Toxic Effect of Cadmium on Soil Microbial Activity and Plant Growth: Influence of Soil Characteristics and Management

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Abstract: In this study, we use biochemical measures related with the soil microbial activity to evaluate the toxic effects of Cd in two semiarid soils of different characteristics, which have been submitted to different management practices. The Cd concentrations which produced a 5, 10 and 50% inhibition of the parameter studied (Ecological Dose values) were calculated after incubation periods of 3 hours, 20 days and 60 days. In addition, *Lolium perenne* was cultivated in both soils containing the different Cd concentrations in order to ascertain the effect of Cd contamination on plant growth. When the dehydrogenase activity and ATP content of the soils were analysed, the calculated ED values were higher in the agricultural soil than in the abandoned soil. The yield of *Lolium perenne* fell gradually with increasing concentrations of Cd in the soil, the lowest decrease being observed in the agricultural soil at 60 days. The concentration of Cd in the plants increased gradually with the total and DTPA-extractable concentration of this heavy metal in the soil.
Keywords: Cadmium, ecological dose, soil microbial activity, plant growth

1 Introduction

Soil contamination by Cd may arise from both the indiscriminate use of fertilizers, pesticides and sewage sludge in agriculture and from industrial and mining activities (Alloway, 1995). The mobility and bioavailability of this element depend on soil characteristics. Thus, the pH, organic matter content, cation exchange capacity and redox potential of a soil and the plant species are factors which affect the mobility and bioabailability of Cd in the soil-plant system (Adriano, 1986). Soil management can also change its physical, chemical and biological characteristics, thus affecting the toxic effect of Cd.

The measurement of soil microbial activity or biomass is a sensitive indicator of Cd toxicity, which has been used by several authors (Moreno *et al*., 1997; Wilke, 1991). The ecotoxicity of heavy metals or other pollutants can be estimated by their "ecological dose" or concentration, which produces a given inhibition of the microbiological parameter studied in relation to a control without contamination (Babich *et al*., 1983). These values significantly vary with soil characteristics and the time elapsing since Cd contamination began (Doelman and Haanstra, 1984; Speir *et al*., 1995). The results of studies on heavy metal toxicity to soil microbial populations and processes have been used to establish the soil concentrations at which heavy metals affect microbial processes for regulatory purposes (Giller *et al*., 1997). However, in heavy metal polluted soils, there is a risk of accumulating fairly large amounts of heavy metals in plants without their showing stress, which increases the potential risk of their consumption in the diet without being noticed (Oliver, 1997). For this, it is necessary to study the effects of heavy metals on plants and the risk of their accumulation in animals and humans.

In this study, we used biochemical measures related with soil microbial activity to evaluate the toxic effects of Cd in two semiarid soils of different characteristics which had been submitted to different management practices. The ecological Cd doses (ED values) which produced a 5, 10 and 50% inhibition of the parameter studied in relation to a control were calculated after soil incubation periods of 3 hours, 20 days and 60 days. In addition, *Lolium perenne* was cultivated in both soils containing different Cd concentrations in order to ascertain the effect of Cd contamination on plant growth.

2 Materials and methods

2.1 Soils

The experiment was carried out with two different soils from Murcia (SE Spain). The soil from Abarán was collected from the 0—30 cm layer of a bare land which had been subjected to agricultural use for many years before being abandoned (for at least 10 years). The Cartagena soil was collected from 0—30 cm layer of an agricultural plot in current use. The characteristics of both soil are showed in Table 1.

Table 1 Soil characteristics

	ABARAN	CARTAGENA
Sand, %	24.54	27.25
Silt, %	54.12	30.65
Clay, %	21.35	42.10
pH (1:2.5 soil:water)	8.72	8.90
CEC meq/100 g	7.38	7.11
EC (1:5 soil:water), S cm^{-1}	72	106
Total $CaCO_3$, %	38.0	58.0
WHC, %	38.20	36
TOC, %	0.8	0.53
Humic substances, g kg^{-1}	0.21	0.31
Water soluble C, g kg^{-1}	0.02	--
Total N, g kg^{-1}	0.16	0.11
Total P, g kg^{-1}	0.45	0.301
Total K, g kg^{-1}	9.79	5.52
Na, g kg^{-1}	0.40	0.31
Fe, g kg^{-1}	12.6	32.3
Mn, mg kg^{-1}	3.93	7.15
Cd, mg kg^{-1}	< 0.05	< 0.05
Cu, mg kg^{-1}	0.24	0.23
Ni, mg kg^{-1}	< 0.1	0.16
Zn, mg kg^{-1}	0.46	2.88

