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Long-term effect of cadmium on the oribatid mite *Archegozetes longisetosus* Aoki, 1965 in laboratory conditions

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Abstract: *Archegozetes longisetosus* Aoki, 1965 was exposed to various concentrations of cadmium in food (25 or 130 or 250 :g/g) for 1-2 generations. The following population parameters were compared among the groups: longevity and fecundity of adults, their age of reproduction, offspring mortality, and number of adults in the F1 generation. The exposure to cadmium for 2 generations (i.e. 7 months) did not increase the tolerance of mites; their longevity and fecundity were similar as in the 1st generation. However, the mites subjected to cadmium for the 2nd generation reproduced quicker than those exposed for the 1st generation. The initial exposure to a small concentration of cadmium (25 :g/g) and in the next generation to a high concentration (130 :g/g) resulted in a higher fecundity, in comparison to the group that received in both generations 130 :g/g. The mortality of juveniles was very high in all groups, which confirms that short tests, focusing on survival of adults only, may underestimate the toxicity of the substances.

Key words: Oribatida, Acari, tolerance, heavy metals

INTRODUCTION

Toxicity tests on laboratory invertebrates are a powerful tool for evaluating adverse effects of various substances, including heavy metals. The duration of such tests is usually limited to a few weeks, and they focus on survival of adults, but this may lead to an underestimation of the toxicity of the tested substances (LOCK & JANSSEN 2002). In field conditions the organisms are usually exposed to toxic substances for a long time and this may change their tolerance, either due to acclimation or adaptation, or a combination of both (POSTHUMA 1992).

Adaptation to heavy metals has been well documented in plants and aquatic invertebrates (see WALKER et al. 2002), while for the soil fauna the multi-generation studies are rather rare. So far they included the springtails (POSTHUMA 1992), earthworms (REINECKE et al. 1999, SPURGEON & HOPKIN 2000) and enchytraeids (LOCK & JANSSEN 2001, 2002). Some of these studies lasted long, e.g. the test on the earthworms was conducted for 3 years and included 10 generations (REINECKE et al. 1999), while in some later experiments on these animals the adaptation was seen already after 2 generations (SPURGEON & HOPKIN 2000).

Laboratory studies on earthworms *Eisenia fetida* (Savigny, 1826) and enchytraeids *Enchytraeus albidus* (Henle, 1837) have demonstrated that after a long-time exposure (3 years and 18 months, respectively) the tolerance of animals increased (REINECKE et al. 1999, LOCK & JANSSEN 2001). In the enchytraeids the increased tolerance was accompanied by an increased level of metallothioneins, i.e. the proteins responsible for the transport of heavy metals to the places where they can be stored in a harmless form. Another explanation for the increased tolerance could be changes in metal kinetics, observed in the springtails (POSTHUMA 1992).

The aim of our study was to test the tolerance of mites to cadmium after 2 generations of exposure.

MATERIALS AND METHODS

Specimens used in this study originated from the laboratory culture started in 1994 from a few individuals kindly provided by Prof. R. A. NORTON (University of Syracuse, USA). His culture descended from a single female from Puerto Rico (for details see SMRŽ & NORTON 2004). *Archegozetes longisetosus* reproduces by parthenogenesis and only females are present in its populations. There is evidence that the offspring may be genetic clones of their mother (PALMER & NORTON 1992). This is an important advantage for using the species in laboratory tests.

The stock-culture was kept in constant climatic conditions (30°C, relative humidity 90%), in a plastic box with the bottom filled with plaster of Paris and charcoal (4:1), and fed with green algae (*Protococcus* sp.) collected together with tree bark in Bydgoszcz forest. During the experiment the conditions were the same as described above but the mites were fed with dry Chinese cabbage (*Brassica chinensis* L.) contaminated in the laboratory with cadmium, by soaking it in various concentrations of $Cd(NO_3)_2$ solutions. The concentration of cadmium in dry Chinese cabbage was determined by using the atomic absorption spectrometry (AAS).

For the experiment, 60 young adult females of the same age, just after the last moult, were selected at random from the stock-culture. They were divided into 6 groups that were exposed to various concentrations of cadmium for 1 or 2 generations (Table 1). Each group consisted of 10 initial females kept in separate culture boxes and treated as replicates.

