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Heavy Metals in Epigeic Fauna: Trophic-Level and Physiological Hypotheses

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One of the most important topics in ecotoxicology for the last few decades has been transfer of pollutants along the trophic chains (see Beyer 1986; Laskowski 1991). In early 1960s Woodwell et al. (1967) showed that DDT-concentrations in organisms dramatically increase up the trophic chains. Then such "biomagnification" was confirmed for other organochloric compounds (cf. Walker 1990). In 1960s and 1970s it was suggested that biomagnification of anthropogenic pollutants is a general property of living organisms (e.g. Collier et al. 1973; Price et al. 1974). However, many studies have not supported that hypothesis (Moriarty 1975; Ernst and Joosse-van Damme 1983; Beyer et al. 1985; Grodzińska et al. 1987; Willamo and Nuorteva 1987). Van Straalen and Van Wensem (1986) have proved that neither the trophic level nor the body weight determined body concentrations of Zn and Cd in terrestrial invertebrates. They suggested that heavy metal levels in animals were determined mainly by the "physiological equipment" of an organisms (see also Grodzińska et al. 1987; Janssen et al. 1991). Some authors indicate, however, that there are important differences in the mobility of both nutritional and xenobiotic heavy metals in terrestrial ecosystems (Tjell et al. 1983; Bengtsson et al. 1986; Hunter et al. 1987a, 1987b, 1987c).

In this paper we examine whether heavy metal concentrations in epigeic fauna in Polish forests can be explained by trophic levels.

MATERIALS AND METHODS

Samples were collected at four sites in Southern Poland, differing in the levels of pollution. Niepołomice Forest (NF), Ojców National Park (ONP) and Ratanica Watershed (RW) represented moderately polluted areas with some

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differences in respect to various heavy metals, while Olkusz-Pomorzany (OP) was the most polluted ecosystem (fig. 1). Niepołomice Forest is an oak-hornbeam forest, situated some 20 km E from the Nowa Huta steel mill and some 30 km E from Cracow. Ojców National Park beech forests are located east from Upper Silesia Industrial Region, about 20 km N-W from Cracow. Ratanica watershed is a small (approx. 2 km^2) valley in the Carpathian foothills, covered mainly with pine-beech forests; it is located at 40 km distance to S-E from Cracow. Olkusz-Pomorzany is a pine forest situated 40 km W from Cracow, and some 5 km from the zinc-and-lead plant. Acidity of the organic soil layer (pH in H_2O and in KCl respectively) was: RW, 3.83 and 3.28; OP, 4.52 and 4.15; ONP, 5.11 and 4.99; NF, 4.28 and 3.97.

Litter macrofauna were collected by means of a sieving method and with common pitfall Barber traps with glycerine as a protective agent. Small mammals were collected with dug-in funnels. The traps were checked every 5 days. Mammals were frozen separately in plastic bags at -10°C. Invertebrates were divided into taxonomic groups used for further chemical analyses and stored in plastic containers with 50% methanol in -10°C. Additionally, 10 random samples of litter ($A_{0L}+A_{0F}+A_{0H}$) were collected from each study site to determine the pollution level at the area. All animals and litter samples were collected during summer seasons 1989 and 1990.

The animals collected were assigned to four trophic levels: detritivores (*Lumbricidae*), carnivores (*Chilopoda, Arachnida, Staphylinidae* and *Carabidae*), top carnivores (*Insectivora*) and herbivores (*Rodentia*). Insectivores belonged to four species: *Neomys fodiens, Sorex minutus, Sorex araneus* and *Sorex alpinus*. Rodents were represented by *Microtus arvalis, Muscardinus avellanarius, Clethrionomys glareolus* and *Apodemus sp*.

Digestive tracts were removed from the mammals. The animals were not washed. Samples were oven-dried at 75°C and ground in a ceramic bowl. Ground samples were digested in a mixture of nitric and perchloric acids (4:1; v:v). Most of the *Sorex* specimens were analyzed whole, while larger mammals were ground and one subsample from every individual was taken. Invertebrates in each taxonomic group were mixed together and subsamples were taken for chemical analysis. One subsample from each forest-floor sample was analyzed for heavy metal content.

Digests were analyzed for Zn, Cu, Pb and Cd concentrations with Perkin-Elmer atomic absorption spectrometer. Blank samples were analyzed for each set of samples. Detection limits for Zn, Cu, Pb and Cd were 0.05, 0.02, 0.1 and 0.01 $\mu g\ ml^{-1}$ respectively. Concentration ranges measured for digests were: Zn, 1.06-115; Cu, 0.24-3.98; Pb, 0.2-57.5; Cd, 0.02-8.1 µg ml^{-1}.

