Errata:

In case you are specifically interested in iron please note that concentrations of it in Table 1 are expressed in mg/g units for litter and soil. Further more, concentration factor for iron is 0.0075 (page 77).

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Heavy metal concentrations in ground beetles, leaf litter, and soil of a forest ecosystem

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Abstract

The objective of this study was to quantify the relationships between heavy metal concentrations in soil, leaf litter, and ground beetles at four sampling sites of a forest ecosystem in Medvednica Nature Park, Croatia. Ground beetles were sampled by pitfall trapping. Specimens were dry-ashed and soil and beetle samples digested with nitric acid. Lead, cadmium, copper, zinc, manganese, and iron were analyzed using atomic absorption spectrometry. Statistically significant differences between plots were found for lead, cadmium, and iron in ground beetles. Correlations between ground beetles and soil or leaf litter were positive for lead and cadmium concentrations and negative for iron concentration. Differences in species metal concentrations were recorded. Higher concentrations of all studied metals were found in female beetles. However, a significant difference between sexes was found only for manganese. Significant differences in species metal concentrations were found for species that differ in feeding strategies and age based on breeding season and emergence of young adults.

Keywords: Arthropods; Lead; Cadmium; Manganese; Copper; Zinc; Iron; Biological indicator; Croatia; Medvednica Nature Park

1. Introduction

Soil concentrations of heavy metals are the result of both natural and anthropogenic activities. Bloemen et al. (1995) reported higher concentrations of Cd, Cu, Pb, and Zn in the topsoil of woodlands than in the topsoil of arable land in Osnabrück and the surrounding countryside. The greatest accumulation of heavy metals in forest ecosystems occurs in the litter layer (Martin et al., 1982). Therefore, organisms inhabiting the humus horizon are often exposed to higher heavy metal levels (Hopkin, 1989). A large proportion of heavy metal particles in dust deposits on leaves is conveyed to the soil by precipitation and shedding of the leaves (Bloemen et al., 1995; Glavač et al., 1987). There are some data on heavy metal accumulation among various arthropods from both laboratory work (Crommentuijn et al., 1994; Janssen et al., 1991; Kramarz, 1999a, b; Nursita et al., 2005) and field studies (Blanuša et al., 2002; Janssen and Hogervorst, 1993; Rabitsch, 1994; Roth, 1993; Van Straalen et al., 2001), with many differences between groups. Ground beetles (Carabidae) are ground-active arthropods in both their larval and their adult stages, spending most of the time in topsoil and/or on the soil or litter layer. With respect to reproduction period, species can be divided into autumn breeders with maximum adult activity during late summer or autumn, spring breeders with maximum activity in spring or early summer, and carabid species with a flexible reproduction period (i.e., *Abax parallelepipedus)*. Larval development of autumn breeders occurs during winter, whereas spring breeder larvae appear in summer (Turin et al., 2003). Most are generalized carnivores, actively chasing their prey. However, some species of the genus *Carabus* are specialized for earthworms and snails, *Cychrus* species for snails and slugs, etc. (Hurka, 1996). Some macromorphological differentiation

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in feeding strategies of ground beetle species can be observed, such as cychrization, abacization, and procerization (Turin et al., 2003). Although ground beetles are second-order consumers, most recent studies have shown that with regard to metals, biomagnification through the food chain is not the obvious contamination pathway for many land animals (Markert et al., 1997; Rabitsch, 1994; Roth, 1993; Van Straalen et al., 2001). Some studies on heavy metal accumulation and decontamination under laboratory conditions have shown that carabids avoid metal poisoning through the rapid discharge of absorbed metals (Kramarz, 1999a) or decreased food intake (Maryanski et al., 2002).

Many studies have monitored ground beetles in extremely contaminated areas (Rabitsch, 1994; Stone et al., 2002; Van Straalen et al., 2001) and some have detected high levels of lead and cadmium in carabids (Rabitsch, 1994; Van Straalen et al., 2001). In field research on Pb levels in soil invertebrates of a forest ecosystem, Roth (1993) reported the lowest levels of Pb in staphylinids, carabids, tipulids, and spiders among the arthropods. Several studies such as that by Roth (1993) were conducted in protected areas with low-level contamination or natural heavy metal concentrations in the environment, reporting the relationship of their levels in ground beetles. This investigation is focused on ground beetles of forest ecosystems in the protected area of Mount Medvednica. According to Galović et al. (2000), contamination levels of Mt. Medvednica are within the normal range for forest soil or are slightly elevated for Pb, Mn, Zn, and Fe on some parts of the mountain. All specimens were determined at the species level, as many authors have suggested that species-specific differences in invertebrates are more important for metal accumulation than is trophic position (Kramarz, 1999a, b; Rabitsch, 1994; Van Straalen et al., 2001). Metal distributions in invertebrates also depend on various parameters such as sex, developmental stage, season, physiological conditions, etc. (Maryanski et al., 2002; Rabitsch, 1994; Stone et al., 2002; Van Straalen et al., 2001). We therefore analyzed metal concentrations in species and sex-determined adult specimens (all collected in the same season) with respect to their feeding preferences, body size, and age based on breeding season and emergence of adults. Mt. Medvednica is located north of Zagreb, the capital of Croatia, and is the country's largest urban and industrial center. The western part of Mt. Medvednica has several forest reserves and was proclaimed a Nature Park by the Nature Protection Act in 1981. Heavy metal concentrations in the topsoil cover reflect the bedrock lithology or old mining activities but can also be affected by deposition of airborne contaminants. The objectives of this study were to determine heavy metal concentrations in different species of ground beetles, litter, and soil of forest ecosystems, to estimate their mutual relationships, and to investigate the extent that ground beetles act as biological indicators of environmental pollution.

