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The biochemical response to different Cr and Cd concentrations in soils amended with organic wastes

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ABSTRACT

The effects of adding municipal solid waste (MSW) or poultry manure (PM) on the biochemical properties of a soil polluted with Cr and Cd were studied. Soil was mixed with $Cr(NO_3)_3$ and $Cd(NO_3)_2$ to give three concentrations (0, 100, and 250 mg Cr kg^{-1} and 0, 100, and 250 mg Cd kg^{-1}) in the soil, which was then treated with MSW at a rate of 10% or PM at a rate of 7.6%. The pH and biochemical parameters were measured at 0 and 120 days. An unamended and no-polluted soil was used as control. Compared with the non-polluted soil, for the 250 mg Cd kg^{-1} treatment the microbial biomass-C, dehydrogenase, urease, β-glucosidase, phosphatase, and arylsulphatase activities decreases 23%, 26.2%, 36%, 34.8%, 18.4%, and 15.8%, respectively, whereas for 250 mg Cr kg^{-1} treatment the biochemical parameters were slightly lowest than for 250 mg Cd kg^{-1} treatment. For 250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil treatment, the inhibition percentages of the biochemical parameters increased. After the application of organic wastes in Cr + Cd polluted soil, the inhibition of biochemical properties was greater with the MSW amendment than with PM, possibly due to its higher humic acid concentration.

1. Introduction

In recent years, pollution of soils by heavy metals has increased steadily as a result of increased application of organic amendments, uncontrolled dumping, etc. Considering the toxicity of heavy metals to organisms, their presence will affect the microbiology of the soil ecosystem. For this reason, in the last decade, great effort has been put into the study of amendments capable of heavy metal adsorption, immobilization or precipitation in order to reduce their bioavailable fractions in the soil solution and, therefore, their negative effect on soil biological properties. In this regard, we emphasize the role of various sorbents, such as calcite [1], goetite [2], birnesite [3], and zeolite [4].

However, the sorbents most widely used, perhaps because of their higher adsorption capacity, are the organic wastes. In this respect, many studies have highlighted the influence of applying different organic wastes, such as sewage sludge, manure, organic fertilizers with ferrous compounds, poultry manure, and compost, to soil in order to immobilize metals and reduce their negative effects on soil microbial populations and thus on their enzyme activities [5–8].

However, we must take into consideration that the behavior of these organic compounds depends on their chemical composition. Therefore, the effective adsorption of heavy metals will differ between different sources of organic matter.

Basically, organic matter consists of humic substances constituted by humic and fulvic acids and defined as a heterogeneous mixture of organic macromolecules with a very complex chemical structure. These acids have a high content of free functional groups (mainly –COOH and –OH) that are complexed and/or chelated with cations.

Tejada et al. [7,8] suggest that humic substances of higher molecular weight (humic acids) have a greater number of active carboxylic groups than those of lower molecular weight (fulvic acids). For this reason, in the soil, the complexation of heavy metals is greater in humic substances rich in humic acids, resulting in a lesser negative effect of these metals on soil organisms. Therefore, the application of organic matter with a higher content of humic acids than of fulvic acids is of interest in the remediation of soils contaminated by heavy metals.

This complexation by soil organic matter is one of the processes that govern the solubility and assimilation of heavy metals. Obviously, by increasing the amount of metal fixed in the humic substances, the concentration of metal in the soil solution, and therefore the negative impact on soil microorganisms, will be lower.

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Table 1
Characteristics of the experimental soil and organic wastes and the mixture soil + organic wastes, before the pollution with different rates of Cr and Cd, and standard errors. Data are the means of four samples.

	Soil	PM	MSW	Soil + PM	Soil + MSW
pH (H_2O)	8.6 ± 0.2	7.1 ± 0.3	6.2 ± 0.3	7.9 ± 0.2	7.1 ± 0.3
CO_3^{2-} (g kg^{-1})	203 ± 12				
Fine sand (g kg^{-1})	142 ± 35				
Coarse sand (g kg^{-1})	387 ± 26				
Silt (g kg^{-1})	242 ± 19				
Clay (g kg^{-1})	229 ± 10				
Clay types	Smectite: 66% Kaolinite: 20% Illite: 14%				
Organic matter (g kg^{-1})	4.1 ± 0.8	614 ± 26	469 ± 15	27.4 ± 2.6	27.3 ± 2.8
Humic acid-C (g kg^{-1})	12.5 ± 2.4	21.4 ± 2.5	26.3 ± 0.7	23.9 ± 1.2	22.7 ± 1.4
Fulvic acid-C (g kg^{-1})	9.8 ± 1.1	57.8 ± 0.2	35.7 ± 1.7	11.7 ± 0.6	17.1 ± 2.3
Total N (g kg^{-1})	0.4 ± 0.1	40.8 ± 0.9	17.3 ± 1.3	1.8 ± 0.2	1.3 ± 0.2
Fe (mg kg^{-1})	35.8 ± 3.7	180 ± 22	815 ± 38	41.9 ± 1.1	74.5 ± 2.5
Cu (mg kg^{-1})	9.7 ± 1.3	1.6 ± 0.3	82.6 ± 9.8	9.7 ± 0.6	13.5 ± 1.2
Mn (mg kg^{-1})	11.3 ± 2.1	4.2 ± 0.9	75.6 ± 8.1	10.9 ± 0.8	14.9 ± 2.1
Zn (mg kg^{-1})	8.1 ± 1.5	3.3 ± 0.8	134 ± 13	8.0 ± 1.1	14.1 ± 1.7
Cd (mg kg^{-1})	6.5 ± 1.2	0.35 ± 0.17	1.1 ± 0.3	6.5 ± 0.9	6.5 ± 1.1
Pb (mg kg^{-1})	0.36 ± 0.11	0.94 ± 0.12	82.4 ± 3.6	0.38 ± 0.11	4.3 ± 1.5
Ni (mg kg^{-1})	2.9 ± 0.7	1.3 ± 0.2	13.6 ± 1.5	2.8 ± 0.3	3.5 ± 1.2
Cr (mg kg^{-1})	5.3 ± 0.6	0.12 ± 0.02	19.4 ± 1.7	5.3 ± 0.4	6.1 ± 0.8

