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The influence of soil properties and worm-related factors on the concentration of heavy metals in earthworms[1])

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With 2 figures

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1. Introduction

Soil pollution by heavy metals is often studied by analyzing heavy metals present in soil organisms. The metal contents of e.g. earthworms in the polluted biotope are then compared with those in areas with relatively little or no pollution (IRELAND 1975; CZARNOWSKA & JOPKIEWICZ 1978; ASH & LEE 1980; WRIGHT & STRINGER 1980). However, the uptake and accumulation of heavy metals may not depend on the extent of soil contamination alone but also on a number of soil and worm-related variables. Species differences in metal accumulation have been reported (IRELAND 1979; ASH & LEE 1980) in addition to seasonal influences (IRELAND & WOOTTON 1976; ANDERSEN 1979). There is, however, no information available on the influence of soil factors on bio-accumulation of heavy metals in earthworms.

The purpose of the present study was to investigate the role of some physico-chemical soil properties in affecting the availability of heavy metals to earthworms and to study the tolerance of these organisms to the toxic action of some of these metals. This was done by exposing earthworms to contaminated soil under laboratory conditions and by studying earthworms in different types of agricultural soils which had been treated with municipal waste compost.

2. Materials and methods

For the laboratory experiments a sandy loam soil was used with 17% clay, 8% organic matter, *p*H (KCl) of 7.3 and a $CaCO_3$ content of 5.5%. The soil was artificially contaminated by thoroughly mixing with the chloride salt of either cadmium, copper, lead or nickel at concentrations between 150 and 3,000 mg kg^{-1} dry soil. During the experiments the soil moisture content was maintained at 35—40%. Quantities of 5 litres of treated soil were kept in fine-meshed nylon netbags, embedded

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

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in uncontaminated soil in large containers. Groups of five adult *Lumbricus rubellus* of a uniform live weight of about 700 mg (with gut load voided) were introduced into each bag. Air-dried alder leaves were added to the soil surface as food. Each metal concentration was tested in five or six replicates. The containers were kept for 6 or 12 weeks in a controlled environment room at 15 ± 1 °C and 85—90% relative air humidity.

In the field study, earthworms were sampled by digging and handsorting of the top 20 cm layer of a field plot with six different types of agricultural soils, which had been annually treated with municipal waste compost over the past decade (De Haan 1981). Each soil type was divided by concrete slabs into sub-plots of 1×1×1 m, with the original profile of each soil type present to a depth of one metre. Treatment was by mixing the compost into the top 20 cm at rates of 0, 20, and 40 t ha^{-1} y^{-1}; each treatment was represented in four replicates. At the time of sampling the plots were sown with perennial ryegrass.

The six soil types comprised three loamy soils and three sandy soils. These consisted of a marine clay loam with 30% clay ("soil A"), a sandy loam with 10% clay ("soil B"), a riverine clay loam with 40% clay ("soil C"), a peaty sand with at least 10% humus in the top layer ("soil D"), a reclaimed sandy podzolized soil with about 7% humus in the top layer ("soil E"), and a Plaggen soil with about 3% humus ("soil F"). Soil samples were collected from the top 20 cm, air-dried and passed through a 2 mm sieve before analysis. Cation exchange capacity was measured according to Chapman (1965), organic matter was determined from loss-on ignition at 600 °C, and *p*H was measured in 1 N KCl. Results of these measurements are shown in Table 1.

Cu, Fe, Zn, Cr and Mn in soil were determined by destruction in nitric acid/sulphuric acid and Cd, Pb, and Ni by extraction with conc. HCl. Earthworm samples consisting of five to seven specimens with guts voided were dry-ashed in a low-temperature plasma-ashing console (IPC) and the ash dissolved in conc. HCl. The dry weights of adult *Allolobophora caliginosa* (guts voided) varied between 60—130 mg and of sub-adults between 50—100 mg; *Lumbricus rubellus* adults had a dry weight of about 100 mg. Metal contents in soil and worm extracts were measured by atomic absorption spectrophotometry. Cd, Pb and Ni were measured with deuterium arc back-ground correction. Extracts used for the estimation of Mn contained lanthanum at 2,000 mg kg^{-1}. Cr was measured in the presence of 0.4% KCl.

3. Results

3.1. Laboratory observations on dose-response relationships

Table 2 presents data on the percentage mortality observed among adult *Lumbricus rubellus* after exposure for 6 and 12 weeks to sandy loam soil contaminated with either Cd, Pu, Pb or Ni. An estimate of the relative toxicity of the various metals was obtained by comparison of the LC-50, the concentration that causes 50% mortality of the test animals.

