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Zinc and Cadmium Regulation Efficiency in Three Ant Species Originating from a Metal Pollution Gradient

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Abstract Highly efficient accumulation of trace metals is often reported in ants, but their metal regulation strategies are poorly understood. This study examined the relationships between Zn and Cd total (tot) and water soluble (ws) concentrations in soil and in workers of three ant species collected along a metal-pollution gradient: *Formica cunicularia*, *Lasius flavus* and *Myrmica rubra*. Regression line comparisons showed the body loads of metals to depend strongly on the metal and the species. *M. rubra* showed the most efficient regulation of Zn, as its average Zn concentration and the regression slope were several times lower than for the other species. Although the species differed in their Cd levels, the slopes of the relationships between Cd concentration in soil and in ants did not differ between species (tot: $p = 0.71$, ws: $p = 0.31$). The very weak relationship for Cd found for all species suggests at least some active Cd regulation. These results can be explained in the context of tissue-specific metal accumulation. High Zn accumulation in mandibles and ovarioles may explain its high accumulation in *F. cunicularia* and *L. flavus*.

Keywords Accumulation · Ants · Trace metals · Pollution gradient

An understanding of the mechanisms of metal regulation in ants can help explain the differences in metal sensitivity between species (see Eeva et al. 2004), and is needed for this group to be used effectively in biomonitoring studies. Among terrestrial invertebrates, ants, especially the Formicinae subfamily, are considered second only to spiders in their efficiency of metal accumulation (Heikens et al. 2000). The literature provides much evidence for this (Migula and Glowacka 1996; Rabitsch 1995; Silva et al. 2006). However, metals cannot be accumulated in the body infinitely; ants should switch to active regulation when the metal storage capacity of their bodies is reached (Rabitsch 1997, see also Rainbow 2002).

A previous study of the ant *Lasius niger* provided evidence of active regulation of Cd (Grzes 2009a). The purpose of this study was to compare Cd and Zn regulation between three ant species collected from meadows along a metal pollution gradient: *Formica cunicularia, Lasius flavus* and *Myrmica rubra*. Body concentrations of Cd and Zn were analyzed and then regressed against total and water-soluble forms of the two metals in the soil of the collection sites. A linear relationship between body and soil metal concentration, which describes accumulation of metal without its elimination or limitation of its uptake, was taken as the null model (Grzes 2009a).

Materials and Methods

The study area is in the vicinity of the Boleslaw zinc and lead smelter near Olkusz in southern Poland. Metal concentrations in the humus layer in this region exceed 9,600 mg kg^{-1} Zn, 1,500 mg kg^{-1} Pb and 80 mg kg^{-1} Cd (Stone et al. 2001). Three ant species were selected: *Formica cunicularia, Lasius flavus* and *Myrmica rubra*. All are common in Poland, and are typical of open habitats (Czechowski et al. 2002). Previous work indicated that the three species are common in the area affected by the smelter (Grzes 2009b). They differ in their dietary preferences. *F. cunicularia* is mainly predaceous and

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scavenging (Czechowski et al. 2002), but may supplement its diet with honeydew (Novgorodova 2005). *L. flavus* feeds mainly on honeydew of specially raised root aphids, while *M. rubra* is predaceous but feeds on honeydew of aphids and scale insects more than other *Myrmica* species do (Novgorodova 2005; Czechowski et al. 2002).

Eight study sites (M1–M8) were established in meadows along the pollution gradient (Table 1). Total soil concentrations of Zn and Cd at sites M4–M8 were assessed by Stefanowicz et al. (2008), and at M1–M3 by me using the same methods. The ants were collected twice during the first week of June 2006. Between one and three independent colonies of each species, >10 m apart, were found at each site (totals: 17 *F. cunicularia* colonies, 21 *L. flavus* colonies, 18 *M. rubra* colonies). About 40 workers were collected with a plastic exhaustor from the mound surface from each colony. Because the main part of *L. flavus* nests is built under the ground, to collect workers the topmost part of the mound was opened slightly until the first workers emerged. The captured ants were transported to the laboratory in plastic boxes and starved for 3 days at 20°C to empty their gut. Ants that survived were killed by freezing, washed in deionized water and stored at −20°C for further analysis. After defrosting, samples containing 15–30 ants were dried at 105°C for 12 h and digested in boiling HNO_3 (Suprapur, Merck, Germany) in acid-washed Pyrex tubes. The samples were analyzed for Cd and Zn concentration by flame (Zn) or graphite furnace (Cd) atomic absorption spectrometry, GF-AAS (AAnalyst 800, PerkinElmer, Boston, MA, USA). Three blank samples and three samples of standard reference material (lyophilized bovine liver CRM 185R, European Commission) accompanied every run. Zn fell within 10% and Cd within 20% of the certified value.

