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The influence of soil characteristics on the toxicity of four chemicals to the earthworm *Eisenia fetida andrei* (Oligochaeta)

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Summary. The acute toxicity of Cd (chloride), chloroacetamide, 3,4-dichloroaniline and pentachlorophenol to the earthworm *Eisenia fetida andrei* was determined using the OECD (1984) artificial soil and contact testing procedures. To investigate the influence of two soil characteristics (pH and organic-matter content), the toxicity of the chemicals was also determined in two natural sandy soils. It is concluded that the filter-paper contact test cannot be recommended to predict earthworm toxicity of these chemicals in soil. Toxicity in soil was influenced by both pH and organic-matter content. Differences between LC_{50} values in the high-organic-matter artificial soil and in an acid, low-organic-matter sandy soil were, however, not greater than a factor of 3–4. The results of this study therefore support the use of a well-defined artificial soil substrate for standardized earthworm toxicity tests.

Key words: *Eisenia fetida* – Acute toxicity – Artificial soil test – Contact test – Soil characteristics – Cd – Chloroacetamide – 3,4-Dichloroaniline – Pentachlorophenol

In recent years two guidelines for acute toxicity testing of chemicals to earthworms have been published (OECD 1984; EEC 1985). Both guidelines recommend the use of *E. fetida* as test organism and a well-defined artificial soil as test substrate. OECD guideline no. 207 additionally describes a filter-paper contact test. Both testing procedures were reviewed in a ringtest (Edwards 1983), which showed that the contact test gave the most reproducible results. He also concluded that this test was difficult to interpret.

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Soil characteristics can have a great influence on the adsorption and therefore on the bioavailability and toxicity of chemicals in soil. Böttger (1976) demonstrated that the adsorption and phytotoxicity of three herbicides was mainly influenced by the amount of organic matter, but also by the clay content and pH of soil. Adsorption of organic chemicals onto soil is often correlated with soil organic-matter content (Brown and Flagg 1981). The adsorption of chlorinated phenols is also influenced by soil pH (Schwarzenbach and Westall 1985), while positively charged ions like those of heavy metals are adsorbed onto clay and organic matter and their adsorption decreases with decreasing soil pH (Bolt and Bruggenwert 1976).

The aim of the present study was to investigate the influence of two soil characteristics, pH and organic-matter content, on the toxicity of chemicals to earthworms. Therefore, in addition to the OECD artificial soil, a natural sandy soil was tested at its own low pH and at the pH of the artificial soil. Four chemicals were selected which are known to show different adsorption behaviour in different soils.

In addition to these soil tests, filter-paper contact tests were conducted with the same chemicals.

Materials and methods

General. The earthworm species used, *E. fetida andrei*, was cultured at an average ambient temperature of 20°–22°C, using horse manure as the food source. The earthworms used in the toxicity tests were adult with a well-developed clitellum, more than 6 weeks old, with an average weight of 300–420 mg.

The chemicals used, cadmium chloride, chloroacetamide, 3,4-dichloroaniline and pentachlorophenol, were at least 95% pure and were purchased from Merck Holland and J. T. Baker Chemical Co.

LC_{50} values were calculated using the method of Litchfield and Wilcoxon (1949).

Contact test. A modification of the OECD (1984) filter-paper contact test was performed. Filter paper (Blauband, 589³, Schleicher &

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Schüll) was placed in 9-cm diameter petri dishes and treated with 1 ml solution of the test substance in distilled water (cadmium chloride and chloroacetamide) or absolute ethanol (3,4-dichloroaniline and pentachlorophenol). After evaporation of the solvent, the filter paper was remoistened with 1 ml distilled water, and one earthworm was placed on it. Dishes were incubated in the dark at 23±2 °C for 48 h. After 24 and 48 h mortality was determined. An earthworm was considered dead if it did not respond to a gentle mechanical touch on the front end.

A range-finding test was carried out, using five replicates for each of the concentrations of 0, 0.1, 1.0, 10 and 100 μg cm^{-2} of the test substance. A narrow, more definitive, range of test concentrations, including concentrations giving 0 and 100% mortality, was used to determine the more specific LC_{50} values. At least five concentrations and a control were evaluated in the definitive test, with 10 replicates for each concentration tested. Individual weights of all (surviving) earthworms were determined at the beginning and at the end of the tests. Before the tests the earthworms were preconditioned for approximately 4 h on wet filter paper at 23±2 °C in the dark.