Table 1. Characteristics of six soil types (A—F) treated with municipal waste compost

Loamy soils				Sandy soils			
Soil	CEC	OM	*p*H	Soil	CEC	OM	*p*H
A1	26.3	5.8	7.1	D1	20.5	12.4	4.7
2	24.5	6.7	7.0	2	19.2	11.2	5.2
3	25.1	8.4	6.9	3	18.3	13.6	5.8
B1	9.4	2.8	6.6	E1	13.5	6.4	5.4
2	10.5	4.0	7.0	2	12.7	7.4	5.4
3	12.3	4.9	7.0	3	23.2	8.1	5.7
C1	26.4	6.9	5.3	F1	5.3	2.8	4.8
2	28.7	9.2	5.8	2	6.1	3.7	5.5
3	28.7	9.7	5.9	3	7.1	4.3	6.0

Note: 1 = untreated control; 2 = 20 t ha^{-1} y^{-1}; 3 = 40 t ha^{-1} y^{-1}. CEC in meq. 100 g^{-1}; organic matter (OM) in g 100 g^{-1}. For a description of soil types, see text.

Table 2. Toxicity of Cd, Cu, Pb and Ni in a sandy loam soil to adult *Lumbricus rubellus* (N = 25—30)

Dosage (mg kg^{-1} dry wt soil)	% Mortality							
	Cadmium		Copper		Lead		Nickel	
	week 6	week 12	week 6	week 12	week 6	week 12	week 6	week 12
Control[1])	4	12	4	12	3	13	3	13
20	0	12	0	8	7	30	10	10
150	3	3	0	8	0	13	7	23
1,000	100	100	52	100	0	17	13	40
3,000	100	100	100	100	7	20	73	100

[1]) The uncontaminated control soil contained 0.5 mg Cd kg^{-1}, 12 mg Cu kg^{-1}, 26 mg Pb kg^{-1} and 17 mg Ni kg^{-1}.

Table 3. Changes in weight of adult *L. rubellus* after 6 and 12 weeks exposure to Cd, Cu, Pb or Ni a in sandy loam soil

Dosage (mg kg^{-1} dry wt soil)	% weight change over 6 or 12 week exposure							
	Cadmium		Copper		Lead		Nickel	
	week 6	week 12	week 6	week 12	week 6	week 12	week 6	week 12
Control	+25	+16	+25	+16	+21	+12	+16	+21
20	+11	+ 6	+15	+10	+17	+16	+15	+10
150	+20	+23	+22	+14	+20	+16	+ 3	+ 5
1,000	—	—	—32	—	+17	+14	— 5	—22
3,000	—	—	—	—	— 7	—29	— 9	—

The 6 week LC-50 of Cd-contaminated soil was between 150 and 1,000 mg kg^{-1} and that of Cu-contaminated soil was 1,000 mg · kg^{-1}. Soil contaminated with Ni showed a 6 week LC-50 of 2,000—2,500 mg kg^{-1} and of more than 3,000 mg kg^{-1} when contaminated with Pb. According to their 6 week LC-50, the metals thus followed the order of increasing toxicity as follows: Pb < Ni < Cu < Cd. This order remained valid when the duration of exposure was extended to a 12 weeks period.

The worms were especially tolerant to Pb, which even at concentrations in soil as high as 3,000 mg kg^{-1} did not induce any mortality during 6 weeks exposure. At 12 weeks of exposure, a slight increase in mortality was observed (Table 2) in addition to a reduced body weight (Table 3). The presence of empty gut contents indicated an inhibited food intake activity among the test animals. In soil contaminated with Pb and Ni, the increase in mortality rate after 6 weeks exposure was preceded by a reduction in body weight (Tables 2 and 3).

Fig. 1 shows that the accumulated amount of metal in the worms increased with increasing extent of soil contamination according to dose-response relationships characteristic for each metal. The ratio of the concentration of a specific metal element in the worm to that in the soil ("concentration factor") was not a constant but varied considerably with the level of soil contamination. Generally, the concentration factor tended to decline with increasing levels of soil contamination. It only remained constant with Pb and Ni at soil concentrations in a range between about 100 and 1,000 mg kg^{-1}, and for Pb tended to increase at soil concentrations greater than about 1,000 mg kg^{-1}. According to the dose--response relationships shown in Fig. 1, the various metals were accumulated in the following order of increasing efficiency: Ni < Pb ≦ Cu < Cd. The concentration of cadmium was consistently greater in worms than in soil at all soil concentrations investigated.

