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Growth and reproduction of the earthworm *Eisenia fetida* after exposure to sublethal concentrations of metals

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With 5 figures

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1. Introduction

The use of land for the treatment and disposal of various wastes has resulted in a desire for more information concerning the effects of these materials on the soil ecosystem. The soil is a complex living system that needs to be managed to avoid severe physical and chemical perturbations. Earthworms are often studied as a representative organism of the soil biota that may be affected by changes in soil environment.

Earthworms have been shown to be affected by a wide variety of man's activities. They have been studied in soils affected by mining (BULL *et al.* 1977, IRELAND 1976 and 1979), smelting (WRIGHT & STRINGER 1980), high applications of animal wastes containing copper (RHEE 1977 and CURRY & COTTON 1980) and land disposal of sludge (ANDERSEN 1979 and MORI & KURIHARE 1979). Metals are a potentially toxic component of many waste materials and their effect on earthworm growth and reproduction has been evaluated (HARTENSTEIN *et al.* 1980 and MALECKI *et al.* 1982).

Little work, however, has been done on the ability of earthworms to recover from the physiological effects of exposure to high concentrations of metals. This information is needed to determine the utility of earthworms for monitoring the effectiveness of remedial action (i.e., clean-up and reclamation) at hazardous waste spills/sites. Biotically available residual contaminants in soil at such sites may pose considerable risk to recolonizing animals. Results of the current research will help determine the value of earthworms as indicator organisms for chemical analysis in identifying general soil conditions at such sites. In addition, the results will assist in the development of procedures for assessing the ability of desirable soil biota such as earthworms to recolonize once the contaminants are removed.

This paper identifies the ability of one earthworm species *Eisenia fetida* (SAVIGNY, 1826), to recover after exposure to sublethal concentrations of five metals: cadmium, copper, lead, nickel, and zinc. Compensation is measured in terms of both growth and reproduction.

2. Methods

The culture methods used in these studies are described in more detail elsewhere (MALECKI *et al.* 1982). The five metals used in these experiments were selected on the basis of their potential toxicity, their presence in nature and their tendency to be concentrated in municipal and industrial wastes. Four water soluble chemical forms of each metal were used: acetate, chloride, nitrate, and sulfate (Fig. 1).

The studies were done in petri dishes (20 × 100 mm) with 30 g (dry mass) of screened soil on the bottom of each dish (Fig. 1). The test chemical was mixed with aged horse manure (75% moisture) to give the desired metal concentration on a mg g^{-1} dry mass basis. The manure was used to provide a uniform food source and was monitored to insure that no drugs were present. The layer of soil in each petri dish was covered with 20 g of the contaminated metal-manure mixture, and two worms (< 10 mg each) were added per dish. Five repetitions were run for a total of ten worms per metal concentration. Controls were run with each set of experiments consisting of the above treatment without metal addition to the manure, and were used to detect any variation in the manure or procedures. All experiments were maintained at 25 °C in a darkened incubator. Information from a previous study (MALECKI *et al.* 1982) was used for determining sublethal metal concentrations tested. The sequence of events used in these experiments is outlined in Table 1.

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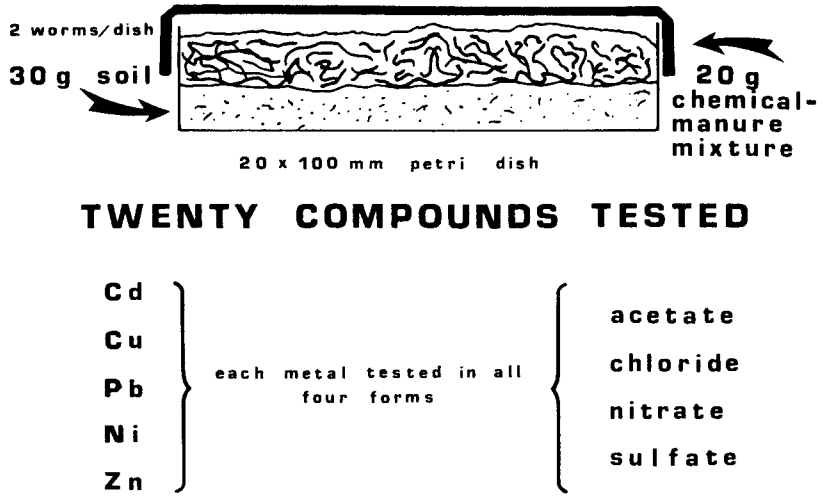

Fig. 1. The physical set-up and the chemical forms of the metals used in the compensatory growth studies.