The current literature indicates that soil enzymatic activities react faster than physical variables and/or after any chemical change in the soil and, therefore, may be useful as early indicators of the various biological changes that may occur in soil [9].

Although there is much information about the adverse effects of heavy metals on soil enzymatic activities, there is not much information on how these activities evolve when the soil is contaminated with various metals at different rates. Therefore, the first objective of this work is to study the effect of two metals (Cr and Cd) very toxic to soil microorganisms, at different concentrations, on some soil biological properties (microbial biomass-C and enzymatic activities associated with the cycles of C, N, and P in soil), which will inform us about the impact of such concentrations of these heavy metals on soil biology. The second objective is to study the adsorption capacity of a poultry manure and the organic fraction of a municipal solid waste in a soil contaminated by Cd and Cr, alone or combined at different concentrations, and their effect on the soil biological properties mentioned previously.

2. Materials and methods

2.1. Soil and organic wastes

The soil used in this experiment is a Plagic Antrosol [10]. Soil samples were collected from the 0–25 cm surface layer. The main soil characteristics are shown in Table 1.

Soil pH was determined in distilled water with a glass electrode (soil:H_2O ratio 1:2.5). Soil texture was determined by Robinson's pipette method [11], and quantification and the dominant clay types were determined by X-ray diffraction. Total carbonates were measured by quantifying the CO_2 produced by HCl addition to the soil [12]. Soil organic matter was determined by the method of Yeomans and Bremner [13]. Humic and fulvic acids were extracted with 0.1 M sodium pyrophosphate and 0.1 M sodium hydroxide at pH 13 [14]. The supernatant was acidified to pH 2 with HCl and allowed to stand for 24 h at room temperature. To separate humic-like acids from fulvic-like acids, the solution was centrifuged and the precipitate, containing humic-like acids, was dissolved with sodium hydroxide [13]. After the removal of humic-like acids, the acidic filtrate, containing the dissolved fulvic acid-like fraction, was passed through a column of XAD-8 resin. The adsorbed fulvic fraction was then recovered by elution with 0.1 M NaOH, desalted using Amberlyst 15 cation-exchange resin, and, finally, freeze-dried. The carbon contents of the humic and fulvic-like acids were determined by the method described. Total N was determined by the Kjeldhal method [12]. After nitric and perchloric acid digestion, the total Ca, Mg, Fe, Cu, Mn, Zn, Cd, Pb, Ni, and Cr concentrations were determined by atomic absorption spectrometry and K was determined by atomic emission spectrometry, according to MAPA methods [12].

The organic wastes applied were the organic fraction of a municipal solid waste (MSW) and a poultry manure (PM). The organic fraction of municipal solid waste is obtained by mechanical treatment of mixed municipal solid waste, which is after biologically treated/stabilized by composting. The general properties of both organic wastes are shown in Table 1.

Organic matter was determined by dry combustion, according to the official methods of the Spanish Ministry of Agriculture [12]. Humic and fulvic acids were extracted, separated, and determined by the methods previously described. Total N was determined by the Kjeldhal method [12]. The phosphorus content was determined by the method of Willians and Stewart, as described by Guitian and Carballas [15]. After nitric and perchloric acid digestion, the total Ca, Mg, Fe, Cu, Mn, Zn, Cd, Pb, Ni, and Cr concentrations were determined by atomic absorption spectrometry and K was determined by atomic emission spectrometry, according to MAPA methods [12].

Table 2 shows the acidic functional group contents of humic acids isolated from both organic wastes. The carboxyl group content was estimated by direct potentiometric titration at pH 8, the phenolic hydroxyl group content was estimated as two times the change in charge between pH 8 and pH 10, and the total acidity was calculated by addition [16].

Table 2
Acidic functional group contents (±standard errors of three laboratory replicates) of humic acids (HAs) isolated from PM and MSW.

	Total acidity (mol kg^{-1})	COOH (mol kg^{-1})	Phenolic OH (mol kg^{-1})
PM	3.99 ± 0.13	2.99 ± 0.09	0.99 ± 0.05
MSW	4.29 ± 0.04	3.19 ± 0.03	1.10 ± 0.03

2.2. Incubation procedure and analytical determinations

Five hundred grams of soil were pre-incubated at 25 °C for 7 days at 30–40% of their water-holding capacity, according to Moreno et al. [6], prior to the treatments. After this pre-incubation period, soil samples were mixed with two solutions of $Cr(NO_3)_3$ and $Cd(NO_3)_2$ to give three final concentrations (0, 100, and 250 mg Cr kg^{-1} soil and 0, 100, and 250 mg Cd kg^{-1} soil), and were then treated with MSW at a rate of 10% or PM at a rate of 7.6%, to ensure that the same amount of organic matter was applied in both treatments. An unamended and non-polluted soil was used as control. Distilled water was added to each soil to bring it to 60% of its water-holding capacity. All treated soil samples were placed in semi-closed microcosms. Treatments were incubated in triplicate, in darkness at 25 °C, inside an incubation chamber for 120 days.

