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Ant species richness and evenness increase along a metal pollution gradient in the Bolesław zinc smelter area

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Summary

Ants are considered to be relatively resistant to metal pollution, but the effect of metal toxicity on ant communities is poorly understood. This work examined the relationship between ant species diversity and heavy metal pollution at 16 meadow and forest sites along a metal contamination gradient in a mining and smelting region near Olkusz, Poland. Menhinick's index was used to estimate species richness. Pielou's index of evenness (*J*), Simpson's index of diversity (*D*) and the slopes of rank-abundance curves were used to estimate of species evenness. Regardless of species composition differences between forest and meadow, the increase in species diversity with increasing metal pollution was very clear in both ecosystems. The more polluted the site, the more species were detected and the more similar in relative abundance they were. Consequently, the extent to which one or a few species dominated a community decreased. This result can be explained by indirect effects of metal pollution, that is, changes in species interactions rather than by changes in abiotic conditions.

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Introduction

Ants are considered useful for biomonitoring studies as they can respond rapidly to environmental changes (reviewed by Underwood and Fisher 2006). The literature on metal effects in ants covers such topics as metal accumulation patterns in different species, sites and seasons (Rabitsch 1995; Rabitsch 1997a–c; Eeva et al. 2004; Grześ 2009), the activity of metabolic and detoxifying enzymes (Migula and Głowacka 1996; Migula et al. 1997), the immune response of wild ant populations (Sorvari et al. 2007), and the species composition,

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relative abundance and colony size of *Formica* s. str. populations (Eeva et al. 2004). The influence of different disturbances such as fire, land conversion, habitat fragmentation and mining area rehabilitation on ant species diversity has been studied by many authors (reviewed by Underwood and Fisher 2006), but the effect of metal toxicity on free-living ant communities is still poorly understood.

Some authors consider ants to be relatively resistant to industrial pollution (e.g., Pętal 1987; Maavara et al. 1994; Eeva et al. 2004). Maavara et al. (1994) suggested that workers are able to inhibit metal transfer to larvae, but the physiological basis of such a mechanism is unclear. Migula and Głowacka (1996) concluded that despite high metal concentrations in ants from metal-polluted sites, cellular energetic processes – measured as the AEC index – were maintained at normal levels. Other authors have demonstrated that metal pollution may decrease colony size and suppress the immune response, leading to higher infection risk in polluted environments (Krzysztofiak 1991; Eeva et al. 2004; Sorvari et al. 2007). These findings suggest that metal pollution can decrease the species diversity of ants in polluted environments.

This study addresses two questions:

1. Does the diversity of ants (species richness and evenness) change along a metal pollution gradient?
2. Does the response of ant communities to pollution differ between ecosystems (Scots pine forest vs. meadow)?

Materials and methods

Study area, sampling and species identification

The study area was in the vicinity of Olkusz city in southern Poland. Metal concentrations in the humus layer at the most polluted sites of this region exceed 9600 mg kg^{-1} zinc, 1500 mg kg^{-1} lead and 80 mg kg^{-1} cadmium (Stone et al. 2001). Sixteen sites were established along the pollution gradient, including eight in mixed Scots pine forests (F1–F8, forest transect) and eight in meadows (M1–M8, meadow transect). The forest transect was established between 1.9 and 31.8 km, while meadow transect between 0.6 and 32.6 km away from the pollution source. The metal concentrations in the sites decreased with increasing distance to the smelter approaching background levels at sites further than about 25 km from the smelter. The forests were on sandy podsolized soil with more humus layer (Zygmunt et al. 2006). The meadows were on sandy or sandy-limestone soils. Total and water-soluble soil concentrations of Zn, Cd and Pb, and other site characteristics, are given in Table 1. As the metal concentrations in soil were highly correlated with each other (Table 2), total zinc concentration was used as the pollution index. Soil Zn and Cd concentrations at sites M4–M8, F2–F5 and F7–F8 are as provided by Stefanowicz et al. (2008), and site F1 as provided by Stone et al. (2001). The soil analyses for sites M1–M3 and F6 are my own, done according to the methods described by Stefanowicz et al. (2008).