Table 1. The time and activity sequence used in the compensatory growth experiment

Time	Activity	Expected Results*
weeks 1-6	Earthworms exposed to various sublethal concentrations of metals in an organic substrate	High concentrations of metals expected to adversely impact growth and cocoon production
weeks 7-12	Earthworms exposed to an organic substrate without metals	Earthworms expected to recover, i.e., growth and cocoon production expected to increase from that which occurred in the first six weeks

* Growth and cocoon production evaluated by counting and weighing worms and counting cocoons every two weeks starting at week 4 and continuing through week 12; controls (without metals) were included in each set of experiments.

The worms were exposed to known metal concentrations in horse manure for the first six weeks (Table 2). For the second six weeks of the study, the worms were exposed to horse manure without any metals added. This schedule gave six weeks exposure to a metal and six weeks to recover from any ill effects it caused. In this test procedure the earthworms were exposed to effects similar to those they might experience at a spill and/or a hazardous waste disposal site with subsequent clean up activity.

Table 2. The concentration of metals used for each metal form tested

Metal*	Concentration Tested**				
Cadmium	0	100	250	500	1,000
Copper	0	500	1,000	2,000	4,000
Lead	0	5,000	10,000	20,000	40,000
Nickel	0	250	500	1,000	2,000
Zinc	0	1,000	2,500	5,000	10,000

* metal forms included acetate, chloride, nitrate, and sulfate

** μg metal per gram of manure (dry mass)

At the end of the twelve-week testing period, the earthworm biomass and cocoon production for each six-week period were analyzed separately using one-way analysis of variance. The biomass was analyzed by evaluating the average mass of the earthworms at the end of the six and twelve week time periods. Reproduction was analyzed by evaluating the total number of cocoons produced for the two six-week periods. If significant differences were found, further analysis was done using Duncan's new multiple range test.

3. Results

Each of the five metals used in the 12-week experiments was tested using the four forms: acetate, chloride, nitrate, and sulfate. The final mass and cocoon production were significantly different from controls for all metals tested after the first six weeks except for lead chloride and lead nitrate (Table 3). The final masses at six weeks for these two chemicals

Table 3. Resulting F values using one-way analysis of variance to compare the different concentrations of the metal forms to the controls for growth and cocoon production from 1—6 and 7—12 weeks

		Form of Metal							
		Acetate		Chloride		Nitrate		Sulfate	
metal	time (weeks)	growth	cocoon production	growth	cocoon production	growth	cocoon production	growth	cocoon production
Ca	1-6	6.79†	15.31†	30.40†	17.52†	30.63†	17.25†	38.57†	6.49†
	7-12	1.56	2.45	0.16	8.20†	2.49	10.62†	0.83	1.42
Cu	1-6	30.72†	9.47†	42.05†	8.31†	32.34†	7.92†	26.68†	10.76†
	7-12	2.08	2.31	1.70	1.37	2.23	0.42	0.98	1.39
Pb	1-6	21.82†	6.08†	1.44	3.84†	2.08	4.93†	10.39†	8.73†
	7-12	2.72	0.77	1.48	2.00	0.56	2.80	2.77	1.23
Ni	1-6	47.94†	24.09†	42.52†	16.34†	60.75†	22.65†	45.90†	29.66†
	7-12	1.17	2.18	2.68	0.62	2.64	5.87†	1.44	13.38†
Zn	1-6	25.00†	13.11†	20.63†	23.43†	14.07†	6.19†	26.81†	16.87†
	7-12	1.07	1.83	1.06	1.00	0.22	0.19	1.40	2.04

† Indicates significance at $F^{4}_{20,\,0.05} = 2.87$

were not significantly different from the control masses. There was, however, a significant detrimental impact of these chemicals on the reproductive ability of the worms at the test concentrations used.

