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Root-induced changes of Zn and Pb dynamics in the rhizosphere of sunflower with different plant growth promoting treatments in a heavily contaminated soil

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ABSTRACT

Root induced changes are deemed to have an important role in the success of remediation techniques in contaminated soils. Here, the effects of two nano-particles [SiO_2 and zeolite] with an application rate of 200 mg kg^{-1}, and two bacteria [*Bacillus safensis* FO-036b(T) and *Pseudomonas fluorescens* p.f.169] in the rhizosphere of sunflower on Zn and Pb dynamics were studied in greenhouse conditions. The treatments reduced the exchangeable Zn (from 13.68% to 30.82%) and Pb (from 10.34% to 25.92%) in the rhizosphere compared to the control. The EC and microbial respiration/population of the rhizosphere and bulk soil had an opposite trend with the exchangeable fraction of Zn and Pb, but dissolved organic carbon followed a similar trend with the more bioavailable fractions. As a result, the accumulation of Pb and Zn in the plant tissues was significantly ($p < 0.05$) reduced by the application of amendments, which might be due to the shift of the metals to immobile forms induced by the nature of the treatments and changes in the rhizosphere process. The empirical conditions of this research produced the intensification of the rhizosphere process because the findings highlight those changes in the rhizosphere EC, pH and dissolved organic carbon can affect the efficiency of zeolite/SiO_2 NPs and bacteria to immobilize Pb and Zn in the soil, depending on the chemical character of the metals and the treatments. Generally, the affinity of the biotic treatment for Pb was more than the abiotic and conversely, the abiotic treatment showed a higher ability to immobilize Zn than the biotic treatment.

1. Introduction

There are many anthropogenic activities, such as agricultural and industrial activities, transportation, coal combustion residues, spillage of petrochemicals and waste disposal which have increased the accumulation of heavy metals such as Zn and Pb in agricultural soils (Jamali et al., 2009; Zhang et al., 2010; Mousavi et al., 2013, 2017). Lead has no metabolic role in living organisms (Kabata-Pendias and Pendias, 1992) and is very toxic and non-biodegradable (Adriano, 2003). However it can be efficiently absorbed and transported within plant tissue (Mousavi et al., 2010b, 2013). Thus, it can readily enter the food chain, resulting in phytotoxicity (Chen and Kao, 2000) and consequently causing serious threats to public health. The maximum acceptable concentration range of Pb in soil and plants respectively is 2–300 mg kg^{-1} and 0.2–20 mg kg^{-1} (Alloway, 1990). By contrast, Zinc (Zn) is an essential micronutrient and has special physiological functions in all living systems, such as the maintenance of structural and functional unity of biological membranes and the promotion of protein synthesis and gene expression (Andreini et al., 2006). Tolerance to environmental stress conditions has a high requirement for Zn to regulate and maintain the expression of genes needed to protect cells from the detrimental effects of the stress (Cakmak, 2000). The concentration range of Zn in soil and plants is normally 1–900 mg kg^{-1} and 1–400 mg kg^{-1}, respectively (Alloway, 1990). After entering the soil and sediment, Zn and Pb may be distributed among soil constituents and connected to them in different forms, which have often been related to as fractionation (Osakwe and Okolie, 2015; Li et al., 2016). To describe the activities of Zn and Pb in soil, knowledge about both total concentration and chemical fractionation is unavoidable. However, for measuring Zn and Pb activity in soil and to find how readily Zn and Pb uptake by plants happen, it is necessary to study the chemical fractionation rather than the total Zn and Pb content, as the former determines the mobility and bioavailability of Zn and Pb (Wu et al., 2006). Sequential extraction methods offer an efficient tool for studying the

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metal forms (Samiei and Bostani, 2016; Rosado et al., 2016). Tessier et al. (1979) presented an extraction method that is the most widely applied technique. It enables the partitioning of the total metal content into five forms: exchangeable, carbonate bound, Fe/Mn oxide bound, organic bound and residual fraction. The procedure was suggested for sediments, but it can also be used for soils.