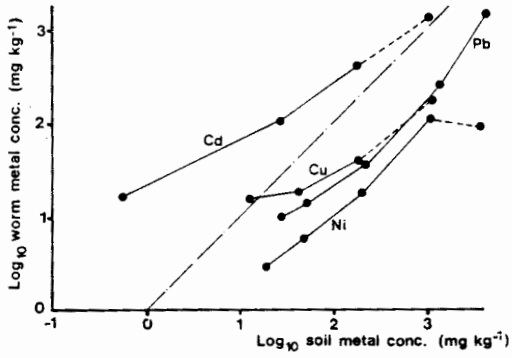


Fig. 1. Metal concentration in adult *L. rubellus* after 12 weeks (———) or 6 weeks (------) exposure to sandy loam soil with added metal. The straight broken line indicates a hypothetical 1:1 relationship.

Table 4. Average total number per m² of earthworms in six soil types and three compost levels; with analysis of variance

Compost level ($t\ ha^{-1}\ y^{-1}$)	Soil type					
	A	B	C	D	E	F
0	36.3	32.8	25.5	21.8	20.3	12.8
20	25.9	30.5	24.5	17.0	22.0	14.3
40	34.8	23.8	25.8	22.5	10.3	20.0

Source of variation	df	SS	MS	F
Soil type	5	2,882	576	4.76***
Compost	2	63	31.5	0.26
Soil type x compost	10	727	72.7	0.60
Error	54	6,520	121	
Total	71	10,192		

*** $P < 0.005$.

Table 5. Distribution of earthworm species as percentage of total numbers per soil type and compost level

Soil type	Compost level ($t\ ha^{-1}\ y^{-1}$)	Earthworm species (%)						
		(1)	(2)	(3)	(4)	(5)	(6)	(7)
A	0	92	0	3	0	2	4	0
	20	86	5	4	0	1	3	0
	40	76	4	11	0	1	9	0
B	0	73	0	2	0	0	27	0
	20	75	0	0	0	1	24	1
	40	58	1	1	0	1	38	1
C	0	87	0	0	0	1	12	0
	20	80	0	5	1	1	14	0
	40	90	0	0	0	0	9	1
D	0	70	1	1	0	2	10	15
	20	87	0	3	0	0	4	6
	40	74	1	0	0	2	9	13
E	0	95	1	0	0	1	3	0
	20	98	0	0	0	0	1	1
	40	95	0	0	0	2	2	0
F	0	92	0	0	0	2	6	0
	20	98	0	0	0	0	2	0
	40	70	14	0	6	4	6	0

Note: (1) *Allolobophora caliginosa*; (2) *A. chlorotica*; (3) *A. rosea*; (4) *A. longa*; (5) *Lumbricus terrestris*; (6) *L. rubellus*; (7) *Dendrobaena rubida*.

3.2. Effect of soil type and compost treatment: field observations

3.2.1. Species composition and worm numbers

Table 4 shows the average total number of earthworms sampled from four replicate plots. In sandy soils (soil D—F) the numbers tended to be lower than in loamy soils (soil A—C). Two-way analysis of variance showed that annual treatment with municipal waste compost did not exert a significant positive or negative influence on earthworm numbers, but that soil type appeared to be a significant determining factor. As shown by the lack of an interacting effect, soil type affected earthworm numbers independent of compost treatment.

Table 5 presents the species composition of the earthworm populations sampled from the field plot with different soil types treated with municipal waste compost. Among a total of seven species found *Allolobophora caliginosa* was clearly dominant in all soils. *L. rubellus* was present as a second dominant species notably in the sandy loam soil (soil B). *Dendrobaena rubida* specifically occurred in the peaty sandy soil (soil D), which agreed with the life-habit of this species as a feeder on litter in woodlands.

3.2.2. Metal concentration in adults and sub-adults

The presence of heavy metals was studied in *A. caliginosa*, which, for chemical analysis, was present in sufficient numbers in all soils. Worm samples were distinguished in clitellated adults and in sub-adults which are characterized by the possession of tubercula pubertatis but which have the clitellum still undeveloped. Fig. 2 shows that the subadult worms generally had lower body concentrations of Cd, Zn and Pb than the adult worms sampled from the same soil. When considered over all soils, the average metal content of sub-adults was 21% lower for Cd, 18% for Zn and 25% for Pb. These differences were significant ($P < 0.001$) when tested by two-tailed Wilcoxon's signed-ranks test. No significant differences between adult and sub-adult worms were found with regard to the body concentration of either Cu, Ni, Fe, Mn or Cr. For these metals, the data on body concentration in adults and sub-adults were pooled.

