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CONTENT OF HEAVY METALS IN BODIES OF FIELD GROUND BEETLES (COLEOPTERA, CARABIDAE) WITH RESPECT TO SELECTED ECOLOGICAL FACTORS

ABSTRACT: The concentration of heavy metals in the bodies of invertebrates is dependent on their physiological equipment and prevalent environmental factors. To verify the effect of some of these factors on the content of metals (Pb, Cd, Zn, Cu, Mn) we analysed and then tested (using RDA, t-test) ten species of field ground beetles (Coleoptera, Carabidae).

A significant effect of Cu and Cd was discovered in terms of the sex; the males accumulated more Cu (27.520 mg kg⁻¹) than females (18.297 mg kg⁻¹) ($P < 0.01$), which, on the contrary, accumulated more Cd (1.495 mg kg⁻¹) than males (0.663 mg kg⁻¹) ($P < 0.02$). The content of all the metals differed significantly ($P < 0.03$) according to the species, unambiguously showing species-specific models of accumulation. The effect of the feeding ecology was evident only on the essential elements; carnivores (Zn – 222.596 mg kg⁻¹, Cu – 27.211 mg kg⁻¹, Mn – 71.929 mg kg⁻¹) had a significantly ($P < 0.03$) higher contents than omnivores (Zn – 168.198 mg kg⁻¹, Cu – 21.116 mg kg⁻¹, Mn – 58.452 mg kg⁻¹). Although there were differences ($P < 0.01$) in the concentrations of Zn and Cu between the spring (Zn – 163.749 mg kg⁻¹, Cu – 19.998 mg kg⁻¹) and autumn (Zn – 202.373 mg kg⁻¹, Cu – 25.496 mg kg⁻¹) species, the effect of the type of reproduction is considered to be only partial. At the same time the time of sampling affected the Zn and Mn ($P < 0.02$) content. An important positive correlation was determined

between the contents of Cu-Zn, Mn-Zn and Mn-Cu.

KEY WORDS: heavy metals, breeding type, carnivores, omnivores, trophic groups, accumulation, seasonal aspects, sex

1. INTRODUCTION

Metals such as Pb, Cd, Zn, Cu and Mn and microelements in general are widely distributed and naturally occurring elements in the environment. For most organisms their low concentrations are harmless; some of them occurring in trace amounts are of vital importance as essential elements for organisms to function (Clarkson 1979, Tyler *et al.* 1989). Increasing their concentrations in the ecosystems as a consequence of human activities (waste, fertilisation, combustion of fossil fuel etc.) may evoke developmental changes and weakening and even extinction of organisms living in these ecosystems (Beijer and Jernelöv 1979, Tyler *et al.* 1989).

Heavy metals in the soil find their way into invertebrates and into higher trophic levels through the food web from soil via detritivores or plants. Although the assumption of biological accumulation of anthropogenic foreign substances from the phytophages

to carnivores was confirmed (Price *et al.* 1974), recent studies (e.g. van Straalen and van Wensem 1986, Lindquist and Block 1997) proved that this pattern cannot be applied as a general rule, at least not for metals (Kramarz 1999).

During the accumulation of metals in the bodies of invertebrates the concentrations do not increase in direct proportion to the trophic level and are not dependent on body size (van Straalen and van Wensem 1986, Lindquist and Block 1997). They are not even similar in the same ecologic groups – predators like in Carabidae and Araneae. Ground beetles have much lower levels of concentrations because of the different strategy in food intake. The concentration of metals in the bodies of invertebrates considerably differs among the taxonomic groups, species and specimens of the same species (Tyler *et al.* 1989). The content of metals in the body of invertebrates is dependent on the type of food and chemical form of the metal in the food (Lindquist and Block 1997), on concrete conditions during food intake (e.g. soil concentration, pH, availability of the metals etc.) and most of all on the physiological equipment of the individual invertebrate species (Ma 1982; Ma *et al.* 1983), which is the assimilation capacity and, above all, excretion of the element (Janssen *et al.* 1991, Lindquist *et al.* 1995; Kramarz 1999).

