

## Metal Kinetics and Respiration Rates in F<sub>1</sub> Generation of Carabid Beetles (*Pterostichus oblongopunctatus* F.) Originating From Metal-Contaminated and Reference Areas

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**Abstract.** We investigated resistance to metals in carabid beetles inhabiting metal-polluted and reference areas. Chronic multigeneration exposure to toxic metal concentrations may potentially result in adaptation through decreased metal uptake rate and/or increased excretion rate. The cost of resistance to pollution could be associated with increased metabolic rate. To test these predictions, laboratory cultured F<sub>1</sub>-generation beetles originating from metal-polluted and reference sites were exposed to food contaminated with zinc and/or cadmium for 10 weeks. After that, uncontaminated food was offered to the animals for another 3 weeks. During the experiment, internal concentrations of Cd and Zn were measured as were respiration rates of the animals. The results obtained show no significant differences in metal accumulation and excretion patterns or respiration rates between the populations. This may suggest that adaptation has not occurred in the beetles chronically exposed to toxic metal concentrations. The possible explanations for the lack of differences between the populations are discussed.

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Chronic exposure to sublethal concentrations of toxic chemicals in the environment can lead to changes at all levels of biologic organization of soil and epigeic invertebrates: biochemistry and physiology of individuals, populations, and whole communities (*e.g.*, Donker 1992; Posthuma and van Straalen 1993; Jones and Hopkin 1998; Lagisz *et al.* 2002c). Recently, one of the most actively discussed problems in ecotoxicology has been the cost of tolerance to pollution. The topic arises from the widely recognized theory of energetic trade-offs in life history assuming that the energetic budget of every organism is limited. Consequently, the available energy must be distributed to all processes in such a way as to maximize the organism's fitness, and the selection pressure is expected to optimize this trade-off (Sibly and Calow 1989).

Detoxification of metals is connected to many energetically costly processes. For example, the production of the metal-binding proteins, metallothioneins, may cause an increase in metabolic rate (Hopkin 1989; Walker *et al.* 2001). It has been observed that respiration rate was increased by 51% in the shrimps *Palaemonetes paludosus* (Rowe 1998), by 175% in the tadpoles of frog *Rana catesbeiana* (Rowe *et al.* 1998), and by 32% in the water snakes *Nerodia fasciata* (Hopkins *et al.* 1999) collected in metal polluted environments compared with individuals of the same species originating from unpolluted sites. Similarly, the pupae of moths *Spodoptera exigua* fed with diet contaminated with zinc for a dozen generations exhibited an increase in respiration rate compared with the control animals (Kramarz and Kafel 2003).

At the same time, animals exposed to increased metal concentrations may suffer physiologic deterioration caused by direct toxicity of the metals, and this can be reflected in decreased respiration rate (Devi 1997). In fact, exposure to copper-contaminated food caused a decrease in oxygen consumption in centipedes *Lithobius mutabilis* (Laskowski *et al.* 1996). A significant decrease in respiration rate was also found in snails *Turbo intercostalis* (Pandeswara and Yallapragada 2000) and in mussels *Mytilopsis sallei* (Devi 1997) intoxicated with cadmium. Decreased metabolic rate was observed in house crickets *Acheta domesticus* exposed to cadmium but not when exposed to zinc or lead (Migula 1986).

Metals, being nondegradable, tend to accumulate in animals. However, numerous investigators have reported that soil invertebrates belonging to the same trophic level and exposed to the same environmental concentrations of metals often display different internal concentrations of metals as result of different strategies of decontamination (*e.g.*, Laskowski 1991). Ground beetles, as predators, are potentially exposed to increased levels of toxicants. At the same time, some species are abundant even at heavy metal-polluted localities and are known for efficient metal-detoxification mechanisms (Janssen 1991; Kramarz 1999a).

