Reference 0034

EFFECTS OF METAL POLLUTION ON THE EARTHWORM *DENDROBAENA RUBIDA* (Sav.) IN ACIDIFIED SOILS

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Abstract. Survival of adult *Dendrobaena rubida*, cocoon production, cocoon viability, and growth of juveniles were examined in laboratory experiments when the worms were reared in acidified and metal polluted soils. Solutions of Cu, Cd and Pb were added to give total concentrations of 10, 100 and 500 $\mu g\ g^{-1}$ in soils with a pH of 4.5, 5.5 and 6.5. Adult tissues, hatchlings and cocoons were analyzed for metals.

D. rubida survived poorly in soils with low pH, especially in combination with Pb or Cu, which accumulated in seminal vesicles and cerebral ganglion. Cocoon production was halved when pH was lowered from 6.5 to 4.5 and metals reduced the cocoon number even more in the most acid soils. Hatching success was lower than 20% in acidified soils. In contrast, the number of hatchlings increased when pH decreased and peaked in soils polluted with Cd. While low pH reduced the embryonic development time, metals prolonged it. Juveniles grew slowly and died early at low pH.

1. Introduction

During the last two decades the impact of pollutants on living organisms has received much attention. Metals have been shown to cause significant reductions in abundance of soil invertebrates and decreases in diversity of various soil fauna groups (Bengtsson *et al.*, 1983b). The impact of lowered soil pH on Enchytraeidae, Collembola, and Acarida has been demonstrated in large-scale projects (Bååth *et al.*, 1980; Hågvar and Amundsen, 1981). However, hardly any studies have emphasized the combined effects of metal pollution and acid precipitation on soil invertebrates.

Soil pH is an important factor controlling the uptake of metals, especially Cd, by plants (Peterson and Alloway, 1979). This is because sorption and solubility of metals in soils are pH dependent; the lower the pH, the less is sorption to organic matter. Generally, the stability of Cu and Pb complexes with organic compounds is considerably stronger than complexes of Cd (Stevenson, 1975), which is a mobile metal in the soil (Tyler, 1981). Comparing the sorption of some metals at gradually lower pH, Kuo and Baker (1980) reported a progressively reduced sorption of Zn and Cd from approximately pH 7.0 and downwards, whereas the sorption maximum of Cu was 1 to 1.5 pH units lower. Hence, the availability of metals to plants increases when pH is lowered (Miller *et al.*, 1975; Andersson, 1977; Hutchinson and Collins, 1978; Cavallaro and McBride, 1980).

Consequently, it seems reasonable to assume that a combination of gradually decreasing pH and a continuous fallout of metal ions in the soil due to emissions may also affect soil invertebrate populations and cause increased mortality, reduced growth and changed reproductive output. Furthermore, invertebrates living in the soil surface environment (in the litter or mor horizons) are more exposed to airborne pollutants than

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invertebrates in the mineral soil, as demonstrated by population density changes in metal polluted soils of earthworms with different vertical distributions (Bengtsson and Rundgren, 1982; Bengtsson *et al.*, 1983b).

These observations were extended into the present study, with the aim to examine some possible explanations for the impoverishment of the soil fauna in acidified and metal polluted environments. The purpose was to use a common, surface living lumbricid earthworm, *Dendrobaena rubida* (Sav.) in a laboratory experiment to demonstrate (1) the effects of low soil pH on the uptake of metals by the earthworm, and (2) reinforcement of the effects of metals on survival, growth, and reproductive output of *D. rubida* by a low soil pH.

2. Material and Methods

2.1. Test organism

A number of criteria was defined to delimit an appropriate test species. It should:

(1) occupy habitats in terrestrial ecosystems that may be subject to acidification and/or metal pollution;
(2) live close to the soil surface and hence be exposed to airborne pollutants;
(3) have a limited migration, i.e., be confined to the same habitat during its lifetime;
(4) have a short generation time and a high reproductive output;
(5) be easily reared under laboratory conditions; and
(6) be an ecologically well known representative of the soil fauna.

Dendrobaena rubida fulfills all these requirements. It is a common species in the Nordic countries, though it occurs sparsely, most often with less than 10 ind m^{-2} (Nordström and Rundgren, 1973) and dominates along with *D. octaedra* (Sav.) in poor soils (podsols or podsoloids) of coniferous woods, in oligotrophic-mesotrophic brown forest soils of deciduous woods or in soils impaired by changed agricultural or silvicultural practices (Rundgren, 1976). The species has been classified as acid-tolerant (Satchell, 1955) and may at low densities inhabit soils with pH as low as 3.5 (Nordström and Rundgren, 1974). It is bound to the uppermost soil layers throughout the year and does not penetrate to depths below about 10 cm (Rundgren, 1975). Maturity is reached after 18 to 20 weeks (Graff, 1953a). Satchell (1967) reported that clitellum of *D. rubida* ssp *subrubicunda* (Eisen) is developed in 100 day old individuals and disappears when the worm is 320 day old. No reappearance of clitellum before death has been shown, indicating that *D. rubida* lives for about 1 yr and has only one, but rather long, period of sexual activity, when it produces 40 to 95 cocoons per individual (Evans and Guild, 1948; Graff, 1953b).

2.2. Soils

A suitable soil was obtained by mixing sand (0.2 to 2 mm particle size) from the C horizon of a coniferous forest soil with well decomposed cattle dung (1:2, vol:vol).

Prior to mixing, the dung was heated (80 °C, 3 days) to kill most soil animals. The pH_{H_2O} of the mixed soil became 5.8, and organic C was 4.5 to 6.9% (d.w.).

Copper, Cd, and Pb were added to the soil as nitrate solutions giving the following series of metal concentrations ($\mu g\, g^{-1}$, d.w.): O, 100 Cu, 500 Cu, 10 Cd, 100 Cd, 100 Pb, and 500 Pb. For each metal concentration, a pH_{H_2O} value of 4.5, 5.5, and 6.5 was obtained by adding aliquots of 0.125 M H_2SO_4 or suspended $CaCO_3$. Soil pH was adjusted every third day during two weeks to avoid a rapid change of the soil chemical and microbial properties. Continuous recordings of soil pH during the experimental time showed that pH gradually changed by approximately 0.2 to 0.3 units mo^{-1}. This was compensated for once a month by adding H_2SO_4 or $CaCO_3$ as above.

Supplying a soil with metal ions, e.g., Pb may lower soil pH (Stevenson, 1975) and be followed by altered soil properties and changed microbial composition. For that reason the soils were left for 4 weeks to recover and stabilize before the experiment started.

Soil moisture was maintained at 45 to 50% (d.w.) by adding distilled water.

2.3. Survival and Reproduction of the P Generation

Adult *D. rubida* were collected in late September under cow droppings in a pine plantation east of Lund, S. Sweden and represented the parent generation.

Cardboard boxes (70 × 70 × 100 mm; 0.5 L) were used as rearing chambers. They were filled to 2/3 with soil. Five replicates of each experimental soil (metal, pH) gave a total of 105 rearing chambers. Five to six worms were added to each chamber. Specimens which did not burrow into the soil by the following day were removed and exchanged for new ones. Rearing took place at 14 °C and 70% RH.

Rearing chambers were inspected for the first time after 3 weeks incubation. The soil was handsorted, surviving adults and produced cocoons were counted and soil and worms were then returned to their respective chamber. Worms were checked for survival and reproduction once a month for another 3 mo.

2.4. Hatching and Growth of the F Generation

Harvested cocoons were transferred to hatching chambers, at least one for each soil treatment and inspection date. Since there is a time lag phase in metal uptake by terrestrial invertebrates (Hartenstein *et al.*, 1980: Bengtsson *et al.*, 1983a) cocoons produced during the first 3 weeks were discarded and excluded from the study.

Hatching chambers were made of plastic jars (Ø 40, h 45 mm) with a 5 mm thick bottom layer of plaster of Paris and pulverized activated carbon (9:1, vol:vol). Chamber lids were perforated to allow for air exchange. Tap water was added until the cocoons were almost immersed. Up to 50 cocoons were incubated in the same chamber in darkness at 20 °C.

The chambers were examined once a week during the first 3 mo after cocoon transfer and less regularly during the next 3 mo. Hatchlings and empty shells were counted and removed. Top water was simultaneously exchanged to obviate microbial growth.

Up to ten randomly chosen hatchlings from each soil treatment were transferred to

growth chambers designed like the hatching chambers. They were filled to 2/3 with soil (approx. 80 g) of the same metal content, pH, and moisture as the parent soil. One chamber was used for each treatment and week of hatching but more than 10 hatchlings were never pooled. The chambers were incubated in darkness at 20 °C and soil was exchanged once a month to obviate food deficit.

The growth (expressed as increase in volume) of filial specimens was determined every second week. The body was approximated to a cylinder when estimating body volume (total length, width at 20th segment) (Nordström and Rundgren, 1972). The worms were anaesthetized by CO_2 producing relatively uniformly slackened specimens.

2.5. Metal analysis

Samples of soils from the rearing chambers were taken at the start and end of the experiment to determine the concentrations of extractable metals (Cu, Pb, Cd). Twenty five grams of fresh soil were continuously shaken in 100 mL 1 M NH_4Ac (pH 7.0) for 2 hr. The extract was filtered and 50 mL of the filtrate was evaporated to dryness. Residues were heated in 20 mL conc. HNO_3 and perchloric acid (4 : 1, vol : vol) and after evaporation stored in a refrigerator at 4 °C until analysis. Duplicates of each soil type were analyzed.

Analyses were made by atomic absorption spectrophotometry (Varian AA6); flame technique for soil samples and graphite furnace for tissues and worms (Bengtsson and Gunnarsson, 1984).