Ground beetles (Coleoptera, Carabidae) occurring in the Czech Republic generally have a one-year developmental cycle and according to the time of reproduction they can be divided into two groups. About two thirds reproduce in spring, their larvae develop in early summer and new imagos hatch in late summer or in autumn and then they overwinter; it is what we call reproduction without larval diapause (with gonad diapause). Characteristic of the remaining species is the development with larval diapause when reproduction takes place in autumn, the larvae overwinter together with the imagos and the new generation hatches from these larvae in spring or early summer (Hůrka 1996). In the temperate zone the ground beetles are a dominant component of the epigeic invertebrate fauna on most of the terrestrial sites. They are mostly non-specific predators who may feed on springtails (*Notiophilus*,

Leistus), earthworms, snails (*Carabus*), molluscs (*Cychnus*), millipedes, larvae and adults of weevils, caterpillars of butterflies (*Calosoma*) etc. or they may behave as omnivores tending more to either herbivorous or carnivorous feeding (*Harpalus*), and/or as specific herbivores feeding on young plants or seeds (*Ophonus*, *Amara*) (Laroche 1990, Hůrka 1996). The consequence of feeding specialisation is a different intake of heavy metals into their bodies.

With the exception of Read *et al.* (1987, 1998) most studies involved in the contents of heavy metals in ground beetles were focused more on the individual species and physiological processes than on communities and ecologic aspects. The objective of the study was to contribute to clarify the relations among the ecologic effects (type of reproduction, feeding ecology, seasonal aspect etc.) in ground beetle populations and their effect on the metal content (Pb, Cd, Zn, Cu, Mn) in bodies of ground beetles.

2. STUDY AREA, MATERIAL AND METHODS

The monitoring area is located in Chrlice (Brno, Czech Republic; 49° 08' 04'' N, 16° 39' 31'' E) on altitude 190 m. It is warm climate district, moderately dry, average annual temperature = 9.9 °C and annual sum of precipitation = 509 mm. It is the sugar-beet production region, with flatland, arable soil (fluvisol, medium heavy sandy loam), cultivation of winter rape (*Brassica napus* L.) (2002) and spring wheat (*Triticum aestivum* L.) (2003). Hazardous elements (Cd – 2.25 mg kg⁻¹, Cr – 21.6 mg kg⁻¹) were in past introduced into the soil with applications of contaminated sludge from the sewage works. The sludge was mixed with the soil by ploughing.

In 2005 we analysed 906 specimens of 10 species of ground beetles entrapped during faunistic-ecological studies in a field biotope (agrocoenosis with winter wheat). The beetles (total number of collected specimens was 4793 belonging to 45 species) were entrapped (using pitfall Barber traps) in the field biotope of the monitoring area of the Central Institute for Supervising and Testing in Agriculture (CISTA) under long-term loading with heavy metals (Purchart and Kula 2005).

The samples were assorted according to species (Table 1), sex and date of collection. The number of specimens in one sample ranged from two specimens (the biggest species) to fifty specimens (the smallest species). After drying and weighing, the samples were subjected to dry mineralization for 14 hours in nitrogen dioxides (NO_x) and ozone at a maximal temperature of 400°C in the Apion mineraliser. The ash was hot-dissolved in 5% nitric acid and analysed using ICP MS (Inductively Coupled Plasma-Mass Spectrometry) (Ortega 2002) (ICP MS spectrometer Elan 6000 – Perkin Elmer, argon flow 0.85 l.min⁻¹, RF regenerator output 1000W; Department of Chemistry and Biochemistry, Mendel University of Agriculture and Forestry in Brno). The contents of Mn, Zn, Cu, Pb and Cd (mg kg⁻¹) were determined.

All the analysed species were classified according to their food preference (carnivorous, omnivorous) and type of reproduction (spring, summer) (Table 1) (Petruška 1967, 1986, Bousquet 1986, Laroche 1990,

Cole *et al.* 2002). The names of taxons of the ground beetles are in accordance with Hůrka (1996).