2. Materials and methods

2.1. Study area and sampling

According to metal concentrations from the geochemical map of Mt. Medvednica stream sediments (Galović et al., 2000), four locations in the Medvednica Nature Park area were selected for sampling of ground beetles, litter, and soil. All four locations (P1, P2, P3, P4) were situated in the same forest community, *Lamio orvale–Fagetum*, with beech as the dominant tree species on all plots to minimize differences in habitat conditions, soil properties, and type of organic matter composition. This provided a higher probability of capturing the same species on all four plots, similar leaf litter composition, and similar soil types. All locations had an acidic brown soil type and were situated on the southern slopes of the mountain (Fig. 1). There are various ore deposits, particularly in the central areas of Mt. Medvednica. In this investigation, two locations (plots P2 and P3) were situated near an old lead/zinc ore mine. Plot P2 was located on the top of the catchment area, closer to the entrance of the old mine, and plot P3 was placed a few hundred meters below, in the same catchment area, to investigate the impact of surface flow on metal concentrations in soil, litter, and ground beetles. Plot P4 was chosen as Galović et al. (2000) found the highest concentrations of studied metals, particularly cadmium, in soil there. Plot P1 served as a control, having the lowest concentrations of heavy metals.

Ground beetles were collected in 10 pitfall traps placed in three rows at the tips of 10-m sided triangles (Fig. 1) for each plot. Traps were emptied every 2 days in June and July 2003. Each beetle was identified at the species and sex levels, and then frozen in plastic bags until heavy metal analysis was conducted. Carabids were identified using specialized keys (Freude et al., 1976; Hurka, 1996; Trautner and Geigenmüller, 1987). From each plot 10 samples of approximately 3 L of leaf litter were collected and from the same locations the top 10 cm of soil under the leaves was collected at two depths (0–5 cm layer A and 5–10 cm layer B). Soil and litter samples were collected in plastic bags and were then ground, sieved through a 0.8-mm mesh, and stored at room temperature until trace element analysis was conducted. Soil pH was determined by adding 10 mL of deionized water to approximately 1 g of dried sample. To determine the percentage of organic matter, 10 g of soil was ashed at 800 °C.

2.2. Trace element analysis

Trace elements iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), lead (Pb), and cadmium (Cd) were measured using atomic absorption spectrometry (AAS), either flame or electrothermal, according to the concentration level of the element.

Air-dried leaf litter was ground, dried at 105 °C, weighed, and dry-ashed at 450 °C in a muffle furnace. Ashes were digested in concentrated nitric acid and adjusted with deionized water to form 0.5% nitric acid solution. Carabids were also dried at 105 °C, weighed, dry-ashed at 450 °C, and digested to form 10 mL 0.5% nitric acid solution. Collected soil samples were extracted with 30% nitric acid solution by the same procedure described earlier (Kučak and Blanuša, 1998). Extracts were filtered and adjusted to 25 mL to form 10% nitric acid solution.

Standard Reference Materials (SRM) were subjected to the same procedures as samples in the present study. San Joaquin Soil (2709, National Institute of Standards and Technology, USA) was used as internal quality control of the method applied to soil samples. This SRM was found to have a content of trace elements and organic matter similar to that of Croatian soils. Orchard Leaves (1571; National Bureau of Standards, USA) were subjected to the same procedure as the leaf litter. Bovine Liver (1577b; National Institute of Standards and Technology, USA) was used to compare the methods used for carabid analysis. All elements except cadmium were measured using flame AAS (AA375, Varian, Australia). Cadmium was measured using electrothermal AAS (AA300; Varian). All measurements were carried out with deuterium background correction. The recoveries of trace elements in Orchard Leaves ranged from 94% to 115%, with the exception of Cd (81%). In

