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An ecological dose-response model approach to short- and long-term effects of heavy metals on arylsulphatase activity in soil

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Summary. The aim of this study was to provide data to evaluate the short- and long-term effects of heavy metals on arylsulphatase activity in five soils. The effects are fitted on a logistic dose-response model and are presented graphically as the ecological dose (heavy metal concentration corresponding to 50% inhibition; ED_{50}) and ecological dose range (heavy metal concentration range corresponding to 10–90% inhibition; EDR). In 7 out of 22 comparable soil-metal combinations the ED_{50} decreased significantly over 6 weeks to 18 months of incubation and in two cases the ED_{50} increased. Toxicity (defined as ED_{50}) was highest in sand and sandy loam and lowest in sandy peat. Cd toxicity in sand, silty loam, and clay varied from 1.08 to 9.04 mmol kg^{-1}. Both Cr and Ni toxicity varied strongly and decreased with time in some soils while increasing in others. The Cu toxicity ranged from 4.51 to 2 mmol kg^{-1} in sand and silty loam, respectively, but remained fairly constant over time. Pb was the least toxic element (14.5 to 59.9 mmol kg^{-1}). The toxicity of Zn ranged from 5.73 to 148 mmol kg^{-1} in sand and sandy peat, respectively. At critical concentrations set by the Dutch Soil Protection Act, Cr, Cu, Ni, and Zn inhibited arylsulphatase by 53, 35, 48 and 97%, respectively.

Key words: Heavy metal toxicity – Soil pollution – Arylsulphatase – Logistic dose effect – Ecological dose (ED_{50}) – Ecological dose range (EDR) – Cd – Cr – Cu – Ni – Pb – Zn

Over the last two decades there have been a number of studies on the adverse effects of heavy metals on processes such as the accumulation of organic matter (Pugh and Williams 1971; Rühling and Tyler 1973; Nordgren et al. 1983), soil respiration (Chaney et al. 1978; Doelman and Haanstra 1984), ammonification and nitrification (Premi and Cornfield 1969) and N_2 fixation (Rother et al. 1982). Effects on soil enzymatic activities have also been investigated, for example urease (Tabatabai 1977; Doelman and Haanstra 1986) and phosphatase (Tyler 1976; Juma and Tabatabai 1977; Mathur and Sanderson 1980; Mathé and Kovacs 1980).

Apart from the studies of Al-Khafaji and Tabatabai (1979) and Nriagu and Soon (1984), little attention has been paid to the effect of heavy metals on arylsulphatase. Al-Khafaji and Tabatabai (1979) studied the inhibition of arylsulphatase in four soils by 21 trace elements, using 25 mmol kg^{-1} of each element. In two of the soils a concentration of 2.5 mmol kg^{-1} was also used. Nriagu and Soon (1984) studied the effects of pH and heavy metals in relatively unpolluted lake sediments. Recently, arylsulphatase activity was monitored in a study on the adverse effects of acid precipitation in peat (Press et al. 1985; Jarvis et al. 1987).

Attempts have been made to fit data to a mathematical model (Tyler 1976; Mathé and Kovacs 1980; Doelman and Haanstra 1986). Models can be helpful in predicting levels of soil pollution at which certain effects can be expected. The relevance of these curves was emphasized by Babich et al. (1983).

The aim of the present study was to provide data on short- and long-term changes in arylsulphatase activity in soils. The effect of seven concentrations of six heavy metals in five different soils over two time periods was measured. The enzyme arylsulphatase was chosen because it is known to be sensitive to heavy metals.