The metal manure mixture was removed from the test dishes after the first six weeks. The worms in the test dishes were fed untreated manure for an additional six-week period. At the end of the second six-week period, the worms were evaluated for differences in final mass and total cocoon production over both six-week periods. There were no significant differences in final masses between the controls and any concentration tested for the five metals (Table 3). The worms exposed to the higher concentrations of cadmium chloride, cadmium nitrate, nickel nitrate, and nickel sulfate resulted in significantly lower numbers of cocoons compared to the controls after the second six weeks without metal being present. All other forms of metal tested did not result in significantly fewer cocoons than the controls for weeks 7—12 of the test period.

Figure 2 shows the average earthworm mass gains achieved at the end of the six and twelve-week periods for the five metals tested. The four forms of each metal were summarized to provide a visual comparison of the information used to develop the data in Table 3. The results indicate that the worms were able to achieve compensatory growth which allowed them to recover to approximately the control mass values after the metal was removed from their food source.

Figure 3 shows the total cocoon production for the six and twelve week periods. The worms generally were not able to compensate as much for reproduction as they were for growth. The cocoon production at the two highest cadmium and nickel concentrations were found to be significantly lower than the final reproductive control values.

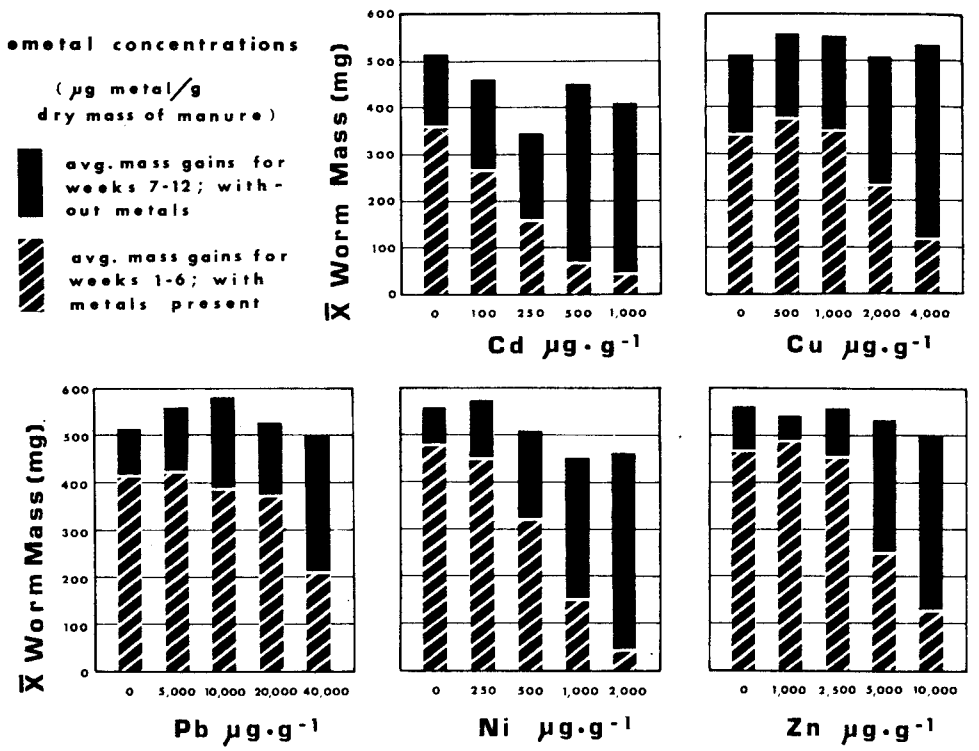

Fig. 2. Average mass gains by *E. fetida* for a twelve week period with the metal concentrations (µg metal/g dry mass) indicated for all four metal forms combined.

