohb. and *Veronica officinalis* L. Generally, the herbaceous plants contained more Zn than browse.

The heavy-metal contents in plants from Rogów were compared with those in plants originating from Białowieża, *i.e.* from a slightly polluted

Table 2

Contents of heavy metals (ppm dry weight) in plants from Rogów area.

Types of vegetation	Fe	Mn	Zn	Cu	Pb
Herb layer					
Viola silvestris Rchb.	397	610	216	7.2	24
Veronica officinalis L.	580	360	106	10.4	16
Oxalis acetosella L.	444	1100	50	9.6	18
Fragaria vesca L.	550	510	44	8.6	18
Dwarf shrubs:					
Vaccinium myrtillus L.	224	2050	41	7.6	18
Cultivated plants:					
Secale cereale L. — tillering	440	200	100	9.6	6
Herb layer $\bar{x}$	439	807	93	8.8	17
Browse					
Rubus sp.	268	930	44	9.4	19
Frangula alnus Mill.	378	1770	44	8.0	22
Evonymus europaea L.	356	350	44	10.0	20
Corylus avellana L.	470	3080	38	9.2	24
Carpinus betulus L.	330	3900	50	5.2	18
Betula verrucosa Ehrh.	98	3310	400 *	10.2	16
Tilia cordata Mill.	184	940	33	10.2	21
Quercus robur L.	188	3620	35	5.6	16
Browse $\bar{x}$	284	2237	86	8.5	20

* not included into the calculation of the mean value.

area as well as from Niepołomice Forest near Kraków, *i.e.* from an area particularly exposed to contamination (Table 3). It was found that Rogów had an intermediate level of heavy metals except for Mn and Fe. The level of Pb in the ground layer plants in Rogów was three times greater than in the plants from Białowieża and two times less than in the plants from Niepołomice. The levels of Zn and Cu were similar in Białowieża and in Rogów, being typical of forest vegetation. However, the level of Mn in the browse, with an average value of 2237 in Rogów, differed drastically from that of the two remaining localities where the average level of Mn was 300 ppm. The level of Fe in Rogów was triple that in Białowieża.

Table 3

Mean heavy metal contents (ppm dry weigth) in plants growing in areas of different degrees of contamination.

Types of vegetation	Fe	Mn	Zn	Cu	Pb
Białowieża (according to Grodzińska *et al.*, 1983)					
Herb layer	178	—	95	7	5
herbs and grasses	338	—	129	8	10
dwarf shrubs (*Vaccinium* sp.)	17	—	60	5	0
Browse	66	310	102	8	5
Rogów (this paper)					
Herb layer	439	807	93	9	17
herbs	482	558	103	9	16
dwarf shrubs (*Vaccinium* sp.)	224	2050	41	8	18
wintercorn (rye)	440	200	100	10	6
Browse	284	2237	86	8	20
Niepołomice (according to Grodzińska *et al.*, 1983)					
Herb layer	2103	—	177	7	35
herbs and grasses	3782	—	172	8	37
dwarf shrubs (*Vaccinium* sp.)	425	—	182	5	33
Browse	336	347	172	9	13

3.3. Alimentary Tract

The heavy-metal level was analyzed in the contents of the rumen, duodenum, small intestine, caecum and colon, and in liver (Fig. 1). The levels of all metals, with the exception of Cu, were markedly lower in the liver than in the alimentary tract. This testified to the positive role of the latter in the removal of superfluous elements from the organism. Fe was especially high in all parts of the alimentary tract and an increase in the amount of Mn was observed starting from the small intestine to the colon (feces). The Zn content was distributed likewise in the alimentary tract.

With the aid of the one way ANOVA significant differences were revealed in the heavy metal occurrence in the alimentary tract of roe-deer (Fe: $F=10.06$, $p<0.001$; Mn: $F=18.59$, $p<0.001$; Zn: $F=3.28$, $p<0.05$; Cu: $F=3.84$, $p<0.01$; Pb: $F=2.83$, $p<0.05$; in all cases $df=5,45$). However, in the majority of analyses the above effect was caused solely by the fact that the content of respective elements in the liver was significantly different from that of the remaining parts of the alimentary tract. Within the latter no differentiation in this respect was found (Duncan test). Small differentiation was in the heavy metal content in the rumen, small intestine, caecum and colon in the specimens under study (Table 4). The above analysis showed that the amounts of Fe and Mn in the liver were different from those in the respective parts of the

