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Short- and long-term effects of heavy metals on urease activity in soils

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Summary. The inhibitory effects of cadmium, chromium, copper, lead, nickel and zinc on urease activity of five different soils during two different periods were investigated, in order to obtain information on the change in heavy metal toxicity with time. The results are presented graphically as logistic dose-response curves. When the ecological dose range was used as a measure of toxicity this value decreased significantly only for copper in the sandy soil. Considering toxicity as the ecological dose-50% (ED50) value, toxicity tended to increase over 1½ years for cadmium, copper and zinc. For nickel and lead, however, the toxicity stabilized in all soils, except in sand and clay. The average ED50 value of zinc varied between 100 and 300 mg kg^{-1} and its toxicity was highest. It is emphasized that these data may help to set limits for the heavy-metal pollution of soils.

Key words: Heavy metal toxicity – Soil pollution – Urease – Logistic dose-response curve

In industrial countries the functioning of biological systems in soil is threatened by increasingly higher concentrations of heavy metals (Tyler 1972, Mayer 1985). The total mineralization of organic matter is carried out by a large community of organisms and involves a wide range of metabolic processes in which organisms as well as processes may relieve each other. Therefore certain specific metabolic steps may be a more-sensitive indicator concerning heavy metal pollution than overall processes such as soil respiration and ammonification. Soil enzyme activities may be considered as specific metabolic processes.

Urease, an important enzyme in nitrogen metabolism (Bremner and Mulvaney 1978), has been extensively studied in relation to its inhibition by heavy metals. Nor (1982) reported a complete inhibition of urea hydrolysis in two soils by 120 mg Cu kg^{-1}. Bremner and Douglas (1971) reported inhibition percentages of less than 5% in two air-dried soils by adding 50 mg kg^{-1} Cr^{3+}, Ni and Pb, while Cu caused an inhibition of 13%-16%. Tabatabai (1977), adding 5 mmol kg^{-1} heavy metals to each of five air-dried soils, found an inhibition range of 49%–67% by Cd, 17%–50% by Cr^{3+}, 51%–94% by Cu, 20%–33% by Ni, 2%–55% by Pb and 23%–31% by Zn. All the inhibition percentages were found after 30 min after addition.

Soils near metallurgic industries or on mine waste are subjected to prolonged heavy metal contamination and the urease activity has been found to be lower than that in sites at a larger distance. For example, a 90% decrease in activity has been reported in Cu- and Zn-polluted soils by Tyler (1974). Pancholy et al. (1975) reported a reduction in urease activity of approximately 85% due to Zn, Pb and Cd, whereas Williams et al. (1977) observed a 99% reduction in Pb- and Zn-polluted sites. These reports on the chronic or long-term effects are based on the questionable assumption that polluted and unpolluted soils differ only in heavy-metal content although information on physiochemical data of the soils involved are scarcely reported. The acute or short-term effects mentioned earlier are carried out in vitro and are not to be compared with the long-term effects.

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No information on the changes of inhibitory effects of heavy metals versus time has been reported so far. The aim of this study was to provide manageable data on long-term changes for the Soil Protection Act, which is in preparation in The Netherlands (Ministerie van Volksgezondheid en Milieuhygiëne 1981). Therefore, the effect of various concentrations of heavy metals on urease activity was measured in five soil types, 6 weeks after addition as well as after 1½ years.

Materials and methods

Measurements of urease activity were carried out on samples of five soil types, collected from various parts of The Netherlands (Table 1). The origin of the soils and the sampling method have been described by Doelman and Haanstra (1984). The soil samples were sieved (2-mm mesh) immediately after collection and stored in darkness during the whole experiment at 20°C and at moistures of 12%, 16%, 20%, 22% and 30%, respectively. To subsamples of each soil type 55, 150, 400, 1000, 3000 and 8000 mg kg^{-1} Cd, Cr, Cu, Ni, Pb and Zn were added. Untreated soil served as control. The heavy metals were added as a finely ground powder of their chloride salts to field-moist soil (55%–70% WHC).

