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Effect of Zinc Contamination on Life History Parameters of a Ground Beetle, *Poecilus cupreus*

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One of the serious environmental problems in a number of highly industrialized and urbanized areas is soil pollution with metals. Being non-degradable, metals tend to accumulate in the surface organic soil layers and enter terrestrial food chains through invertebrates inhabiting soil and litter. From abundant field data it is well known that different groups of terrestrial invertebrates differ widely in their body concentrations of metals (Beyer *et al.,* 1986; Hunter *et al.,* 1987; Laskowski and Maryánski, 1993). Much less is known about the connection between metal contamination and susceptibility to metal poisoning, especially regarding sublethal effects of long-term exposure. Even though some standard laboratory tests consider sublethal effects (e.g., *Eisenia foetida* reproduction test), all of these are based on short-term experiments. Such tests may be satisfactory for evaluating degradable chemicals, such as pesticides, but are of limited use for detecting sublethal effects of those chemicals which can accumulate in the body throughout the organism's lifetime. In the latter situation, two strategies are represented among terrestrial invertebrates: (1) accumulation of pollutants in the body, and (2) decontamination by intense excretion (see Hopkin, 1989 for more details). The first option probably has the advantage of requiring less energy then decontamination processes, but the obvious disadvantage is the potential for accumulating harmful substances to toxic levels. The second option, although protecting an organism from direct toxicity, may require the expenditure of a significant amount of energy.

The Carabidae (ground beetles) is a taxonomic group of special interest for at least two reasons. First, as predators, they represent the second trophic level at least potentially exposed to elevated levels of toxicants through the biomagnification effect (this refers mostly to organic chemicals). At the same time they are soil invertebrates with the lowest concentrations of heavy metals, even in heavily polluted regions (Hunter *et al.*, 1987; Laskowski and Maryánski, 1993; Maryánski *et al.*, 1995). Second, ground beetles are considered 'beneficial' insects owing to their role as predators of many invertebrate pest species. Thus, the knowledge of pollutant effects on fitness in carabids has both scientific and applied value. In this work, we present the results of a 250-day long experiment on the effects of Zn pollution on *Poecilus cupreus* L. - a meadow-living ground beetle.

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MATERIALS AND METHODS

Adult carabids for the experiment were obtained from a laboratory stock culture and were prepared for reproduction according to the method of Heimbach (1992). Five males and five females were put into each of 4 breeding boxes, and the eggs were removed every 3rd day. Individual eggs were put into cells of tissue culture boxes with a piece of wet filter paper. At the outset, each box was assigned to a particular treatment. The eggs collected on one day were distributed at random among all treatments. Due to expected high mortality, to the highest treatment approximately twice as much eggs was assigned as to the control. Every individual had its own record card with the following information: individual symbol, egg collection date, date of eclosion (start of the experiment), date of imago emergence, sex, body mass at emergence from pupae, body mass after overwintering, date of mating and mate's symbol, number of eggs laid to the end of experiment (fecundity), time between mating and first oviposition, number of larvae hatching, and time of death of an individual if occurring before the termination of the experiment.

Immediately after hatching, the larvae were exposed to the experimental treatments. They were fed housefly pupae reared on an artificial medium contaminated with 400 (Zn400) or 6400 (Zn6400) mg Zn kg^{-1} (on a dry weight basis). The control group was offered pupae of housefly reared on uncontaminated food. After hatching, larvae were transferred to plastic vials (30 cm^3) filled with crushed wet garden peat. The larvae were fed *ad libitum*, and uneaten remains were removed every 3rd day. After emergence, adults were sexed and weighed. After 2 weeks, the adults were put in their peat-tilled vials into a refrigerator for overwintering (ca. 7°C complete darkness). After 90 days, all beetles were transferred to long day conditions (16 h light) at 20°C and 15 days later, 20 pairs per treatment were selected for further study and weighed. Four pairs were taken at random from each of the 5 tissue culture boxes per treatment, representing 'population replicates' for each treatment.