Tests in soil. The artificial soil comprised (by dry weight) 10% < 0.5 mm ground sphagnum peat, 20% kaolin clay, 69% fine sand and 1% calcium carbonate. In the toxicity tests the water content was adjusted to 35% dry weight.

Gilze soil (low-organic-matter sand) was collected from the top 15 cm of a meadow near Gilze, The Netherlands, and sieved (< 4 mm) to remove stones and plant roots. Gilze soil + pH was derived from Gilze soil by adjusting the pH to 7.0 using calcium carbonate. Both the natural soils, Gilze and Gilze + pH, were tested at a water content of 22% dry weight. Table 1 gives the main characteristics of the soils used in this study.

Cadmium chloride and chloroacetamide were added to the soils as aqueous solutions. Pentachlorophenol and 3,4-dichloroaniline are not readily soluble in water and were therefore mixed with a small amount of dry soil and subsequently added to the rest of the soil. The soils were mixed intensively with a household mixer, and moisture contents were raised to the desired levels by the addition of distilled water. Amounts equivalent to 0.5 kg dry artificial soil or 0.6 kg dry natural soil were placed in 1-l glass jars and 10 adult earthworms were added. The jars were loosely covered with polypropylene lids, allowing exchange of air, and incubated at 23±2 °C and a cycle of 12 h light and 12 h dark. Mortality was assessed as described above after 7 and 14 days.

A range-finding test on concentrations of the test substances of 0, 1, 10, 100 and 1000 mg kg^{-1} dry soil was carried out to determine concentrations causing 0 and 100% mortality. A definitive test was performed at five test concentrations and a control. The range-finding test had one jar per concentration and the definitive test had two, all containing 10 worms per jar. The earthworms were preconditioned by keeping them for 24 h under the same conditions in the untreated soil before starting a definitive test.

Soil pH and moisture content, and individual weights of all (surviving) earthworms were determined at the beginning and at the end of the definitive tests.

Table 1. Characteristics of soils used in this study

Characteristics	Artificial soil	Gilze + pH soil	Gilze soil
pH (1 *N* KCl)	7.0	7.0	4.1
Organic matter (%)	7.7	1.7	1.7
Clay (%)	10.4	4.3	4.3
CEC (meq 100 g^{-1})	10.8	–	5.5

CEC, cation exchange capacity

Results

Table 2 shows the results of both the filter-paper contact test and the soil tests in terms of LC_{50} values and 95% confidence limits.

For several tests no exact LC_{50} could be calculated because of a very steep dose–response relationship. Determination of exact LC_{50} values > 1000 mg kg^{-1} was considered unnecessary. Therefore no exact LC_{50} values are given for Cd in the high pH soils.

Chloroacetamide and 3,4-dichloroaniline had lost most of their toxicity after 7 days. Further incubation for another 7 days did not increase the mortality. Mortality due to pentachlorophenol and Cd (chloride) did increase with increasing exposure time.

The individual earthworm weights decreased in all tests including the untreated controls. In soils, worm weights decreased by 3% up to 45%, and only in a few cases (Cd in Gilze soil, pentachlorophenol and 3,4-dichloroaniline in Gilze and Gilze + pH soils) was there an apparent dose-related decrease. In contact tests, the weight decrease was dose-related for all chemicals tested, and ranged from 5%–11% in controls up to 12%–29% in treated groups.

Table 2. Toxicity of four selected chemicals to the earthworm *Eisenia fetida andrei* in three soils and from a filter-paper contact test

Chemical	Test substrate			
	Filter paper (LC_{50} μg cm^{-2}, 95% CL)	Artificial soil	Gilze + pH soil (LC_{50} mg kg^{-1}, 95% CL)	Gilze soil
Chloroacetamide	> 1 < 1.8[b]	40 (36–44)	> 32 < 56	> 10 < 18
Cd (chloride)[a]	4.8 (3.8–6.1)	> 1000	> 1000[c]	> 320 < 560[d]
3,4-Dichloroaniline	3.1 (2.1–4.5)	> 180 < 320	> 100 < 180	> 100 < 180
Pentachlorophenol	1.8 (1.1–2.9)	28.5 (23–36)	16 (14–18)	52 (44–61)

