Effect of iron oxide nanoparticles on germination and early growth of *Raphanus sativus*

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Abstract: - Iron oxide nanoparticles (IO-NPs) were prepared by both coprecipitation and partial oxidation with and without citrate ions. These routes yielded IO-NPs with typical sizes and modified surfaces. The synthetized IO-NPs were suspended in water and used for the study of their effect on the germination of radish seeds. It was found that IO-NPs do not exhibit inhibitory effects in the germination of radish. Additionally, the IO-NPs obtained through co-precipitation and citrate modified exhibit greater effects on the development of radish stems. X-ray fluorescence measurements indicated that IO-NPs increased the concentration of iron in the radish cotyledons. Additionally, magnetic measurements detected that radish cotyledon grown in presence of IO-NP exhibit superparamagnetic properties. It suggests that IO-NPs remain superparamagnetic in the radish cotyledons.

Key-Words: Iron oxide, nanoparticles, radish.

1 Introduction

Currently, nanoscience and nanotechnology have attracted great attention; it is due to the fascinating properties of matter at nanoscales. Particularly, iron oxide nanoparticles (IO-NPs) are a focus of interest because of their potential biomedical and environmental applications. One extensive use of IO-NPs is in water remediation processes, such as arsenic removal from water [1, 2]. It may be expected that some living organisms -especially those that interact with treated water such as algae, plants, and fungi- will be affected as a result of exposure to IO-NPs added in remediation processes [3]. Plants are an essential basis of all ecosystems because they interact with air, water, and soil. It has been demonstrated that plants play a decisive role in the fate and transport of NPs through absorption and bioaccumulation [4, 5].

In the last few years, the effect of different IO-NPs on the development of plants is a focus of intense research [6-11]. It has been reported the phytotoxicity of different metal oxide NPs such as Al_2O_3, Fe_3O_4, SiO_2, and ZnO on the growth of plants. The case of Arabidopsis thaliana has been studied because of its rapid germination and short life, which facilitates the study throughout its life cycle [8]. This study concludes that the most phytotoxic NPs were ZnO followed by Fe_3O_4, Al_2O_3, and finally SiO_2 with no toxic effects [8]. However, some studies have reported opposite effects in another kind of plants; it has been reported the increment in development of cereals [9, 10]. Additionally, it has been found that suitable concentrations of magnetite NPs increase the levels of chlorophyll "A" concentration [9]. Furthermore, IO-NPs has been used for soybean growth, it was reported that a concentration of 0.75 g/L of NPs increased the dry weight of leaves, and a concentration of 0.5 g/L yielded an increase of 48% compared with control [7]. Additionally, it has been found that carbon coated IO-NPs were able to penetrate tissues and migrate into different regions of a plant, such as a root, stem and leaves [6, 11]. Consequently, this opens a wide range of possibilities for the use of IO-NPs in plant research and agronomy.

2 Materials and methods

2.1 Synthesis of iron oxide nanoparticles

The IO-NPs used in this work were synthesized by different techniques, which yielded a particular characteristic; please see Table 1 and the references therein.

Table 1. Characteristics of the iron oxide nanoparticles synthetized in this work.

Nanoparticle	Method	Size distribution (nm)	Addition of citrate	Reference
NP1	Coprecipitation		Yes	[12]
NP2	Partial oxidation	70 a 100 nm	Yes	[13]
NP3	Partial oxidation	30 a 90 nm	No	[13]
NP4	Coprecipitation		No	[12]

All the synthetized IO-NPs exhibited the magnetite crystal phase; however, the oxidizing conditions of the environment promote their transformation to a core-shell like the structure of magnetite-maghemite. The synthesized IO-NPs were washed several times and subsequently were suspended in deionized water. The obtained suspensions were used to evaluate their effects in radish germination as is described below.

2.2 Soil characteristics and seeds preparation

Germination tests were realized in forest soil with the following characteristics: pH of 5.8, water holding capacity of 44.1%, the content of organic matter 3.1 %, and a sandy clay loam texture, with 63% sand, 22% clay and 15% silt. The radish (Raphanus sativus) seeds were kept under dry and dark conditions before use. Seeds were sterilized in a 5% sodium hypochlorite solution for 10 minutes and subsequently rinsed with deionized water.