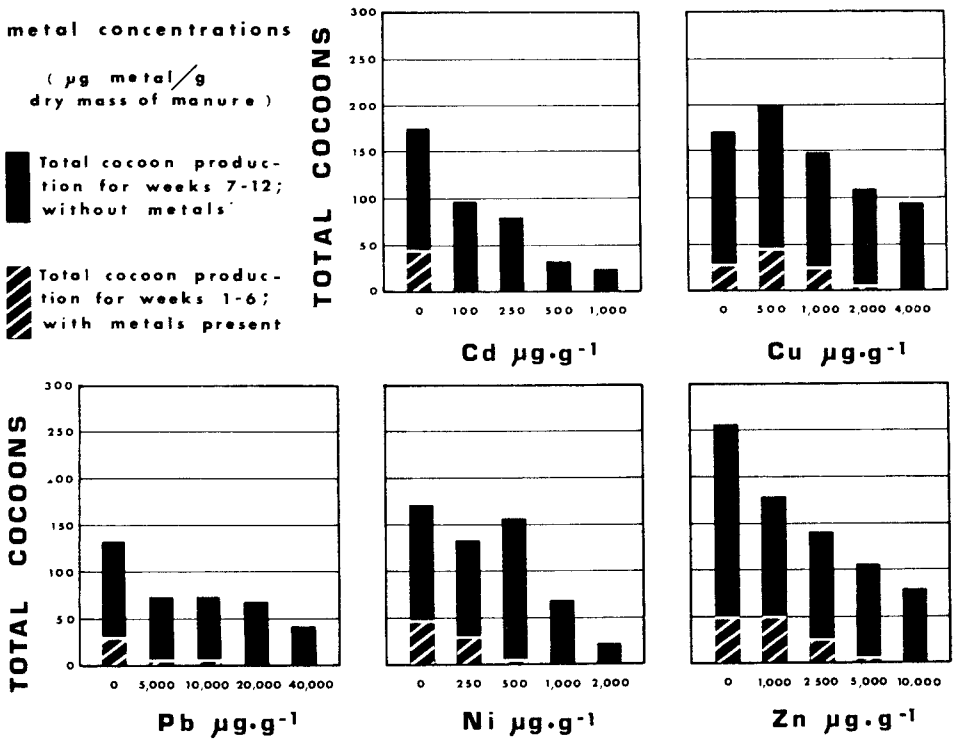

Fig. 3. Total cocoon production by the earthworm *E. fetida* for a twelve week period with the metal concentration (µg metal/g dry mass) indicated for all four metal forms combined.

4. Discussion

The comparison of growth and reproduction over the twelve-week period was made with four different forms of five metals to see if the different metal compounds had a discernible effect. Previous work in our laboratory (Malecki *et al.* 1982) has demonstrated that the solubility of the metal may be a key factor in assessing the effects of metals on earthworm survival, growth, and reproduction. The insoluble forms of the metals were shown to have less impact on growth and reproduction than the soluble forms. All metal forms used in this study were highly soluble in water and no significant differences were found between any of the forms tested for any specific metal.

Figure 4 identifies the pattern of mass gain that occurred over the twelve weeks of the study. Different rates of mass gain occurred when the worms were exposed to a metal than when they were not. In Figure 4 the numbers indicate the slopes of the mass increase for the worms grown for the first six week period with zinc present and for the second six weeks without any additional zinc in the manure. With no difference between the treated and control worms, the slope would be equal to one. A slope of less than 1 indicates that the treated worm is not growing as fast as the control. As can be seen from Figure 4, the higher the concentration of zinc to which the worms were exposed, the lower the slope, i.e., the lower the rate of mass gain. After the worms were permitted to grow an additional six weeks without metals being present, the worms that were exposed to the highest metal concentrations demonstrated the greatest mass gain. The worms exposed to high concentrations of metals were able to gain mass daily at a rate two to three fold that demonstrated by the control worms during the second six week test period (Table 4). The worms exposed to the highest concentration of metals were, at the end of twelve weeks, able to achieve a final

