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Different responses of epigeic beetles to heavy metal contamination depending on functional traits at the family level

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Beetles are the most diverse group of animals in the world, with various functions in all types of terrestrial ecosystems. The aim of the paper was to propose a simple method for evaluating the functioning of forest ecosystems on the basis of changes in life traits of beetles along a gradient of heavy metal contamination. A total of 16 families of beetles (14,142 individuals) were recorded in 18 sample-sites (rows of pitfall traps), along a zinc-contamination gradient (166-3,957 mg/kg dw). NMDS ordination analysis based on the Bray-Curtis coefficient indicated a tendency towards differentiation of beetle families between contamination levels (ANOSIM comparison). SIMPER analysis showed that three groups (Geotrupidae, Carabidae and Silphidae) were more abundant in the reference sites than the polluted sites, whereas Curculionidae, representing herbivores, and small predators of the Staphylinidae family were more abundant on heavily contaminated sites. A generalized linear model showed that pollution class and season were responsible for most of the variation in family abundance. Geotrupidae were most sensitive to contamination. Their occurrence on heavily contaminated sites was extremely low and seemed to have resulted from random colonisation from other regions. Our results indicated that beetles at the family level are good indicators of heavy-metal concentration. Their appearance reflects both direct and indirect effects of heavy metals. They can accumulate a large amount of pollutants that reduce their survival rate and may also be good indicators of ecological processes such as impoverishment of food-web chains and reduced decomposition rates. Beetle abundance at the family level underscores the usefulness of these organisms in heavy-metal monitoring. Moreover, simple methods of sampling and taxonomic identification as well as variation in life traits along a disturbance gradient, make it possible to obtain valuable information on the condition of forest ecosystems contaminated with heavy metals.

Key words: beetles, ecological indicators, heavy metals, functional traits

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INTRODUCTION

Beetles are the most diverse group of animals in the world and have a variety of functions in all types of terrestrial ecosystems (Crowson 1981). Such functional variation is visible at the family level. In most research, functional traits are defined as properties of species that affect individual fitness and govern species sensitivity to environmental changes (Southwood 1977). However, immense variation among invertebrates and difficulties in taxonomic identification due to the vast diversity of these organisms (Giller 1996) severely limit analysis of life traits in actual ecosystems studied in field research. Soil invertebrates play a major role in forest-ecosystem dynamics, including carbon sequestration, nutrient turnover, and soil properties (Lavelle et al. 2006). There is a need to estimate the influence of soil disturbances on functional traits of ecosystem elements, particularly given the growing negative impact of anthropogenic disturbances in forest ecosystems on many levels. Heavy metals are one of the major threats to terrestrial and aquatic ecosystems, and the impact of contamination in forest ecosystems is poorly understood. Hence the use of bioindicators in this type of research is a valuable tool for ecotoxicologists and forest managers. In a contaminated forest the body size of epigean invertebrates changes along the contamination gradient (Ribera et al. 2001, Urlich et al. 2009, Skalski et al. 2010). Sometimes, however, invertebrate diversity may increase in more contaminated soils (Skalski et al. 2011), while indicating weaker competitive interactions between species (Bednarska 2013, Skalski et al. 2015). Life traits of invertebrates usually do not respond directly to heavy-metal concentration but depend on changes in soil properties such as organic-matter content (Hedde et al. 2012, Kędzior et al. 2014).

Most studies of invertebrate responses to contamination have focused on single groups with more or less similar trait variation, e.g. predatory ground beetles (Blake et al 1994, Skalski et al. 2010, 2012, Purchart et al. 2010), springtails (Spurgeon and Hopkin 1996), earthworms (Decaens et al. 2008, Pospiech and Skalski 2006), and ants (Grzes 2009). They usually have similar food preferences and play very similar roles in the ecosystem. In contrast, more general identification of invertebrates, e.g. to the order, allows for the specification of a number of environmental functions characterising taxa with different life traits (Nahmani and Rossi 2003). We still need simple indicators that can be used to evaluate the situation in the ecosystem (Skalski and Pospiech 2006).