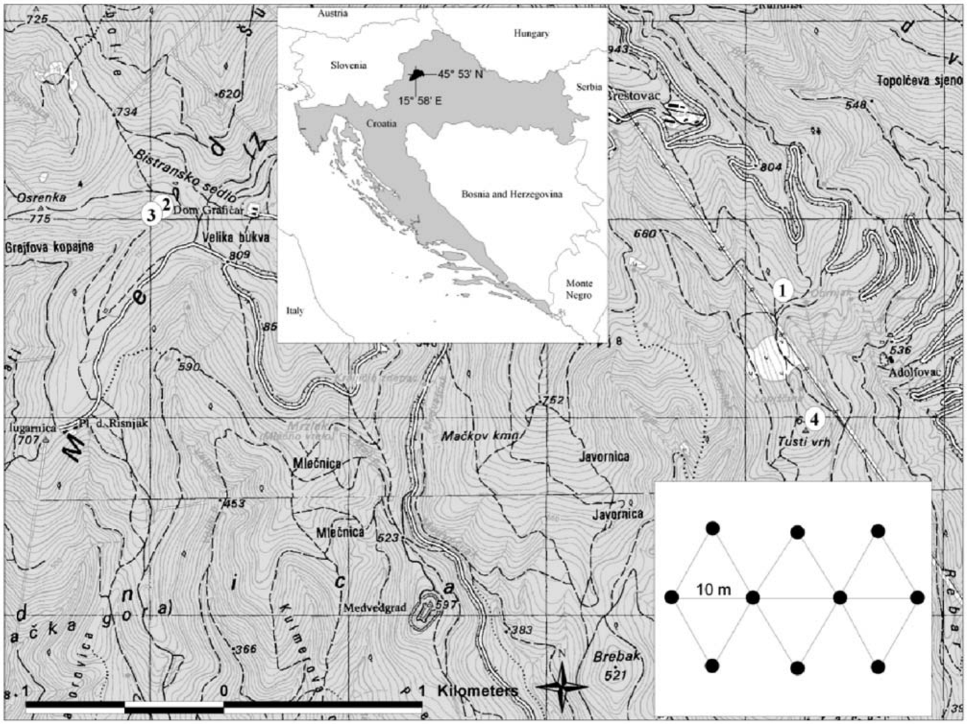

Fig. 1. Map of the sampling area with four sampling sites (denoted as 1, 2, 3, 4). Upper smaller map, position of the Medvednica Nature Park in Croatia; lower smaller map, distribution of pitfall traps per plot.

Bovine Liver, recoveries were between 91% (for Cd) and 112% (for Zn). In San Joaquin Soil, the extraction with nitric acid resulted in different recoveries from 35% for Fe and Zn to 61% for Cd, depending on the solubility of the inorganic salts in soil. Despite the low recoveries, the results of soil extraction in this study were not corrected because metal concentrations in San Joaquin soil tend to be rather low.

2.3. Statistical analysis

Statistical analyses were performed using the Statistica package, Ver. 6 (StatSoft Inc., Tulsa, OK, USA). Differences between sites were evaluated by the ANOVA test ($P = 0.05$). Heavy metal concentrations in the soil and litter were compared between sites and between layers and in the beetles were compared between sites, species, feeding strategies, body size, breeding season, and sexes. This was followed by a multiple comparison of the means using Tukey's honest significant difference (HSD) method. Statistical differences between the two soil layers, between the sexes, and between the two species of carabids common on all four plots were calculated using the Students t-test ($P = 0.05$). Pearson's product-moment correlation ($P = 0.05$) was performed between trace elements, acidity, and organic matter contents in soil, leaf litter, and ground beetles.

3. Results

3.1. Soil and leaf litter analysis

According to the ANOVA test, significant differences ($P = 0.05$) were found for all analyzed variables (i.e., heavy metal concentrations, organic matter content, and pH H_2O). Using Tukey's test, we isolated 12 groups of variables with significant differences between plots (Table 1).

Organic matter content in soil was significantly higher in plot P3 than in the remaining plots, and a positive significant correlation was found for Pb and Cd in layer A, contrary to negative correlations with the concentrations of other metals in both layers (Table 2).

The concentration of Fe in leaf litter varied significantly on all plots in comparison to the control plot P1, which had the lowest concentration of Fe (Table 1). The concentration of iron in soil either negatively correlated with other metals or was not significant (Table 2). Concentrations of Pb were significantly higher in leaf litter and layer A on plot P3 and in layer B on plot P4 in comparison to the control plot P1 (Table 1). The highest concentration of Cd was found on plot P4 in leaf litter and both soil layers with significantly different concentrations in relation to other plots (Table 1).

Based on the t-test between two soil layers, significant differences were found for Pb and Cd, with higher concentrations in the top layer A on plots P1, P2, and P3.

Concentrations of Zn, Mn, Pb, and organic matter content differed significantly between plots P2 and P3 both in the soil layers and in the leaf litter. Although plot P3 was more distant from the lead mine than P2, it is possible that higher lead concentrations and higher organic matter content in soil and leaf litter on P3 are the result of surface flow, as P3 lies below P2 in the same catchment area.

