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The ecological dose value (ED_{50}) for assessing Cd toxicity on ATP content and dehydrogenase and urease activities of soil

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Abstract

Two soils of contrasting texture, organic matter content and pH were treated with $CdSO_4$ solutions to give a Cd concentration range of 0–4000 mg kg^{-1} soil. The content of ATP and dehydrogenase and urease activities of soils were assayed after 3 h, and 7 and 28 days of Cd contamination. The relative ED_{50} values were calculated by two kinetic models (model 1 and model 2) used by Speir et al. (1995) and by the sigmoidal dose–response model (model 3) employed by Haanstra et al. (1985). Model 1 was the most successful in calculating the ED_{50} values for the ATP content, urease and dehydrogenase activities when both soils were contaminated by Cd. Similar ED_{50} values were predicted by model 1 (describing the full inhibition) and model 3 only when the correlation coefficients r^2 were higher than 0.9. The ED_{50} values of ATP calculated by model 1 were markedly higher than those calculated by model 2 (describing partial inhibition) when both models gave correlation coefficients higher than 0.9. This behavior was due to the high asymptote values obtained using model 2. According to model 2, some of the enzyme activities responsible for the ATP synthesis were probably not inhibited at the highest Cd concentrations. The inhibitory effect of Cd on the ATP content and both enzymatic activities was lower in the Castelporziano soil, which had the highest total organic carbon content.

Keywords: ED_{50}; Cd contamination; Soil ATP; Soil dehydrogenase activity; Soil urease activity

1. Introduction

Soil contamination with heavy metals has been reported to affect negatively the composition and activity of soil microflora (Bååth, 1989; Brookes, 1995; Nannipieri et al., 1997). Sources of this contamination are repeated applications of sewage sludges, municipal wastes and animal slurries, the activity of smelting industries, impurities in fertilizers and deposition of air pollutants derived from burning of fossil fuels and various industrial activities. It is important to ascertain the maximum amounts of heavy metals that can be supported by a soil without any effect on its quality. In order to quantify easily the influence of pollutants on microbe-mediated processes in soil, Babich et al. (1983) developed the concept of an "ecological dose 50%" (ED_{50}), which is the concentration of a toxicant that inhibits a microbe-mediated ecological process by 50%.

The ED_{50} can also be useful in determining which factors affect the toxicity of the heavy metal. These factors can include the time (Doelman and Haanstra, 1986) and soil abiotic properties (Doelman and Haanstra, 1984) affecting the speciation and mobility of heavy metals in soils and controlling their availability and residence time. In addition, the ED_{50} can be used to establish which microbiological and biochemical properties of soil are most sensitive to heavy metal contamination (Speir et al., 1995).

Different mathematical models have been used to calculate the ED_{50} values. Speir et al. (1995, 1999) employed a Michaelis–Menten kinetic approach to model inhibition of soil biological properties by Cr (VI) and As (V). Haanstra et al. (1985) proposed a sigmoidal dose–response model relating the tested parameters to the natural logarithm of the heavy metals concentration.

The aim of this study was to compare the above-mentioned approaches to assess the inhibition of the ATP content and dehydrogenase and urease activities of two different soils by Cd pollution. These soil parameters were assayed at different periods in order to assess the effect of duration of exposure to Cd pollution.

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2. Materials and methods

2.1. Soil samples and handling

Soils were surface sampled (0–10 cm) from two different areas of Italy: Montepaldi (15 km SW of Florence) and Castelporziano (30 km W of Rome). The two soils were characterized by different physico-chemical properties (Table 1). The field-moist soil samples were sieved (<2 mm) and stored in plastic bags at 4°C. Before the experiment, soil samples were pre-incubated for 7 days at 25°C to stabilize microbial activity.