Materials and methods

Measurement of arylsulphatase activity were made on samples of five soils, collected in various parts of the Netherlands (Table 1). The origin of the soils and the sampling method have been described earlier (Doelman and Haanstra 1984). The soil samples were sieved (2 mm mesh) immediately after collection and stored before analysis in darkness at 20°C at moisture contents ranging from 120 to 300 ml kg^{-1}. This moisture range represented 55–70% water-holding capacity and was comparable to field moist conditions. For the analyses, 55, 150,

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Table 1. Physicochemical characteristics, indigenous concentrations of metals, and arylsulphatase activity in five soils

	Sand	Sandy loam	Silty loam	Clay	Sandy peat
pH (H_2O)	7.0	6.0	7.7	7.5	4.4
pH (KCl)	7.7	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 (mEq 100 g^{-1})	1 – 2	10 – 12	16	30	50 – 55
WHC (%)	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
Arylsulphatase[a] (t_0)	2.86	5.30*	2.58	5.89*	44.6*
Arylsulphatase[a] (t_1)	2.64	1.81	3.29	3.28	19.3

* $P < 0.05$ versus t_1; CEC, cation exchange capacity; WHC, water-holding capacity

[a] Mean arylsulphatase activity expressed in mmol h^{-1} kg^{-1} dry soil; t_0, measured after 6 weeks; t_1, measured after 18 months

400, 1000, 3000, and 8000 mg kg^{-1} dry weight Cd, Cr, Cu, Ni, Pb, and Zn (as chlorides) were added as finely ground powder to field-moist soil subsamples (55 – 70% water-holding capacity). For mutual comparison these concentrations were converted to mmol kg^{-1}. Untreated soil was used as a control. The addition of heavy metals had no significant acidic effects (pH changes less than 0.5) on any soil except for the highest concentration (8000 mg kg^{-1}) 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.

Arylsulphatase activity was measured by the method of Tabatabai and Bremner (1970), using p-nitrophenylsulphate as the substrate. Soil (2.5 g) was incubated with 5 ml of 10 mmol l^{-1} p-nitrophenylsulphate without toluene and buffer for 2 h at 30°C in an overhead shaker (25 rpm). The arylsulphatase activity was measured in triplicate with a suitable control and was expressed as mmol p-nitrophenol formed per kg dry soil per h.

Initial measurements confirmed that the production of p-nitrophenol was linear with time. This indicated zero-order reaction kinetics and showed that shaking with water did not extract materials and significantly influence the reaction velocity. On the assumption that heavy metals need some time to settle in soil, the first set of measurements ($t = 0$) was made 6 weeks after the heavy metals were added to the soil. The timing of the second set of measurement ($t = 1$) was arbitrarily selected as 18 months after the addition of heavy metals. The pH-H_2O and pH-KCl values were determined in a 1 : 2 (w : v) suspension of soil and demineralised water or soil and 1 M KCl, respectively. Organic matter was determined as loss of weight on ignition (12 h at 600°C), expressed as a percentage of the total weight. Cation exchange capacity was determined with ammoniumacetate at pH 7. The water-holding capacity was expressed as ml kg^{-1} of dry soil. 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-nitrophenylsulphate, using the Eadie-Hofstee transformation (velocity versus velocity/concentration).

The relationship between the means of triplicate measurements as dependent variables and the logarithm of the added concentrations as independent variables was described by a logistic response model, similar to that described for urease activity in soil (Doelman and Haanstra 1986). This relationship is expressed as:

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

where Y is the observed arylsulphatase activity at the logarithm of the heavy metal concentration X and c is the calculated initial (uninhibited) level of arylsulphatase activity, determined by the activity of the control sample and of samples with low concentrations. The parameter b is a slope parameter indicating the inhibition rate, and is equal to $4.39/(0.1\,c - 0.9\,c)$. The parameter a is the logarithm of the concentration at which the arylsulphatase activity is half the uninhibited level ($a = 0.5\,c$). E is the stochastic error term, describing the deviations of the observations around the model response curve. For clarity, the logistic curve with the parameters a, b, and c is shown in Fig. 1. As the logarithm of the added heavy metal concentration of the blanks cannot be calculated ($\ln 0 = -\infty$), it was substituted by the logarithm of a small value (e.g. 10^{-3} mmol kg^{-1}, essential for the curve but not for the heavy metal content), where the curve reaches its asymptotic value. The use of the total heavy metal content of both blanks and samples eliminates this substitution but obscures the calculation of the effect of the heavy metal 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 the parameter a by taking its inverse logarithm. The EDR is defined as the dose range in which activity decreases from 90 to 10% of the calculated uninhibited arylsulphatase activity. Details of the use of the logistic response model and the EDR are described by Haanstra et al. (1985).

