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Short- and long-term effects of heavy metals on phosphatase activity in soils: An ecological dose-response model approach

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Summary. The aim of this study was to provide manageable data to help establish permissible limits for the pollution of soil by heavy metals. Therefore the short- and long-term effects of heavy metal pollution on phosphatase activity was studied in five different soil types. The results are presented graphically as logistic dose-response curves. It was possible to construct a curve for sand and silty loam soil but it was more difficult to establish a curve for sandy loam and clay soil and nearly impossible (except for Cu) for peat. The toxicity of the various metals can be compared on the basis of mmol values. In clay soils, for Cd, Cr, Cu, and Zn, the 50% effective ecological dose (ED_{50}) values were comparable (approximately 45 mmol kg^{-1}), but the ED_{10} values were very different, at 7.4, 41.4, 15.1, and 0.55, respectively. At the ED_{50} value, toxicity did not decrease with time and, in sandy soils, was approximately 2.6 mmol kg^{-1} dry soil for Cd, Cu, and Zn. In four out of five soils, the Cd toxicity was higher 1.5 years after the addition of heavy metal salts than after 6 weeks. Toxicity was least in the sandy loam, silty loam, and clay soil, and varied in general between 12 and 88 mmol kg^{-1}. In setting limits, the criteria selected (no-effect level, ED_{10} or ED_{50}) determine the concentration and also the toxicity of the sequence. It is suggested that the data presented here could be very useful in helping to set permissible limits for heavy metal soil pollution.

Key words: Heavy metal toxicity – Soil pollution – Phosphatase – Logistic dose-effect curve – Effective ecological dose (ED_{50})

The adverse effects of heavy metal pollution on nutrient mineralization in soil have been known for at least 20 years. The ultimate result of this irreversible pollution, the accumulation of organic matter, due to either Hg pollution, or Cu and Zn, or Cu, Zn and Pb, or Zn, Fe, Pb and Cd, has been documented by Pugh and Williams (1971), Ruhling and Tyler (1973), Nordgren et al. (1983) and Strojan (1978). Effects on soil respiration (Chaney et al. 1978; Doelman and Haanstra 1984), on ammonification and nitrification (Premi and Cornfield 1969), urease activity (Tabatabai 1977; Doelman and Haanstra 1986) and N_2 fixation (Rother et al. 1982) have also been reported. The inhibitory influence of heavy metal pollution on enzymes in the S cycle was recorded by Al-Khafaji and Tabatabai (1979) and in the P cycle by Tyler (1976), Mathur and Sanderson (1980), Juma and Tabatabai (1977), and Mathé and Kovacs (1980).

So far these data, based on soil microbiological research, have contributed little to the setting of permissible limits for pollution of soil by heavy metals. Most research has not started with the aim of soil protection. Further, the biological functioning of soil and the behaviour of heavy metals in it (e.g. availability) is poorly understood. Most of the investigations were carried out on several heavy metals, using only a few concentrations for convenience, and therefore no dose-response trend could be derived. Mathé and Kovacs (1980) and Doelman and Haanstra (1986), in different ways, did provide dose-response curves. Babich et al. (1983) had stressed the relevance of the dose-response curve, which reflects the combined response of numerous populations of soil microbiota and the influence of soil physicochemical properties in the effective dose values. The curves can set limits for soil pollution since they show at what level a certain adverse effect can be expected.

The aim of this study was to provide manageable data on short- and long-term changes in soil phosphatase activity, by measuring the effect of seven concen-

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trations of six heavy metals in five soil types over two periods of time.

We studied the phosphatase enzyme, which influences the release of inorganic P from organically bound P, because data on its sensitivity to heavy metals are limited even though it is a key enzyme in the maintenance of the P cycle in soils (Speir and Ross 1975).