Table 1
Properties of soils used in Cd toxicity tests

	Montepaldi	Castelporziano
Sand (%)	66.0	87.5
Silt (%)	21.0	8.0
Clay (%)	13.0	4.6
CEC (meq 100 g^{-1})	17.3	6.7
$CaCO_3$ (%)	3.5	0.08
TOC (%)	1.7	2.27
pH (H_2O)	8.1	4.8
Total N (%)	0.18	0.09
Cd (mg kg^{-1} dry soil)	7.51	1.44
Ni (mg kg^{-1} dry soil)	51.53	12.62
Cu (mg kg^{-1} dry soil)	194.66	4.48
Zn (mg kg^{-1} dry soil)	88.91	27.5

2.2. Experimental design

The soils (100 g) were treated with 5 ml of $CdSO_4$ solutions to give a Cd concentration ranging from 3 to 4000 mg kg^{-1} soil (20 different Cd concentrations in soils). Untreated soils served as controls. The soil moisture was adjusted to 55% of the WHC with distilled water and then the soils were incubated under controlled conditions (25°C and darkness) for 3 h, and 7 and 28 days.

2.3. Soil analyses

ATP was extracted from the soils by the procedure of Webster et al. (1984) and determined as reported by Ciardi and Nannipieri (1990). Soil dehydrogenase activity was determined using *p*-iodonitrotetrazolium chloride (INT), as reported by Von Mersi and Schinner (1991), and expressed as μg iodonitrotetrazolium formazan (INTF) h^{-1} g^{-1} soil. Soil urease activity was determined by the buffered method, as reported by Kandeler and Gerber (1988), and expressed as μg NH_4^+–N h^{-1} g^{-1} soil. Water-soluble Cd was determined in the following way: 20 g soil were shaken for 2 h; the mixture was then centrifuged (5000 rpm) and the supernatant filtrated through Watman 42 filter paper. Cadmium concentration was measured using a Perkin-Elmer 1100B atomic absorption spectroscope.

2.4. Mathematical analysis of data

The two kinetic models proposed by Speir et al. (1995) and the sigmoidal dose–response model proposed by Haanstra et al. (1985) were used to assess the inhibition of soil biological and biochemical properties by Cd. The algebraic expressions of kinetic models were $\nu = c(1 + bi)$ (model 1) and $\nu = c(1 + ai)/(1 + bi)$ (model 2). The constants a, b and c were always positive, with $b > a$. Model 1 describes the full inhibition of ν (tested parameter) by i, the concentration of inhibitor (Cd concentration), and model 2 describes the partial inhibition. For data fitting model 1 it was possible to calculate the ecological dose by the relationship $ED_{50} = 1/b$. In the case of model 2 the relationship was $ED_{50} = (1 - a/b)/(b - a)$. Model 2 describes a concave rectangular hyperbolic relationship between ν and i, with asymptote ca/b parallel to but above the x-axis. The equation for the sigmoidal dose–response model was $y = a/(1 + e^{b(x-c)})$, where y is the tested parameter, x is the natural logarithm of Cd concentration, a is the uninhibited value of y, b is a slope factor value and c is the natural logarithm of ED_{50}. Diagrammatic representations of these three models are presented in Speir et al. (1995) and Haanstra et al. (1985).

3. Results

3.1. Water-soluble Cd

Water-soluble Cd was higher in Castelporziano than in Montepaldi soil throughout (Table 2). In both soils water solubilized a lower percentage of the added Cd in the

Table 2
Ranges in the percentages of water-soluble Cd values

Treatment range (mg Cd added kg^{-1} soil)	Incubation time		
	3 h	7 days	28 days
Montepaldi (%)			
3–1000	0.04–0.55	0–0.45	0.02–0.57
1500–4000	1.01–6.24	1.69–11.40	0.58–3.37
Castelporziano (%)			
3–1000	0.82–4.09	0.86–4.62	1.04–3.43
1500–3000	5.80–10.	6.27–24.12	5.43–15.29

Table 3
The ED_{50} values of ATP, and urease and dehydrogenase activities in Montepaldi and Castelporziano soils after 3 h and 7 and 28 days of Cd pollution. N.F. indicates no fit of the data to the model; r^2 and asymptote values, although calculable, are not valid. N.I. indicates no inhibition of urease activity by Cd amendment. Model 1 describes full inhibition with the algebraic form $\nu = c/(1 + bi)$. Model 2 describes partial inhibition with the algebraic form $\nu = c(1 + ai)/(1 + bi)$. Model 3 describes a sigmoidal dose–response curve with the algebraic form $y = a/(1 + e^{b(x-c)})$

