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Cadmium regulation by *Lasius niger*: A contribution to understanding high metal levels in ants

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Abstract Ants (Formicinae, Hymenoptera) are considered efficient accumulators of Zn and Cd. In this study the relationship between Cd concentrations in soil and in *Lasius niger* workers was assessed for 20 colonies located along a gradient of Cd pollution. As soil Cd concentration increased from 1 to 8 mg/kg, ant body Cd concentration increased rapidly to about 40 mg/kg, and remained stable at this level, through a progressive increase of soil Cd concentration from 8 to 21 mg/kg. The phase of rapid increase confirms the high ability of *L. niger* for Cd accumulation, while the stabilization of Cd body concentration indicates either increasing elimination rate or decreasing rate of uptake. Therefore it can be concluded that *Lasius niger* has a high ability to accumulate Cd, but simultaneously an ability for Cd regulation was detected.

Key words accumulation, ants, elimination, heavy metals, pollution gradient, regulation

Introduction

High body concentration of heavy metals, especially of Zn and Cd in ants, is a well known phenomenon reported by many authors (Stary & Kubiznakowa, 1987; Nuorteva, 1990; Migula & Głowacka, 1996; Rabitsch, 1997a, b; Eeva *et al.*, 2004). It has been demonstrated that metal levels in ants differ among species, seasons, life stages and sites. Regarding ants originating from unpolluted areas, Zn and Cd concentrations vary in the range of 128–1 392 mg/kg and 0.3–90.1 mg/kg, respectively (Stary & Kubiznakowa, 1987; Ylä-Mononen, 1989 after Nuorteva, 1990; Nuorteva, 1990). These levels may be even higher in ants originating from metal polluted areas, indicating a high ability of ants for Zn and Cd accumulation. Examples of Zn and Cd concentrations in ants reported from the literature are provided in Table 1.

Although accumulation of metals in ants has been widely investigated, we still do not know how ants deal with excessive metal uptake in polluted environments. Theoretically, high accumulation may result from a regulation strategy, called immobilization, which is based on metal storage in metabolically inactive forms in the body (see Vijver *et al.*, 2004). However, as noted by Rabitsch (1997b), metals cannot be accumulated indefinitely because of limited storage capacity, and therefore a switch from accumulation to elimination should take place when maximum storage capacity is reached. It was shown that invertebrates can balance body concentration of metals by restricted assimilation from food and/or by metal excretion with feces (e.g. Janssen *et al.*, 1991, Babič *et al.*, 1997, Spurgeon & Hopkin, 1999). Accumulation combined with excretion of excessive metal load is a well known regulation strategy in many terrestrial and aquatic arthropods, especially for essential metals (cf. Janssen *et al.*, 1991; Rainbow, 2002).

Accumulation ability of ants living in metal-polluted areas is therefore likely to be combined with metal elimination or with uptake restriction. Empirical data supporting this hypothesis are limited because accumulation of Zn and Cd usually has been compared between polluted and unpolluted sites: an approach which does not give much insight into the relationship between body concentration and pollution level. Assessing this relationship would help

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Table 1 Zn and Cd levels in species of ants reported by various authors.

Species	Zn (mg/kg)	Cd (mg/kg)	Locality
Formica fusca[†]	545	14.9	Austria, Arnoldstein
Formica lumbris[‡]	662	15.1	Czechoslovakia, Spalenec
Formica aquilonia[§]	550	10.1	Finland, Harjavalta
Formica polyctena[‡]	528	6.5	Finland, Perniö
Formica polyctena[‡]	784	40.2	Czechoslovakia, Kvetna
Formica polyctena[‡]	716	28.2	Czechoslovakia, Spalenec
Formica pratensis[‡]	1392	90.1	Czechoslovakia, Kvetna
Formica rufa[‡]	550	6.9	Finland, Espoo
Formica rufa[‡]	382	6.7	Finland, Perniö
Formica rufa[‡]	319	26.1	Czechoslovakia, Kvetna
Formica sanguinea[‡]	600	6.9	Finland, Espoo
Formica truncorum[‡]	590	9.0	Finland, Espoo
Lasius flavus[‡]	190	5.9	Finland, Bromarv
Lasius platytotax[‡]	276	28.6	Austria, Arnoldstein
Myrmica rubra[‡]	128	0.3	Finland, Perniö
Myrmica ruginodis[‡]	128	0.6	Finland, Perniö
Myrmica sabuleti[†]	435	7.2	Austria, Arnoldstein
Tetramorium caespitum[†]	211	3.7	Austria, Arnoldstein

[†]Rabitsch, 1995, polluted site.