The total metal contents (Cu, Pb, Cd) of 1.000 g dried rearing soil of each treatment (24 hr, 105 °C) were measured after heat digesting in conc. HNO_3 and perchloric acid (4 : 1, vol : vol). Duplicates of each soil treatment were analyzed.

Metal content of four adult worms was measured at the start of the experiment and of 1 to 4 adults (depending on survival) randomly chosen from each soil after 3 and 16 weeks rearing. The worms were kept for 4 days at 4 °C on moist filter paper in plastic jars to empty the intestines before dissection. The pharynx, a part of the muscles (just behind the clitellum), seminal vesicles, and cerebral ganglion were saved for analysis. Cocoons, hatchlings (0 to 7 day old), and juveniles (50 and 100 day old) were analyzed without dissection. Tissues and individuals were dried (24 hr, 85 °C) and weighed on a Cahn micro balance (± 0.1 μg). A micromethod (Bengtsson and Gunnarsson, 1984) was used to digest the tissues and individuals in conc. HNO_3.

3. Results

3.1. Metals in soil

The sandy soil mixed with dung had lower total concentrations of the studied metals than the average for Swedish soils reported by Andersson (1977), viz. 14.6 μg g^{-1} Cu, 15.9 μg g^{-1} Pb, and 0.22 μg g^{-1} Cd (Table I). Less than 11% of the total Cu and less than 21% of total Pb content were extractable by NH_4Ac. The sorption of Cd to the soils was not that effective; 31 to 79% was extracted. These figures exceed the average

TABLE I

Metal concentration (μg g^{-1}, d.w.) (total, A, and exchangeable, B) in soils of different pH and supplied with nitrate solutions of Cu, Cd, and Pb. Mean and SD (n = 2) are given and, within brackets, percentage exchangeable of total concentration.

	A			B					
				at start			after 4 mo		
Metals added to soil μg g^{-1}\pH	4.5	5.5	6.5	4.5	5.5	6.5	4.5	5.5	6.5
	Cu								
0 Cu	3.6 ± 0.4	<0.5	1.3 ± 1.8	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
100 Cu	102 ± 2	98 ± 2	122 ± 1	5.5 ± 0.2 (5)	4.5 ± 0.2 (5)	4.6 ± 0.2 (4)	2.0 ± 0.6 (2)	1.5 ± 2.1 (2)	1.0 ± 1.3 (1)
500 Cu	464 ± 33	512 ± 8	456 ± 30	38 ± 5 (8)	37 ± 6 (7)	27 ± 2 (6)	n.t.	58 ± 8 (11)	35 ± 3 (8)
	Cd								
0 Cd	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
10 Cd	12 ± 1	15 ± 0	14 ± 4	6.5 ± 0.1 (56)	5.7 ± 0.1 (39)	5.6 ± 0 (39)	9.2 ± 0.7 (79)	6.0 ± 0.5 (40)	4.6 ± 0 (32)
100 Cd	92 ± 2	91 ± 5	101 ± 8	36 ± 1 (40)	34 ± 1 (37)	31 ± 1 (31)	59 ± 3 (64)	50 ± 0 (54)	36 ± 1 (35)
	Pb								
0 Pb	1.2 ± 0	4.6 ± 2.6	3.0 ± 2.6	1.2 ± 0.2	0.9 ± 0	0.7 ± 0.2	2.1 ± 0.3	1.6 ± 0.4	1.0 ± 0
100 Pb	130 ± 4	134 ± 12	152 ± 8	21 ± 0 (16)	15 ± 2 (11)	14 ± 1 (10)	26 ± 5 (20)	14 ± 2 (11)	9 ± 0 (6)
500 Pb	590 ± 59	564 ± 13	560 ± 78	91 ± 3 (16)	83 ± 8 (15)	62 ± 4 (11)	122 ± 11 (21)	96 ± 5 (17)	60 ± 0 (11)

values given for concentrations of extractable metals naturally present in cultivated soils in Sweden; 1.8% Cu, 6.5% Pb, and 41% Cd (Andersson, 1977), probably because the metals in the present study were added purely in inorganic form.

Soil pH had a minor effect on the concentration of extractable Cu, whereas the proportions of extractable Pb and Cd were higher at lower pH than at pH 6.5 (Table I). The concentration of extractable Cd and Pb may become up to twice as high in a soil as pH drops one unit. In soils where metals are less loosely bound, an increase by a factor of 1.3 may be more realistic (cf. Pb at 0 $\mu g\ g^{-1}$ in Table I).

The metal extractability increased during the course of the experiment (Table I). It is not clear from our data what mechanisms caused the increase, but probably a combination of fewer charged sites in the soil matrix as the organic matter decomposed and/or changes in the chemical state of metals, e.g. from local precipitates to charge bound.

3.2. Metals in the P generation

Low concentrations of Cu, Cd, and Pb were initially found in adult *D. rubida* (Table II). All tissues studied had higher concentrations of Cu than of Pb or Cd.

If the differences in concentration of NH_4Ac extractable metals are also indicative of the availability of metals to earthworms, one would suggest that earthworms reared at pH 4.5 should have about twice as high tissue metal concentrations as earthworms reared at pH 6.5 after 4 mo (cf. Table I). However, the highest tissue metal concentrations were not always associated with the lowest soil pH (Table II). Peak Cu concentrations at 100 $\mu g\ g^{-1}$ Cu in soil occurred in seminal vesicles at pH 4.5 and in other tissues at pH 5.5. Most tissues from worms in 10 $\mu g\ g^{-1}$ Cd had the highest concentrations at pH 4.5 after 4 mo in the soil, whereas the Cd distribution in tissues was independent of pH at 100 $\mu g\ g^{-1}$ Cd in the soil.

Lead uptake was pH dependent but highest concentrations were not invariably found at the lowest comparable pH. No worms survived for 4 mo in Pb polluted soils at pH 4.5, which was probably due to lethal Pb concentrations, as indicated by the rapid rise in ganglion Pb concentrations in 3 weeks.

The uptake of metals and their concentrations in tissues were dependent on the time of exposure to the metal polluted soil. The concentration of Cu increased substantially during the experiment when the worms were reared at 100 $\mu g\ g^{-1}$ Cu, whereas the concentration in worms at 500 $\mu g\ g^{-1}$ evidently reached lethal levels since few animals survived to the end of the experiment (Table II). Cadmium was rapidly incorporated in the worms and the concentration in muscles increased 15 to 30 times during the first three weeks at 10 $\mu g\ g^{-1}$ Cd but little thereafter. Lead was slowly taken up, and rarely reached the same levels as the other metals.

Muscle and pharynx concentrations of metals increased as soil metal concentrations were raised; whereas concentrations in seminal vesicles and cerebral ganglion were sometimes lower at higher concentrations (Table II). The ratio of the concentration of metal in muscles to that in the soil was approximately the same after rearing the worms for 3 weeks at 100 and 500 $\mu g\ g^{-1}$ Pb at the three different pH levels (0.06 to 0.09 : 1.0).

TABLE II

Metal concentration (μg g^{-1}, d.w.) in different tissues of the earthworm *Dendrobaena rubida* exposed to metal contaminated soils of different acidities. Mean and SD are given for 4 samples, or for 3 (a) and 2 samples (b) at start of experiment and after rearing the worms for 21 and 113 days, respectively, n.t. = not tested (mostly because of low survival).

Metal in soil	pH in soil	pharynx			muscles			seminal		vesicles	cerebral ganglion		
		0	21	113	0	21	113	0	21	113	0	21	113
Cu													
100 μg g^{-1}	4.5		107 ± 16$^{b)}$	2184 ± 1740$^{a)}$		50 ± 8$^{a)}$	809 ± 759$^{a)}$		852 ± 622$^{a)}$	3820 ± 4285$^{b)}$		32	294 ± 13$^{b)}$
	5.5		161 ± 162$^{a)}$	3843 ± 4392		96 ± 102	1647 ± 1974		543 ± 340$^{a)}$	943 ± 375$^{b)}$		41 ± 30$^{b)}$	379 ± 32$^{b)}$
	6.5		259 ± 197$^{a)}$	306 ± 74		57 ± 52	102 ± 42		304 ± 31$^{a)}$	255 ± 118$^{a)}$		51	263 ± 143$^{a)}$
		22 ± 15			20 ± 7			85 ± 103$^{a)}$			100 ± 52$^{b)}$		
500 μg g^{-1}	4.5		155 ± 81$^{a)}$	n.t.		145 ± 93$^{a)}$	n.t.		n.t.	n.t.		127 ± 35$^{b)}$	n.t.
	5.5		188 ± 94	n.t.		64 ± 8$^{a)}$	n.t.		158	n.t.		60	n.t.
	6.5		134 ± 28$^{b)}$	n.t.		68 ± 30$^{a)}$	n.t.		754	n.t.		30 ± 43$^{b)}$	n.t.
Cd													
10 μg g^{-1}	4.5		251 ± 108	303 ± 77$^{a)}$		90 ± 17	108 ± 22		252 ± 134$^{b)}$	540 ± 439		116 ± 36$^{a)}$	290 ± 0$^{b)}$
	5.5		154 ± 6$^{b)}$	n.t.		50 ± 17	n.t.		353	n.t.		76 ± 1$^{b)}$	161 ± 23$^{a)}$
	6.5		166 ± 27	273 ± 69$^{a)}$		51 ± 13	72 ± 25		257 ± 158	362 ± 207		69 ± 32	254 ± 58
		5 ± 2			3 ± 1			6 ± 1$^{a)}$			17 ± 7$^{b)}$		
100 μg g^{-1}	4.5		288 ± 67$^{a)}$	762 ± 308		127 ± 42$^{b)}$	204 ± 48		626 ± 132$^{a)}$	1033 ± 405		267 ± 55$^{a)}$	490 ± 200$^{b)}$
	5.5		238 ± 40	948 ± 185$^{a)}$		100 ± 34	212 ± 38		416 ± 158	1051 ± 285		265 ± 9	740 ± 224
	6.5		430 ± 93	766 ± 141		178 ± 23	238 ± 118		400 ± 562$^{a)}$	1115 ± 146$^{a)}$		193 ± 37$^{a)}$	888 ± 216$^{b)}$
Pb													
100 μg g^{-1}	4.5		13 ± 5$^{b)}$	n.t.		9 ± 5	n.t.		81 ± 71$^{a)}$	n.t.		196 ± 142$^{a)}$	n.t.
	5.5		8 ± 7	24 ± 19$^{a)}$		9 ± 4	18 ± 12$^{a)}$		86	304 ± 254$^{a)}$		32 ± 39$^{b)}$	142 ± 109$^{b)}$
	6.5		6 ± 5	15 ± 14		6 ± 4	32 ± 48		29 ± 15$^{b)}$	89 ± 116		44 ± 22$^{a)}$	240 ± 144$^{a)}$
		8 ± 5			13 ± 5			24 ± 22$^{a)}$			56 ± 9$^{b)}$		
500 μg g^{-1}	4.5		38 ± 5	n.t.		45 ± 14	n.t.		88	n.t.		202 ± 82$^{a)}$	n.t.
	5.5		30 ± 11	40 ± 25		30 ± 6$^{a)}$	79 ± 45		132	237 ± 59		114 ± 140$^{b)}$	116 ± 52
	6.5		20 ± 6	39 ± 14$^{a)}$		33 ± 16	73 ± 13		104 ± 26$^{a)}$	165 ± 118		100 ± 5$^{b)}$	127 ± 52$^{b)}$