Ants were sampled with pitfall traps; 1056 pitfall traps (66 per site) were set out in 10 m × 5 m grids during the period of highest seasonal abundance, from late May until late June 2005. The traps were filled with a mixture of water and antifreeze (Borygo, Tesco Poland), and were emptied five times during the sampling period, every 7–10 days. Pitfall trapping is an effective (King and Porter 2005) and commonly used sampling technique (e.g., Andersen 1991; Hoffmann et al. 2000; Lindsey and Skinner 2001), recommended for ant species diversity studies (Underwood and Fisher 2006; Bestelmeyer et al. 2000). It provides information about species richness, species composition and relative abundance, and can be used for brief (days) or continuous sampling (Underwood Fisher 2006; Bestelmeyer et al. 2000). The accuracy of results from trapping may suffer from differences in species activity, creating a bias towards the most active ants (Bestelmeyer et al. 2000). In this study, less active species such as *Stenamma debile* or *Lasius flavus* were captured along with the active species, probably due to the long sampling period and large number of traps per site. The ants from all sampling dates and traps at one site were pooled, so that each site was represented by one sample. Only the worker caste was used for species identification, as most taxonomy is based on workers and since the presence of a worker is clear evidence of colony establishment (Longino et al. 2002). The very small number of males and queens captured were excluded from the analyses. Species were identified according to Czechowski et al. (2002). The national reference collection was consulted with a specialist (Dr. W. Czechowska, Polish Academy of Sciences) for accurate identification.

Data analysis

To test the relationship between pollution and ant species richness and evenness, diversity indices

Table 1. Concentrations of total and water soluble (ws) metals in the upper soil level in meadows (M1–M8) and forests (F1–F8) located along the "Boleslaw" smelter pollution gradient.

Site	Location N/E	Distance from the smelter (km)	Zn_{total} (mg kg^{-1})	Zn_{ws} (mg kg^{-1})	Cd_{total} (mg kg^{-1})	Cd_{ws} (μg kg^{-1})	Pb_{total} (mg kg^{-1})	Pb_{ws} (mg kg^{-1})
M1	50°17′/ 19°32′	4.3	4644.5	7.2	21.00	80.5	2110	1.97
M2	50°16′/ 19°28′	0.6	2229.5	11.1	20.33	79.5	814	1.57
M3	50°18′/ 19°33′	5.5	1245.6	0.69	4.80	34.6	119	0.01
M4	50°20′/ 19°33′	8.6	272.85	1.15	3.67	16.6	126	0.31
M5	50°18′/ 19°29′	4.1	238.90	2.36	2.50	25.9	670	3.25
M6	50°25′/ 19°38′	20.0	154.00	1.82	1.54	25.4	96.0	0.52
M7	50°22′/ 19°32′	12.8	101.96	1.4	2.24	25.64	92.9	0.16
M8	50°32′/ 19°38′	32.6	85.53	0.30	1.14	11.1	79.7	0.18
F1	50°17′/ 19°29′	3.5	10.454	19.82	82.9	594	2635	3.43
F2	50°17′/ 19°29′	1.9	6151	36.4	71.4	297	2209	7.94
F3	50°18′/ 19°29′	3.9	1763	23.1	39.1	401	1244	13.8
F4	50°19′/ 19°30′	5.3	1253	13.6	14.7	220	1236	10.1
F5	50°19′/ 19°32′	7.9	755	8.5	12.2	132	756	1.7
F6	50°25′/ 19°38′	19.6	224	2.86	4.03	37.6	330	0.81
F7	50°22′/ 19°32′	12.3	134	2.15	1.09	39.93	149	0.37
F8	50°32′/ 19°39′	31.8	109	2.11	1.48	36.48	157	0.64

Soil Zn and Cd concentrations at sites M4–M8, F2–F5 and F7–F8 are given as provided by Stefanowicz et al. (2008), and site F1 by Stone et al. (2001).