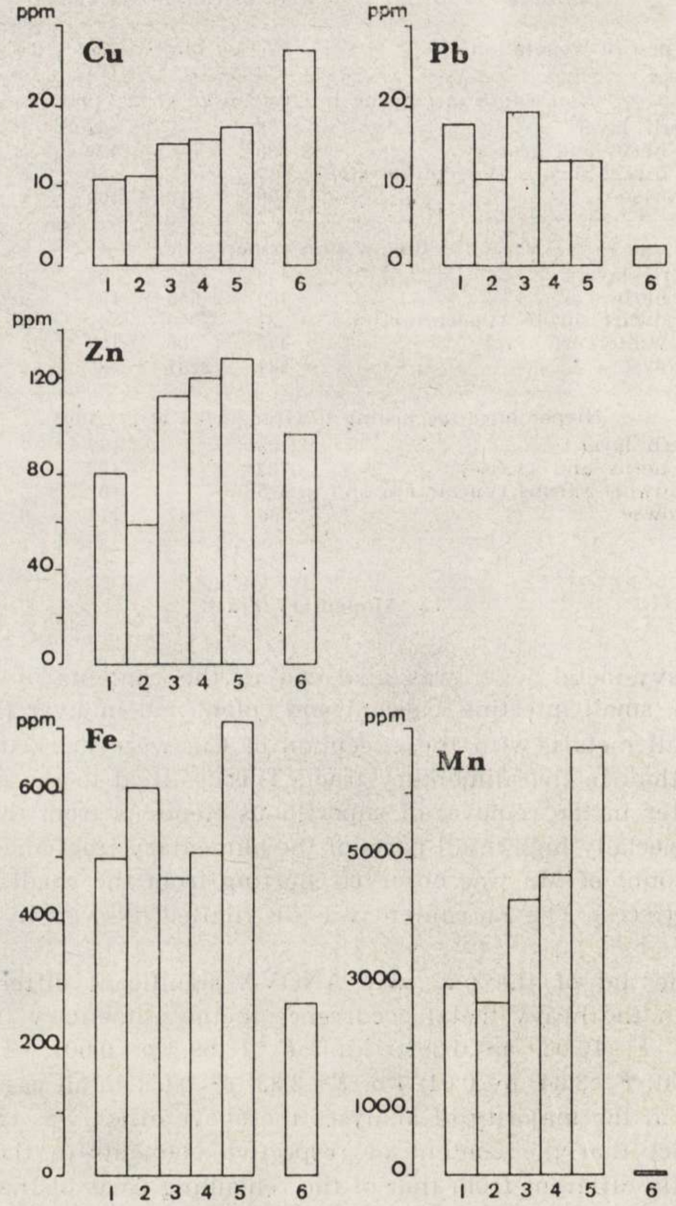

Fig. 1. Contents of heavy metals (ppm, dry weight) in different parts of the alimentary tract of roe deer from Rogów (winter 1983/1984); 1 — rumen, 2 — duodenum, 3 — small intestine, 4 — caecum, 5 — large intestine, 6 — liver.

Table 4

The results of Duncan tests applied to heavy metal contents (Fe, Zn, Mn) in roe deer liver and intestine tract. Ru — rumen, D — duodenum, SI — small intestine, C — caecum, LI — large intestine, L — liver. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns — not significant.

	Fe					Zn					Mn				
	D	SI	C	LI	L	D	SI	C	LI	L	D	SI	C	LI	L
Ru	ns	ns	ns	**	**	ns	ns	ns	*	ns	ns	ns	*	***	***
D		*	ns	ns	***		*	**	**	ns		*	*	***	***
SI			ns	**	**			ns	ns	ns			ns	**	***
C				**	**				ns	ns				**	***
LI					***					ns					***

alimentary tract. However, there were no differences in Zn. Likewise, no significant differences were found in the amounts of heavy metals with respect to the age of specimens. The rumen analysis of food content showed that the amounts of Fe and Mn were higher in the rumens containing only the angiospermous plants than in those with an admixture of coniferous-plant. These differences were significant for Fe only (Table 5).

When comparing the rumen levels of Fe and Mn between the specimens shot in summer (Table 5) and in winter (Fig. 1), it may be seen that the amount of Fe was ten times higher, and that of Mn two times higher in winter than in summer.