Materials and methods

Samples of five soil types (Table 1), collected from various parts of the Netherlands as described by Doelman and Haanstra (1984), were sieved (mesh 2 mm) immediately after collection and then stores in the dark at 20 °C and at 12%, 16%, 20%, 22%, and 30% moisture. These moisture levels represent 55% – 70% water-holding capacity and are comparable to field-moist soil. To subsamples of each soil type, 55, 150, 400, 1000, 3000, and 8000 mg kg^{-1} of Cd (112.4 g mol^{-1}), Cr (52 g mol^{-1}), Cu (63.5 g mol^{-1}), Ni (57.8 g mol^{-1}), Pb (207.2 g mol^{-1}), and Zn (65.4 g mol^{-1}) was added. For comparison the concentrations were converted to mmol kg^{-1}. Untreated soil served as a control. The heavy metals (as chlorides) were added as finely ground powders. There was no serious acidification effect in any soil (pH changes less than 0.5), except for the highest concentration of Cr in sandy loam, clay, and sandy peat where the pH-KCl decreased from 5.1 to 2.6, from 6.8 to 3.1, and from 4.3 to 2.8, respectively.

The pH-H_2O and pH-KCl were determined in a suspension of an equivalent of 25 g dry soil in 50 ml demineralised water and 50 ml 1 *M* KCl, respectively. Organic matter was determined as loss on ignition (12 h at 600 °C), expressed as a percentage of total weight. Cation exchange capacity (CEC) was determined with ammonium acetate at pH 7.

Phosphatase activity was measured by the method of Tabatabai and Bremner (1969), using *p*-nitrophenylphosphate (p-NPP) as a substrate. Half a gram of soil was incubated with 5 ml 10 mmol·l^{-1} p-NPP without either toluene and buffer for 1 h in an overhead shaker (25 rpm) at 20 °C. The phosphatase activity was measured in triplicate with a blank at 6 weeks ($t = 0$) and 18 months ($t = 1$), after amending the soils with heavy metals, and was expressed as mmol p-NPP formed per kg dry soil per hour.

The values of K_m and V_{max} were determined by measuring the reaction velocity at concentrations of 2.0, 0.5, 0.2, 0.1, 0.05, 0.025, 0.015, and 0.010 mmol·l^{-1} p-NPP and using the Eadie-Hofstee transformation (velocity versus velocity/concentration).

The relationships between the means of the triplicate phosphatase activity measurements as dependent variables and the logarithm of the added concentrations as independent variables were described using a logistic model as described for urease (Doelman and Haanstra 1986). The relationship is expressed as:

$$Y = \frac{c}{1+e^{b(X-a)}} + E$$

where Y is the observed phosphatase activity at the (natural) logarithm of the heavy metal concentration X; c is the calculated initial (uninhibited) enzyme level as determined by the control sample and samples with low concentrations of heavy metals; b is a slope parameter indicating the inhibition rate and is equal to $4.39/(0.1c-0.9c)$, a is the logarithm of the concentration at which the phosphatase activity is half the uninhibited level ($a = 0.5c$); and E is a stochastic error term, describing the deviations of the observations around the model-response curve (Fig. 1). As the logarithm of the heavy metal concentration of the blanks cannot be calculated (ln $0 = -\infty$), it has been replaced by the logarithm of a very small value (e.g. 10^{-3} mg kg^{-1}) where the curve reaches its asymptotic value. This substitution is essential for the curve but not for the heavy metal content. Use of the total heavy metal content of both blanks

Table 1. Physicochemical characteristics, indigenous concentrations of metals and phosphatase levels in five soil types