We used the redundancy analysis (RDA) in CANOCO for Windows 4.5 to find the connection between the content of metals (species data) in bodies of ground beetles and the type of reproduction, feeding ecology, seasonal aspects, concrete species and sex (environmental data). Due to an insufficient number of samples the effect of sex at the level of the species was not determined. CANOCO tested the significance of the effect of environmental factors using the Monte Carlo Permutation test. In our case the RDAs were run with CANOCO's default options: scaling focused on inter-species correlations, species scores divided by the standard deviation, species data were log transformed, species centred by species scores, samples not centred. Concrete environmental factors were tested separately and the significance of their effect on the content of the individual metals was explored. To confirm the RDA

Table 1. Body content of metals in analysed species of Carabidae (mg kg⁻¹; S.D. = standard deviation; N = number of analysed specimens; n = number of samples; α = 0.05), trophic groups (o = omnivores; c = carnivores) and breeding type (sb = spring breeder; ab = autumn breeder).

| Species | N | n | Trophic group | Breeding type | Pb | | Cd | | Zn | | Cu | | Mn | |
|---|-----|---|---------------|---------------|-------|-------|-------|-------|---------|--------|--------|--------|--------|--------|
| | | | | | mean | S.D. | mean | S.D. | mean | S.D. | mean | S.D. | mean | S.D. |
| <i>Anchomenus dorsalis</i> (Pontoppidan, 1763) | 364 | 8 | o | sb | 1.295 | 0.432 | 0.901 | 0.408 | 179.241 | 32.603 | 23.931 | 4.745 | 78.44 | 25.786 |
| <i>Calathus erratus</i> (C. R. Sahlberg, 1827) | 84 | 8 | c | ab | 2.155 | 1.890 | 1.221 | 0.599 | 221.132 | 19.791 | 21.59 | 5.924 | 65.532 | 17.073 |
| <i>Calathus fuscipes</i> (Goeze, 1777) | 70 | 4 | c | ab | 0.657 | 0.173 | 1.185 | 1.041 | 216.553 | 5.156 | 33.628 | 16.672 | 83.763 | 32.163 |
| <i>Calosoma auropunctatum</i> (Herbst, 1784) | 8 | 4 | c | ab | 0.691 | 0.326 | 0.389 | 0.079 | 193.228 | 29.537 | 21.665 | 3.029 | 62.845 | 26.611 |
| <i>Harpalus affinis</i> (Schränk, 1781) | 133 | 8 | o | sb | 0.711 | 0.352 | 0.304 | 0.122 | 148.699 | 30.247 | 21.489 | 10.357 | 49.105 | 13.181 |
| <i>Harpalus distinguendus</i> (Duftschmid, 1812) | 19 | 8 | o | sb | 2.847 | 2.115 | 2.731 | 4.680 | 173.173 | 53.189 | 17.686 | 7.911 | 47.781 | 15.176 |
| <i>Leistus ferrugineus</i> (Linnaeus, 1758) | 33 | 4 | c | ab | 3.093 | 3.368 | 0.889 | 0.416 | 260.938 | 47.687 | 37.583 | 11.603 | 81.975 | 32.632 |
| <i>Poecilus cupreus</i> (Linnaeus, 1758) | 77 | 8 | o | sb | 0.604 | 0.262 | 0.488 | 0.173 | 153.885 | 41.845 | 16.885 | 5.783 | 62.803 | 26.652 |
| <i>Pseudoophonus rufipes</i> (De Geer, 1774) | 68 | 8 | o | ab | 1.150 | 0.463 | 0.489 | 0.208 | 166.624 | 24.050 | 20.155 | 6.277 | 57.883 | 20.817 |
| <i>Pterostichus melanarius</i> (Illiger, 1798) | 50 | 8 | o | ab | 0.648 | 0.329 | 1.805 | 1.343 | 187.566 | 41.943 | 26.551 | 12.066 | 54.700 | 14.106 |

Table 2. Content of metals in Carabidae (mg kg⁻¹; S.D. = standard deviation; N = number of analysed specimens; n = number of samples; $\alpha = 0.05$) in respect to trophic groups, time of reproduction and sex.