3.4. Feces

The results of analyses indicate that the quantity of metals in question in the roe-deer feces was 2 to 6 times higher in Rogów than in Białowieża and that the maximum values were observed in the case of Fe,

Table 5

Contents of heavy metals (ppm dry weight) in the rumen of roe-deer culled in the Rogów area in summer 1980.

Element	Rumen contents: Angiosperm plants ($n=5$)	Rumen contents: Angiosperm and gymnosperm plants ($n=5$)
Fe	508.0±232.8*	261.2± 70.6*
Mn	1468±562.6	1362.6±754.2
Zn	81.4± 10.4	80.6± 15.5
Cu	10.0± 1.6	11.4± 3.1
Pb	21.6± 6.6	17.7± 7.2

* $p<0.05$.

Mn and Pb (Table 6). The contents of Fe and Mn were lowest in spring and their maximal values occurred in winter while the higher contents of Zn, Cu and Pb were determined at the beginning of the vegetation season.

Table 6

Contents of heavy metals (ppm dry weight) in roe-deer feces.

Season	Fe	Mn	Zn	Cu	Pb
		Rogów			
Spring	1785	3900	164	27.7	25.2
Summer	1210	4115	189	41.7	18.5
Autumn	1375	8125	143	30.0	19.0
Winter	7113	6718	128	16.9	12.7
Annual mean	2871	5714	156	29.1	18.8
		Białowieża			
Autumn	223	2943	128	14.9	5.3

4. DISCUSSION

Results of the study made in a small woodland surrounded by fields in central Poland permit the following conclusions to be drawn. First, the study area was only moderately contaminated and the contents of heavy metals were on an intermediate level between the heavily polluted areas, Niepołomice Forest, Dulowska Forest (Grodzińska *et al.*, 1983; Sawicka-Kapusta *et al.*, 1981) and the unpolluted ones — Białowieża Forest (Grodzińska, 1971, 1978; Makomaska, 1978, and Sawicka-Kapusta, 1978). Second, the above relationships concern the soil, litter and plants, and the diet of roe-deer. However, the content of Mn and Fe in the area of Rogów markedly exceeded that encountered in the other two areas, due to heavy acid soil reaction (pH=3.3—4.0).

High levels of Mn and Fe in the soil were reflected by the amounts of these metals in the plants which showed a tendency to cumulate Mn and Fe in their tissues (Faber, 1977). In this study a lot of Mn was found in *Oxalis acetosella, Vaccinium myrtillus, Carpinus betulus, Corylus avellana, Betula verrucosa, Quercus robur* and *Frangula alnus* (Table 2). Studies by Sarosiek and Wachowska (1963) have shown that the more soluble Mn in the soil, the more this element is taken up by a plant.

Plants in the highly acidic environment of the Rogów Forest accumulated large quantities of Mn when compared to values given in literature, *i.e.* from 14 to 150 ppm dry weight (Kabata-Pendias & Pendias, 1979). This leads to the disproportion between the amounts of Fe and Mn in plants while the Fe/Mn ratio averaged about 0.12—0.45 : 1. At the normal growth of plants the above ratio is 1.5—2.5 : 1. However, the excess of Mn is being precipitated in the form of MnO_2 and stored in the non-assimilatory tissues in plants, *i.e.* in hair and on the surface of leaves and stems. This is why plants show great tolerance to excessive amounts of Mn (Kabata-Pendias & Pendias, 1979).

The amount of heavy metals in plants influences their amount in the organism of a herbivorous animal. Heavy metals are cumulated in only some organs and the remaining part is excreted through the alimentary tract to the environment (Friberg *et al.*, 1979). The liver is one of the organs that cumulates the heavy metals.

Roe-deer from the vicinity of Rogów (Fig. 1) accumulated 2—3 times more Fe and about 2 times more Pb in their livers than the animals from Niepołomicka Forest (Sawicka-Kapusta & Kozłowski, 1984). The level of Mn also seems to be very high but in literature, there are no data available concerning the amount of Mn in the liver of game. The data obtained for Mn can be compared with those obtained in other mammals. According to Underwood (1971), the content of Mn in human liver oscillates from 1.7 to 4.7 ppm (in a fresh sample). Włostowski (1984) reported only 4 ppm d.w. of Mn, in the liver of field mice *i.e.* about 10 times less than in roe-deer from the Rogów area.