Table 2. Pearson correlation coefficients for metal concentrations from the 16 study sites along the pollution gradient.

	log Cd tot	log Cd ws	log Pb tot	log Pb ws	log Zn tot
log Cd tot	1				
log Cd ws	0.9014	1			
log Pb tot	0.8963	0.8613	1		
log Pb ws	0.7460	0.8225	0.8282	1	
log Zn tot	0.9519	0.8278	0.8640	0.6283	1

All correlation coefficients are significant at $p<0.001$.
tot – total metal concentration, ws – water soluble metal fraction.

were calculated and then correlated with soil Zn concentration along the pollution gradient. From the many indices used in diversity surveys (see Magurran 2004a), a set of species richness and evenness measures were selected. Due to the great variation of sample size, Menhinick's index was used to estimate species richness (Magurran 2004b). Pielou's index of evenness, the Simpson index of diversity and slopes of rank-abundance curves (expressed as beta parameter) provided estimates of species evenness (May 1975). The effect of ecosystem type was tested by comparing regression lines. Factors that appeared non-significant were withdrawn from the analysis. Total soil zinc concentrations were log-transformed and standardized to the least-polluted site.

Cluster analysis was performed to group the sites by similarity of species composition, with single linkage as the grouping method. Because the samples were dominated by a small number of very abundant species, distances between objects were measured by Pearson's $1-r$ correlation distance. This method should be the most appropriate as, unlike other popular distance measuring methods (e.g., Euclidean or Chebychev distance), Pearson's correlation distance is not biased by the measurements with the highest values (McGarigal et al. 2000).

The relationship between sites and the abundance of each species was estimated with RDA analysis, using Canoco ver. 4.0 (ter Braak and Smilauer, 1998). Total Zn concentration at the sites was taken as the ordination parameter, while ecosystem type (i.e., forest and meadow) was the covariate.

Results

Of the 94 ant species reported for Poland, 22 species belonging to six genera were represented by the 71,288 ants collected. The species and their relative abundance at each site are listed in Table 3. The most diverse genera in both forest and meadow were *Myrmica* and *Lasius*. Total individuals captured per site ranged from 674 to 15,825 individuals. The number of individuals collected from meadows was distinctly higher than that collected from forests. The species structure of ant communities also differed between forest and meadow, as each community had its unique species. Seven of the 20 species found in forests were not found in meadows, while 2 of the 15 species collected from meadows were not found in forests. Cluster analysis confirmed this general difference between forests and meadows, showing forest and meadow as the most distinct groups (Figure 1). The sites did not form clusters according to soil Zn concentration, indicating that cluster analysis did not show Zn pollution as a major factor determining species composition at the study sites. In contrast to this result, RDA (Figure 2) showed a

Table 3. Relative species abundance in percent of total number of ants found in meadows (M1–M8) and forests (F1–F8) located along the "Boleslaw" smelter pollution gradient.

Species	Number of individuals captured		Relative abundance (%)	
	Forests	Meadows	Forests	Meadows
Formica cinerea	7	1	0.08	0.00
Formica cunicularia	0	1582	0.00	2.60
Formica fusca	461	130	5.05	0.21
Formica polyctena	292	0	3.20	0.00
Formica rufa	335	0	3.67	0.00
Lasius alienus	33	10	0.36	0.02
Lasius distinguendus	1	5	0.01	0.01
Lasius flavus	7	33	0.08	0.05
Lasius niger	220	46,565	2.41	76.45
Lasius platythorax	1931	0	21.14	0.00
Leptothorax acervorum	14	3	0.15	0.00
Leptothorax muscorum	4	2	0.04	0.00
Myrmica lobicornis	280	0	3.01	0.00
Myrmica lonae	68	0	0.74	0.00
Myrmica rubra	886	9242	9.70	15.18
Myrmica ruginodis	4411	0	48.28	0.00
Myrmica rugulosa	0	2119	0.00	3.479
Myrmica sabuleti	144	104	1.58	0.17
Myrmica scabrinodis	4	564	0.04	0.93
Myrmica schencki	10	443	0.11	0.73
Stenamma debile	23	0	0.25	0.00
Tetramorium caespitum	5	101	0.05	0.17
Total	9136	60,904	100%	100%