The standard deviation of the difference between 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 affecting the rate of the enzymatic reaction, we determined the K_m and V_{max} values of the unamended soils (Table 2). As expected, large differences were found, but the substrate concentration used (10 mmol l^{-1}) was more than 5× K_m, as recommended by Burns (1978). Our K_m values are lower than those reported by Tabatabai and Bremner (1971) and Jarvis et al. (1987), higher than those reported by Han and Yoshida (1982) and in the same order of magnitude as those observed by Perucci and Scarponi (1984). These differences reflect on the variability of the assay (buffer, toluol, temperature) and the vastly different soils studied.

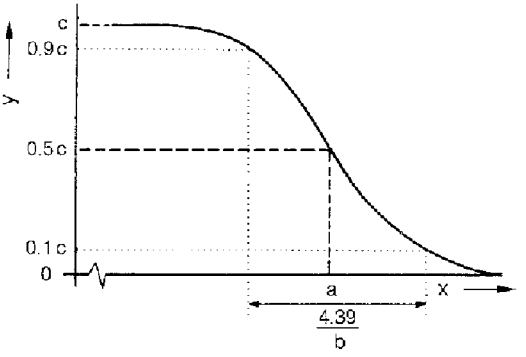

Fig. 1. The logistic response curve with parameters a, b, and c

In Table 1 shows arylsulphatase activities in the unamended soils at t_0 and t_1. After 18 months the activity of the unamended samples was significantly reduced in the sandy loam, clay, and sandy peat, while no significant changes were observed in the others. In the unamended soils at $t = 0$, enzyme activity values were similar to values found by Sarathchandra and Perrot (1981), but higher when compared with most other studies, a finding that may reflect the different methodologies used.

Table 2. Kinetic values of soil arylsulphatase activity in five different soils

Soil	K_m (mmol l^{-1})	V_{max} (mmol $kg^{-1} h^{-1}$)
Sand	0.113 ± 0.078	0.014 ± 0.005
Sandy loam	0.164 ± 0.036	0.026 ± 0.004
Silty loam	0.397 ± 0.150	0.101 ± 0.024
Clay	0.220 ± 0.134	0.039 ± 0.014
Sandy peat	0.625 ± 0.158	0.368 ± 0.075

Table 3 shows the ED_{50} and the EDR while in Fig. 1 showns the influence of the six metals on the enzyme activity in five soils at t_0 (6 weeks after heavy metal addition) and t_1 (18 months after heavy metal addition). For clarity the measuring points were left out. In 10 out of 60 soil-metal-time combinations the iteration process did not converge, due to a too large spread or a close similarity in the data. In 22 cases (out of 30 possibles) we were able to compare the ED_{50} at t_0 with that of t_1. In 16 (9 not significant, 7 significant; $P < 0.05$) the ED_{50} was higher at t_0 than at t_1. In 2 significant and 3 not significant cases the reverse was found. To compare our results with those of Al-Khafaji and Tabatabai (1979) we calculated the percentage of inhibition at the concentrations they have used (25 and 2.5 mmol kg^{-1}). The results showed higher inhibition percentages than those reported by Al-Khafaji and Tabatabai (1979) for all metals in all types of soils, except for sandy peat, with the most pronounced difference in toxicity found for Cu. Probably, this discrepancy was caused by an increase in toxicity with time, since Al-Khafaji and Tabatabai (1979) measured the

