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Response of *Eisenia fetida* to the application of different organic wastes in an aluminium-contaminated soil

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ABSTRACT

The effects of amending Al-contaminated soils with municipal solid waste compost (MSW), poultry manure (PM) and cow manure (CM) on Al toxicity and morphological alterations and biochemical enzymes of the earthworm *Eisenia fetida* were investigated in an incubation spiking experiment. Al(NO₃)₃ was added to a soil at rates equivalent to 0, 50 and 100 mg Al kg⁻¹. In order to apply the same amount of organic matter (OM), soil samples were mixed with MSW, PM and CM at a rate of 10%, 7.6% and 5.8%, respectively. Earthworm cocoon number, average weight of cocoon and number of juveniles per cocoon were measured after 30 days of incubation, whereas weight, Al concentration and available Al, as well cellulase and glutathione-S-transferase activities of earthworms were measured after 3, 15, 30, 60, 90 and 120 days. Reproductive and enzymatic activities of earthworms decreased with increasing the rate of Al applied. The inhibition of weight, enzyme activities and Al concentration of earthworm was lower in organically amended soils than in unamended soils. At the end of the incubation, earthworm weight and enzymatic activities in highly contaminated soils increased and Al concentration in earthworm tissues decreased as the relative amount of humic acids applied to soil increased, following the order unamended soil < CM-amended soil < PM-amended soil < MSW-amended soil. These results suggest that the application of organic amendments with higher content of humic acids may be more beneficial for remediation of Al-polluted soils.

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1. Introduction

Environmental problems associated with the presence and accumulation of aluminium (Al) in soil has become an issue of intensive discussion in developed countries because this heavy metal has toxic effects on soil biology, plants, animals and humans (Tennakone et al., 1992; Phillips and Bolger, 1998; Van Gestel and Hoogerwerf, 2001; Matus et al., 2009). The chronic effect of Al compounds is often connected with mortality of some animals and Alzheimer or some other human neurodegenerative diseases (Matus and Kubova, 2002, 2008).

Currently, there has been no satisfactory disposal process for Al containing materials, and their burning release polluted dusts and unusable wastes, which may impact on soil biology, plants and animals in case of their improper disposal. Also, in several world's areas there is no organic recycling or controlled disposal practices for such wastes. Therefore, it is necessary to establish environmental strategies that eliminate or reduce these problems.

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Two aspects of Al chemistry are pertinent to environmental studies. First, the Al cationic species (e.g., "free" Al³⁺, [Al(OH)]²⁺, [Al(OH)₂]⁺, [AlO₄Al₁₂(OH)₂₄]⁷⁺) are responsible for its toxic effects. The toxicity of [AlSO₄]⁺ ions is not always accepted. On the other hand, complexes of Al with organic ligands are considered practically non-toxic (Matus and Kubova, 2008; Matus et al., 2009).

The "in situ" remediation of heavy metal polluted soils is mainly based on the extraction or stabilization of the contaminants (Gray et al., 2006). During the last decade, the possibility of heavy metal immobilization in soils through the addition of different amendments or sorbents has been extensively investigated in order to reduce the risk of groundwater contamination, plant uptake and exposure to living organisms (Castaldi et al., 2005; Tandy et al., 2009). Binding of heavy metals by OM, especially by humic substances (HS), is one of principal process that govern the solubility and the bioavailability, and thus the fate, of heavy metals in the environment. However, the effective adsorption of heavy metals will differ according to the different OM and heavy metals features (e.g., humification degree, formation of inner...). Although humic acids show a lower average content of acidic functional groups compared with fulvic acids,

the former have a higher molecular weight, degree of polycondensation and recalcitrance (Stevenson, 1994) that allow humic acids to form more stable complexes with metals.

Very little is known about the benefits of applying different organic amendments to immobilize Al in contaminated soils and thereby to reduce its toxic effects. Therefore, this study could provide new information about the toxic behavior of this metal in soils amended with OM of different source and composition.

The impact of heavy metals on ecosystems is generally measured using standardized ecotoxicological tests, based on observing whether any detrimental effects are caused to a set of model organisms playing key roles in ecosystem structure and function (Tejada et al., 2007; Moreno et al., 2009; Harris-Hellal et al., 2009). In particular, the use of earthworms for toxicity testing is highly recommended (EC, 2004) and is considered as preferred biotest for assessing the environmental health status of chemical pollution (Reinecke and Reinecke, 2007; Chakra Reddy and Venkateswara Rao, 2008). The activities of enzymes such as oxidoreductases (e.g., superoxide dismutase, catalase), transferases (glutathione-S-transferases), and hydrolases (acetylcholine esterase) determined in worms exposed to contaminated soils provide a measure of pollutant bioavailability and toxic effects (Chakra Reddy and Venkateswara Rao, 2008). It is also easy to quantify different life-cycle parameters of earthworms, such as growth, reproduction, accumulation and excretion of pollutants (Shi et al., 2007).

The use of biomarkers is a concept in earthworm toxicity testing. Of the potential biomarkers, earthworm glutathione-S-transferase and cellulase enzymes are shown to respond to toxin exposures (Xiao et al., 2006). The glutathione-S-transferase is an important detoxification enzyme and its activity has been used as a potential bioindicator and biomarker of earthworms to heavy metals (Lukkari et al., 2004) and pesticide exposure (Booth et al., 2001; Xiao et al., 2006). Cellulase activity of earthworms indicates their role in the decomposition of plant litter and other cellulosic materials. It was used as a biomarker of a pesticide contamination on earthworms (Luo et al., 1999; Xiao et al., 2006).