Data were log-transformed to normalize the distributions. The homogeneity of variances was tested with the Cochran test. Significantly different variances were detected for one-third of all comparisons, nevertheless we decided do use Tukey test for separating means. In the first step, data were analyzed with a multifactor ANOVA (Sokal and Rohlf, 1981) with taxonomic group and location as factors. Because of the highly significant interactions in the case of Zn, Cu and Pb, in the second step, data were analyzed with one-way analysis of variance (Sokal and Rohlf, 1981) with taxonomic groups or location as factors.

RESULTS AND DISCUSSION

For all metals and sites studied we found no increase in concentrations with trophic level. In most cases concentrations of heavy metals in top carnivores were lower or equal to the concentrations in animals at lower trophic levels. For eleven cases out of 16 the concentrations of heavy metals in carnivorous mammals did not differ significantly from the concentrations in herbivorous mammals (tab. 1).

In all cases except Cu in ONP and NF, and Pb in NF and RW, body-concentrations of heavy metals in carabids did not differ significantly from the concentrations in insectivores, which represent higher trophic level than carabids (Hunter et al. 1987c). Arachnids generally had significantly higher levels of heavy metals than in carabids, although both groups occupy a similar trophic level. Significant differences in heavy metal concentrations between lumbricids and arachnids were found only in five cases (three for Cu), even though these groups belong to separate trophic levels (tab. 1).

A between-site comparison yielded no significant differences in Zn levels in chilopods, carabids, rodents and insectivores, although highly significant differences in Zn concentrations in the forest litter were detected. Heavy Zn pollution in OP was reflected in significantly higher Zn levels in lumbricids, arachnids and staphylinids from OP (fig. 1). For all but one (*Arachnida*) case no significant differences were found in Cu levels in animals from different plots, despite the fact that there were highly significant ($P<0.0001$) differences in the litter Cu-levels. The differences in Pb and Cd concentrations in the forest litter were much smaller (although still statistically significant) than were the differences in Zn concentrations. However, those differences resulted in the significant between-plot differences in

Table 1. Average concentrations of heavy metals in litter and in animals clustered by taxonomic groups; groups marked with the same letter are homogeneous accordingly to 95% Tukey HSD intervals; for all sites $P<0.001$; n = average number of individuals per one sample; number of samples in brackets (same for all metals); ONP = Ojców National Park, OP = Olkusz-Pomorzany, NF = Niepołomice forest, RW = Ratanica Watershed; li = litter, Lu = *Lumbricidae*, Ch = *Chilopoda*, Ar = *Arachnida*, St = *Staphylinidae*, Ca = *Carabidae*, In = *Insectivora*, Ro = *Rodentia*.

litter, taxonomic group/n	average concentration µg g^{-1} dry wt			
	ONP	OP	NF	RW
		Zn		
li	82^{A} (10)	2116^{E} (10)	178^{A} (10)	146^{A} (10)
Lu/4	615^{C} (2)	1895DE (2)	464^{C} (5)	236^{A} (4)
Ch/26	440^{C} (4)	784^{CD} (3)	528^{C} (5)	536^{BC} (5)
Ar/56	438^{C} (5)	1021DE (5)	488^{C} (5)	612^{C} (5)
St/66	252^{BC} (2)	465^{BC} (5)	384^{BC} (5)	291^{ABC} (4)
Ca/6	138^{AB} (5)	200^{A} (5)	139^{A} (6)	192^{A} (6)
In/1	277^{BC} (13)	169^{A} (12)	254^{AB} (12)	214^{A} (11)
Ro/1	139^{AB} (4)	331^{AB} (3)	229^{AB} (10)	270^{AB} (5)
		Cu		
li	5.8^{A}	26.6^{C}	12.6^{BC}	23.2^{C}
Lu	13.5^{BC}	28.7^{BCD}	15.8^{BCD}	25.3^{BC}
Ch	55.4^{E}	58.6^{CD}	33.1^{E}	52.6^{DE}
Ar	41.2^{DE}	102.9^{D}	42.9^{E}	93.9^{E}
St	21.8^{CD}	25.8^{C}	18.4^{D}	29.6^{CD}
Ca	21.7^{C}	20.1^{BC}	18.1^{CD}	18.8^{BC}
In	10.9^{B}	10.5^{B}	11.6^{B}	11.1^{AB}
Ro	8.7^{AB}	6.0^{A}	8.6^{A}	8.4^{A}
		Pb		
li	42.0^{BC}	443.4^{B}	41.4^{BCD}	161.8^{CD}
Lu	106.3^{C}	840.9^{B}	61.4^{ABC}	408.1^{D}
Ch	16.2^{ABC}	329.9^{AB}	702.0^{CDE}	93.5^{ABC}
Ar	64.5^{C}	231.0^{AB}	417.8^{E}	339.7^{BCD}
St	56.3^{ABC}	389.3^{B}	248.4^{CDE}	13.4^{AB}
Ca	29.1^{ABC}	177.1^{AB}	336.9^{DE}	215.8^{CD}
In	15.3^{AB}	47.3^{A}	19.6^{AB}	5.6^{A}
Ro	5.7^{A}	23.7^{A}	6.2^{A}	5.1^{A}
		Cd		
li	4.8^{BC}	34.5^{CDE}	8.8^{BC}	3.8^{BC}
Lu	21.1^{C}	121.3^{E}	23.2^{C}	13.6^{BC}
Ch	4.2^{BC}	14.1^{BCDE}	32.8^{BC}	2.5^{B}
Ar	15.0^{C}	58.4^{DE}	31.2^{C}	32.2^{C}
St	3.3^{BC}	12.0^{CD}	8.5^{BC}	2.4^{BC}
Ca	1.9^{B}	9.0^{BC}	8.4^{BC}	2.6^{B}
In	3.1^{B}	4.6^{AB}	2.9^{B}	1.9^{B}
Ro	0.2^{A}	0.8^{A}	0.1^{A}	0.1^{A}