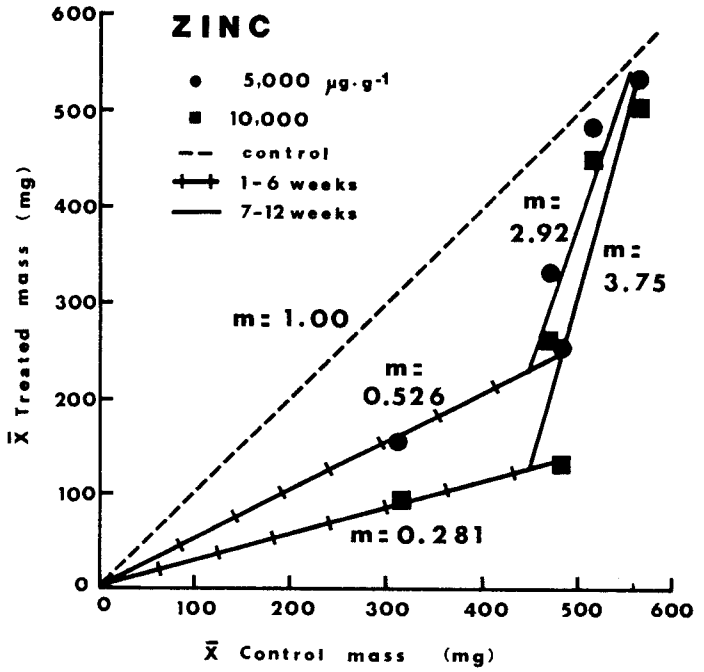

Fig. 4. Earthworm mass, treated compared to controls for all forms of zinc tested.

Table 4. Comparing earthworm growth rate for treated (weeks 1—6) and untreated (weeks 7—12 to controls for all four forms of the five metals tested

Metal	Conc. (μg·g⁻¹)	Slope (m)[t] treated	Slope (m)[t] untreated
Cadmium	100	0.68	1.18
	250	0.41	1.54
	500	0.19	2.27
	1,000	0.12	2.26
Copper	500	1.12	1.10
	1,000	1.03	1.16
	2,000	0.65	1.47
	4,000	0.33	2.11
Lead	5,000	1.01	1.23
	10,000	0.92	1.80
	20,000	0.88	1.50
	40,000	0.62	2.24
Nickel	250	0.96	1.29
	500	0.70	1.95
	1,000	0.32	3.14
	2,000	0.11	4.84
Zinc	1,000	1.01	0.87
	2,500	0.93	1.57
	5,000	0.53	2.92
	10,000	0.28	3.75

[t] Ratio of slope at concentration indicated to slope of control.

Table 5. Comparing earthworm cocoon production for treated (weeks 4—6) and untreated (weeks 10—12) to controls for all four forms of the five metals tested

Metal	Conc. (μg·g⁻¹)	Slope (m)[t] treated	Slope (m)[t] untreated
Cadmium	100	0.02	0.94
	250	0.00	1.02
	500	0.00	0.70
	1,000	0.00	0.62
Copper	500	1.34	1.08
	1,000	0.71	1.11
	2,000	0.06	1.04
	4,000	0.00	1.14
Lead	5,000	0.16	0.71
	10,000	0.31	0.72
	20,000	0.03	0.76
	40,000	0.02	0.44
Nickel	250	0.92	1.13
	500	0.13	1.21
	1,000	0.00	0.95
	2,000	0.00	0.64
Zinc	1,000	1.05	0.95
	2,500	0.53	0.91
	5,000	0.10	1.08
	10,000	0.00	1.08

[t] Ratio of slope at concentration indicated to slope of control.

mass that was not statistically significant from the control (Table 3). The worms exposed to the highest concentration of metals had the slowest growth rates during the treatment period and had the fastest growth rates during the recovery period.

The data in Figure 5 summarizes cocoon production of worms exposed to varying concentrations of cadmium and zinc. If there was no difference between the treated cocoon production and controls, the lines would have a slope of one (dashed line). Concentrations of 1,000 and 2,000 mg g^{-1} Zn appeared to have no effect on reproduction. Table 5 compares the slopes of cocoon production for all the metals for the treated and untreated time periods. These were determined using data taken from weeks 4 to 6 in the first six week period. During the second six week period, the slopes were determined using data taken from weeks 10 to 12. The first period was chosen to allow the worms to reach maturity while still being treated. The latter time period was used to give the worms time to recover from the effects of the metals and grow before evaluating cocoon production in the recovery period.

Table 5 shows that the higher the metal concentration to which the worms were exposed during the treatment period, the lower the rate of cocoon production. When the metals are removed. the rate of cocoon production recovered to approximately a slope of 1.0. Unlike the growth rate data, in which the treated worms added mass at 2 to 3 times the normal growth rate after they no longer were exposed to the metals, the treated worms were not able to produce cocoons at an increased rate. Thus *E. fetida* appears to possess a limited reproductive rate and seems unable to compensate for a period with a decreased reproductive potential following exposure to metals.