We aimed to study accumulation and excretion of cadmium, zinc, and a mixture of both—as well as the cost of metal detoxification expressed as respiration rate, body mass change, and survival probability—in the carabid beetle *Pterostichus oblongopunctatus* F. This species was chosen because of its

high abundance in the vicinity of the zinc- and lead smelter “Boleslaw” in Southern Poland, which has been highly polluted with zinc, cadmium, and lead for at least 50 years. *P. oblongopunctatus* is a generalist predator, and its diet consists of seasonally available invertebrates and plants; therefore this species is potentially exposed to a broad range of metal concentrations in its diet. Zinc and cadmium were chosen for the experiment on the basis of their different physiologic functions: cadmium is regarded as a xenobiotic (nonessential) metal, whereas zinc is a micronutrient (essential). Both are bound by metallothioneins; however, cadmium has higher affinity to them and may displace zinc. The concentrations of metals used in the experiment were chosen in accordance to the earlier study by Mozder *et al.* (2003) on the same species. To clarify if multigenerational intoxication with metals may lead to genetically based changes in mechanisms of metal regulation, we studied the F<sub>1</sub>-generation of beetles reared from the animals collected from two sites: unpolluted and metal-polluted.

## Materials and Methods

### Study Animals

Adult beetles were trapped at two sites: reference (Zn: 151 ± 35 mg kg<sup>-1</sup> dry humus; Cd: 0.84 ± 0.4 mg kg<sup>-1</sup> dry humus; Cu: 10.7 ± 1.0 mg kg<sup>-1</sup> dry humus; Pb: 136.0 ± 8.8 mg kg<sup>-1</sup> dry humus) and polluted (Zn: 5104 ± 729 mg kg<sup>-1</sup> dry humus; Cd: 51.1 ± 19.3 mg kg<sup>-1</sup> dry humus; Cu: 37.6 ± 3.7 mg kg<sup>-1</sup> dry humus; Pb: 1832 ± 215 mg kg<sup>-1</sup> dry humus). The contaminated site was located 2.5 km away from a zinc and lead smelter, whereas the control site was established at a 30-km distance from the source of pollution. Beetles were mated, and hatched larvae were reared on uncontaminated food (cat food Royal Canin Kitten 36). After emerging from the pupa, imagines were overwintered to reach sexual maturity for 2 months at 12°C ± 1°C in a photoperiod of 8 hours light to 16 hours of darkness. Adults of the F<sub>1</sub>-generation process were kept in plastic vials (approximately 150 ml volume) filled with a 1 cm-thick layer of wet peat, maintained at 20 ± 2°C at a photoperiod of 16 hours light to 8 hours of darkness.

At the beginning of the accumulation experiment the beetles were randomly assigned to one of four artificial mediums: (1) control, (2) contaminated with 50 mg Cd kg<sup>-1</sup>, (3) contaminated with or 500 mg Zn kg<sup>-1</sup>, (4) contaminated with a mixture of 500 mg Zn kg<sup>-1</sup> and 50 mg Cd kg<sup>-1</sup>. Approximately the same number (*n* = 19 to 21) of female and male animals in each treatment from each site were used in the experiment. The artificial medium comprised dried chicken meat mixed with metal-salt solutions (CdCl<sub>2</sub> \* 2.5 H<sub>2</sub>O GR-pure [Merck, Poland], ZnCl<sub>2</sub> anhydrous [a.r. Ubichem plc, UK], or mixture of both) to contaminate the food to the desired level. Nominal and actual concentrations of metals in the food for all treatments are listed in Table 1. Animals were fed every 3 days, all the remains of old food were removed; and survival of the beetles was checked and recorded.

