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Influence of pH on the accumulation of cadmium and lead in earthworms (*Aporrectodea caliginosa*) under controlled conditions

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This study was conducted with soils from the vicinity of Oulu, northern Finland to investigate the effect of pH on the accumulation of Cd and Pb in earthworms. The earthworms (*Aporrectodea caliginosa*) were maintained in wooden boxes in two series of experiments. The calcium, pH and heavy metal content (Pb and Cd) were adjusted in order to follow the influence of pH on the Pb and Cd uptake by the worms. The cadmium uptake seems to clearly depend on the pH of the soil and the accumulation becomes faster at a lower pH. The same trend is also seen in lead accumulation. The concentration ratio for cadmium over a period of 230 days varied from 13 to 33.

1. Introduction

Earthworms are one of the most important organisms responsible for the mechanical mixing of the soil and play a major role in maintaining physical soil characteristics and processes like aeration, water permeability and mineral turnover (Satchell 1967). They can also be used for monitoring the bioavailability of elements contained in soil. Their availability and size, and their limited horizontal migration, enhance their suitability for this purpose (Helmke et al. 1979, Morgan 1986).

Previously we reported on lead and cadmium concentrations in earthworms in the city of Oulu, northern Finland (Pulliainen et al. 1986). Increased lead concentrations were found in the worms *Aporrectodea caliginosa* and *Lumbricus rubellus* at Ainola, Rusko and Huonesuo, all sites close to highways. Since the soil here is often acid and may become more acidic as a result of acidic wet deposition, we decided further to investigate the role of pH on the uptake of lead and cadmium in earthworms under controlled laboratory conditions. The present study describes the results of breeding experiments carried out in 1986–87.

2. Material and methods

2.1. Earthworms and soils

The earthworms (*Aporrectodea caliginosa*) were maintained in wooden boxes of dimensions 73×23×22 cm. Two series of experiments were performed. The worms for the first set were collected on 13th and 25th of May 1986 south of the University Hospital within the City of Oulu, and those for the second one at the end of August and beginning of September in 1986 in a garden area south of Kainuuntie, about 8 km east of the centre of Oulu.

Experiment 1

Nine treatments were prepared for the earthworms. The soil was first sieved with a net of mesh size 5 mm, homogenized and analyzed before use. The soil originated from the city garden and contained an undetectable concentration of lead and cadmium, while the calcium concentration was 1210 mg/kg. The pH was 3.6. About 15–16 kg of fresh soil was added to each box. The pH of the soil was adjusted with dilute nitric acid or sodium hydroxide solutions, and the concentrations of cadmium, calcium and lead were adjusted with the addition of aqueous solutions of their nitrates. Each reagent solution was thoroughly mixed into the soil for each required treatment. The boxes were ready on June 27th, 1986 and were allowed to stabilize for seven days, before the worms were put into them. The soil was covered with a layer of hay in order to retain moisture, which was maintained at about 31% (± 3%). The proportion of organic matter of the dry weight was about 21%. The boxes were maintained in a temperature-conditioned room at 12°C, and the pH, organic matter content and the levels of the metals in the soil in each box were determined several times in the course of the experiment (see Tables 1, 2).

On July 4th, 1986 110–130 (of which about 2/3 were adults) worms were put into each box. The initial concentrations of the metals studied were Cd 3.7, Pb 4. 0 and Ca 2920 mg/kkg. The worms put into boxes I, VI and IX (with a high concentration of calcium nitrate) did not burrow into the soil but stayed on the surface and died. The illumination in the room followed a rhythm of dark between 00–08 hours and light between 08–24 hours. The boxes were covered by a net to prevent the worms escaping. At the end of the experiments, on August 5th, the lights were completely taken away. Due to a failure in the machinery halfway through the experiment, the temperature varied between +12 and +22°C towards the end. In addition, there was a breakdown between July 26th and 29th and during this period the temperature dropped to eleven degrees below zero. This will be referred to below as 'the accident'.

Experiment 2

Here only five treatments were used, which were identical to those described above, and the soil of each box was treated in the same way as earlier, except that birch leaf litter was used to cover the soil instead of hay and the soil used was more sandy. The amount of organic matter with respect to the dry weight was about 5%. The concentrations of cadmium and lead in the soil were lower than the detection limits of the methods used. The calcium concentration was 1490mg/kg. The humidity was maintained at around 15% (± 2%). About 22 kg of soil was weighed into each box. Calcium sulphate dihydrate powder was used instead of a solution of calcium nitrate for adjusting the calcium concentration in the boxes (Table 3). The boxes were ready on September 19th, 1986 and were allowed to stabilize for five days.