3.2. Ground beetle analysis

Altogether, 282 specimens belonging to nine species (*Carabus convexus*, *Carabus coriaceus*, *Carabus violaceus*, *Carabus nemoralis*, *Carabus intricatus*, *Carabus ullrichi*,

Table 1
Trace element concentrations (mg/kg dry weight), pH H_2O, and organic matter content in soil, litter, and ground beetles at four locations (results are presented as arithmetic means $\pm$ SD)

	n	pH	Org. matt. (%)	Pb	Cd	Mn	Cu	Zn	Fe
Plot P1									
Litter	10			$11.3^{3b} \pm 4.42$	$0.21^{6b} \pm 0.07$	500 ± 85.8	6.93 ± 0.85	41.5 ± 5.25	$0.47^{10a} \pm 0.24$
Soil (0–5 cm)	10	4.33 ± 0.27	$26.3^{1b} \pm 4.75$	$61.8^{4b} \pm 6.91$	$0.23^{7b} \pm 0.16$	534 ± 410	12.4 ± 1.52	65.8 ± 11.4	$13.4^{11a} \pm 0.90$
Soil (5–10 cm)	10	4.22 ± 0.15	$21.4^{2b} \pm 2.83$	$47.7^{6b} \pm 6.72$	$0.25^{8b} \pm 0.10$	353 ± 306	11.9 ± 1.82	60.5 ± 7.94	$14.4^{12a} \pm 1.57$
Ground beetles	38			$0.29^{b} \pm 0.19$	3.04 ± 2.63	68.9 ± 97.7	$25.1^{b} \pm 11.7$	$154^{b} \pm 43.3$	166 ± 109
Plot P2									
Litter	10			$19.3^{3b} \pm 9.13$	$0.24^{6b} \pm 0.07$	540 ± 157	9.28 ± 1.41	51.8 ± 7.01	$1.42^{10b} \pm 1.13$
Soil (0–5 cm)	10	3.97 ± 0.17	$29.2^{1b} \pm 4.33$	$55.0^{4b} \pm 13.1$	$0.34^{7b} \pm 0.17$	652 ± 299	10.1 ± 1.32	49.9 ± 4.39	$21.0^{11b} \pm 1.89$
Soil (5–10 cm)	10	3.95 ± 0.11	$22.6^{2b} \pm 3.27$	$40.8^{5b,6b} \pm 11.9$	$0.19^{8b} \pm 0.09$	521 ± 201	10.1 ± 1.58	47.9 ± 4.34	$24.0^{12b} \pm 1.23$
Ground beetles	24			$0.59^{b} \pm 1.17$	1.79 ± 1.92	114 ± 147	27.6 ± 14.2	132 ± 28.3	148 ± 77.8
Plot P3									
Litter	10			$40.4^{3a} \pm 20.7$	$0.26^{6b} \pm 0.07$	385 ± 80.6	8.03 ± 1.46	39.1 ± 5.17	$2.04^{10b} \pm 2.30$
Soil (0–5 cm)	10	3.52 ± 0.17	$48.9^{1a} \pm 14.7$	$77.7^{4a} \pm 16.0$	$0.40^{7b} \pm 0.11$	32.8 ± 18.8	11.7 ± 2.15	32.1 ± 11.6	$17.9^{11b} \pm 6.68$
Soil (5–10 cm)	10	3.61 ± 0.18	$37.0^{2a} \pm 12.1$	$58.7^{5a} \pm 11.0$	$0.26^{8b} \pm 0.14$	26.9 ± 12.5	11.2 ± 1.95	24.0 ± 8.48	$26.5^{12b} \pm 9.11$
Ground beetles	17			$1.71^{a} \pm 2.67$	2.56 ± 1.57	83.3 ± 93.0	29.2 ± 18.9	136 ± 20.0	126 ± 41.1
Plot P4									
Litter	10			$23.9^{3b} \pm 10.6$	$0.39^{6a} \pm 0.09$	1070 ± 244	9.72 ± 1.57	54.2 ± 8.70	$2.97^{10b} \pm 2.40$
Soil (0–5 cm)	10	5.35 ± 0.28	$19.7^{1b} \pm 3.96$	69.4 ± 11.7	$0.41^{7a} \pm 0.11$	5883 ± 1529	22.4 ± 5.01	85.7 ± 14.2	$20.1^{11b} \pm 3.20$
Soil (5–10 cm)	10	5.12 ± 0.18	$15.3^{2b} \pm 1.89$	$65.3^{6a} \pm 11.0$	$0.42^{8a} \pm 0.15$	5772 ± 1829	21.5 ± 4.32	81.0 ± 14.4	$20.9^{12b} \pm 3.36$
Ground beetles	55			$0.21^{b} \pm 0.15$	3.47 ± 3.90	78.9 ± 120	$20.6^{a} \pm 9.34$	$134^{a} \pm 26.6$	155 ± 65.9

Significant differences in heavy metal concentrations in sites and in layers found with Tukey's HSD test are indicated with different superscripts (same number with different letters denotes significantly different concentrations at the 0.05 level) for soil layers and leaf litter. For ground beetles, only letters were used.