### Experimental Setup

During the accumulation period of the experiment at 0, 7, 28, 49, and 70 days, two male and female animals from each group (population/treatment) were randomly selected. After 70 days, the decontamina-

**Table 1.** Concentrations of Zn and Cd in food used as a treatment for beetles (actual concentrations: average ± SD)<sup>a</sup>

Food Treatment	Nominal concentrations		Actual concentrations	
	Zn [mg kg <sup>-1</sup> ]	Cd [mg kg <sup>-1</sup> ]	Zn [mg kg <sup>-1</sup> ]	Cd [mg kg <sup>-1</sup> ]
Control	0	0	22.3 ± 1.3	0.01 ± 0.03
Zn	500	0	481.1 ± 58.7	0.08 ± 0.02
Cd	0	50	22.0 ± 0.9	43.9 ± 3.3
Zn and Cd	500	50	486.2 ± 34.9	39.2 ± 2.7

<sup>a</sup> *N* = 5 for all values.

tion phase began. All of the remaining animals were offered only uncontaminated food and were selected for analysis at days 77 and 98. After 48 hours of starvation, respiration rates were measured in the selected animals. Each individual was weighed to the nearest 0.1 mg (Radwag WPA 180/k, Poland). Two individuals from each group (population/sex/treatment/day) were then placed in a separate 50-ml flask connected to an individual channel of a computer-controlled, closed-circuit, 30-channel respirometer (Micro-Oxymax, Columbus Instrument). Each flask (with an Eppendorf-type tube with distilled water and a hole in the lid to maintain high humidity) was put in a chamber with controlled conditions (20°C ± 0.5°C at a photoperiod of 16 hours light to 8 hours of darkness). The respiration rates were measured for 72 hours at 4-hour intervals. The animals were not provided with food during the measurements. After the measurement the beetles were refrigerated for further analyses.

For chemical analyses, individual animals were dried at 105°C for 12 hours and weighed and digested in 2 ml boiling Suprapur-grade Merck nitric acid. After cooling to room temperature, each sample was diluted to 10 ml with deionized water. Samples of artificial medium were prepared for analyses in a similar way. All samples were analyzed for Zn concentration using flame atomic absorption spectrometry (AAS), and Cd content was measured with graphite furnace AAS (Perkin-Elmer AAnalyst 800). Three blank samples and two samples of standard reference material (bovine liver BCR no. 185, reference material, Sweden; certified concentrations; Zn = 142 ± 3 mg kg<sup>-1</sup> and Cd = 0.298 ± 0.035 mg kg<sup>-1</sup>) accompanied every run of analysis and were used for correction of obtained data.

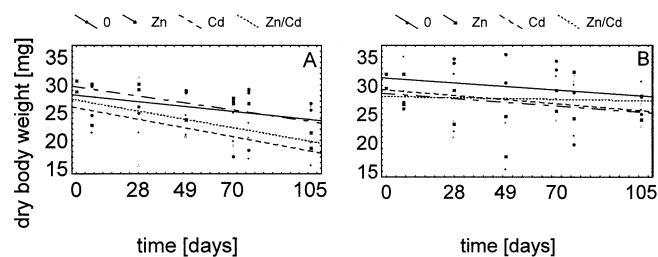
### Statistical Analysis

When analyzing survival, body mass, and metal kinetics, each specimen was treated as an independent replicate. Because we found no significant differences in survival between male and female animals data for both sexes were pooled together to increase the power of analyses. The treatments were compared for their effects on the animals' survival using Gehan's generalized Wilcoxon test for multiple samples (Statistica 6.0, StatSoft), whereas site of origin and treatment were treated as discrete/grouping variables. Body mass was analyzed using Generalized Linear Models (Statistica 6.0).