The aim of this paper was to propose a simple method for evaluating the impact of heavy-metal contamination in forest ecosystems on the basis of variation in the life traits of beetles at the family level. Strong specificity of food preferences and high dependence on nutrient turnover or soil structure create a good opportunity to unveil changes in an ecosystem chronically contaminated by heavy metals.

MATERIAL AND METHODS

The sampling area was located in the vicinity of Cracow at various distances from a zinc smelter (Skalski et al. 2010, Skalski et al. 2011, Lagisz et al. 2012). In this region, the typical forest association is subatlantic fresh pine forest (Leucobryo-Pinetum) with elements of Sambuco-Salicion and eutrophic beech (Fagion sylvaticae), which has been managed for many years. A single complex of the forest was selected, supporting a uniform age, plant composition, and tree structure, but containing three groups of localities differing in heavy-metal contamination (L, M, and H). The group of reference sites with a low contamination level (L) had a mean zinc concentration of 166 mg/kg dw in the humus layer, whereas moderately contaminated sites (M) were characterized by zinc at a level of 1,000 mg/kg dw and heavily contaminated sites (H) of 3,957 mg/kg dw. In each locality, three replicates of pitfall trap rows located randomly were set up to collect epigaeic beetles. The distance between trap rows exceeded 500 m. Overall, there were 180 traps (10 in each row, 10m between traps) during the sampling period. Standard plastic cups (10 cm diameter) filled with esthylene glycol without protecting roof were used as a trap. Trapped individuals were collected twice a month in the spring and autumn of 2012. All beetles were identified to the family level using standard keys (Freude et al 1976) while the nomenclature of the families was based on Fauna Europaea (http://www.faunaeur.org/).

The relationship between the level of zinc concentration and abundance of each family were tested using a generalized linear model of Poisson distribution and a log-link function included in the Statistica 10.0 software (Statsoft, 2010). Non-metric multidimensional scaling was used to show the differences in beetle assemblage composition on the family level between contamination categories (L, M and H). The Bray-Curtis similarity index was applied as a measure of similarity distances between assemblages along a season and pollution gradient. The significance of distances between assemblages was tested using ANOSIM with Bonferroni corrrection. SIMPER analysis (similarity percentages) was performed to detect the most influential family for each of the three contamination levels. All multivariate analyses were provided using Past v. 2.17 (Hammer et al. 2001).

RESULTS

A total of 16 families of beetles (14,142 individuals) were recorded in the pitfall traps in 18 locality rows. Nearly 9,000 specimens belonged to the family Geotrupidae, which has only 3 species. Ground beetles (Carabidae) were represented by 3,300 individuals. Another 3 beetle families, Curculionidae, Staphylinidae, and Silphidae, were represented by about 700 specimens while the remaining families were represented by markedly fewer specimens (Tab. 1).

NMDS ordination analysis based on the Bray-Curtis coefficient indicated a tendency towards differentiation of beetle families between zinc-contamination levels (Fig. 1). The results of ANOSIM incorporating the 3 contamination levels were congruent with this trend and statistically significant (Tab. 2). SIMPER analysis showed that 3 groups (Geotrupidae, Carabidae, and Silphidae) were more abundant in the reference (L) than the other (M and H) sites (Tab. 3). Their contribution to the dissimilarity of NMDS was over 90 %. Only Curculionidae, representing a herbivorous group, and the small predators of the Staphylinidae family were more abundant on heavily contaminated sites, but their contribution to the dissimilarity was under 8 %. The remaining families scarcely spread out, sharing only about 1 % of the dissimilarity.