Means from different sites bearing different superscripts (same number and different letter) differ significantly from each other at the 0.05 level (example: 1a differs significantly from 1b). Different numbers with the same or different letters do not differ signifficantly.

Table 2
Coefficient of correlations (*r*) between heavy metal concentrations, pH H_2O, and organic matter content in soil

Variable	Fe	Zn	Cu	Mn	Cd	Pb	Org. matt.	pH
Fe		−0.50	NS	NS	NS	NS	NS	NS
Zn	NS		0.75	0.72	0.54	NS	−0.62	0.85
Cu	NS	0.77		0.79	0.60	0.52	−0.43	0.80
Mn	NS	0.77	0.83		0.63	0.46	−0.51	0.86
Cd	NS	NS	NS	0.32		0.63	NS	0.47
Pb	−0.36	NS	NS	NS	0.41		NS	0.35
Org. matt.	−0.38	−0.56	−0.36	−0.51	0.33	0.55		−0.62
pH	NS	0.80	0.74	0.86	NS	NS	−0.62	

Coefficients of correlation obtained for soil layer A (0–5 cm) are in the lower left section and those for soil layer B (5–10 cm) in the upper right section. Only coefficients with $P < 0.05$ are shown (NS, not significant).

Abax parallelepipedus, *Abax parallelus*, and *Cychrus attenuatus*) were collected (Table 3). Of the nine studied species on four plots, three were common to all plots: *C. violaceus*, *C. intricatus*, and *C. attenuatus* (Table 3).

The ANOVA with Tukey's HSD tests showed significantly lower concentrations of Cu and Zn in carabids on plot P4 than in carabids on the control plot P1, although the highest Cu and Zn concentrations were measured in the soil and leaf litter of this plot (Table 1). The overall carabid Pb concentrations were significantly higher on plot P3 than on other plots (Table 1).

The element concentration factor, calculated as the ratio of the concentrations in ground beetles and soil, showed the ability of ground beetles to take up most of the analyzed metals irrespective of the sampling location. In this study, cadmium was concentrated with a factor of 8.7, iron with 7.5, zinc with 2.5, and copper with 1.8. Factors for lead and manganese were 0.02 and 0.05, respectively.

Concentrations of Cd and Pb in carabids correlated positively while Cu and Zn correlated negatively with those concentrations in the soil ($r = 0.19$ for Cd, $r = 0.15$ for Pb, $r = -0.26$ for Cu, $r = -0.14$ for Zn), although

Table 3
Trace element concentrations (mg/kg dry weight) in different species of ground beetles at four locations

Species	fs, bs	mbl	Plot	*N*	Pb	Cd	Mn	Cu	Zn	Fe
Carabus convexus	pr, s	18	1	8	0.32±0.13	0.40±0.36	36.4±19.1	21.6±7.20	120±19.2	135±64.7
			4	10	0.32±0.19	0.34±0.21	43.6±19.9	15.9±2.31	102±11.8	158±84.4
Carabus coriaceus	pr, a	36	1	7	0.30±0.20	3.30±2.80	67.4±28.8	33.3±23.8	148±63.5	158±84.1
			4	10	0.13±0.10	5.69±4.40	58.1±42.6	30.7±16.6	145±29.5	182±84.1
Carabus violaceus	pr, a	30	1	42	0.32±0.20	4.89±1.90	112±154	27.1±6.80	193±25.6	220±162
			2	31	0.87±1.48	2.55±2.19	174±167	30.8±12.1	147±21.1	176±90.8
			3	16	2.31±3.10	3.05±1.53	77.2±86.7	23.6±6.40	138±17.8	120±41.7
			4	20	0.14±0.07	5.78±4.20	173±249	22.2±3.90	149±17.9	178±58.8
Carabus nemoralis	pr, a	26	1	3	0.19±0.06	6.28±1.65	26.1±3.40	17.8±1.28	139±16.8	162±48.6
			4	4	0.15±0.05	10.6±9.90	73.3±68.8	19.5±0.99	120±19.3	155±0.63
Carabus intricatus	cy, s	30	1	8	0.20±0.07	0.61±0.32	61.3±64.5	22.1±6.42	122±16.8	113±28.7
			2	10	0.16±0.06	0.84±0.60	16.5±6.80	19.2±1.35	102±19.6	107±29.4
			3	8	0.49±0.25	0.85±0.21	32.4±13.2	20.7±4.64	127±19.4	122±25.6
			4	22	0.24±0.21	0.80±0.70	59.9±52.4	17.8±4.40	126±18.7	115±31.4
Carabus ullrichi	pr, s	28	1	3	0.37±0.32	2.40±0.21	19.0±17.6	14.4±3.10	130±28.7	117±13.7
			4	22	0.20±0.17	3.42±1.61	36.9±18.1	13.1±5.10	113±18.0	164±63.0
Abax parallelepipedus	ab, s and a	20	1	2	0.27±0.01	0.27±0.01	29.0±11.9	19.4±1.74	129±7.14	151±26.3
			4	27	0.17±0.07	1.92±1.25	59.3±81.0	15.1±2.12	154±25.7	113±37.2
Abax parallelus	ab, s	16	1	2	0.52	0.64	42.2	38.1	177	157
			2	2	0.25	0.21	29.5	13.9	140	110
			4	13	0.39	0.45	56.4	15.8	129	193
Cychrus attenuatus	cy, a	16	1	3	0.29	0.31	69.8	31.4	103	123
			2	2	NR	1.03	117	69.7	120	113
			3	5	0.85	3.35	219	76.9	148	171
			4	2	1.06	4.72	249	83.2	129	152

