The impact of cadmium and mercury contamination on reproduction and body mass of earthworms

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ABSTRACT

The accumulation of heavy metals in the tissues of earthworms is a helpful indicator of environmental contamination. The degree of substrate contamination can be additionally evaluated on the basis of survivability, reproduction and body mass of earthworms. In this study *Eisenia fetida* Sav. earthworms were exposed to a series of increasing concentrations of cadmium and mercury. The numbers of animals and their body mass were checked after 4 and 8 months. The strongest impact of substrate contamination was exerted upon the number of young individuals and cocoons. The cadmium contamination did not affect adversely the mass of earthworms, whereas in the mercury-contaminated group the decline in body mass was evident. After 8 months of experiments, the content of heavy metal in the bodies of earthworms was determined. An evident relationship between the cadmium and mercury contents in the substrate and their accumulation in earthworms' tissues was found. The concentration in the bodies exceeded the level in the substrate.

Keywords: *Eisenia fetida* Sav.; survival rate; body mass; metal toxicity; heavy metals

As indicated by various studies, earthworms are capable of accumulating heavy metals from contaminated substrate (Spurgeon and Hopkin 1996b, Rosciszewska et al. 2003). The species *Eisenia fetida* Sav. is used as a model organism in the risk assessment for chemicals and their effect on terrestrial invertebrates (Spurgeon et al. 2003). On the other hand, the earthworms themselves represent an important link in the food chain. Thus they can accumulate and transfer the hazardous elements from the soil to higher levels of the trophic system (Hendriks et al. 1995, Spurgeon and Hopkin 1996a). In the case of earthworms used as an additive to fodder given to poultry or fish (Toboga 1980, Popek et al. 2003), there is a danger that the trophic chain might end with human.

The contamination of the substrate can affect the reproductive capability of invertebrates, which is a sensitive indicator of the animals' condition. Over a longer time, this factor will determine the ultimate survival of populations (Van Straalen et al. 1989). Depending on the stress resulting from the contamination, the outcome may be described through survivability, reproduction or growth rates of individuals.

The objective of the undertaken studies was to determine the effect of substrate contamination by mercury and cadmium on the body of earthworms, their survivability and the accumulation of heavy metals in their tissues.

MATERIAL AND METHODS

The experiments were carried out in laboratory conditions on earthworms of the *Eisenia fetida* Sav. species, kept in a substrate contaminated by mercury and cadmium. These elements, in the form of $CdSO_4$ and $HgClCH_3$, were introduced into the substrate by spraying and mixing. The control pot had no modified background levels of cadmium and mercury concentrations (Cd – 1.41 μg/g, Hg – 0.08 μg/g).

For each metal, five pots filled with 10 kg of cattle manure were prepared. In these pots, increasing series of concentrations of heavy metal compounds were added (2, 4, 6, 8, 10 μg/g of dry mass). Into each pot, 100 sexually mature earthworms (individuals with clitellum) were then introduced. The conditions were the same in all pots (pH ~ 7.0, moisture content ~ 70%). No additional food was provided throughout the duration of the experiments.

After four months of the experiment, manual sorting was applied to determine the number of individuals and age structure, represented by three age classes: adults, young, and cocoons.

The same procedure was repeated after the next four-month cycle.

After eight months, the earthworms were removed from the substrate, cleaned of mucus and manure particles, and weighed. In order to replace the content of the digestive tracts of earthworms, the animals were kept for three days in containers filled with wet potato flour layers separated by sheets of cellulose wadding. The bodies of earthworms were dried and mineralised with mixture of concentrated hydrochloric and nitric acids. Next, samples were analysed using atomic absorption spectrophotometry (AAS). The obtained numerical data were then submitted to statistical analysis, where the normal distribution was tested by using the Kolmogorov-Smirnov test, in which the Pearson correlation coefficients were calculated, while differences between groups were checked with a two-way analysis of variation and post-hoc Tukey test (StatSoft Inc. 2001).

RESULTS AND DISCUSSION

Demographic parameters

The data obtained in the experiments were used to calculate the correlation between the numbers of earthworms and the concentrations of the studied elements in the substrate. In most cases the correlation values were negative (Table 1). The comparison of the results obtained after 4 and 8 months in experiments with cadmium-contaminated substrates, showed a decrease in the numbers of adults and cocoons, with a simultaneous increase in the number of young earthworms. In the group subjected to mercury contamination, these trends were less evident. The coefficient of correlation indicates the adverse effect of cadmium and mercury on the survival rate among earthworms. The reduction in the number of earthworms exposed to the long-term effects of heavy metals is a result of increased mortality (Khalil et al. 1996) and the occurrence of defects in their reproductive cells, which affect the reproduction (Reinecke and Reinecke 1997).