Soil type	Sand	Sandy loam	Silty loam	Clay	Sandy peat
pH-H_2O	7.0	6.0	7.7	7.5	4.4
pH-KCl	6.7	5.1	7.4	6.8	4.3
Organic matter (%)	1.6	5.7	2.4	3.2	12.8
Clay, <2 µm (%)	2	9	19	60	5
Silt, 2 – 50 µm (%)	5	26	74	0	13
Sand, >50 µm (%)	93	65	7	40	82
CEC[a]	1 – 2	10 – 12	16	30	50 – 55
WHC[b]	23	25	33	34	51
$CaCO_3$ (%)	2.6	0.1	9.6	0.1	0.1
Total N (%)	0.04	0.15	0.10	0.15	0.58
Total P (%)	0.08	0.11	0.13	0.25	0.05
Fe (mg kg^{-1})	3990	2760	26600	33900	4400
Mg (mg kg^{-1})	1380	310	9500	7920	490
Mn (mg kg^{-1})	97	240	820	963	57
Cd (mg kg^{-1})	0.4	0.5	1.0	0.5	0.4
Cr (mg kg^{-1})	4	2	34	76	11
Cu (mg kg^{-1})	4	6.5	22	52	5.5
Ni (mg kg^{-1})	8	2	25	39	4
Pb (mg kg^{-1})	32	13	42	130	26
Zn (mg kg^{-1})	14	17	103	226	38
Phosphatase (t_0)[c]	50	342	79	183	538
Phosphatase (t_1)[c]	50	192	84	164	435

[a] Cation exchange capacity
[b] Water-holding capacity
[c] Mean phosphatase level expressed in mmol h^{-1} kg^{-1} dry soil; t_0, measured after 6 weeks; t_1, measured after 18 months

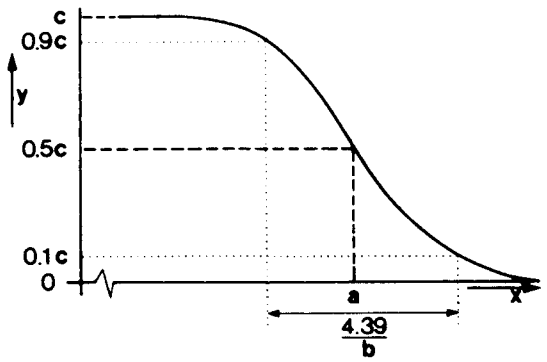

Fig. 1. The logistic response curve with parameters *a*, *b* and *c*

and samples would avoid the need for the substitution but would obscure the calculation of the effect of addition, especially at low concentrations. For the computations we used the iterative least square procedure supplied by the statistical program package GENSTAT (Alvey et al. 1982).

The ED_{50} as discussed by Babich et al. (1983) is easily calculated from parameter *a* by taking its inverse logarithm. The ecological dose range is defined as the dose range in which activity decreases from 90% to 10% of the calculated uninhibited activity. Details of the use of the logistic response model and the ecological dose range can be found elsewhere (Haanstra et al. 1985).

The standard deviation of the difference between the measurements after 6 weeks and after 18 months was calculated as the square root of the sum of the estimated variances of the parameters.

Results and discussion

Since substrate concentration is an important factor in determining the velocity of the enzymatic reaction, we determined the K_m and V_{max} values of the unamended soils (Table 2). As expected, large differences were observed, but the substrate concentration used (10 mmol l^{-1}) was far in excess of the five times K_m recommended by Burns (1978). Calculated K_m values were low for the sand and the silty loam and in the same order of magnitude as the data reported by Tabatabai and Bremner (1971).

Table 1 gives the phosphatase activities of the unamended soils at t_0 and t_1. Only in the sandy loam did the enzyme activity clearly differ (342 versus 192 mmol h^{-1} kg^{-1}) within 18 months of amendment with heavy metals. Harrison (1983) suggested that there is a positive relationship between phosphatase and organic-matter content, probably because the enzymes become bound in the humic-protein complex (Ladd and Butler 1975; Burns 1982; Nannipieri et al. 1988), and are therefore protected against degradation. This may explain why the highest activity was found in the sandy peat soil (organic matter 12.8%). A negative correlation between the clay + silt content of soils and the phosphatase level was reported by McLaren (1975). This may explain the rather high phosphatase activity in the sandy loam.