Results are presented as arithmetic means ± SD, if available (fs, feeding strategies; pr, procerus group; cy, cychrus-group; ab, abax group; bs, breeding season; s, spring; a, autumn; mbl, mean body lenght/mm; *N*, number of caught specimens; NR, there are no results because measurement failed).

not significantly. Correlation factors for Mn and Fe were very low.

The analysis of metals in relation to species produced the following results: on plot P1, no statistically significant differences between the species were found; on plot P2, species differed in concentrations of Mn, Cu, Zn; and on plot P3, they differed only in Pb concentrations; and on plot P4, the species differed in Cd concentrations. The ANOVA test showed no significant difference in metal concentrations on the four plots for *C. intricatus*. For *C. violaceus*, statistically significant differences in Pb concentrations were found for plot P3 and the control plot and for plots P3 and P4. Higher Pb concentration in *C. violaceus* on plot P3 was accompanied by higher Pb concentration in leaf litter and soil layer A. The ANOVA test was applied to compare the Cd concentrations in different carabid species on each plot. With regard to Cd concentrations on plot P4, there were statistically significant differences between *C. intricatus* and *C. coriaceus,* between *C. nemoralis* and *C. violaceus*, and between *A. parallelepipedus* or *C. convexus* and *C. nemoralis*. The concentrations of Cd in *C. convexus* (collected on plots P1 and P4) and *C. intricatus* (collected on all four plots) did not correspond to Cd concentrations in the soil. On all plots, the concentrations of most of the studied metals were higher in females than in males; however, these differences were not significant according to the *t* test, with the exception of manganese. Species were divided into three groups according to feeding preferences (Table 3): the specialist cychrus group feeding on smaller snails, the procerus group feed on larger snails and earthworms, and the abax group feeding on different invertebrate groups and carrion (Turin et al., 2003). The ANOVA test followed by the Tukey's HSD test showed significant differences between the procerus group and the other two groups with regard to Cd concentrations (both $P<0.005$, Fig. 2). The mean body size of the species ranged from 16 to 36 mm (Table 3). The Tukey's HSD test revealed significant differences only in cadmium concentrations in the 26-mm body size (represented by one species, *C. nemoralis*) and the other carabids ($P<0.003$).

According to the ANOVA and Tukey's HSD tests, autumn breeders contained significantly higher concentrations of Fe, Zn, Cu, Mn, and Cd than spring breeders and of Cd than animals with flexible breeding periods ($P<0.001$) (Fig. 3).

4. Discussion

Heikens et al. (2001) ranked taxonomic groups of terrestrial invertebrates according to metal accumulation.

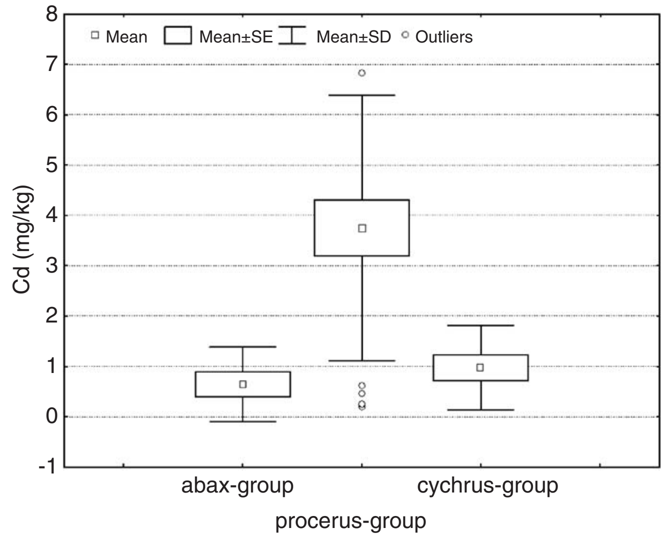

Fig. 2. Cadmium concentrations in ground beetles according to feeding strategies (results are presented as arithmetic means ± SE and SD).

