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# Maghemite nanoparticles and ferrous sulfate for the stimulation of iron plaque formation and arsenic immobilization in *Phragmites australis*☆

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ABSTRACT

Wetland plants are considered as suitable biofilters for the removal of metal(loid)s and other contaminants from waters and wastewaters, due to their ability to accumulate and retain the contaminants in their roots. The iron plaque (IP) on the root surface influences the metal(loid)s retention processes. The stimulation of the IP development on roots of *Phragmites australis* by the external supply of a novel synthetic nanomaterial (nanomaghemite, $nFe_2O_3$) and $FeSO_4$ (alone or in combination) was studied. An hydroponic experiment was carried out to evaluate the iron plaque formation after external iron addition, as well as their influence on arsenic immobilization capacity. Microscopic and spectroscopic techniques were utilized to assess the distribution of Fe and As in the roots. The addition of Fe stimulated the generation of the IP, especially when $FeSO_4$ was involved. The nanoparticles alone were not efficient with regard to IP formation or As adsorption, even though they adhered to the root surface and did not enter into epithelial root cells. The combination of $FeSO_4$ and $nFe_2O_3$ was the most effective treatment for improving the As removal capacity, and it seems to be an effective way to enhance the rhizofiltration potential of *P. australis* in As contaminated (waste)waters.



## 1. Introduction

Wetland plants can alter the redox conditions in the proximity of roots and rhizomes (rhizosphere) through the release and transport of oxygen within the root aerenchyma (a porous tissue that provides a low-resistance internal pathway for the exchange of gases between aerial and submerged tissues) for respiration (Visser et al., 2000). Under anaerobic conditions, which can appear in wetlands, this process induces the oxidation and consequent precipitation of elements like metal(loid)s present in the water and also in soil and sediments (Zhao et al., 2009). Therefore, a highly active region for $Fe^{2+}$ oxidation and $Fe^{3+}$ reduction is commonly created in the oxygenated rhizosphere, which promotes the precipitation of iron oxides and the formation of a natural iron plaque on the surface of the roots of some plant species (Otte et al., 1989; Liang et al., 2006). This iron plaque consists of a mixture of crystalline and amorphous iron (oxyhydr)oxides, composed mainly of $Fe^{3+}$ minerals such as lepidocrocite, goethite, or ferrihydrite that present a high metal(loid)s adsorption capacity (Blute et al., 2004; Seyfferth, 2015). Their presence has been reported in the roots of some mono and dicotyledonous wetland species such as *Oryza sativa*, *Typha latifolia*, *Phragmites australis*, *Juncus bulbosus*, *Aster tripolium*, or *Spartina alterniflora* (Visser et al., 2000), reaching up to 10% of root dry weight and extending for near 20 μm in the rhizosphere (Hansel and Fendorf, 2001).

Several studies have shown that the iron plaque of aquatic plants can sequester nutrients and metal(loid)s, including As, influencing their availability, dispersion in the ecosystem, and plant uptake (Chen et al., 2005; Liang et al., 2006; Wu et al., 2012). For example, high concentrations of As bound to the iron plaque of *Typha* sp. (cattail) and the presence of As in less available forms in

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rhizosediments, in comparison with bulk sediments, in wetlands impacted by mine tailings have been reported (An et al., 2011). The oxidizing capacity of plant roots and the decrease in As uptake and translocation to aboveground tissues in the presence of iron plaque have also been reported (Liu et al., 2004; Wu et al., 2012; Pan et al., 2014). The high adsorption affinity of As(V) for iron (oxyhydr)oxides provokes strong retention of As on the iron plaque, and this effect is considered even more important than As co-precipitation on the surface of plant roots (Blute et al., 2004).

Since some aquatic macrophytes have well recognized bioaccumulation properties (Bonanno and Giudice, 2010), their use as biofilters for the removal of toxic elements from polluted water has gained interest in recent decades in relation to natural and constructed wetlands and wastewater treatment facilities (An et al., 2011; Allende et al., 2014; Salem et al., 2014). Constructed wetlands are engineered systems that have been designed to utilize processes occurring in natural wetlands but do so within a more controlled and efficient environment (Vymazal and Březinová, 2016). In this sense, taking into account the influence of the iron plaque on As tolerance and uptake, the stimulation of its formation on root surfaces may increase the efficiency and applicability of rhizofiltration techniques to remediate As contaminated waters. The oxidizing capacity of plant roots is considered the most important biotic factor controlling iron plaque formation (Deng et al., 2010; Pan et al., 2014; Atulba et al., 2015); but the quantity and mineral composition depend also on the characteristics and conditions of the growing medium (Allende et al., 2014; Seyfferth, 2015). It has been proven that iron plaque formation can be increased in rice roots by the supply of $Fe^{2+}$ (Liang et al., 2006; Ghassemzadeh et al., 2008) and $Fe^{3+}$ to the culture solution (Shaibur et al., 2015).

The use of novel iron nanomaterials has recently received wide attention because of their elevated sorption capacity for contaminants (Waychunas et al., 2005; Komárek et al., 2013; Martínez-Fernández et al., 2016a). It has been demonstrated that nano iron oxides are important scavengers of the most toxic forms of As(III) (Zhang et al., 2010; Lin et al., 2012; Tuutijarvi et al., 2012), Cr(VI) (Akhbarizadeh et al., 2014; Jiang et al., 2013), Cd(II) (Roy and Bhattacharya, 2012; Komárek et al., 2015), Cu(II) (Chen and Li, 2010; Akhbarizadeh et al., 2014), Pb(II) (Nassar, 2010; Xu et al., 2012), and Zn(II) (Chomoucka et al., 2012; Martínez-Fernández et al., 2016b) due to their surface modifiability, excellent biocompatibility, and, especially, their higher reactivity and relatively large specific surface area (tens to hundreds of $m^2\ g^{-1}$) compared to greater particle size materials (Komárek et al., 2013). Synthetic nanomaghemite ($nFe_2O_3$) is a promising material for the removal of inorganic contaminants as it is well known to be an important sorbent for metals and metalloids and it is readily available, inexpensive, and can be easily separated and recovered because it is magnetic (Komárek et al., 2015). In addition, nanomaghemite is an Fe(III) nano-oxide and one of the possible oxidation products of nanozerovalent iron, which has been used for the immobilization of As in contaminated soils (Zhang et al., 2010).