The obtained body concentrations of Cd and Zn for each colony were regressed against total and water-soluble forms of Zn and Cd in the soil of the study sites using simple regressions in four independent analyses with colony as data point (body Zn vs. soil total Zn, body Zn vs. soil water-soluble Zn, body Cd vs. soil total Cd, body Cd vs. soil water-soluble Cd).

The regression line slopes and intercepts were compared between species using the procedure in Statgraphics Centurion XV (StatPoint, Inc.) in four independent analyses with colony as data point (body Zn vs. soil total Zn, body Zn vs. soil water-soluble Zn, body Cd vs. soil total Cd, body Cd vs. soil water-soluble Cd).

Results

Table 2 gives the average species-specific body concentrations of Cd and Zn calculated by pooling the results of all colonies of a species from each study site.

Comparison of the regression lines for body Cd vs. both total (tot) and water-soluble (ws) concentrations of Cd in soil showed significant differences in intercepts between species ($p < 0.0001$ for both tot and ws), meaning that the species had different levels of Cd already at the control site, following the order *M. rubra* < *F. cunicularia* < *L. flavus*. The differences in slopes were not significant, (tot: $p = 0.71$, ws: $p = 0.31$, Fig. 1). The slopes were very shallow (Table 3), indicating that body Cd in all species depended only weakly on soil Cd. Nevertheless, both models were significant at $p < 0.0001$.

Comparison of the regression lines for Zn showed significant differences in slopes (tot: $p = 0.017$, ws: $p = 0.022$) and intercepts (tot: $p < 0.0001$, ws: $p < 0.0001$, Fig. 2). *M. rubra* had the lowest Zn concentration at the control site, and the lowest regression slope across the pollution gradient (Table 3). Both models were significant at $p < 0.0001$.

Discussion

The results showed that metal regulation efficiency depended on the metal and the species. For both metals

Table 1 Concentrations of total and water soluble (WS) metals in the upper soil layer at the study sites M1–M8

Site	Location N/E	Distance from the smelter (km)	Zn_{total} (mg/kg)	Zn_{ws} (mg/kg)	Cd_{total} (mg/kg)	Cd_{ws} (µg/kg)	Pb_{total} (mg/kg)	Pb_{ws} (mg/kg)
M1	50°17′/19°32′	4.3	4,644.5	7.2	21.00	80.5	2,110	1.97
M2	50°16′/19°28′	0.6	2,229.5	11.1	20.33	79.5	814	1.57
M3	50°18′/19°33′	5.5	1,245.6	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

Table 2 Averages body concentrations of Zn and Cd in investigated species

Species	Site	Nest number	Cd (mg/kg)	Zn (mg/kg)
Formica cunicularia	M1	3	29.25	907.66
	M3	3	10.49	456.61
	M4	3	19.39	802.90
	M5	2	30.38	320.11
	M6	3	23.75	388.79
	M7	2	19.90	389.13
	M8	1	19.54	335.25
Lasius flavus	M1	3	82.06	882.31
	M2	3	62.65	575.42
	M3	1	62.78	299.87
	M4	2	60.59	514.66
	M5	2	53.54	285.72
	M6	3	37.93	279.72
	M7	3	59.96	318.30
	M8	3	41.71	292.19
Myrmica rubra	M2	3	5.51	199.62
	M3	3	6.39	192.17
	M4	2	0.92	195.14
	M5	1	4.69	185.97
	M6	2	1.96	201.48
	M7	3	5.95	180.98
	M8	3	3.26	181.42

there were significant differences in intercepts, meaning that at the least polluted site (control) the species differed in their metal accumulation. Differences in slopes were found only for Zn, indicating that regulation was most efficient in *M. rubra* (Fig. 2); *F. cunicularia* and *L. flavus* showed stronger dependence of body Zn on soil Zn. The slopes for Cd were very shallow and did not differ significantly between species, meaning that all species were able to maintain almost stable levels of this metal. Assuming that stability of body load of metal along the pollution gradient indicates active regulation of it, regulation efficiency was highest for *M. rubra*. The very weak relationship for Cd found for all species shows effective Cd regulation.