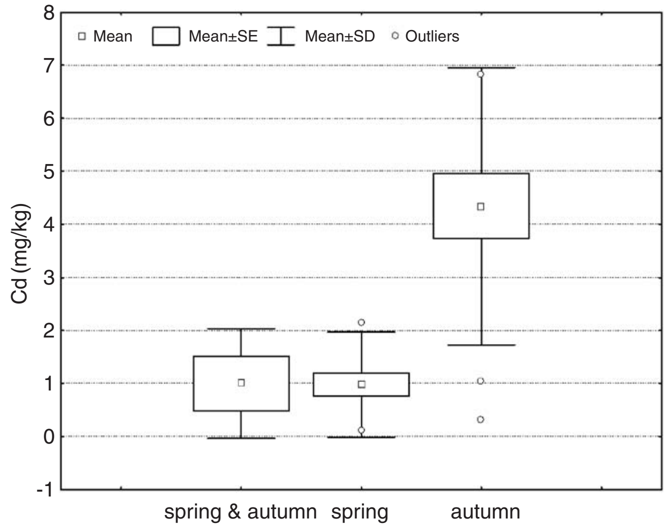

Fig. 3. Cadmium concentrations in ground beetles according to their breeding season (results are presented as arithmetic means ± SE and SD).

High metal concentrations have been associated with isopods and low concentrations with Coleoptera: Pb, Cd, and Cu concentrations increased with soil concentrations for most taxonomic groups in the following order: Pb > Cd > Cu. Van Straalen et al. (2001) reported high concentrations of Cd and Pb in ground beetles, oribatid mites, and earthworms and low concentrations in springtails, centipedes, and spiders near a metallurgical factory. Concentration factors obtained in this study compared to those in a previous study with woodlice (Blanuša et al., 2002) showed that the cadmium factor is much higher and lead factor lower in ground beetles. This indicates that each group has its own uptake and ability to concentrate trace elements. Due to higher cadmium concentrations in ground beetles, cadmium measurements are much easier. Therefore, ground beetles seem to be better indicators of cadmium presence in the environment than, e.g., woodlice.

In this investigation, Cd and Pb concentrations in ground beetles corresponded to those concentrations in the soil and leaf litter, which was not the case for other metals. Carabids sampled on plots P2 and P3, where the highest Pb concentations in soil and litter were found, had higher Pb concentrations than specimens from the remaining two plots.

Stone et al. (2002) showed that Cu concentrations in ground beetles seemed to be efficiently regulated regardless of metal levels in soil. Rabitsch (1994) observed that regulatory mechanisms for Cu appeared to be established in most cases. The concentration of nutritional metals (i.e., Cu, Zn) can be regulated more efficiently than that of xenobiotic metals (i.e., Pb or Cd) (Kramarz, 1999a,b). Kramarz (1999a) showed that the ground beetle *Poecilus cupreus*, when exposed to elevated concentrations of Zn in food, maintained constant internal Zn concentrations, while in beetles fed with Cd-contaminated food Cd concentrations after rapid initial increase are stabilized. The beetles excreted Cd efficiently when switched to non-Cd-contaminated food. The results of this study confirm that finding and show that the concentrations of essential metals such as Zn, Cu, Fe, and Mn in ground beetles do not correspond to concentrations in soil and litter, which suggests a regulation efficiency of these metals different from those of Pb and Cd. Stone et al. (2002) found significant differences in carabid female enzyme activity along a metal gradient, which was not recorded for males. Rabitsch (1994) revealed elevated concentrations of non-essential elements in females of two carabid species, while males accumulated more of the essential elements Zn and Cu. A similar trend was evident in our investigations, where females accumulated more Cd and Pb than Zn and Cu; however, males showed no trend in accumulating more Zn and Cu. Statistically significant differences between the sexes were seen for Mn among the studied metals, although there were no significant correlations between concentrations in ground beetles and in soil and litter.

Rabitsch (1994) confirmed the accumulation of metals in litter and plant species- and element-specific concentration patterns. The leaf litter sampled during this investigation originates from sampling sites in the same forest ecosystem and consists mainly of beech leaves, which reduces the influence of the plant species on the concentration of metals. The concentrations of Pb in carabids, unlike other metals, correspond well to Pb concentrations in leaf litter (Table 1). The lowest concentration of lead, in comparison to other samples, was recorded in carabids sampled on plot P4, despite the higher Pb concentrations sampled in soil layer B. This indicates that the leaf litter rather than the soil layers may have the greatest impact on Pb concentrations in carabids. However, Cd concentrations in carabids correspond well to Cd in soil layer B on all four plots. Carabids are most active on the soil surface between the soil and the litter layer (Hurka, 1996). They spend their inactive stages (egg, pupa, hibernation) primarily in the soil or under decaying wood.