The same ratio was higher after rearing the worms at 10 μg g^{-1} Cd (5.0 to 9.0 : 1.0) than at 100 μg g^{-1} Cd (1.0 to 1.8 : 1.0) (Table II). Studies of the behavior of metals in invertebrates have suggested concentration factors ($\bar{x}$ μg g^{-1} metals in body: $\bar{x}$ μg g^{-1} metals in the environment/medium) above unity for Cd but mostly below unity for Pb and Cu (for references concerning Pb and Cd see Wright and Stringer, 1980: Table 5 and for Cu Hartenstein *et al.*, 1980 and Ma, 1982). Using the values in Table II for metal concentrations in muscles (approximately equivalent to whole body) calculated concentration factors had a ratio above unity for Cd, but below unity for Pb. In Cu supplied soils (100 μg g^{-1} Cu) a concentration factor above unity was obtained after 4 mo rearing of the worms.

Metals were unevenly distributed within the worm body. Seminal vesicles had higher concentrations of all metals than muscles, and cerebral ganglion higher concentrations of Cd and Pb than muscles. After 4 mo rearing the Cd concentrations were highly differentiated between the worm tissues studied, most elevated in seminal vesicles, where a 60-to-90 fold increase of the initial concentration was found when the worms were reared at 10 μg g^{-1} Cd, and a 185-fold increase at 100 μg g^{-1} Cd. The increase of the Pb concentration in different tissues was moderate, but also highest in seminal vesicles, where 4 to 14 times higher concentrations were found in worms reared in Pb supplied soil. Copper was most easily concentrated in seminal vesicles and pharynx (where minor soil particles may remain even after a careful rinsing and cause irrepresentative peaks in metal concentrations).

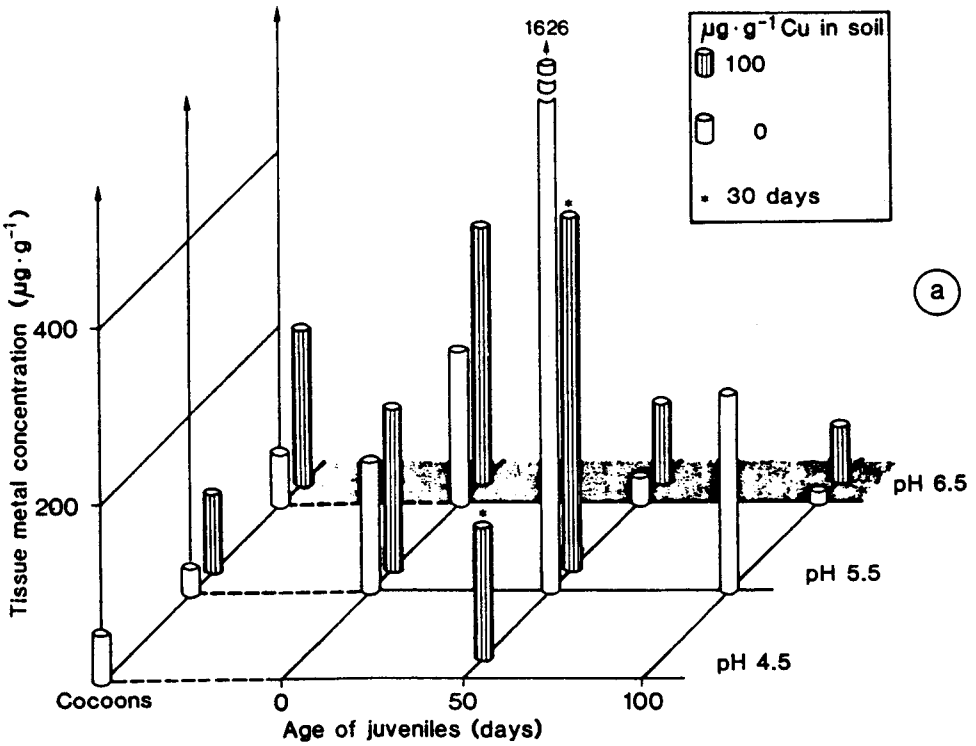
3.3. Metals in the F Generation

As indicated in Figure 1, metals seemed to be transferred from one generation to another. Cocoons produced in 100 μg g^{-1} Cu or 100 μg g^{-1} Cd soils carried most metal ions; the concentrations were on average 134 μg g^{-1} Cu (range 77 to 240) and 131 μg g^{-1} Cd (range 83 to 200), respectively, whereas cocoons from Pb polluted soils had lower metal concentrations, less than 83 μg g^{-1} (range 13 to 83). However, the ratio between the concentrations of Cd or Pb in the muscles of adults reared for 4 mo and the concentrations in the cocoons were close to unity, whereas the relationship was not so clear for Cu. The cocoon production by adults reared at 500 μg g^{-1} Cu almost failed and no cocoons could be saved for metal analysis.

There was no clear relationship between soil pH and metal concentrations of cocoons when they were produced in Cd or Pb supplied soils, whereas the Cu concentration of cocoons was twice as high when they were deposited at 100 μg g^{-1} Cu, pH 6.5 compared with 100 μg g^{-1} Cu, pH 5.5 (Figure 1). Increasing the amount of Cd 10 times or of Pb 5 times in the soil was followed by a 5- and 2-fold higher concentration of respective metal in the cocoons deposited. Cocoons produced in unpolluted soils (less than 4 μg g^{-1} Cu) contained about 50 μg g^{-1} Cu.

Generally, hatchlings had higher concentrations of metals than cocoons. Presumably the metal ions were carried by the worm itself and not incorporated in the shell or associated with the excreted products remaining in the cocoon.