2.2 Soil contamination and incubation

Two hundred grams of both soils were put into semi-closed microcosms (Naseby and Lynch, 1998) and treated with different amounts of Cd. Control microcosms lacking Cd treatment were prepared. The Cd treatments consisted of adding $CdSO_4$ solutions to achieve final concentrations in soil of 3, 50 , 200, 600, 1,000, 2,000, 4,000, 5,000 and 8,000 mg Cd kg^{-1} soil. All treatments were realized in triplicate. The microcosms were incubated in the dark at 25℃ and 70%—80% humidity for three periods of time (3 hours, 20 days and 60 days). The soil moisture content was adjusted to 50%—60% of its water-holding capacity (WHC) during the incubation time as needed. When the different time periods had concluded, 50 g of homogenised soil from each microcosm was put into plastic bags which were stored at 4℃ before use.

2.3 Lolium perenne crop

In the remaining 150 g of soil from each microcosm, 2 g of ryegrass (*Lolium perenne* L.) seeds were sown. The crop was grown in controlled conditions (28℃, 70%—80% of relative humidity and 14 hours of light/10 hours of darkness) for 19 days, after which the crop was harvested.

2.4 Soil biochemical parameters

Soil ATP and dehydrogenase activity were measured in triplicate and, the means of the triplicate measurement were used to calculate ED values.

The ATP was extracted from soil using the Webster *et al*. (1984) procedure and measured as recommended in Ciardi and Nannipieri (1990). To one gram of soil were added 20 ml of a phosphoric acid extractant, and the closed flasks were shaken in a cool bath. The mixture was filtered through Whatman paper and an aliquot was used to measure the ATP content by means of the luciferin-luciferase assay in a luminometer (Optocomp 1, MGM Instruments, Inc.)

Soil dehydrogenase activity (DH) was determined using 1 g of soil, and the reduction of p-iodonitrotetrazolium chloride (INT) to iodonitrotetrazolium formazan (INTF) was measured as a modification of the method reported by Von Mersi and Schiner (1991). DH activity was expressed as µg INTF h^{-1} g^{-1} soil.

2.5 Chemical analyses

Total organic C (TOC) was determined by Yeomans and Bremner's (1988) method. The C contents of the liquid fractions (one extractable by Na pyrophosphate and the other a water-soluble fraction, both with a solid/liquid ratio of 1/10) were determined spectrophotometrically after addition of $K_2Cr_2O_4$ and H_2SO_4 (digestion at 150℃ for 15 min.) according to Sims and Haby's method (1971).

The total content of heavy metals and micronutrients in the nitric-perchloric digestion extract, and the Cd extractable with diethylenetriaminopentacetic acid (DTPA; 0.005 M DTPA, 0.01 M $CaCl_2$ and 0.1 M trietanolamine solution, pH: 7.3; Lindsay and Norwell, 1978) were measured by atomic absorption spectrometry using a Perkin-Elmer 5500 spectrophotometer. The total contents of Na and K in the nitric-perchloric digestion extract were measured by flame emission using a Jenway PFP 7 photometer. The total P content was determined by the method of Murphy and Riley (1962) and the total N content was determined using the Kjeldahl method.

2.6 Mathematical models

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 calculate the ED values and to evaluate the suitability of these models to describe the inhibition of soil biochemical properties by Cd. The algebraic expressions of the kinetic models were: $v = c/(1 + bi)$ (Model 1) and $v = c(1 + ai)/(1 + bi)$ (Model 2). The constants *a*, *b* and *c* were always positive, with $b > a$. Model 1 describes the full inhibition of *v* (tested parameter) by *i*, the concentration of inhibitor (Cd concentration) and Model 2 describes the partial inhibition. From the equations of both Model 1 and Model 2 which best predicted the experimental data, it was possible to calculate the ecological dose values using the following relationships: $ED_{50} = 1/b$; $ED_{10} = 1/9b$; and $ED_5 = 1/95\ b$.

The mathematical equation for the sigmoidal dose-response model (Model 3) was: $y = a/\{1 + \exp[b(x - c)]\}$, 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 parameter indicating the inhibition rate, and *c* is the natural logarithm of ED_{50}. The ED values were calculated using the following expressions: $ED_{50} = \exp c$; $ED_{10} = \exp [c – (2.2/b)]$; and $ED_5 = \exp [c – (2.9/b)]$. Model 3 describes a logistic curve, which is the relationship between the measured activity and the natural logarithm of the inhibitor concentration. Diagrammatic representations of these three models are presented in Speir *et al*., (1995) and Haanstra *et al*., (1985). The values of the constants *a*, *b* and *c* of the models were estimated using Marquadt's iterative search algorithm of the statistical program STATGRAPHICS Plus version 2.1 (Statistical Graphics Corp.). The value of the regression coefficient (r^2) of the non-linear regression was only determined at $P < 0.05$.

