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Toxicity of metals to the earthworm *Eisenia fetida*

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Summary. Development of methods to measure the effect of man's residuals on soil ecosystems is desirable. Earthworms, as one of the largest and most easily obtained components of the soil biota, are suitable for evaluating perturbations to soil ecosystems.

The impact of five metals (Cd, Cu, Ni, Pb, and Zn) on the survival of the earthworm *Eisenia fetida* (Savigny) was evaluated using the contact and artificial soil tests. There was no difference in toxicity between the different metal salts (acetate, chloride, nitrate, and sulfate) of each of the five metals using the contact test. In the contact test, the order of toxicity, from most toxic to least toxic, was Cu > Zn > Ni ≈ Cd > Pb. The nitrate salt of the five metals was tested using the artificial soil test and the order of toxicity, from most toxic to least toxic, was Cu ≈ Zn ≈ Ni > Cd > Pb. Stress on earthworm populations, as shown by weight loss, was shown to increase with increasing concentrations of metal salts.

These studies indicate that: (a) earthworms can be a suitable biomonitoring tool to assist in measuring the effect of metals in wastes added to soils and (b) the contact and artificial soil tests can measure the biological impacts.

Key words: *Eisenia fetida* – Artificial soil test – Contact test – Acute toxicity

Man's activities have led to increasing amounts of metals in soil ecosystems such as land treatment and waste disposal sites. Interest has developed in the use of earthworms as indicators of environmental contamination. This has led to different earthworm testing procedures, including two developed by the European Economic Community (EEC 1982). These tests are designed to demonstrate the impact that a chemical or a mixture of chemicals would have on earthworms in soil ecosystems.

Work has been done on the effect on earthworm growth with the addition of metals to aerobically digested sludge (Hartenstein et al. 1980a; Hartenstein et al. 1980b; Hartenstein et al. 1981). Work in our laboratory (Malecki et al. 1982), using growth and reproductive tests, has shown that *Eisenia fetida* (Savigny) could not differentiate between the soluble salts of five metals tested, but could differentiate between the metals. The study started with young worms that were grown in a food source to which the metal salts were added.

The study discussed in this paper was designed to evaluate the toxicity of five metals to earthworms using the contact and artificial soil tests. The results were analyzed statistically to determine differences between the four salts of the five metals. The results also were compared with other information on the toxicity of metals to earthworms.

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Materials and methods

General

The worms used in these tests, *E.fetida*, were grown in the author's laboratory at room temperature (20°–22°C) on a consistent organic food source, horse manure. Care was taken to ensure that manure from medicated animals was not used.

Water-soluble salts (acetate, chloride, nitrate, and sulfate) of five metals (Cd, Cu, Ni, Pb, and Zn) were selected for evaluation on the basis of their potential toxicity and presence in many municipal and industrial wastes that may be applied to soils. All 18 metals tested were Fisher-certified chemicals.

Data analysis to determine LC_{50} values used the method of Litchfield and Wilcoxon (1949). Results are expressed in terms of LC_{50} values and 95% confidence intervals.

Contact test

The contact test procedures described by the EEC (1982) were followed. Glass vials (3 oz) were lined with 12 × 6.7 cm filter paper to give an area of 80 cm^2. Fresh aqueous salt solutions were made for each series of tests. One milliter of water was added to the filter paper to provide enough moisture for earthworm survival. One adult earthworm (300–500 mg) was added per vial and the vials were kept on their side at 20°C in a darkened incubator. Only one worm is added per vial because the death of one worm in the vial could adversely affect others if present.

After 48 h, the viability of the earthworms was determined. Earthworms were considered dead if they did not respond to a gentle mechanical stimulus. Worm mortality was related to the concentration of the chemical to which the worm was exposed.

The approximate LC_{50} for each chemical was obtained using a series of tests to determine the range of chemical concentrations that had an adverse impact. This permitted the use of a narrow range of concentrations from 0% to 100% that included the LC_{50}. This was accomplished by using chemical concentrations that varied over several logs. At least five concentrations were evaluated in the definitive test, with ten or more replicates for each concentration tested. Controls with everything except test chemical were included in each series of experiments.

Artificial soil test.

The EEC tentative artificial soil test method (EEC 1982) was followed. A medium which consists of 10% finely ground sphagnum peat, 20% kaolinite clay, 69% fine sand, and 1% pulverized calcium carbonate (all measured on a dry weight basis) was used. pH was adjusted to 6.0 ± 0.5, moisture content raised to 25% of the dry weight of the four components, and the test metal was dissolved in enough water to bring the final moisture content of the artificial soil to 35%.