	Montepaldi			Castelporziano		
	3 h	7 days	28 days	3 h	7 days	28 days
ATP						
Model 1						
ED_{50}[a]	1194.4	4347.8	1250	9090.9	12500	33333.3
r^2	0.963	0.9778	0.982	0.9773	0.968	0.988
Model 2						
ED_{50}	N.F.	76.92	1000	250	500	N.F.
r^2	–	0.995	0.983	0.986	0.972	–
Asymptote[a]	–	29.6	5.26	34.4	14.38	–
Model 3						
ED_{50}	1156.4	2835.6	1224.2	18215	32306	16155
r^2	0.813	0.82	0.87	0.49	0.19	0.10
Dehydrogenase						
Model 1						
ED_{50}	909.1	3703.7	3846.2	2702.7	1234.6	5555.6
r^2	0.976	0.955	0.874	0.992	0.978	0.982
Model 2						
ED_{50}	N.F.	N.F.	N.F.	1265.8	1123.6	N.F.
r^2	–	–	–	0.993	0.978	–
Asymptote	–	–	–	60.6	8.71	–
Model 3						
ED_{50}	994.3	2751.8	2275.6	2751.8	1248.9	4402.8
r^2	0.903	0.676	0.519	0.88	0.848	0.593
Urease						
Model 1						
ED_{50}	1538.5	1162.8	1190.5	4166.7	909.1	N.I.
r^2	0.914	0.99	0.987	0.982	0.937	–
Model 2						
ED_{50}	N.F.	N.F.	N.F.	N.F.	1000	N.I.
r^2	–	–	–	–	0.938	–
Asymptote	–	–	–	–	0.065	–
Model 3						
ED_{50}	1860.2	1236.4	1331.4	4265.4	914.1	N.I.
r^2	0.685	0.949	0.951	0.654	0.712	–

[a] ED_{50} and asymptote values are expressed as mg Cd kg^{-1} soil.

3–1000 than in the 1500–3000 mg Cd kg^{-1} soil treatment range.

3.2. Effect of Cd pollution on soil ATP content and dehydrogenase and urease activities

The ED_{50} values calculated with the three models are shown in Table 3. Some typical plots showing the fitting of the measured parameters to the models used are presented in Figs. 1–3. With the exception of the urease activity of Castelporziano soil after 28 days of contamination, the inhibition of the tested parameters by Cd concentration in both soils was described by at least one of the tested models. Model 3, which describes a sigmoidal dose–response curve, was less successful than models 1 and 2 in fitting the experimental data. Only in three cases (dehydrogenase activity of Montepaldi soil at 3 h and urease activity of Montepaldi soil at 7 and 28 days) the r^2 values of model 3 were higher than 0.9 (Table 3).

The ED_{50} values of ATP were always found to be higher in Castelporziano soil than in Montepaldi soil when the results of the same model were compared. This was not the case for dehydrogenase and urease activity (Table 3).

Generally the ED_{50} values of dehydrogenase and urease activities predicted by model 1 and model 3 were similar when their correlation coefficients (r^2) were higher than 0.9 (Table 3). After 7 days of Cd contamination the ED_{50} values of dehydrogenase activity of Castelporziano soil, predicted