Mites were provided with fresh food every other day, and at the same time the old food was discarded from the boxes and observations of mites were carried out. The following population parameters were compared among the groups: longevity of initial adults, fecundity of initial adults, their age of reproduction (in most cases the eggs were well hidden by the females, so we recorded the age when the first larvae appeared), offspring mortality, and number of adults obtained in the F1 generation. The experiment was conducted until all mites from the offspring generation became adults. The statistical calculations (ANOVA/MANOVA analyses followed by a post-hoc Tukey test) were carried out with STATISTICA 6.

Table 1. Experimental groups of *Archegozetes longisetosus* exposed to cadmium for 1 or 2 generations

Group	Exposure to cadmium (µg/g)	
	1st generation	2nd generation
1	25	-
2	25	130
3	130	-
4	130	130
5	250	-
6	130	250

RESULTS

Most parameters, e.g. longevity of initial adults, depended on the concentration of cadmium, but not on exposure time (Fig. 1). A significant decrease in longevity was observed in the group that received cadmium in food, at the concentration 250 µg/g. Mites from this group lived on average for about 30 days, i.e. half as long as the mites from groups exposed to lower concentrations of cadmium.

Cadmium affected also the fecundity of initial adults. With the increasing concentration of cadmium the fecundity of mites decreased. In the group exposed to 25 µg/g, the average fecundity was 55 individuals per initial female, while at concentrations 130 µg/g and 250 µg/g, it was respectively 27 and 14 individuals per initial female. In the group exposed in the 1st generation to a small concentration of cadmium (25 µg/g) and in the 2nd generation to 130 µg/g, the fecundity was higher than in the group that received in both generations 130 µg/g. The mortality of juveniles was very high in all groups and reached the highest level (ca. 90%) in the group exposed to the highest concentration of cadmium.

The exposure of mites to cadmium at the concentration 130 µg/g for 2 generations resulted in a quicker reproduction. In the 1st generation, first larvae were noted on average after 24 days, while in the 2nd generation after 15 days. The standard deviation in the former group was higher (SD = 7.6) than in the latter group (SD = 1.4).

DISCUSSION

Cadmium is one of the most toxic heavy metals. Admittedly some adult mites are able to survive extremely high concentrations of this metal, e.g. *Nothrus silvestris* Nicolet, 1855, *Rhysotritia duplicata* (Grandjean, 1953) and *Steganacarus magnus* (Nicolet, 1855) were exposed for 8 weeks to 1000 µg/g without any harmful effect (LUDWIG et al. 1991, 1993). However, the reproduction of adults or survival of juveniles were disturbed by much lower concentrations. For example, in *Archegozetes longisetosus* exposed to cadmium in food (green algae, *Protococcus* sp.), the