3 Results and discussion

For soil DH activity and ATP content, ED values calculated with the three models were higher in the Cartagena soil than in the Abarán soil (Tables 2 and 3), meaning that the toxic effect of Cd on these two

biochemical parameter was lower in the former soil. This may have been due to the higher content of clay, $CaCO_3$ and Fe or Mn oxyhydroxides of the Cartagena soil as regards Abarán soil (Table 1). It is known that a high clay, $CaCO_3$ and Fe or Mn oxyhydroxide content decreases the Cd solubility and mobility (Adriano, 1986) and so its Cd bioavailability to soil microorganisms also decreases. However, another factor which may have been important for the toxic effect of Cd on these two biochemical parameters was the way in which the two soils have been managed. The Cartagena soil was an agricultural soil and its microbial communities may be different to those of the bare soil (Abarán soil) The regression coefficient (r^2) values of the models which fitted the inhibition of DH activity by Cd, were high in all cases. However the inhibition of ATP by Cd was poorly described by the three models used in some cases. Soil DH is an index of overall microbial activity (Trevors, 1984) and is very sensitive to changes produced in this microbial activity by heavy metals (Brookes, 1995). Soil ATP is highly correlated with soil microbial biomass C, when the soil is preincubated under controlled conditions before determination; but the measurement of ATP content immediately after sampling is assumed to be an index of the microbial activity in soil (Nannipieri, 1997). Thus, a high ATP content may result from stress conditions such as a high concentration of Cd. The ED values increased after 60 days of incubation compared with those after 3 hours of incubation in most cases, probably due to the growing insolubilization of Cd in soil with time. The increase of ED values with incubation time has also been observed for Cr and As in other related studies (Speir *et al*., 1995 and 1999).

Table 2 Ecological dose values and regression coefficient (r^2) for inhibition by Cd of dehydrogenase activity in Abaran and Cartagena soils 3 hours, 20 days and 60 days after Cd treatment

	Abaran			Cartagena		
	3 hours	20 days	60 days	3 hours	20 days	60 days
Model 1						
ED_5	58.5	25.1	75.2	65.8	175.4	263.2
ED_{10}	125.5	52.9	158.7	138.9	370.4	555.6
ED_{50}	1,111.1	476.2	1,458.6	1,250.0	3,333.3	5,000.0
r^2	0.935,2	0.860,8	0.838,9	0.966,5	0.805,7	0.783,2
Model 2						
ED_5	58.5	17.0	75.2	65.8	175.5	87.7
ED_{10}	123.5	35.8	158.7	138.9	370.4	185.2
ED_{50}	1,111.1	322.6	1,428.6	1250.0	3,333.3	1,666.7
r^2	0.935,0	0.875,6	0.838,0	0.966,5	0.807,5	0.802,0
Model 3						
ED_5	112.3	3.9	207.1	112.2	2,650.9	146.1
ED_{10}	207.1	10.1	336.1	211.6	2,053.6	365.9
ED_{50}	1,254.3	236.5	1,308.9	1,365.3	4,050.8	5,440.4
r^2	0.925,6	0.937,9	0.909,2	0.963,4	0.934,5	0.841,5

Increasing Cd concentration in the two soil resulted in lower ryegrass yield in both soils (Figure 1). Cd concentration higher than 2000 mg kg^{-1} in the Abarán soil and 5000 mg kg^{-1} in the Cartagena soil inhibited seed germination. Macnicol and Beckett (1985) found that a Cd concentration higher than 30—35 mg kg^{-1} dry matter produced a depletion of ryegrass yield. Cadmium is a heavy metal of significant concern because its accumulation in plants may be harmful to human health (Mortvedt, 1996). In this study, the Cd content of ryegrass increased as the content of DTPA-extractable Cd increased in the two soils, and there was a linear correlation between these two variables (Table 4). The measurement of DTPA-extractable Cd fraction has been used previously to estimate the fraction available to plants (Singh and Narwal, 1984).

