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Effects of *Glomus deserticola* inoculation on *Prosopis*: Enhancing chromium and lead uptake and translocation as confirmed by X-ray mapping, ICP-OES and TEM techniques

Jack A. Arias[a], Jose R. Peralta-Videa[b], Joanne T. Ellzey[c], Minghua Ren[d], Marian N. Viveros[c], Jorge L. Gardea-Torresdey[a,b,*]

[a] Environmental Science and Engineering Ph.D. Program, University of Texas at El Paso, 500 West University Ave., El Paso, TX 79968, USA
[b] Chemistry Department, University of Texas at El Paso, 500 West University Ave., El Paso, TX 79968, USA
[c] Biology Department, University of Texas at El Paso, 500 West University Ave., El Paso, TX 79968, USA
[d] Geology Department, University of Texas at El Paso, 500 West University Ave., El Paso, TX 79968, USA

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi contribute to plant growth, mediating the uptake of mineral elements. In polluted areas, AM also binds toxic heavy metals to roots. In this study, mesquite plants (*Prosopis* sp.), associated with *Glomus deserticola*, were treated for 15 days (in hydroponics) with lead at 0, 10, 50, or 100 mg L^{-1}, and chromium(III) and (VI) at 0, 20, 40, 75, or 125 mg Cr L^{-1}. All Cr ion concentrations and the highest Pb concentration reduced shoot size compared to the control. Toxic effects (yellowish leaves, leaf decay) were observed after seven treatment days. However, Pb and Cr(III) treated plants recovered upon conclusion of experimental period. Total amylase activity in leaves increased upon the addition of Pb and Cr. The inductively coupled plasma-optical emission spectroscopy results showed that plants treated with Pb at 50 mg L^{-1} accumulated in roots, stems, and leaves: 61947, 9584, and 478 mg Pb kg^{-1}; whereas plants treated with Cr(III) and Cr(VI) at 125 mg L^{-1} accumulated 28815, 6055, and 647; and 13767, 5010, and 2530 mg Cr kg^{-1}. The transmission electron microscopy (TEM) micrographs showed the presence of *G. deserticola* within roots. X-ray mapping demonstrated higher Cr and Pb deposition in xylem and phloem cells. Results suggest that *G. deserticola* improves metal tolerance/accumulation in mesquite.

1. Introduction

Chromium (Cr) and lead (Pb) are widely distributed in nature. However, human activities have raised the concentration of these elements to dangerous levels in a number of sites around the world. Chromium exists in several oxidation states, but the trivalent [Cr(III)] and hexavalent [Cr(VI)] are the most stable and abundant forms in the environment (Chandra and Kulshreshtha, 2004; Rai et al., 2007). Lead is found in nature complexed with organic matter, adsorbed on clays and oxides, and precipitated as carbonates, hydroxides and phosphates (Epstein et al., 1999).

At low concentrations, Cr(III) is considered essential while Cr(VI) is toxic and carcinogenic for animals and humans. However, neither Cr(III) nor Cr(VI) has known functions in plants (James, 1996). In humans, excessive Cr may produce ulcers, allergic dermatitis, lung cancer, renal insufficiency, or liver necrosis (Srivastava et al., 2002). Chromium is used for leather tanning, paints, corrosion inhibition, chrome plating, steel production, and wood preservation. In the presence of manganese oxide excess, Cr(III) oxidizes into Cr(VI), which is more soluble in water and more toxic than other Cr forms (Dai et al., 2009). In animals, lead has no known function in metabolic processes and is toxic even when absorbed in small amounts (Peralta-Videa et al., 2009). In humans, lead toxicity affects the skin, internal organs, the nervous system, and may cause sterility and mental retardation (Peralta-Videa et al., 2009). Lead contamination is produced by mining, smelting, burning of fossil fuels, and the manufacture of pesticides and fertilizers (Hu and Zhang, 2005).

