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ORIGINAL ARTICLE

Effects of hematite and ferrihydrite nanoparticles on germination and growth of maize seedlings

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Abstract Engineered iron oxide nanoparticles (IO-NPs) have been used extensively for environmental remediation. It may cause the release IO-NPs to the environment affecting the functions of ecosystems. Plants are an important component of ecosystems and can be used for the evaluation of overall fate, transport and exposure pathways of IO-NPs in the environment. In this work, the effects of engineered ferrihydrite and hematite NPs on the germination and growth of maize are studied. The germination and growth of maize were done with treatments at different concentrations of hematite and ferrihydrite NPs, namely 1, 2, 4, and 6 g/L. Biological indicators of toxicity or stress in maize seedlings were not observed in treatments with engineered hematite and ferrihydrite NPs. In contrast, the NPs treatments increased the growth of maize and the chlorophyll content, except for hematite NPs at 6 g/L, where non-significant effects were found. The translocation of engineered ferrihydrite and hematite NPs in maize stems was demonstrated using confocal laser scanning microscopy.

1. Introduction

Currently, nanoscience and nanotechnology have attracted great attention; it is because nanomaterials exhibit fascinating properties arising from their small scale. Particularly, iron oxide (IO) nanoparticles (NPs) are a focus of interest because of their potential applications in environmental remediation and biomedicine (Di Bona et al., 2015, 2014; Faraji et al., 2010; Indira and Lakshmi, 2010). Specific environmental applications of IO-NPs are arsenic removal technologies

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(Giménez et al., 2007; Richmond et al., 2004), and Fenton-like catalysts for the degradation of aqueous organic solutes (Bokare and Choi, 2014; Xue et al., 2009). Iron is an essential element for living organism and is the fourth most common element in the Earth's crust. However, due to the release of engineered IO-NPs to the environment, they may affect the functions of ecosystems.

Plants are an important component of ecosystems and can be used for the evaluation of overall fate, transport and exposure pathways of NPs in the environment (Bombin et al., 2015; Zhu et al., 2008). A few works have reported the effects of IO-NPs on the germination and growth of plants. For example, it has been reported that daily additions of Fe_3O_4-NPs in the presence of static magnetic fields, increased the growth of *Zea mayz* and the levels of chlorophyll (Răcuciu and Creangă, 2007). Increased chlorophyll levels have also been reported in soybean seedlings treated with 9 nm Fe_3O_4-NPs applied in a concentration based on iron concentration needed for plant growth; no trace of toxicity but translocation into soy bean stems was reported (Mahmoudi, 2013). Furthermore, it has been found that aqueous suspensions of Fe_3O_4-NPs can be translocated throughout pumpkin plant tissues and accumulated into the roots and leaves (Zhu et al., 2008).

Additionally, the biological effects of 9 and 18 nm γ-Fe_2O_3-NPs towards watermelon seedlings have been evaluated; it was found that the treatments with NPs increased different biological indicators such as seed germination, seedling growth, and larger activities of catalase, peroxidase (POD) and superoxide dismutase (SOD) were reported (Li et al., 2013). Otherwise, the phytotoxicity of Fe_2O_3-NPs of 20–40 nm in concentrations from 0 to 5 g/L was evaluated for lettuce, radish and cucumber seeds, where no significant phytotoxic effects were reported (Wu et al., 2012). In addition, the phytotoxicity of 6 nm γ-Fe_2O_3-NPs in concentrations from 0 to 2 g/L on rice plants has been reported; a significantly higher root elongation in treated plants with respect to the control was found (Alidoust and Isoda, 2014). It was concluded that the phytotoxicity of γ-Fe_2O_3-NPs is small and even lower than the phytotoxicity of bulk γ-Fe_2O_3 (Alidoust and Isoda, 2014). In a very recent work, the response of transgenic and conventional rice to γ-Fe_2O_3-NPs was studied (Gui et al., 2015). It was found that upon exposure to γ-Fe_2O_3-NPs, the activities of SOD and POD of transgenic rice were notably higher than the control; otherwise, in non-transgenic rice, their activities varied slightly but not significantly among treatments (Gui et al., 2015).

