Removal of phyto-accessible copper from contaminated soils using zero valent iron amendment and magnetic separation methods: Assessment of residual toxicity using plant and MetPLATE™ studies[☆]

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A B S T R A C T

Zero valent iron (ZVI) has been widely tested and used in remediation of both contaminated soils and groundwater, and in general, the *in situ* amendment of the contaminated media is used as remediation approach. However, concerns remain as to the potential detrimental effects of both the immobilized ZVI and the adsorbed pollutants as the treated system could undergo transformations over time. Accordingly, plans for soil remediation by *in situ* immobilization of sorbents should include a long-term monitoring of the treated systems. Here, we report on a comparative study in which artificially Cu-contaminated sandy and organic soils characterized by different metal binding capacities were treated by either *(i) in situ* immobilization of ZVI in the soils, or *(ii)* by a ZVI amendment followed by magnetic retrieval of formed ZVI-Cu complexes prior to plant growth studies. The latter relies on the combination of the high metal adsorption capacity and magnetism of ZVI. Two plant species, *Lactuca sativa* (lettuce) and *Brassica juncea* (Indian mustard) were used to assess the efficiency of the two treatment methods in eliminating the bioavailable fraction of Cu. Overall, the results showed that, if soil remediation by *in situ* immobilization reduces the bio-accessible fraction of Cu, treatment using ZVI amendment followed by magnetic separation performs better. The latter resulted in less Cu accumulated in the shoots and roots of plants. In parallel to the plant growth study, we used MetPLATE™, a short-term bioassay based on the inhibition of the β-galactosidase enzyme by the bioavailable fraction of heavy metal cations, to predict the efficiency of the two treatment methods with regard to the elimination of Cu phyto-toxicity. The results of the bioassay confirmed the trends of phyto-toxicity results, suggesting that MetPLATE™ could be an adequate alternative to the more expensive, labor intensive, and time consuming plant growth studies.

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1. Introduction

The contamination of natural systems by heavy metals has been linked to several anthropogenic activities including mining, smelting, electroplating, energy production, military operations, and sewage sludge disposal (Lim et al., 2004; Nedelkoska and Doran, 2000; Quartacci et al., 2006; Zhou et al., 2013). For example, sewage sludge from industrial sources often contains toxic metals such as lead, cadmium, nickel, chromium, copper and zinc (Mani et al., 2015; Wani et al., 2007), which can persist in the top cultivated layer of soils if land applied. In fact, metals such as cadmium, chromium, and zinc can be very mobile in soil environments (Madejon et al., 2004; Mani et al., 2015; Wuana and Okieimen, 2011), and would accumulate in plants, posing health threats to grazing animals and humans (Boularbah et al., 1996).

The high cost of traditional soil remediation techniques such as excavation and landfilling and the limited resources allocated to the remediation of contaminated soil sites have led to the development of alternative techniques that are cost-effective and less disruptive to the environment (Kumpiene et al., 2008). Such techniques include phytoremediation and soil amendments with pollutant degrading (for organic pollutants) or stabilizing agents.

The bioavailability of metals to plants is determined by a combination of physicochemical factors including metal speciation (Notten et al., 2005), soil pH and texture, organic matter and clay content, cation exchange capacity, soil's redox status (Planquart

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et al., 1999), as well as the contact time between metals and their binding sites. By using the concept of "*effective concentration or* C_E" which accounts for the concentrations of metals in soil solution as well as the metal fractions that can be supplied over time by the solid phase, a good correlation was found between copper (Cu) accumulated in plants and C_E (Zhang et al., 2001). In fact, plants have been used quite widely to indicate or monitor environment contamination by heavy metals (Mertens et al., 2005; Pugh et al., 2002). In a study by Madejon et al., the concentrations of Cd and Zn in leaves of white poplar were positively correlated with the concentrations of the same metals in the operationally defined and chemically extractable soil bioavailable fraction (Madejon et al., 2004). On the other hand, the bioaccumulation of metals in plants depends on both plant species used and the type of metal of concern (Bonanno, 2013; Galal and Shehata, 2015; Naidu et al., 2003; Nawab et al., 2015). In Phytoremediation, plants of great interest are hyper-accumulators. This is a group of plant species which rely on a metal tolerance strategy other than exclusion (Meharg, 2005). The potential of hyper-accumulators in the remediation of metal contaminated soils has been widely investigated (Ali et al., 2013; Anjum, 2012; Hasan et al., 2007; Ma et al., 2001; Nedelkoska and Doran, 2000; Pollard et al., 2014; Pongrac et al., 2009; Sahi et al., 2002; Salt et al., 1998; Sura-de Jong et al., 2015, Tang et al., 2003) However, if phytoremediation is perceived as one of the most promising, economical, and environment friendly remediation approaches, it remains prone to some limitations. In fact, the efficiency of phytoremediation can be negatively impacted when metals are strongly immobilized in soils and the removal limited to metals interacting with root surfaces (Padmavathiamma and Li, 2007; Sharma et al., 2013; Wu et al., 2004). The majority of hyper-accumulators tend to have very low biomass production, requiring a long time to remediate large scale contaminated areas (Tang et al., 2003, Tran and Popova, 2013). In addition, the disposal of the harvested metal-contaminated plants is another concern (Chigbo and Batty, 2013; Sas-Nowosielska et al., 2004). Overall, the above non-exhaustive list of limitations of the phytoremediation approach allows for continued exploration of the *in situ* immobilization (or stabilization) and degradation techniques, for which, zero valent iron (ZVI) is one of the commonly used sorbents for both contaminated soils and groundwater.

