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The effect of metals on the growth and reproduction of *Eisenia foetida* (Oligochaeta, Lumbricidae)[1])

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With 4 figures

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1. Introduction

Man's development and redistribution of the many inorganic and organic chemicals used in commerce and industry have had a noticeable impact on the environment. Many studies have identified the impact of these chemicals on air and water quality, but little work has been done to quantify the effects of such chemicals on life in the soil. Earthworms are a major component of soil fauna (EDWARDS & LOFTY 1977) and as such can be used as possible indicators of the impact of man's activities on soil ecosystems (BROMENSHENK & STATES 1980). Earthworms also have a potential use in waste conversion systems (NEUHAUSER et al. 1980b), with protein production as a possible by-product (SABINE 1978).

To evaluate the use of earthworms as a biomonitoring tool and in waste conversion and disposal systems, information is needed on how various chemicals affect earthworms. Since metals can limit biological activity, studies to estimate the tolerance of earthworms to potentially toxic metals are needed. This study evaluates the effects of five metals upon the growth and reproduction of *Eisenia foetida*.

Previous work with other earthworm species by the authors has shown *E. foetida* to be a highly desirable species for work of this nature. This species grows and reproduces rapidly (HARTENSTEIN *et al.* 1979) and is amenable to growth under a variety of substrates (NEUHAUSER *et al.* 1980a). Studies with other species of earthworms (NEUHAUSER *et al.* 1979 and KAPLAN *et al.* 1980) have demonstrated that this species is the most suitable for the current objectives noted in this paper.

2. Methods

2.1. General

The earthworms used in these experiments were obtained from cultures in the authors' laboratory. The cultures of *Eisenia foetida* (SAVIGNY, 1826) are maintained under controlled conditions at room temperature (21—24 °C) with cocoons being harvested periodically. Horse manure was used as an earthworm food source because it is consistent in food value and readily available from the Cornell horse barns. The manure was obtained from horses receiving no medication. Young worms, < 2 weeks of age and < 10 mg each, were used in all experiments because young worms of known age and size give more consistent results in screening tests than adult worms of random age and size.

The five metals chosen for these experiments were selected on the basis of their potential toxic effect and because they are found widely in nature and are generally concentrated in municipal and industrial wastes. Six chemical forms of each metal were chosen to cover a broad range of solubility and to represent the forms likely to be found in the soil.

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1) Contribution to the DARWIN Centenary Symposium on Earthworm Ecology, Grange-over-Sands (1981).

2) The authors are respectively: Research Support Specialist, Research Associate, and Liberty Hyde Bailey Professor of Engineering.

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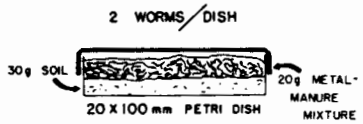

SHORT TERM STUDIES

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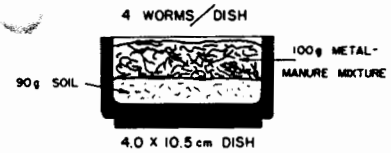

LONG TERM STUDIES

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Fig. 1. The physical set-up for eight-week growth studies.

Fig. 2. The physical set-up for twenty-week growth and reproductive studies.

2.2. Growth studies

The short term growth studies (Fig. 1) were conducted in petri dishes (20 × 100 mm) with a manure and metal mixture (20 g) placed on a layer of screened soil (30 g). The metal to be tested was mixed with a known quantity of horse manure to achieve the desired metal concentration on a ppm dry weight basis. Five dishes per metal concentration were tested with two young worms being placed in each dish, resulting in a total of ten worms exposed to each concentration. Controls, which were run for each screening, consisted of the above protocol without any metal added to the manure. The worms were checked for survival and weight gains at four, six and eight weeks. The manure-metal mixture was easily separated from the soil layer, removed and replenished at four and six weeks.

These studies started with metal concentrations ranging from 10,000—40,000 ppm. In successive concentration tests, the level of precision regarding toxic effects was increased. Three ranges of concentrations were evaluated for each metal type tested, except for acetates and chlorides, which had four ranges of concentrations. At the end of each evaluation, the earthworm weights were analyzed using one-way analysis of variance to test for significance among means. If significance was found, Duncan's new multiple range test was used to determine where the differences occurred between means.