Table 2. Kinetic values of soil phosphatase in five different soils

Soil	K_m (mmol l^{-1})	V_{max} (mmol kg^{-1} h^{-1})
Sand	0.051 ± 0.007	0.230 ± 0.016
Sandy loam	0.664 ± 0.060	1.700 ± 0.125
Silty loam	0.082 ± 0.019	0.969 ± 0.134
Clay	0.239 ± 0.088	1.582 ± 0.404
Sandy peat	0.351 ± 0.185	4.471 ± 1.630

Table 3 presents the ED_{50} and the ecological dose range, while Fig. 2 gives the effects of the six heavy metals in the various soil types at t_0 (6 weeks after addition) and t_1 (18 months after addition). The coefficient of variation of the ED_{50} values was generally larger 18 months after the addition of the heavy metals than after 6 weeks. Since the number of measuring points was minimal, a large spread was expected.

In setting acceptable limits on pollution the ED_{50} values are often taken as a base. For safety, therefore, a security factor was added (e.g., the ED_{50} value divided by 10 or 1000, depending on the statistical reliability of the value and on whose safety is involved). For the least buffered (CEC = 1 – 2 mEq 100 g^{-1} soil) sandy soil after the 18-month period, these values were lowest for Cd (2.9 mmol kg^{-1}), Cu (2.7) and Zn (2.6). The Ni value was approximately four times higher, and the Cr and Pb values were extremely high at 385 and 381 mmol kg^{-1} (Table 3), respectively. In the sandy loam the ED_{50} values for Cd, Cr, Cu, Ni, and Zn were at least 10 times higher than in the sand, between 29.8 and 137 mmol kg^{-1}, while for Pb no ED_{50} value could be calculated. In the heavier silty loam, after 18 months of incubation, the ED_{50} values were lower than in the sandy loam, with the exception of Zn and Cr (Table 3). For Cd and Cu these values had decreased, after 18 months, from 48.8 and 101 to 2.1 and 11.7 mmol kg^{-1}, respectively. In the clay soil the ED_{50} values for Cd, Cr, Cu and Zn were of similar magnitude, between 43 and 51 mmol kg^{-1} (Table 3). The Ni and Pb values could not be calculated, but after 6 weeks of incubation the values varied between 22 and 111 mmol kg^{-1}. Except for Cu in the sandy peat, no adequate dose-response curve could be obtained.

Taking the sand as an example, the relative toxicity of the heavy metals towards phosphatase, after 18 months of incubation, was Zn > Cu > Cd > Ni > Pb > Cr according to the ED_{50} levels. In sandy loam this sequence was Cu > Zn > Cr > Cd > Ni > Pb and in silty loam, Cd > Cu > Ni > Pb > Cr > Zn. The position of Zn is particularly unexpected in silty loam. In their short-term experiments Juma and Tabatabai (1977) also reported toxicity values of 25 mmol kg^{-1} and an average sequence of Hg > Cd > Cu > Zn > Cr > Ni > Pb

Table 3. Effects of heavy metals on soil phosphatase activity 6 weeks after addition and after 18 months of incubation