Urease activity, expressed as millimoles of urea hydrolysed at 37°C kg^{-1} dry soil $hour^{-1}$, was determined by a procedure essentially equivalent to the non-buffer method of Zantua and Bremner (1975a). A concentration of 10 mmol urea l^{-1} was used. The use of toluene and a buffer solution was excluded in order to measure the urease activity at conditions as close as possible to the conditions in the soil.

During the 5-h incubation, an increase in pH (7.02 to 7.49) in the sandy soil was observed. In the other soils the increase of the pH never exceeded 0.3 units. This supports the choice of the non-buffer method. After 5 h of incubation non-hydrolysed urea was determined colorimetrically with a sulphuric acid-diacetylmonoxime-thiosemicarbazide mixture (Guidi et al. 1973). Since we found that Cu was interfering with the colorimetric method used, it was precipitated with 8-hydroxy-quinoline (0.05%) after extraction of the non-hydrolysed urea. No interference was found with Cd, Cr, Ni, Pb and Zn at concentrations smaller than 1 mmol l^{-1}.

After 6 weeks ($t = 0$) and after 18 months ($t = 1$) urease activity was measured in the soil samples. All measurements were carried out in triplicate with a blank, prepared by adding urea to the sample after stopping hydrolysis with phenylmercuriacetate. The relation of the means of the triplicate measurements as dependent variables and the logarithm of the added concentrations as independent variables were described using a logistic response model. This relationship is expressed as:

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

where Y is the observed urease activity at the (natural) logarithm of the heavy metal concentration X and c is the calculated initial (uninhibited) level of urease activity, determined by the urease activity of the control sample and of samples with low concentrations of heavy metals. The parameter b is a slope parameter indicating the inhibition rate and is equal to $4.39/(0.1c–0.9c)$. The parameter a is the logarithm of the concentration at which the urease activity is half the uninhibited level ($a = 0.5c$). E is the stochastic error term, describing the deviations of the observations around the model response curve. For reasons of clarity the logistic curve with the parameters a, b and c is shown in Fig. 1. As the

Table 1. Physicochemical characteristics and indigenous concentrations of metals in soils

Soil type	Sand	Sandy loam	Silty loam	Clay	Sandy peat
pH–H_2O[a]	7.0	6.0	7.7	7.5	4.4
pH–KCl[b]	7.7	5.1	7.4	6.8	4.3
Organic matter[c]	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[d]	1–2	10–12	16	30	50–55
WHC[e]	23	25	33	34	51
$CaCO_3$ (%)	2.6	0.1	9.6	0.1	0.1
N, total (%)	0.04	0.15	0.10	0.15	0.58
P, total (%)	0.08	0.11	0.13	0.25	0.05
Fe[f]	3990	2760	26 600	33 900	4400
Mg	1380	310	9500	7920	490
Mn	97	240	820	963	57
Cd	0.4	0.5	1.0	0.5	0.4
Cr	4	2	34	76	11
Cu	4	6.5	22	52	5.5
Ni	8	2	25	39	4
Pb	32	13	42	130	26
Zn	14	17	103	226	38

[a] Twenty-five grams soil in 50 ml water
[b] Twenty-five grams soil in 50 ml 1 *M* KCl
[c] Determined as loss of weight on ignition (2 h at 600°C) expressed as percentage of total weight
[d] Cation exchange capacity in mEq 100 g^{-1} determined with ammonium acetate at pH 7
[e] Water-holding capacity expressed as percentage of dry soil
[f] Metal contents expressed in milligrams per kilogram