The percentage distribution of age classes in the group subjected to cadmium contamination displayed an increase in the proportions of adult earthworms and cocoons in the set, with a simultaneous decrease in the proportion of young individuals in the pots with higher concentrations of cadmium in the substrate. This tendency was more manifest in the data obtained after 8 months. The increased production of cocoons in the substrate contaminated by cadmium could be a defence reaction against a harmful factor. Similar reactions were found by Phillips and Bolger (1998), who reported that at pH close to neutral, the increased contamination of the substrate with aluminium provoked earthworms to produce increased numbers of cocoons.

In the mercury-contaminated group, no evident changes in the percentage proportions of age classes were found, and their distribution was close

Table 1. The number and age structure of earthworms bred in contaminated substrate

Metal	Contamination (μg/g)	Number of individuals after 4 months						Number of individuals after 8 months					
		adults		young		cocoons		adults		young		cocoons	
		individuals	(%)	individuals	(%)		(%)	individuals	(%)	individuals	(%)		(%)
Control		111	45	112	42	36	13	81	43	93	49	15	8
Cd	2	134	29	309	66	21	5	92	29	221	69	8	2
	4	62	38	84	52	16	10	54	28	134	68	8	4
	6	102	59	48	27	25	14	88	35	145	58	18	7
	8	107	79	17	12	13	9	91	41	91	41	39	18
	10	84	42	87	44	28	14	83	40	98	47	27	13
	r	–0.32	0.53	–0.70	–0.63	0.28	0.71	0.19	0.92*	–0.88*	–0.90*	0.82	0.86
Hg	2	113	49	71	31	46	20	100	43	109	46	25	11
	4	95	55	24	14	53	31	64	50	53	41	12	9
	6	99	35	158	57	23	8	115	46	120	49	13	5
	8	75	55	35	26	26	19	56	51	47	44	5	5
	10	76	37	120	58	11	5	66	43	74	47	15	10
	r	–0.92*	–0.39	0.30	0.53	–0.89*	–0.64	–0.47	0.04	–0.37	0.26	–0.59	–0.34

r – Pearson coefficient of correlation; *statistically significant to that presented in the control group. However, a general decrease in the numbers of each age class was observed in line with the increased contamination. This result is well corroborated by the results obtained from many other field studies, where lower numbers of earthworms were found in the sites closer to the source of heavy metal contamination (Terihivuo et al. 1994).

Body mass

After 4 months, in all pots with cadmium- as well as mercury-contaminated substrate, an increase in the body mass of earthworms was noted compared with the initial values (0.38 g, SD = 0.11). After 8 months, the statistically significant differences between the initial and current body mass were found in earthworms from Cd 2 and Cd 8 pots. In the case of earthworms from the Cd 2 pot, it was lower than the initial mass. In mercury-contaminated substrate the earthworms' mass was still higher than at the beginning of the experiment (Table 2).

When comparing the results in the same pots after 4 and 8 months using the Tukey test, statistically significant differences in reduced body masses of adults were found in Cd 2 and Cd 6 pots, and in all pots contaminated with mercury.

The decrease in body mass might be associated with a lower availability of food. In an experiment involving the effect of the contamination of the substrate on the mass of *E. fetida* Spurgeon et al. (1994) noted that in the initial period of exposure there was an increase in the mass of earthworms, followed later by their decrease. Their studies did not reveal statistically significant differences between masses of earthworms exposed to various concentrations of heavy metals.

In order to estimate the relationship between the contamination of substrate and the body mass of earthworms, correlations were calculated for each of the metal at the confidence level $p < 0.05$. The average body mass of earthworms from the cadmium-contaminated substrate after 4 months did not show a statistically significant correlation with the level of contamination and the coefficient of correlation was $r = 0.238$. After 8 months the coefficient was $r = 0.411$ and the correlation was statistically significant. This might result from the inhibition in the development of cocoons and young earthworms and survival of adults; this is one of the conclusions, supported by quantitative data, obtained in the study. However, the studies of Malecki et al. (1982) and Neuhauser et al. (1984) indicated that the body mass of earthworms' decreases with the increase in the contamination of the substrate; among the metals they studied, cadmium had the strongest reducing effect on the body mass of earthworms. Nevertheless, it should be noted that the maximum concentrations employed in their studies were several times higher than those used in the present study. In the range of concentrations used in this study, no differences were observed.

Table 2. The body mass of earthworms kept in contaminated substrate

Metal	Concentration (µg/g)	After 4 months			After 8 months		
		number of individuals	mean body mass (g)	SD	number of individuals	mean body mass (g)	SD
Control		20	0.59	0.04	15	0.46	0.04
Cd	2	13	0.56^{a}	0.04	10	$0.30^{a, A}$	0.03
	4	15	0.57	0.05	13	0.41	0.04
	6	11	0.67^{b}	0.05	12	0.44^{b}	0.05
	8	12	0.73	0.03	15	0.58^{A}	0.05
	10	12	0.59	0.06	18	0.42	0.02
Hg	2	11	$0.74^{B, C}$	0.06	16	0.55	0.04
	4	14	$0.43^{B, D}$	0.03	12	0.49	0.03
	6	13	0.56	0.05	15	0.43	0.03
	8	15	0.65^{D}	0.06	15	0.56	0.04
	10	15	0.47^{C}	0.05	10	0.38	0.03