| species group | | | Pb | | Cd | | Zn | | Cu | | Mn | |
|-----------------|-----|----|-------|-------|-------|-------|---------|--------|--------|--------|--------|--------|
| | N | n | mean | S.D. | mean | S.D. | mean | S.D. | mean | S.D. | mean | S.D. |
| omnivores | 711 | 48 | 1.209 | 1.178 | 1.120 | 2.085 | 168.198 | 38.906 | 21.116 | 8.532 | 58.452 | 21.613 |
| carnivores | 195 | 20 | 1.750 | 2.016 | 0.981 | 0.663 | 222.596 | 33.838 | 27.211 | 11.429 | 71.929 | 24.743 |
| spring breeders | 593 | 32 | 1.364 | 1.387 | 1.106 | 2.439 | 163.749 | 40.611 | 19.998 | 7.709 | 59.532 | 23.675 |
| autumn breeders | 313 | 36 | 1.372 | 1.576 | 1.055 | 0.902 | 202.373 | 40.805 | 25.496 | 10.787 | 64.979 | 22.849 |
| males | 450 | 34 | 1.636 | 1.896 | 0.663 | 0.519 | 179.876 | 49.081 | 27.520 | 10.470 | 60.061 | 22.680 |
| females | 456 | 34 | 1.100 | 0.837 | 1.495 | 2.414 | 188.517 | 40.419 | 18.297 | 6.428 | 64.770 | 23.868 |

results, the significance of differences in average values for the respective variables (environmental factors) was assessed using the t-test (for the independent variables) in the STATISTICA Cz 6.1. programme, in which also the correlation coefficients were determined (Pearson's correlation coefficient) for correlations among the metals.

3. RESULTS

3.1. Lead

The content of lead in the soil of the locality was 31.6 mg kg⁻¹ and was in the range of the natural concentration of Pb in the soil (2 – 200 mg kg⁻¹) (Tsuchiya 1979). In zoophages the content of lead was slightly higher (average of 1.750 mg kg⁻¹) than in omnivores (average of 1.209 mg kg⁻¹) but the difference was insignificant (Tables 2, 3, 4). The level of lead was the same in species reproducing in the autumn (average 1.372 mg kg⁻¹) and in spring (average 1.364 mg kg⁻¹) (Table 2). The effect of sex and time of sampling on the Pb content were also insignificant. A significant effect (RDA: $F = 5.044$, $P = 0.001$) on the concentration of lead in the bodies of ground beetles was detected only at the level of species; nevertheless significant differences were detected only among the omni-

vores (RDA: $F = 8.173$, $P = 0.001$) (Table 3). The highest absolute value was 8.11 mg kg⁻¹ in a *Leistus ferrugineus* (Duftschmidt) male, while the lowest was noticed in a *Harpalus affinis* (Schrank) male – 0.31 mg kg⁻¹.

3.2. Cadmium

In study area the cadmium concentration in the soil was not very high (2.44 mg kg⁻¹) compared to the natural concentration (<1 mg kg⁻¹) reported by Friberg *et al.* (1979). The cadmium content was significantly higher in omnivores (average 1.120 mg kg⁻¹) than in carnivores (average 0.981 mg kg⁻¹) (Tables 2, 3, 4). No significant differences were detected among the carnivorous or omnivorous species. The content was slightly higher (insignificant) in spring species (average 1.106 mg kg⁻¹) than in autumn species (average 1.055 mg kg⁻¹) (Tables 2, 3, 4). In general a significant effect on the cadmium concentration appeared only in terms of sex (RDA: $F = 5.491$, $P = 0.017$; t-test: $P = 0.053$) (Tables 3, 4) and pertinence to a concrete species (RDA: $F = 2.432$, $P = 0.018$). The effect of the seasonal aspect was not proved. The highest absolute value was detected in a *Harpalus distinguendus* (Duftschmidt) female – 13.43 mg kg⁻¹ and the lowest in a *Calathus fuscipes* (Goeze) male – 0.10 mg kg⁻¹.