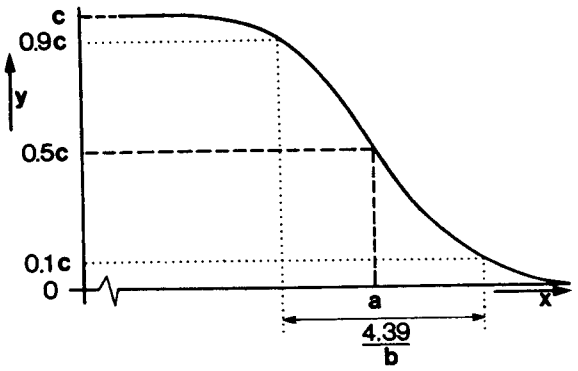

Fig. 1. The logistic response curve with parameters *a*, *b*, and *c*

logarithm of the heavy metal concentration of the blanks could not be calculated ($ln\ o = -\sim$), it was substituted by the logarithm of a very small value (e.g. 10^{-3} mg kg^{-1}, essential for the curve but not for the heavy metal content), where the curve reaches its asymptotic value. For the computations we used the iterative least square procedure supplied by the statistical program package GENSTAT (Alvey et al. 1982).

The ecological dose-50% (ED50) as discussed by Babich et al. (1983) is easily calculated from the parameter *a* simply by taking its inverse logarithm. The ecological dose range (EDR) 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 EDR are described 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.

Table 2. Effects of heavy metals on soil urease activity 6 weeks after addition and after 1 1/2 years

		Six weeks after addition				One and a half year after addition			
Metal	Soil	ED_{50}[a]	CV[b]	EDR[c]	Uninhibited level[d]	ED_{50}[a]	CV[b]	EDR[c]	Uninhibited level[d]
Cd	Sand	340	3	150– 770	1.17 ± 0.08	120	8	40– 380	1.76 ± 0.29
	Sandy loam	A*				30	13	< 1– 1470	1.70 ± 0.09
	Silty loam	970	2	360– 2600	0.69 ± 0.04*	520	3	280– 960	3.58 ± 0.29
	Clay	4460	3	950–21 000	1.41 ± 0.08	520	25	3–85 100	2.30 ± 0.53
	Sandy peat	3260	1	1980– 5370	6.23 ± 0.24*	490	72	< 1– > 10^5	3.24 ± 1.27
Cr	Sand	3970*	4	1880– 8350	1.23 ± 0.11*	630	2	390– 1010	2.76 ± 0.09
	Sandy loam	B				< 1	212	< 1– > 10^5	2.53± 2.85
	Silty loam	4470	2	2050– 9740	0.79 ± 0.04*	1110	3	890– 1390	0.32 ± 0.06
	Clay	490	3	280– 860	1.34 ± 0.09	420	1	350– 510	1.63 ± 0.14
	Sandy peat	1360	2	360– 5140	3.46 ± 0.18	–			
Cu	Sand	260	3	80– 830*	0.98 ± 0.06*	680	10	20–25 500	2.99 ± 0.36
	Sandy loam	570	6	120– 2830	2.78 ± 0.29	B			
	Silty loam	A				1990	13	340–11 800	0.35 ± 0.07
	Clay	1370	2	440– 4260	1.32 ± 0.07*	1080	5	520– 2220	2.14 ± 0.32
	Sandy peat	4200	11	60– > 10^5	6.72 ± 0.81*	1970	21	210–18 500	3.03 ± 1.00
Ni	Sand	100*	6	30– 340	1.10 ± 0.14*	410	5	120– 1370	3.18 ± 0.33
	Sandy loam	2040	4	860– 4830	2.60 ± 0.20*	2790	2	2300– 3390	0.86 ± 0.11
	Silty loam	1650	5	130–20 800	1.02 ± 0.08*	1740	12	14– > 10^5	0.55 ± 0.07
	Clay	3380*	2	610–18 700	1.54 ± 0.07*	370	6	90– 1510	1.14 ± 0.13
	Sandy peat	3030	3	1100– 8390	4.49 ± 0.28*	2320	10	540–10 000	2.51 ± 0.44
Pb	Sand	–				1590	3	860– 2940	2.14 ± 0.11
	Sandy loam	5060	2	1400–10 600	3.00 ± 0.13*	2870	1	2440– 3390	0.70 ± 0.04
	Silty loam	7190	2	2780–18 600	0.71 ± 0.03*	8130	1	6860– 9650	0.46 ± 0.02
	Clay	5730	1	696–47 900	1.54 ± 0.04*	1340	9	80–23 100	3.42 ± 0.49
	Sandy peat	6230	3	2300–16 900	5.59 ± 0.43*	7050	1	6000– 8400	3.11 ± 0.29
Zn	Sand	420	4	70– 2490	1.47 ± 0.12*	290	2	160– 530	2.28 ± 0.11
	Sandy loam	480	5	30– 8320	3.77 ± 0.21*	110	14	1–17 400	1.96 ± 0.17
	Silty loam	1030	7	30–38 200	1.80 ± 0.16	B			
	Clay	1780*	4	460– 6820	1.60 ± 0.11*	90	8	8– 980	3.06 ± 0.26
	Sandy peat	–				70	22	5– 930	5.37 ± 0.50