Nanoparticle materials are important supporting materials in remediation of soil contamination due to their large surface area and their physical/chemical reactivity. According to Dutta et al. (2000) a nanoparticle is defined as particle with an average characteristic dimension less than 100 nm. These materials show completely new or improved properties based on size, distribution, morphology, and phase compared with larger particles of the bulk material they are made of (Perez al, 2004). Most of the atoms of a nanoparticle on the surface are unsaturated and can easily cohere with other ions, thus possessing considerable chemical reactivity (Liang et al., 2000).

The rhizosphere is a microenvironment where physical, chemical and biological properties may drastically differ in many respects from those in the bulk soil (Marschner and Rmheld, 1996) due to root-induced processes in the vicinity of the root, such as the release of exudates, adsorption and desorption of elements, removal of water and the physical forces of the plant roots. Therefore, the dynamics, transformations, phytoavailability and toxicity of heavy metals are expected to differ obviously from those in bulk soil (Bravin et al., 2012; Houben and Sonnet, 2012). Furthermore, soil application of different amendments for phytostabilization may also change the rhizosphere reactions, which may, in turn, impact the transformation, mobility and bioavailability of trace metals remarkably in response to change in soil pH, rhizodeposition and microbial community (Park et al., 2011).

Silicon from different sources has been demonstrated to reduce the harmful effects of trace metals on plants growing in contaminated soils (Rizwan et al., 2012; Shen et al., 2014; Keller et al., 2015). Zeolites are a group of alumino-silicates with a negative charge (Mohamed, 2001) which are commonly applied as toxic metal-immobilizing agents (Bolan et al., 2014; Navel and Martins, 2014). The fractionation of metals in the soil solution can be changed by Si through the formation of silicate complexes (Putwattana et al., 2010). Some researchers have reported that Fe-Mn oxides and organic matter fractions are the dominant geochemical fractions in soils treated with Si (Chen et al., 2000; Liang et al., 2005), which consequently decreases the exchangeable fraction, thereby reducing trace metal bioavailability in the soil. However, little attention has been paid to the effects of nano-sized SiO_2 and zeolite on the chemical behavior of trace metals in the rhizosphere.

Bacteria that colonize plant roots and promote plant growth are referred to as plant growth-promoting rhizobacteria (PGPR) (Beneduzi et al., 2012). The ability of these bacteria in the alleviation of plant stress in metal-contaminated soils is well demonstrated (Mani et al., 2015; Kamran et al., 2016). Release of chelating agents and siderophores, acidification, phosphate solubilization, and redox changes are known as the main strategies of PGPRs to affect trace metal mobility and bioavailability (Ma et al., 2011). However, studies on PGPRs effects on the fate and behavior of trace metals in rhizosphere soil and in the presence of mineral amendments are still limited. Therefore, the aim of this work was to gain better insight about how the soil application of nanoparticles (SiO_2 and zeolite) and bacteria (*Bacillus safensis* FO-036b (T) and *Pseudomonas fluorescens* p.f.169) in the contaminated soil affect the rhizospheric reaction and, in turn, the fractionation and the bioavailability of Zn and Pb in the rhizosphere of sunflower (*Helianthus annuus*L.). Sunflower is commonly known as a heavy metal-tolerant plant and effective in phytoremediation (January et al., 2008; Huicheng et al., 2012), which is one of the most important oilseed crops in the world, and especially in Iran