2.3. Reproductive studies

Low concentrations (50—10,000 ppm) of the metal acetates and chlorides were evaluated for twenty weeks to determine growth and reproductive effects. The earthworms, manure and chemicals for these studies (Fig. 2) were the same as described in the growth studies with the exception that a larger container and quantity of manure-metal mixture and soil was used; 100 g of the manure-metal mixture was placed on 90 g of soil in a 4.0 × 10.5 cm stacking dish. In addition, four worms, < 10 mg each, were placed in each dish. There were three repetitions for each concentration tested, with five concentrations tested for each metal acetate and chloride.

The dishes were checked for earthworm growth and cocoon production every two weeks. At the end of the testing period, earthworm final weights and cocoon production were analyzed using one-way analysis of variance. The number of cocoons produced by each repetition was summed at the end of the experiment and divided by the number of worms present in the repetition. Results were calculated in terms of total cocoon production/worm/experiment and were analyzed for differences between means. If significant differences were found using one-way analysis of variance for either weight or reproduction, differences between means were analyzed using Duncan's new multiple range test.

3. Results

3.1. Growth studies

The final weights, expressed as a percentage of control weights, were plotted against the concentration of the metal in the food source for each of the five metals tested. Fig. 3, showing the effect of increasing cadmium concentrations upon weight at eight weeks, is representative of the data obtained.

The carbonate forms of cadmium (Cd), nickel (Ni) and zinc (Zn) and the oxide forms of copper (Cu) and nickel showed much less of an effect on growth than was demonstrated by the acetates, chlorides, nitrates and sulfates. A possible reason for this could be the relatively insoluble nature of the carbonate and oxide forms (Table 1). There were no differences observed between the effects of any of the six chemical forms of lead (Pb) tested. This undoubtedly was due to the low solubility and toxicity of all the lead compounds tested.

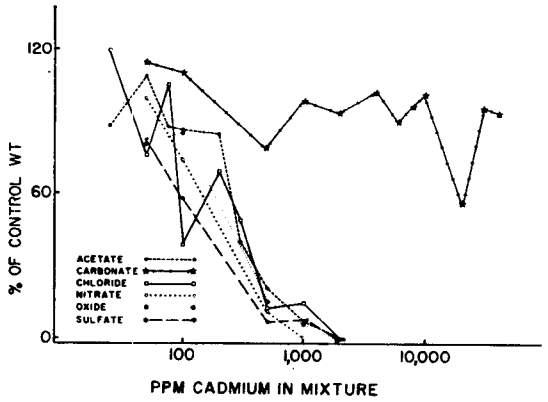

Fig. 3. The final weight reached, expressed as a percentage of control weight, for specific concentrations and cadmium compounds indicated.

Table 1. The solubilities of a saturated solution of the 30 metal compounds tested and concentrations (ppm dry weight) at which significant differences from a control first occurred in the growth studies

Metal compound	Solubility mg or g/100 g of H_2O at *p*H 7	Concentrations at which significant differences occurred	
		Growth	Reproduction
Cadmium acetate	66.0 g	100	25
Cadmium carbonate	0.39 mg	(40,000)[1])	20,000
Cadmium chloride	54.7 g	100	75
Cadmium nitrate	61.3 g	500	100
Cadmium oxide	0.48 mg	500	50
Cadmium sulfate	76.7 g	50	100
Copper acetate	6.8 g	1,000	2,000
Copper carbonate	30.0 mg	1,000	2,000
Copper chloride	43.3 g	500	2,000
Copper nitrate	60.1 g	100	100
Copper oxide	0.2 mg	40,000	20,000
Copper sulfate	18.5 g	4,000	100
Lead acetate	35.6 g	12,000	4,000
Lead carbonate	0.1—0.2 mg	40,000	10,000
Lead chloride	1.1 g	(40,000)[1])	14,000
Lead nitrate	59.7 g	20,000	4,000
Lead oxide	12.4 mg	20,000	10,000
Lead sulfate	4.0 mg	16,000	10,000
Nickel acetate	16.0 g	500	300
Nickel carbonate	1.1 mg	500	2,000
Nickel chloride	39.6 g	200	200
Nickel nitrate	50.0 g	500	500
Nickel oxide	0.9 mg	(40,000)[1])	40,000
Nickel sulfate	40.8 g	500	500
Zinc acetate	41.6 g	4,000	2,000
Zinc carbonate	20.6 mg	(40,000)[1])	500
Zinc chloride	81.2 g	2,000	2,000
Zinc nitrate	56.1 g	2,000	2,000
Zinc oxide	0.4 mg	4,000	4,000
Zinc sulfate	63.5 g	2,000	500

1) Not significant to highest level tested; highest level tested indicated by parenthesis.