3.3.3. Soil factors affecting metal accumulation in worms

Fig. 2a and b show that the body burden of Cd and Zn tended to be greater in *A. caliginosa* in sandy soils in loamy soils than, although the soil concentration of Cd and Zn was higher in the latter. Also Pb was clearly better accumulated by worms in sandy than in loamy soils. In addition to soil type, the rate of compost application also had a significant influence on the body metal content of *A. caliginosa*. In general the body metal content increased with increasing rates of compost application, but in some cases the reverse was true. In the Plaggen soil (soil F), for instance, the Cd content of the worms tended to decrease rather than increase with increasing rates of compost application. Annual treatment with municipal waste compost caused an increase in organic matter and cation exchange capacity, and also raised the *p*H of the soil, although the variation in these soil parameters remained smaller within than between soil types (Table 1).

As a consequence of the combined influence of soil type and compost application the concentration factor of the various metal elements showed a considerable variability (Table 6). The concentration factor generally tended to be larger in sandy than in loamy soils and to decline with increasing rate of compost application. The difference between sandy and loamy soils was greatest for Cd and Zn, but also considerable for Pb, Ni, Fe and Cu. Only Cr behaved similarly in all soils, but this metal element was hardly accumulated at all by the worms. Among the various metal elements, Cd and Zn were the only two metals consistently showing an enrichment in the worms. Both metals had concentration factors which were several times greater than unity in all soils. The concentration factor of Ni, Fe, Mn and Cr never exceeded unity. These metals were accumulated more weakly than Cu or Pb. In order of increasing concentration factor the general sequence in all soils was as follows: $Cr < Mn \leqq Fe < Ni < Pb \leqq Cu < Zn < Cd$.