[a] LC_{50} values expressed as μg Cd cm^{-2} or mg Cd kg^{-1}
[b] 10% mortality at 1.8 μg cm^{-2}, test carried out at 25±2 °C
[c] 5% mortality at 1000 mg kg^{-1}
[d] Test carried out in the dark

Soil moisture contents did not change significantly during the 14-day testing period. Soil pH values did change, especially in soils which were amended with calcium carbonate. In both the artificial soil and the Gilze + pH soil, pH values (0.01 *N* KCl) increased from 6.6 – 6.9 at the beginning to 7.2 – 7.5 at the end of the definitive tests. This pH increase was not due to earthworm activity, since the same increase in pH was also measured after 14 days in soil without earthworms. In the Gilze soil, pH values (0.01 *N* KCl) ranged between 4.4 and 4.6 at the beginning of the tests and had not increased by more than 0.2 pH units at the end of the tests.

Discussion

LC_{50} values obtained in this study for the earthworm toxicity of chloroacetamide, Cd (chloride) and pentachlorophenol, in both a contact test and a test in three soils, are in close agreement with those reported for *E. fetida* by other authors (Edwards 1983; Heimbach 1984; Neuhauser et al. 1985a, b; Roberts and Dorough 1984). LC_{50} values for these chemicals in the filter-paper contact test are somewhat lower than those reported in the literature, probably because a slightly different method of contact testing was used. No data on the toxicity to earthworms of 3,4-dichloroaniline were found in the literature.

Comparison of the results of the contact test with those obtained in soils clearly demonstrates that the contact test has no predictive value for the toxicity in soil. Differences between the lowest and highest LC_{50} values in the contact test are only 3- to 4-fold while in soils they are over 30-fold. Heimbach (1984) and Neuhauser et al. (1986) confirm this conclusion, finding only a poor correlation between the results obtained with the contact test and a test using an artificial soil substrate for 18 and 10 chemicals, respectively.

When the LC_{50} values determined in both high-pH soils are compared, the toxicity of 3,4-dichloroaniline and pentachlorophenol is higher in the natural soil. This is probably due to its lower organic-matter content, as indicated by Brown and Flagg (1981) and Böttger (1976).

Differences between LC_{50} values determined for both natural soils demonstrate that soil pH influences earthworm toxicity for all the chemicals tested except 3,4-dichloroaniline. For the OECD and EEC reference substance chloroacetamide this finding contradicts the results of Heimbach and Edwards (1983), who found that soil pH had no effect on the toxicity to *E. fetida*. The microbiological degradation of chloroacetamide may be retarded at a low soil pH. Chloroacetamide was chosen for this study because it is only poorly adsorbed onto soil.

The influence of pH on Cd toxicity was expectec since, as discussed before, its adsorption decrease: with decreasing soil pH. Ma (1982) demonstrated tha Cd has an increasing bioavailability, measured as ar increased bioaccumulation in earthworms, at decreas ing soil pH.

The decreased toxicity of pentachlorophenol a low soil pH can be explained by its low pKa valu (4.74). At a soil pH of about 7.0 this compound i mainly present as an anion which adsorbs poorly ont soils. At a soil pH as low as 4.5 mainly the phenoli form will be present, exhibiting stronger adsorption The anionic form is said to be most toxic. Th phenolic form, however, is more lipophilic and ha therefore a much greater tendency to be taken up b living organisms. At low soil pH adsorption seems t dominate bioaccumulation and toxicity.

From the point of view of standardization the us of a well-defined substrate, like the artificial soil, recommended for standardized toxicity tests wit earthworms (Dean-Ross 1983). The adsorptio behaviour of the four chemicals tested in the prese study is influenced by soil pH and/or organic-matt content, and consequently, variation of these sc characteristics affects earthworm toxicity, as shown b the results in Table 2 and discussion above.

Table 2 indicates that differences between LC values for individual chemicals due to variations soil pH and organic-matter content are, however, rel tively small, and are not greater than a factor of 3 – In fact, differences between results obtained by d ferent laboratories carrying out toxicity tests accor ing to the same methods and using the same substrat and chemicals are often much larger (Edwards 198 It must therefore be concluded that the results of o study support the use of a well-defined artificial s substrate for standardized earthworm toxicity testir

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