The pairs were kept in 11 x7.5x4.5 cm plastic boxes, covered with loose plastic lids, at 20°C, relative humidity ≥80%, and a 1ight:dark regime of 16h:8h. The bottoms of the boxes were cut-out and replaced with soft plastic net of 1 mm mesh. The boxes were filled with ca. 3 cm layer of expanded clay granules used for hydrocultures (Heimbach, 1992). Eggs from each couple were washed-out every 3rd day and each egg was put into a separate cell of a tissue culture box. The experiment was terminated after 69-75 days of egg laying,

For statistical analysis of treatment effects on individual life-history traits, each specimen was treated as an independent replicate. The treatments were compared for their effects on the length of larval stage, adult mass at emergence from pupae, adult mass after overwintering, fecundity (number of eggs laid per female) and hatching success. Because most results seriously deviated from the normal distribution, the nonparametric Kruskal-Wallis test was used. To calculate coefficients of population growth rate, the 'population replicates' were used, that is the average survival and reproduction rates for all individuals and pairs originating from one tissue culture box.

RESULTS AND DISCUSSION

Out of the 603 eggs used at the start of the experiment, 455 larvae hatched and were used in the study. Of these, 373 individuals survived the larval and pupal stages, and one adult did not emerge successfully from the peat.

There were initially 76 individuals in the control treatment, 97 in Zn400, and 200 in Zn6400. Because preliminary analysis detected a number of outliers, all data were 10% truncated (both tails). Thus, the final analysis of treatment effects on length of the larval stage and imago mass at emergence was made on 60 control individuals, 77 from Zn400 and 160 from Zn6400. Effect on body mass after overwintering was studied on 32 beetles from each treatment, and on fecundity - on 16 mating pairs per treatment.

A significant treatment effect was found for the length of the larval stage (p=0.003) but the significant difference appeared only between the Zn400 and Zn6400 treatments, with neither differing from the control (Fig. 1). The two possible explanations are that either this was a random effect detected by a pure chance (type I error; Sokal and Rohlf, 1981) or the Zn400 treatment resulted in fact in a slight decrease in development time, while the highest treatment caused slightly delayed pupation. In the latter case, one may speculate about reasons for such a distribution of this trait among differently treated populations. Decreased development time could result from the phenomenon called ‘hormesis’ which, although not fully understood yet, has been observed in a number of experiments at low-level treatments (cf. Hoffmann and Parsons, 1994). In contrast, the prolonged development at Zn6400 could reflect increased costs of detoxification, resulting in less resources available for growth. As there was no significant difference between treatments in adult mass at emergence, this would suggest a shift in a trade-off curve in the direction to maintain productivity at the expense of time needed to reach maturity (cf. Sibly and Calow, 1989).

Although no difference was detected between treatments in the adult body mass immediately after their emergence from pupae, there was a noted decrease in body mass of Zn-treated animals after overwintering (p=0.038; Fig. 1). This result suggests that the animals poisoned with Zn, especially at 6400 mg kg^{-1}, have gathered lower energy resources for overwintering and/or were not able to feed during winter season as effectively as the control beetles (all animals were offered food during winter). Nevertheless, this did not result in increased mortality among treated individuals, and no significant effect of Zn poisoning on survival was detected until the end of the experiment.

The number of eggs (fecundity) laid during the experiment was generally low when compared to some other data (Heimbach, 1992) and extremely variable: 2 to 68 eggs per pair in the control, 0 to 56 for Zn400 and 0 to 37 for Zn6400. The effect of treatment was highly significant (p=0.0004), with an average of 22.8 eggs per female in the control, 7.9 in Zn400 and 4.7 in Zn6400. The median values were, respectively, 18, 1.5 and 0 (Fig. 1). No significant effect of treatment on hatching success (p>0.8) was found.

Having replicate populations allowed us to summarize treatment effects on population growth rate by calculating R_0 values. For each population, its specific sex ratio,