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

		6 weeks after addition			18 months after addition		
	Metal	ED_{50}[a]	CV[b]	EDR[c]	ED_{50}[a]	CV[b]	EDR[c]
Sand	Cd	19.7*	34	7.29 – 53.2	1.08	36	0.03 – 35.6
	Cr	0.33	186	< 0.01 – > 1000	3.90	52	0.04 – 376
	Cu	6.14	2	5.22 – 7.23	4.51	58	0.10 – 212
	Ni	36.1	16	6.34 – 206	1.68	159	0.02 – 140
	Pb	40.0	2	34.3 – 45.7	42.4	18	1.33 – > 1000
	Zn	13.9*	13	1.62 – 119	5.73	5	4.79 – 6.86
Sandy loam	Cd	A			16.0	52	0.03 – > 1000
	Cr	5.95	14	0.89 – 39.7	0.23	210	0.02 – 3.65
	Cu	15.2	1	12.8 – 17.9	8.61	23	5.46 – 13.6
	Ni	40.0	3	10.4 – 155	B		
	Pb	B			14.5	1	12.8 – 16.4
	Zn	33.4*	11	11.2 – 99.9	14.5	2	12.3 – 17.1
Silty loam	Cd	16.8*	7	1.27 – 223	1.22	245	0.06 – 29.4
	Cr	B			7.91	24	1.60 – 39.2
	Cu	235	24	1.59 – > 1000	12.0	23	4.51 – 32.1
	Ni	92.0*	2	37.6 – 225	1.57	329	0.03 – 71.3
	Pb	44.1	2	35.3 – 55.0	21.9	18	7.89 – 61.0
	Zn	19.8*	10	2.33 – 168	66.5	10	41.6 – 106
Clay	Cd	84.7*	4	39.5 – 182	9.04	7	0.25 – 321
	Cr	5.41	17	0.82 – 35.6	11.05	7	5.30 – 23.1
	Cu	42.8*	4	9.75 – 188	76.3	3	42.0 – 139
	Ni	96.4	4	18.2 – 510	41.5	4	4.61 – 374
	Pb	B			59.9	3	9.19 – 390
	Zn	85.0*	3	36.2 – 200	43.4	1	15.6 – 121
Sandy peat	Cd	28.4	1	23.7 – 34.0	B		
	Cr	114	3	64.2 – 201	61.6	2	52.5 – 72.3
	Cu	140	1	135 – 166	110	3	52.3 – 229
	Ni	A			138	1	120 – 154
	Pb	A			A		
	Zn	A			148	3	122 – 180

* $P < 0.05$ versus 18 months

[a] ED_{50}, heavy metal concentration (in mmol kg^{-1}) at which arylsulphatase activity is half of the uninhibited level

[b] CV, coefficient of variation $(S_d/a) \cdot 100$

[c] EDR, heavy metal concentration range at which arylsulphatase activity decreases from 90% to 10% of the maximum (in mmol kg^{-1})