Copper was rapidly taken up by the growing juveniles at pH 5.5 and a peak was


1626
µg·g⁻¹ Cu in soil
100
0
* 30 days
a
Tissue metal concentration (µg·g⁻¹)
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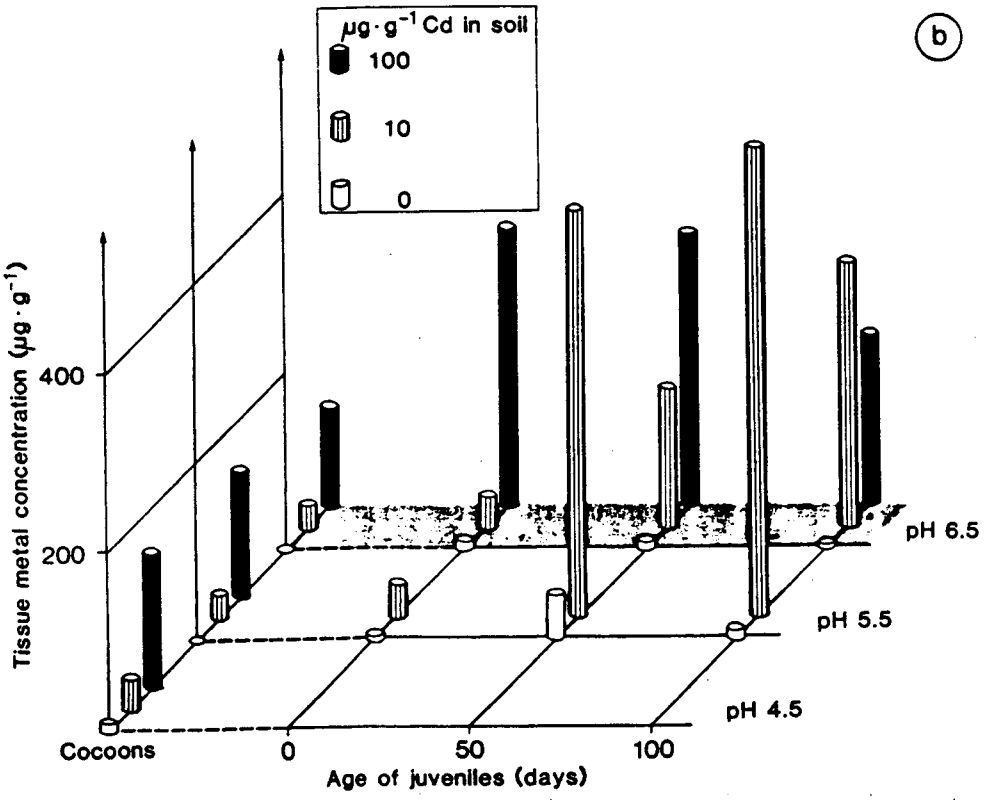
Age of juveniles (days)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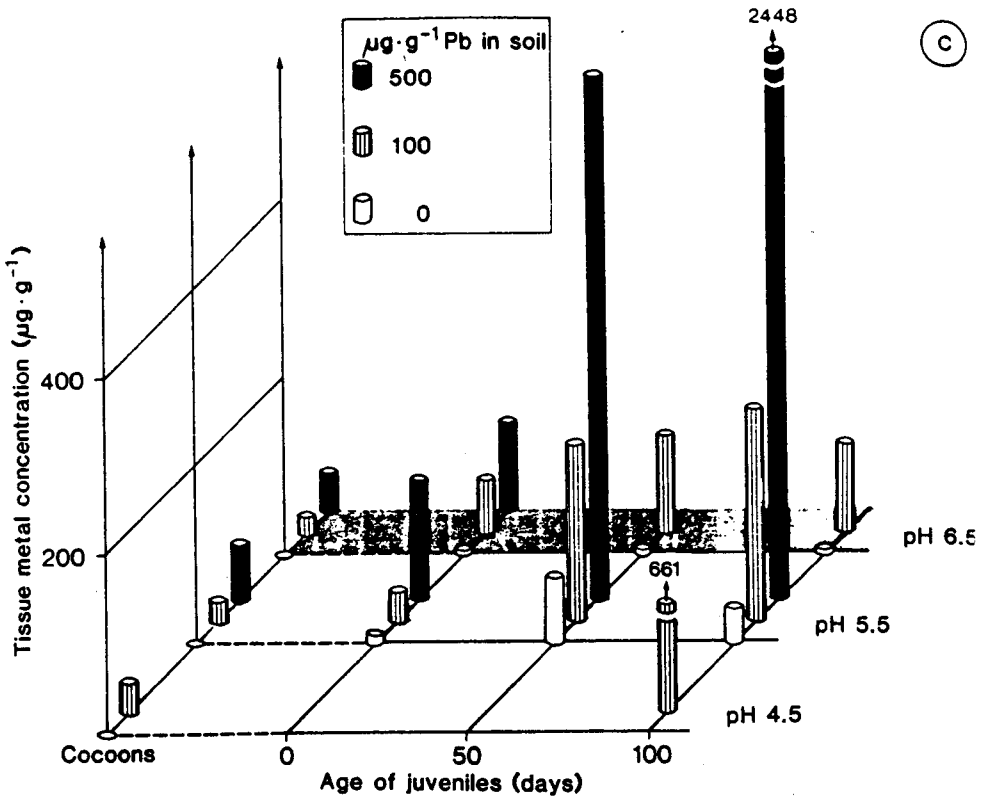


Fig. 1. Concentration of Cu (a), Cd (b), and Pb (c) in cocoons and juvenile of *Dendrobaena rubida* reared in soils of different pH and metal concentration. Juvenile and adult worms were always reared in soils of the same treatment (metal content, pH, moisture).

reached after 1 to 1.5 mo (Figure 1). Juveniles growing at pH 6.5 quickly reached an equilibrium and were able to maintain a Cu concentration of about 25 $\mu g\, g^{-1}$ (at 0 $\mu g\, g^{-1}$ Cu) and 75 $\mu g\, g^{-1}$ (at 100 $\mu g\, g^{-1}$ Cu) throughout the study, i.e., the same values as found in muscles of the parent generation under the same circumstances (cf Table II). These findings and the fact that Cu occurred in cocoons in unpolluted soils, indicate a certain Cu demand in these earthworms.

Cadmium and Pb were also rapidly taken up by the growing worm and the uptake was pH dependent. Significantly higher Cd concentrations were recorded in 50 day old juveniles at pH 5.5 than at pH 6.5 ($p < 0.01$. Student's t-test) when the juveniles were reared in 100 $\mu g\, g^{-1}$ Cd soil and the uptake continued throughout the study. Very high values of Pb were recorded in specimens reared in 500 $\mu g\, g^{-1}$ Pb, pH 5.5 soil. During the whole study period Cd and Pb concentrations in the juvenile body were considerably higher than the levels found in muscles of adult worms (cf Table II). Apparently, juveniles of *D. rubida* lack the ability to actively excrete these metals or the excretion may be less efficient than the uptake during the first 100 days of growth.

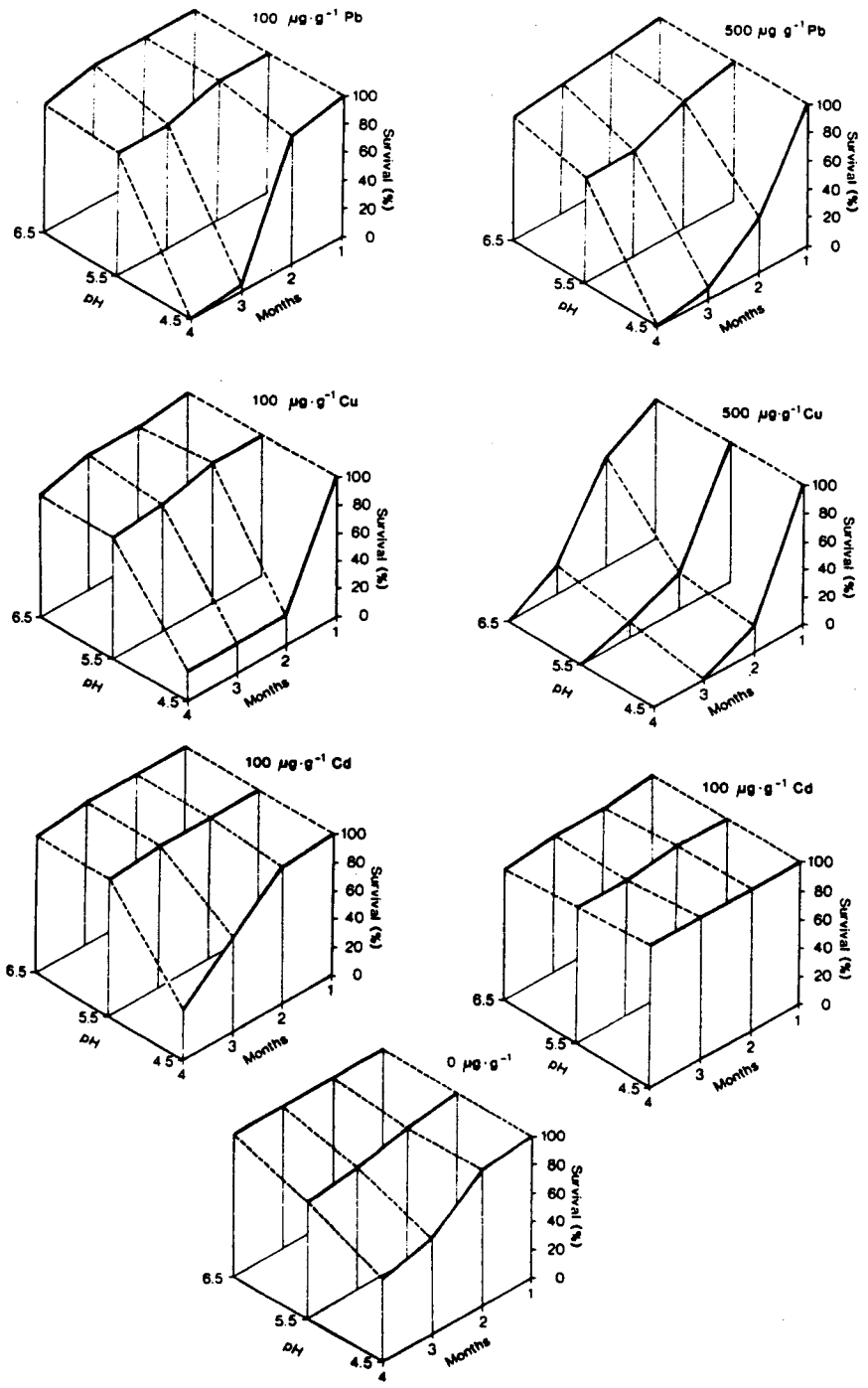


Fig. 2. Survival of adult *Dendrobaena rubida* reared in soils of different pH and concentration of Cu, Cd, and Pb during a 4 mo study period.

3.4. Effects on Earthworms Performance

3.4.1. *Survival of the Parent Generation*

Survival of adult *D. rubida* was pH-dependent; the population at pH 4.5, 0 $\mu g\, g^{-1}\, M^{+}$ was reduced by 52% within 4 mo (Figure 2). The reduction was even greater when a low pH was combined with Cu or Pb. The results of adding Cd to pH 4.5 soils were unclear and may illustrate the high degree of variability in susceptibility to metals in a population. Irrespective of pH, 500 $\mu g\, g^{-1}$ Cu in the soil rapidly caused a collapse of the populations and the survival was significantly lower each month than in the control soils ($p < 0.05$); worms still alive were seen sticking firmly to the lid.