[a] ED_{50} heavy metal concentration (in mg kg^{-1}) at which urease activity is half of the uninhibited level
[b] CV, coefficient of variation (Sa/a) × 100
[c] EDR, heavy metal concentration range at which urease activity decreases from 90% to 10% of the maximal (in mg kg^{-1})
[d] in millimoles hydrolyzed urea per kilogram per hour

A, calculation process did not converge, all measurements at the same level. ED_{50} > 8000 mg kg^{-1}; B, calculation process did not converge due to excessive spread; *, significantly different from the corresponding value 1 1/2 years after addition; –, no observations

Results and discussion

In Table 1 physicochemical properties of the various soils are presented. In Table 2 the ED50, the EDR and the uninhibited urease activity values are presented. The coefficient of variation (CV) of the ED50 values is larger 1½ years after addition of heavy metals than 6 weeks after addition. Since the number of measuring points is minimal, a large spread may be expected. To become more pronounced, most measuring points should be in the concentration range around the ED50. This should also lead to a better estimation of the parameter *b*. As a consequence we were able to detect a significant change in the EDR in only one case (Cu in sandy soil). If the parameters *a* and *b* were estimated precisely, we would expect the EDR of metals to change with time in more soils.

In sand and clay soil, the uninhibited level of urease activity was higher after 1½ years of storage at field conditions at 20°C while in sandy loam, silty

