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Arbuscular mycorrhizal fungi alleviate the heavy metal toxicity on sunflower (*Helianthus annuus* L.) plants cultivated on a heavily contaminated field soil at a WEEE-recycling site

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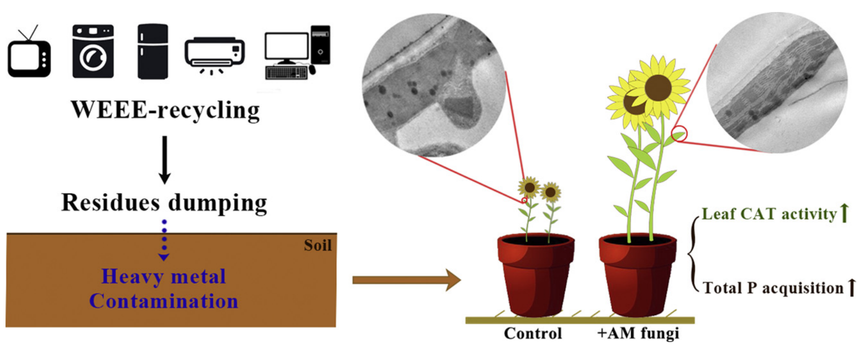
HIGHLIGHTS

- WEEE-recycling induced serious heavy metal (HM) contamination on soil and sunfower.
- AM fungal inoculation (+M) enhanced both P absorbtion and plant growth of sunfower.
- +M significantly decreased HM levels in the shoot, but not the root, of sunfower.
- +M increased leaf catalase activity and reduced HM toxicity on thylakoid structure.
- +M promoted HM uptakes, suggesting its potential application for phytoextraction.

GRAPHICAL ABSTRACT



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ABSTRACT

An 8-week pot experiment was conducted to investigate the growth and responses of sunflower (*Helianthus annuus* L.) to arbuscular mycorrhizal (AM) fungal inoculations on a heavily heavy metal (HM)-contaminated (H) soil and a lightly HM-enriched (L) soil, both of which were collected from a waste electrical and electronic equipment (WEEE)-recycling site. Compared with the L soil, the H soil induced significantly larger ($P < 0.05$) concentrations of Cd, Cu, Pb, Cr, Zn and Ni in sunflower (except for root Cr and shoot Ni), which impaired the thylakoid lamellar folds in leaves. The biomasses and P concentrations of shoots and roots, as well as the total P acquisitions per pot were all significantly decreased ($P < 0.05$). Both *Funneliformis mosseae* (*Fm*) and *F. caledonium* (*Fc*) inoculation significantly increased ($P < 0.05$) root mycorrhizal colonization. For the L soil, AM fungal inoculations had no significant effects on the soil-plant system, except for a decrease of soil pH and increases of soil available P and DTPA-extractable Zn concentrations with the *Fm*-inoculated treatment. For the H soil, however, AM fungal inoculations significantly increased ($P < 0.05$) the biomasses and P concentrations of shoots and roots, as well as the total P acquisitions per pot, and significantly reduced ($P < 0.05$) the concentrations of HMs in shoots (except for Cu and Pb with *Fm*- and *Fc*- inoculated treatments, respectively) and alleviated the toxicity symptoms of HMs in thylakoid structure of leaves. AM fungal inoculations in the H soil also

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significantly increased ($P < 0.05$) the shoot uptake of HMs (except for Cr), and tended to decrease the total concentrations of HMs in soils. This suggests the potential application of AM fungi for both reducing HM stress and promoting phytoextraction of HM-contaminated soils caused by WEEE recycling.

1. Introduction

Waste electrical and electronic equipment (WEEE) refer to end-of-life electronic products, including television sets, washing machines, air conditioners, computers, mobile phones, and others, which are composed of sophisticated blends of plastics and metals, among other materials (Xu et al., 2015). WEEE contain reusable precious metal resources, such as Au, Ag, Pd and Pt (Zhao et al., 2015). Due to the cheap labor cost and the weak legislation system, a number of WEEE-recycling workshops have been assembled in some cities of developing countries, such as Guiyu and Taizhou of China, Delhi and Bengaluru of India, Lagos of Nigeria, and Accra of Ghana (Xu et al., 2015). In these areas, WEEE are recycled by local villagers using primitive disassembly methods, without appropriate facilities to prevent environmental pollution (Damrongsiri et al., 2016). Therefore, tens of millions of tons of WEEE-recycling residues were dumped in workshops, yards, roadsides, open fields, wastelands, irrigation canals, ponds, rivers and riversides every year (Huo et al., 2007). Hazardous chemicals, such as heavy metals (HMs), can be released from the disposal or recycling processes of WEEE, polluting the environment (Zhang et al., 2014). In fact, extensive research has reported heavy pollutions of HMs (such as Cd, Cu, Pb, Cr, Zn and Ni) in soils (Tang et al., 2010; Wu et al., 2015; Zhao et al., 2015), vegetables (Luo et al., 2011), and crops (Fu et al., 2008) in neighboring fields due to WEEE recycling. Large concentrations of HMs in the soil may inhibit seed germination and/or increase the HM accumulation risks of plants, and even influence nutrient metabolism or reduce the photosynthetic and growth rates when the accumulation exceeds a certain threshold (Andrade et al., 2010; Subba et al., 2014; Gill et al., 2015; Gupta et al., 2017). In fact, damage to plant organs due to large concentrations of HMs has been widely observed, such as the thinning of the cell wall, the formation of intercellular spaces, and amoeboid protrusions and folds, and the appearance of immature nucleus and ruptured thylakoid membranes (Kaur et al., 2013; Gill et al., 2015). In addition, plants treated with large concentrations of HMs usually show a significant reduction of chlorophyll and carotenoid contents (Subba et al., 2014), as well as the generation of redox imbalance (Gupta et al., 2017).