[‡]Stary & Kubiznakowa, 1987; Ylä-Mononen, 1989 after Nuorteva, 1990; Nuorteva, 1990, unpolluted sites.

[§]Eeva *et al.*, 2004, polluted site.

to test this hypothesis based on the assumption that if accumulation dominates, body metal concentration should increase gradually along the gradient of pollution. If instead metal elimination or limited uptake takes place, this should result in stabilization of body level along the pollution gradient.

In this study adult workers of the ant *Lasius niger* collected along a metal pollution gradient were analyzed for body Cd concentration. Body concentrations were then plotted against total Cd concentration in soil and the relationship between the two variables was analyzed using many models of simple regression. The linear relationship was treated as the null model, which describes accumulation without elimination or limited metal uptake.

Materials and methods

The study area is located in the vicinity of "Boleslaw" zinc-and-lead smelter near Olkusz in southern Poland. Metal concentrations in the humus layer in this region exceed 9 600 mg/kg of Zn, 1 500 mg/kg of Pb and 80 mg/kg of Cd (Stone *et al.*, 2001). The ant *Lasius niger* (L.) is a common species in Poland, typical of both forests and open habitats (Czechowski *et al.*, 2002). Previous studies indicated that *L. niger* is the most common ant species in the area affected by the smelter (Grześ, unpublished data, 2005).

Seven study sites (S1 – S7) located on meadows were established along the pollution gradient. Total Zn and Cd soil concentrations and water-soluble concentrations of Cd in sites S3 – S7 were assessed by Stefanowicz *et al.* (2008); the metal concentrations of sites S1 and S2 were analyzed by the author using the methods described in Stefanowicz *et al.* (2008). Concentrations of total and water-soluble forms of Cd are positively correlated ($P < 0.000\,1$). Sampling of ants was performed during one day in the beginning of September 2005. At each site three independent colonies were selected (21 colonies in total, distance between colonies >10 m). About 30 adult workers were collected from the mound surface of each colony using a plastic exhauster. After catching the ants, they were transported to the laboratory in plastic boxes and starved for 3 days at 20 °C to empty their guts. Ants that survived were killed by freezing, washed in deionized water and stored at

Table 2 Zinc and Cd concentrations in 10 cm-deep topsoil and other characteristics of the seven study sites in the vicinity of the "Boleslaw" smelter (sites S3 – S7, after Stefanowicz *et al.*, 2008).

	Site						
	S1	S2	S3	S4	S5	S6	S7
Distance from smelter (km)	4.3	5.5	0.6	8.6	4.1	20.0	32.6
Geographical coordinates							
N	50° 17'	50° 18'	50° 16'	50° 20'	50° 18'	50° 25'	50° 32'
E	19° 32'	19° 33'	19° 28'	19° 33'	19° 29'	19° 38'	19° 38'
Total Cd (mg/kg)	21.00	4.800	20.33	3.667	2.497	1.540	1.138
Water-soluble Cd (μg/kg)	80.5	34.6	79.5	16.6	25.9	25.4	11.1
Total Zn (mg/kg)	4 644.5	1 245.6	2 229.5	272.85	238.90	154.00	85.53

–20 °C for further analysis. After being defrosted, samples containing 7–20 ants were dried at 105 °C for 12 h and digested in a mixture of boiling HNO_3 and $HClO_4$ (7:1) suprapure-grade acids (Merck, Darmstadt, Germany) in acid-washed Pyrex tubes. The samples were analyzed for Cd concentration using graphite-furnace atomic absorption spectrometry (GF-AAS: PerkinElmer, Boston, MA, USA). One sample was excluded from the analysis due to a mistake during sample preparation. Three blank samples and three samples of reference-standard material (lyophilized bovine liver CRM 185R, European Commission) accompanied every run of the analysis. The trend in body Cd concentration along the soil pollution gradient was analyzed by a set of simple regression models and the final model was selected based on the highest R^2 and a non-significant lack-of-fit test.