A, iteration process did not converge, all measurements at the same level

B, iteration process did not converge due to excessive spread

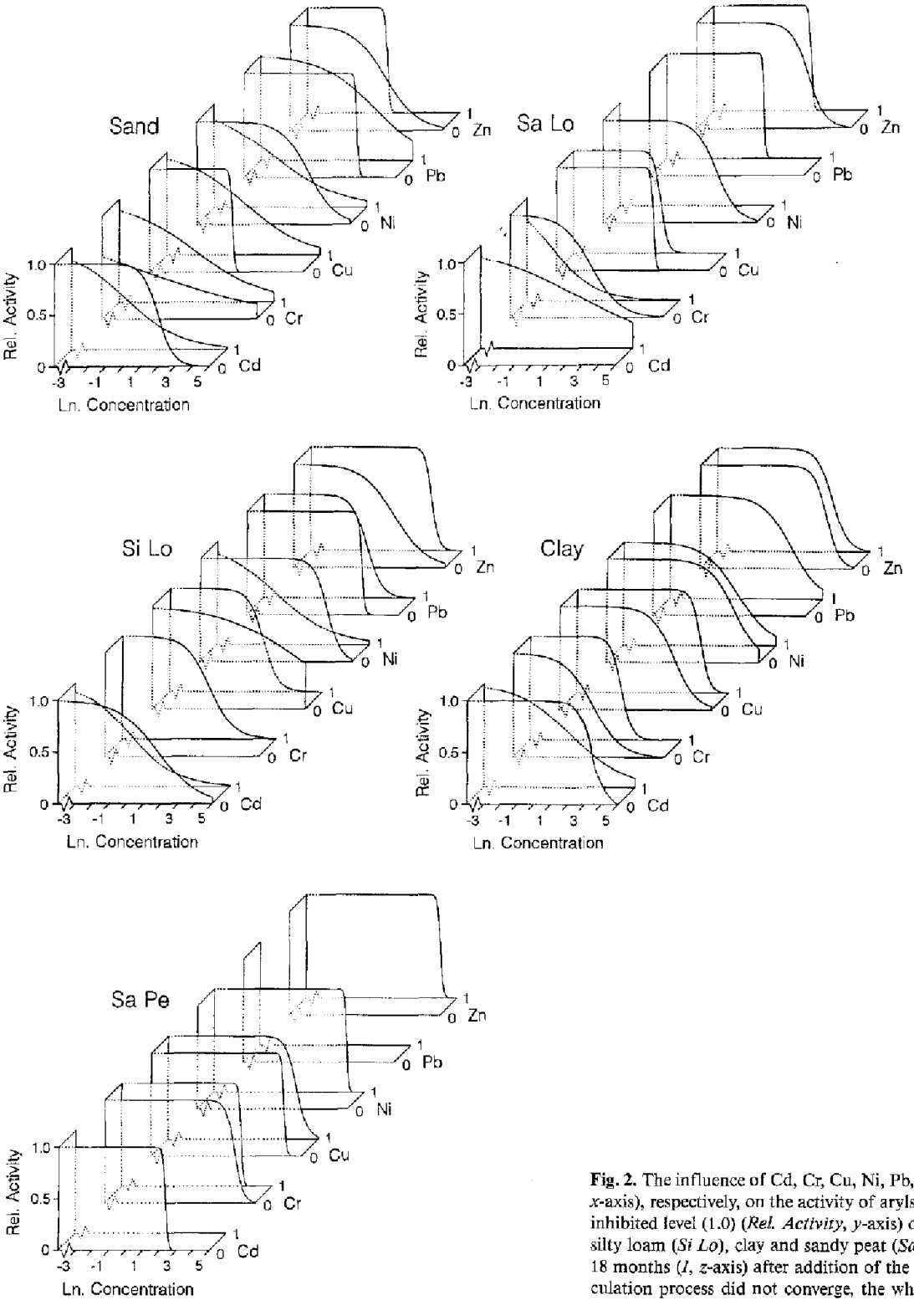

Fig. 2. The influence of Cd, Cr, Cu, Ni, Pb, and Zn (*Ln. Concentration*, *x*-axis), respectively, on the activity of arylsulphatase, relative to the uninhibited level (1.0) (*Rel. Activity*, *y*-axis) of sand, sandy loam (*Sa Lo*), silty loam (*Si Lo*), clay and sandy peat (*Sa Pe*) 6 weeks, (*0*, *z*-axis) and 18 months (*1*, *z*-axis) after addition of the heavy metals. When the calculation process did not converge, the whole curve was left out

activity immediately after the heavy metal treatment. Their relative toxicity order at 25 mmol kg^{-1} was Cr > Cd > Zn ≥ Cu > Ni ≥ Pb, whereas the present heavy metal series was Cr > Cd ≥ Cu > Zn > Pb > Ni. Nriagu and Soon (1984) found the following toxicity order in Lake Erie sediments Cu > Zn > Ni; but it another sediment they found that Ni was more toxic than Cu.

In appears that the toxicity of heavy metals increases with time. This has also been reported for Ni in sandy loam (Haanstra et al. 1985), where respiration was measured weekly for up to 13 weeks. The EDR values in the present study (Table 3) did not show a significant change over time. However, due to a rather poor estimate of the parameter *b*, no precise or meaningful comparison between EDR values at 6 weeks and those at 18 months can be made.