It has been shown that IO-NPs (Fe_3O_4 and γ-Fe_2O_3) could affect positively different plant growth performance indicators such as root and stem elongation, increased chlorophyll levels, and larger activities of catalase, POD and SOD. Thus the use of IO-NPs opens a wide range of possibilities in plant research and agronomy (González-Melendi et al., 2008). However, to the best of our knowledge, any work has been reported for the evaluation of the effects of other IO-NPs on the growth of plants. Hematite (α-Fe_2O_3) and ferrihydrite ($5Fe_2O_3{\cdot}9H_2O$) NPs were selected here because these engineered NPs are widely applied in environmental remediation of water and soils. It is important to mention that hematite is the most stable iron oxide polymorph, and ferrihydrite is a metastable poorly crystallized phase which occurs in natural media in form of NPs (Jambor and Dutrizac, 1998; Schwertmann and Cornell, 2000). In addition, despite metastability of ferrihydrite, in natural environments, the presence of soluble silicate species and organic matter inhibits its transformation to more crystalline IOs. Naturally occurring ferrihydrite NPs have been detected in a great variety of agriculturally productive terrains such as loess, peat bog, and paddy fields (Jambor and Dutrizac, 1998).

Given the importance of both hematite and ferrihydrite NPs, it is imperative to study their effects on the growth of different plants. For this study, maize was selected because it constitutes a staple food in many regions of the world, especially in Mexico, where maize is a central ingredient in Mexican food. The aim of this work is to study the effects of engineered hematite and ferrihydrite NPs on different biological indicators, their uptake, and translocation on maize seedlings.

2. Experimental details

2.1. Synthesis and characterization of IO-NPs

All chemicals were of analytical grade and used as received without further purification. The 2-line ferrihydrite NPs were prepared following a slightly modified reported procedure Schwertmann and Cornell, 2000. Firstly, under constant stirring, 6 mL of 6.0 M NaOH was added to 100 mL of 0.3 M $FeCl_3$. In order to reach a pH of 8.0, some drops of 1.0 M NaOH were added to the reaction media. Once obtaining the ferrihydrite NPs, they were washed several times with deionized water and dried at room temperature (RT) for 48 h. For the preparation of the hematite NPs, the suspension of freshly prepared ferrihydrite NPs was stirred for 10 min. After that, 0.16 g of $FeSO_4{\cdot}7H_2O$ (atomic ratio of Fe(II)/Fe(III) = 0.02) was added, this decreased the pH to 6.5, then the reaction medium was carefully adjusted to pH 9.0 with 1.0 M NaOH. Subsequently, 5 mL of a pH buffer of 1 M $NaHCO_3$ was added. The reaction was stirred at 95 °C for 120 min; it yielded hematite NPs, which were washed by the same procedure described above and dried at RT for 48 h.

The structural properties of the synthesized powders were studied by X-ray diffraction (XRD) and transmission electron microscopy (TEM). For XRD, a Philips diffractometer X'Pert with the Cu(Kα) radiation in a 2-θ range of 20–80° was used. For the TEM analyses, samples were prepared by dispersing the NPs in ethanol; this dispersion was dropped onto 300 mesh holey lacey carbon grids and observed in a FEI Titan microscope operated at 300 kV.

2.2. Seed germination and early growth

Maize (*Z. Mays*) seeds were donated by the Antonio Narro Agrarian Autonomous University, Saltillo, Mexico. In order to evaluate the effects of IO-NPs on germination of maize, the seeds were washed with deionized water (DIW) and placed at 4 °C before experiments. Furthermore, the suspensions of IO-NPs were prepared by ultrasonicating different amounts of ferrihydrite and hematite in DIW for 30 min (Lin and Xing, 2007); it yielded suspensions of 1, 2, 4 and 6 g/L, which were used as treatments. After this, the seeds were submerged for 1 h in the suspensions of IO-NPs, and subsequently 10 seeds were placed on sterile paper in Petri dishes along with 10 mL of the respective suspension. For the control, only distilled water was added to the Petri dishes (U.S. Environmental