In this study the level of Mn, Fe, Cu and Pb in the liver was considerably lower than in the alimentary tract (Fig. 1). This testifies to the fact that most of the heavy metals pass through the alimentary tract with food and only small amounts of the metals is imbibed and, afterwards, cumulated in the organism. As Underwood (1971) cites, an average of 5 to 20% of metals taken up with food is imbibed by the organism. It is difficult to assess the amount of elements excreted with feces since in this work, only the level of heavy metal cumulation in the liver was studied and, as Sawicka-Kapusta (1978) showed roe-deer antlers are also important in heavy metal cumulation.

A relatively small amount of heavy metals in the liver is explained by Włostowski (1984) who thinks that the environmental contamination does not increase the total content of metals in the liver on account of the considerable growth of mass of this organ.

The content of heavy metals in roe-deer reflected their amount in plants (Sawicka-Kapusta *et al.*, *1981)*. So, the great content of Mn and Fe in the plants determines the level of these elements in the liver, *e.g.* in roe-deer of Rogów the amount of Fe was 2—3 times greater than in roe-deer from the Niepołomicka Forest area (Bobek *et al.*, 1984). On the other hand, in feces, the level of metals under study was 2 to 6 times higher in roe-deer of Rogów than in those from Białowieża. But, at the same time, the level of Pb, Cu and Zn in feces from Dulowska Forest (polluted area), exceeded the level of these metals found in feces in Rogów (Sawicka-Kapusta *et al.*, 1981). In this study, however, the samples of plants were taken in November. Thus the applicability of results is restricted.

The roe-deer originated from a small woodland surrounded by fields. They penetrated the neighbouring fields to the average distance of 500 m from the forest and fed on the available and abundant forage (Aulak & Babińska-Werka, in prep.). In the annual cycle, the crop plants and weeds constituted from 55 to 60% of the biomass of the rumen content, excluding autumn where this value is only 40% (Babińska-Werka, in prep.). Since roe-deer feed on equivalent amounts of forage originating from the forest and from the fields it takes part in the transfer of heavy metals in the environment. This follows through feces that contain most of the metals taken up with food. According to studies by Aulak & Babińska-Werka (in prep.) an overwhelming amount of roe deer feces in the study area were found in the forest. Since the forest plants contain more heavy metals than rye — the main crop in the agrocoenoses under study — the amount of heavy metals carried by roe-deer from the fields to the forest should be insignificant.

This study was conducted in an area with relatively extensive farming. It seems that with more intensive farming, and more mineral fertilizers and pesticides applied the roe-deer will obviously transfer considerably greater quantities of heavy metals from the fields to the forest.

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METALE CIĘŻKIE W WĄTROBIE I PRZEWODZIE POKARMOWYM SARN W ZALEŻNOŚCI OD ZAWARTOŚCI W GLEBIE I POKARMIE

Streszczenie

Na terenie lasów Leśnego Zakładu Doświadczalnego Szkoły Głównej Gospodarstwa Wiejskiego w Rogowie, przeprowadzono badania na zawartością metali ciężkich (Pb, Zn, Cu, Mn, Fe) w pokarmie i wątrobie oraz przewodzie pokarmowym sarn, *Capreolus capreolus* (Linnaeus, 1758), zamieszkujących mały las (ok. 200 ha) otoczony polami uprawnymi. Aby prześledzić przepływ metali ciężkich w układzie roślina-roślinożerca, zbadano ich zawartość w glebie, ściółce, roślinach, stanowiących potencjalny pokarm sarn, a także w poszczególnych częściach przewodu pokarmowego badanych zwierząt i w ich kale.

Stwierdzono, że w glebie, ściółce i roślinach zawartość analizowanych metali układała się na poziomie pośrednim między terenami niezanieczyszczonymi — Puszczą Białowieską, a bardzo zanieczyszczonymi — Puszczą Niepołomicką. W Rogowie jedynie ilości Mn i Fe były bardzo wysokie, co związane było z silnie kwaśnym odczynem gleby. Zjedzone z pokarmem, niepotrzebne pierwiastki sarna usuwa z kałem (Fig. 1). Duża ilość Mn i Fe w pokarmie odbiła się na ich zawartości w kale (Tabela 6).