Table 2. Statistical analysis of the growth data-final earthworm weight (mg) and cocoons produced for five metal acetates

Chemical	Control	25 ppm	50 ppm	75 ppm	100 ppm	200 ppm	300 ppm	500 ppm	1,000 ppm
Cadmium acetate	ab[1]) 471.0 ± 36.1	bcd 412.6 ± 66.9	a 512.6 ± 113.1	bc 416.0 ± 49.1	cd 409.6 ± 61.0	d 355.2 ± 53.2	187.4 ± 48.6	100.4 ± 22.4	34.8 ± 10.4
Cocoons	8.8 ± 2.1	a 4.2 ± 2.3	a 5.6 ± 2.1	a 3.8 ± 1.5	a 4.4 ± 2.7	0	0	0	0
Cupric acetate	ab 550.4 ± 47.9	bc 466.0 ± 108.4	ab 564.2 ± 75.2	ab 575.4 ± 151.2	a 580.4 ± 92.8	abc 471.6 ± 82.4	abc 515.2 ± 55.6	abc 523.0 ± 45.9	cd 430.0 ± 39.9
Cocoons	ab 8.2 ± 1.3	b 6.2 ± 3.2	a 10.2 ± 1.5	ab 7.6 ± 2.3	b 5.4 ± 2.3	b 6.4 ± 3.0	ab 8.2 ± 3.9	b 6.2 ± 0.4	b 6.0 ± 1.9
Nickel acetate	a 595.0 ± 104.2	ab 503.0 ± 58.1	ab 517.4 ± 55.6	b 479.2 ± 98.7	a 601.2 ± 92.8	b 460.0 ± 133.8	ab 506.6 ± 76.2	269.8 ± 46.1	c 81.2 ± 28.4
Cocoons	ab 10.2 ± 0.8	abc 8.6 ± 1.4	a 11.6 ±3.8	abc 7.8 ± 3.1	abc 8.4 ± 6.2	a 8.8 ± 3.1	c 6.0 ± 0.7	1.2 ± 0.4	0
Zinc acetate	ab 477.4 ± 54.5	ab 469.0 ± 57.6	ab 442.8 ± 76.2	ab 498.2 ± 70.4	a 506.4 ± 112.3	bc 432.4 ± 40.8	a 525.0 ± 117.7	bc 401.4 ± 59.6	a 505.4 ± 103.7
Cocoons	abc 8.8 ± 1.3	ab 9.6 ± 5.6	a 11.0 ± 4.8	cd 6.6 ± 1.1	ab 9.6 ± 2.3	abc 8.8 ± 4.1	bc 7.8 ± 1.1	bcd 7.6 ± 2.7	a 10.4 ± 3.9
	Control	**50 ppm**	**100 ppm**	**500 ppm**	**1,000 ppm**	**2,000 ppm**	**4,000 ppm**	**6,000 ppm**	**8,000 ppm**
Lead acetate	ab 450.4 ± 31.9	c 370.6 ± 69.1	a 480.6 ± 84.6	ab 452.2 ± 114.5	a 485.4 ± 73.0	560.2 ± 66.7	ab 447.6 ± 80.0	a 447.6 ± 52.3	b 405.4 ± 71.3
Cocoons	ab 10.8 ± 1.9	c 7.4 ± 3.0	bc 8.6 ± 1.8	bc 8.0 ± 4.4	a 11.6 ± 5.0	a 12.2 ± 1.9	d 5.4 ± 3.6	d 3.6 ± 1.1	de 1.6 ± 1.1