Arbuscular mycorrhizal (AM) fungi can form symbiotic associations with most terrestrial plant species in a wide range of soils (Hassan et al., 2013), and can improve plant performance through increased defenses against environmental stresses, both biotic and abiotic, such as drought, salinity, and HM toxicity (Ferrol et al., 2016). In the extreme environment with large concentrations of various HMs, AM fungi can survive (Sanchez-Castro et al., 2017) and are known to have beneficial effects on host plants. On the one hand, AM fungi can make a considerable contribution to nutrient (notably P) uptake to promote plant growth (Smith and Read, 2008). On the other hand, AM fungi can alleviate HM toxicity on plants in polluted soil by the reduction of HM acquisitions (Hu et al., 2014), the biological dilution of HMs (Hu et al., 2013), and the decrease of oxidative stress (Neagoe et al., 2014). Consequently, there may be a potential of using AM fungi to enhance plant resistance to environmental stress caused by HM contamination. For example, *Funneliformis mosseae* (*Fm*) could reduce the concentrations of Pb, Cd and As of horse tamarind (*Leucaena leucocephala* (Lam.) de Wit) (Zhan et al., 2016), while *F. caledonium* (*Fc*) could decrease shoot Cd, Cu, Pb, and Zn concentrations of maize (*Zea mays* L.) (Wang et al., 2007). However, different AM fungal species may induce differential effects on the absorption of elements by the same plant. For example, there was a significant difference in Cu-sorption capacity between the extraradical mycelium of *Fc* and *Fm* (Gonzalez-Chavez et al., 2002), and inoculation with *Fc* and *Fm* could induce differential U and Cd absorption by Chinese brake fern (*Pteris vittata* L.) and Alfred stonecrop (Sedum alfredii Hance), respectively (Chen et al., 2006; Hu et al., 2013).

To be a potential material for phytoremediation that can survive in heavily contaminated soils with various HMs, ideal plants should have a considerable capacity for HM tolerance and a fast growth rate with a large biomass. Sunflower (*Helianthus annuus* L.), a food, oil and fuel bioenergy plant species (Fozia et al., 2008), grows in a wide range of soils throughout the world (Forte and Mutiti, 2017). Its stem can grow as high as 3 m tall with the flower head reaching up to 30 cm within 2 or 3 months (Adesodun et al., 2009; Jadia and Fulekar, 2008), and it can accumulate relatively large concentrations of Cd (Lopes Júnior et al., 2014), Cu (Forte and Mutiti, 2017), Pb (Batista et al., 2017), Cr (Cutright et al., 2010), Zn (Adesodun et al., 2009), and Ni (Shaheen and Rinklebe, 2015). However, most former studies focused on the growth of sunflower on mild or moderate contaminated (only by one to two kinds of HMs) soils (de Andrade et al., 2008; Fozia et al., 2008; Adesodun et al., 2009; Jarrah et al., 2014). There is limited information available for the role of AM fungi on plant growth in heavily contaminated soil with various HMs, such as caused by WEEE recycling. Thus, it was hypothesized that AM fungal inoculation could alleviate the stress of HMs on plants via decreasing HM concentrations, and different AM fungal species may induce differential effects on the absorption of various HMs by sunflower. The objective of this experiment was therefore to investigate the growth of sunflower on a heavily HM-contaminated (H) soil and a lightly HM-enriched (L) soil, both of which were collected from a WEEE-recycling site, and the plant's responses to the inoculations of two different AM fungal species. This study may contribute to developing application strategies of AM fungi to reduce HM stress and promote plant growth in HM-contaminated soils around WEEE-recycling sites.

2. Materials and methods

2.1. Mycorrhizal inoculum

Two AM fungal inocula were used in this experiment. *Funneliformis mosseae* (Nicol. & Gerd.) Gerd. & Trappe M47V (*Fm*) was obtained from the International Bank for Glomeromycota (IBG), France. *F. caledonium* (Nicolson & Gerd.) Walker & Schüßler 90036 (*Fc*) was deposited at the Institute of Soil Science, Chinese Academy of Sciences, China. The two AM fungal inocula and the non-mycorrhizal inoculum were prepared using the same substrate under the same conditions. After plant harvesting, all inocula were air-dried and passed through a 2-mm sieve.

2.2. Soil preparation

Two soil samples were collected from a residue-dumping (RD) site (28°30′47″N, 121°24′16″E), which was used for the dumping of WEEE-recycling residues, and a neighboring field (NF) site (28°30′42″N, 121°24′21″E), which was an arable agricultural land 210 m far away from the RD site, in Taizhou City, Zhejiang Province, one of the three major WEEE-recycling bases in China. The soil samples were air-dried, ground with a wooden pestle, and homogenized by sieving through a 5-mm sieve. Subsamples of soils were separately sieved through a 2 mm sieve for analyzing soil properties. Soil pH (soil: deionized water = 1:5) was measured with a pH meter (Thermo Scientific Orion Star A211, USA). Soil available P was extracted with sodium