Ten adult *E.fetida* (300–500) mg were added to 400 g (dry weight) of the test mixture in covered round glass dishes 6.5 cm high and 12.5 cm in diameter. The dishes were placed in an incubator at 20°C and mortality was assessed after 2 weeks. Earthworms were considered dead if they did not respond to a gentle mechanical stimulus.

Concentrations of 0% to 100% mortality were determined using a range-finding test that included chemical concentrations varying over several logs. Controls were run in all tests and consisted of everything except the test chemical. A definitive test was performed thereafter with at least five chemical concentrations, and four replicates per concentration. The 400 g dry weight provides enough soil so that the death of one worm will not adversely affect any other worms.

Table 1. The earthworm contact test LC_{50} values for the metals tested

	Metal salt (LC_{50}, $\mu g \cdot cm^{-2}$, 95% Cl)				
Metal	Acetate	Chloride	Nitrate	Sulfate	All salts
Cadmium	20 (16–25)[1] [a]	18 (12–25)[1]	24 (12–45)[1]	26 (17–39)[1]	22 (18–26)[3] [b]
Copper	6.7 (5.3–8.5)[2]	4.9 (4.0–5.9)[2]	7.4 (3.8–14.4)[2]	6.3 (4.6–8.6)[2]	6.2 (5.3–8.0)[1]
Lead	50 (30–83)[3]	NT[c]	64 (45–92)[3]	NT	59 (42–82)[4]
Nickel	17 (13–22)[4]	17 (12–24)[4]	25 (19–34)[4]	20 (16–25)[4]	20 (16–25)[3]
Zinc	13 (10–16)[5]	12 (6–24)[5]	10 (8–12)[5]	13 (7–26)[5]	12 (10–14)[2]

[a] Means with a common number in a row are not significantly different, $P < 0.05$

[b] Means with a common number in a column are not significantly different, $P < 0.05$

[c] NT, not tested

Results

Contact test

The four salts (acetate, chloride, nitrate, and sulfate) of the five metals (Cd, Cu, Ni, Pb, and Zn) were tested to determine whether the salts of the metals exhibit different toxicities. Table 1 lists the LC_{50} and 95% confidence interval (CI) for all the metal salts tested. Using the methods of Litchfield and Wilcoxon (1949), the salts of each metal were compared with each other to see whether the LC_{50} values were significantly different. Table 1 indicates that each of the salts tested for each metal was not statistically different from any of the others.

The individual metal salt results for each metal in Table 1 were combined to give a contact test LC_{50} value for each metal. A comparison of the combined contact test values (Table 1) shows that Cu was the most toxic metal tested, followed by Zn. No difference was found for the toxicity of Cd and Ni, while Pb was shown to be the least toxic metal tested.

Thus, while the test could not distinguish copper acetate from any of the other copper salts tested, it could distinguish copper from any of the other four metals tested.

Artificial soil test

The artificial soil test is much more labor intensive than the contact test. The results from the contact test show there was no difference in toxicity for any of the metal salts. To save time and effort in the artificial soil test, only the nitrate salt of each metal was tested.

Table 2 lists the values obtained with the nitrate salt of the metal tested. The test was not able to distinguish in toxicity between Cu, Ni, and Zn and all three were the most toxic metals tested. Cd was the next most toxic metal tested, followed by Pb as the least toxic metal tested.

Table 2. The artificial soil test LC_{50} values for the nitrate salts of the metals tested

Metal	Artificial soil test (LC_{50}, mg kg^{-1}, 95% CI)
Cd	1843 (1660–2045)[b]
Cu	643 (549– 753)[a]
Pb	5941 (5292–6670)[c]
Ni	757 (661– 867)[a]
Zn	662 (574– 674)[a]

Means with a common letter are not significantly different, $P < 0.05$

Discussion

Metals

The contact and artificial soil tests are short-term designed to evaluate the impact of a specific chemical or substance on earthworm survival. Previous work on the toxicity of metals to earthworms (Gish and Christensen 1973; Van Hook 1974; Ireland 1979; Ash and Lee 1980) concentrated on evaluating the concentration of metals in the earthworm and the soil. Such studies provide no indication of the actual toxicity of the metals to the worms, but indicated the concentrations of metals that adult worms can tolerate.

