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Interaction between arbuscular mycorrhizal fungi and vermicompost on copper phytoremediation in a sandy soil

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ABSTRACT

Arbuscular mycorrhizal fungi and vermicompost may decrease the deleterious effects of copper on plants. The goal of the present study was to evaluate the effect of inoculation with the fungus *Rhizophagus clarus* and the addition of grape bagasse vermicompost on phytoremediation by *Canavalia ensiformis* of a sandy soil with high Cu concentration. Soil was contaminated with 100 mg Cu kg^{-1}, fertilized with vermicompost at levels equivalent to 0, 10, 20, 40 and 80 mg P kg^{-1}, and cultivated with *C. ensiformis* with and without inoculation with *R. clarus*. Availability of Cu and other nutrients in the soil and in the soil solution, shoot and root accumulation of Cu and other nutrients, plant growth, and Cu phytotoxicity—using photochemical efficiency, concentration of photosynthetic pigments, and oxidative stress enzyme activities as indicators of Cu phytotoxicity—were evaluated. Phytostabilization showed better performance with the addition of the vermicompost level equivalent to 20 mg P kg^{-1} and in the presence of *R. clarus*. Phytoextraction was higher with the addition of the vermicompost level equivalent to 40 mg P kg^{-1} and without *R. clarus* inoculation. However, *C. ensiformis* was not a good phytoextractor because less than 100 mg Cu kg^{-1} accumulated in the shoot. The system *C. ensiformis*–vermicompost–*R. clarus* exhibited potential for Cu phytostabilization in sandy soils.

1. Introduction

Soil Cu contamination is a common problem in many agricultural regions worldwide (Andrade et al., 2010). In Southern Brazil, the humid subtropical climate propitiates the occurrence of fungal diseases in vines, and control of such diseases is often performed through frequent application of Bordeaux mixture, causing Cu accumulation in soils (Mirlean et al., 2009). This problem is aggravated by the fact that many vineyards in Brazil are established in sandy soils, with low concentrations of organic matter, resulting in low Cu-sorption capacity and higher environmental contamination potential. Miotto et al. (2014) measured levels of 62.5 mg Cu kg^{-1} at the top 20 cm of a vineyard sandy soil, whereas natural concentrations are 3.2 mg Cu kg^{-1} (USEPA 3050B method). In the same soil, Brunetto et al. (2013) observed Cu concentrations 70 times higher in the 0-10 cm layer and 40 times higher in the 10-20 cm layer compared to adjacent soil without vines (method 0.01 mol L^{-1} EDTA + 1.0 mol L^{-1} NH_4CH_3COO).

Plant cultivation in soils with Cu accumulation normally results in increased heavy metal concentration in plant tissues and higher production of reactive oxygen species (ROS) in plant cells (Briat and Lebrun 1999). ROS, such as the superoxide anion radical (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$) (Kohen and Nyska 2002), cause oxidative damage to lipids, proteins, and nucleic acids. Excess Cu can also inhibit photosynthesis and production of chlorophyll *a* and *b* (Baglyas and Pólos 2014; Cambrollé et al., 2014). As a result of these physiological damages, ground-cover plants and vine seedlings established in old vineyards in Southern Brazil exhibit chlorosis, necrosis, root growth inhibition, and decreased dry matter production (Miotto et al., 2014). This damage results in lower soil cover and increased erosion, Cu contamination of surface water and groundwater (Besnard et al., 2001), and economic losses for wine growers.

However, some plants are able to grow in environments with high soil heavy metal concentrations and to accumulate heavy

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metals in the shoot and/or roots (Dipu et al., 2012). These plants exhibit enzymatic and non-enzymatic defense mechanisms against the detrimental effects of heavy metal toxicity. Increases in carotenoid production, metal retention on the roots, organic acid complexation, and several changes in cellular metabolism are some of the non-enzymatic defense mechanisms exhibited by these plants (Clemens 1999; Bowler and Fluhr 2000; Resende et al., 2003; Andrade et al., 2009). Jack bean (*Canavalia ensiformis* (L.) D. C.) is a tropical legume, used as a ground-cover crop, and exhibits potential to be used in phytoremediation (Andrade et al., 2010). This species forms symbiotic associations with N-fixing bacteria and arbuscular mycorrhizal fungi (AMF), which increase the capacity of these crops to tolerate water and nutritional deficiencies as well as heavy metal phytotoxicity (Watts-Williams et al., 2013; Medina et al., 2015; Ferreira et al., 2015).

Phytoremediating plants usually have difficulty establishing themselves in soils with high heavy metal concentrations. Phytoremediation efficiency may therefore be increased through mycorrhizal fungi inoculation and addition of organic fertilizers to the soil (Jadia and Fulekar 2008; Fernández-Gómez et al., 2012). Symbiotic associations with arbuscular mycorrhizal fungi were observed to increase the growth of *Tagetes erecta* and Cu phytoextraction in environments with high Cu concentration (Castillo et al., 2011). Moreover, higher Cu extraction by *Oenothera picensis* was observed following addition of organic compounds to the soil (González et al., 2014)

Organic fertilizers can improve the efficiency of phytoremediation by reducing the bioavailability of heavy metals due to their high concentrations of heavy metal-binding functional groups, promoting plant growth in the contaminated areas, reducing copper migration into the soil and increasing phytostabilization (Galende et al., 2014). In contrast, the addition of organic fertilizers can increase the levels of soluble forms of carbon that promote Cu desorption and increase the bioavailability of Cu in soil, which favors phytoextraction (Karami et al., 2011). Grape bagasse is a residue of wine production that can be used for production of organic fertilizers. This process represents a more environmentally friendly use of grape bagasse, contributes to the replenishment of part of the nutrients in the vineyard soil, and improves the soils' chemical, physical, and biological properties, especially for sandy soils (Fernández-Bayo et al., 2007). However, an interaction between arbuscular mycorrhizal fungi and vermicompost in the phytoremediation of a heavy metal-contaminated soil by *Trifolium repens* was not observed (Fernández-Gómez et al., 2012). The synergistic effect of mycorrhizal fungi and vermicompost in phytoremediation may depend on the plant species, fungal species, and the soil and vermicompost characteristics. The goal of the present study was to evaluate the effect of inoculation with *Rhizophagus clarus* and addition of grape-bagasse vermicompost on phytoremediation by *C. ensiformis* of a sandy soil with high Cu concentration.