Chromium(III) and lead persist in the environment due to their low solubility and bioavailability, which changes when the pH is greater than 4.5. Chromium(VI) exists predominantly at a pH greater than 6 (Bartlett, 1988). In general, the availability of Cr and Pb depends on their oxidation state, pH, and complexation (Bartlett and James, 1983). Studies have demonstrated that both Cr species, as well as Pb reduce plant growth, alter enzymatic activity, and mineral nutrition (Shanker et al., 2005; Lopez et al., 2005, 2007).

* Corresponding author at: Chemistry Department, University of Texas at El Paso, 500 West University Ave., El Paso, TX 79968, USA. Tel.: +1 915 747 5359; fax: +1 915 747 5748.
E-mail address: jgardea@utep.edu (J.L. Gardea-Torresdey).

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One of the enzymes altered by Cr and Pb toxicity is amylase (Lopez et al., 2007).

The U.S. EPA has recommended and often enforced different techniques, including physical and chemical methods, for the restoration and management of areas contaminated with heavy metals (Mulligan et al., 2001). However, these techniques are often expensive, labor consuming, soil disturbing and improper for large areas (Chaney et al., 1997). In the last three decades it has been documented that plants and associated microorganisms could be considered a harmless option to remove heavy metals excess from polluted sites (Horne, 2000; Fischerova et al., 2006). However, the response of desert plants associated with arbuscular mycorrhizal (AM) fungi under exposure to chromium and lead has not been reported.

Mesquite is a fast proliferating shrub that grows in seemingly harsh conditions such as heavy metals in soil and water scarcity. It produces large biomass, is a source of stock food, and is used for erosion control (Jeffrey and March, 1995). Sinha (1999) and Fischerova et al. (2006) have reported that tree species can uptake high amounts of metals, produce greater biomass, and are easier to harvest and manipulate compared to shrubs and herbs. The ability of *Prosopis* sp., *Salsola kali*, and *Convolvulus arvensis* to uptake Cr and Pb has been previously reported (Jeffrey and March, 1995; Aldrich et al., 2003; de la Rosa et al., 2005; Montes-Holguin et al., 2006). However, to our knowledge, the accumulation of Cr and Pb by mesquite associated with arbuscular mycorrhizal (AM) fungi has not been studied. In this association, the fungus contributes to plant health through active nutrient absorption and resistance against pathogen attacks (Rufyikiri et al., 2002). In turn, the host plant releases metabolites critical for the fungal growth and development (Tawaraya et al., 1996). Furthermore, studies have demonstrated that the symbiotic interaction between plants and AM fungi has an effect on the tolerance and uptake of heavy metals, but little is known about it (Anderson et al., 1993). To the knowledge of the authors, there are no reports on the effect of AM fungi on enzyme activity in mesquite. In order to obtain insight about the metabolic state of mesquite associated with endomycorrhizal fungi, in response to Cr and Pb stress, total amylolitic activity (TAA) was assayed in leaves at the end of the experimental period.

The objectives of the present work were to determine the response of mesquite plants associated with the AM fungus *Glomus deserticola* under Cr and Pb stress by measuring the growth of plants, elemental absorption, metal distribution and deposition, and total amylase activity (TAA). The working hypothesis was that the AM fungi would either increase the mesquite tolerance to Cr and Pb toxicity or increase the uptake of both toxic elements. Plants were treated with 0–125 mg L^{-1} of Cr(III) or Cr(VI), and with 0–100 mg L^{-1} of Pb in hydroponics. Instrumental techniques utilized included inductively coupled plasma/optical emission spectroscopy (ICP-OES), electron scanning microprobe microscopy (ESM), transmission electron microscopy (TEM), scanning transmission electron microscopy (STEM), and ultraviolet–visible spectroscopy (UV/VIS) to determine the element concentrations, metal distribution and deposition, and the amylase activity.