Soil	Heavy metal	6 weeks after addition			18 months after addition		
		ED_{50}[a,d]	CV[b]	EDR[c]	ED_{50}[a,d]	CV[b]	EDR[c]
Sand	Cd	7.47	10	0.08 – 672	2.94	11	0.14 – 62.0
	Cr	61.7	2	21.0 – 182	385	22	13.9 – > 1000
	Cu	2.20	6	0.05 – 100	2.68	10	0.13 – 56.5
	Ni	18.9	9	0.63 – 565	13.1	14	0.70 – 250
	Pb	B			381	19	0.97 – 483
	Zn	3.37	9	0.06 – 167	2.60	6	0.08 – 91.8
Sandy loam	Cd	B			87.8	1	71.8 – 108
	Cr	106	3	53.5 – 212	53.7	5	16.5 – 174
	Cu	B			29.8	4	6.9 – 127
	Ni	96.9*	1	36.1 – 261	137	1	119 – 157
	Pb	A			A		
	Zn	51.1	7	20.5 – 127	45.4	1	8.72 – 236
Silty loam	Cd	48.8*	17	12.5 – 190	2.05	6	0.12 – 33.8
	Cr	71.7	2	14.0 – 367	79.6	13	5.38 – > 1000
	Cu	101*	6	29.0 – 354	11.7	6	2.68 – 49.9
	Ni	72.1	2	6.81 – 753	36.3	13	4.26 – 307
	Pb	39.5	1	34.4 – 45.5	36.7	6	9.51 – 142
	Zn	45.3	1	40.1 – 51.1	74.5	15	4.59 – > 1000
Clay	Cd	87.0*	1	1.25 – > 1000	47.2	3	7.38 – 300
	Cr	22.5*	3	1.00 – 512	51.0	1	41.4 – 62.9
	Cu	43.3	1	4.56 – 414	43.6	3	15.1 – 125
	Ni	111	2	6.47 – > 1000	B		
	Pb	53.9	2	18.0 – 157	B		
	Zn	55.4		2.45 – > 1000	43.5	17	0.55 – > 1000
Sandy peat	Cd	A			B		
	Cr	61.7	2	7.31 – 530	B		
	Cu	41.5	2	1.56 – > 1000	38.4	13	0.91 – > 1000
	Ni	B			B		
	Pb	A			B		
	Zn	A			B		

[a] ED_{50}, heavy metal concentration (in mmol kg^{-1}) at which phosphatase activity is half of the inhibited level
[b] CV, coefficient of variation (SD of ED_{50}/ED_{50}) × 100
[c] EDR, heavy metal concentration range at which phosphatase activity decreases from 90% to 10% of the maximal (in mmol kg^{-1})
[d] A, calculation process did not converge, with all measurements at the same level; B, calculation process did not converge due to excessive spread
* Significantly different from the corresponding value 18 months after addition

for acid phosphatase and Cd > Hg > Zn > Cr > Pb > Cu > Ni for alkaline phosphatase. In their results Cu ranks low in toxicity. For phosphatase, Cd, Zn and Cu are generally the more toxic compounds. The inconsistency in the data may be caused by the different chemical forms of the heavy metals influencing the microbiota. These differences may be caused by soil compounds such as clay, silt, or organic matter.

Since heavy metal pollution is irreversible we believe that the ED_{10} value is more useful in protecting soil quality.

The ecological dose range (Table 3) indicates the concentrations that cause a 10% and a 90% inhibition. In the sandy soil the 10% values are extremely low. The Cd, Cu, Ni, and Zn values were 0.08, 0.05, 0.63, and 0.06 mmol kg^{-1}, respectively, 6 weeks after the metals were mixed through the soil, and after 18 months the values were 0.14, 0.13, 0.70, and 0.08 mmol kg^{-1}. These low inhibitory levels are similar to data from Chaney et al. (1978) and Walter and Stadelmann (1979), who reported inhibition of respiration at concentrations ranging between 6 and 28 mg kg^{-1} Cd or Zn in black forest soil and sandy soil, respectively. Reber (1989) also reported a significant inhibition of respiration in sandy soil at 5 mg kg^{-1} Cd. In the present study, similarly low ED_{10} data were found only in the silty loam for Cd, in the clay for Zn, and in the sandy peat for Cu (0.91 mmol kg^{-1}) 18 months after the addition of the heavy metals. In the sandy loam the ED_{10} value for Cd and Ni were surprisingly high (71.8 and 119 mmol kg^{-1}, respectively) while for Cu and Zn the ED_{10} values were 6.9 and 8.7, respectively, and for Cr 16.5 mmol kg^{-1}. In the silty loam all these values were lower, indicating that this