2. Materials and methods

2.1. Soil, vermicompost, and plant growth conditions

The soil used to grow the plants was collected from an area of natural pasture having no history of cultivation. This area was located within 20 m of a vineyard in the state of Rio Grande do Sul, Brazil (30°48′27″S and 55°22′42″W). The soil, classified as Typic Hapludalf (Soil Survey Staff, 2010), was collected from a depth of 0.0–0.2 m; vegetation and litter were removed. The soil was autoclaved to eliminate possible viable spores from native arbuscular mycorrhizal fungi. After 60 days, the soil pH was adjusted to 6.0 by adding lime, and 100 mg Cu kg^{-1} soil was added in the form of copper sulfate (33.34%) and copper chloride (66.33%). The soil was incubated for 30 days, and the following chemical and physical characteristics were analyzed (methods are shown in parentheses): 140 g kg^{-1} clay (densimeter), 9.0 g kg^{-1} organic matter (Walkley–Black), pH 5.6 (water 1:1), 4.5 mg dm^{-3} P (Mehlich-1), 84.0 mg dm^{-3} K (Mehlich-1), 94.2 mg dm^{-3} Cu (Mehlich-1), 1.6 mg dm^{-3} Zn (Mehlich-1), 44.8% base saturation, and 0% Al saturation. Field capacity was determined in a tension table by saturating the soil samples for 48 h and subjecting them to 10 kPa for 4 days (Klute, 1986).

Vermicompost was produced from grape bagasse subjected to aerobic composting and subsequently to vermicomposting with Eisenia andrei Bouché (1972) worms. The vermicompost was autoclaved and was chemically analyzed after 60 days (Table 1). Seven days before sowing, a filtrate (without AMF propagules) of non-sterile soil or vermicompost was added to reestablish the native microbial populations in the sterile soil and vermicompost (Haymann and Mosse, 1971).

C. ensiformis (L.) D.C. (jack bean) was grown in a greenhouse (29°41′11.46″S and 53°43′8.28″W). The experimental units consisted of 5-L pots with 3.5 kg soil. The soil moisture was controlled through daily weighings and kept at 70% field capacity by adding distilled water. Five seeds inoculated with *Bradyrhizobium elkanii* were sown on each pot. Thinning was performed eight days following germination, keeping two seedlings per pot.

AMF inoculation was performed using 100 spores of *R. clarus* (Nicolson and Schenck, 2010) C. Walker & A. Schüßler per pot, placed close to the root, following multiplication in trap cultures under laboratory conditions. Spore extraction was performed by wet sieving (Gerdemann and Nicolson, 1963), followed by centrifugation in water at 412 *g* for three minutes and in 45% sucrose (m/v) at 309 *g* for two minutes. Selection and counting of viable spores was performed using a stereomicroscope.

2.2. Experimental design

The experimental design was completely randomized, with a 5×2 factorial arrangement and three replicates. Five levels of vermicompost were tested: 0, 4.8, 9.7, 19.4, and 38.8 g vermicompost kg^{-1} soil, supplying 0, 10, 20, 40, and 80 mg P per kg soil (0PV, 10PV, 20PV, 40PV, and 80PV), respectively. Half of the plants from all treatments were inoculated with arbuscular mycorrhizal fungus (+AMF), and the other half were not inoculated (−AMF). Based on a previous experiment, 6.38 mg P kg^{-1} were added as a KH_2PO_4

Table 1
Chemical parameters (in dry weight) of grape-bagasse vermicompost and limits established by the Brazilian legislation.

Parameters	Units	Vermicompost	Limits[e]
Nitrogen[a]	g kg^{-1}	32.0	min. 5.0
Phosphorus[b]	g kg^{-1}	3.0	–
Potassium[b]	g kg^{-1}	12.0	–
Carbon[a]	g kg^{-1}	346.0	min. 150.0
C/N ratio	–	10.5	max. 14.0
pH	–	8.0	min. 6.0
Mercury[c]	mg kg^{-1}	< 0.01	max. 0.4
Copper[d]	mg kg^{-1}	39.0	max. 70
Zinc[d]	mg kg^{-1}	27.2	max. 200
Cadmium[d]	mg kg^{-1}	< 0.2	max. 0.7
Nickel[d]	mg kg^{-1}	4.0	max. 25
Chrome[d]	mg kg^{-1}	7.0	max. 7.0
Lead[d]	mg kg^{-1}	3.0	max. 45
Molibdenum[d]	mg kg^{-1}	0.2	–

[a] Determined using an Elemental Analyzer (Flash 1112, Thermo Finnigan, Italy).
[b] Acid digestion in sulfuric acid and determination in Atomic Absorption Spectrophotometer (AAS) (GBC, 932 AA, USA), according to EMBRAPA (1997).
[c] Method EPA7471A.
[d] Method EPA3050.
[e] Normative instruction n° 46, 10/06/2011, MAPA (Brasil, 2011).