For each treatment and at the beginning and at the end of the incubation period, three soil sub-samples were taken (30 g of soil per sub-sample). In each soil sub-sample, soil microbial biomass-C was determined using the $CHCl_3$ fumigation-extraction method [17]. Soil dehydrogenase activity was determined in 1 g of soil: the reduction of *p*-iodonitrotetrazolium chloride (INT) to *p*-iodonitrotetrazolium formazan was measured following the method reported by García et al. [18]. Soil urease activity was determined by the method of Kandeler and Gerber [19], using urea as substrate. Alkaline phosphatase activity was measured using *p*-nitrophenyl phosphate as substrate [20]. The β-glucosidase activity was determined using *p*-nitrophenyl-β-D-glucopyranoside as substrate [21]. Arylsulfatase activity was determined using *p*-nitrophenylsulfate as substrate [22].

Also, for each treatment at 15 and 120 days after the soil pollution, the Cr and Cd extractable were determined using diethylenetriaminepentaacetic acid (DTPA) by shaking 0.5 g of soil with 25 ml of a solution of 5 mM DTPA and 10 mM $CaCl_2$ in an end-over-end shaker for 1 h [23]. Then, the suspension was centrifuged for 30 min at 5000 × *g*, and filtered on Whatman No. 1 filter paper. The Cr and Pb concentrations were measured by atomic absorption spectrometer.

Soil sub-samples were stored in sealed polyethylene bags at 4 °C for 15 days, until chemical analysis.

2.3. Statistical analysis

To compare statistically the effect of each parameter (pH and biochemical properties) a factorial ANOVA was used, considering the time of incubation and the treatments used as independent variables and each soil parameter as the dependent variable. For this, the Statgraphics v. 5.0 software package [24] was used. With the results obtained, a study of multiple ranges was conducted, obtaining the homogeneous groups between the studied treatments enable us to establish significant differences ($P < 0.05$) between them. For the ANOVA, triplicate data were used for each treatment and every experimental season. The values that appear in the tables are the averages of three readings.

3. Results

3.1. Cr and Pb extractable in soils and pH

Table 3 shows the evolution of the Cr and Cd extractable in soils for all treatments at 15 and 120 days after the soil pollution. The results suggested that the addition of organic matter to the soil decreased the concentration of both metals. However, this decrease was higher in soils amended with MSW than for PM.

Table 4 shows the evolution of soil pH after 120 days incubation. Considering the non-organic and non-polluted soils, the pH evolution remained constant. However, when the different doses of heavy metals were applied, the pH decreased at the end of the experiment, significant differences existed among the treatments. The 250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil treatment had the lowest pH value at the end of the incubation, its value being decreased by 12.9% with respect to the control soil.

In contrast to the previous case, when the two organic matter sources were applied to the polluted soils, the pH tended to descend slightly. In both cases, the greatest decrease was seen at the end of the experiment, for the 250 mg Cr kg^{-1} + 250 mg Cd kg^{-1} treatment. Nevertheless, for the polluted soil with the highest rates of Cr + Cd and the PM amendment, the pH decreased by 13.8% relative to the non-polluted soil amended with PM, while for the polluted soil with the highest rates of Cr + Cd and the MSW amendment, the pH decreased by 12.5% with respect to the non-polluted, MSW-amended soil.

3.2. Biological parameters

Table 4 shows the initial and final values of the microbial biomass-C in the soils polluted with both heavy metals at different doses, both amended with organic matter and non-amended. For the non-amended soils, the control soil (non-polluted) exhibited the highest values of this parameter. When the metals were added alone at the highest dose, the soil microbial biomass-C was affected more negatively by Cd than by Cr: it decreased by 23% when Cd was added to the soil at the highest concentration and by 20% when Cr was added at the highest concentration, compared with the non-polluted soil. When the metals were combined, the decrease in the soil microbial biomass-C was greater: at the end of the incubation period, the microbial biomass-C was lowest for the combination 250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil, being 41.9% less than in the non-polluted soil.

When organic matter was added to the soil, the microbial biomass-C increased progressively in the non-polluted soil (Table 4), possibly due to organic matter mineralization. This increase was significantly ($P < 0.05$) higher in the soil amended with PM than in that amended with MSW. In the soils amended organically, the decrease in the microbial biomass-C was lower than in the non-amended soils, indicating a favorable effect of the organic matter on the microbial biomass-C in heavy metal-polluted soils. However, the percentage decrease depended on the type of organic matter applied. At the end of the incubation period, when the soil was contaminated by Cr alone at the highest dose, the microbial biomass-C was decreased by 16.7% for the MSW-amended soil and by 15.8% for the PM-amended soil, compared with the organic matter amended-, non-polluted soils. For Cd, the decrease of this biological parameter was greater, 19.7% for the MSW-amended soil and 21.6% for the PM-amended soil. The percentage reduction of the microbial biomass-C was higher in the amended soils polluted with the highest rate of Cr + Cd: the greatest decrease was for the 250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil combination, reductions at the end of the incubation period being 33.1% for the polluted, MSW-amended soil and 35.1% for the polluted, PM-amended soil. The statistical analyses show significant differences among the treatments studied during the experimental period.