In this work, we studied the effects of amending Al-contaminated soils with municipal solid waste compost (MSW), poultry manure (PM) and cow manure (CM) on Al toxicity and morphological alterations and enzyme activities of earthworms, in order to assess the use of these organic amendments for remediation.

2. Materials and methods

2.1. Soil and organic amendments

The soil used in this experiment is a Haplic Acrisol (FAO, 1977) located in the region of Sierra Morena (Córdoba, Spain). Soil samples were collected from the surface layer (0–25 cm). The main soil characteristics are shown in Table 1.

Soil pH was determined in distilled water with a glass electrode (soil:H₂O ratio 1:2.5). Soil texture was determined by Robinson's pipette method (SSEW, 1982), and quantification and the dominant clay types were determined by X-ray diffraction. Soil organic matter was determined by the method of Yeomans and Bremner (1988). Humic and fulvic acids were extracted with 0.1 M sodium pyrophosphate and 0.1 M sodium hydroxide at pH 13 (Kononova, 1966). The supernatant was acidified to pH 2 with HCl and allowed to stand for 24 h at room temperature. To separate humic acids-like (HA-like) from fulvic acids-like (FA-like), the solution was centrifuged and the precipitate, containing humic-like acids, was dissolved with sodium hydroxide (Yeomans and Bremner, 1988). After the removal of HA-like, the acidic filtrate, containing the dissolved FA-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 (MAPA, 1986). After nitric and perchloric acid digestion, the total Fe, Cu, Mn, Zn, Cd, Al, Pb and Ni concentrations were determined by ICP-AES.

The organic wastes applied were the organic fraction of a municipal solid waste (MSW), poultry manure (PM) and cow manure (CM). 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 (MAPA, 1986). Humic and fulvic acids were extracted, separated, and determined by the methods previously described. Total N, Fe, Cu, Mn, Zn, Cd, Al, Pb and Ni were determined by the methods previously described.

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 (Ritchie and Perdue, 2003).

2.2. Incubation procedure

Seven 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. (2003), prior to the treatments. After this pre-incubation period, soil samples were mixed with a solution of Al(NO₃)₃ to give three final concentrations (0, 50 and 100 mg Al kg⁻¹ soil), and treated with MSW at a rate of 10% or PM at a rate of 7.6% or CM at a rate of 5.8%, respectively, in order to applying the same amount of organic matter to the soil. An unamended soil was used as control.

Table 1

Main physico-chemical features of the experimental soil, organic wastes and the mixture soil+organic wastes, before the pollution with Al. Data represent the means of four samples. Standard errors are also reported.

	Soil	MSW	PM	CM	Soil+MSW	Soil+PM	Soil+CM
pH (H ₂ O)	4.6 ± 0.1	6.2 ± 0.3	7.1 ± 0.3	8.1 ± 0.4	5.1 ± 0.4	5.8 ± 0.3	6.2 ± 0.3
Sand (g kg ⁻¹)	350 ± 14						
Silt (g kg ⁻¹)	362 ± 25						
Clay (g kg ⁻¹)	288 ± 12						
Clay types	Illite: 73%						
	Kaolinite: 27%						
Organic matter (g kg ⁻¹)	2.1 ± 0.4	469 ± 15	614 ± 26	804 ± 35	469 ± 12	469 ± 13	469 ± 10
Humic acid-C (mg kg ⁻¹)	5.8 ± 1.3	1030 ± 17	672 ± 14	461 ± 13	104 ± 6	60.8 ± 2.3	28.2 ± 1.1
Fulvic acid-C (mg kg ⁻¹)	10.4 ± 2.1	711 ± 6	775 ± 10	638 ± 24	79.4 ± 1.6	78.6 ± 1.9	41.6 ± 2.2
Total N (g kg ⁻¹)	0.5 ± 0.1	17.3 ± 1.3	40.8 ± 1.9	29.9 ± 2.3	2.4 ± 0.8	3.9 ± 1.1	2.1 ± 0.7
Total Fe (mg kg ⁻¹)	379 ± 13	815 ± 38	180 ± 22	420 ± 30	442 ± 17	383 ± 13	401 ± 20
Total Cu (mg kg ⁻¹)	32.4 ± 2.5	82.6 ± 9.8	1.6 ± 0.3	26 ± 2.8	38.3 ± 2.3	35.9 ± 1.7	34.1 ± 1.5
Total Mn (mg kg ⁻¹)	69.8 ± 3.2	75.6 ± 8.1	4.2 ± 0.9	13.2 ± 1.4	72.4 ± 1.7	33.1 ± 1.1	32.9 ± 1.3
Total Zn (mg kg ⁻¹)	19.9 ± 1.8	134 ± 13	3.3 ± 0.8	11.8 ± 1.7	28.2 ± 1.3	21.1 ± 1.7	20.2 ± 1.4
Total Cd (mg kg ⁻¹)	6.3 ± 1.4	1.1 ± 0.3	0.35 ± 0.17	0.30 ± 0.09	6.4 ± 1.1	6.3 ± 1.3	6.3 ± 1.4
Total Al (mg kg ⁻¹)	77 ± 8	4.9 ± 0.8	0.48 ± 0.11	1.3 ± 0.2	78 ± 10	77 ± 7	77 ± 11
Total Pb (mg kg ⁻¹)	1.2 ± 0.4	13.6 ± 1.5	1.3 ± 0.12	5.1 ± 2.3	2.3 ± 0.8	1.4 ± 0.5	1.6 ± 0.3
Total Ni (mg kg ⁻¹)	3.6 ± 0.8	1.3 ± 0.2	1.3 ± 0.2	1.0 ± 0.06	3.7 ± 0.9	3.6 ± 0.7	3.6 ± 0.5

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