ZVI (or Fe^0) has been used as soil amendment to decrease the leaching of potentially toxic trace elements, and therefore their bioavailability, through a combination of sorption processes. Although, this remediation method is not new and is extensively discussed in the literature (Cundy et al., 2008; Kumpiene et al., 2008; Tiberg et al., 2016), soil amendment with ZVI continues to be explored, especially with the discovery of new potentials offered by the use of nano-sized particles (Fu et al., 2014; Machado et al., 2013; Tang and Lo, 2013). On the other hand, the remediation of metal contaminated soils by magnetic separation has been studied, focusing primarily on the magnetic and density characteristics of the treated soils (Jobin et al., 2016; Sierra et al., 2014,2013).

Based on the well-established ability of ZVI to adsorb metals and its magnetic characteristics, in this study, we assessed the suitability of magnetic separation in a stepwise approach as alternative method to the simple *in situ* immobilization for remediation of metal contaminated soils. A comparative approach was used to explore the possibility of mitigating Cu toxicity to plants, not only through *in situ* immobilization, but also through a method that combines soil amendment with ZVI fillings and magnetic separation of ZVI from soils prior to plant growth experiments. An enzyme based bioassay, MetPLATE™, which is specific for the detection of the bioavailable fraction of metals (Bitton et al., 1994), was used to assess the efficiency of the two tested ZVI-based soil treatment methods to eliminate the bio-accessible Cu in artificially contaminated sandy and organic soils.

2. Material and methods

2.1. Soil sample collection, characterization, and spiking with copper

Sandy and organic soils were selected for this study. The sandy soil was sampled from the top 4 feet at the McCarty Woods Reservation Area on the University of Florida campus. This soil is representative of soils prevailing in North-Central Florida. In contrast, the organic soil used in this study was purchased from a local landscaping store and similar to the sandy soil, characterized before use. In the laboratory, the two soils were first air dried and then sieved (sieve #10; 2.0 mm particles). Next, selected soil physicochemical parameters relevant to metal sorption were determined. The pH of the soils was measured in a 1:1 mass/volume soil suspension in deionized water (or DI-water) after an equilibration period of 4 h, using a pH-meter model 240 (Corning). The organic carbon (Sura-de Jong et al.) content was determined according to the Walkley-Black method, in which OC is oxidized by potassium dichromate (Walkley and Black, 1934). The organic matter (OM) content was estimated based on loss on ignition (LOI) and determined as sample's percent weight loss after combustion at 550 °C for 2 h. This is one of the most widely used procedures for obtaining a good estimate of the oxidizable OM fraction in soils (Hesse, 1971). The effective cation exchange capacity (CEC_e), which is a measure of the quantity of sites on soil surfaces that can retain cations by electrostatic forces, was determined as described by Sumner and Miller (Sumner et al., 1996). Briefly, CEC_e was determined by extracting base cations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) and aluminum (Al^{3+}) with ammonium chloride, the concentrations of which were then determined by the Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), and the calculated CEC_e expressed in centimoles of charge per kg of soil ($Cmol_c/kg$).

In this study, we used soils artificially spiked with Cu. Although the use of soils collected from metal polluted sites would have been ideal, the use of the spiking approach in this preliminary study was driven by the need to control metal concentration gradients in soils, and to take advantage of soils with pre-determined heavy metal binding capacities, which were readily available to us. To produce Cu-contaminated soil samples, concentrated solutions of Cu were first prepared by dissolving a pre-weighed amount of $CuSO_4 \cdot H_2O$ in DI-water and used to spike the tested soils. Based on the pre-determined and previously published soil heavy metal binding capacity (SHMBC) of the two soils used (Feng et al., 2007), the sandy soil was spiked to obtain a final Cu concentration of 100 mg Cu/kg soil, while the organic soil was spiked to reach a final concentration of 500 mg Cu/kg soil. In both cases, the produced soil slurries were well-homogenized using plastic spatula and let to air dry in the fume hood for 10 days. At the end of the drying period, the spiked soils were ground and sieved (#10; 2.0 mm particles), and were then ready for use in different laboratory experiments.

2.2. Preparation of ZVI fillings and soil amendments

40-mesh ZVI fillings purchased from Fisher contained metal impurities including 2503 mg Cu/kg. Therefore, they were thoroughly washed with acidified DI-water (1% HCl), and then immersed in a 1 M NaOH solution for 72 h. The above steps were followed by thorough rinsing with DI-water until the pH of the washing water was down to ~7. This treatment reduced the Cu impurity content of the ZVI down to ~100 mg/kg. For the amendment experiments, 80 mL of DI-water were added to 100 g of either non-spiked (controls) or Cu-spiked soils, and the produced soil