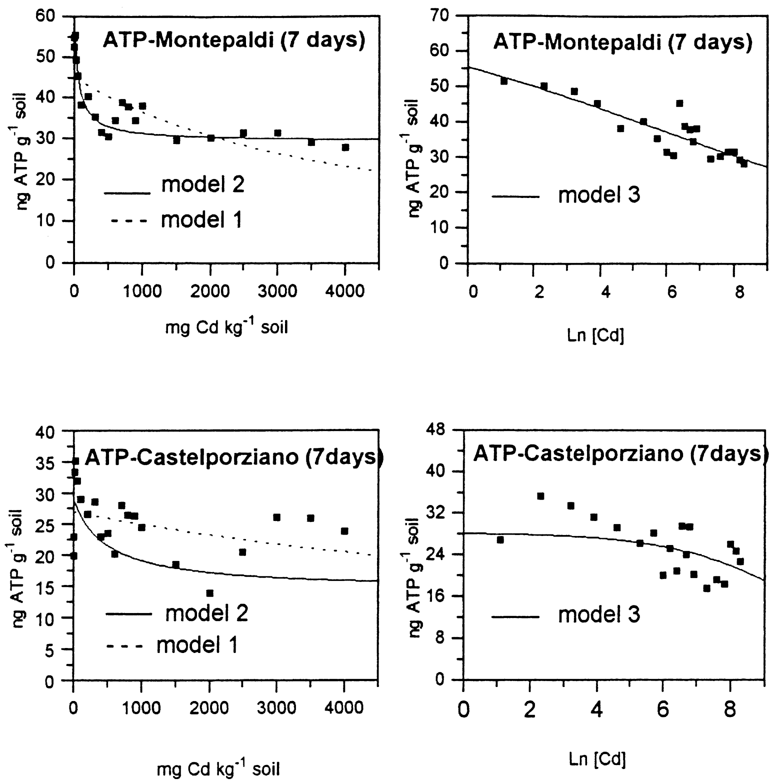

Fig. 1. Experimental data and calculated plots of the relationship between ATP content and total Cd concentration according to model 1 and model 2, describing full and partial inhibition, respectively, and the sigmoidal dose–response model (model 3).

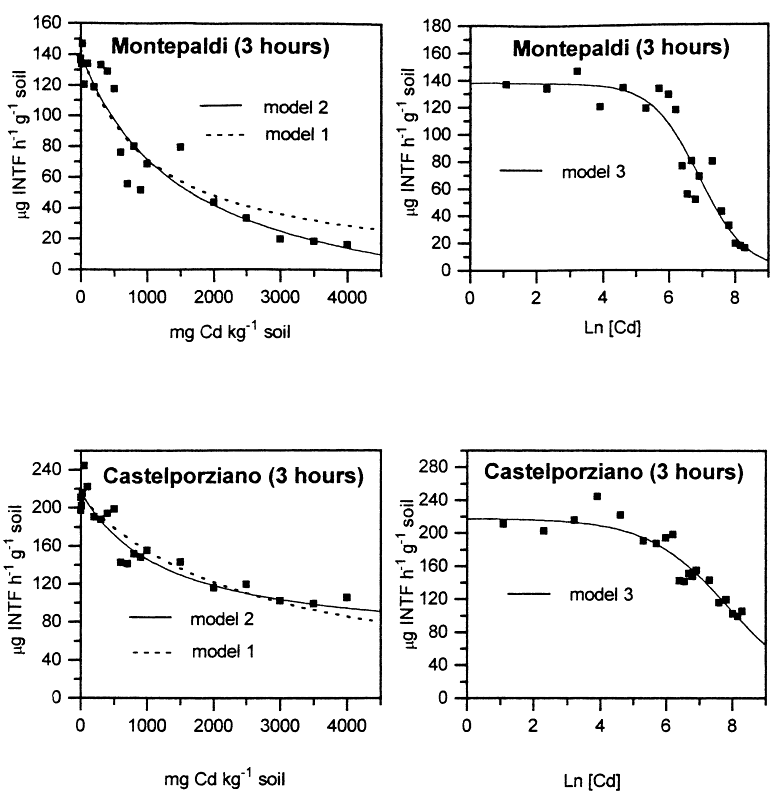

Fig. 2. Experimental data and calculated plots of the relationship between dehydrogenase activity and total Cd concentration according to model 1 and model 2, describing full and partial inhibition, respectively, and the sigmoidal dose–response model (model 3).