2.3 Germination tests

The experiments were realized with a complete factorial treatment design with three replications. In order to study the effect of IO-NPs on the germination of radish seeds, different IO-NPs treatments were used, one used as a reference (no application of IO-NPs), and a set of IO-NPs suspensions. The suspensions were prepared by using the NP1, NP2, NP3 and NP4 at six different concentrations, i.e., 0.3, 0.75, 1.5, 3.0, 6.0, and 15.0 g/L. For each treatment set, 10 seeds were placed in a Petri dish and covered with 50 g of dry soil; subsequently, 33 mL of water (for the reference) or 33 mL of IO-NPs suspensions were aggregated to the Petri dish. The germination of the seeds was realized inside of a box, which led to developing roots and stems and also prevents the loss of water. The germination of the seeds was completed in a range of 2 to 3 days. Subsequently, plants were grown in an incubator at room temperature for 4 days with cycles of 16 h light and 8 h dark. After the growth period, the length of root and stem were measured. Statistical analysis of the data was performed using the STATISTICA 8 software package, which conducted the analysis of variance; the means were compared by the multiple range test of Duncan ($p<0.05$).

2.4 Chemical analysis

In order to determine the translocations of IO-NPs in radish, X-ray fluorescence (XRF) analysis realized in an EDXFR-AMPTEK equipment and magnetic properties were conducted in a MicroMagTM 2900 alternating gradient magnetometer (AGM). Before both determinations, radish cotyledon samples were dried and ground in an agate mortar.

3 Results y discussion

The effects of NPs on the development of plants can be quantified by measuring the germination percentage and some characteristics of their early growth. The concentration of NPs in soil is an important factor for the evaluation of different effects because different concentrations may promote opposite effects.

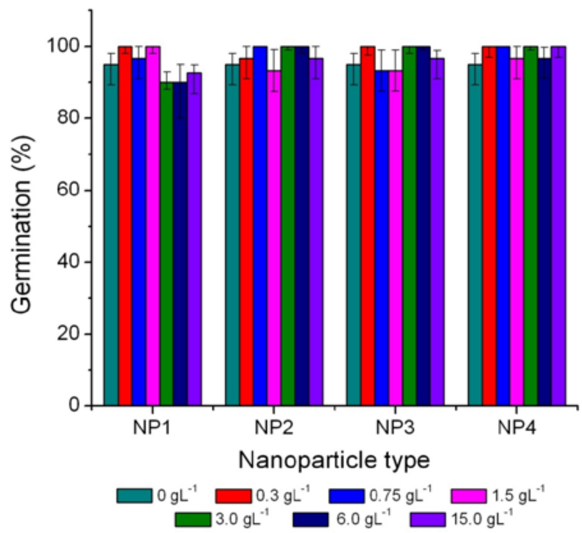

Figure 1. Germination percent of radish seeds treated with four nanoparticle suspension types at the concentrations indicated in the graph.

Figure 1 shows the results of germination percentage of radish seeds; the measurements were realized in the reference (no addition of IO-NPs) and in presence of IO-NPs suspensions with concentrations ranging from 0.3 to 15 g/L. It can be observed that for all treatments, the germination is greater than 90%. Additionally, no significant differences ($p > 0.05$) among reference and IO-NPs treatments were detected.

It can be observed that the suspensions of NP1 at high concentrations shown a slight decrease in the germination percentage. It may be due to the small size of the NPs which easily penetrates the seeds barrier; the other factor is that these NPs are coated with citrate. These characteristics will promote a higher translocation of NPs and the subsequent dissolution inside the plant. This may promote the development of the toxic effects of free iron during germination [14-16].

It can be concluded that the used IO-NPs do not display inhibitory effects in the germination of radish seeds. As a comparison, it has been reported that no inhibitory effects were detected in germination of cucumber and lettuce seeds in presence of Au, Ag, and Fe_3O_4 NPs at concentrations of 62, 100 and 116 mg/L, respectively [17]. As a comparison, concentrations up to 15 g/L of IO-NPs do not exhibit inhibitory effects in the radish seeds germination.

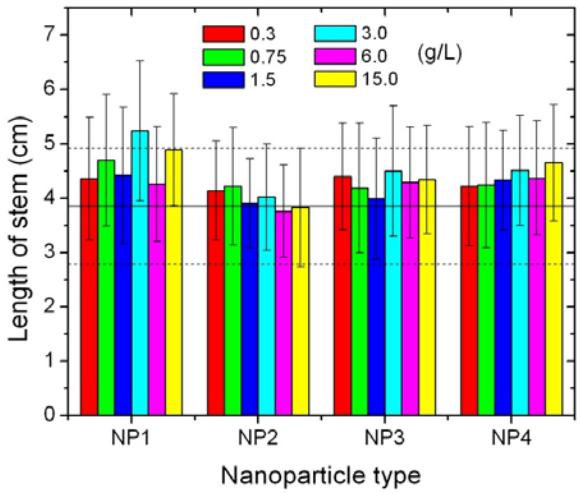

Figure 2. Length of stems of radish seedlings grown in presence of four nanoparticle suspension types at the concentrations indicated in the graph. The horizontal lines indicate the length of stems measured without NPs, -filled line average and dashed lines standard deviation-.