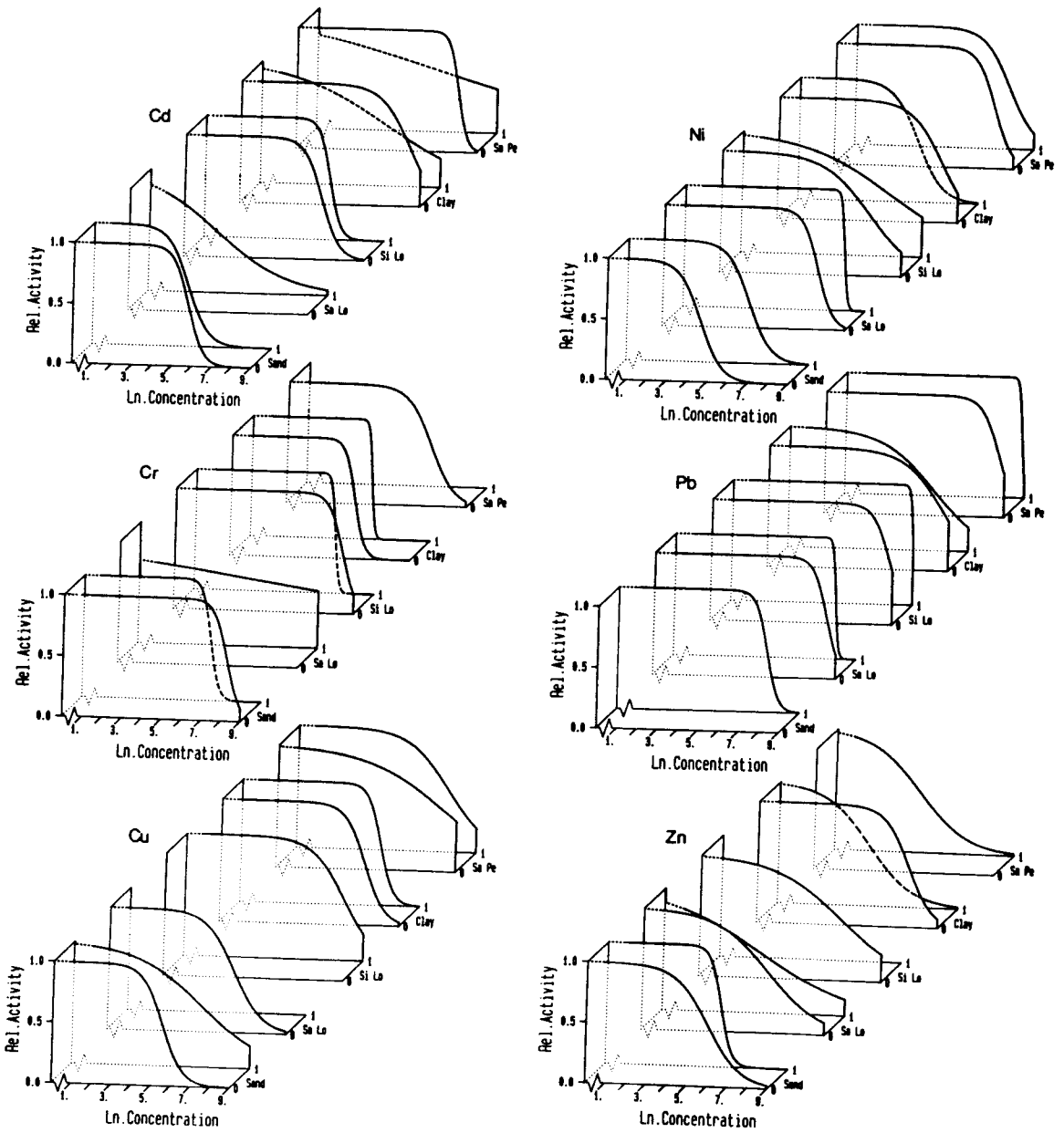

Fig. 2. The influence of Cd, Cr, Cu, Ni, Pb and Zn (*Ln. Concentration*, *x*-axis) respectively on the urease activity (*Rel. Activity*, *y*-axis) of sand, sandy loam (*Sa Lo*), *silty loam (Si Lo)*, clay and sandy peat (*Sa Pe*) *soil, 6 weeks (O, z*-axis) and 1½ yaers (*l, z*-axis) after the addition of the metals. When the calculation process did not converge, the whole curve was left out

loam and sandy peat soil the level after 1½ years was lower. According to Zantua and Bremner (1975b) the level of urease activity remains constant under these conditions for a very long time and also Nannipieri et al. (1983) have found a constant level of microbial biomass and urease activity under the same conditions. A decrease in urease rather than an increase was expected since no organic matter was added during storage. The levels of urease activity reported here are similar to those in the soils studied by Dalal (1975) and Zantua and Bremner (1975b) although the physicochemical properties of their soils differ from our soils. The total activity of an enzyme in soil is a composite of activities associated with various compounds (living cells, cell residues, clay and humic colloids), which proportionally are hard to quantify (Burns 1982; Nannipieri 1984). As the number of microorganisms varies in different soils, and within one soil they may fluctuate over time (Jagnow 1971), a soil may vary in its urease activity.