Species with narrow pronotum, head, and mandibles are able to prey on small-sized snails entering the shell (cychrization, i.e., *C. attenuatus*, *C. intricatus*) and most are specialists. Species with broader head and pronotum can prey on larger snails (procerization, i.e., *C. coriaceus*), and some also feed on earthworms. Our results showed that carabids of the procerus group concentrate more Cd than carabids of the cychrus and abax groups. For *C. violaceus* of the procerus group, great preferences were recorded for juvenile slugs appearing in September (Paill, 2000). During the spring and early summer they may consume alternative foods such as earthworms. Juvenile earthworms appear from spring to autumn (Mršić, 1997) and feed more on soil organic matter than adults. As such, they are exposed to greater metal concentrations in their diet because metals tend to be associated with the organic rather than the inorganic components of the soil (Hopkin, 1989). Also, earthworms from the upper soil layer and litter layer are likely more available prey for carabids.

Species that reproduce in autumn have overwintering larvae and young adults appear in the spring. Spring breeders hibernate as young adults and are active in the following spring. In this investigation, all specimens were collected during late spring and early summer, and therefore autumn breeders were younger than spring breeders. According to this investigation, autumn breeders accumulate more Cd than spring breeders which were overwintering adult species. Those results indicate differences in metal concentrations in ground beetles due to food preferences and age. Further investigations should be aimed at revealing the relationships between metal concentrations in ground beetles and life history and type of hibernation.

Species-specific differences were also observed. For *C. intricatus*, no significant differences in the concentrations of metals on the four plots were recorded. One difference between *C. intricatus* and the other two species common on all four plots is their different habitat range. In the literature (Casale et al., 1982; Hurka, 1996), *C. intricatus* is classified as a stenotopic species. Eurytopic species are found in a wide range of habitats, whereas stenotopic species are specific to one or only a few habitats (Eversham et al., 1996). Conservation efforts due to environmental changes are focused on habitat-specific species, which can become extinct when their habitats are destroyed or degraded. According to Eversham et al. (1996), most stenotopic species among the ground beetles have declined in Great Britain, and a similar trend has been found for several other countries. Low concentrations in *C. intricatus* in comparison to those in other species may be a consequence of elimination mechanisms activated on a lower body concentration of metals due to high sensitivity to environmental factors, as this species is accustomed to very specific habitat conditions. Janssen et al. (1991) recorded high cadmium assimilation efficiencies with a high excretion rate, which resulted in a low equilibrium concentration in the carabid *Notiophilus biguttatus*. Crommentuijn et al. (1994) revealed differences in bioavailability in species on the basis of lethal body concentrations. Species with the highest concentrations did not run the highest risk.

As data on the impact of environmental pollutants on the population structure and spatial distribution of terrestrial invertebrates exist for certain heavy metals (Faulkner and Lochmiller, 2000; Read et al., 1998; Spurgeon and Hopkin, 1999), this study should be supplemented with research on variation in the composition and abundance of ground beetle communities depending on abiotic factors. Future research should take into account species-specific differences and include more extensive laboratory studies to establish the physiological foundations and ecological determination of these differences. Laboratory research on Cd and Pb mechanisms of elimination should be performed on a number of species. Depending on the composition of leaf litter, the influence of lead in leaf litter on carabids should also be examined in other plant communities.

Knowledge of ground beetle biology and their reactions under natural conditions is necessary to foresee the conditions of the organisms and their responses to potential changes and negative influences in the environment. Due to the fact that this study was conducted in a protected area with a low pollution level, but also in the vicinity of one of the country's largest urban and industrial centers, there is a need for permanent monitoring of this area in the best possible way and, if necessary, for additional protection.

5. Conclusions

Among the studied metals, lead and cadmium showed differing trends of concentrations in carabids. Concentrations of lead and cadmium in carabids generally corresponded to those in the soil and leaf litter.

Due to the configuration of the terrain, surface flow should be considered, as the lower parts of the catchment area showed higher concentrations of lead in soil or leaf litter and carabids. The concentration of lead in the leaf litter suggests a stronger relationship to Pb concentrations in carabids than does the concentration of lead in soil. Due to high cadmium concentration in ground beetles, they can be used as indicators of cadmium presence in the environment.

This study has shown differences in metal concentrations in different species, which suggests that attention should be paid not only to indicator groups but also to indicator species that are more susceptible to changes in the environment and hence more subject to possible negative impacts of heavy metals such as lead and cadmium. Feeding habits and age of the beetles have significant impacts on metal concentrations, particularly on cadmium. The concentrations of studied metals in *C. intricatus* showed no increase with the gradient in the soil and in the leaf litter on all four plots as compared to those in *C. violaceus*. It would therefore be necessary to investigate the ecological reasons and the physiological grounds for such reactions in a particular species.

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