On September 24th, 1986 95 worms were put into each box, now mostly adults. The initial concentration of the studied metals were Cd 10.0 mg/kg, Pb 8.0 mg/kg and Ca 3270 mg/kg (13 worms). The boxes were kept in a warehouse at approximately room temperature (+20°C), and light was allowed in through a skylight in the roof.

2.2. Analytical methods

For each analysis 7–15 worms (4–10 in the second experiment), both juveniles and adults, were taken. The worms were then kept in plastic jars, covered with rye flour and wet paper towels at

12°C (experiment 1) or on two dampened paper towels at room temperature (experiment 2) for 7 days to allow depuration of their gut contents. After that they were washed twice with distilled water and dried gently on paper towels.

The earthworm and soil samples were dried in an oven at 105°C for 24 hours before analysis. The samples were digested in a boiling mixture of concentrated nitric and perchloric acids (7:3 v/v). The soluble metal concentration was estimated by extraction of soil 2–5 gdw) with fifty millilitres of a mixture of water (1100 ml), ammonium acetate (100 g) and acetic acid (100 ml) (pH 4.8); the soil-solution slurries were shaken for one hour in a Griffen Flask Shaker. Finally, the solutions were filtered before the determination of lead, cadmium and calcium.

Cadmium, calcium and lead were determined by flame atomic absorption spectrophotometry using deuterium lamp background correction (Pye Unicam SP 9). To avoid interference in the determination of calcium, either strontium or lanthanum was used as a releasing agent. Some control determinations were also carried out with direct current plasma atomic emission spectrometry (SpectraSpan III B). All concentrations are expressed on a dry weight basis.

The organic material content of the soil samples was determined by igniting the dried samples for four hours at 500°C. The pH of the soil samples was determined from fresh soil –0.1 m KCL slurries (w:v, 1:25) which were allowed to equilibrate for 90 minutes. A Radiometer PHM 64 research pH meter equipped with a glass electrode was used for the pH measurements.

The experiments were begun by determining the pH of the soil in the breeding boxes, using two ratios of soil to KCl solution, namely 1:1 (25 ml of both soil and KCl solution) and 1:2.5 (20 ml soil and 50 ml KCl solution). Since practically no difference existed between the resulting pH values, a volume ratio of 1:2.5 was selected for the future work.

2.3. Statistical methods

The meaning of time in the uptake of the metals was described by simple linear regression between days and uptake (r's and b's presented in legends of figures). The significance of the differences between the accumulation of metals in different pH values was tested by comparing the slopes with the t-test.

3. Results

Ammonium acetate extractions showed that the solubility of lead in soil is lower than that of cadmium. In addition the solubility of both metals decreases with increasing pH of the soil, especially in the case of lead (Tables 1 and 2).

In the first experiment cadmium accumulated rapidly in the worms, and reached a ten-fold concentration with respect to the soil in 30 days. The cadmium uptake seems to clearly depend on the pH of the soil (Figs. 1 and 2). The accumulation is faster at a lower pH. The following statistics were obtained: I/IV t = 0.1245 P = NS; I/VII t = 9.83***; IV/VII t = 1.79 NS; II/V t = 2.279 NS; II/VIII t = 6.927***; V/VIII t =

Table 1. pH (first and last values, 3.7.86 to 11.8.86), organic matter (OM, %), and metal concentrations (mg/kg, average of three determinations) in the earthworm boxes in the first experiment.

Box	pH	OM	Cd	Pb	Ca
I	4.88–4.71	17.8	3.3	20.7	2360
II	5.04–5.18	16.2	36.3	101	2200
III	4.97–5.03	15.5	34.0	93.5	9040
IV	5.97–5.41	18.9	3.4	21.0	2370
V	5.84–5.86	16.0	37.2	97.8	2410
VI	5.71–5.87	15.5	33.5	91.5	8540
VII	6.82–5.95	18.2	3.0	19.3	2310
VIII	6.92–6.07	17.3	38.7	105	2490
IX	6.83–6.82	15.7	31.9	99.6	8630

Table 2. Soluble (%, two determinations) metal concentrations in the earthworm boxes at 11.8.1986 (33 days after the beginning of the experiment). For total metal concentrations and pH values see Table 1.