Small letters – statistically significant differences among results from the same group after 4 and 8 months ($P < 0.05$); capital letters – statistically significant differences among groups with different level of contamination ($P < 0.05$); SD – standard deviation

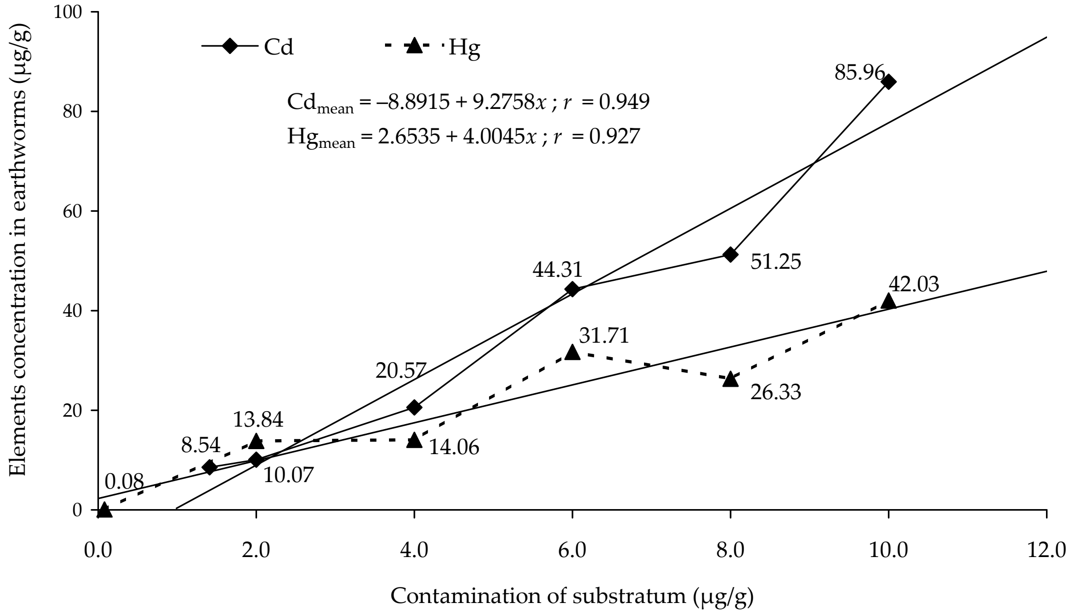

Figure 1. Average concentrations of cadmium and mercury in the tissues of earthworms (μg/g in dry mass ± SD)

In the mercury-contaminated group, after 4 and 8 months, the correlation coefficients were negative (r = –0.111 and r = –0.297, respectively). In this case, the correlation after 8 months was statistically significant. This fact confirms the particularly high toxicity of this element, which is regarded as one of the most dangerous substances contaminating the environment (Mazurek 2001).

Accumulation of heavy metals

The relationship determined by this study between the level of contamination in the substrate and the accumulation of heavy metals in the tissues of earthworms, is supported by many other field and laboratory studies (Mariño et al. 1992, Spurgeon and Hopkin 1999). The increase in heavy metal contamination was reflected in statistically significant differences ($p < 0.05$) in the levels of these elements, in the tissues of earthworms from the pots where subsequent concentrations were applied. The concentrations of the studied metals in the tissues of *E. fetida* exceeded many times the concentrations in the substrate (Figure 1). These results confirmed the data from literature (Rebanova et al. 1995, Lapinski et al. 2002).

A similar relationship as was found in this study for cadmium accumulation in earthworms was obtained by Brewer and Barret (1995). In their study, with the concentration of cadmium of ca. 2 μg/g, the level of its concentration in the tissues of earthworms was almost 40 times higher than that in the soil. Heikens et al. (2001), who studied the concentrations of heavy metals in earthworms, also found that the increased accumulation in the tissues of these annelids was in line with the increased contamination.

The analyses showed that for each of the studied elements, the correlations between the concentration of the element in the substrate and its accumulation in the bodies of earthworms was statistically significant. The Pearson coefficient for cadmium accumulation was r = 0.949, for mercury r = 0.927.

To conclude, it was observed that in substrates with a higher contamination of Cd or Hg, the survivability of earthworms is lower. The increase in cadmium contamination provoked earthworms to produce more cocoons. The contamination of substrate by mercury caused a decrease of body mass of earthworms.

With the concentrations applied in the presented study, a rapid increase in the concentrations of cadmium and mercury in earthworms' tissues was observed. Unlike during the accumulation of the metals that perform certain biological functions in the body (e.g. copper or zinc) (Spurgeon and Hopkin 1999), there is no saturation of tissues with cadmium or mercury. Taking into account the high toxicity of these elements, this poses a serious danger to the organisms on the next level of the trophic chain.

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