The kinetics of metals in the beetles during the accumulation and decontamination periods was described with the one-compartment model (Janssen 1991). It was assumed that a metal is assimilated at a constant rate *a* (mg day<sup>-1</sup>) and eliminated at a constant rate *k<sub>a</sub>* (day<sup>-1</sup>). The following equation was used to estimate the parameters,

$$C_t = C_0 + \frac{a[1 - e^{-k_a t}]}{k_a},$$

where *C<sub>t</sub>* is the concentration of a metal in the beetle (mg kg<sup>-1</sup>) at time *t*; *C<sub>0</sub>* (mg kg<sup>-1</sup>) is the average concentration of the metal before the start of the experiment (*t*<sub>0</sub>); *t* is time in days; and *e* is the base of the natural



**Fig. 1.** Changes in dry body weight in the carabid beetle *P. oblongopunctatus* originating from a contaminated site and exposed to four treatments: fed with uncontaminated food (0) and exposed for 70 days to food contaminated, respectively, with zinc (Zn), cadmium (Cd), and mixture of zinc and cadmium (Zn/Cd). After day 70, all animals were fed with uncontaminated food of the same type. (A) Male animals. (B) Female animals

logarithm. For the decontamination period, only the excretion rate ( $k_e$ ) was studied, and the simplified exponential model was used:

$$C_t = C_{70}e^{-k_e[t-t_0]}$$

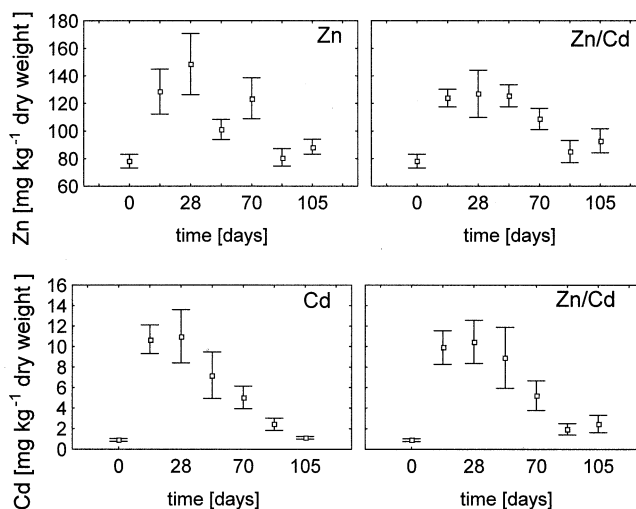
where  $C_{70}$  ( $\text{mg kg}^{-1}$ ) is the metal concentration at  $t = 70$  days (end of accumulation period);  $k_e$  is the excretion rate for the decontamination phase; and  $t_0$  (days) is the time of switching to uncontaminated food (Kramarz 1999). The parameters were estimated from untransformed data using the Levenberg-Marquardt method as implemented in the Statistica 6.0 software package (StatSoft). Because most of the fitted models were statistically insignificant ( $p > 0.05$ ), probably because of the high variation of the obtained data, and did not allow for comparisons between the populations, samples for male and female animals as well as populations were pooled to calculate uptake and excretion rates according to the models described.

Additionally, data on metal concentrations during days 7 to 70 (accumulation experiment) were pooled, log transformed to get normal distributions within groups (population/treatment/sex), and analyzed with analysis of variance (ANOVA). Similarly, data from days 77 and 98 (decontamination experiment) were pooled, log transformed, and also analysed with ANOVA. The influence of cadmium and zinc on each other's accumulation and excretion rates were checked by comparing concentrations during the accumulation and decontamination periods between one-metal and two-metal treatments (Student *t*-test on pooled data).

The data concerning respiration rates were analyzed separately for the accumulation period (days 0, 7, 28, 49, and 70) and the decontamination period. In the case of decontamination period, statistical analyses included the last day of accumulation to check for possible increases or decreases in metabolic rate (*i.e.*, days 70, 77, and 98). The analyzed variables were oxygen consumption, carbon dioxide production, and respiration quotient (RQ). RQ was calculated according to the following equation,

$$RQ = \frac{VCO_2}{VO_2},$$

where  $VCO_2$  is produced carbon dioxide and  $VO_2$  consumed oxygen. Because the obtained values were measured for two individuals at the same time, oxygen consumption and carbon dioxide production were calculated per gram of body mass (and per hour). For the data representing the animals from each group (population/sex/treatment), simple regression was calculated (Statgraphics Plus 5.0, Statistical Graphics) to check if there was any trend in respiration rate during a given period.