Box	Cd	Pb	Ca
II	85, 82	50, 47	50, 50
V	78, 74	43, 43	43, 48
VIII	77, 68	35, 32	58, 38

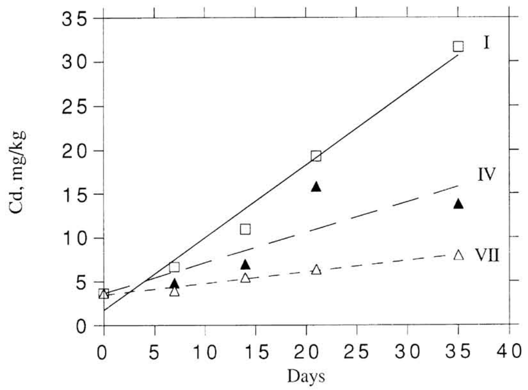

Fig. 1. Effect of pH on the accumulation of cadmium in *Aporrectodea caliginosa* in the presence of a low Cd concentration (about 3 mg/kg) in soil. Statistics (r = correlation coefficient, b = slope): I r = 0.989**, b = 0.826**; IV r = 0.857*, b = 0.346NS; VII r = 0.989**, b = 0.13**; For further information, see Table 1.

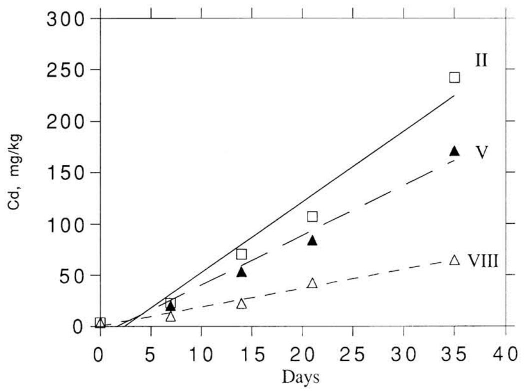

Fig. 2. Effect of pH on the accumulation of cadmium in *Aporrectodea caliginosa* in the presence of a high Cd concentration (about 37 mg/kg) in soil. Statistics (see also Fig. 1): II; r = 0.981**, b = 6.89**; V r = 0.989**, b = 4.855**; VIII r = 0.99**, b = 1.841**. For further information, see Table 1.

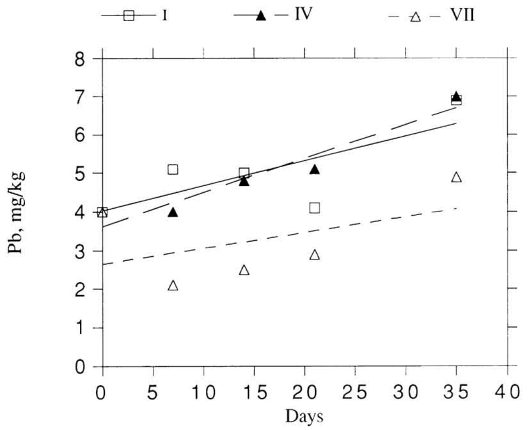

Fig. 3. Effect of pH on the accumulation of lead in *Aporrectodea caliginosa* in the presence of a low Pb concentration (about 20 mg/kg) in soil. Statistics (see also Fig. 1): I r = 0.745 NS, b = 0.064 NS; IV r = 0.964**, b = 0.088**; VII r = 0.477 NS, b = 0.041 NS. For further information, see Table 1.

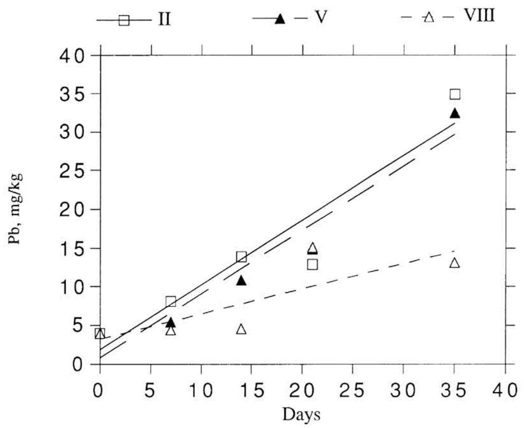

Fig. 4. Effect of pH on the accumulation of lead in *Aporrectodea caliginosa* in the presence of a high Pb concentration (about 100 mg/kg) in soil. Statistics (see also Fig. 1): II r = 0.944**, b = 0.837*; V r = 0.969**, b = 0.825**; VIII r = 0.813*, b = 0.326 NS. For further information, see Table 1.