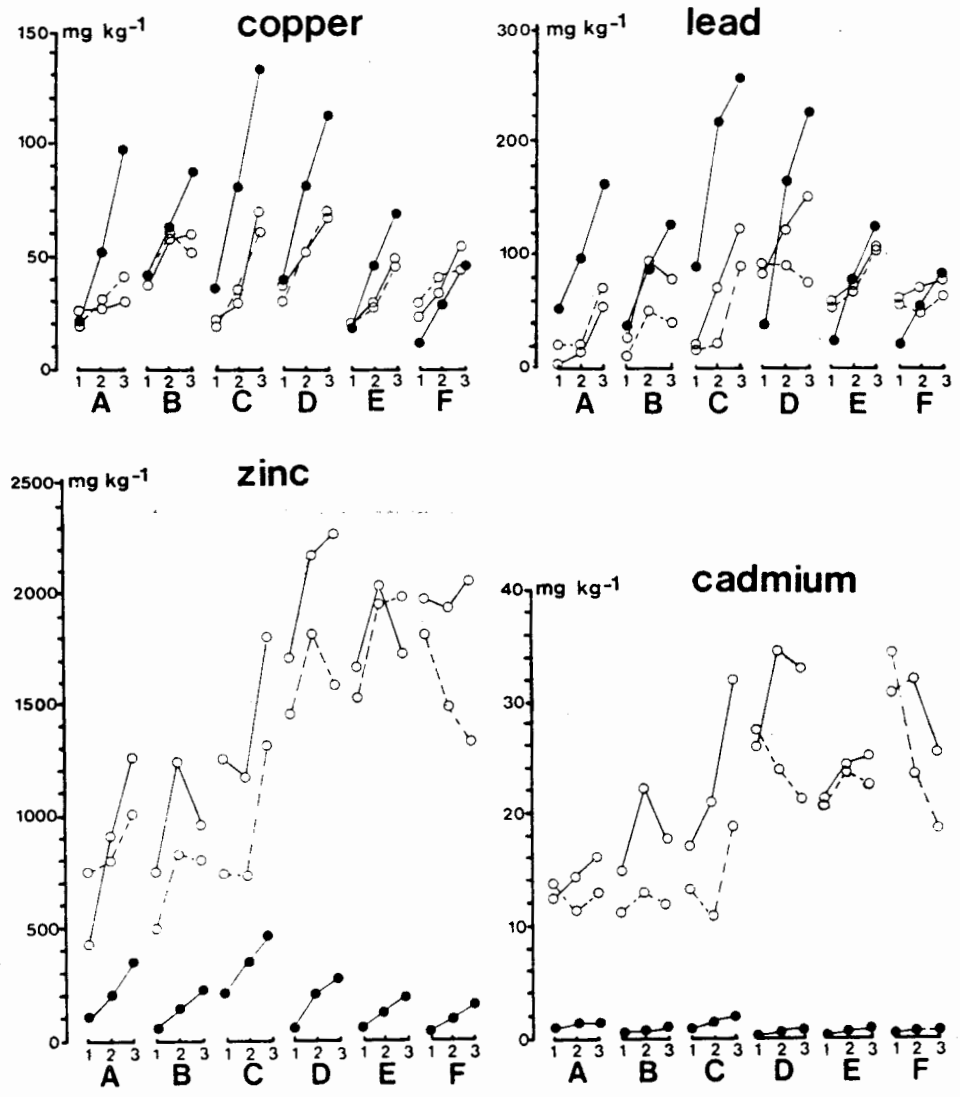
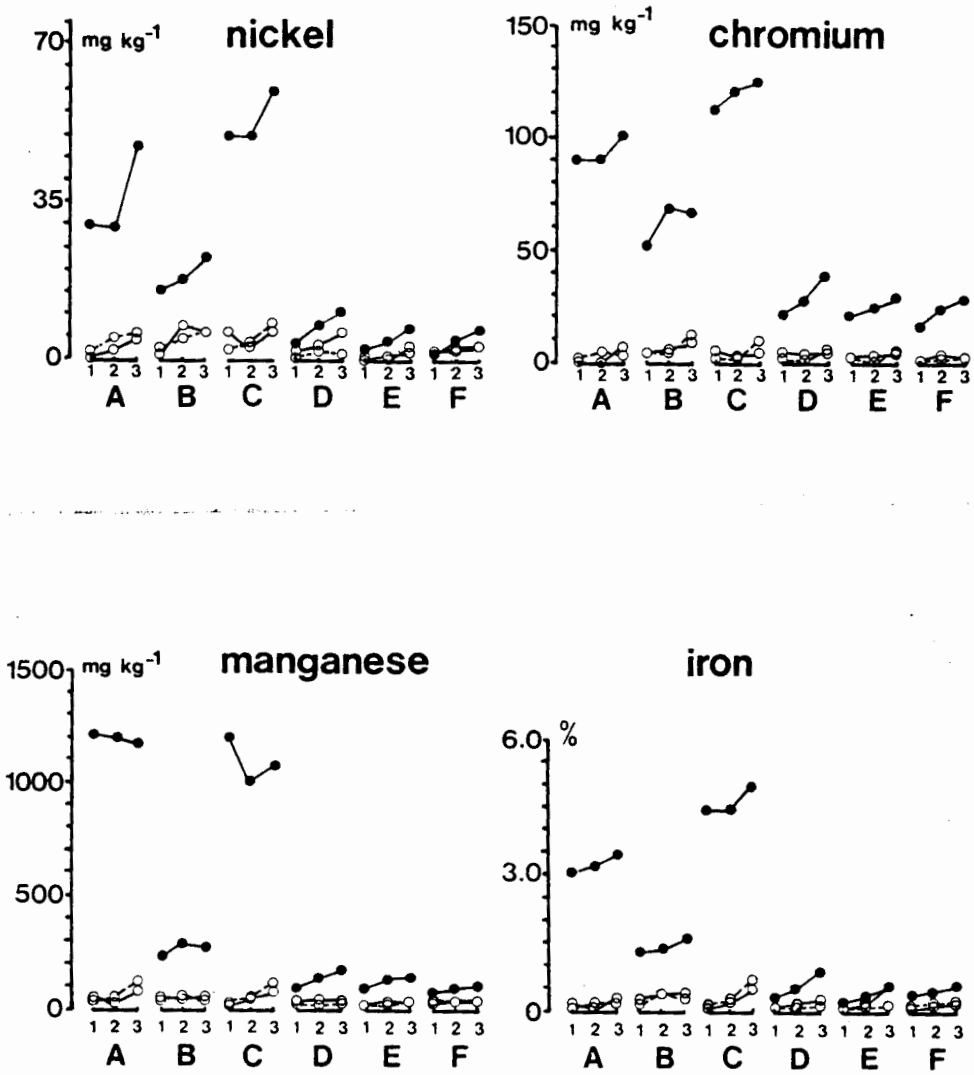


Fig. 2. Metal concentration in *A. caliginosa* (○) and in different soils (●) treated with municipal waste compost. Fe in g · 100 g^{-1} dry wt. Adult (———); sub-adult (------). 1 = untreated soil; 2 = 20 t ha^{-1} y^{-1}; 3 = 40 t ha^{-1} y^{-1} of compost.