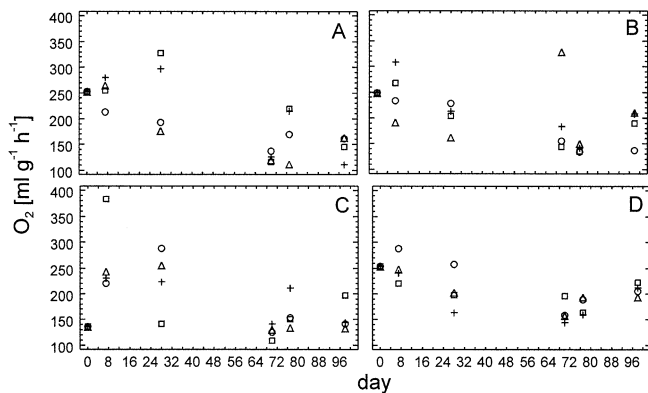


**Fig. 2.** Changes in the internal body concentrations of Zn and Cd (mean values and SE) in the beetles exposed to food contaminated respectively with zinc (Zn), cadmium (Cd), and mixture of zinc and cadmium (Zn/Cd). After day 70 all animals were fed with uncontaminated food of the same type

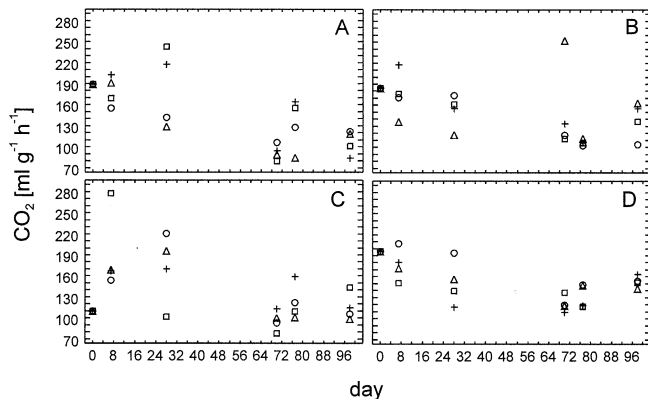
## Results and Discussion

Out of the 315 animals used at the start of the experiment, 88 died before the end of it, and 224 were killed for AAS analysis. The initial dry body mass was significantly lower in the animals originating from the contaminated site ( $p = 0.0002$ ), and female animals were larger than male animals in both populations ( $p < 0.0001$ ). At the beginning of the experiment, there was no difference in body mass between the treatments ( $p > 0.05$ ,  $N = 8$ ). Survival analysis showed that neither sex nor treatment nor population type had any influence on the probability of death during the experiment. However, a general pattern was found showing that animals with larger initial body mass were more likely to survive longer ( $p = 0.0073$ ,  $N = 24$ ). During the experiment, average body mass tended to decrease during all treatments ( $p < 0.001$ ,  $N = 224$ ); however, this effect was most pronounced in the male animals originating from contaminated site (Fig. 1).

The initial concentrations of zinc and cadmium in the animals were similar to those reported by Stone *et al.* (2002) for *P. oblongopunctatus* collected from unpolluted sites, *i.e.*, on average  $78.15 \pm 13.92 \text{ mg kg}^{-1}$  dry weight for Zn and  $0.89 \pm 0.39 \text{ mg kg}^{-1}$  dry weight for Cd ( $N = 8$ ). After the next 70 days of feeding with contaminated food, metal loads in animals treated with metals were significantly higher ( $p < 0.001$ ,  $N = 128$ ) and showed no differences between populations from different sites of origin ( $p = 0.151$  for zinc and  $p = 0.424$  for cadmium;  $N = 128$ ) (Fig. 2). Average zinc concentration for zinc-treated animals was  $125.50 \pm 46.50 \text{ mg kg}^{-1}$  dry weight ( $N = 32$ ), and this result is consistent with numbers reported by Stone *et al.* (2002) for beetles captured at the metal-contaminated sites. This level was reached at the end of the first week, and the concentrations remained high but variable until the end of accumulation period. Female animals accumulated significantly more zinc per unit body mass than male animals ( $p = 0.003$ ). The animals fed with cadmium-