7.812***. The accumulation of lead was not so pronounced, but again higher lead concentrations were noted at lower pH values (Figs. 3 and 4). The following statistics were obtained: I/IV t = 2.279 NS; IV/VII t = 3.219*; I/VII t = 1.039 NS; II/V t = 0.058 NS; II/VIII t = 2.366 NS; V/VII t = 2.742*.

Soluble lead and cadmium concentrations seem to decrease as a function of time, especially in the case of lead. The proportion of soluble

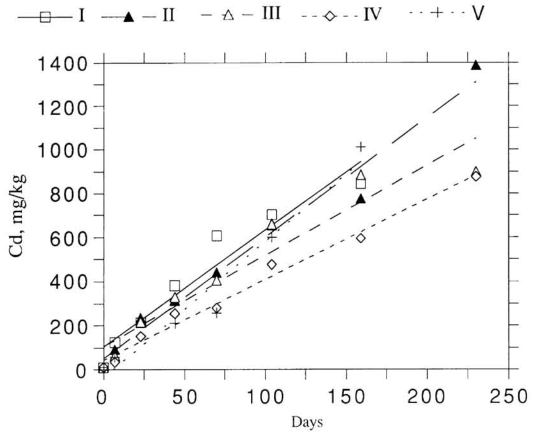

Fig. 5. Effect of pH on the accumulation of cadmium in *Aporrectodea caliginosa*. Statistics (see also Fig. 1). I $r = 0.965^{**}$, $b = 5.271^{***}$; II $r = 0.981^{**}$, $b = 4.815^{***}$; III $r = 0.957^{**}$, $b = 4.114^{***}$; IV $r = 0.992^{**}$, $b = 3.651^{***}$; V $r = 0.98^{**}$, $b = 6.163^{***}$. For further information, see Table 3.

cadmium did not depend upon the pH, while the lowest solubility of lead was recorded in the box with the highest pH (Tables 3 and 4).

The second experiment again showed a significant accumulation of cadmium in the worms (Fig. 5), the concentration ratio at the end of the experiment being in the range 13–33 (over a period of 230 days). The following significant statistics were obtained: II/IV $t = 2.477^{*}$; V/IV $t = 3.859^{**}$; I/IV $t = 2.425^{*}$. Accumulation was faster at a low pH at the beginning, but later the influence of pH bacame more obscure. The following results were obtained as means ±*SD* for individual worms (five worms from box no. 2, 159 days): Cd 812 ±165 mg/kg and Pb 49.7 ±12 mg/kg (Ca 4020 ±560 mg/kg).

The accumulation of lead in the worms was slow, and the figures scarcely exceeded the lead concentrations in the soil. Lead accumulation was here also lowest at the highest pH values, but some individual high values were obtained in the determinations (for which no reason can be given) which made the results difficult to interpret. No statistical differences between pH values were found.

A certain proportion of the worms died in the accident described above, and to our astonishment

Table 3. pH (first and last value), organic matter (OM, %), and total metal concentrations (mg/kg average of four determinations) in the earthworm breeding boxes in the second experiment. Sampling dates are shown in Table 4.

Box	pH	OM	Cd	Pb	Ca
I	4.33–4.29	4.2	40.4	107	1400
II	4.34–4.27	4.7	41.5	108	9260
III	5.25–5.05	5.1	43.7	104	8920
IV	6.29–5.95	5.0	45.2	104	10280
V	6.94–5.90	4.9	42.6	101	1580

Table 4. Soluble (%, two determinations) metal concentrations in the earthworm breeding boxes in second experiment. For total metal concentrations and pH values see Table 3.

Box /Date	Cd	Pb	Ca
I			
1.10.86	90, 85	62, 63	26, 25
7.11.86	92, 97	53, 61	25, 28
6.1.87	73, 62	45, 37	25, 20
4.3.87	81, 82	56, 57	31, 30
II			
1.10.86	90, 85	60, 60	106[a], 79
7.11.86	123[a], 102[a]	62, 59	88, 92
6.1.87	86, 87	48, 54	88, 87
4.3.87	86, 83	51, 46	82, 94
III			
1.10.86	80, 87	51, 62	88, 98
7.11.86	86, 92	64, 64	90, 101
6.1.87	72, 83	43, 52	68, 97
4.3.87	74, 83	40, 46	77, 111[a]
IV			
1.10.86	91, 91	69, 66	89, 75
7.11.86	80, 94	66, 52	74, 79
6.1.87	81, 83	59, 50	80, 91
4.3.87	78, 79	46, 46	84, 88
V			
1.10.86	78, 76	40, 40	29, 30
7.11.86	95, 93	45, 43	33, 32
6.1.87	78, 84	33, 33	34, 37
4.3.87	77, 73	31, 28	38, 36