Table 5 shows the dehydrogenase activity during the incubation period. As for microbial biomass-C, the control soil (non-polluted) exhibited the highest values. When the soil was contaminated with the metals added singly, the highest percentage inhibition of dehydrogenase occurred at the end of the incubation for 250 mg Cd kg^{-1} soil (26.2% with respect to the non-polluted soil), while for Cr, the greatest inhibition was 18.6% (compared with the non-polluted soil), at 250 mg Cr kg^{-1} soil. The combined contamination by both metals produced a greater inhibition of this enzyme. In this respect,

Table 3
Evolution of Cr and Cd extractable in soils (mean ± st. error) during the incubation period.

	Cr (mg kg^{-1} soil)		Cd (mg kg^{-1} soil)	
	15	120	15	120
0 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil	1.7 ± 0.3	1.5 ± 0.2	2.0 ± 0.4	1.8 ± 0.3
100 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil	80.6 ± 3.6	83.3 ± 4.8	2.1 ± 0.5	1.9 ± 0.4
250 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil	225 ± 11	231 ± 18	2.1 ± 0.4	1.8 ± 0.5
0 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil	1.8 ± 0.4	1.6 ± 0.3	87.7 ± 6.3	86.5 ± 4.2
0 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil	1.8 ± 0.5	1.5 ± 0.5	235 ± 20	238 ± 15
100 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil	79.9 ± 4.2	81.4 ± 5.8	86.2 ± 4.9	87.1 ± 7.0
100 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil	81.7 ± 6.2	82.1 ± 5.3	229 ± 17	226 ± 19
250 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil	218 ± 15	223 ± 10	86.2 ± 7.2	84.6 ± 5.1
250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil	220 ± 12	225 ± 16	236 ± 13	239 ± 17
0 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + MSW	1.3 ± 0.2	1.2 ± 0.4	1.6 ± 0.4	1.4 ± 0.3
100 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + MSW	70.1 ± 4.1	69.0 ± 6.2	1.5 ± 0.3	1.28 ± 0.3
250 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + MSW	210 ± 10	203 ± 15	1.5 ± 0.4	1.2 ± 0.3
0 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + MSW	1.5 ± 0.5	1.3 ± 0.3	82.0 ± 3.9	80.1 ± 5.2
0 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + MSW	1.5 ± 0.3	1.4 ± 0.5	220 ± 11	215 ± 14
100 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + MSW	68.3 ± 5.2	67.1 ± 3.4	81.0 ± 5.1	79.2 ± 3.6
100 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + MSW	69.3 ± 5.5	66.0 ± 4.2	218 ± 13	210 ± 16
250 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + MSW	206 ± 17	200 ± 14	84.0 ± 6.0	82.2 ± 4.8
250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + MSW	204 ± 15	199 ± 11	223 ± 20	210 ± 14
0 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + PM	1.5 ± 0.3	1.4 ± 0.2	1.7 ± 0.04	1.5 ± 0.04
100 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + PM	73.4 ± 4.8	72.0 ± 5.5	1.5 ± 0.03	1.3 ± 0.06
250 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + PM	214 ± 17	207 ± 11	1.6 ± 0.3	1.4 ± 0.3
0 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + PM	1.6 ± 0.2	1.5 ± 0.3	84.3 ± 4.9	83.0 ± 2.9
0 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + PM	1.7 ± 0.3	1.6 ± 0.2	224 ± 14	218 ± 15
100 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + PM	70.4 ± 3.5	69.3 ± 4.8	84.1 ± 3.2	81.2 ± 3.9
100 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + PM	71.7 ± 5.0	70.0 ± 6.2	223 ± 15	219 ± 11
250 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + PM	210 ± 11	203 ± 14	85.4 ± 7.1	83.3 ± 7.2
250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + PM	211 ± 17	202 ± 15	227 ± 19	216 ± 11

at the end of the experimental period, the dehydrogenase activity was inhibited by 41.9% compared with the non-polluted soil.

When organic matter was added to the soil, the dehydrogenase activity of the non-polluted soil increased progressively during the incubation period (Table 5). However, like microbial biomass-C, the increase in the dehydrogenase activity depended on the type of organic matter applied: the increase was bigger in the soil amended with PM than in that receiving MSW. The application of organic matter to the polluted soils also produced differences in the enzyme inhibition percentages. In this respect, when Cd was added as the

Table 4
pH and microbial biomass-C (mean ± st. error) in control soil, soil + MSW, and soil + PM at different Cr and Cd rates, at the beginning and at the end of the incubation period.