Table 3. Metal content in Carabidae: Results of redundancy analysis (RDA); x = compared with, n = number of samples, NS = non significant ($P > 0.05$).

| | n | Pb | | Cd | | Zn | | Cu | | Mn | |
|-------------------------|----|-------|-------|-------|-------|--------|-------|--------|-------|--------|-------|
| | | F | P | F | P | F | P | F | P | F | P |
| omnivores x carnivores | 68 | | NS | | NS | 26.916 | 0.001 | 6.132 | 0.015 | 5.915 | 0.023 |
| carnivores x carnivores | 20 | | NS | | NS | 3.678 | 0.038 | 3.288 | 0.043 | | NS |
| omnivores x omnivores | 48 | 8.173 | 0.001 | | NS | | NS | | NS | | NS |
| spring b. x autumn b.* | 68 | | NS | | NS | 15.964 | 0.001 | 6.769 | 0.006 | | NS |
| male x female | 68 | | NS | 5.491 | 0.017 | | NS | 21.829 | 0.001 | | NS |
| species | 68 | 5.044 | 0.001 | 2.432 | 0.018 | 4.407 | 0.002 | 2.821 | 0.011 | 2.435 | 0.021 |
| aspects | 68 | | NS | | NS | 5.073 | 0.005 | | NS | 11.489 | 0.001 |

* spring b. - spring breeders, autumn b. - autumn breeders

Table 4. Metal content in Carabidae: Results of t-test (x = compared with, n = number of samples, NS = non significant).

| | n | Pb | Cd | Zn | Cu | Mn |
|------------------------|----|----|-------|--------|--------|-------|
| omnivores x carnivores | 68 | NS | NS | <0.001 | 0.018 | 0.028 |
| spring b. x autumn b.* | 68 | NS | NS | <0.001 | <0.001 | NS |
| males x females | 68 | NS | 0.053 | NS | 0.001 | NS |

* spring b. - spring breeders, autumn b. - autumn breeders

3.3. Zinc

Natural concentrations of zinc in the soil ranged from 10 to 30 mg kg⁻¹ (Elinder and Piscator 1979), while the Zn values detected in study area (214.2 mg kg⁻¹) were approximately 10 times higher. The difference between males and females was not significant. On the contrary, the feeding habit, whether omnivores or carnivores (RDA: $F = 26.916$, $P = 0.001$; t-test: $P < 0.001$), type of reproduction (RDA: $F = 15.964$, $P = 0.001$; t-test: $P < 0.001$), pertinence to a concrete species (RDA: $F = 4.407$, $P = 0.002$) and time of sampling (RDA: $F = 5.073$, $P = 0.005$) significantly affected the content of Zn (Tables 3, 4). Significant differences were discovered among carnivorous species (RDA: $F = 3.678$, $P = 0.038$); no significant differences were found among the omnivorous species

(Table 3). The *L. ferrugineus* female contained the highest level of Zn – 292.16 mg kg⁻¹ and the lowest levels were detected in the *H. distinguendus* male – 89.63 mg kg⁻¹.

3.4. Copper

Natural concentrations of copper in unpolluted soils vary from 2 to 250 mg kg⁻¹ (median 30 mg kg⁻¹) (Bowen 1985). The detected content of copper in the soil (containing the sludge) in study area (82.43 mg kg⁻¹) was within the range of natural concentrations. The effect of the seasonal aspect on Cu concentration in the insect bodies was not significant. The effect was significant in the case of trophic groups (omnivores and carnivores) (RDA: $F = 6.132$, $P = 0.015$; t-test: $P = 0.018$), type of reproduction (RDA: $F = 6.769$, $P = 0.006$; t-test: $P < 0.001$), sex (RDA:

$F = 21.829$, $P = 0.001$; t-test: $P = 0.001$) and species (RDA: $F = 2.821$, $P = 0.011$) (Tables 3, 4). Much like Zn, significant differences in the level of Cu were detected among the carnivores (RDA: $F = 3.288$, $P = 0.043$) (Table 3). As with Pb the *L. ferrugineus* male had the highest amount of Cu – 51.68 mg kg⁻¹ and a *Poecilus cupreus* (L.) female had the lowest amount – 7.84 mg kg⁻¹.