3.4.2. *Cocoon Production*

D. rubida deposited significantly fewer cocoons per adult worm at pH 4.5 in 0 $\mu g\, g^{-1}\, M^{+}$ soils than at higher pH ($p < 0.05$) and the production was obviously pH-dependent also when metals where added (Figure 3). Metals caused the low

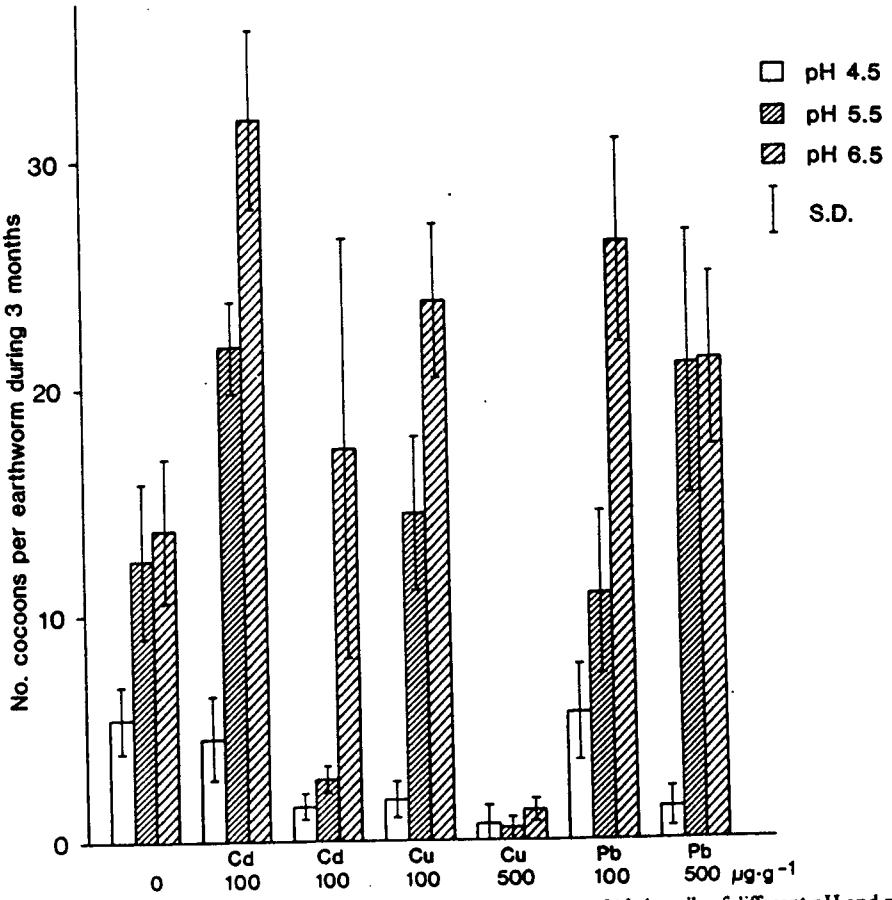


Fig. 3. Number of cocoons deposited during 3 mo by *Dendrobaena rubida* in soils of different pH and meta concentration. Mean and standard deviation are shown.

›duction at pH 4.5 to drop further, and significantly fewer coccons were deposited 100 μg g^{-1} Cu, 500 μg g^{-1} Cu, 100 μg g^{-1} Cd, and 500 μg g^{-1} Pb than at ;g g^{-1} M^{+} ($p < 0.05$). The influence of metals on cocoon production in pH 5.5 soils ;fered depending on the metal and its concentration. Ten μg g^{-1} Cd led to a significant ..rease ($p < 0.05$), whereas 100 μg g^{-1} Cu, 500 μg g^{-1} Cu, and 100 μg g^{-1} Cd 'sulted in a significant decrease ($p < 0.05$). Adding 10 μg g^{-1} Cd to pH 6.5 soil further ;ressed the positive effect that low concentrations of Cd seem to have on cocoon ;oduction. Copper and Pb, the availability of which was pH-dependent, had in 100 μg g^{-1} Cu or Pb, pH 6.5 soils a similar effect, and the cocoon production in these ases was higher than in unpolluted soils ($p < 0.05$). The unhealthy animals at 00 μg g^{-1} Cu deposited a negligible number of cocoons per adult worm, irrespective f soil pH.

Cocoons produced at 100 μg g^{-1} Cd and 500 μg g^{-1} Pb were often aberrantly :olored or, in case of 100 μg g^{-1} Cd, occasionally abnormally shaped.

Most cocoons were produced during the third rearing month in soils without metals ;dded and the pattern was the same irrespective of pH (Figure 4). Generally, :00 μg g^{-1} Cu or 100 μg g^{-1} Pb resulted in a cocoon production that was more evenly

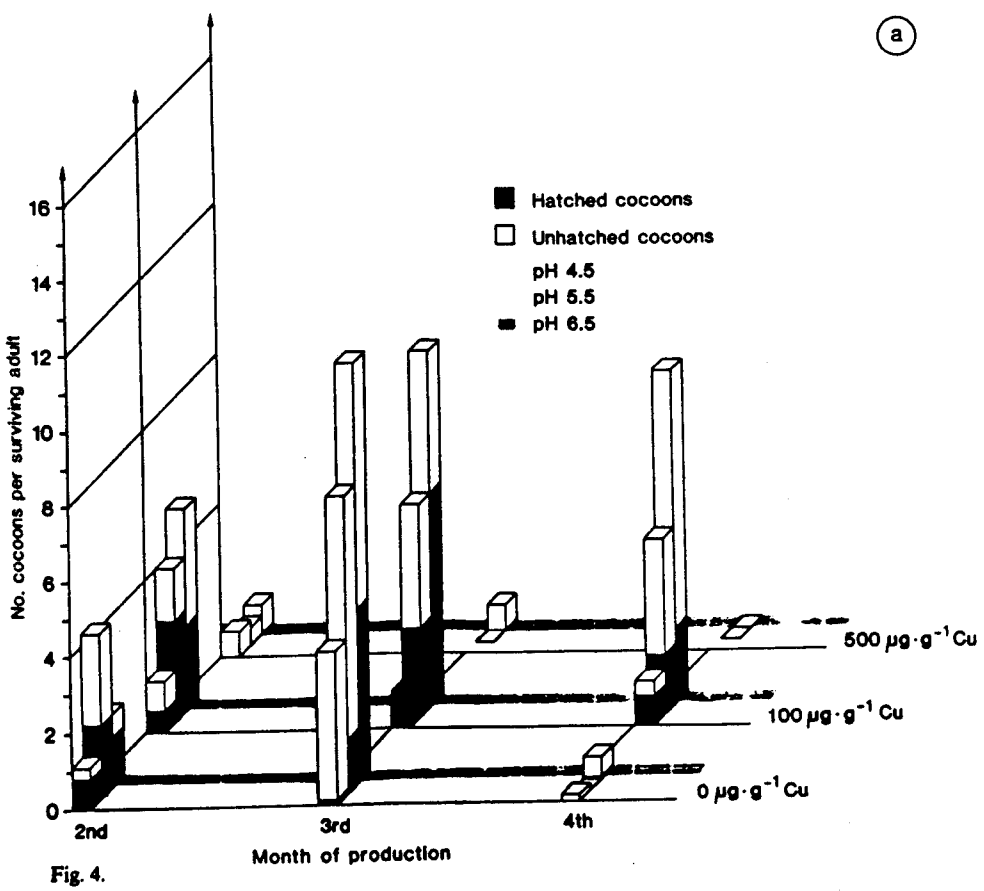


Fig. 4.

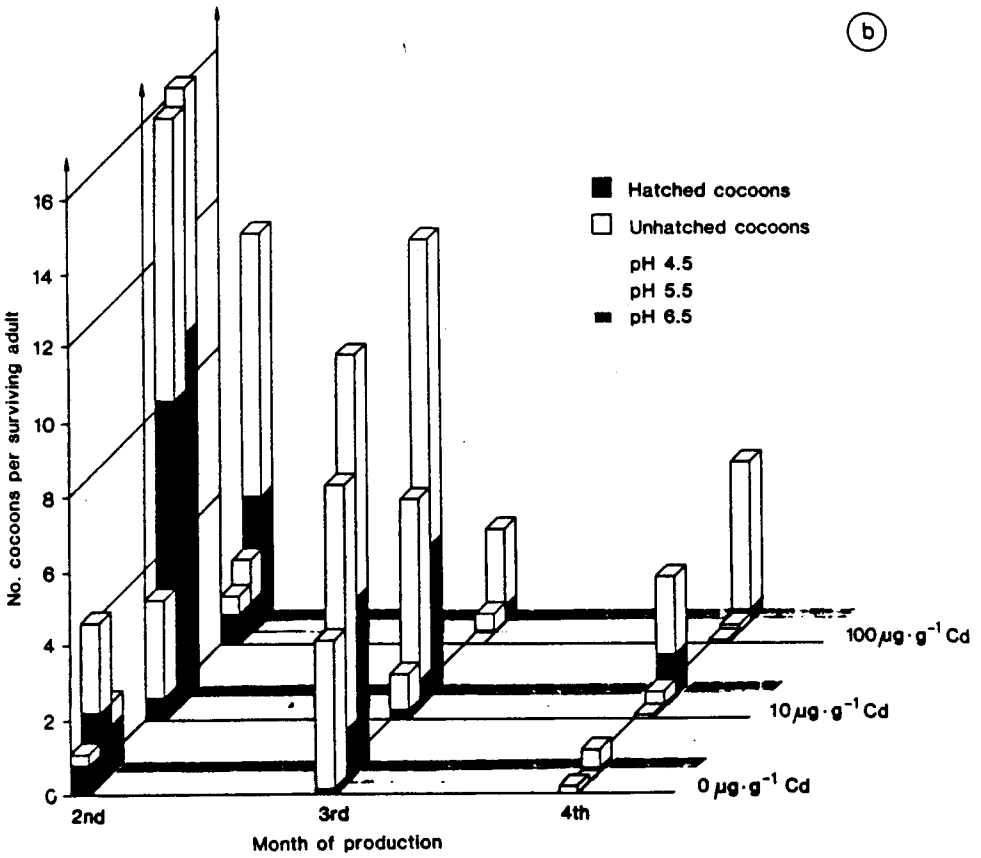


Fig. 4 cont.

distributed over the time studied. Cadmium seemed to force the worms to advance their production and 50% or more of the cocoons were deposited during the second month.