Pb body-levels in earthworms, staphylinids, carabids, rodents and insectivores, and in Cd body-levels in earthworms, staphylinids, carabids and rodents (fig. 1). Highly significant interactions between taxonomic groups and sites in a multifactor ANOVA for Zn, Cu and Pb suggest that, for these metals, the diversified patterns of their translocation are possible, depending on the pollution level. This implies that indicating taxonomic groups which "generally" accumulate high levels of these metals, and groups which "generally" have low concentrations, is not fully justified. However, data presented in tab. 1 and fig. 1 indicate that in most cases lumbricids, chilopods and arachnids have the highest levels of Zn, while chilopods and arachnids have the highest levels of Cu. Insectivores and rodents are usually those with the lowest concentrations of heavy metals. For Cd it is possible, on the basis of the multifactor ANOVA, to arrange animals studied in the order from those with the lowest levels to those with the highest Cd levels:

Rodentia<Insectivora≤Carabidae≤Chilopoda≈
≈Staphylinidae<Lumbricidae≈Arachnida

One of the possible explanations for diversified patterns of Zn, Cu and Pb distribution in epigeic fauna depending on the area pollution level is regulation of body levels for these metals. While this agrees with other results obtained for Zn and Cu (very few statistically significant differences in between-plot comparisons), this contradicts some other results for Pb. For Pb we found the most numerous cases of significant differences in between-plot comparisons. Thus, we suggest that some factors other than Pb concentration in litter can modify Pb distribution in epigeic fauna. One of the possibilities could be the Ca-soil status which was not determined in our experiment (Van Straalen et al. 1987).

Results obtained support earlier suggestions that the importance of various heavy metals for the ecotoxicology of epigeic animals does not depend on metals' concentrations in soil alone (Hunter et al. 1987a, Hopkin 1989). At present it seems to be agreed that most terrestrial organisms can control Zn body-levels quite efficiently (e.g. Johnson et al. 1978, Beyer and Cromartie 1987, Willamo and Nuorteva 1987). Many authors suggest that Cd level is much more poorly controlled, being thus of a special concern as anthropogenic pollutant (Tjell et al. 1983, Hunter et al. 1987a, 1987b, 1987c, Van Straalen et al. 1987, Hegestrom and West 1989, Hopkin 1989). Nevertheless, also Cd body-level can be controlled efficiently by some soil invertebrates (Janssen et al. 1991).

Whether Cu and Pb are regulated is more controversial. Our results suggest efficient Cu body level regulation in animals studied. This agrees with Beyer et al. (1985), Van Straalen et al. (1987) and Alberici et al. (1989). However, Hunter et al. (1987c) found Cu regulation in mammals, while detritivores and herbivores had biomagnification factors in the range of 2-4 (Hunter et al. 1987a, 1987b). Our results suggest that the differences in Cu concentrations among various groups of animals studied can be explained exclusively by physiological mechanisms. We found the lowest concentrations in mammals, which use iron in haemoglobin, and do not require high Cu levels. Chilopods and arachnids had the highest Cu body concentrations, and they use the hemocyanin Cu-based oxygen carrying system. Staphylinids and carabids are equipped with a trachea system which is usually accompanied with a high content of Cu-based cytochrome (Wigglesworth 1977). Thus, insects require higher levels of Cu than mammals, but lower than myriapods and some arachnids. Earthworms can control Cu levels in a way similar to mammals (Streit 1984), although they are more endangered by Cu pollution because of the direct contact with soil solution.