2. Materials and methods

2.1. Materials

The studied soil, from Calcixerepts on the USDA system, was sampled (surface layer, 0–30 cm) from the vicinity of the National Iranian Lead and Zinc Company (NILZ) in Zanjan, Iran (36° 36' 40" and 36° 38' 40" N; 48° 37' 33" and 48° 38' 48" E) on Feb, 9, 2016, and after air-drying, it was sieved (2 mm) to determine some biological (microbial population (Alexander, 1982), microbial respiration (Anderson and John, 1982)), physical (soil texture (Gee and Bauder, 1986)) and chemical properties (pH (Klute, 1986), EC (Bremner, 1982), CEC (Sumner and Miller, 1996), OC% (Walkley and Black, 1934), Calcite % (Loeppert and Suarez, 1996), Si (Hurney, 1973), N (Bremner, 1996), P (Olsen et al., 1954), K (Helmke and Sparks, 1996), Zn and Pb concentration (Page, 1982)). The results of analysis are as follows; microbial population 150,000 MPN g^{-1}; microbial respiration 0.25 mg g^{-1}; soil texture, loamy; pH 7.19; EC 4.64 dS m^{-1}; CEC 17.94 meq. 100 g^{-1}; OC 0.39%; Calcite 15.25%; Si 158.98 mg kg^{-1}; N 0.07%; P 8.6 mg kg^{-1}; K 344 mg kg^{-1}; total Zn 7027.54 mg kg^{-1}; extracted Zn by DTPA 311.3 mg kg^{-1}; total Pb4752.48 mg kg^{-1} and extracted Pb by DTPA 174.23 mg kg^{-1}. Because of many adverse effects which are induced by sterilization of soil, especially in elemental composition, carbonate and organic compounds (Perkins et al., 2013), we decided to use non-sterilized soil in this work.

A natural clinoptilolite-zeolite powder (< 60 μm with purity > 95%) supplied by Afrand Tusca Co., Tehran, Iran, was used for this work. It was then changed into nano-sized particles (< 100 nm) by using a Planetary Ball Mill (PM 600) at the Material and Energy Research Center (https://en.merc.ac.ir/) in Karaj, Iran. Its initial characteristics were found as follows (unit %): SiO_2 66.5, Al_2O_3 11.8, CaO 3.1, K_2O 2.1, Na_2O 2, Fe_2O_3 1.3, MgO 0.8, TiO 0.3, MnO 0.04, P_2O_5 0.01 and its CEC was 160–180 meq. 100 g^{-1}. The studied SiO_2-NPs was obtained from Sigma-Aldrich (Purity > 95%). The morphology and characteristics of the zeolite/SiO_2-NPs were evaluated by field emission scanning electron microscopy (FE-SEM; Hitachi S-4700, Tokyo, Japan), and energy-dispersive X-ray spectra (EDS) were prepared using FE-SEM.

Two native strains of bacteria, namely *Bacillus safensis* FO-036b(T) and *Pseudomonas fluorescens* p.f.169, were separated and purified from the soils in the vicinity of a zinc and lead mine in Arak city, Markazi province, Iran, at longitude 35° 48′ 35″E and latitude 50° 58′ 18″N (Motesharezadeh and Savaghebi-Firoozabadi, 2010; Mohammadzadeh et al., 2014), and considered as biotic treatment, and their resistance to high levels of Pb and Zn in respect to colonization, were tested on plate (Saikia et al., 2015). For this test, the metals Pb and Zn were applied as $PbNO_3$ and ZnO. The salt solutions were prepared in high concentrations (Pb 9.652 mM and Zn 38.238 mM) and then added to a sterilized nutrient agar medium. Plates were then spot inoculated with the *Bacillus safensis* and *Pseudomonas fluorescens* and incubated at 37 °C. Colony forming units were determined after 2 days (Saikia et al., 2015).

To examine the other characteristics of the selected strains, the standard and routine protocols were used. After inoculating the plates and 2 days of incubation at 37 °C, the shape and colors of the colonies were examined under the microscope after Gram staining (Saikia et al., 2015). The isolates were also biochemically tested for siderophore in supernatants of culture fluids, and their characterization was performed directly on the agar plates. Orange halos around the colonies on blue agar were indicative of siderophore excretion (Schwyn and Neilands, 1987). Activity of ACC-Deaminase was determined, according to Penrose and Glick (2003), using 9 cm petri dishes, containing Rhizobial Mineral Medium (RMM) in three replicates. In order to study the potential of the strains in respect to indole-3-acetic acid production based on the Patten and Glick (2002) method, the selected strains were propagated overnight in 5 ml of DF salts minimal medium (Dworkin and Foster, 1958). After an incubation period of 42 h, the density of each