3.4.3. *Hatching Success*

Successful hatching increased with higher pH in soils without metals added; few cocoons hatched at pH 4.5 (Table III). Metals added to pH 4.5 soils affected hatching differently. Generally, hatching success improved but some of the high values could perhaps partly be explained by few observations. Hatching failed entirely at 500 $\mu g\, g^{-1}$ Cu or Pb, pH 4.5. Hatching was significantly better in soils of pH 5.5 when 100 $\mu g\, g^{-1}$ Cu or 10 $\mu g\, g^{-1}$ Cd had been added compared with unpolluted soi ($p < 0.05$). Except for 500 $\mu g\, g^{-1}$ Cu, the metal concentrations of pH 6.5 soils did no change the hatching success appreciably.

Cocoons deposited in the unpolluted soils at the earliest phase of the rearing perioc had the best chances for successful hatching , while those harvested the last month (presumably at the end of the reproductive period) hatched less successfully (Figure 3)

Cocoons deposited during the second month always produce more hatchlings pe hatched cocoon regardless of soil acidity or metal content than those deposited late

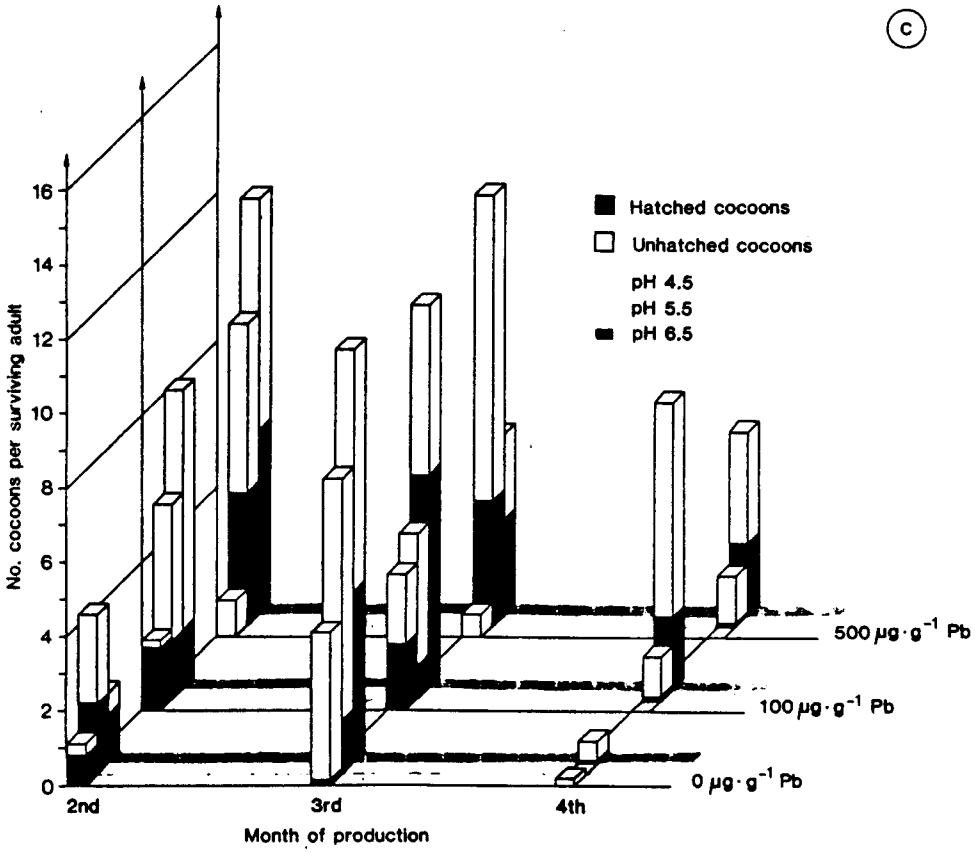


Fig. 4. Number of cocoons deposited by *Dendrobaena rubida* during the second, third and fourth month of rearing in soils of different pH and concentration of Cu (a), Cd (b), and Pb (c). The proportions of hatched and unhatched cocoons are shown in the bars.

TABLE III

Percentage of successfully hatched cocoons and, within brackets, number of cocoons produced in soils at different pH and metal concentrations.

Metal	pH 4.5		5.5		6.5	
$\mu g\ g^{-1}$	%	*n*	%	*n*	%	*n*
0 M⁺	18.2	(77)	25.2	(250)	43.5	(299)
100 Cu	55.6	(9)	47.3	(184)	43.9	(408)
500 Cu	0	(2)	0	(1)	0	(6)
10 Cd	19.3	(57)	40.8	(488)	47.1	(696)
100 Cd	56.0	(25)	31.6	(57)	23.0	(352)
100 Pb	84.2	(19)	23.2	(168)	35.9	(434)
500 Pb	0	(9)	33.3	(339)	44.7	(443)

TABLE IV

Number of hatchlings per hatched cocoon of *D. rubida* and total number of hatchlings at different soil treatments.

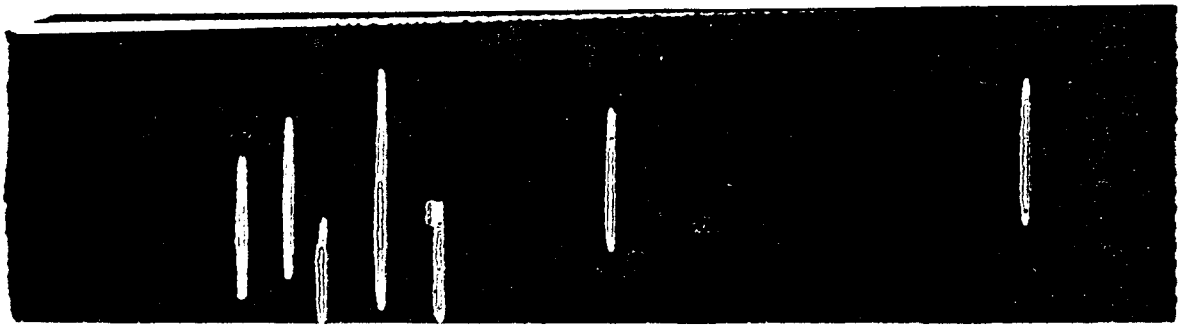
Metal added $\mu g\, g^{-1}$	Soil pH	Month of production				No. of hatched cocoons	Total no. of hatchlings
		2	3	4	$\bar{x}$		
0	4.5	1.33	1.00	–	1.29	14	19
	5.5	1.11	1.04	–	1.08	63	68
	6.5	1.04	1.02	1.02	1.02	130	133
100 Cu	4.5	1.00	1.00	1.00	1.00	5	5
	5.5	1.25	1.16	1.00	1.17	87	102
	6.5	1.24	1.20	1.09	1.20	179	214
500 Cu	4.5	–	–	–	–	0	–
	5.5	–	–	–	–	0	–
	6.5	–	–	–	–	0	–
10 Cd	4.5	1.44	1.00	–	1.36	11	15
	5.5	1.13	1.12	1.00	1.13	199	224
	6.5	1.16	1.04	1.00	1.12	328	366
100 Cd	4.5	1.57	–	–	1.57	14	24
	5.5	1.00	1.00	–	1.00	18	18
	6.5	1.16	1.11	1.00	1.15	81	93
100 Pb	4.5	1.07	1.00	–	1.06	16	17
	5.5	1.42	1.25	1.00	1.36	39	53
	6.5	1.15	1.12	1.00	1.11	156	173
500 Pb	4.5	–	–	–	–	0	–
	5.5	1.23	1.13	1.00	1.18	113	133
	6.5	1.07	1.04	1.08	1.06	198	210

($p < 0.05$) (Table IV). The later the cocoon was deposited, the fewer hatchlings emerged from each hatched cocoon; in 9 out of 11 cases the ratio of hatchlings to hatched cocoon was equal to 1.00. More hatchlings emerged per cocoon at pH 4.5 than at higher pH values both in 0 $\mu g\, g^{-1}$ M^+ soils ($p < 0.05$) and in Cd supplied soils ($p < 0.05$ at 10 $\mu g\, g^{-1}$ Cd; $p < 0.01$ at 100 $\mu g\, g^{-1}$ Cd), whereas the highest numbers of hatchlings per hatched cocoon were obtained at higher pH levels in Cu or Pb treated soils.

The total production of hatchlings was more affected by low pH than by metals added ($p < 0.05$) (Table IV). A minor surplus of Cd in the pH 5.5 and 6.5 soils did, however, increase the total output of hatchlings by 2 to 3 times.

3.4.4. *Embryonic Development*

The fate of each cocoon was followed for 40 weeks and hatching time was recorded. Hatching at pH 5.5, 0 $\mu g\, g^{-1}$ M^+ fell within the first 12 weeks of incubation, mostly (53%) during the second month.



Metals caused a longer embryonic developmental time. Cocoons deposited at pH 5.5, 0 $\mu g\ g^{-1}\ M^+$ had always on average a shorter developmental time than those deposited in pH 5.5 soils supplied with metals. The embryonic development was significantly longer for 100 $\mu g\ g^{-1}$ Cu, 10 $\mu g\ g^{-1}$ Cd and 500 $\mu g\ g^{-1}$ Pb soils ($p < 0.001$ for Cu and Pb, and $p < 0.01$ for Cd, Student's t-test). A prolonged developmental time was even more obvious when metals had been supplied to pH 6.5 soils; all embryos in cocoons deposited in metal polluted soils, but 100 $\mu g\ g^{-1}$ Cd, needed a significantly longer time to develop ($p < 0.001$).