[a] Value must be due to a small scale nonhomogenity of the soil.

the percentage of these varied in a regular manner as follows:

Box	pH	% dead worms
I	4.80	8
II	5.19	13
IV	5.45	20
V	5.82	38
VII	6.06	48
VIII	6.26	49

Thus, a clear positive correlation seems to occur with increasing pH, and the worms in the more acid soils survived much better.

4. Discussion

Most reports dealing with lead and cadmium concentrations in earthworms revealed a greater concentration factor for cadmium than for lead (Hook 1974, Andersen 1979, Helmke et al. 1979, Ireland 1979, Hartenstein et al. 1980, Beyer et al. 1982, Ma et al. 1983, Pietz et al. 1984, Kruse & Barrett 1985, Morgan & Morgan 1988a), although opposite results have sometimes been obtained (Pulliainen et al. 1986, Beyer & Cromartie 1987). Investigations dealing only with lead have nevertheless confirmed its bioaccumulation in earthworms (Gish & Christensen 1973, Wielgus-Serafinska & Kawka 1976, Ireland 1977, Wielgus-Serafinska 1979), and established that this is closely related to the concentration of lead in the soil (Ma 1982).

Significant differences exist between worm species and their accumulation of metals (Ireland & Richards 1977, Ireland 1979, Andersen 1979, Beyer & Cromartie 1987), and the age of the worms also affects their heavy metal accumulation to some extent (Andersen & Laursen 1982, Honda et al. 1984), as well as the composition of the soil itself. The concentration factor seems to be larger in sandy than in loamy soil (Ma 1982). The activity of a worm also has a great influence on its metal uptake (Ireland 1977), but this might be a less important factor in the present study because of the controlled experimental conditions. However, the pH of the soil may influence the activity of worms.

Ma (1982) noticed that when the mean lead concentration factors were plotted against the mean total soil calcium, a negative correlation was obtained. This may indicate that high soil Ca concentrations inhibit the Pb accumulation in the worms reported also by Morgan & Morgan (1988b). In our second experiment the Ca content in box soils II, III and IV (Table 3) was high, but the lead concentration in worms seems to vary randomly. Lead and calcium are to some extent chemically related, and seem to be connected with the cation exchange balance in soils. The low pH and low soil Ca concentrations combine to increase the Pb bioavailability (Morgan 1986).

The influence of pH on lead accumulation was unclear in our experiments, although a slight increase could be seen in the most acid treatments. Ma (1982), demonstrated with *Aporrectodea caliginosa* that the lead burdens in worms are determined primarily by the soil Pb and soil pH, and to a lesser extent by the cation exchange capacity of the soil. Ma et al. (1983) derived an equation in which the soil pH and soil Pb explained >70% of variance in earthworm Pb. Morgan & Morgan (1988a) also stated that the soil pH and soil Ca had a major influence on Pb accumulation.

Both of our experiments pointed to an increasing accumulation of cadmium at lower pH values. Beyer et al. (1982) found that liming the soil reduced Cd concentrations in earthworms slightly ($P<0.05$), and stated that at least this does not preclude the possibility that pH could be important in alkaline or very strongly acid soils. Ma (1982) found that the soil pH had the most significant influence on the concentrations of Cd and Zn in worms and proposed that lowering of the pH leads to an increased desorption of metal cations due to competition with H^+ ions. In the results obtained by Ma et al. (1983) Cd appeared to be accumulated by *Lumbricus rubellus* in greater quantities when present in soil of low pH. In addition Ireland & Richards (1981) showed that 68% of the total body wall cadmium in worms incubated for 26 days was surface bound.

Our results seem to confirm earlier observations on the importance of a combination of factors for heavy metal burdens in earthworms, soil concentration, pH and soil Ca. Altogether, the considerable influence of the pH on the bioaccumulation of cadmium constitutes a serious warning with respect to acid rain. The enrichment of cadmium in food chains involving worms will accelerate continually and may approach toxic limits.

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