The EDR values (Table 3) show the heavy metal concentration range at which enzyme activity decreases from

10 to 90% of the control value. Except for those in sandy peat, we found low ED_{10} values. The present values for Cd (0.03–0.25 mmol kg^{-1}) are in the same range as those (0.04 mmol kg^{-1}) inhibiting respiration in a sandy soil (Reber 1989). Values reported for the inhibition of soil respiration by Chaney et al. (1978) and Walter and Stadelman (1979) are in the same order of magnitude (0.05–0.25 mmol kg^{-1}). With Zn, the concentrations (0.09–0.43 mmol kg^{-1}, Doelman and Haanstra 1984) that inhibited respiration were lower than those (4.8–42 mmol kg^{-1}) that caused a 10% decrease in arylsulphatase activity (Table 3).

The toxic effect was strongest in sand and sandy loam, although Cd and Ni in silty loam and Cd in clay were also very toxic. The toxicity of Zn for arylsulphatase was moderate compared with its toxicity for urease (Doelman and Haanstra 1986). But since the EDR is narrow in most cases, once a certain concentration is reached any further pollution will have strong inhibitory effects.

It is remarkable that in 10 out of 60 curves, the same number found for urease (Doelman and Haanstra 1986) in the same soils, the results could not be interpreted because the calculation did not converge. For phosphatase determined in the same soils, 16 out of 60 curves gave uninterpretable results (Doelman and Haanstra 1989). Most of the uninterpretable curves for arylsulphatase and phosphatase occurred in the sandy peat samples (organic matter 12.8%), less in the sandy loam samples (organic matter 5.7%), and only in a few cases in the other soils (organic matter 1.6–3.2%). Humic matter has a strong influence on the chemical form of heavy metals in soil, but the effects on toxicity are largely unknown. Moreover, humic matter can be seen as a backbone that supports enzyme molecules (Burns 1982). At the microsite level, environmental factors such as pH, surface charges, and redox conditions are of crucial importance for enzyme activity (Nannipieri 1984), and can markedly influence environmental conditions in the area surrounding the protein. These different influences affect the phosphatase activity in a more unpredictable way than urease and arylsulphatase activity but the reason for this remains obscure. As enzyme activity in soil changes according to environmental factors (Burns 1982; Nannipieri 1984) intracellular enzymes and enzymes adsorbed by soil colloids are probably the most important types for measuring toxicity. In the first case the heavy metal has to pass through the cell wall and the cell membrane before it can react with the enzyme. In the second case, with no cellular barriers, the action of heavy metal is more direct. The contribution of intracellular and of adsorbed enzymes to the total enzyme activity can only be determined when methods become available to determine the various types of absorbed enzymes and to measure their activities.

Heavy metals are adsorbed by clay and form complexes with various organic molecules present in soil organic matter, thereby changing their toxicity. The nature of these organometallic complexes is poorly understood, but, in the present study, the ED_{50} increased with an increase in both the cation exchange capacity CEC and the organic matter (OM) content ($r_{CEC} = 0.66$, $r_{CEC} = 0.81$; $r_{OM} = 0.48$; $r_{OM} = 0.75$; $P < 0.05$, after 6 weeks and 18 months, respectively) and not with an increase in the clay content ($r = 0.27$, $r = 0.03$, after 6 weeks and 18 months, respectively), and we assume that, in general, heavy metals complexed with organic molecules are less toxic to arylsulphatase. The increase in the correlation between the ED_{50} and both the cation exchange capacity and the organic matter content over time is probably caused by a slow formation of organic complexes.

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. The *B* value implies that above this concentration research must be undertaken to investigate whether the metabolic properties of the soil have been disrupted. The *B* values for Cr, Cu, Ni, and Zn need reconsidering, since arylsulphatase activity in sand is inhibited at these concentrations by 53, 35, 48, and 97%, respectively. In sandy soils especially, pig manure containing high concentrations of Cu and sewage sludge often containing appreciable amounts of Zn are frequently used as organic fertilizers. As long as the organic matter is present, the effects of the heavy metals will be partly alleviated, but after the organic matter is mineralized the increase in heavy metal content may disrupt the metabolic properties of the soil. As soil polluted with heavy metals is very hard to clean, greater care should be taken to prevent soil pollution with heavy metals.

With these data, based on logistic dose-response curves, we hope to supply a scientific basis for a revision of critical heavy metal concentrations in the Netherlands.

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