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Arbuscular mycorrhizal fungi-assisted phytoremediation of a lead-contaminated site

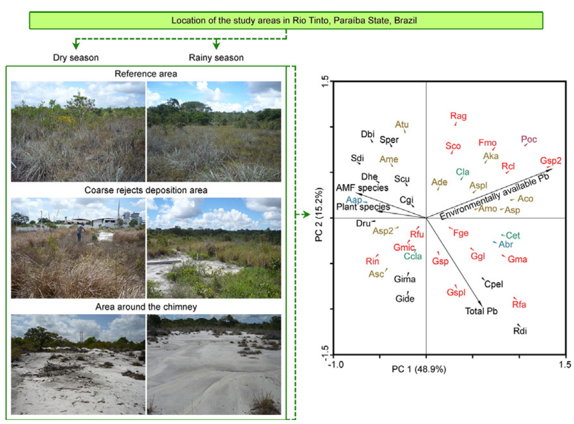
Jerusa Schneider [a,b,*], Jochen Bundschuh [c,d,e], Clístenes Williams Araújo do Nascimento [b]

[a] School of Civil Engineering, Architecture and Urban Design – FEC Sanitation and Environment Dept. – DSA, State University of Campinas – Unicamp, P. O. Box 6143, 13083-889 Campinas (SP) Brazil
[b] Agronomy Department, Federal Rural University of Pernambuco (UFRPE), Recife, Pernambuco 52171-920, Brazil
[c] Deputy Vice-Chancellor's Office (Research and Innovation), University of Southern Queensland, West Street, Toowoomba, 4350, QLD, Australia
[d] Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, 4350, QLD, Australia
[e] KTH-International Groundwater Arsenic Research Group, Department of Sustainable Development, Environmental Science and Engineering, KTH Royal Institute of Technology, Teknikringen 76, SE-10044 Stockholm, Sweden

HIGHLIGHTS

- AMF community diversity and activity depends on soil Pb contamination level.
- Plants diversity in Pb contaminated soils is related to AMF colonization.
- AMF colonization plays a role in the vegetation establishment on Pb-contaminated sites.

GRAPHICAL ABSTRACT



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ABSTRACT

Knowledge of the behavior of plant species associated with arbuscular mycorrhizal fungi (AMF) and the ability of such plants to grow on metal-contaminated soils is important to phytoremediation. Here, we evaluate the occurrence and diversity of AMF and plant species as well as their interactions in soil contaminated with lead (Pb) from the recycling of automotive batteries. The experimental area was divided into three locations: a non-contaminated native area, a coarse rejects deposition area, and an area receiving particulate material from the chimneys during the Pb melting process. Thirty-nine AMF species from six families and 10 genera were identified. The *Acaulospora* and *Glomus* genera exhibited the highest occurrences both in the bulk (10 and 6) and in the rhizosphere soils (9 and 6). All of the herbaceous species presented mycorrhizal colonization. The highest Pb concentrations (mg kg^{-1}) in roots and shoots, respectively, were observed in *Vetiveria zizanoides* (15,433 and 934), *Pteris vitata* (9343 and 865), *Pteridim aquilinun* (1433 and 733), and *Ricinus communis* (1106 and 625). The diversity of AMF seems to be related to the area heterogeneity; the structure communities of AMF are correlated with the soil Pb concentration. We found that plant diversity was significantly correlated with AMF diversity ($r = 0.645$; $P > 0.05$) in areas with high Pb soil

* Corresponding author.
E-mail address: jerusasch@gmail.com (J. Schneider).

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concentrations. A better understanding of AMF communities in the presence of Pb stress may shed light on the interactions between fungi and metals taking place in contaminated sites. Such knowledge can aid in developing soil phytoremediation techniques such as phytostabilization.

1. Introduction

The increase in industrial and mining activities has altered the natural geochemical cycle of heavy metals. The increasing prevalence of these elements in the biosphere makes environmental cleanup a challenge. Lead (Pb) is classified as the second most dangerous element on the priority list of the United States Environmental Protection Agency (ATSDR, 2013). The global automobile battery industry uses approximately 70% of the Pb consumed worldwide (Paoliello et al., 2002); battery recycling is one of the primary forms of Pb soil contamination (Cecchi et al., 2008). The waste originating from this activity may contaminate the recycling facility itself as well as the surrounding areas due to effluent and ash emissions that pose a serious risk to animals and humans (Cecchi et al., 2008). Due to the potential toxicity and high persistence of Pb, soils polluted with this metal are an environmental problem requiring an effective and economically viable solution (Nascimento and Xing, 2006).

Current methods to ameliorate Pb-contaminated soils include soil removal, soil washing, and physical stabilization. However, these methods are associated with high costs and are environmentally unfriendly (Tu and Ma, 2002). Therefore, there is an ongoing search for soil remediation methods that are cost effective and less harmful to the environment.