In Fig. 2 dose-response curves are given for every metal in the different soils, whereas the separate measuring points are omitted for reasons of clarity. Whenever the calculation process did not converge, the whole curve was left out. Striking results with Cd are: a rather strong inhibitory effect in sand and sandy loam soil and a tendency towards increasing toxicity during 1½ years in all the soil types. For Cd in the sand, the silty loam and the clay soil comparable curves were obtained where the toxicity either increased with time or remained approximately constant. Only in the case of Cr treatment was an acidification effect also observed; the pH-KCl decreased from 5.1 to 2.6, from 6.8 to 3.1 and from 4.3 to 2.8 in sandy loam, clay and sandy peat respectively (Doelman and Haanstra 1984). For Cu the toxicity strongly decreased in the sand soil when the urease activity was measured after 1½ years, which was also shown in a significant change of the EDR (Table 2), while in the clay and sandy peat soil time hardly affected toxicity. Striking for Ni is that for all soils the data fit the logistic dose-response curves; the toxicity decreased in the sand and sandy loam soil, was maintained approximately at a constant level in the silty loam and sandy peat and increased in clay. For Pb the toxicity was low and generally did not change significantly in 1½ years. For Zn the toxicity generally, especially in the silty loam, clay and sandy peat soil, was high and comparable to that of Cd.

For Cd and Zn the toxicity towards urease after an extended period increased rather than decreased. Especially for Zn, the ED50 values after 1½ years average between 100 and 300 mg kg^{-1}, which is rather low considering the Zn content in soil material such as sewage sludge. In 337 samples the average content was 1647 mg Zn kg^{-1}, with extreme values of 639 and 4770 mg kg^{-1} (Haan 1978). Although sludges are supposed to be mixed with soil the speciation of Zn may differ from that of this present study. Therefore a certain caution is required before extrapolating the results to sludge-treated soils.

The dose at which 50% inhibition of urease occurs varies in the different soils. Generally in the sand and sandy loam soils the toxicity of heavy metals (except for Zn in the clay and sandy peat soil) was strongest. It should be noticed that the silty loam soil, with the highest Fe and Mn content, in the long run was generally more resistant to heavy metals than the clay and sandy peat soils were.

In comparable studies clay (for Cd and Zn) and organic matter (for Cd) were the main abiotic factors responsible for differences of the inhibition of respiration (Doelman and Haanstra 1984). These two soil fractions strongly bind extracellular urease (Dalal 1975; Beri et al. 1978) and may be the main fractions of binding heavy metals. However, a correlation between the inhibition of urease and the organic matter and clay content could not be found. Due to the high number of adsorbing sites present on humic molecules it is probably unlikely that heavy metals can interact directly with the active sites or with other parts of the enzyme so as to cause inhibition. The reported resistance of urease-humus complexes to denaturating agents (Nannipieri et al. 1978) may also be true for heavy metals.

This research elaborates the "ecological dose" concept concerning the impact of heavy metals on some microbe-mediated ecological processes in soil by Babich et al. (1983). According to them, this concept has the advantage that it reflects the combined response of numerous populations of soil microorganisms. Soil urease activity is a combined response of a large part of the soil microflora. Another advantage is that the influence of soil physicochemical properties is incorporated in the ED values.

By presenting the results as in Fig. 2, not only ED50 values as suggested by Babich et al. but other values can be estimated and used for the formulation of a Soil Protection Act. Soil microbiologists must indicate which ED value is an acceptable value to ensure the maintainance of the soil mineralization capacity. The first distinct measurable inhibitory dose should be considered as an important ecological value, since from that point a gradual increase in the reduction of mineralization of organic matter will occur as described by the EDR.