	pH Incubation days		Microbial biomass-C (μg C g^{-1} dry soil) Incubation days	
	0	120	0	120
0 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil	8.6a† ± 0.2	8.5a ± 0.2	192ab† ± 15	172ab ± 13
100 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil	8.5a ± 0.6	7.9a ± 0.2	190ab ± 17	143a ± 11
250 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil	8.5a ± 0.3	7.9a ± 0.1	190ab ± 21	140a ± 19
0 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil	8.5a ± 0.8	7.9a ± 0.2	188ab ± 22	138a ± 15
0 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil	8.5a ± 0.3	7.8a ± 0.2	189ab ± 14	127a ± 18
100 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil	8.5a ± 0.5	7.7a ± 0.1	187ab ± 10	122a ± 15
100 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil	8.4a ± 0.2	7.6a ± 0.3	189ab ± 29	115a ± 19
250 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil	8.4a ± 0.7	7.6a ± 0.2	188ab ± 25	109a ± 12
250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil	8.4a ± 0.6	7.4ab ± 0.2	186ab ± 20	100a ± 16
0 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + MSW	7.1ab ± 0.3	7.2ab ± 0.2	239b ± 21	299bc ± 14
100 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + MSW	7.0ab ± 0.6	6.8ab ± 0.1	237b ± 17	260b ± 13
250 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + MSW	7.0ab ± 0.4	6.8ab ± 0.3	237b ± 23	249b ± 19
0 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + MSW	7.0ab ± 0.5	6.8ab ± 0.2	236b ± 22	256b ± 14
0 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + MSW	7.0ab ± 0.6	6.6ab ± 0.3	238b ± 10	240b ± 18
100 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + MSW	6.9ab ± 0.3	6.5b ± 0.2	238b ± 11	234b ± 16
100 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + MSW	6.9ab ± 0.4	6.4b ± 0.3	236b ± 15	213ab ± 15
250 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + MSW	6.9ab ± 0.5	6.4b ± 0.2	236b ± 20	207ab ± 18
250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + MSW	6.8ab ± 0.9	6.3b ± 0.1	235b ± 27	200ab ± 17
0 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + PM	7.9a ± 0.3	8.0a ± 0.2	289b ± 26	379c ± 17
100 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + PM	7.8a ± 0.2	7.6a ± 0.2	288b ± 15	321bc ± 20
250 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + PM	7.8a ± 0.4	7.5ab ± 0.1	288b ± 23	319bc ± 11
0 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + PM	7.8a ± 0.6	7.4ab ± 0.2	288b ± 25	315bc ± 14
0 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + PM	7.7a ± 0.4	7.2ab ± 0.4	287b ± 21	297bc ± 16
100 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + PM	7.6a ± 0.3	7.2ab ± 0.2	288b ± 19	278b ± 17
100 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + PM	7.7a ± 0.6	7.1ab ± 0.3	287b ± 16	264b ± 19
250 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + PM	7.7a ± 0.8	7.1ab ± 0.3	286b ± 20	253b ± 21
250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + PM	7.6a ± 0.5	6.9ab ± 0.2	286b ± 29	246b ± 18

† Different letters following the figures indicate a significant difference at $P < 0.05$.

Table 5
Dehydrogenase and urease activities (μg INTF g^{-1} h^{-1}) (mean ± st. error) in control soil, soil + MSW, and soil + PM at different Cr and Cd rates, at the beginning and at the end of the incubation period. The results are reported in dry matter basis.

	Dehydrogenase activity (μg INTF g^{-1} h^{-1}) Incubation days		Urease activity (μg NH_4^+ g^{-1} h^{-1}) Incubation days	
	0	120	0	120
0 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil	1.5b† ± 0.3	1.0a ± 0.2	1.5b† ± 0.7	1.0a ± 0.4
100 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil	1.4b ± 0.9	0.85a ± 0.16	1.4b ± 0.5	0.86a ± 0.21
250 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil	1.4b ± 0.4	0.80a ± 0.11	1.4b ± 0.4	0.73a ± 0.16
0 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil	1.4b ± 0.5	0.83a ± 0.16	1.4b ± 0.9	0.81a ± 0.18
0 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil	1.3b ± 0.2	0.77a ± 0.15	1.4b ± 0.8	0.64a ± 0.14
100 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil	1.4b ± 0.8	0.74a ± 0.11	1.4b ± 0.7	0.58a ± 0.11
100 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil	1.3b ± 0.7	0.71a ± 0.16	1.3b ± 0.6	0.54a ± 0.17
250 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil	1.3b ± 0.4	0.67a ± 0.14	1.3b ± 0.5	0.51a ± 0.12
250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil	1.3b ± 0.5	0.61a ± 0.16	1.2b ± 0.7	0.46a ± 0.13
0 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + MSW	4.8c ± 1.6	5.8c ± 1.7	2.7c ± 1.2	3.2c ± 1.6
100 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + MSW	4.7c ± 1.8	5.2c ± 1.3	2.6c ± 1.6	3.0c ± 1.4
250 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + MSW	4.7c ± 1.3	4.9c ± 1.5	2.6c ± 1.3	2.8c ± 1.3
0 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + MSW	4.7c ± 1.6	5.1c ± 1.2	2.6c ± 1.5	2.9c ± 1.5
0 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + MSW	4.7c ± 1.1	4.7c ± 1.1	2.6c ± 1.1	2.6c ± 1.4
100 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + MSW	4.6c ± 2.0	4.5c ± 1.3	2.5c ± 0.9	2.5c ± 1.4
100 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + MSW	4.6c ± 1.9	4.2c ± 1.3	2.5c ± 1.3	2.3c ± 1.2
250 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + MSW	4.6c ± 1.2	4.1c ± 1.1	2.5c ± 1.0	2.2c ± 1.1
250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + MSW	4.6c ± 1.0	3.9c ± 1.2	2.5c ± 1.1	2.0c ± 1.3
0 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + PM	5.6c ± 1.3	7.4d ± 1.9	3.6c ± 1.6	4.7d ± 1.2
100 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + PM	5.5c ± 1.1	6.5d ± 1.4	3.5c ± 1.3	4.2d ± 1.6
250 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + PM	5.5c ± 1.2	6.1c ± 1.6	3.5c ± 1.5	3.8c ± 1.4
0 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + PM	5.5c ± 1.3	6.3d ± 1.5	3.4c ± 1.4	4.0d ± 1.5
0 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + PM	5.5c ± 1.9	5.8c ± 1.3	3.5c ± 1.7	3.6c ± 1.2
100 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + PM	5.5c ± 1.5	5.5c ± 1.1	3.4c ± 1.6	3.4c ± 1.4
100 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + PM	5.5c ± 1.4	5.3c ± 1.4	3.4c ± 1.2	3.1c ± 1.5
250 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + PM	5.4c ± 1.3	5.2c ± 1.5	3.3c ± 1.5	2.9c ± 1.2
250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + PM	5.4c ± 1.0	4.9c ± 1.3	3.3c ± 1.1	2.7c ± 1.6

INTF: 2-*p*-iodo-3-nitrophenyl.
† Different letters following the figures indicate a significant difference at $P < 0.05$.