The abundance of beetles in the forest differed significantly between zinc-contamination levels (Tab. 4). The generalized linear model showed that contamination level and season were responsible for most of the variation. There was a significant shift in the seasonal activity of beetles between the reference (L) and highly contaminated (H) assemblages. The reference (L) and moderately contaminated (M) assemblages had two peaks of activity at the end of June and in the middle of September. On heavily contaminated localities, however, seasonal variation never reached a clear peak, with abundances remaining at a level of up to 50 specimens (Fig 2). A strong relationship between the contamination level and mean abundance was revealed for most of the families (Tab. 4), but the response to contamination differed between them. The most sensitive to contamination were Geotrupidae, whose occurrence on heavily contaminated sites was extremely low and seemed to be the result of random colonisation from other regions. The seasonal pattern of the L and M sites had two peaks, in late spring and early autumn. Similar patterns of seasonal distribution were revealed for scavengers of the family Silphidae and small predators and scavengers of the family Staphylinidae. They, too, have two peaks in abundance in late spring and early autumn, with silphids showing a decrease in abundance on contaminated sites and the opposite being true for staphylinids. Carabidae, which represent mainly predators, had significantly higher abundances on the L sites

Table 1. Total density and frequency of occurrence of beetle families in three groups of contamination localities (L - low concentration of Zink. M - medium concentration of Zink. H - high concentration of Zink)

Family	Total abundance			Total frequency		
	L	M	H	L	M	H
Anthicidae	1	0	0	0.021	0.000	0.000
Aphodiidae	1	1	0	0.021	0.021	0.000
Cerambycidae	0	1	0	0.000	0.021	0.000
Chrysomelidae	1	2	2	0.021	0.042	0.042
Coccinellidae	1	0	1	0.021	0.000	0.021
Cryptophagidae	1	0	0	0.021	0.000	0.000
Elateridae	1	1	21	0.021	0.021	0.208
Geotrupidae	4971	3811	121	1.000	1.000	0.500
Histeridae	14	7	1	0.146	0.125	0.021
Nitidulidae	0	0	1	0.000	0.000	0.021
Scarabaeidae	5	1	1	0.042	0.021	0.021
Silphidae	331	379	14	0.771	0.729	0.188
Staphylinidae	26	189	291	0.271	0.583	0.646
Tenebrionidae	1	0	0	0.021	0.000	0.000
Curculionidae	198	254	208	1.000	1.000	1.000
Carabidae	1613	849	824	1.000	1.000	1.000

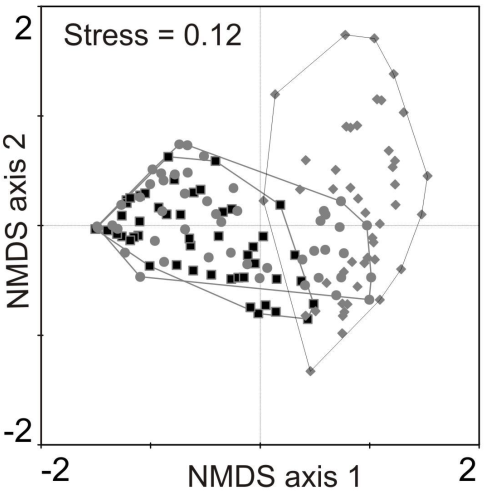

Fig 1. Diagram of non-metric multidimensional scaling of beetle assemblages classified to three groups of contamination (square- almost uncontaminated sites, circle- moderately contaminated sites, diamond- highly contaminated sites)

Table 2. R coefficient of ANOSIM analysis for three contamination groups of beetle assemblages. The significant differences were estimated with Bonferroni correction (* p<0.05. ***p<0.001)

Contamination level	Low	Medium	High
Low	-	-	-
Medium	**0.05***	-	-
High	**0.60*** **	**0.39*** **	-

during spring. The opposite response to contamination was characteristic for herbivorous Curculionidae, whose abundance in early spring was higher on the H sites and decreased over the subsequent weeks (Fig 2).

DISCUSSION

Good ecological indicators are mainly used to assess the condition of the environment and to estimate environmental change after a disturbance (Niemi and McDonald 2004). This study clearly demonstrated that some beetle families were negatively affected by heavy-metal contamination. Both detritivore and scavenger families – Geotrupidae and Silphidae – were severely affected in the heavily polluted forest, indicating the breakdown of decomposition processes in the forest ecosystem. Heavy metals slow microbial decomposition of organic matter, interrupting regular processes in the soil and restricting overall soil productivity (Gadd 2010). Self-evidently, microbes also decompose dead plants and animals which are the primary food source for scavenger and detritivore beetles. Their absence or a change in their composition may lead to various changes in food quality, limiting food availability for both groups of beetles. The activity density of detritivores on heavily contaminated sites during the season is random and has no seasonal dynamics.

