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The influence of earthworm and mycorrhizal co-inoculation on Cd speciation in a contaminated soil

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ABSTRACT

This experiment was conducted to establish how earthworms and arbuscular mycorrhizal fungi (AMF) interactively impact cadmium (Cd) chemical fractions in a calcareous soil artificially contaminated with Cd. The chemical forms of Cd using the Sposito's sequential extraction procedure were determined in a soil spiked with Cd (10 and 20 mg Cd kg^{-1}), inoculated or un-inoculated with earthworm (*Lumbricus rubellus* L. species) and AMF (*Glomus intraradices* and *Glomus mosseae* species) under greenhouse conditions for two months. Results showed that Cd concentrations of the non-residual fraction (i.e., soluble/exchangeable, organic bound and inorganic bound forms) increased with earthworm addition and the residual Cd fraction tended to decrease. Arbuscular mycorrhizal inoculation decreased the inorganic bound Cd fraction with a concurrent increase in the residual Cd fraction. However, no significant interactions between earthworm and AMF were observed for the non-residual Cd fraction, suggesting the combined influence of both soil organisms on the easily and potentially available Cd was largely independent or additive. While the presence of both earthworms and AMF resulted in an antagonistic interaction on the residual Cd fraction at high Cd level, this interaction was additive at low Cd level. Cadmium addition increased its uptake into the body of earthworms, which was significantly greater at high than low Cd levels. However, bioaccumulation factor for Cd accumulation in the earthworm body was lower at high than low Cd levels, indicating the low transfer of Cd from the soil environment to earthworm tissues at higher exposure levels. It is concluded that earthworms affects the chemical Cd fractions more than AMF.

1. Introduction

Cadmium (Cd), a potentially toxic metal, enters the food chain through plant uptake from polluted soils (Kirkham, 2006; Smith, 2009).Undoubtedly, a high level of this highly mobile and toxic metal in the soil is a major danger to both the environmental quality and human health in the long-term (Ali et al., 2013). Cadmium is well-known to have an adverse impact on all the living organisms in soil and terrestrial plants in Cd-polluted environments. However, the potential toxicity of Cd for soil macro- and micro-biota, and crop growth often is more closely correlated with the concentration of bio-available Cd fraction than with the total metal concentration (Vig et al., 2003; Kirkham, 2006). Thus, it is indispensable to understand changes in Cd mobility and bioavailability, which depends largely on its chemical forms in the soil rather than on the total Cd level (Sposito et al., 1982; Ma and Rao, 1997).

The sequential extraction or solid phase speciation techniques for Cd fractionation offer a powerful means for assessing its distribution with different bioavailability in soil (Sposito et al., 1982; Ma and Rao, 1997). Cadmium fractionation usually results in operationally defined fractions or forms that are closely linked to its bioavailability to soil organisms and plants (Nannoni et al., 2011; Bai et al., 2008). Several abiotic and biotic factors such as soil pH, soil organic matter (SOM) and dissolved organic carbon (DOC) contents, cation exchange capacity, fertilizer application and soil organisms directly or indirectly affect Cd speciation and consequently its mobility and bioavailability (Adriano, 2001; Vig et al., 2003; Bolan et al., 2003; Renella et al., 2004; Kirkham, 2006; Pelfrêne et al., 2012).

Among soil organisms, arbuscular mycorrhizal fungi (AMF) and earthworms are the reported biotic factors which may have an influence on the mobility and bioavailability of Cd and other heavy metals in soil–plant systems, largely owing to changes in their

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distribution among various soil fractions (Tao et al., 2003; Wen et al., 2004; Dai et al., 2004; Lukkari et al., 2006; Huang et al., 2005, 2008; Bai et al., 2008; Ruiz et al., 2011). Specifically, both earthworms (Wen et al., 2004; Ruiz et al., 2009; Nannoni et al., 2011) and mycorrhizal fungi (Giasson et al., 2005; Huang et al., 2008) have been found to influence soil Cd behavior and chemistry with changes in its immobilization and mobilization or its speciation in Cd-polluted soils. However, the individual effects of earthworms and AMF on the availability and speciation of Cd and other toxic metals are inconsistent in the literature when earthworms (Wen et al., 2004; Sizmur et al., 2011; Ruiz et al., 2009; Natal-da-Luz et al., 2011; Kizilkaya, 2004; Ruiz et al., 2011) or AMF (Munier-Lamy et al., 2007; Bai et al., 2008; Huang et al., 2008; Subramanian et al., 2009) were present.

Earthworms may often modify individually the availability and speciation of Cd and other toxic metals in soil by changing the soil characteristics such as pH and DOC, and the stimulation of microbial activity (Ma et al., 2002; Wen et al., 2004; Sizmur et al., 2011). Ma et al. (2002) reported that available Pb and Zn concentrations increased in earthworm-worked soils. The addition of earthworms affected Zn, Pb, As and Cu fractionations, to different extents, depending on soil type and heavy metals (Cheng and Wong, 2002; Kizilkaya, 2004; Sizmur et al., 2011). An increase of Cd and other metals (Cr, Co, Ni, Zn, Cu, and Pb) in the water soluble, exchangeable and carbonate fractions was reported when *Eisenia fetida* was present (Wen et al., 2004). The activity of *E. fetida* in soils polluted by mining activities changed the chemical forms of Cd and other metals (Pb, Zn, Cu) with a significant increase in the non-residual fractions (Ruiz et al., 2009). On the other hand, the activity of earthworms (*Pheretima* sp.) declined the concentration of exchangeable and carbonate Zn fractions, however, the significance of the changes depended upon the soil type (Ma et al., 2002). The influence of *Lumbricus terrestris* on Pb and Zn speciation was minor, with a significant increase only in organically bound heavy metals in mining soils (Ruiz et al., 2011).