Table 3 Ecological dose values and regression coefficient (r^2) for inhibition by Cd of ATP content in Abaran and Cartagena soils 3 hours, 20 days and 60 days after Cd treatment

	Abaran			Cartagena		
	3 hours	20 days	60 days	3 hours	20 days	60 days
Model 1						
ED_5	175.4	131.6	65.8	526.3	526.3	526.3
ED_{10}	370.4	277.8	138.9	1,111.1	1,111.1	1,111.1
ED_{50}	3,333.3	2,500.0	1,250.0	10,000.0	10,000.0	10,000.0
r^2	0.799,6	0.874,9	0.878,6	0.646,3	0.800,1	0.840,6
Model 2						
ED_5	21.9	131.6	65.8	175.4	526.3	526.3
ED_{10}	46.3	277.8	138.9	370.4	1,111.1	1,111.1
ED_{50}	416.7	25,000.0	1,250.0	3,333.3	10,000.0	10,000.0
r^2	0.831,9	0.874,9	0.878,4	0.655,3	0.800,9	0.641,3
Model 3						
ED_5	5.1	140.7	28.8	256.9	713.4	1,391.7
ED_{10}	25.2	283.8	67.0	593.8	1,238.3	2,443.0
ED_{50}	2,683.6	2,235.9	1,103.5	6,974.4	6,265.4	12,778.3
r^2	0.886,1	0.914,0	0.870,6	0.649,4	0.823,3	0.700,6

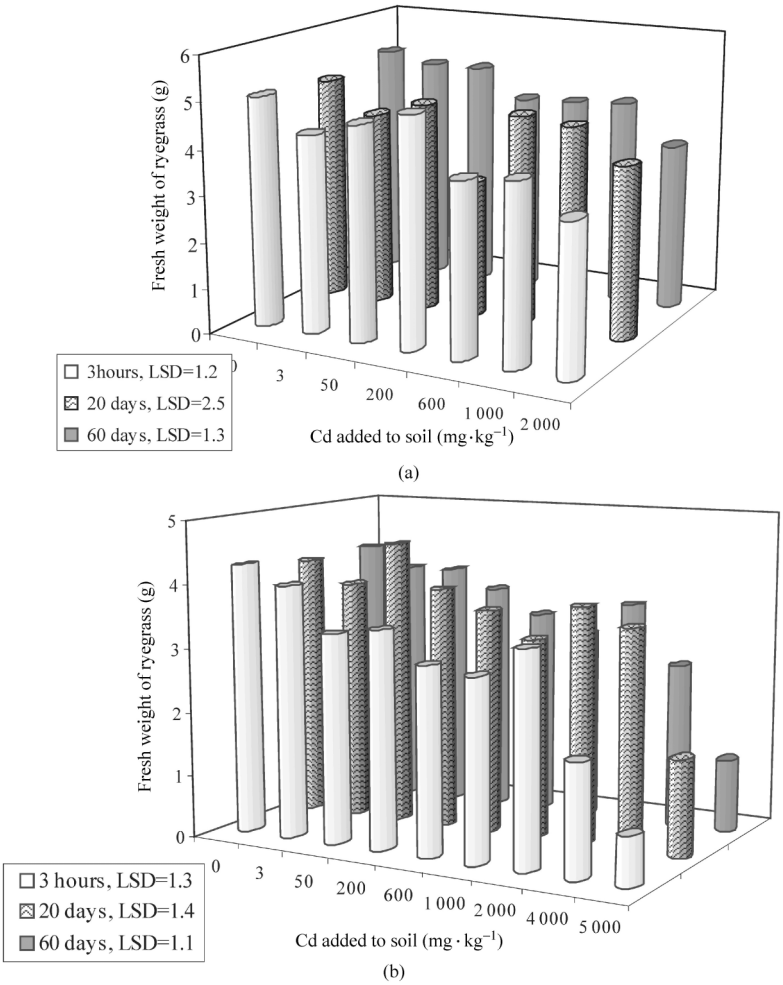

Fig.1 Yield of ryegrass in Abarán soil (a), and in Cartagena soil (b) with different concentrations of Cdafter 3 hours, 20 days and 60 days of incubation

Table 4 Linear correlation between Cd extractable with DTPA from the two soils and Cd content in plant after 3 hours and 60 days of incubation

	r^2	Significance[1]	n
Abaran soil, 3 hours	0.9892	**	5
Abaran soil, 60 days	0.9469	*	5
Cartagena soil, 3 hours	0.8917	*	6
Cartagena soil, 60 days	0.9898	**	6

[1] Significance: * $p<0.01$; ** $p<0.001$

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