Table 3. Individual family contributions to the difference in taxonomic composition between unpolluted moderately polluted and highly polluted sites from SIMPER analysis

Taxa	Average abundance		% Contribution
	Low contamination	High contamination	
Geotrupidae	104	2.52	61.36
Carabidae	33.6	17.2	24.31
Curculionidae	4.13	4.33	4.976
Silphidae	6.9	0.292	4.871
Staphylinidae	0.542	6.06	3.697
	Low contamination	Medium contamination	
Geotrupidae	104	79.4	59.67
Carabidae	33.6	17.7	23.22
Silphidae	6.9	7.9	7.119
Curculionidae	4.13	5.29	6.217
Staphylinidae	0.542	3.94	3.207
	Medium contamination	High contamination	
Geotrupidae	79.4	2.52	55.47
Carabidae	17.7	17.2	19.77
Curculionidae	5.29	4.33	9.171
Staphylinidae	3.94	6.06	7.413
Silphidae	7.9	0.292	7.212

Changes in microbial activity and diversity may provide an explanation in the case of herbivores. In contrast to detritivores, their abundance was higher in the heavily contaminated sites. Here, certain microbes may affect insect-plant relationships. Elimination of certain microbes leads to higher nutritional content and biomass of plants while reducing the defence compounds against phytophages (Hol et al. 2010). Moreover, higher concentrations of heavy metals decrease the survival rate of trees (Kiikkilä 2003). Tree species usually invest considerable energy in the production and accumulation of defence compounds. In heavily contaminated sites, some of this energy must be expended on detoxification processes and metal accumulation. Under these circumstances, trees become more susceptible to herbivore attacks. Both of the factors mentioned above are probably responsible for the increased weevil abundance in the highly contaminated forest sections.

The reduction of the nutrient pool in heavily contaminated forest sections probably also affects carnivores. In our example, ground beetles were less abundant in contaminated sites. Some authors have documented that heavy metals can be transferred from soils to invertebrates and from herbivores to