Why does *M. rubra* regulate Zn better than the other species, and why is Cd regulated differently from Zn? Rabitsch (1997) investigated tissue-specific accumulation patterns in three ant species collected at a polluted site: *F. pratensis*, *F. polyctena* and *Camponotus ligniperda*. Both Zn and Cd appeared to be accumulated mainly in the midgut and Malphigian tubes, but very high levels of Zn were also found in ovarioles and mandibles. Metals stored in the midgut and Malphigian tubes may be eliminated easily with feces (Rabitsch 1997; Lidqvist et al. 1995;

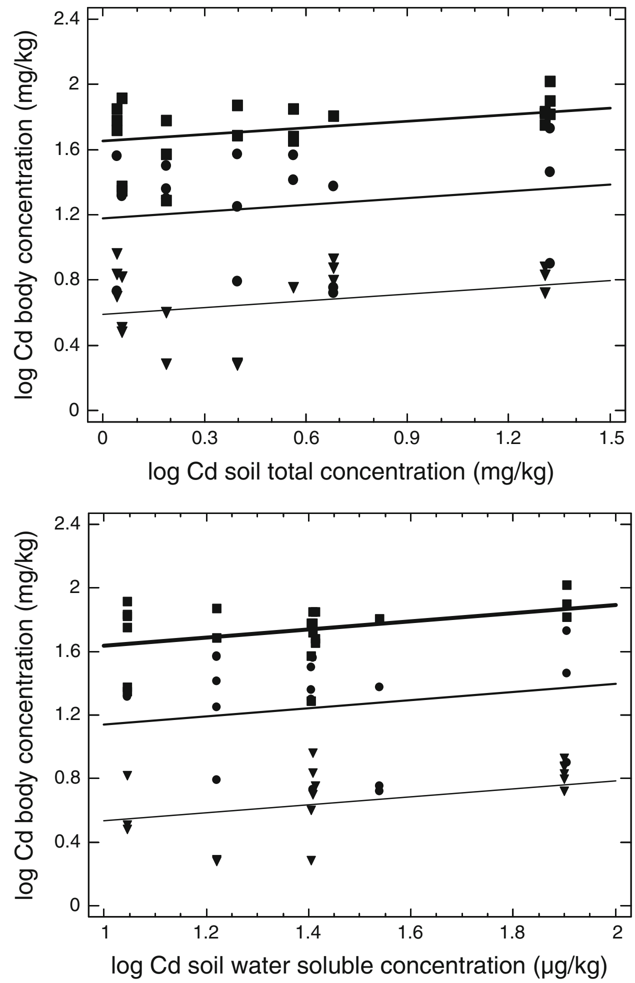

Fig. 1 Relationship between total and water soluble soil Cd concentration and Cd concentration in the three ants species: *Lasius flavus* (filled square; thickest line) *Formica cunicularia* (filled circle; middle-thickness line) *Myrmica rubra* (filled triangle; thinnest line) The differences in slopes were not significant in both cases ($p = 0.71$, $p = 0.31$) and were withdrawn from the models. Each point indicates one colony

Hopkin 1989), allowing body loads of metals to kept stable regardless of the ambient levels. Binding and short-term storage of metals in the midgut and Malphigian tubes may account for Cd regulation in ants. High Zn or Cd concentrations in the midgut and Malphigian tubes have been found in other insects as well (Craig et al. 1998; Sohal and Lamb 1997; Warchalowska-Sliwa et al. 2005).