The concentration factor of certain metal elements were significantly related to soil characteristics such as organic matter content, *p*H and cation exchange capacity (CEC) of the soil. CEC equals the total amount of cations exchangeably adsorbed by the exchange complex. Simple correlation coefficients determined for the linear regression between concentration factor and soil characteristics are shown in Table 7. Significant negative correlations were found between the concentration factor and the *p*H of the soil for the metals Cd, Zn and Pb. For Ni the correlation coefficient was almost significant ($0.05 < P < 0.1$). The concentration factor of all metal elements, except Cr, showed a significant negative correlation with the cation exchange capacity of the soil, while for Cu an additional negative correlation was found with soil organic matter.



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The relationship between the metal content in worms and soil was investigated for all soils through stepwise multiple linear regression analysis with CEC and soil pH as second and third independent variables. Table 8 shows the proportion of the total variance in body metal content accounted for when one or more variables were included into the regression model. The rate of Cd or Zn addition accounted for little of the variance of worm Cd or worm Zn, even when including CEC into the regression equation. Soil pH had the most significant influence on the concentration of Cd or Zn in the worms. The best-fit equation for worm Zn (mg kg^{-1}) was found to be as follows: worm Zn = 4,918 + 2.74 soil Zn — 40 2 CEC — 536 pH, which accounted for 72% of the variance in worm Zn. The best-fit equation for worm Pb was: worm Pb = 165 + 0.54 soil Pb — 2.64 CEC — 17.9 pH, which accounted for 68% of the variance in worm Pb concentration. These equations indicated that at a given soil concentration of Zn or Pb the concentration of these metals in the worms is elevated as soil pH and CEC decrease.

Table 6. Concentration factors (ratio of worm metal concentration in mg kg^{-1} to soil metal concentration in mg kg^{-1}) for heavy metals in six different soil types amended with municipal waste compost

Soil	Cd(a)	Cd(s)	Cr	Cu	Fe	Mn	Ni	Pb(a)	Pb(s)	Zn(a)	Zn(s)
A1	12.3	13.9	.01	1.15	.05	.04	.05	.00	.22	4.0	7.1
2	10.7	8.0	.03	.57	.05	.04	.11	.16	.11	4.4	3.8
3	11.3	8.5	.07	.34	.11	.10	.12	.30	.45	3.6	2.9
B1	26.2	18.6	.11	.96	.17	.19	.20	.73	.21	12.2	8.1
2	30.4	17.6	.10	.94	.27	.23	.38	1.20	.61	8.8	5.9
3	17.5	11.7	.15	.66	.23	.21	.29	.60	.28	4.4	3.6
C1	15.3	12.3	.03	.56	.03	.02	.07	.21	.16	6.0	3.5
2	13.6	6.8	.02	.41	.06	.05	.08	.34	.10	3.4	2.1
3	15.9	8.9	.07	.48	.14	.11	.12	.51	.34	3.8	2.8
D1	112.2	120.0	.08	.90	.22	.23	.34	2.16	2.51	32.0	27.4
2	60.9	42.1	.03	.64	.21	.22	.36	.77	.57	10.9	9.2
3	40.5	26.1	.10	.60	.22	.19	.31	.67	.33	8.4	5.9
E1	79.6	77.0	.09	1.10	.32	.15	.21	2.62	2.72	43.8	40.2
3	42.6	41.8	.03	.62	.30	.15	.29	.93	.85	16.5	15.8
3	30.7	27.7	.13	.69	.22	.22	.40	.83	.85	8.9	10.2
F1	140.0	156.4	.00	2.45	.36	.32	1.86	2.63	2.45	81.9	74.9
2	59.4	43.3	.11	1.37	.38	.39	.55	1.24	.85	23.8	18.3
3	40.5	29.7	.13	1.08	.36	.32	.38	.88	.73	13.3	8.6

Note: 1 = untreated control; 2 = 20 t ha^{-1} y^{-1}; 3 = 40 t ha^{-1} y^{-1}. *A. caliginosa*, adults (a) and sub-adults (s).