Table 6
β-Glucosidase and phosphatase activities (μmol PNP g^{-1} h^{-1}) (mean ± st. error) in control soil, soil + MSW, and soil + PM at different Cr and Cd rates, at the beginning and at the end of the incubation period. The results are reported in dry matter basis.

	β-Glucosidase activity (μmol PNP g^{-1} h^{-1}) Incubation days		Phosphatase activity (μmol PNP g^{-1} h^{-1}) Incubation days	
	0	120	0	120
0 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil	2.9b† ± 0.4	2.3ab ± 0.4	4.7b† ± 1.8	3.8ab ± 1.2
100 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil	2.8b ± 0.7	2.0a ± 0.5	4.6b ± 1.5	3.4a ± 1.1
250 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil	2.8b ± 0.9	1.7a ± 0.4	4.6b ± 1.2	3.2a ± 1.2
0 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil	2.8b ± 0.5	1.9a ± 0.4	4.5b ± 1.4	3.3a ± 1.0
0 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil	2.8b ± 0.3	1.5a ± 0.5	4.6b ± 1.3	3.1a ± 1.1
100 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil	2.7b ± 1.0	1.4a ± 0.4	4.5b ± 1.5	2.9a ± 0.7
100 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil	2.7b ± 0.7	1.3a ± 0.3	4.5b ± 1.8	2.7a ± 0.8
250 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil	2.7b ± 0.6	1.2a ± 0.3	4.4b ± 1.2	2.6a ± 0.9
250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil	2.6b ± 0.5	1.1a ± 0.3	4.4b ± 1.1	2.4a ± 0.9
0 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + MSW	4.3c ± 1.1	5.1c ± 1.4	8.7c ± 1.7	10.3c ± 1.9
100 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + MSW	4.2c ± 1.6	4.8c ± 1.1	8.6c ± 1.3	9.7c ± 1.6
250 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + MSW	4.1c ± 1.4	4.4c ± 1.3	8.6c ± 1.4	9.3c ± 1.2
0 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + MSW	4.1c ± 1.0	4.6c ± 1.4	8.6c ± 1.1	9.5c ± 1.4
0 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + MSW	4.2c ± 1.2	4.1c ± 1.5	8.5c ± 1.0	9.1c ± 1.5
100 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + MSW	4.1c ± 1.1	4.0c ± 1.2	8.5c ± 1.8	8.7c ± 1.2
100 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + MSW	4.1c ± 1.5	3.8c ± 1.3	8.5c ± 1.3	8.3c ± 1.4
250 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + MSW	4.0c ± 1.3	3.7c ± 1.5	8.4c ± 1.6	7.9c ± 1.5
250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + MSW	4.0c ± 0.8	3.6c ± 1.4	8.4c ± 1.3	7.6c ± 1.7
0 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + PM	5.1c ± 1.3	6.5d ± 1.6	9.7c ± 1.6	12.3d ± 1.9
100 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + PM	5.0c ± 1.2	5.9d ± 1.4	9.5c ± 1.1	11.3d ± 2.1
250 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + PM	5.0c ± 1.5	5.5c ± 1.2	9.5c ± 1.3	10.9c ± 1.8
0 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + PM	5.0c ± 1.1	5.7d ± 1.3	9.6c ± 1.4	11.0d ± 2.0
0 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + PM	4.9c ± 1.9	5.1c ± 1.2	9.5c ± 1.5	10.7c ± 1.7
100 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + PM	4.9c ± 1.3	4.9c ± 1.4	9.4c ± 1.2	10.1c ± 1.8
100 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + PM	4.7c ± 1.6	4.6c ± 1.5	9.4c ± 1.0	9.5c ± 1.6
250 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + PM	4.8c ± 0.9	4.4c ± 1.3	9.4c ± 1.8	9.1c ± 1.9
250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + PM	4.7c ± 1.0	4.1c ± 1.4	9.3c ± 1.6	8.6c ± 1.4

PNP: *p*-nitrophenol.
† Different letters following the figures indicate a significant difference at $P < 0.05$.

Table 7
Arylsulfatase activity (μmol PNF g^{-1} h^{-1}) (mean ± st. error) in control soil, soil + MSW, and soil + PM at different Cr and Cd rates, at the beginning and at the end of the incubation period. The results are reported in dry matter basis.