Metals also tended to cause a less synchronized hatching period. For instance, only 24% hatched successfully during the second month when 100 $\mu g\ g^{-1}$ Cu had been added to pH 5.5 soil, which should be compared with 53% for 0 $\mu g\ g^{-1}\ M^+$. The less synchronized hatching period was traced in the significantly higher variance in developmental time for cocoons produced in soils of 100 $\mu g\ g^{-1}$ Cu (pH 5.5, 6.5), 10 $\mu g\ g^{-1}$ Cd (pH 4.5, 6.5), 100 $\mu g\ g^{-1}$ Cd (pH 6.5), and 100 $\mu g\ g^{-1}$ Pb (pH 6.5) than for those deposited at pH 5.5, 0 $\mu g\ g^{-1}\ M^+$ ($p < 0.05$, one-way ANOVA). For instance, successful hatching was still recorded in metal polluted soils after 30 to 34 weeks incubation.

3.4.5. *Volumetric Growth*

Low pH caused a slow volumetric growth and an early mortality of juvenile *D. rubida* (Figure 5). In general, juveniles grew most rapidly in soils of pH 6.5. Juveniles reared at pH 6.5, 100 $\mu g\ g^{-1}$ Cu displayed the most rapid volumetric growth and reached the size of an adult *D. rubida* within 80 days; a result that emphasizes the importance of Cu for growth of earthworms. 100 $\mu g\ g^{-1}$ Cd or Pb in the soil led to a significantly more rapid growth at pH 6.5 than at pH 6.5, 0 $\mu g\ g^{-1}\ M^+$ in soil ($p < 0.05$).

4. Discussion

Most studies dealing with the effects of pollutants on soil organisms have presented LD_{50} figures but ignored estimating the chances of a population to survive in a habitat subject to pollution at sublethal levels. Obviously, acidification and increased metal concentration of the soil affect a *Dendrobaena rubida* population and the lethal and sublethal effects are manifested in:

(1) lower survival of adults;
(2) reduced numbers of cocoons deposited;
(3) reduced cocoon viability;
(4) changed numbers of hatchlings per cocoon;
(5) less synchronized hatching and a longer time needed for embryonic development; and
(6) reduced volumetric growth of juveniles.

It is evident from our 4 mo study that *D. rubida* survives and grows poorly in soils of low pH, especially when Cu or Pb have polluted the soil. We supplied considerably lower amounts of Cu to the soils than Hartenstein *et al.* (1980) who reported that of all

metals studied (Cd, Cu, Pb, Zn, Ni at different concentrations) only Cu was toxic to *Eisenia foetida* (Sav.), and that more than 2.500 $\mu g\ g^{-1}$ Cu was required for 100% mortality in a 1 week experiment. However, earthworms that live more than 1 week, which most of them do, may in the long run experience some difficulties to survive and reproduce in considerably less polluted environments. Normally, senescence of *D. rubida* (= clitellum regresses) is recognized at the age of 300 to 350 day and at that time the body also starts to loose weight (Satchell, 1967). These characteristics were observed in our experiments only on adults reared in soils of low pH and in all soils supplied with 500 $\mu g\ g^{-1}$ Cu or Pb, whereas adults in the control soil were still in good shape when the experiments were terminated. Thus, a combination of acidification and metal pollution leads to a rapid aging of the short-lived *D. rubida* and a shorter postreproductive period.

The number of cocoons produced per individual in the soils at pH 5.5 and 6.5 without metals added were well above or comparable with those reported elsewhere (Evans and Guild, 1948; Graff, 1953b). Breeding experiments with *Allolobophora caliginosa* (Sav.), briefly reported by van Rhee (1975) have suggested a reduced cocoon production and egg viability in soils supplied with 110 $\mu g\ g^{-1}$ Cu. Our results clearly differ from his findings. Evidently low Cu concentrations (here 100 $\mu g\ g^{-1}$) in the soils are beneficial for the cocoon production of *D. rubida*. The contradictory results may partly be attributed to differences in response between the two species studied, but presumably mostly to differences in the amount of Cu available to the worms in the two experimental soils. That small amounts of available Cu in the medium are necessary for successful reproduction is known from various invertebrate groups. For instance, the springtail *Onychiurus armatus* (Tullb.) reproduced significantly better when the medium contained 45 $\mu g\ g^{-1}$ Cu than 0 $\mu g\ g^{-1}$ Cu (Bengtsson *et al.*, 1985). It must, however, be stressed that a further increase of the total amount of Cu (by adding more Cu ions to the soil) or of the amount of available Cu (by lowering pH) reduces the cocoon production of *D. rubida*.

If Cu is essential for cocoon production, can a shortage be compensated for by Pb? The results shown in Table III and similar studies on egg production in Collembola (Bengtsson *et al.*, 1985) support this assumption. *D. rubida* raised its cocoon production in soils at pH 6.5 and 100 $\mu g\ g^{-1}$ or 500 $\mu g\ g^{-1}$ Pb, and *Onychiurus armatus* produced more eggs when the medium lacked Cu but contained Pb (15 and 45 $\mu g\ g^{-1}$ Pb).

The viability of *D. rubida* cocoons was low even in the unpolluted soil and usually less than 50% of the cocoons hatched successfully (Table III). The mechanisms resolving the loss are unclear, but at least three factors were prominent in our experiments.

(1) The weight of *D. rubida* cocoons declines over the reproductive period and hatchability reduces as the adult worm gets older (Evans and Guild 1948), possibly because fewer eggs or less nutrients are transferred to the cocoon. As mentioned earlier, senescence of worms advanced in soils with low pH, affecting viability of the embryo, as illustrated by declined cocoon hatching over time at pH 4.5 and 5.5, 0 $\mu g\ g^{-1}\ M^{+}$ (Figure 4). Acidified soils apparently are detrimental for hatchability through early aging of the adults.

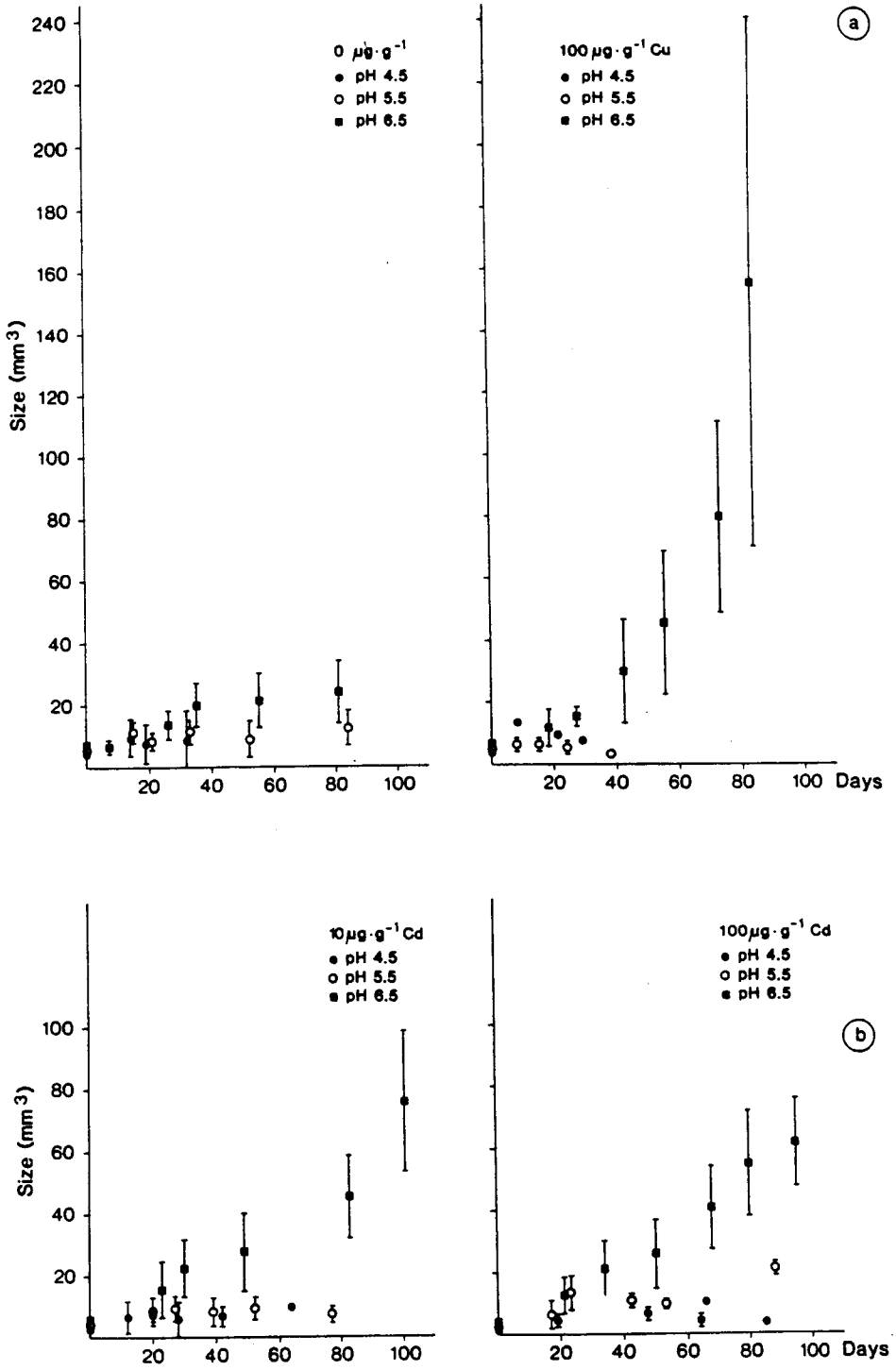
(2) Available Cu at relatively low concentrations was advantageous for cocoon viability and partly counterbalanced the low pH effects. It is obvious that adults transferred Cu to the next generation (Figure 1) even when the extractable Cu concentration was less than 0.5 $\mu g\ g^{-1}$ (Table I). Cadmium or Pb at 100 $\mu g\ g^{-1}$ in the soil substrate can apparently compensate for a Cu deficit.