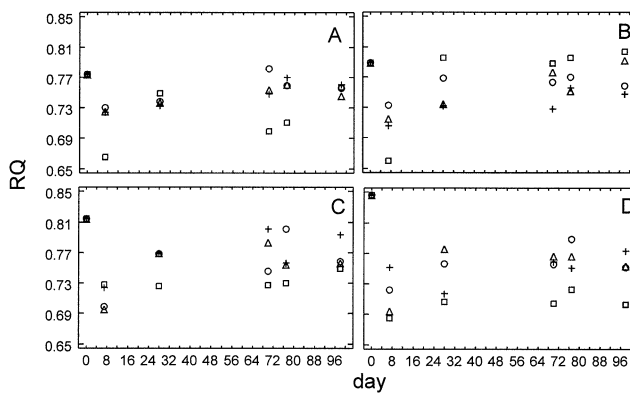
**Fig. 3.** Average oxygen consumption rates of beetles during the course of the experiment. After day, 70 all animals were fed with uncontaminated food of the same type. (A) Female animals from control site. (B) Male animals from control site. (C) Female animals from polluted site. (D) Male animals from polluted site. Square-control treatment; cross-zinc treatment; circle-cadmium treatment; triangle-mixture of zinc and cadmium



**Fig. 4.** Average carbon dioxide production rates of beetles during the experiment. After day 70, all animals were fed with uncontaminated food of the same type. (A) Female animals from control site. (B) Male animals from control site. (C) Female animals from polluted site. (D) Male animals from polluted site. Square-control treatment; cross-zinc treatment; circle-cadmium treatment; triangle-mixture of zinc and cadmium

contaminated food during the accumulation period had average internal Cd concentrations of approximately  $8.41 \pm 5.61 \text{ mg kg}^{-1}$  dry weight ( $N = 32$ ), which was similar to the range reported by Stone *et al.* (2002) for animals collected from polluted sites. At the same time, there was no difference in cadmium concentrations between male and female animals ( $p = 0.512$ ). Already after 7 days of feeding with uncontaminated food (decontamination period), the levels of metals in animals decreased to the values close to those observed at the beginning of the experiment (Fig. 2).

In the case of the accumulation period, all accumulation and elimination coefficients calculated from the pooled data were nonsignificant. The coefficient of elimination ( $k_e$ ), calculated for the decontamination period for animals exposed



**Fig. 5.** Average RQ values of beetles during the experiment. After day 70, all animals were fed with uncontaminated food of the same type. (A) Female animals from control site. (B) Male animals from control site. (C) Female animals from polluted site. (D) Male animals from polluted site. Square-control treatment; cross-zinc treatment; circle-cadmium treatment; triangle-mixture of zinc and cadmium, RQ-respiration quotient