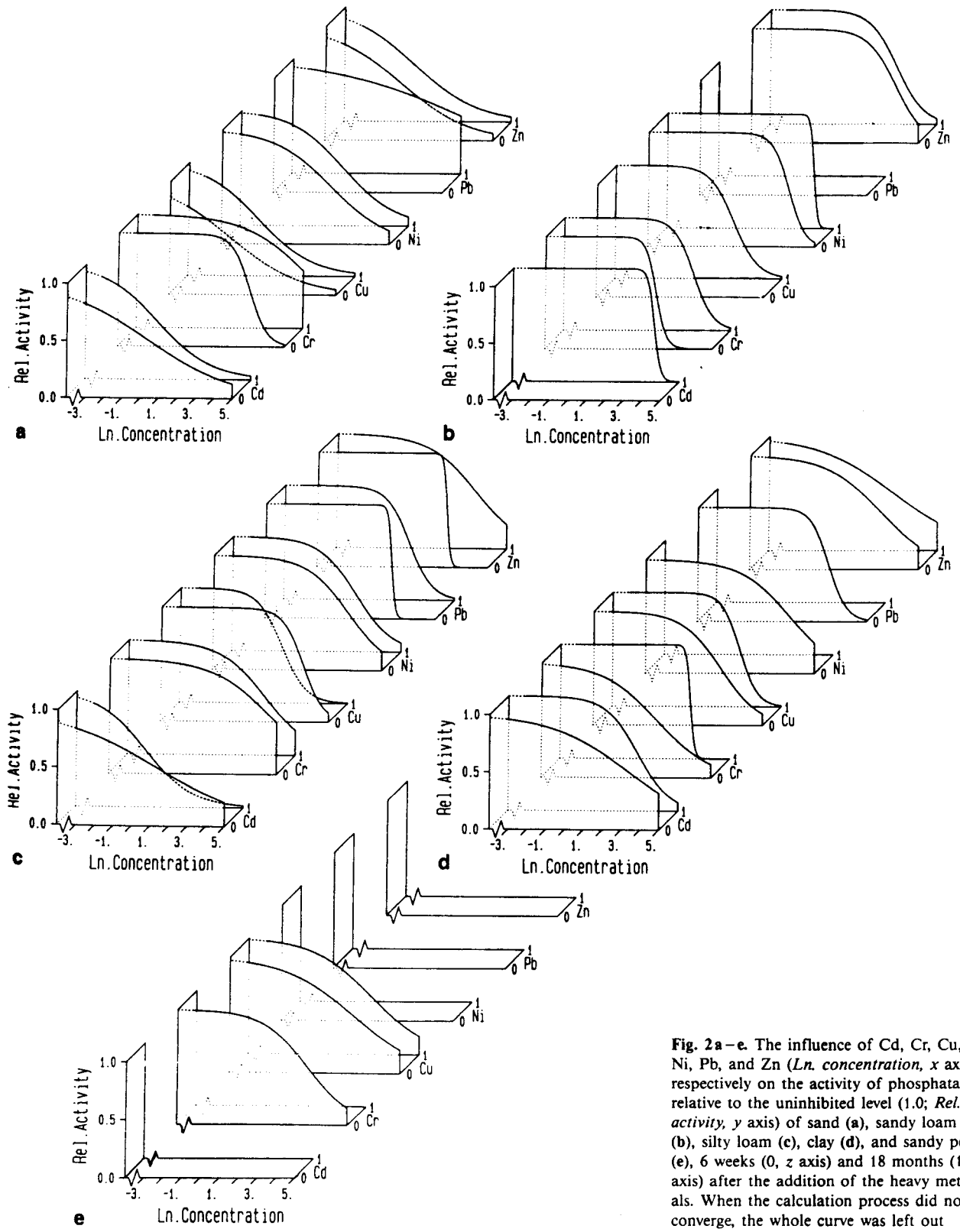

Fig. 2a–e. The influence of Cd, Cr, Cu, Ni, Pb, and Zn (*Ln. concentration, x* axis) respectively on the activity of phosphatase, relative to the uninhibited level (1.0; *Rel. activity, y* axis) of sand (**a**), sandy loam (**b**), silty loam (**c**), clay (**d**), and sandy peat (**e**), 6 weeks (0, *z* axis) and 18 months (1, *z* axis) after the addition of the heavy metals. When the calculation process did not converge, the whole curve was left out