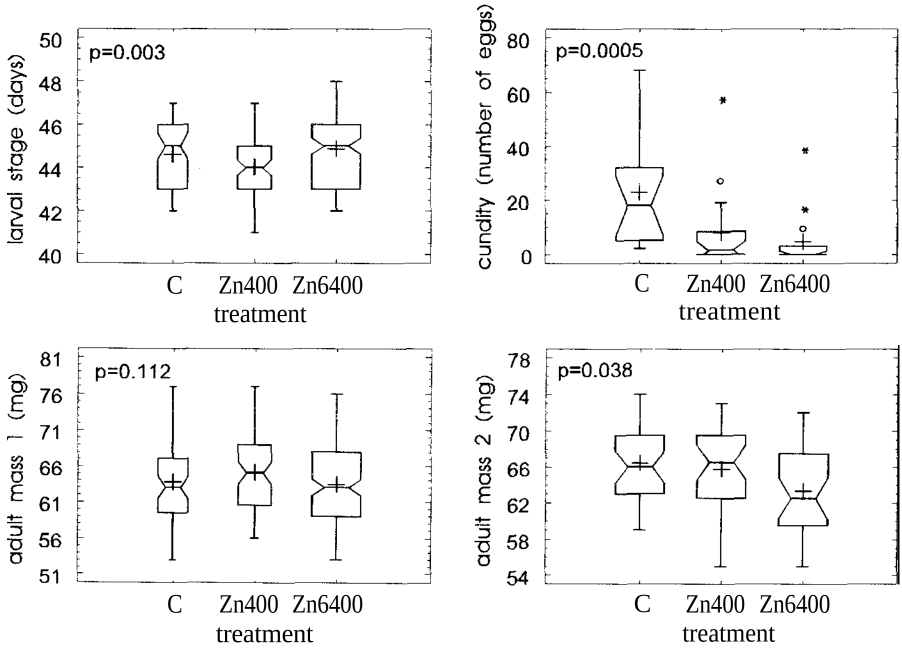

Figure 1. Effect of Zn treatment on the length of larval stage, fecundity, adult mass after emergence (adult mass 1) and after overwintering (adult mass 2). Means are marked with crosses, notches indicate approximate 95% confidence intervals around median, asterisks mark outliers.

probability of survival from egg to adult and fecundity was used. The resultant R_0s were highly variable, mostly due to high variability in fecundity (cf. above), and the broad confidence intervals (C.I.) do not allow for strong conclusions about significance of the overall treatment effects on the population level. Nevertheless, the highest average R_0 was found for control populations (1.98f ± 1.0, average±95% C.I.), followed by a slightly lower value for Zn400 (1.92±2.2), and the lowest for Zn6400 (1.13±1.6). It is also noteworthy that the R_0s in control populations exhibited the lowest variability among all treatments and there were no non-reproducing populations (R_0 = 0.92 to 3.88). In contrast, in Zn400 two populations did not reproduce at all (R_0 = 0.0) but the highest value was as high as 4.79. In Zn6400, there were three non-reproducing populations (R_0= 0.0), and the higher of the two positive values was 4.17. This may suggest that although clear negative effects of Zn treatment, especially at 6400 mg kg^{-1}, were found for average values, there are some individuals/populations particularly resistant to poisoning with this metal. It remains to be studied, however, how common such resistance is and whether it is heritable in carabids.

The calculated R_0 values may suggest increased variability of some traits in populations exposed to Zn pollution. In fact, the coefficients of variability (CV) of R_0s increased from 58% for control populations to 131% for Zn400 and 160% for Zn6400. Among particular traits, increased variability in Zn-treated populations was found in time to pupation (p=0.017) and fecundity (difference nonsignificant; Tab. 1). In contrast, variability of the adult mass at emergence and after overwintering were slightly lower among treated animals, but the differences were nonsignificant at p=0.05 (Tab. 1).

Table 1. Variability of selected life history traits in populations of *P. cupreus* under different experimental treatments.

Treatment	Time to Pupation CV% (n)	Adult Mass at Emergence CV% (n)	Adult Mass After Overwintering CV% (n)	Fecundity CV% (n)
Control	4.3 (6)	15.9(6)	11.3 (5)	123.4 (5)
Zn400	4.3 (7)	14.8 (7)	12.0(5)	127.7 (5)
Zn6400	5.5 (15)	12.8 (15)	9.9(5)	174.4 (5)
significance level	0.017	0.344	0.289	0.210

Coefficients of variability (CV) are given in percent, number of replicate populations in brackets. In the lowest row of the table, the significance levels for between-treatment differences in CV are reported (Kruskal-Wallis test).

Depledge (1990) pointed out that interindividual variability is usually overlooked in ecotoxicological studies as a possible measure of sublethal effects of toxicants. In fact, Forbes at al. (1995) showed that exposure to cadmium increased the variability in growth rate within sexual and asexual gastropod populations. Also, the ranking of means and variances among populations were changed after Cd treatment (Forbes *et al.*, 1995). Our results conform to those studies, and collectively these data suggest that in ecotoxicological experiments, especially in research on sublethal effects, changes in the shape of distribution may be as important as effects observed on any measure of a central tendency (mean, median, mode).

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