significant explanatory effect of Zn pollution on species composition (Monte Carlo permutation test, $p = 0.004$ after 499 permutations). This was confirmed by simple regression, which showed all the diversity indices to be correlated significantly with Zn pollution. Menhinick's species richness index and Pielou's index gradually increased with soil Zn (Menhinick's index: $P = 0.030$, $r = 0.54$; J: $p = 0.014$, $r = 0.598$). This indicates an increase of species richness and evenness with increasing pollution. Simpson's index decreased while the slopes of rank-abundance curves increased with Zn concentration, both indicating a decrease of species dominance with increasing pollution (Simpson index: $P = 0.008$, $r = 0.636$; slopes of rank-abundance curves $P = 0.008$, $r = 0.638$). Comparison of regression lines (Figure 3), done to test for differences between forest and meadow ant community response to pollution, showed a significant difference in regression intercepts ($P < 0.0001$) for Menhinick's index, although the difference in slopes was not significant ($P = 0.1617$). A similarly significant difference in intercepts ($p = 0.0132$) and insignificant difference in slopes ($P = 0.4395$) was found for Pielou's index. Both models were significant at $P < 0.0001$ (Menhinick's index) and $P = 0.0036$ (Pielou's index). This indicates that although forests had generally higher Menhinick's and Pielou's indices, metal contamination affected the ant communities of both ecosystem types to a similar extent. For parameter beta of species rank-abundance curves and Simpson's index (D), neither differences in slopes ($P = 0.5549$ and 0.4042, respectively) nor in intercepts ($P = 0.2131$ and 0.0598, respectively) were significant. After withdrawing insignificant factors, both models were significant at $P = 0.0081$ (beta) and $P = 0.0078$ (D).

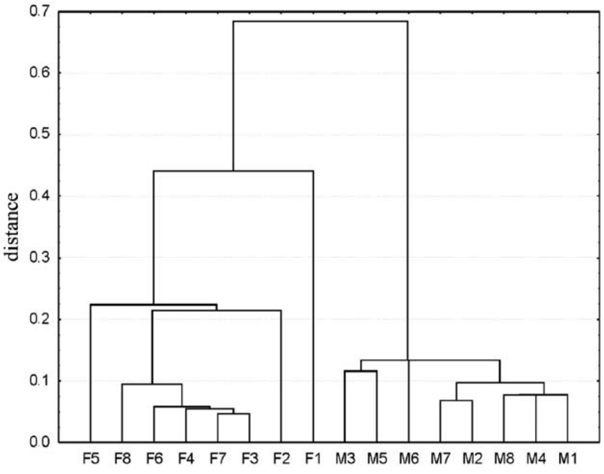

Figure 1. Cluster analysis of ant species composition in meadows (M1–M8) and forests (F1–F8) located along the "Boleslaw" smelter pollution gradient.