2. Materials and methods

2.1. Seed germination, fungal inoculation, and treatment application

Mesquite (*Prosopis* sp.) seeds were obtained from Wild Seeds (Tempe, Arizona). Before performing an experiment, the seeds were sterilized in commercial sodium hypochlorite solution [diluted to 4% with sterilized deionized water (DI)] for 30 min and rinsed three times with sterilized DI. For germination, the seeds were placed in sterilized paper towels dampened with Murashige and Skoog nutrient solution as described by Carrillo-Castaneda et al. (2005). Five grams of clay containing the *G. deserticola* spores provided by Reforestation Technologies International (RTI) (Salinas, CA) were ground and diluted in 5 mL of DI. An aliquot of 1 mL of the fungal–clay solution was added to the seeds in each paper towel. When the radicle–hypocotyl axis (the axial part below cotyledons) and the *G. deserticola* mycelia appeared (5 days), seedlings were placed in 400 mL capacity Mason jars containing the MS nutrient solution in an ENVIRCO laminar flow hood (Environmental Air Control, Albuquerque, NM). In this experiment, Cr(III) [from ($CrNO_3$)], Cr(VI) [from K_2CrO_4], and Pb [from Pb (NO_3)$_2$] (Sigma–Aldrich, St. Louis, MO) were used. The Cr ions were used at 0, 20, 40, 75, and 125 mg L^{-1} and Pb at 0, 5, 10, 50, and 100 mg L^{-1}. These concentrations were selected based on previous studies performed in our research group without AM fungi (Aldrich et al., 2003, 2004). Three replicates (15 seedlings each) were used for each metal ion concentration. The glassware and DI were sterilized at 120 °C and a pressure of 1.25 kg cm^{-2} for 45 min to avoid fungal and microbial contamination (Market Forge, Albertville, MN). The jars containing the seedlings with the respective treatments were set for 15 days at 27 °C, 12 h photoperiod, and light output of 53 μmoles m^{-2} s^{-1}.

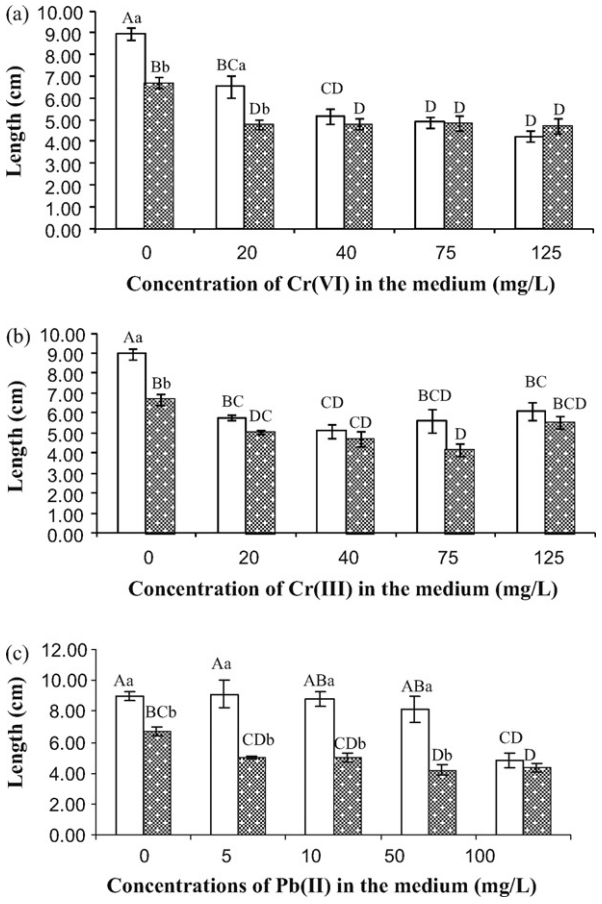

Fig. 1. Average length of mesquite roots (□), and stems (■) treated for 15 days in hydroponics with (a) chromium(VI) at 0, 20, 40, 75, and 125 mg L^{-1}, (b) chromium(III) at 0, 20, 40, 75, and 125 mg L^{-1}, and (c) lead at 0, 5, 10, 50, and 100 mg L^{-1}. Uppercase letters stand for significant differences ($p < 0.05$) between treatments for the same tissue. Lowercase letters indicate significant differences ($p < 0.05$) between tissues of the same treatment. Error bars stand for SE.