	Incubation days	
	0	120
0 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil	2.6b† ± 0.9	1.9a ± 0.6
100 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil	2.4b ± 0.7	1.8a ± 0.4
250 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil	2.4b ± 1.0	1.6a ± 0.5
0 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil	2.5b ± 0.4	1.7a ± 0.8
0 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil	2.4b ± 0.5	1.6a ± 0.6
100 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil	2.4b ± 0.3	1.5a ± 0.8
100 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil	2.3b ± 0.7	1.5a ± 0.5
250 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil	2.3b ± 0.9	1.4a ± 0.3
250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil	2.2b ± 0.8	1.4a ± 0.6
0 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + MSW	4.8c ± 1.7	5.7c ± 1.2
100 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + MSW	4.6c ± 1.1	5.5c ± 1.3
250 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + MSW	4.6c ± 1.3	5.1c ± 1.4
0 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + MSW	4.5c ± 1.2	5.3c ± 1.3
0 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + MSW	4.4c ± 1.9	5.0c ± 1.1
100 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + MSW	4.4c ± 1.2	4.9c ± 1.2
100 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + MSW	4.4c ± 1.5	4.8c ± 1.4
250 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + MSW	4.3c ± 1.0	4.6c ± 1.5
250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + MSW	4.3c ± 1.3	4.4c ± 1.2
0 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + PM	6.1c ± 1.9	7.6d ± 1.8
100 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + PM	6.0c ± 1.5	7.2d ± 1.3
250 mg Cr kg^{-1} soil + 0 mg Cd kg^{-1} soil + PM	6.0c ± 1.2	6.7c ± 1.4
0 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + PM	5.9c ± 1.4	6.9d ± 1.5
0 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + PM	6.0c ± 1.3	6.6c ± 1.5
100 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + PM	5.9c ± 1.3	6.4c ± 1.3
100 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + PM	5.8c ± 1.9	6.2c ± 1.6
250 mg Cr kg^{-1} soil + 100 mg Cd kg^{-1} soil + PM	5.8c ± 1.4	6.1c ± 1.3
250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} soil + PM	5.9c ± 1.1	5.7c ± 1.4

NF: *p*-nitrophenyl.
† Different letters following the figures indicate a significant difference at $P < 0.05$.

only polluting metal at its highest dose, the dehydrogenase activity decreased until the end of the incubation period, by 21.6% and 18.9% for the soils amended with PM and MSW, respectively, compared with the non-polluted, amended soils. For Cr, the inhibition was lower: when it was added alone at its highest dose, dehydrogenase activity was decreased at the end of the incubation period by 17.6% and 15.5% for the soils amended with PM and MSW, respectively, compared with the non-polluted, amended soils. The combined application of both metals to the amended soils gave the highest inhibition of dehydrogenase activity. The application of 250 mg Cr kg^{-1} soil + 250 mg Cd kg^{-1} inhibited this enzyme by 33.8% and 32.7% for the PM and MSW treatments, respectively, compared with the non-polluted, amended soils.

The trend of the hydrolase enzymes was very similar to that of dehydrogenase activity (Tables 5–7). Again, when the metals were applied singly, the greatest inhibition occurred for the Cd than for Cr-polluted soils. When the highest rates of the metals were applied together, the urease, β-glucosidase, phosphatase and arylsulfatase inhibition percentages were 54%, 52.2%, 46.9% and 26.4%, respectively, compared to the control soil. The results reveal a decreased percentage of hydrolase enzymes inhibition when organic matter was added to the soil. As for the enzymes described previously, this decrease was greater for the MSW-polluted soil than for PM. Also, when the amended soils were contaminated with the highest rates of the metals without combining them, the enzymatic inhibition was more pronounced for the Cd-polluted soil than for that polluted by Cr. At the end of the experimental period, when the highest rates of the metals were applied together on soil organic-amended, the highest inhibition of urease, β-glucosidase, phosphatase and arylsulfatase occurred for the 250 mg Cd kg^{-1} soil + 250 mg Cr kg^{-1} soil + PM treatment (42.6%, 36.9%, 30.1% and 25%, respectively) with respect to the 0 mg Cd kg^{-1} soil + 0 mg Cr kg^{-1} soil + PM treatment, while for the 250 mg Cd kg^{-1} soil + 250 mg Cr kg^{-1} soil + MSW treatment (37.5%, 29.49%, 26.2% and 22.8%, respectively) with respect to the 0 mg Cd kg^{-1} soil + 0 mg Cr kg^{-1} soil + MSW treatment.

4. Discussion

Our results indicate that soil biochemical properties are of great utility for understanding the negative impacts of heavy metals in soils. In this respect, enzymatic activities permit the immediate detection of changes in the quality of soils resulting from their contamination by heavy metals, since these activities are linked closely to the cycles of nutrients [25].

Also, our results indicate that soil microbial biomass-C and the diverse enzymatic activities studied were inhibited by Cr and Cd, when applied individually or when mixed at different rates. These results are in agreement with Tejada et al. [7], Akmal et al. [26] and Wang et al. [27], who demonstrated the toxic effects of heavy metals on soil biology.

In accordance with Renella et al. [28,29], ours results indicate that when Cr and Cd were applied to the soil mixed at the highest rates, the values of the soil biochemical parameters decreased more than when each heavy metal was applied individually. These results suggest that the combination of different heavy metals can have a more-highly toxic effect on the soil biological properties.

However, the toxic effect of these heavy metals on the soil biochemical parameters differed greatly, depending on the type of polluting metal. Our results suggest that when the metals were applied individually, Cd gave rise to a greater inhibition of the biochemical parameters than did Cr. According to the Irving-Williams series (that refers to the relative stability of the complexes formed by the metallic ions), Cr forms complexes with colloids that are more stable than those of Cd. This suggests that Cd, at an equal applied dose, might be more abundant in the soil solution than Cr, and this could explain the greater toxicity of Cd; this would be in agreement with the results of Guo et al. [30].