3.5. Manganese

Average concentrations of Mn in the soil range between 500 and 900 mg kg⁻¹ (Piscator 1979). The amount monitored in the study area locality was on the lower limit of natural concentration (453.3 mg kg⁻¹). The difference between the spring (average 59.532 mg kg⁻¹) and autumn (average 64.979 mg kg⁻¹) species (Table 2) and the sex was insignificant. As significant we see the effect of trophic groups (RDA: $F = 5.915$, $P = 0.023$; t-test: $P = 0.028$) and pertinence to species (RDA: $F = 2.435$, $P = 0.021$); highly significant was the effect of the time of sampling (RDA: $F = 11.489$, $P = 0.001$) (Tables 3, 4). No differences were discovered among the carnivores and omnivores. The highest absolute value of Mn was noticed in the *L. ferrugineus* female – 129.81 mg kg⁻¹ and the lowest in the *H. distinguendus* male – 26.79 mg kg⁻¹.

4. DISCUSSION

The differences in the levels of Cd, Cu and Zn are dependent more on the type of food and chemical form of the metal in the food than on the trophic level, and although significant differences between males and females appear on localities under low pollution load, at the level of sex they do not show specific accumulation models (Lindquist and Block 1997). In study area (Chrlice in the Czech Republic) the differences between the sexes were significant only in the content of Cd (RDA: $P = 0.017$; t-test: $P = 0.05$), with higher concentrations in females, and Cu (RDA: $P = 0.001$; t-test: $P = 0.001$), with higher concentrations in males (Table 2). *Pseudoophonus rufipes* (De Geer), *Pterostichus melanarius* (Illiger) and *Anchomenus dorsalis* (Pontoppidan) gave the same results (Novák 1985, 1988, 1989). *Pterostichus ni-*

ger (Schaller) females had a higher content of Cd and males had a higher content of Cu (Lindquist and Block 1997). The content of Zn did not differ significantly between the males and females; however the higher content of Zn in females corresponds with results discovered in *Pterostichus oblongopunctatus* (F.) (Lagisz *et al.* 2005). In the vicinity of the cadmium-zinc smelting plant in Austria, Rabitsch (1995) investigated the content of metals in invertebrates. Out of the studied ground beetles (*Aptinus bombardarda* (Illiger), *Abax parallelepipedus* (Piller et Mitterpacher), *Carabus hortensis* (L.), *P. rufipes*, *Poecilus versicolor* (Sturm), *Pterostichus burmeisteri* Heer, *P. oblongopunctatus* and *Calathus erratus* (C. R. Sahlberg)) only *A. bombardarda* and *A. parallelepipedus* showed a distribution model of accumulation between sexes. *A. bombardarda* males had a significantly higher content of Cu and Zn, and the level of Pb and Cd was higher in females. A higher content of Pb, Cd and Zn was detected in *A. parallelepipedus* females and a higher content of Cu in the males. Species-specific distribution models of accumulation between sexes appeared in some species only. The only exception is Cu, where a higher accumulation was discovered almost exclusively in males, independent of the species, feeding ecology or type of reproduction.

In the study area significant interspecies differences in all the studied elements were detected (RDA: Pb – $P = 0.001$; Cd – $P = 0.018$; Zn – $P = 0.002$; Cu – $P = 0.011$; Mn – $P = 0.021$). The content of microelements in the animal bodies is dependent on the species-specific equipment and their content in food (Ma 1982, Ma *et al.* 1983, Pokarzhevskii and van Straalen 1996). Essential elements, to which Zn, Cu and Mn belong (Elinder and Piscator 1979, Piscator 1979a, 1979b), are accumulated in the body in relatively constant amounts and all surplus is rapidly released from the body; their amounts in the body are dependent particularly on the excretion abilities (Kramarz 1999), while elements such as Cd are accumulated particularly in dependence on their amount in food and effectiveness of their assimilation (Janssen *et al.* 1991). Nuorteva (1999) pointed out the interspecies differences in Cd accumulation in

ground beetles; they were discovered in our studies too.