The other important difference between Zn and Cu body levels regulation can be attributed to the possible differences in assimilation of these two metals from the digestive tract. Requirements for Zn and Cu in animals are similar, but Zn is much more abundant in the environment (in forest litter 50-300 $\mu g\ g^{-1}$ for Zn *vs* 10-20 $\mu g\ g^{-1}$ for Cu; Hopkin 1989). This should have forced animals to evolve very efficient active mechanisms for Cu assimilation, while Zn assimilation could remain less efficient (Hopkin 1989). Nevertheless, in the case of both Cu and Zn some regulation of intake probably does exist. Some studies indicate that metallothionein is an important factor in this regulation (see Friberg et al. 1979, Hopkin 1989). On the other hand it is known that metallothionein can bind not only Cu and Zn but also some other metals, especially Cd (Friberg et al. 1979, Hopkin 1989). It is possible that some non-essential metals can be transported from the digestive tract into the body by mechanisms evolved for the assimilation of essential metals. The most endangered by intoxication with xenobiotic metals would thus be animals with especially efficient mechanisms for assimilation of some chemically similar (i.e. those binding to the same proteins) essential metals.

Our results indicate that physiological differences in the requirements and assimilation of essential metals can be of special importance in determining the distribution of heavy metals in epigeic fauna. Janssen et al. (1991) have found that the body concentration of Cd depends to

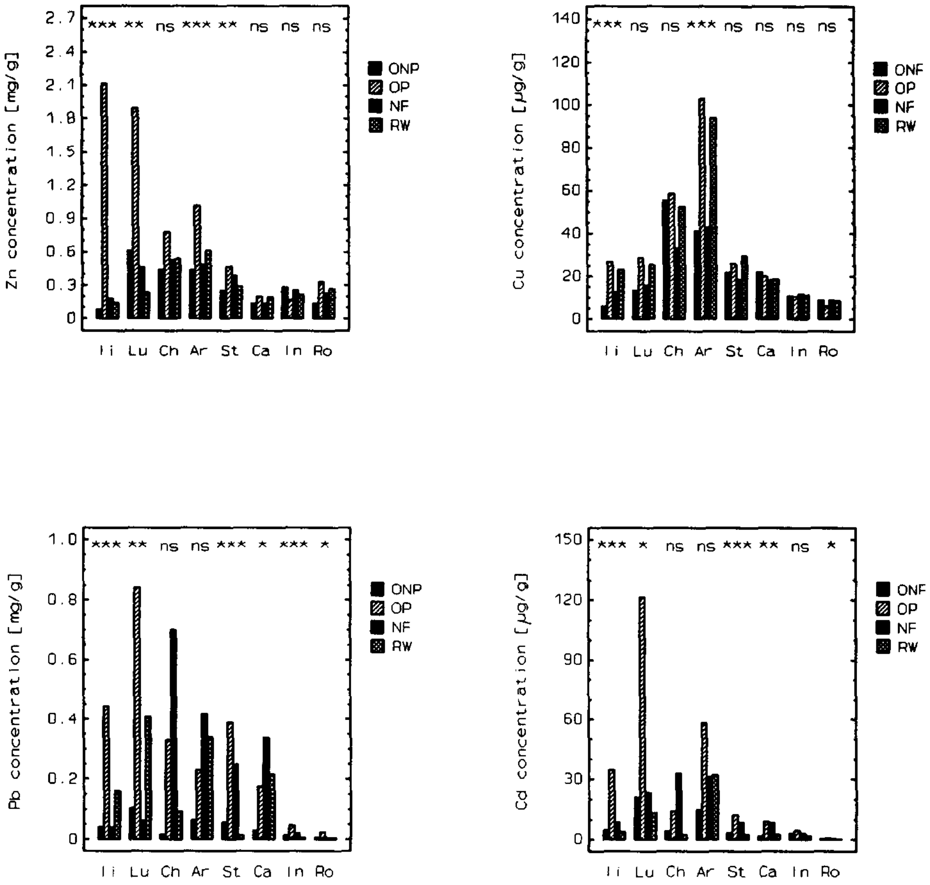

Figure 1. Between-plot comparison of zinc (Zn), copper (Cu), lead (Pb) and cadmium (Cd) levels in litter and in epigeic animals from four study sites; for descriptions of symbols see tab. 1.
*** - $P<0.001$
** - $P<0.02$
* - $P<0.05$
ns - not significant

a greater extent on excretion ability than on assimilation efficiency. The heavy metal content in terrestrial animals could, thus, be explained better by physiological factors than by trophic levels.

It seems that some regulation mechanisms pertain to all metals studied, but essential metals are controlled more efficiently than xenobiotic ones. Similar suggestions can be found in Van Straalen et al. (1987), Hegstrom and West (1989), Hopkin (1989) and Laskowski (1991). One should be cautious, however, interpreting results for Pb: its concentration in epigeic fauna seems to be influenced by some environmental factors other than Pb level in the soil organic layer.

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