The effect of adding metals to sludge on the accumulation of metals and growth of *E.fetida* (Hartenstein et al. 1980a; Hartenstein et al. 1980b; Hartenstein et al. 1981) has been evaluated. In addition, Malecki et al. (1982) identified the concentrations of the five metals tested in these experiments that inhibit earthworm growth and reproduction. The ability of *E.fetida* to recover from sublethal concentrations of the five metals tested in these experiments and resume normal growth and reproduction was found by Neuhauser et al. (1984). Malecki et al. (1982) and Neuhauser et al. (1984) found that Cd was the most toxic of the metals tested, in contrast to the results of the contact and artificial soil tests reported in this paper, which showed Cu, Ni, and Zn to be as toxic or more toxic than Cd to earthworms. Cadmium requires a period of time to permit toxic symptoms to develop (Doull and Klaassen 1980), one of the possible reasons why the contact and artificial soil tests did not show the toxic effects of Cd. While Cu is an essential metal in most organisms, the range between deficiency and toxicity is low in those species without effective barriers for controlled absorption, such as earthworms (Doull and Klaassen 1980).

In the artificial soil test, the worms were weighed at the beginning and end of each 14-day experiment. The change in weight of survivors (Fig. 1), as a function of the amount of metal tested, indicates that weight loss was related to the type and amount of metal tested. The lines noted in Fig. 1 are lines of best fit of the data. The worms exposed to the three most toxic metals tested: Cu, Zn, and Ni, all had similar weight losses. Regression equations were calculated only up to 50% weight loss, because very few worms survived the 2-week test with more than 50% weight loss. Such weight loss data can indicate the stress the worms are under at the different concentrations of each metal.

Testing procedures such as the earthworm contact and artificial soil test are useful indicators of acute toxicity. At nonacute concentrations of the five me-

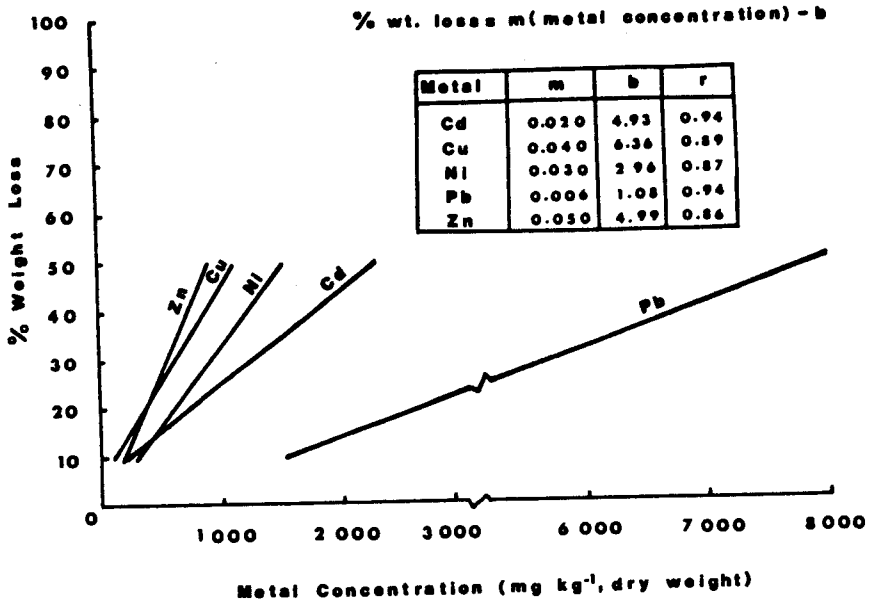

Fig. 1. Weight loss of surviving worms as a function of metal concentration in the artificial soil test

tals tested, adverse effects on growth and reproduction occur. While chemical concentrations that do not kill the adult earthworm but impact reproduction and young earthworms are much more difficult to obtain, they probably are more useful from a long-term ecological perspective.

In conclusion, there was no difference in toxicity between the different metal salts (acetate, chloride, nitrate, and sulfate) of the five metals tested (Cd, Cu, Ni, Pb, and Zn) to the earthworm *Eisenia fetida* in the contact and artificial soil tests. In the contact test the order of toxicity, from most toxic to least toxic, was Cu > Zn > Ni ≈ Cd > Pb. In the artificial soil test the order of toxicity, from most toxic to least toxic, was Cu ≈ Zn ≈ Ni > Cd > Pb.

The artificial soil test provides information regarding the concentrations of metals in soils that could actually cause the reduction or eradication of an earthworm population in soil ecosystems. While it is recognized that each soil will have different chemical and physical parameters that affect the availability of metals, the artificial soil test permits standardized testing.

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