The effects of IO-NPs in the initial growth of radish can be quantified by measuring their stem and root lengths. The measurements of the shoots are shown in Figure 2. It can be observed that the radish plants are grown in presence of NP1 suspensions, the stem length is larger than the other treatments. For NP1, stem lengths were statistically different ($p < 0.05$) compared to the reference (no addition of IO-NPs). For treatments with NP2 suspensions, the stem lengths were not statistically different ($p > 0.05$) compared to the reference. For both NP3 and NP4 suspensions, all the considered concentrations increased the stem length when compared to a reference and were statistically different ($p < 0.05$). It can be concluded that smaller citrate coated IO-NPs exhibit a greater effect on the length of stem, it may be possible to the higher penetration of nanoparticles into the plants.

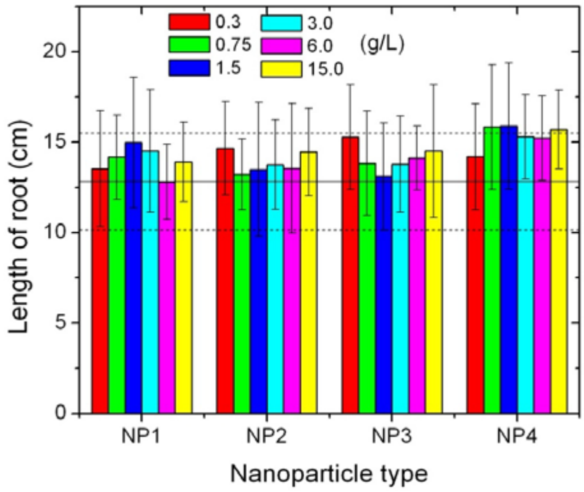

Figure 3. Length of roots of radish seedlings grown in presence of four nanoparticle suspension types at the concentrations indicated in the graph. The horizontal lines indicate the length of stems measured without NPs, -filled line average and dashed lines standard deviation-.

For the length of roots, Figure 3 shows the determined measurements for all the treatments. It can be observed that for most of the NPs suspensions, the root lengths are larger than the determined in the reference (no addition of IO-NPs). However, it can be highlighted that the larger root lengths were found at greater concentrations of NP4, where root lengths were statistically different ($p < 0.05$) compared to the reference (no addition of IO-NPs).

The determined stem and root lengths indicate that the IO-NPs have a positive effect on the initial growth of radish plants. Similar results have been observed in a study of the effect of magnetite NPs on the growth of pumpkin, where root length increased in presence of magnetite NPs [6]. Other studies have found that the application of IO-NPs and ZnCuFe-oxide NPs increase the stem length and root of mung bean [18].

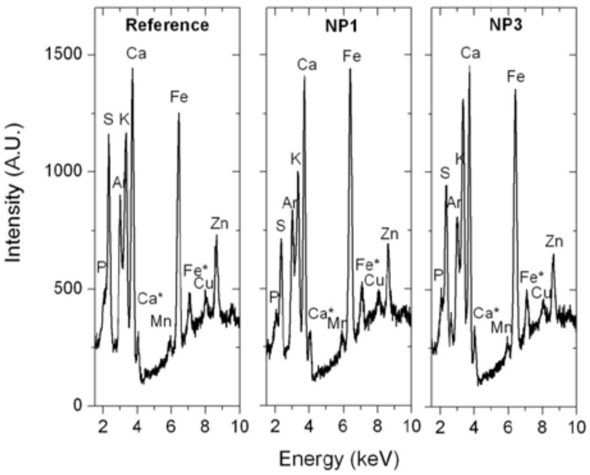

Figure 4. XRF spectra of radish cotyledons grown in water (reference) and in presence of NP1 and NP2 suspension. All indicated emissions are the K-α lines, except the indicated with *, which indicate the K-β lines.

The translocation of IO-NPs during radish germination was determined through XRF and AGM. The XRF spectra are shown in Figure 4, the samples analyzed were the cotyledons of the reference (no addition of IO-NPs), and cotyledons obtained in samples grown in suspensions with 3 g/L of NP1and NP2. It can be observed in Figure 4 that the intensity of the Fe-K□ peaksdepends on the treatment; for the reference, a shorter peak is observed. Table 2 shows the quantitative analysis obtained from the spectra. It can be observed in Table 2 that the highest content of iron was found in treatments with NP1 suspensions, and NP3 exhibited a higher iron content than the control treatments. These results show that the presence of IO-NPs during germination of radish increase the content of iron in cotyledons. Additionally, it can be observed that the highest iron content was found in treatments with smaller sized IO-NPs, it relates to the greater impact on both germination percent and length of stems found in NP1 treatments.