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References

Alvey N, Galway N, Lane P (1982) An introduction to GENSTAT. Academic Press, London

Babich H, Bewley RJE, Stotzky G (1983) Application of "Ecological Dose" concept to the impact of heavy metals on some microbe-mediated ecological processes in soil. Arch Environ Contam Toxicol 12:421–426

Beri V, Goswami KP, Brar SS (1978) Urease activity and its Michaelis constant for soil systems. Plant and Soil 49: 105–115

Bremner JM, Douglas LA (1971) Inhibition of urease activity in soils. Soil Biol Biochem 3:297–307

Bremner JM, Mulvaney RL (1978) Urease activity in soils. In: Burns RG (ed). Soil enzymes. Academic Press, London, pp 149–196

Burns (1982) Enzyme activity in soil: Location and a possible role in microbial ecology. Soil Biol Biochem 14:423–427

Dalal RC (1975) Urease activity in some Trinidad soils. Soil Biol Biochem 7:5–8

Doelman P, Haanstra L (1984) Short-term and long-term effects of cadmium, chromium, copper, nickel, lead and zinc on microbial respiration in relation to abiotic soil factors. Plant and Soil 79:317–327

Guidi G, Poggia G, Petruzzelli G (1973) Determinazione automatica dell'urea nel terreno. Proceedings Simposio Internazionale Technicon, Milano

Haan S de (1978) Sewage sludge as organic fertilizer or as a means for soil-improvement in grassland (in Dutch). Boerderij/Veehouderij 62:30–37

Haanstra L, Oude Voshaar JH, Doelman P (1985) The use of sigmodial dose response curves in soil exotoxicological research. Plant and Soil 84:293–297

Jagnow G (1971) Seasonal amounts of fungal mycelium, numbers of aerobic bacteria and bacterial spores and numbers of sacharolytic anaerobic bacteria and bacterial spores in a beech and spruce forest soil of the Solling. Proceedings 4th International Colloquium for Soil Zoologie, INRA, Dijon, France, pp 303–312

Mayer R (1985) Ein Bodenkundler warnt: In Schwermetall-Anreicherung plus saurem Niederschlag liegt die stärkste Bedrohung. Bild der Wissenschaft 3:63–70

Ministerie van Volksgezondheid en Milieuhygiëne (1981) Regulations concerning soil protection: the soil protection act (in Dutch). Staatsuitgeverij, 's Gravenhage, p 133

Nannipieri P, Ceccauti B, Cervelli S, Sequi P (1978) Stability and kinetic properties of humus-urease complexes. Soil Biol Biochem 10:143–147

Nannipieri P, Muccini L, Ciardi C (1983) Enzyme activity and microbial biomass: production and persistence. Soil Biol Biochem 15:679–685

Nannipieri P (1984) Microbial biomass and activity measurements in soil: Ecological significance. In:Klug MJ, Reddy CA (eds) Current perspectives in microbial ecology. American Society of Microbiology, Washington DC, pp 515–521

Nor YM (1982) Soil urease activity and kinetics. Soil Biol Biochem 14:63–65

Olson U (1921) Über Vergiftung der Amylase durch Schwermetalle und organische Stoffe. Z Phys Chem 78:509–516

Pancholy SK, Rice EL, Turner JA (1975) Soil factors preventing revegetation of a denuded area near an abandoned zinc smelter in Oklahoma. J Appl Ecol 12:337–342

Tabatabai MA (1977) Effects of trace elements on urease activity in soils. Soil Biol Biochem 9:9–13

Tyler G (1972) Heavy metals pollute nature, may reduce productivity. Ambio 1:52–59

Tyler G (1974) Heavy metal pollution and soil enzymatic activity. Plant and Soil 41:303–311

Williams ST, McNeilly T, Wellinton EMH (1977) The decomposition of vegetation growing on metal mine waste. Soil Biol Biochem 9:271–275

Zantua MJ, Bremner JM (1975a) Comparison of methods of assaying urease activity in soils. Soil Biol Biochem 7:291–295

Zantua MJ, Bremner JM (1975b) Preservation of soil samples for assay of urease activity. Soil Biol Biochem 7:297–299

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