AMF may also influence Cd mobility and toxicity by increasing soil pH (Shen et al., 2006), sequestering Cd inside extra-radical mycelium (Janoušková and Pavlíková, 2010) and binding Cd ions to glomalin (González-Chávez et al., 2004; Aghababaei et al., 2014), a glycoprotein produced by AMF. Arbuscular mycorrhizal hyphae can change Cd from a carbonate to a water-soluble form, since fungal hyphae exude simple organic acids like citric and oxalic acids that facilitate the solubility of heavy metals (Giasson et al., 2005). AMF inoculation resulted in the transformation of unavailable Zn forms into available forms by increasing the organically bound Zn fraction and reducing crystalline oxide and residual Zn fractions (Subramanian et al., 2009). The speciation of heavy metals (Cu, Zn and Pb) was changed by AMF symbiosis from bio-available to non-bio-available forms (Huang et al., 2005). In contrast, mycorrhizal inoculation by *Glomus mosseae* species did not affect Se speciation (Munier-Lamy et al., 2007). All these discrepancies in the published results could stem from differences in earthworm and fungal species, pollution mode, heavy metals and soil conditions, and therefore cannot be an overall impact of earthworm and AMF inoculation.

Although the overall influences of earthworms and AMF alone on the speciation of Cd and other metals have been studied in contaminated soils with variable results, to our knowledge no study was conducted to show their interactive effects. The presence of earthworm activity and AMF has been proposed as a practice for both bioremediation of polluted soils and plant protection against metal toxicity, but their combined effects on toxic metal speciation are not clear. The influence of these organisms on metal speciation might be particularly crucial in contaminated soils, since they may concurrently change heavy metal availability and mobility with a consequence for their uptake by plant and phyto-extraction in contaminated environments (Yu et al., 2005; Ma et al., 2006; Hua et al., 2010). However, all these studies did not attempt to determine changes in the metal speciation with earthworm activity and AMF inoculation. Specifically, we should know how the biotic interactions between these two soil organisms affect Cd availability and toxicity, and whether the combined effects are additive or interactive. This can further enhance our understanding of the interactions between earthworms and AMF and their significant role in either protecting plants against the phyto-toxicity of metals or in promoting phyto-remediation efficiency in soils polluted with toxic metals. Therefore, the main aim of the current study was to investigate changes in Cd speciation in a calcareous soil artificially spiked with 10 and 20 mg Cd kg^{-1} and inoculated with earthworm (*Lumbricus rubellus*) and AMF (*Glomus intraradices* and *G. mosseae* species) under glasshouse conditions. We hypothesized that earthworms and AMF would interactively affect Cd speciation and transformation. It is assumed that earthworm activity would modify AMF effects on soil Cd fractionation pattern or AMF inoculation would change earthworm effects on the distribution of Cd among soil solid phases.

2. Materials and methods

2.1. Experimental lay-out

The experiment was 3 × 2 × 3 factorial with Cd level, earthworm and AMF inoculation arranged in a completely randomized design with three replications. Treatments consisted of a full factorial combinations of three Cd levels (0, 10 and 20 mg kg^{-1}) applied as $CdCl_2$, two earthworm treatments (no earthworm, NE, and with *L. rubellus* earthworm, WE) and three AMF treatments (*G. intraradices*, *G. mosseae*, and non-mycorrhizal control, NM) in a calcareous soil cropped with maize (*Zea mays* L.) under glasshouse conditions for two months.

2.2. Soil preparation and treatment

A typical calcareous sandy loam soil, classified as Typic Calcixerepts (Soil Survey Staff, 2010), from the 0–30 cm layer was obtained in a cropland field without pollution history. The soil was air-dried, passed through a 2 mm sieve and autoclaved at 121 °C for 2 h. A subsample of the study soil was analyzed for general chemical and physical properties. The soil had the following physical and chemical properties: pH (in H_2O) 8.1, ECe 0.20 dS m^{-1}; CEC 19.7 cmol (+) kg^{-1}; $CaCO_3$ 190 mg g^{-1}; organic C 2.8 mg g^{-1}, total N 0.6 mg g^{-1}, available P and K 5.7 and 168 mg kg^{-1}, respectively; clay 16%, silt 12% and sand 72%. We used plastic pots with the bottom covered with nylon net to prevent earthworm escape. In total, 54 pots were prepared and filled with 8 kg (fresh weight) autoclaved soil. To establish and reactivate soil microorganisms, 50 ml soil suspension was added to each pot to inoculate the autoclaved soil with fresh microorganisms. To obtain the soil suspension, 500 g fresh soil was suspended in 1.5 L de-ionized water and filtered through a 25 μm for eliminating AMF spores (Schroeder and Janos, 2004). The soils were artificially contaminated with Cd (as cadmium chloride) at the following rates: 0 (control), 10 and 20 mg kg^{-1} on a dry weight basis. The control treatments were watered with distilled water and others with appropriate aliquots of aqueous solutions of Cd chloride to obtain the above concentrations. Soils were mixed thoroughly for an even distribution of added Cd in the soil matrix. De-ionized distilled water was added to the soil to achieve a moisture content of about 60–70% of field capacity. The soils were incubated at room temperature (about 20 °C) for 4 months, allowing Cd to distribute into various fractions,