In spite of their aggregation capacity and high reactivity, it is not known if the presence of $nFe_2O_3$ or other iron nanoparticles could result in a bigger and more efficient iron plaque on the roots of plants in constructed wetlands. The high reactive capacity of nanomaghemite has been shown to favor its adhesion to the root surface through accumulation in the epithelial root cell wall, its internalization and upward transport to the shoots being insignificant (Martínez-Fernández et al., 2016b). For that reason, the aim of this experiment was to investigate the stimulation of iron plaque development on the roots of *Phragmites australis* (common reed) by external additions of Fe, including $Fe^{2+}$ as $FeSO_4$ and $Fe^{3+}$ as $nFe_2O_3$ nanoparticles, as a pre-treatment before its use in rhizofiltration. The As immobilization in the iron plaque of so treated *P. australis* plants (one of the most studied wetland macrophyte used in rhizofiltration; Erlonger, 2009; Vymazal and Březinová, 2016) was also tested. The spatial distribution of the root plaques (transverse and longitudinal) was evaluated by scanning electron microscopy (SEM-EDS) after a hydroponic experiment. The influence of the iron treatments on the adsorption and As retention capacity of the plants was also evaluated.

## 2. Materials and methods

A hydroponic experiment was designed to evaluate iron plaque formation and its effectivity for the immobilization of arsenic. The experiment was carried out in two consecutive phases: *Phase 1* consisted of the stimulation of the iron plaque development on the roots of *P. australis* by different sources of Fe; and *Phase 2* involved exposure to As of the iron plaque formed on the roots and As accumulation in the different plant tissues.

### 2.1. Plants collection and propagation

Fresh rhizomes of *Phragmites australis* ((Cav.) Trin. ex Steudel., Poaceae) were taken from adult indigenous plants growing in the Segura river basin (Murcia, Spain). Live rhizomes, approximately 20 cm in length, were washed and hand-separated from the sediment, and placed in plastic trays with a 4-cm layer of vermiculite that was saturated weekly with water, enriched 2:1 with an NPK solution (1 M $NH_4NO_3$, 1 M $KNO_3$, and 1 M $KH_2PO_4$). The rhizomes were kept in a growth chamber under controlled conditions (25/18 °C day/night, 80% humidity). Four weeks later, once new roots and shoots had developed, uniform healthy seedlings were selected, the old rhizomes were cut off, and the plants were transferred to 1.7-l opaque plastic containers (15 cm × 15 cm) for hydroponic culture with a modified nutritive solution (pH 6.5, 2 mM $Ca(NO_3)_2$, 2 mM $KNO_3$, 1 mM $NH_4NO_3$, 1 mM $KH_2PO_4$, 0.5 mM $MgSO_4$, 12.5 μM $H_3BO_3$, 1 μM $MnSO_4$, 1 μM $ZnSO_4$, 0.25 μM $CuSO_4$, 0.05 μM $(NH_4)_6Mo_7O_{24}$, 25 μM NaCl, and 10 μM FeEDDHA). Five seedlings were placed in each container and left for three weeks in the nutritive solution (replaced every 5 days) in a greenhouse with controlled conditions (25/20 °C day/night, 60% humidity) and natural illumination (spring). To recreate the natural hypoxic conditions under which *P. australis* usually lives, the nutritive solution was not aerated - in order to favor the reduction of the dissolved oxygen concentration and the creation of aerenchyma tissues in the roots. The increase in the specific surface area of roots in stagnant conditions, compared to aerated conditions, enhances the release of $O_2$ into the rhizosphere and thereby provides the conditions adequate for iron plaque formation (Liang et al., 2006; Deng et al., 2010; Wu et al., 2012).

### 2.2. Development of the iron plaque and the As retention test

Before the stimulation of the iron plaque on *P. australis* roots, the solution of each container was replaced with deionized water, in which all the seedlings were left for 48 h to wash the roots and minimize any interference with the Fe forms to be added. Then, 50-day-old seedlings were exposed to the corresponding treatment solutions in the containers for 5 days (*Phase 1*): (i) *CT*: control treatment (deionized water without Fe); (ii) *CT-N*: nutritive solution with 10 μM FeEDDHA; (iii) *Fe II*: 25 mg $l^{-1}$ of $Fe^{2+}$ added as $FeSO_4 \cdot 7H_2O$; (iv) *Fe III*: 25 mg $l^{-1}$ of $Fe^{3+}$ added as nanomaghemite ($nFe_2O_3$); (v) ½ *Fe II + III*: 12.5 mg $l^{-1}$ of $Fe^{2+}$ plus 12.5 mg $l^{-1}$ of $Fe^{3+}$; and (vi) *Fe II + III*: 25 mg $l^{-1}$ of $Fe^{2+}$ plus 25 mg $l^{-1}$ of $Fe^{3+}$. The iron nanomaterial used in the experiment had been characterized previously (nanopowder of γ-$Fe_2O_3$, Sigma Aldrich (Germany),