soil is more sensitive to heavy metals even though its CEC is higher (16 mEq $100\,g^{-1}$). In the clay soil (CEC 30 mEq $100\,g^{-1}$) the toxicity values, with the exception of Zn, were higher than in the sandy or loamy soils. In the sandy peat the only detectable ED_{10} value was very low at 0.9 mmol kg^{-1}. This corresponds with the observations of Mathur and Sanderson (1980) on acid-organic soils (Histosols) where concentrations of Cu between 0.45 and 4.5 mmol kg^{-1} inhibited acid phosphatase. However, in the sandy peat the effects of the heavy metals on phosphatase activity were not consistent and therefore no ED_{10} or ED_{50} values could be obtained. However, Tyler (1975, 1976) showed that heavy metals have a fairly consistent negative effect on the phosphatase level in organic soil; a linear regression line was obtained between phosphatase activity and log Cu+Zn. This discrepancy with the present data may be ascribed to inaccuracies in the statistical analysis, since the present study has only 4° of freedom where Tyler (1976) has 39°. In Fig. 3 the separate measuring points were omitted for clarity. Whenever the calculation process did not converge, the whole curve was left out. In general, for fairly homogeneous agricultural soils such as sand, sandy loam, silty loam and clay soils, the inhibitory effects of the heavy metal range applied were so regular that reasonable curves were obtained. In the sandy peat (high organic-matter content) the data were consistent in only 4 (Cd, Cr, and Cu at t_0 and Cu at t_1) of the 12 pollution series. This is in contrast with the results found in the present study for urease in the sandy peat where 10 out of 12 curves were interpretable (Doelman and Haanstra 1986). This finding is explained if the humic matter is seen as a backbone supporting not only enzyme molecules, proteins, amino acids, and carbohydrates (Burns 1982) but also heavy metals. Environmental factors such as pH, water, surface charges, and redox conditions at the microsite level are of crucial importance for the activity of various forms of phosphatases with different characteristics (Nannipieri 1984) and also for the speciation and the availability of heavy metals. However, these environmental factors are subject to changes over time. It is not unlikely that these changes, caused by a diversity of microbial and enzymatic activities, will be in a delicate balance. Therefore, small changes in environmental conditions can have unpredictably large results. Small differences in environmental conditions during long-term incubation are unavoidable and may have caused the large spread in the experimental data. It is remarkable that with the measurements of urease (Doelman and Haanstra 1986) and arylsulphatase (Haanstra and Doelman, in preparation) on the same samples, most curves did not show such a large spread. This supports the view that the complex nature of phosphatase is responsible for the spread found in sandy peat. This explanation is not entirely satisfactory but it emphasises the need for rigid standardisation of sample handling during incubation.

As shown by the ED_{10} and ED_{50} values and by examination of the whole curve, the negative impact of toxicity to phosphatase is strongest in sandy soil. Since the heavy metals are compared on a molecular weight basis in every soil, the differences in toxicity of the different heavy metals is due to intrinsic properties of the metal ion. The toxicity of Zn and of Cu at higher levels should be a serious warning, since pig manure contains high concentrations of Cu and sewage sludge often contains high concentrations of Zn. Those two materials are often used as organic fertilizers.

In the Netherlands, critical heavy metal concentrations (*b* values) are set at 5, 250, 100, 100, 150, and 500 mg kg^{-1} for Cd, Cr, Cu, Ni, Pb, and Zn, respectively. Above these values research is required to determine whether the soil can still function. For Cu and especially for Zn these criteria should be reconsidered, because in sandy soils phosphatase activity is inhibited to approximately 60% and 30% of the initial activity at these concentrations. This is particularly important because sewage sludge and pig manure, often containing heavy metals, are often applied to sandy soils to improve them.

We hope that the data presented above, set out in a dose-response curve, can be used as resource material for a Soil Protection Act.

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