Carnivorous insect species take up metals in various forms from different parts of the food and their relative amounts differ in dependence on the type of food and on the feeding behaviour of their preys (Lindquist and Block 1997). Carnivores had significantly higher values of Zn (RDA: $P = 0.001$; t-test: $P < 0.001$), Cu (RDA: $P = 0.015$; t-test: $P = 0.018$) and Mn (RDA: $P = 0.023$; t-test: $P = 0.028$), and also of Pb (insignificant). A slightly higher (insignificant) concentration of Cd was noticed only in omnivores (Table 2). Significant differences in Zn accumulation were discovered in carnivorous species (RDA: $P = 0.038$) and Cu (RDA: $P = 0.043$). In omnivorous species the differences in the concentration of metals were significant only in the case of Pb (RDA: $P = 0.001$). The generally significant differences in Pb concentration among the species can be due to the highly significant differences in the content of Pb among the omnivores. In carnivorous species these differences were insignificant (Table 3). Apparently the omnivorous species are exposed to much differentiated values of taken up Pb, due to the wider spectrum of food and greater variability in food preferences, than the carnivorous species. These differences are probably due to the plant component of the food, which contains less energy than animal-based food (Roth 1993). To obtain the same amount of energy one omnivorous species feeding plant-based food must therefore take up more food (and thus also Pb) than other omnivorous species feeding animal-based food.

The type of reproduction affected only Zn (RDA: $P = 0.001$; t-test: $P < 0.001$) and Cu concentration (RDA: $P = 0.006$; t-test: $P < 0.001$); the values of both metals were significantly higher in species reproducing in the autumn than in the spring species (Table 2). As was mentioned above the values of Zn and Cu were significantly higher in carnivores and so these differences can at least partly be explained by the composition of the tested species spectrum, which consisted of autumn omnivores and carnivores and only spring omnivores (Table 1). On top of that significant differences in Zn and Cu concentrations were discovered only within

the group of carnivorous species (Table 3). According to Novák (1985) nutrients (Zn, Cu) stored in the fat body are exhausted during overwintering. This could also be the reason why the Cu and Zn values are higher in the autumn species (overwintering in the larval stage) than in the spring species (wintering in the stage of imago). This may mean that the Zn and Cu content in ground beetles is dependent on the type of reproduction only partly and that the concentration of metals is affected by the feeding mode of the individual species.

The effect of the seasonal aspect (time of sampling) was important for Zn (RDA: $P = 0.005$) and Mn (RDA: $P = 0.001$). In ground beetles Hunter *et al.* (1987) discovered significant differences in Cu and Cd accumulation during the vegetation period. This finding, however, was not confirmed in our study. In accordance with our results Novák (1985) noticed changes in Mn concentrations in populations of *P. rufipes*. The Mn content in the bodies of ground beetles changed with the length of their life and, in addition, it was dependent on the species pertinence and food ecology (Table 3). Rabitsch (1995) noted that the time of imago entrapping had a significant effect on Pb concentration. Novák (1988) indicated that the concentration of lead (and other metals) is higher in older specimens exposed to a polluted environment for a longer period of time. Novák (1985) also pointed out that the age composition of the populations had a considerable effect on the annual dynamics of metals in the bodies of the ground beetles. Especially in the period of increased feeding activity the supplies of nutrients are stored in the fat body; on the other hand, during sexual activity and during wintering these nutrients are drawn away. In addition, the population composed of juvenile specimens, feed-active or reproducing individuals may have a considerable effect on the annual dynamics of the metals.

We found a significant ($P < 0.01$) positive correlation between Cu-Zn, Mn-Zn and Mn-Cu (Table 5). These three metals belong to the essential elements (Elinder and Piscator 1979, Piscator 1979a, 1979b, Dmuchowski and Bytnerowicz 1995), the lack of which may cause changes in the

Table 5. Correlation coefficient between metals (significant values are marked with bold letters; $P < 0.001$, $n = 68$) in Carabidae.

| | Pb | Cd | Zn | Cu | Mn |
|----|-------|-------|-------------|-------------|------|
| Pb | 1.00 | | | | |
| Cd | 0.02 | 1.00 | | | |
| Zn | 0.17 | 0.18 | 1.00 | | |
| Cu | -0.09 | -0.15 | 0.37 | 1.00 | |
| Mn | -0.11 | 0.04 | 0.59 | 0.33 | 1.00 |

growth of the beetles (Hopkin 1989). Due to the discovered correlations it is possible that bio-accumulation of these elements interacts, similarly as Cd and Zn in *Porcellio laevis* (Isopoda) (Odendaal and Reinecke 2004). It was also discovered that the uptake and excretion of Cd and Zn in *Porcellio scaber* (Isopoda) is affected by the presence of other metals (Witzel 2000).

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