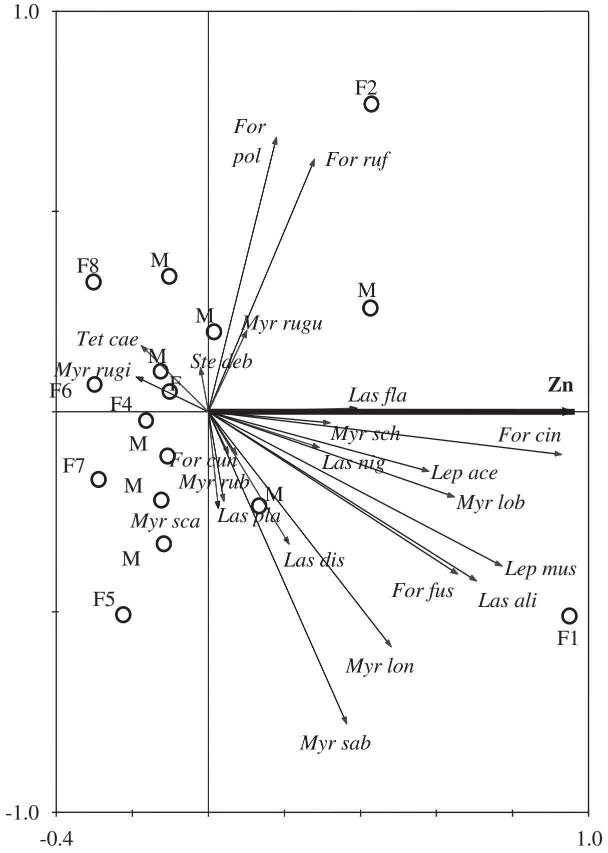

Figure 2. RDA tri-plot of ant community composition in meadows (M1–M8) and forests (F1–F8) located along the "Boleslaw" smelter pollution gradient. For species names see Table 3.

Discussion

The use of two transects in this study allows stronger inferences about pollution-driven changes in the diversity of ant communities than would be possible with the more common single-transect approach (e.g., Pedersen et al. 1999; Brändle et al. 2001) or with a simple comparison of polluted vs. unpolluted sites. Although the transects are not completely independent as they are both located in the same area polluted by the same smelter, this approach allowed us to determine whether the same trend in community diversity changes can be observed regardless of the differences between the two ecosystem types. The difficulty with proper replication is inherent in this kind of study in which the effects of specific pollutants are studied in the field. As there are no two identical smelters located in similar environments, proper replication is