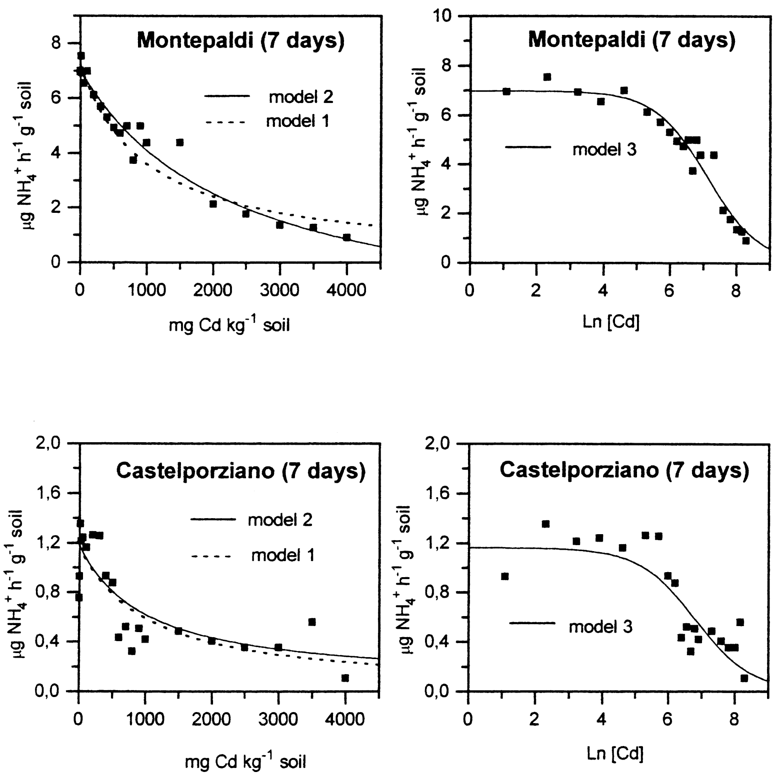

Fig. 3. Experimental data and calculated plots of the relationship between urease activity and total Cd concentration according to model 1 and model 2, describing full and partial inhibition, respectively, and the sigmoidal dose–response model (model 3).

by model 1 and model 2, were similar (Table 2) because the asymptote value of model 2 was close to zero (data not shown). The same was observed for the urease activity in Castelporziano soil after 7 days of Cd pollution (Fig. 3).

The ED_{50} values for the ATP content of Montepaldi soil peaked after 7 days whereas in Castelporziano soil the highest value was reached after 28 days when the values were calculated by model 1 (Table 3). Calculation by model 3 gave the highest values at 7 days. Usually the ED_{50} values of dehydrogenase were the highest at 28 days in both soils, whereas those of urease remained generally constant in Montepaldi and decreased at 7 days in Castelporziano soil, with no inhibition at 28 days.

4. Discussion

Water-soluble Cd, which may indicate the amount of Cd available to microbial populations, was greater in Castelporziano soil than in Montepaldi soil (Table 2). Precipitation of Cd as carbonates and hydroxides could occur in Montepaldi soil due to the presence of carbonates and alkalinity (pH value of 8.1). In addition to a lower pH value, the Castelporziano soil was also characterized by higher sand and organic matter contents and a lower clay content than Montepaldi soil (Table 1). Several processes affect heavy metal solubility in soil besides pH and presence of carbonates (McBride, 1989; Alloway, 1995). Negative charges on both organic and inorganic colloids surfaces hold cations by non-specific electrostatic forces, which depend only on the charge and hydration of the cation. However, the amount of non-exchangeable forms generally prevails over the exchangeable forms (McBride, 1989). Metal oxides and hydroxides as well as amorphous aluminosilicates provide surface sites for chemisorption of heavy metals. Usually Cd^{2+} has higher affinity than Mg^{2+} but lower affinity than Pb^{2+}, Cu^{2+}, $Zn^{2+}$and Ni^{2+} for these surfaces. The content of soluble organic ligands may have been higher in Castelporziano soil than in Montepaldi soil not only for the higher organic C content but also for soil use. Castelporzaino, being a forest soil, is not plowed every year as the arable Montepaldi soil. Organic matter of no-till soil is richer in water-soluble organic compounds but less aromatic than organic matter of plowed soils (Arshad et al., 1990) Complexation of Cd with low-molecular-weight organic ligands in the soil solutions can decrease Cd sorption by soil colloids (Alloway, 1995; Krishnamurti et al., 1997).