Chemical	2,000 ppm	4,000 ppm	6,000 ppm	8,000 ppm	10,000 ppm	20,000 ppm	30,000 ppm	40,000 ppm	F value
Cadmium acetate	0	0	0	0	0	0	0	0	$F^{3}_{41} = 46.02$
Cocoons	0	0	0	0	0	0	0	0	$F^{4}_{25} = 5.65$
Cupric acetate	de 275.2 ± 95.1	e 112.2 ±27.3	0	0	0	0	0	0	$F^{10}_{56} = 9.01$
Cocoons	0	0	0	0	0	0	0	0	$F^{8}_{51} = 3.63$
Nickel acetate	c 106.7 ± 76.6	0	0	0	0	0	0	0	$F^{7}_{48} = 14.18$
Cocoons	0	0	0	0	0	0	0	0	$F^{7}_{42} = 6.22$
Zinc acetate	a 512.4 ± 90.4	c 370.2 ±82.4	c 335.8 ±149.0	d 194.6 ±75.7	d 231.8 ±41.3	de 153.4 ±25.7	e 87.5 ±10.4	0	$F^{15}_{78} = 17.18$
Cocoons	d 5.6 ± 1.1	e 1.2 ± 1.3	e 0.6 ± 0.5	0	0	0	0	0	$F^{11}_{58} = 9.86$
	10,000 ppm	**12,000 00m**	**14,000 ppm**	**16,000 ppm**	**18,000 ppm**	**20,000 ppm**	**30,000 ppm**	**40,000 ppm**	**F value**
Lead acetate	a 478.8 ± 87.1	de 171.4 ±65.4	c 255.2 ±64.9	de 168.8 ±74.3	cd 230.2 ±96.2	c 281.3 ±71.8	e 140.0 ±60.9	e 113.5 ±30.9	$F^{16}_{82} = 22.75$
Cocoons	e 0.6 ± 0.5	0	0	0	0	0	0	0	$F^{9}_{55} = 11.39$

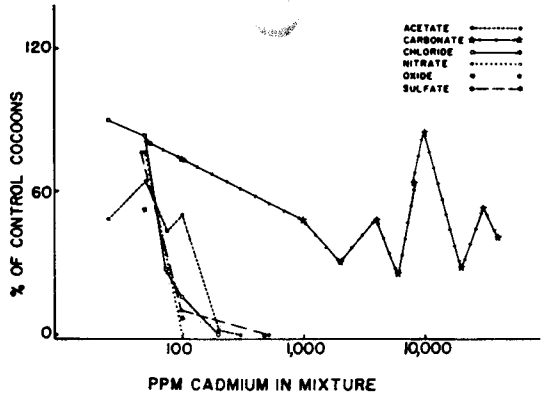

Fig. 4. The number of cocoons produced, expressed as a percentage of control cocoons, for specific concentrations of cadmium compounds.

All final weight data points were analyzed for differences between means. The acetate, chloride, nitrate and sulfate form of each metal gave similar detrimental results, while the carbonate and oxide forms often had little effect upon growth. Table 2 presents the statistical analysis for the metal acetates as representative of the total statistical analysis.

Table 1, which identifies the concentration at which a significant difference from the control first occurred, and Table 2, which provides a statistical analysis of the acetate data, were used to compare the relative toxicities of the five metals. Carbonate and oxide data were not used in these comparisons due to their minimal impact.

Cadmium had the greatest effect on growth rates, causing a decreased growth rate at a concentration of 100 ppm for cadmium acetate and chloride and 50 ppm for cadmium sulfate. The concentration of Ni, the next most toxic element tested, reached 200 ppm (nickel chloride) before a significant difference from the control occurred. In comparison, Cu had to reach 500 ppm and Zn 2,000 ppm before final weights differed significantly from controls. Pb showed the least effect, having to reach concentrations of 12,000 ppm before statistically different final weights occurred.

At the end of the eight-week studies, the number of cocoons present were measured. This time period was adequate to estimate the reproductive effect because the reproductive phase of *E. foetida* usually begins at five weeks of age.

Fig. 4 shows the number of cocoons produced, expressed as a percentage of control cocoons, when Cd was added to the manure. This illustrates the most extreme effect of a tested metal upon reproduction, due to the high toxicity of Cd. The carbonate and oxide forms of the metals tested did not cause the same reduction of cocoon production shown by the other four forms. The minimal impact of carbonate and oxide also was observed with the growth studies.