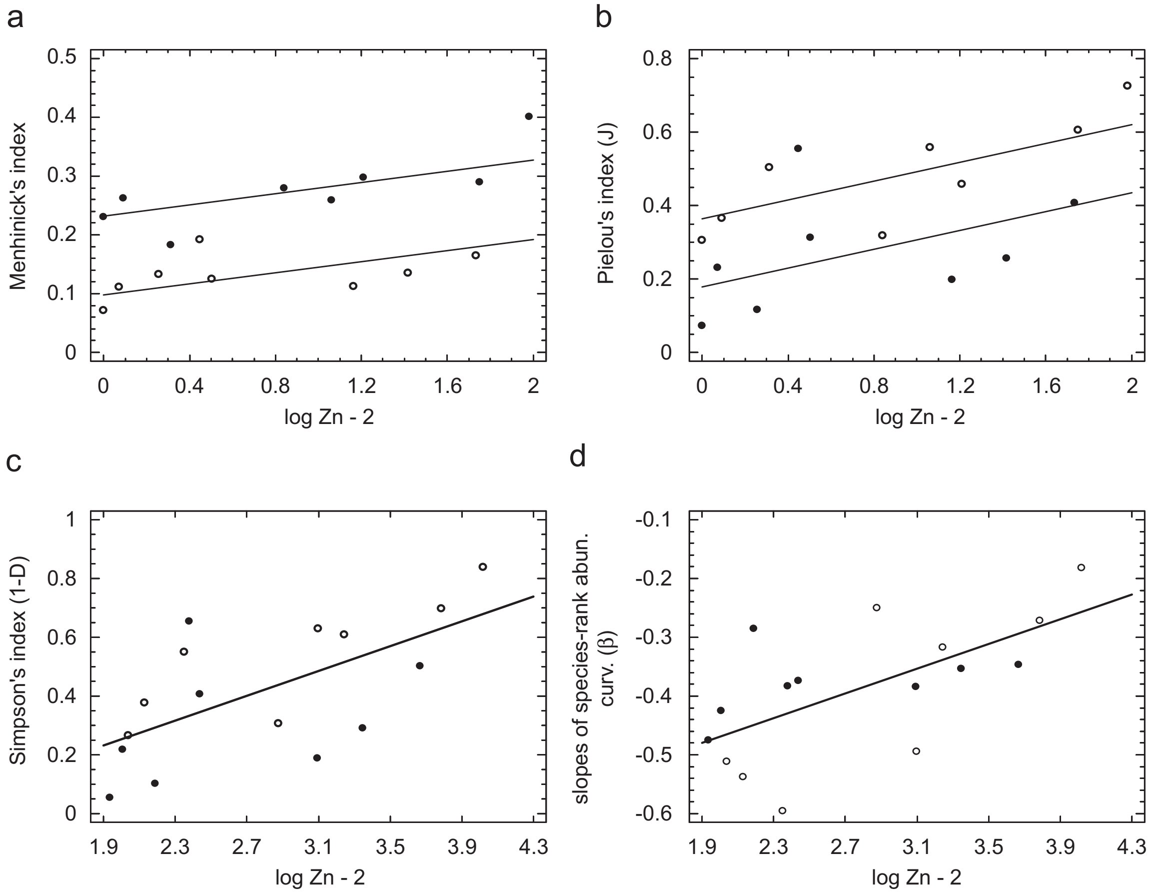

Figure 3. Effect of Zn contamination on (a) Menhenick index (b) Pielou's index of evenness (*J*), (c) Simpson's index of diversity (1-D) and (d) slopes of species-rank abundance curves. ● = forests; ○ = meadows. The single line in c and d represents the effect of withdrawing the insignificant difference in intercepts between forests and meadows. *P*-values for models and differences in slopes and intercepts of regression lines are given in the text.

usually not possible, so techniques other than comparison of (for example) communities from polluted and unpolluted sites should be used. Among these, studies on large spatial scales followed by cluster and/or regression analyses seem the most plausible approach (Wickham et al. 1997; Ren et al. 2007; Stefanowicz et al. 2008) by avoiding the problem of pseudo-replication (cf. Hurlbert 1984).

Regardless of the differences between forests and meadows in ant species composition, the increase in species diversity with increasing Zn pollution was very clear. The more polluted the site, the more species were detected and the more similar were the species in their relative abundance. Consequently, the extent to which one or a few species dominated a community decreased with increasing pollution. Comparison of regression lines confirmed this finding, and also indicated that the impact of metal pollution on the two ecosystems was comparable.

The literature on the influence of metal pollution on ant community diversity is sparse. For example, Migliorini et al. (2004) included ants in a study comparing the responses of different arthropod communities to heavy metal pollution, mainly Pb and Sb, but found no clear relationship. Despite the long sampling period (three months) in that study and an effective sampling technique (pitfall traps), only 56 ants were captured, suggesting an overall low abundance of ants in the investigated area. Eeva et al. (2004) compared the colony frequency, colony size and species composition of five *Formica* species between a clean area and one polluted mainly with Cu, Zn, Ni, Pb and As. All species had smaller colonies in the polluted area, but changes in colony frequency differed considerably between species – depending on the species, colony abundance in the polluted area was higher, lower or unchanged. In their study, for three investigated species the site characteristic, an index combining forest age and amount of available foraging substrate, was a more important determinant of occurrence than the pollution level. The changes in colony frequency of the other two species were attributed to metal tolerance, but the authors did not rule out the influence of other unmeasured factors.