Therefore, the physical-chemical characteristics of the polluted soil also play a fundamental role in metal complexation and, in consequence, in the effects on the soil biochemical properties. Recently, many authors have shown that the soil pH plays a fundamental role in this process [27,31]. These authors suggested that an acid soil pH exercises a higher toxic effect on the soil microorganisms than a basic pH, because at acid pH the heavy metals would be more soluble. However, this depends of the heavy metal considered. The literature indicates that the Cr metal is more soluble in a basic pH, while the Cd is more soluble in a acidic pH. In our study, the non-organic amended soil polluted with the different heavy metals had basic pH values during the incubation period. However as discussed above, Cd gave rise to a greater inhibition of the biochemical parameters than did Cr probably due that the Cr forms complexes with colloids that are more stable than those of Cd.

There currently exist diverse interpretations that try to explain the negative interactions between heavy metals and the soil biochemical properties, the great majority of them indicating that these interactions do not depend directly on the soil pH. Renella et al. [29] found that negative effects on the biological properties of soil contaminated by heavy metals can possibly be a consequence of a decrease in the time that substrates are available to the microorganisms, a lower synthesis and/or liberation of the extracellular enzymes of the soil microorganisms, or the inhibition of extracellular enzymes. On the other hand, it is known that the different metallic ions differ in their capacity to act as inhibitors of diverse soil enzymes [7,8]. In general, the inhibition by heavy metals follows non-competitive kinetics; that is to say, there is no relationship between the quantity of inhibitor and the substrate concentration. The metallic ions can inactivate enzymes by reacting

with sulfhydric groups, a reaction similar to the formation of a metal sulfide. The sulfhydric groups of the enzymes can serve as an integral part of the active catalyst that participates in the maintenance of a correct structure in the enzyme–protein relationship.

With respect to the hydrolase enzymes studied, it is important to highlight that urease exhibited a higher inhibition percentage compared with β-glucosidase, phosphatase, and arylsulfatase. This inhibition was greater in the soils polluted with the highest Cr + Cd rate, followed by the highest Cd rate, and lastly that of Cr. This is in agreement with the results of Cifuentes [32], who indicated that urease is more sensitive to contamination by a combination of heavy metals in comparison with their individual contamination. Nevertheless, it is necessary to keep in mind that other authors [33] suggest that β-glucosidase also could be used as a good indicator of soil heavy metal contamination, because this enzyme is also very sensitive to these elements.

The results obtained in this work also suggest that dehydrogenase can serve as a good indicator of heavy metal contamination. This enzyme has a behavior very similar to that of the other enzymes described previously. Since this enzyme has an intracellular origin, it is found fundamentally in the viable microorganisms resistant to heavy metal contamination. Thus, its activity is related to the presence of live microorganisms and their oxidative capacity [7,8].

Since soil contamination by heavy metals supposes a serious environmental problem, in recent years different techniques have been developed for their immobilization or elimination. Usually, techniques such as phytoremediation or immobilization with diverse organic substances [8,34] are employed. However, the immobilization of these metals by organic substances, due to their great adsorption power, is of special interest, since this adsorption will depend on the chemical characteristics of the organic matter. In all cases, this immobilization of the metals implies a decline in their concentration in the soil solution and, as a consequence, in their mobility.

It has been observed that humic substances influence the biological properties of toxic ions, acting as an accumulation phase for heavy metals following the formation of metal–humate complexes (chelates) with different degrees of stability, probably as a result of the humic substances containing several major functional groups, such as carboxyl, phenolic, alcohol, and carbonyl [35].

Nevertheless, it is necessary to point out that the origin and quality of this organic matter will be decisive in the formation of chelates. In this respect, Tejada et al. [7,8] found that the adsorption of heavy metals increased when the humic acid content increased in the organic matter, relative to the fulvic acid content, due mainly to the fact that humic acids possess a higher number of carboxylic groups than do fulvic acids.

In our experiment, the application of PM or MSW to the soil decreased the inhibition of enzymes compared with the non-amended and contaminated soil. Also, the higher humic acid content in MSW than in PM (Table 2) makes one think that metal adsorption should be higher in MSW than in PM, explaining why the inhibition of enzymatic activities was lower in the polluted soils amended with MSW than in those receiving PM. These results are in agreement with those obtained by other authors who applied different sources of organic matter (cotton gin compost, sewage sludge, etc.) to soils polluted by diverse heavy metals (Ni, Pb, Cd) [7,8].

When the two heavy metals were added together to the organically-amended soil, the inhibition of enzymatic activities was greater for the soil amended with PM than for that receiving MSW. Also, this inhibition was higher in the polluted soils with the highest Cr–Cd rate, followed by the highest Cd rate and lastly that of Cr. Possibly, the Cr complexes formed were more stable than those of Cd, resulting in a more toxic effect of Cd.

5. Conclusions

The toxic effects of Cr and Cd on the soil biochemical parameters measured were very different, depending on the particular metal considered. When the metals were applied individually, Cd was more inhibitory than Cr, possibly due to the fact that Cr forms more stable complexes than Cd with the colloids. The application of MSW or PM, at the doses studied, to a soil polluted with Cr + Cd under laboratory conditions improved the soil enzymatic activities compared with soil non-treated with organic materials. The enzyme activities studied were inhibited by Cr + Cd and both organic amendments decreased significantly the Cr + Cd toxic effects. However, the beneficial effect was higher with MSW than with PM. Our results suggest that the addition of MSW or PM may be considered a good strategy for remediation of heavy metal-polluted soil, and that the addition of organic materials with a higher concentration of humic acid than of fulvic acid is advisable.

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