Table 7. Simple correlation coefficients for the linear regression between the concentration factor of a metal and either soil organic matter (OM), cation exchange capacity (CEC) or soil *p*H

Metal element	OM	CEC	*p*H
Cd(a)	—.01	—.51*	—.73***
Cd(s)	—.04	—.44	—.70**
Cr	—.13	—.39	+.22
Cu	—.57*	—.66**	—.32
Fe	—.30	—.86***	—.40
Mn	—.30	—.82***	—.34
Ni	—.35	—.59*	—.44
Pb(a)	—.14	—.57*	—.61**
Pb(s)	—.02	—.39	—.63**
Zn(a)	—.28	—.55*	—.60**
Zn(s)	—.27	—.50*	—.58**

Note: (a) adult; (s) sub-adult; other data were pooled for adult and sub-adults. * $P < .05$; ** $P < 0.01$, *** $P < .001$.

Table 8. Proportion (%) of variance in metal concentration in *A. caliginosa* accounted for by variation in soil metal content and soil properties in 18 different soils

Metal	Soil metal content	Soil metal + CEC	Soil metal + CEC + *p*H
Cd	—	—	46
Cr	18	30	33
Cu	55	79	78
Fe	30	34	35
Mn	34	30	41
Ni	29	41	43
Pb	35	56	68
Zn	—	7	72

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Table 9. Data for the partial non-linear regression between concentration factor (y) and either soil *p*H or CEC (x) according to the equation $y = a \cdot e^{bx}$

Metal	x	a	b	r
Cadmium	*p*H	2,722	—0.752	—0.75***
	CEC	87.7	—0.059	—0.63**
Lead	*p*H.	60.3	—0.765	—0.60**
	CEC	2.72	—0.082	—0.66**
Zinc	*p*H	1,150	—0.796	—0.68**
	CEC	40.9	—0.079	—0.70**

*** $P < .001$; ** $P < .01$.

The CEC variable also exerted a negative effect on the level of worm Cu, but a negative *p*H effect could not be observed. Soil *p*H apparently did not contribute to the relationship between the level of worm Cu and soil Cu. Substitution of CEC for organic matter did not alter the percentage variance in worm Cu accounted for. The concentration of soil Cu remained a major single source of the variance in worm Cu, accounting for 55% of the variance in worm Cu. In this respect Cu differed from all other metals. Inclusion of CEC into the regression model increased the proportion of the variance accounted for to 79%, corresponding with a best-fit equation for worm Cu as follows: worm Cu = 31.7 + 0.44 soil Cu — 0.99 CEC. The regression data of all best-fit equations obtained were statistically significant (t-test).

4. Discussion

The body concentration of a metal element in earthworms is determined by the soil concentration present, the intrinsic rate of bio-accumulation, and the tolerance of the organisms to that element. It also depends on the influence of soil factors determining the availability of the metal for uptake by the earthworms. When taking all abiotic conditions constant, each of the various metal elements shows its characteristic dose-response relationship (Fig. 1). These relationships illustrate the fact that the concentration factor may vary considerably with the extent of soil contamination (see also Table 6). Such variability should be considered when comparing data on concentration factors for heavy metals (e.g. WRIGHT & STRINGER 1980).

As judged from overt toxicity effects with regard to growth and survival, the adult worms of *L. rubellus* were most tolerant to Pb, allowing the body concentration of Pb to increase to levels of about 500 mg kg^{-1}. Much higher levels, however, of 3,600 mg kg^{-1} have been reported to occur in *L. rubellus* from a lead mining site in Wales (IRELAND 1979). The possibility that in such highly polluted environments the earthworms have developed an increased tolerance to lead cannot wholly be excluded. It seems likely from the present results that the accumulation of very high body levels of Pb is facilitated by an acid condition of the polluted soil and by the fact that the concentration factor of Pb tends to be greater in highly polluted than in less polluted sites (Fig. 1: Tables 7 and 8).

The field study has indicated that soil *p*H is a prime factor for predicting the uptake and accumulation of Cd, Zn as well as Pb. Lowering of *p*H leads to increased desorption of metal cations due to competition with H^+ ions. Soil *p*H is the most important single factor determining the solubility of zinc in the soil solution (SHUMAN 1980). The general importance of the availability of metal elements rather than their total concentration in soil also appears from the negative correlation between the concentration factor and the cation exchange capacity of the soil. This was significant for all metal elements except Cr, which was almost not taken up (Table 6). The CEC provides an estimate of the capacity of the soil to adsorb heavy metals and, as shown by the present results, gives a measure of the ability of soils to retain these metals against uptake by earthworms.