Castelporziano has a higher microbial biomass C content than Montepaldi (570 and 380 μg C g^{-1} soil, respectively). Microorganisms can solubilize heavy metals in soil by producing water-soluble organic compounds or ligands that form soluble metal complexes (Francis et al., 1980) or by releasing microbial metabolic products that change the physico-chemical conditions of the soil environment (Charmugathas and Bollag, 1987). Microorganisms can also render heavy metals insoluble in soil by producing water-insoluble organic compounds or ligands that form insoluble metal complexes. A bacterium isolated from a loamy soil released a protein forming a water-insoluble Cd compound (Kurek et al., 1991). No data are available on the persistence of this water-insoluble Cd–protein complex in soils where a highly diverse microbial population is present (Torsvik et al., 1996).

In spite of the fact that water-soluble Cd values were higher in Castelporziano soil than in Montepaldi soil, the inhibitory effect of Cd on ATP was lower in Castelporziano soil as evidenced by the ED_{50} values. This behavior could be due to differences in the composition of soil microbiota with differences in sensitivity to metal toxicity (Giller et al., 1998).

The ED_{50} values were calculated using the Cd concentrations added to soil because the tested models were not fitted using the water-soluble Cd data. It may be questionable to use the kinetic models to calculate the ED_{50} value of a parameter such as ATP content, expressing a concentration value and not an enzyme activity. However the ATP content depends on various enzyme activities involved in either ATP synthesis or degradation. Cd can inhibit one or more of these enzyme activities and thus it can affect the soil ATP content indirectly.

The ED_{50} values of the ATP content predicted by model 2 were much smaller than those calculated from model 1, probably due to the high asymptote values. By fitting the data according to model 2, the ATP content did not fall to zero but to an asymptote parallel to but above the *x*-axis (Fig. 1). It may be possible that some of the enzyme activities involved in the ATP synthesis were not inhibited by the increase in Cd concentration. In this case it can be erroneous to calculate the ED_{50} value because the decrease in ATP content by Cd pollution was not higher than 50% of the initial value. When the asymptote values tend to zero, model 1 and model 2 tend to equality and the ED_{50} values were similar, such as in Montepaldi soil after 28 days (Table 3). The ED_{50} values predicted by model 1, which describe a total inhibition, were also higher than those calculated by model 3.

No inhibition of urease activity was observed in Castelporziano soil at 28 days whereas the ED_{50} values of dehydrogenase activities generally peaked at 28 days. Possibly microbial and/or chemical processes made the Cd less available to soil microorganisms, and/or resistant microbial species were selected on prolonging the Cd contamination. Other authors have observed a consistent effect of duration of heavy metals exposure on the ED_{50} values (Doelman and Haanstra, 1986; Haanstra and Doelman, 1991; Speir et al., 1995) but they have used longer incubation periods than those used in this work.

The soil ATP content is an indicator of soil microbial biomass when the samples are pre-incubated under controlled conditions before the analysis (Jenkinson, 1988). Dehydrogenase is an intracellular enzyme, which is supposed to reflect microbial activity (Skujins, 1976; Nannipieri, 1994); on the other hand, the urease activity of a soil can reflect the contribution of both intracellular and extracellular enzymes, which can be adsorbed on inorganic colloids or enclosed in humic complexes. The decrease in ATP content or the inhibition of dehydrogenase activity by Cd may be due to the negative effects of the heavy metal on the activity of microbial species sensitive to Cd pollution. This can also be true for the inhibition of urease activity by Cd; however, in this case the possibility that Cd inhibits the activity of extracellular ureases stabilized by soil colloids can not be excluded.

In conclusion, model 1 was the most successful one in calculating the ED_{50} values for the ATP content and urease and dehydrogenase activities when Cd contaminated both soils. In spite of the higher water-soluble Cd concentration, the ED_{50} values of Castelporziano soil were higher than those of Montepaldi soil, probably due to the presence of more sensitive soil microbiota in the latter than in the former soil. The ED_{50} values generally increased with the duration of Cd exposure, probably due to the selection of more resistant microbial populations. A better understanding of the meaning of the ED_{50} values of metals-polluted soils requires determination of the composition of the microbial population, and how it changes with the duration of the exposure to heavy metal also.

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