Results

Body concentration of Cd averaged 32.36 mg/kg ($n = 20$). As soil Cd concentration increased from 1 to 8 mg/kg, ant body Cd concentration increased rapidly to approximately 40 mg/kg and remained stable at this level despite progressive increase of soil Cd concentration from 8 to 21 mg/kg (Fig. 1). The linear model was not adequate (lack-of-fit test $P = 0.014$). Of the other models tested (reported in Table 3), the S-curve model [$y = \exp(a + b/x)$ where a, b are estimated parameters] yielded the highest R^2 value (66.21%) and was significant at $P < 0.000\,1$ (lack-of-fit test $P = 0.80$).

Table 3 Results of fitting simple regression models to the data on log Cd body concentrations of *Lasius niger*.

Model	Correlation	R^2
S-curve model	–0.8137	66.21%
Square root-Y reciprocal-X	–0.8068	65.09%
Square root-Y logarithmic-X	0.7750	60.06%
Logarithmic-X	0.7722	59.62%
Reciprocal-X	–0.7641	58.38%
Double reciprocal	0.7479	55.93%
Multiplicative	0.7421	55.08%
Square root-X	0.7047	49.66%
Squared-Y logarithmic-X	0.7035	49.49%
Double square root	0.6882	47.36%
Squared-Y square root-X	0.6730	45.29%
Logarithmic-Y square root-X	0.6401	40.97%
Squared-Y reciprocal-X	–0.6365	40.52%
Linear	0.6237	38.90%
Squared-Y	0.6192	38.34%
Square root-Y	0.5952	35.43%
Exponential	0.5401	29.18%
Double squared	0.5308	28.18%
Reciprocal-Y square root-X	–0.5109	26.10%
Squared-X	0.5089	25.90%
Square root-Y squared-X	0.4719	22.27%
Logarithmic-Y squared-X	0.4152	17.24%
Reciprocal-Y	–0.4117	16.95%
Reciprocal-Y squared-X	–0.2986	8.92%
Logistic	no fit	–

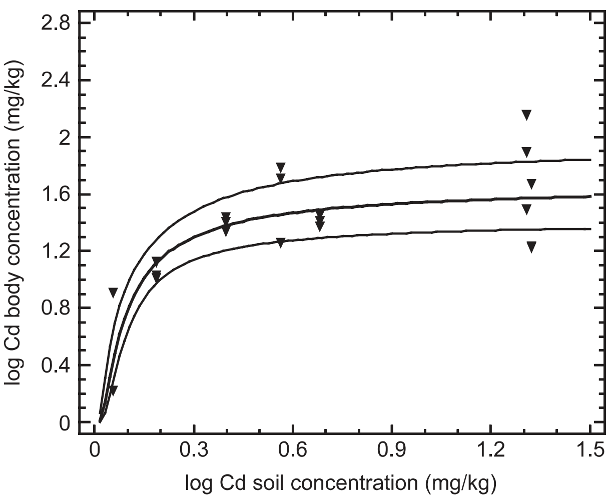

Fig. 1 Relationship between total soil Cd concentration and total Cd concentration in the bodies of *Lasius niger* originating from the vicinity of the "Boleslaw" smelter [simple regression, S-curve model, $y = \exp(0.508 - 0.074/x)$, $P < 0.000\,1$, $R^2 = 66.21\%$, $n = 20$]. The thinner lines denote 95% confidence limits.

Discussion

The Cd levels found in *L. niger* from polluted "Boleslaw" sites were comparable to those reported for closely related *L. platythorax* from a polluted area in Arnoldstein, Austria (Rabitsch, 1990; Table 1). However, in addition to earlier studies, stabilization of Cd concentration was observed along the pollution transect, meaning that *L. niger* is capable of cadmium regulation. This conclusion matches the expectations described in the Introduction; however, the mechanism of that regulation (restricted uptake or elimination) remains unknown. Further implications of this result are as follows.

(i) *L. niger* is not a suitable species for biomonitoring programs determining Cd availability to arthropods. Because of rapid accumulation on the one hand and efficient regulation at high pollution levels on the other, Cd concentration in ants would not reflect differences in soil contamination level.

(ii) Possible adaptations of *L. niger* to polluted environments may be based on a higher production of metallothioneins and type B granules. These structures are known to play a key role in both Cd immobilization and elimination in invertebrates (Hopkin, 1989). It would require further studies to sort out how Cd is immobilized and stored in ants and how it happens that efficient regulation seems to be "turned on" at a certain Cd level (40 mg/kg, Fig. 1).

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