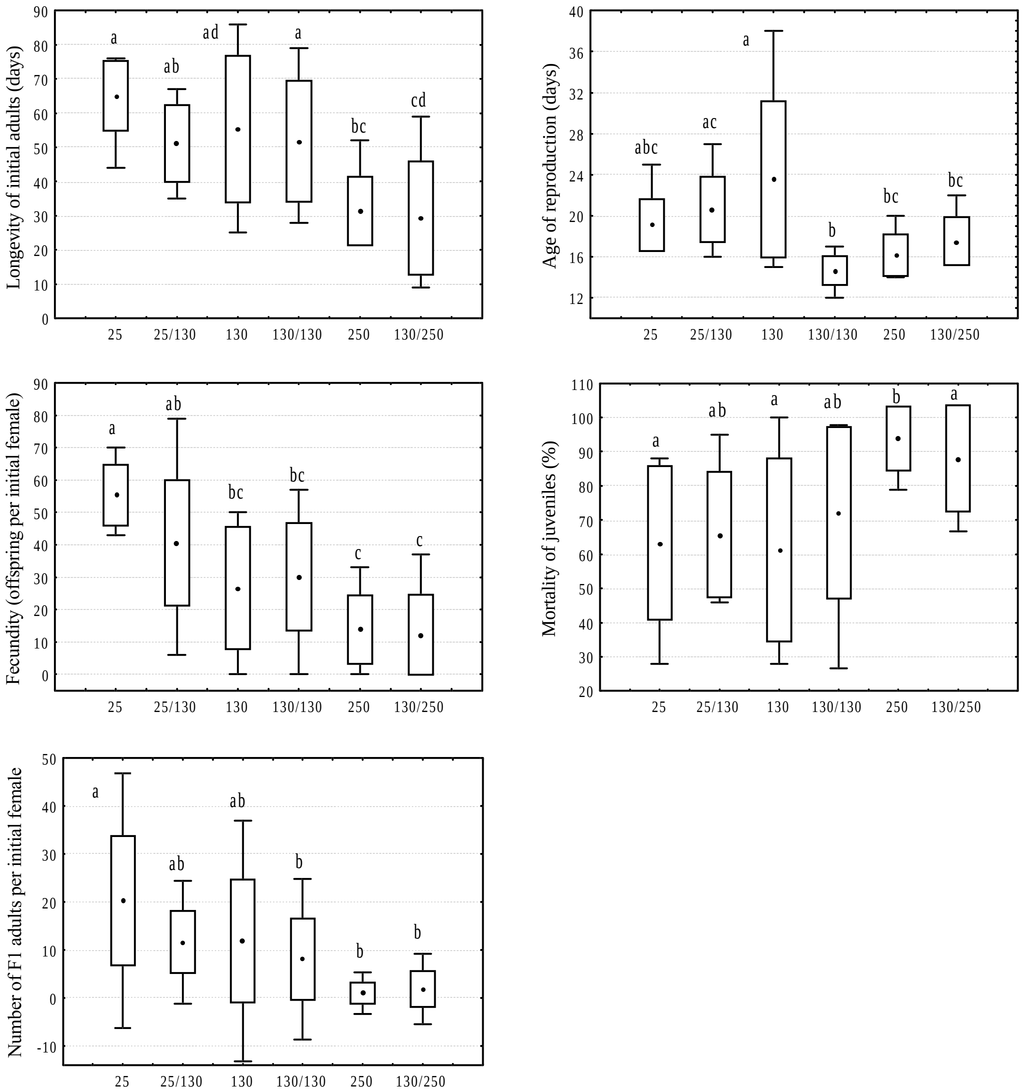

Fig. 1. Life-history parameters of *Archegozetes longisetosus* exposed to various concentrations of cadmium (25, 130 or 250 µg/g) for 1 or 2 generations. Different letters indicate significant differences between the groups at $P < 0.05$ (Tukey test in STATISTICA 6)

harmful effect of cadmium on the fecundity of adults and survival of juveniles was observed at the concentration 250 µg/g (SENICZAK & SENICZAK 2002). *Heminothrus peltifer* (Koch, 1839) was even more sensitive and its reproduction decreased when food contained cadmium at the concentration 8 µg/g (VAN STRAALEN et al. 1989). This study confirmed that survival of adult mites is not a sufficient parameter for evaluating the toxicity of substances.

The mortality of juvenile stages was very high, even at the lowest applied concentration of cadmium (25 μg/g), while survival of adults was affected only by its highest concentration (250 μg/g). These differences were probably caused by feeding habits that are different in developmental stages of *A. longisetosus* (HAQ & PRABHOO 1976). Juveniles probably fed on the fungi growing on the cabbage, and as fungi are known to accumulate very high amounts of heavy metals, juveniles were probably exposed to higher concentrations of cadmium than adults. It is also possible that juvenile stages were simply more sensitive to cadmium than adults. Additionally the juveniles have a higher metabolic rate and consume more than adults (BERTHET 1963).

The exposure of *A. longisetosus* to cadmium for 2 generations (i.e. 7 months) did not result in an increased tolerance: the fecundity and longevity of adults were similar as after exposure for 1 generation. In contrast, in the earthworms exposed to high concentrations of copper and zinc, the increased tolerance was observed already after 2 generations (SPURGEON & HOPKIN 2000). Similarly, in the enchytraeids, an increased tolerance to metals was observed after 2 generations, but due to the variability of the toxicity data from the literature, LOCK & JANSSEN (2002) did not consider this increase as significant and stated that 1-generation toxicity tests on the enchytraeids were sufficient. The fact that mites did not increase their tolerance after 2 generations of exposure to cadmium, while other invertebrates did, may be related to their mode of reproduction. *A. longisetosus* reproduces by thelytokous parthenogenesis and therefore might be unable to adapt so rapidly to changing environments as sexually reproducing species (WALTER & PROCTOR 1999).

However, it was interesting that after exposure for 2 generations, the mites reproduced quicker, which confirms the observations of POSTHUMA (1992) on the springtails. Quicker reproduction can be considered as an adaptive feature that can compensate for the adverse effect of the metal on fecundity and could help the population to persist in the polluted environment.

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