The treatment of Pb-contaminated soils may be accomplished by various techniques. Phytoremediation, an emerging technique that uses plants to clean up soil and water, has gained technical attention and public acceptance thanks to its low cost and minimal impact on soil properties (Arias et al., 2015; Krämer, 2005; Raskin and Ensley, 2000). Among the phytoremediation strategies, phytostabilization involves the reduction of metal mobility in soil and accordingly decreases wind-blown dust, minimizes soil erosion, and reduces the contaminant solubility or bioavailability to the food chain. The success of this technique, however, is highly dependent on the establishment of a plant community in such stressful conditions. In this context, a diverse and functional microbial population may play an important role in plant survival and growth. For the optimal selection of plant species, it is important to consider their capacity for association with arbuscular mycorrhizal fungi (AMF), which increase nutrient and water uptake by plants (Bhalerao, 2013; Cabral et al., 2015). Mycorrhizal associations may lead to an increase up to 80% of P, 60% of Cu, 25% of N, 25% of Zn, and 10% of K absorbed by plants (Jeffries et al., 2003; Soares and Siqueira, 2008).

Arbuscular mycorrhizal fungi have also been shown to be important in the revegetation process of degraded soils, and various mechanisms to this effect have been suggested in the literature (Cabral et al., 2015; Harms et al., 2011; Sessitsch et al., 2013) to dilute heavy metals in plant tissues based on increased plant growth (Christie et al., 2004), exclude uptake by precipitation or metal chelation in the rhizosphere (Kaldorf et al., 1999), and reduce uptake due to the retention and immobilization of metals in fungal structures (González-Chávez et al., 2002; Khan et al., 2000; Zhu et al., 2001) with a consequent reduction in the root elements transferred to shoots (Christie et al., 2004; Joner et al., 2000).

A high Pb concentration in soil may selectively affect AMF populations, favoring those more adapted to stressful conditions. A high Pb concentration may also have an impact on vegetation that differs as a function of isolate identity or fungal species (Pouyu-Rojas et al., 2006). Several studies have reported positive relationships between diversities of plants and AMF (Chen et al., 2015; Grime et al., 1987; Schneider et al., 2013; van der Heijden et al., 1998). Such an association may later the patterns of uptake, accumulation, and translocation of Pb in plants (Arias et al., 2015; Bafeel, 2008; Souza et al., 2012).

Arbuscular mycorrhizal fungi ecology in Pb-contaminated soils is of significant research interest because Pb affects both the community and the activity of AMF; these effects are related to varying isolate tolerances (Yang et al., 2016). It is also important to introduce fungi species/isolates to promote AMF-aided phytostabilization (Schneider et al., 2013; Souza et al., 2012). The AMF contribution to plants under Pb stress depends on the diversity, abundance, persistence, and efficiency of the populations, all of which vary as a function of site and environmental conditions. Therefore, it is essential to consider edaphic characteristics such as pH and level of soil fertility, especially regarding P availability, humidity/aeration, temperature, and vegetation cover (Leigh et al., 2009; Zhang et al., 2010).

Assessing the impact of Pb contamination on AMF populations is an essential step toward phytostabilization of metal-contaminated sites. Taking into account the hypothesis that soil contamination with Pb interferes in both the community and the activity of AMF, being these effects related to the differential tolerance of AMF isolates, the goal of this study was to evaluate the occurrence and diversity of AMF native species in a site contaminated by the recycling of automobile batteries. The data that we collected can be used to assist phytoremediation processes, which can decrease risks to human health and the ecosystem.

2. Material and methods

2.1. Location and characteristics of the study area

The present work was carried out in a site contaminated with Pb due to automobile battery recycling activities. The site was located in Rio Tinto municipality, State of Paraíba, Brazil (6°48′11″S and 35°04′50″ W). The soil in the area was classified as a Ferrihumiluvic Spodosol. The mean annual rainfall in the region was 1634 mm, and two different forms of vegetation were found in the region: semi-deciduous rainforest and open savanna woodland (Oliveira-Filho, 1993).

We studied three subareas (roughly 1000 m^2 each; 50 × 20 m): a natural vegetation (Atlantic Forest fragments) presenting a natural concentration of Pb taken as a background reference (REF), an area dedicated to the disposal of coarse rejects (CR), and an area affected by the deposition of particulate material from the emission of smoke through chimneys during the melting process of Pb (PM). Owing to area heterogeneity and high spatial variability of the Pb concentrations, we carried out soil sampling in lines within the subareas (Fig. 1).

We collected five soil samples from the surface layer (0–0.2 m in depth) of each sampling point. These samples were combined and homogenized to form a composite sample for analysis. Each subarea consisted of three sampling lines (1, 2, and 3) in which six composite samples were obtained. In other words, we studied a total of 18 composite samples from each subarea (i.e., 54 samples in all) (Fig. 1).

2.2. Sampling and soil chemical analyses

We performed the soil sampling in December 2011 (dry season) and June 2012 (rainy season). The soil samples were divided into two parts. One part was used to determine the physical-chemical attributes of the soil; the other part was stored at 6 °C until AMF spore extraction. The