In sandy soils the effect of CEC may be largely derived from soil organic matter. Soil organic matter was found to be an important factor specifically in the relationship between the level of Cu in worms and soil (Tables 7 and 8). This may be explained from the fact that soil organic matter binds Cu^{2+} more strongly than other trace element cations (THORNTON 1979). It may be envisaged that Cu^{2+} ions are rendered unavailable to earthworms by ion exchange and complexation to soil organic matter. The uptake and accumulation of Cu remained, however, uninfluenced by soil *p*H (Tables 7 and 8). The body content of Cu was largely determined by the concentration of Cu in soil and less by other soil factors (Table 8). For metals other than Zn, Cu and Pb only a relatively low proportion of variance in body metal content could be accounted for (Table 8), suggesting the involvement of other factors not measured here. It is also possible that a greater effect of the measured soil variables would have been observed if the range in soil metal concentrations had been greater.

The laboratory and field observations showed that the various metal elements are accumulated by the worms in the following order of increasing efficiency: Cr < Mn ≦ Fe < Ni < Pb ≦ Cu < Zn < Cd. This order may reflect the relative availability of the metal elements in soil to uptake by earthworms. In clay/loam soils the specific adsorption of metal cations has been reported to follow the affinity order Pb > Cu > Zn > Cd (FARRAH & PICKERING 1977; HARMSEN 1977), which is the reverse of the order at which these metals are accumulated by earthworms.

Cd and Zn are the only two metal elements which are enriched in the worms, showing a concentration factor greater than unity at all CEC and *p*H levels and at all levels of soil metal contamination (Fig. 1; Table 6). The chemical similarity of Cd and Zn is reflected by their strongly correlated concentration factors, with r = 0.92 and 0.93 for adult and sub-adult worms respectively (Table 6). On average, Cd was three times more strongly accumulated than Zn.

For Pb it can be estimated from the relationships shown in Table 9 that the concentration factor increases to values greater than unity when the *p*H decreases to a level below about 5. Over a *p*H range decreasing from 6.5 to 4.5 the concentration factor for both Cd, Pb and Zn would increase about five-fold, it also increases considerably when the CEC of the soil is lower. The occurrence of higher levels of Cd in "mature adults" than in "immature adults" of *L. rubellus* has been reported by CARTER *et al.* (1980). The present study has shown that also adults worms of *A. caliginosa* accumulate more Cd, but also Zn and Pb, per mass unit than sub-adult (Fig. 2a; Table 6). It is clear that such variables, in addition to other biotic and abiotic influences, should be taken into account when body metal contents in earthworms are taken as an index of soil pollution.

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MA, WEI-CHUN, 1982. The influence of soil properties and worm-related factors on the concentration of heavy metals in earthworms. Pedobiologia **24**, 109—119.

The uptake and accumulation of heavy metals by earthworms was studied under uniform laboratory conditions and in a field plot with six agricultural soil types treated with municipal waste compost. Adult *Lumbricus rubellus* used in the laboratory study showed increased body metal concentrations with increasing soil levels of added metal according to relationships characteristic for each metal. The rate at which metals were accumulated by *L. rubellus* and, in the field study, by *Allolobophora caliginosa* followed the order Cr < Mn ≦ Fe < Ni < Pb ≦ Cu < Zn < Cd, with adult worms accumulating more Cd, Zn and Pb per mass unit than sub-adults. The potential levels that can be attained are determined by the metal's toxicity, which, according to the 6 week LC-50, followed the order Pb < Ni < Cu < Cd. Thus, the observation that Pb can attain much higher body levels in earthworms than Cu could be ascribed to intrinsic differences in toxicity rather than to differences in rate of uptake and accumulation.

Municipal waste compost treatment significantly altered soilprope rties and soil metal levels but did not adversely affect the species composition or densities of earthworm populations, soil type remaining the most important determining factor. The ratio of the metal concentration in worms to that in soil (concentration factor) correlated negatively with the cation exchange capacity (CEC) of the soil for both Cd, Cu, Fe, Mn, Ni, Pb and Zn, but not for Cr which was only poorly taken up. This together with the observed selective order of uptake, indicated that the body metal content is at least partly a measure of the availability of metals in soil. This was further substantiated by a negative *p*H effect on the uptake of Cd, Zn and Pb. Multiple comparisons between variables revealed that soil *p*H was more important than CEC in affecting body contents of Cd or Zn, whereas *p*H and CEC were equally important in affecting the body content of Pb. The body content of Cu was affected by CEC but not by *p*H. Soil Cu content remained the most important factor affecting the level of Cu in the worms.

Key words: Heavy metals, toxicity, availability, bio-accumulation, concentration factor, *p*H, cation exchange capacity, organic matter, Cd, Zn, Pb, Cu, Ni, Mn, Fe, Cr.