(3) *D. rubida* cocoons are subject to infestation by the nematode *Rhabditis reciproca* Sudhaus (Gunnarsson and Rundgren 1985). Its infestation rate was shown to depend on soil pH and metal concentration, and fewer cocoons were infested at low pH or high metal concentrations in the soil. Hence, the reduced hatching success at pH 4.5 with or without metals added was not due to the parasite, which rather contributed to the 50% or higher cocoon mortality at higher soil pH.

According to Evans and Guild (1948), an average of 1.43 hatchlings emerge per hatched cocoon of *D. rubida* ssp *subrubicunda* – a figure that was only recorded in this study for cocoons produced in the early reproductive phase when Cd or Pb were added to pH 4.5 or 5.5 soils (Table IV). Temperature and parental density (more viable eggs per cocoon are produced at low densities) affect the number of hatchlings per cocoon (Tomlin and Miller, 1980). Since the worms were incubated at constant temperature and parental density was highest at the start of the experiment, other factors may have influenced the number of eggs hatched. Evidently, a lowered pH and available Cd and Pb concentrations slightly elevated above background levels tend to constrain reproductive worms to produce high quality cocoons soon after exposure to pollutants, and the increased numbers of hatchlings emerging per cocoon may compensate for the severe physiological stress. Although this compensation may only peripherally contribute to hatching success at low soil pH, it is clearly beneficial in neutral soils with a certain metal increase. When 10 $\mu g\ g^{-1}$ Cd, 100 $\mu g\ g^{-1}$ Cu, 100 $\mu g\ g^{-1}$ Pb, or 500 $\mu g\ g^{-1}$ Pb were added to soils at pH 6.5, the hatching success expressed as number of hatched worms, was better than in the unpolluted soil (Table IV).

The embryonic development in this study took place under ideal conditions; temperature (cf Graff, 1953a) and moisture were optimal, and the development time for cocoons produced in pH 6.5 soils was in accordance with earlier findings (Evans and Guild, 1948). Whereas a lower soil pH shortened and concentrated the development time at this temperature, metals delayed and dispersed it. A shorter development time may be advantageous for *D. rubida* in the natural environment, where most hatching is concentrated to autumn and ceases when the soil temperature falls (Rundgren, 1977). A long embryonic development under favorable laboratory conditions may be fatal for the cocoons in a metal polluted soil in the field when the temperature drops. Hatchlings emerging in late autumn may suffer from a very short feeding period which may reduce their chances to survive the winter.

The reduced earthworm activity found in acidified soils (cf. Laverack, 1961) may include low feeding activity as well and results in slow growth of juveniles at pH 4.5 and 5.5 (Figure 5). Why the worms grow faster in presence of Cu is not known, but this Cu effect has been observed in other studies of soil invertebrates. The values given by Neuhauser *et al.*, (1984) indicate a better mass gain in the earthworm *E. foetida* (Sav.)


a
0 μg·g⁻¹
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• pH 4.5
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▪ pH 6.5
Size (mm³)
Days
b
10μg·g⁻¹ Cd
• pH 4.5
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Size (mm³)
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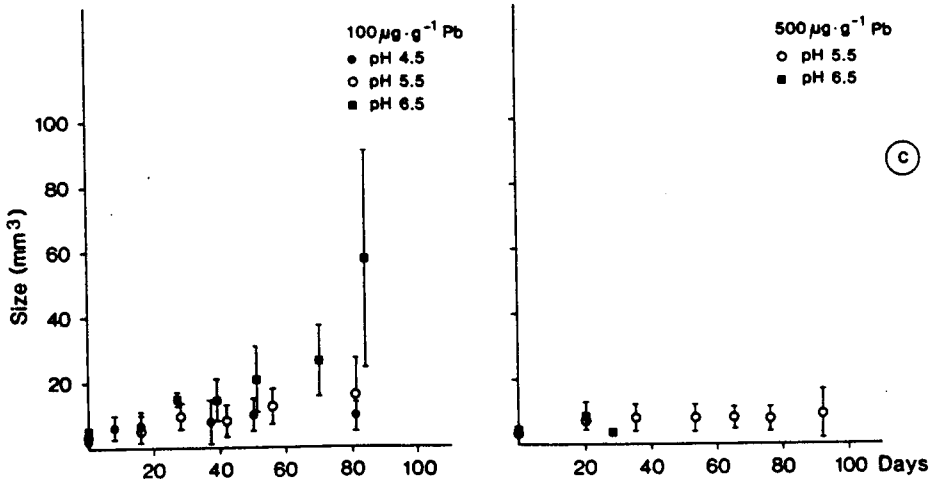

Fig. 5. Volumetric growth of *Dendrobaena rubida* reared for 3 mo in soils of different pH and concentrations of Cu (a), Cd (b), and Pb (c). Mean and standard deviation are shown.

reared in manure supplied with Cu (500 $\mu g\, g^{-1}$ Cu) than in a control soil, and the springtail *O. armatus* grows better when Cu is present in its food (Bengtsson *et al.*, 1983a).

Cadmium is obviously rapidly taken up by *D. rubida* (Table II); the Cd concentration of muscle tissues of adult worms increased 15- to 30-fold within 3 mo. The data presented for metals in *D. rubida* support the general statements in the introduction, namely (1) Pb and Cu are less mobile than Cd in the soil, (2) soil acidity differently influences sorption and solubility of different metals, and (3) the metal availability increases when pH is lowered. However, the concentration factor for metals dropped when the total metal contents of the soil increased (cf Ma, 1982) and the increased availability of Cd and Pb at lower pH did not result in higher metal levels in muscle tissues. These observations may be biased by the sampling procedure, since specimens at the low range of metal concentrations will survive better in the polluted soils and hence have a higher probability of appearing in the samples.

But could improved excretion ability or lowered feeding activity be responsible for a constant or even declining concentration factor as the soil becomes more polluted? Ireland (1975) suggested that Pb can be excreted by substituting Ca in the normal excretion from the calciferous glands. Andersen and Laursen (1982) emphasized the importance of the waste nodules concentrated to the posterior parts (structures not included in the tissue analyses in Table II) in detoxifying processes and excretion of Pb. Thus, the excretion capacities mentioned could partly explain the differences in accumulation found between Pb and Cd. However, it is uncertain whether earthworms have an excretion system that is more efficient as the metal concentration in the soil increases or the juvenile becomes older. The high soil metal concentrations apparently

affect the activity of the aging worms, suggesting that a lower concentration factor is partly associated with a lowered feeding activity.

The metal analyses showed high concentrations of metals in cerebral ganglion and seminal vesicles (organs that regulate or participate in reproduction), and the coefficients of variation for metal concentrations in seminal vesicles were high (Table III). These variations were related to the highly variable size of seminal vesicles. A worm that has quite recently copulated has emptied its sacs and a senescing adult may most likely produce less seminal and prostatic fluids. Emptied seminal vesicles were not analysed, but the analysed material consisted of sacs of varying state. Nevertheless, concentrations of Cu and Cd increased over time in seminal vesicles, most rapidly in worms reared in Cu supplied soils of low pH. We were not able to establish whether high Cu concentrations affected cocoon viability, since the observations at pH 4.5, 100 $\mu g\, g^{-1}$ Cu were too few. Cocoon viability was obviously not reduced at pH 5.5, 100 $\mu g\, g^{-1}$ Cu, although the Cu concentration was 500 $\mu g\, g^{-1}$ in cocoons produced after 3 weeks and 950 $\mu g\, g^{-1}$ in those produced after 4 mo. Furthermore, Cu concentrations of 300 to 800 $\mu g\, g^{-1}$ have been found in seminal vesicles of *Dendrobaena octaedra* (Sav.) performing well at sites 1000 to 7800 m from a brass mill in SE Sweden (Bengtsson *et al.*, 1983b). The high concentrations of Cd (400 to 1100 $\mu g\, g^{-1}$ Cd) in the seminal vesicles may, however, contribute to the reduced cocoon viability. Whether Cd was transferred to the sac content by prostatic fluid is unknown, but the presence of Cd lowered the egg viability. The high concentrations of Cd in the cerebral ganglion are noteworthy. *D. rubida* is chiefly amphimictic and a successful cross-mating requires a complicated behavior which could be perturbated by accumulated metals in the nerve tissues.

Proximately, the combination of acidification and metal pollution will reduce *Dendrobaena* populations in the most contamined areas. Some other lumbricid species, such as *A. caliginosa* and *Lumbricus rubellus* Hoffm. that are less tolerant to low pH but sometimes are found in coniferous habitats, will probably suffer hard from acidification and metal pollution, although due to an overall deeper vertical distribution in the soil, may avoid the most devastating effects of the pollution which hits the soil surface.

However, Ireland (1979) has reported extremely high levels of Pb occurring in *L. rubellus* living in a Welsh mining area. *D. rubida* is unambiguously able to survive in soils of low pH (Nordström and Rundgren, 1974). Whether this species, or earthworms in general, can adapt to the combined effects of acidification and metal pollution is uncertain but worthwhile to study.

Acknowledgement

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