The results indicate that concentrations at which reproduction is suppressed are lower than the concentrations of metals required to suppress growth. Thus, the reproductive potential of earthworms is likely to be affected by metals before growth is affected.

The relative order of effect was Cd, Ni, Cu, Zn and Pb, with the first metal being the most detrimental. Table 2 summarizes the actual number of cocoons obtained over the eight week period and the statistical analysis for the acetate form of the metals tested.

3.2. Reproductive studies

Table 3 summarizes the final weights and statistical analysis for the long-term (twenty-week) studies. The concentrations used in these studies were those that would ensure survival of the worms and permit evaluation of cocoon production.

Table 3. Final weights (mg) and cocoon production achieved at the end of twenty weeks in the reproductive studies

	Metal Concentration in the Mixture					
Cadmium acetate	**0 ppm**	**50 ppm**	**100 ppm**	**200 ppm**	**400 ppm**	**F Value**
Weight[1])	686 ± 22.0	a 542 ± 86.5	a 569 ± 44.4	328 ± 24.5	186 ± 56.1	$F^4_{10} = 44.41$
Cocoons	25.49 ± 1.87	19.28 ± 2.70	15.98 ± 0.77	2.77 ± 1.67	0	$F^4_{10} = 126.01$
Copper acetate	**0 ppm**	**250 ppm**	**500 ppm**	**1,000 ppm**	**2,000 ppm**	**F Value**
Weight	ab 630 ± 142.2	a 728 ± 115.8	a 757 ± 153.9	bc 538 ± 23.4	c 422 ± 62.7	$F^4_{10} = 4.62$
Cocoons	a 25.58 ± 5.93	a 26.86 ± 0.75	a 25.80 ± 2.07	19.43 ± 3.78	3.83 ± 1.71	$F^4_{10} = 24.46$
Lead acetate	**0 ppm**	**500 ppm**	**1,000 ppm**	**5,000 ppm**	**10,000 ppm**	**F Value**
Weight	a 522 ± 51.5	a 671 ± 53.6	a 710 ± 40.8	479 ± 20.5	b 574 ± 43.5	$F^4_{10} = 13.72$
Cocoons	a 22.87 ± 6.86	a 23.21 ± 4.44	a 25.69 ± 2.90	b 16.41 ± 2.36	b 13.24 ± 3.88	$F^4_{10} = 4.27$
Nickel acetate	**0 ppm**	**50 ppm**	**100 ppm**	**200 ppm**	**400 ppm**	**F value**
Weight	a 590 ± 31.2	a 676 ± 54.3	a 712 ± 312.2	a 535 ± 93.7	a 410 ± 56.1	$F^4_{10} = 1.81$
Coccoons	ab 22.17 ± 5.60	ab 23.10 ± 5.37	a 25.66 ± 8.76	b 17.14 ± 3.38	8.11 ± 2.61	$F^4_{10} = 4.66$
Zinc acetate	**0 ppm**	**500 ppm**	**1,000 ppm**	**2,500 ppm**	**5,000 ppm**	**F value**
Weight	b 535 ± 37.1	a 723 ± 141.1	a 681 ± 67.1	a 736 ± 100.0	b 546 ± 47.2	$F^4_{10} = 3.71$
Cocoons	ab 22.53 ± 1.66	a 27.10 ± 4.67	ab 22.22 ± 6.79	b 19.86 ± 3.30	10.55 ± 1.46	$F^4_{10} = 6.72$

[1]) Means with a common letter are not significantly different, $P < 0.05$.
All values $\bar{x} \pm SD$, replications/treatment = 3, $F^4_{10,0.05} = 3.48$

Table 3 also summarizes the statistical analysis of cocoon production for the twenty-week period. The acetates and chlorides were the only forms selected for reproductive studies. The carbonates and oxides were not used due to their insolubility. The nitrates and sulfates gave values similar to the acetates and chlorides for growth and reproduction in the short-term studies and therefore were not used in these studies.