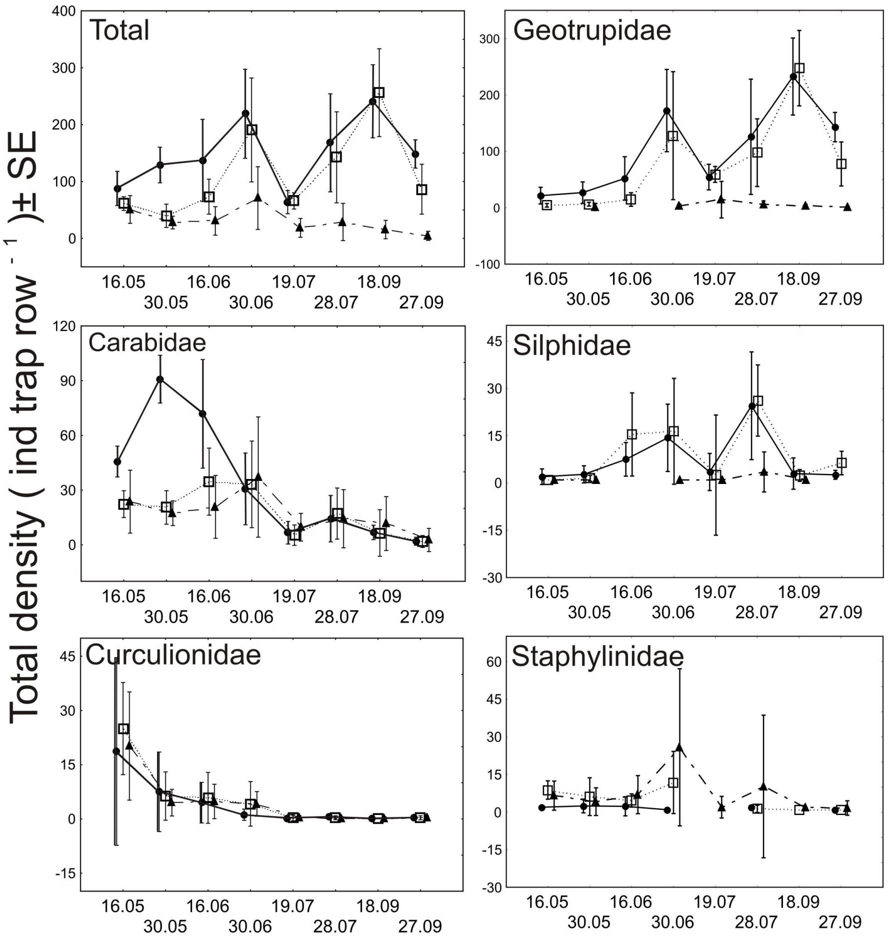

Fig 2. Mean total density ± SE of the most frequently occurring groups of beetles in three classes of contaminations along the season (circle- almost uncontaminated sites, square- moderately contaminated sites, triangle- highly contaminated sites).

carnivores in food webs (e.g. Green et al. 2010). Ground beetles exposed to heavy metal contaminated food show alterations in body size due to less available energy (Maryański et al. 2002). Carnivores have a higher content of zinc and copper than omnivorous beetles (Purchart and Kula 2007). Stone et al. (2001, 2002) also showed significantly elevated levels of heavy metals in carabid beetles on polluted sites as well as greater susceptibility to additional stressors such as starvation. Growth of larvae feeding on contaminated food is also slowed significantly (Mozdzer et al. 2003). Heavy metal contamination may reduce the locomotion behaviour as a result of internal structure damage during larval development decreasing the fitness of the animal under field conditions (Bailey et al. 1995). However, these results do not fully explain the negative effect of heavy metals on various life traits in this beetle family. Ground beetles are regarded as poor heavy metal accumulators and exhibit a high degree of plasticity in the face of such disturbances in the field (Laskowski and Maryański 1993; Butovsky 2011), but they exhibit very high heavy metal concentration in the short period after contamination in the laboratory conditions

(Laskowski et al 2010, Pizzolotto et al 2013).When we bring into consideration lower abundance of their food, e.g. springtails and earthworms in contaminated soils and increasing risk of starvation, the decreased survival rate in the forest ecosystem seems to be clear.

Our results indicate that beetles at the family level are good indicators of heavy-metal contamination. Their occurrence reflects both direct and indirect effects of heavy-metal contamination. They can accumulate large quantities of pollutants that reduce their survival rate and may also be good indicators of ecological processes such as impoverishment of food chains and reduced decomposition rates. Beetle families are useful indicator groups for heavy-metal monitoring because they are relatively easy to both identify and measure while they vary in their responses to disturbances. Moreover, from an economical point of view, the low cost of sampling adds to their usefulness (Noss 1990, Gerald et al. 2004).

Table 4. Generalized linear model for family abundance of beetles along pollution level

	df	Wald's statistic	p
Total			
Residual	1	33187.44	0.0000
Zn mg/kg dw	1	8.13	0.0043
Date	7	1397.21	0.0000
Contamination level	2	831.83	0.0000
Contamination level*Date	14	861.34	0.0000
Carabidae			
Residual	1	5232.57	0.0000
Zn mg/kg dw	1	14.50	0.0001
Date	7	859.32	0.0000
Contamination level	2	23.10	0.0000
Contamination level*Date	14	354.10	0.0000
	df	chi-squared statistic	p
Geotrupidae			
Zn mg/kg dw	1	2301.56	0.0000
Date	7	5114.90	0.0000
Contamination level	2	1392.97	0.0000
Contamination level*Date	12	422.05	0.0000
Silphidae			
Zn mg/kg dw	1	48.46	0.0000
Date	7	478.73	0.0000
Contamination level	2	64.11	0.0000
Contamination level*Date	12	28.47	0.0047
Curculionidae			
Zn mg/kg dw	1	7.48	0.0062
Date	7	1112.13	0.0000
Contamination level	2	28.11	0.0000
Contamination level*Date	12	21.95	0.0380

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