Table 2 shows additional data are of interest, it can be observed that the content of phosphorus, sulfur, and potassium decreased when IO-NPs are present during germination. These three elements are crucial in plants nutrition, their variation in the treatments with IO-NPs indicates that their metabolism is modified. It could be due to the translocation of IO-NPs and their subsequent dissolution. More research on this topic is needed. Additionally, the concentration of calcium and some micronutrients such as manganese, copper, and zinc do not exhibit a direct relation with the use of IO-NPs.

Table 2. The quantitative analysis determined by XRF in radish cotyledons grown in presence of different IO-NPs.

Element	Reference (%)	NP1 (%)	NP3 (%)
P	1.822	1.243	1.556
S	2.211	1.084	1.622
K	0.724	0.310	0.471
Ca	0.585	0.501	0.505
Mn	0.007	0.011	0.007
Fe	**0.101**	**0.146**	**0.133**
Cu	0.006	0.004	0.005
Zn	0.014	0.016	0.018

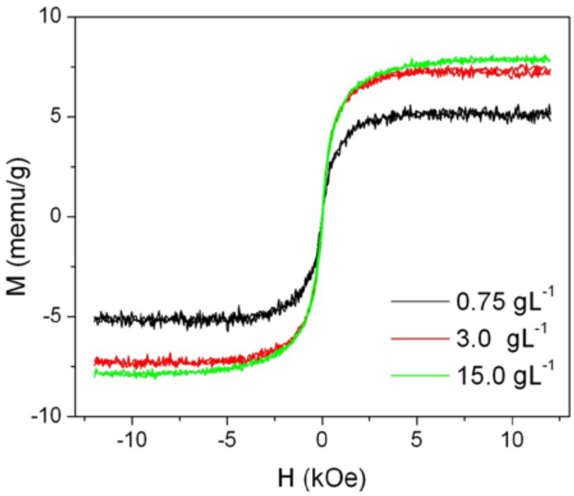

Figure 5. Magnetic measurements realized in dried radish cotyledons grown in presence of NP1 suspensions at three different concentrations. M and H are the magnetization and magnetic field, respectively.

An additional characterization was carried by AGM, which determines the magnetic properties of the samples. Figure 5 shows the magnetization curve determined for cotyledons grown in presence of NP1 suspensions at different concentrations. It can be observed that the samples exhibit a magnetization loop typical of superparamagnetic materials, with no coercive field and remanent magnetization. These measurements indicate that the IO-NPs enter the plant without dissolution, or almost some NPs remain in the plant with superparamagnetic properties. It can be observed in Figure 5 that the saturation magnetization (Ms) increases with the concentration of IO-NPs in the germination treatment; however, Ms reaches a limit, it is because no difference in Ms was detected in treatments with 3 and 15 g/L of IO-NPs suspensions.

It is known that plants store iron in form of IO-NPs, these natural IO-NPs exhibit the following crystal phases: magnetite (Fe_3O_4), ε-Fe_2O_3, and hematite (α-Fe_2O_3) with nanometer sizes ranging from 1 to 50 nm, and agglomerates can measure up to 4 microns [19]. Iron is mineralized in a protein called phytoferritin, which allows incorporation and release of iron in plant cells [14, 15]. In a similar way, the synthesized IO-NPs incorporated during germination of radish seeds enter to the seedlings and supplied iron.

4 Conclusion

Iron oxide nanoparticles prepared by different routes do not exhibit inhibitory effects in the germination of radish. Additional studies of the early grown of radish shown that IO-NPs obtained through co-precipitation and citrate modified exhibit greater effects on the development of radish stems. X-ray fluorescence measurements indicated that IO-NPs increased the concentration of iron in the radish cotyledons. Additionally, magnetic measurements detected that radish cotyledon grown in presence of IO-NP exhibit superparamagnetic properties. It suggests that IO-NPs remain superparamagnetic in the radish cotyledons. Otherwise, the IO-NPs used in the early grown of radish modified the concentration of micronutrients such as sulfur and potassium; it indicates that the IO-NPs modifies their metabolism. It can be concluded that although IO-NPs do not interfere in the germination percentage, they accelerate the grown of seedlings and modifies their macronutrients incorporation

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