The effect on long-term reproduction was similar to that found in the short-term growth studies: Cd, Ni, Cu, Zn and Pb with the first metal being the most inhibitory. Cd inhibited reproduction at 50 ppm, Ni at 400 ppm, and Cu at 2,000 ppm. High concentrations ($\geq$ 5,000 ppm) of both Zn and Pb were needed to inhibit reproduction.

4. Discussion

Previous work studying the interaction of earthworms and metals have considered how concentrations of metals in the environment affected earthworms. The metals could reach the environment in many ways: as a component of sewage sludges applied to land (HELMKE *et al.* 1979 and ANDERSON 1979), in orchard soils contaminated by farming practices (RHEE 1977), in soil contaminated by air pollution (ASH & LEE 1980; CZARNOWSKA & JOPKIEWICZ 1978; GISH & CHRISTENSEN 1973) and by mining and smelting operations (MARTIN & COUGHTREY 1976; ROBERTS & JOHNSON 1978; and IRELAND 1979). There were two studies that added metals to sewage sludge and measured the effects of such metals on earthworms. MORI & KURIHARA (1979) used a single chemical form of cadmium, chromium, copper and zinc and HARTENSTEIN *et al.* (1980) used a single chemical form of cadmium, copper, lead, nickel and zinc.

This study evaluated actual concentrations which affect earthworm growth and reproduction. Previous work examined either the ambient levels of metals found in the soil and the presence or absence of worms or the addition of a limited number of metals to a food source. This paper provides a comparison of the toxic and inhibitory concentrations of five metals under identical conditions. Cd was shown to be the most toxic of the five metals studied, followed by Ni, Cu, Zn and Pb.

An individual metal will probably be present in the soil in a number of different chemical forms. The usual method of analyzing for metals in soils involves a strong acid digestion, with results expressed as the total metal present. Usually no analysis is made of the individual chemical forms present in the soil. Using six different chemical forms of each metal, with different solubilities, permits some insight on the effect that solubility has on the inhibitory effect of metals to soil biota. The different forms of metals in the soil helps explain the range of individual metals reported toxic to earthworms in the literature. These studies have demonstrated that the form of the metal in the soil is of major consideration in judging the effect of a particular metal level on soil biota.

The effect of a metal on soil biota is shown not only by the toxic effects on individual survival and growth, but also by the effect of the metals on reproduction. Previous work has not studied the effect of sub-lethal levels of metals upon reproduction. The sub-lethal concentrations of metals that caused inhibition of reproduction followed the same pattern as that demonstrated by results of the growth effect study, namely Cd was the most inhibitory, followed by Ni, Cu, Zn and Pb.

These studies demonstrate that earthworms respond selectively to individual metal compounds, both in terms of growth and reproduction. Further work is underway on the response of earthworms to more complex mixtures of chemicals.

5. Acknowledgements

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Synopsis: *Original scientific paper*
MALECKI, M. R., E. F. NEUHAUSER & R. C. LOEHR, 1982. The effect of metals on the growth and reproduction of *Eisenia foetida*. Pedobiologia **24**, 129—137.

An experimental methodology determined the short-term effects (eight weeks) of five metals on the growth of the earthworm *Eisenia foetida*. The metals were cadmium, copper, lead, nickel and zinc and were present in six forms (acetate, carbonate, chloride, nitrate, oxide, and sulfate). The concentrations tested ranged from 25 to 40,000 ppm dry weight.

Results of the short-term studies indicated that cadmium had the most detrimental effect upon earthworm growth. The other metals had a lesser impact with the order of decreasing effects being nickel, copper, zinc and lead. The effects of these metals are partially related to the solubility of compounds tested. Both carbonates and oxides generally showed a reduced effect as a result of their low solubility in water. The other four forms tested showed a greater detrimental effect. The short-term studies provided estimates of the effect of these metals on reproduction.

Long-term studies (twenty weeks) determined the reproductive effects of sub-lethal concentrations of the acetate and chloride forms of the five metals. These studies also showed that cadmium had the most deleterious impact on reproduction, followed by nickel, copper, zinc and lead in order of decreasing effect.
Key words: effect, metal, cadmium (Cd), copper (Cu,) lead (Pb), nickel (Ni), zinc (Zn), earthworm (Oligochaeta, Lumbricidae), growth, reproduction.