In contrast to Cd, very high levels of Zn can be stored by ants in ovarioles and mandibles (Rabitsch 1997). High concentrations of Zn in these organs have also been found in other insects (Hillerton and Vincent 1982; Schmidt and Ibrahim 1994) and in crabs (Teixeira et al. 2008). In ants, however, as Rabitsch (1997) noted, the workers are sterile females, their ovarioles do not produce eggs, and only the queen is responsible for reproduction in a colony. This means that ant workers can safely store higher concentrations

Table 3 Regression coefficients estimated for plotting Cd and Zn concentrations in the bodies of three ant species vs. total (tot) and water soluble (ws) forms of metals in soil using simple regression

Species	Cd_{tot}		Cd_{ws}		Zn_{tot}		Zn_{ws}	
	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope
Formica cunicularia	1.235***	0.033	1.303*	−0.03	2.286***	0.159*	2.515***	0.423*
Lasius flavus	1.627***	0.177*	1.396***	0.243	1.972***	0.238***	2.406***	0.324**
Myrmica rubra	0.578***	0.164	0.012	0.434**	2.224***	0.021	2.262***	0.028

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

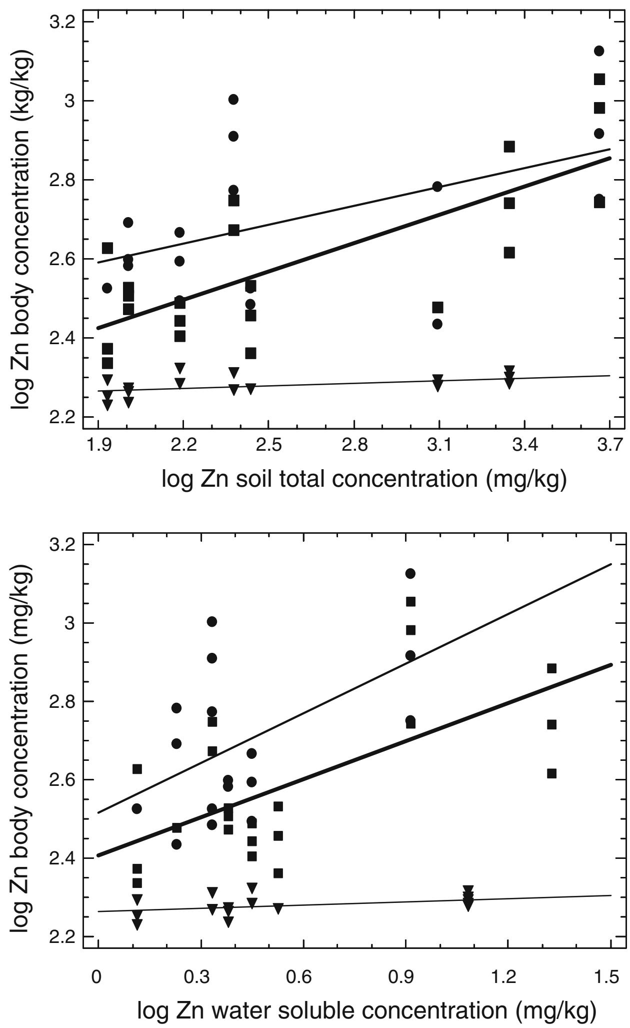

Fig. 2 Relationship between total and water soluble soil Zn concentration and Zn concentration in the three ants species: *Lasius flavus* (filled square; thickest line) *Formica cunicularia* (filled circle; middle-thickness line) *Myrmica rubra* (filled triangle; thinnest line). Each point indicates one colony

of Zn in their ovarioles than other insects can. Long-term accumulation of Zn in ovarioles and mandibles might be responsible for the increased body loads of Zn that I found in *F. cunicularia* and *L. flavus* along the studied pollution gradient. It would be interesting to compare Zn accumulation in these organs in these two species and in *M. rubra*. Rabitsch (1997) reported such differences for other species.

As mentioned, the investigated species differ in their feeding preferences. The metal concentration in the diet was not investigated here; the extent to which differences in diet influenced the accumulation pattern found is beyond the scope of this study. Of the three species investigated, *M. rubra* and *F. cunicularia* have the most similar diet preferences. Both are general predators and honeydew consumers to some extent, but their Zn accumulation patterns differed substantially. Differences in physiology rather than feeding preferences may be more important in explaining those accumulation patterns.

The investigated species differed in their metal regulation efficiency, but all showed some ability to regulate Cd. Of the three species, *M. rubra* regulated Zn most efficiently. The high accumulation of Zn in the other species may be due to long-term storage of metals in ovarioles and mandibles. To elucidate the surprisingly large interspecific differences in metal accumulation, more information is needed on the role of ovarioles in metal accumulation in ants.

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