The sensitivity of invertebrates other than ants to heavy metal pollution has been demonstrated in a number of studies (e.g., Sandaa et al. 1999; Spurgeon and Hopkin 1999; Smit et al. 2002; Gongalsky et al. 2007). A common result is a decrease in species diversity (measured as species diversity and/or species evenness), sometimes due to shifts in community composition that eliminate more sensitive species and promote tolerant (Del Val et al. 1999; Beyrem et al. 2007), and/or opportunistic (Syrek et al. 2006) or invasive species (Piola and Johnston 2008). However, a clear increase in species abundance, richness or evenness is not commonly found. Among fifty publications that described the influence of metal pollution on terrestrial and aquatic invertebrates, vascular plants and algae, only three reported increased diversity with increasing pollution. Migliorini et al. (2004) found that the abundance of Protura, Diplura and Collembola increased with metal pollution, while Symphyla abundance decreased considerably. Russell and Alberti (1998) confirmed Protura as metal tolerant, as this group was the only one found at the most contaminated sites. The increase in species abundance seems to be a consequence of competitive release whereby sensitive groups such as Symphyla decrease while populations of tolerant species increase. Nahmani and Lavelle (2002) also found that the abundance of some groups of arthropods (Hoplinae larvae, Coleoptera Staphylinidae) was positively correlated with metal pollution. Although a direct reason for that pattern was not proposed, the authors pointed to the possible importance of interactions among soil fauna.

The ant species of the investigated community have generally comparable feeding behavior. All are nonspecialized predators, and all except for the genus *Leptothorax* genus are honeydew consumers, with higher or lower percentages of honeydew in their diet (Czechowski et al. 2002). Thus, it can be assumed that the dietary niches of the ants found in the investigated area of the present study overlap. In the context of the competitive release notion described above, the decrease in abundance of behaviorally dominant species would explain the increase in species diversity found. *Formica polyctena* (in forests) and *Formica cunicularia* (in meadows) were considered as behaviorally dominant species due to their aggressive behavior (Driessen et al. 1984; Czechowski et al. 2002). However, RDA analysis did not confirm any distinct decrease in abundance of this species along the pollution gradient (Figure 2). No significant relationship between the abundance of these two species and pollution level was found (*F. polyctena* abundance vs. log Zn: $P = 0.259$, $r = 0.453$; *F. cunicularia* abundance vs. log Zn: $P = 0.359$, $r = 0.375$).

Studies on spiders and carabids would be very helpful in explaining the present results, as they may act as both predators and competitors of ants (Reznikova and Dorosheva 2004; Pekár 2005; Dorosheva and Reznikova 2006). Earlier studies in wet and dry meadows along the pollution gradient in Bolesław showed a weak but non-significant decrease of spider abundance with increasing pollution (Żmudzki et al. 2008). Thus, the release of predation pressure or reduced competition from spiders as a cause of the trend observed in these ant communities seems unlikely. Along the pollution gradient in Bolesław, however, abundance of carabid beetles decreased (Skalski et al. 2002; see also Zygmunt et al. 2006), so it is possible that reduced predation pressure from ground beetles or reduced ant/beetle competition contributed to an increase of ant species diversity. Krzysztofiak (1991) found a negative relationship between ants and their predators/competitors; the density of *Lasius niger* colonies was positively correlated with metal pollution and was due to a decrease in the proportion of other groups of predatory invertebrates (Araneae, Carabidae and Staphylinidae).

Metal pollution may influence communities indirectly by changing abiotic conditions such as temperature or moisture. Hypothetically, the diversity of ants may increase along a pollution gradient if pollution decreases vegetation density and thereby promotes thermophilic ant species (see Czechowski et al. 2002). On the other hand, metal pollution may promote moisture-preferring species by reducing microbial activity, allowing more litter to accumulate (Stefanowicz et al. 2008). As neither temperature nor humidity or vegetation density were recorded in this study, the importance of these effects in explaining the observed pattern remains an open question, but phytological maps (Woch, 2006, unpublished) covering a large part of the sites used in the present study show no distinct relationship between pollution and vegetation density in either the meadows or the forests of the area.

This study provides clear evidence for increased diversity of ant species with increasing metal pollution. This result can probably be explained by indirect effects of metal pollution, that is, changes in species interactions, rather than by changes in abiotic conditions. Other studies cited here suggest that decreased predation and competition from ground beetles may at least partly explain the results.

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