to zinc, amounted to  $0.008 \pm 0.004$  (asymptotic standard error [SE])  $\text{day}^{-1}$  ( $p < 0.04$ ), which equals an excretion rate of about  $1 \text{ mg day}^{-1} \text{ kg}^{-1}$  dry weight. Cadmium  $k_e$  was  $0.047 \pm 0.022$  (asymptotic SE)  $\text{day}^{-1}$  ( $p < 0.05$ ), which yields an excretion rate of approximately  $1.5 \text{ mg day}^{-1} \text{ kg}^{-1}$  dry weight. In the closely related carabid *Poecilium cupreus*, Kramarz (1999) reported the following excretion coefficients ( $k_e$ ):  $0.002 \pm 0.002 \text{ day}^{-1}$  for zinc and  $0.300 \pm 0.019 \text{ day}^{-1}$  for cadmium. Similar cadmium excretion rates those obtained by Kramarz (1999), *i.e.*,  $0.375 \text{ day}^{-1}$ , were also found by Janssen (1991) in *Notiophilus biguttatus*. The observed differences between species may result from species biology as well as from the experiment's setup, *e.g.*, the use of different medium for feeding beetles. However, the results obtained in our study showed that *P. oblongopunctus* is efficient in decontaminating metals and is able to control internal metal concentrations by excretion mechanism.

The differences between metal concentrations in carabids treated with single metals or with a mixture of zinc and cadmium were nonsignificant, which indicated that zinc and cadmium probably did not affect each other's uptake at the levels tested in our study. These results are in agreement with the study by Kramarz (1999).

In our study, we did not find any significant differences in metal assimilation and excretion ability between animals originating from sites with different contamination histories. This suggests that no adaptation in terms of detoxification processes was developed in animals chronically exposed to increased levels of metals. On the contrary, the rapid weight loss observed during the experiment in  $F_1$ -generation male animals originating from the metal-polluted site could be an indirect cue that male animals became more susceptible to additional stresses (*e.g.*, nonnatural food). However, we still cannot exclude the existence of maternal effects, which could influence the characteristics of offspring from animals exposed to metals (Schmidt *et al.* 1992). At the same time, we observed higher levels of accumulated zinc in female than in male animals, which may indicate the existence of different accu-

mulation patterns for this metal in both sexes of *P. oblongopunctatus*.

Comparison of metabolic rates between the two populations also did not show that adaptations were developed in the population from the metal-polluted site. In the case of either toxic effect or acclimation and adaptation to the metal-polluted environment, we should have observed differences in measured values between animals originating from the two sites. In fact, neither respiration rate nor RQ depended on site, treatment, or sex ( $p > 0.05$ , Figs. 3 through 5). Additionally, we did not observe any trend in respiration rate during the experiment ( $p > 0.05$ , Figs. 4 and 5). This outcome correspond well to the study by Lagisz and Laskowski (2002a) in which no differences in respiration rate of *P. oblongopunctatus* collected along the metal contamination gradient (including the sites studied here) were found. Similarly, no changes in metabolic rate were revealed in the populations of the isopod *Porcellio scaber*, which originated from metal-polluted sites (Khali *et al.* 1995) or in the pupae of the moths *S. exigua* exposed for 15 generations to cadmium (Kramarz and Kafel 2003).

This study was run in parallel with a larger project aimed at finding effects of both temporary and long-term pollution with metals on carabid beetles at the biochemical, physiologic individual, population, and community levels. Results obtained so far show that beetles trapped from contaminated sites exhibit higher susceptibility to additional stressors such as food deprivation and contamination as well as insecticide treatment (Stone *et al.* 2001). We observed the effects of contamination on population parameters in P-generation animals, and some differences in characteristics of the F<sub>1</sub>-generation animals, while working on populations originating from contaminated and uncontaminated sites (Lagisz *et al.* 2002; Kramarz *et al.* 2001; Lagisz and Laskowski 2002a, 2002b). We have also conducted experiments on metal dynamics on the F<sub>1</sub>-generation larval stage of *P. oblongopunctatus* originating from the same two sites as used in this study (Mozdzer *et al.* 2003). A significant difference was found between treatments in growth rate and metal accumulation, but not between populations, which is in agreement with the results of our previous experiment (Mozdzer *et al.* 2003). These results indicate that in terms of accumulation rates and resistance to metals in food, no adaptations have occurred in the beetles *Pterostichus oblongopunctatus* inhabiting chronically metal-polluted areas.

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