Previous studies involving land treatment of wastes considered earthworms as an immobile part of the soil biota. However, earthworms have been shown to be somewhat mobile in the soil profile, both vertically to a depth of 2.5 metres and horizontally up to 10 metres per year (Edwards & Lofty 1977). Since earthworms have the ability to move in the soil profile, they may be exposed to high isolated concentrations of potentially toxic

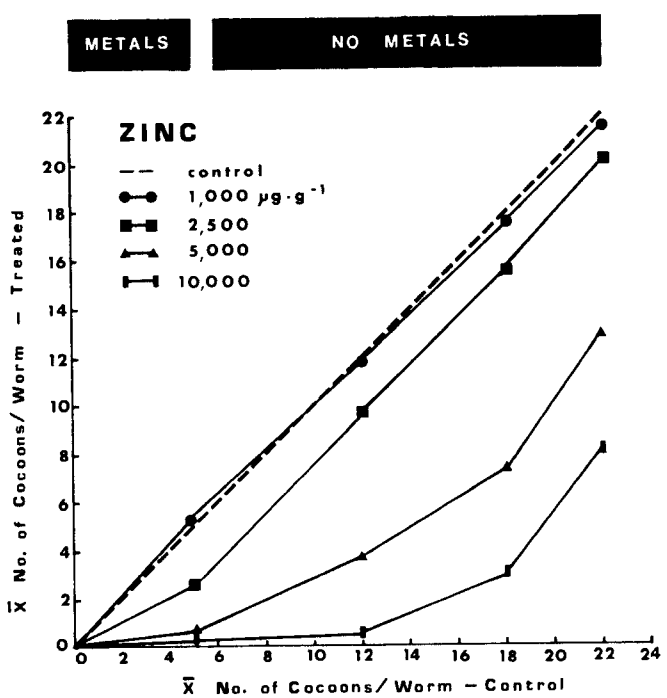

Fig. 5. Cocoon production, treated compared to controls, for all forms of zinc tested.

chemicals. The impact of these chemicals can be severe enough to inhibit the further growth and reproduction of the worm if it remained in soil with a high concentration of chemical. This study demonstrates that earthworm populations have the ability, after being severely affected by metals, to recover when provided with an environment that does not have high metal concentrations.

Table 4 shows that the earthworms exposed to the highest concentrations of each metal tested had the larger percentage increase in growth during weeks 7—12. This type of mass change, to levels that are similar to the unstressed organism, is known as compensatory growth. This type of growth has been shown to occur in cattle (BERG & BUTTERFIELD 1976) and plants (MCNAUGHTON 1983). During the compensatory period, the previously stressed organism gains mass rapidly in comparison with the normal organism of the same age. The same phenomena did not occur with cocoon production, perhaps because the worms were not given sufficient time to achieve a size that would permit reproduction.

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Synopsis: *Original scientific paper*

NEUHAUSER, E. F., M. R. MALECKI & R. C. LOEHR, 1984. Growth and reproduction of the earthworm *Eisenia fetida* after exposure to sublethal concentrations of metals. Pedobiologia **27**, 89—97.

Earthworm growth and reproduction were compared during and after the worms were exposed to sublethal concentrations of five metals: cadmium, copper, lead, nickel and zinc. Most concentrations of metals led to a decreased rate of growth. *Eisenia fetida* was capable of compensatory growth which allowed it to recover to approximate control mass values after the metal was removed from its food source. *E. fetida* appeared able to compensate for growth more than for reproduction. This study demonstrates that metal impacted earthworms have the ability to resume normal growth and reproductive processes when they are removed from environments highly contaminated with metals and placed in an environment that does not contain excessive metal concentrations.

The test procedures exposed the earthworms to concentrations similar to those they might experience under conditions at a spill and/or a hazardous waste site with subsequent reclamation and clean-up activity. These results demonstrate that earthworms may be valuable indicator organisms for identification of soil contamination. The results could assist in assessing the potential of earthworm field populations to recover once contaminants are removed.

Key words: earthworms, Eisenia fetida, cadmium, copper, lead, nickel, zinc, compensatory growth, reproduction.