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Influence of alpha and gamma-iron oxide nanoparticles on marine microalgae species

Veysel Demir^a, Mehmet Ates^{b,c,*}, Zikri Arslan^c, Mustafa Camas^b, Fatih Celik^b, Corneliu Bogatu^d, and afak Seyhaneyildiz Can^b

^aDepartment of Environmental Engineering, Faculty of Engineering, Tunceli University, Tunceli, 62000, Turkey

^bDepartment of Bioengineering, Faculty of Engineering, Tunceli University, Tunceli, 62000, Turkey

^cDepartment of Chemistry and Biochemistry, Jackson State University, PO Box 17910 Jackson, MS 39217

^dDepartment of Civil and Environmental Engineering, Jackson State University, PO Box 18540 Jackson, MS 39217

Abstract

The effects of alpha-iron oxide (α -Fe₂O₃) and gamma-iron oxide (γ -Fe₂O₃) nanoparticles (NPs) on marine microalgae species (*Nannochloropsis* sp. and *Isochrysis* sp.) were investigated in this study. Both Fe₂O₃ NPs covered the surface of algae with the agglomerates of the nanoparticles. This form of physical NP toxicity significantly decreased the sizes of phytoplankton. Both NPs were toxic to the tested algal species, while α -Fe₂O₃ showed less toxicity than γ -Fe₂O₃ NPs for both algal species. A comparative analysis of growth data of the two algal species treated with α -Fe₂O₃ or γ -Fe₂O₃ NPs revealed that *Isochrysis* sp. are more sensitive than *Nannochloropsis* sp. Toxicity of these widely used NPs to primary producers forming the base of the food chain in aquatic environments might result in widespread adverse effects on aquatic environmental health.

Keywords

Phytoplankton; alpha and gamma-iron oxide nanoparticles; nanotoxicity; marine

1. Introduction

Nanoscale particles are widely used in various products and applications in electronics, optics, textiles, medical devices, catalysts, biosensors, cosmetics, food packaging, water treatment technologies, telecommunications, pharmaceuticals, and fuel cells (Aitken et al. 2006; Brody et al. 2006). Nano forms of many metal oxide particles, including the oxidized states of iron nanoparticles (NPs) like maghemite (γ -Fe₂O₃) and hematite (α -Fe₂O₃) are employed in many technological applications. Like γ -Fe₂O₃, α -Fe₂O₃ has only one type cation, Fe³⁺, however, it has no periodic vacancies. The other difference is the crystal

*Corresponding author: mehmetates@tunceli.edu.tr.

structures. While α -Fe₂O₃ has a rhombohedral crystal structure, γ -Fe₂O₃ has cubic cells (Randrianantoandro et al. 2001; Can et al. 2012). Fe₂O₃ NPs can be used as an adsorbent in the removal of metals from aqueous solutions (Grover et al. 2012; Ge et al. 2012; Gonzalez et al. 2012). A catalytic property of Fe₂O₃ NPs which are widely used in laboratory tests and in the treatment of wastewater has also been reported (Perez, 2007). However, extensive production and application of Fe₂O₃ NPs will inevitably result in a greater exposure risk in various environmental segments and the aquatic environment may act as a sink for the entry of these NPs (Farre et al., 2009; Scown et al., 2010; Gaiser et al., 2012; Remya et al., 2015)

Phytoplankton (primary producers) which are mostly very small, often minute organisms ranging in size from 2 to 100 μ m stay suspended in the water column and form the base of the food chain in aquatic environments and primary food source containing proteins, starches, fatty acids and oils for zooplankton (secondary producers) (Tapping 2010). Because phytoplankton producing organics and oxygen is the first level of the trophic food pyramid and plays an important role in the equilibrium of aquatic environments, algae toxicity tests are extensively applied to assess the effects of hazardous substances in sea and fresh water (Sadiq et al. 2011).

The aim of the current study was to investigate the effects of α -Fe₂O₃ and γ -Fe₂O₃ NPs on microalgae species: *Nannochloropsis* sp. and *Isochrysis* sp. These species are chosen since they are widely used as food in aquaculture and they are two of the most frequently used species in aquaculture (Hemaiswarya et al. 2011). For characterization of Fe₂O₃ NPs, several physical–chemical factors like composition, size, stability and solubility were determined using inductively coupled plasma mass spectrometry (ICP-MS), Fourier transform infra-red spectroscopy (FTIR), transmission electron microscopy (TEM), dynamic light scattering (DLS), and Zeta potential measurement. Also the effects of particle size and solubility were comparatively examined on the toxicity of α -Fe₂O₃ and γ -Fe₂O₃ NPs to these commonly used marine microalgae species in aquaculture.

2. Materials and methods

Nano size α -Fe₂O₃ (20–40 nm) and γ -Fe₂O₃ (20–40 nm) particles were purchased from Skyspring Nanomaterials Inc., in Houston, TX, USA. All other chemicals were obtained from Sigma–Aldrich (St. Louis, MO, USA). The NP stock solutions were prepared as described by Wang et al. (2009). For characterization of NPs, all samples were prepared by an identical procedure for FT-IR, TEM, DLS and zeta potential measurements according to the protocols described in our previous studies (Ates et al. 2013a).

The algal species cultures (*Nannochloropsis* sp. and *Isochrysis* sp.) were obtained from Pentair Aquatic Eco-Systems Inc, Apopka, FL, USA. A preliminary experiment was conducted in triplicate to evaluate the algal species' response to different concentrations (0, 1, 5, 10, 25 and 50 mg/L) of α -Fe₂O₃ and γ -Fe₂O₃ NPs separately under the same light intensity for 72 hour exposure. Acute micro-algae toxicity tests were performed for each of the NPs according to the Organization for Economic Cooperation and Development testing guidelines (OECD 1984). Both NPs separately dispersed to avoid aggregation with sonication for 10 minutes. Then, six different concentrations of dispersed NPs immediately

transferred to sterile Erlenmeyer flasks and inoculated with 1 mL of the freshly prepared algal suspension to maintain initial algal concentration of 10,000 cells/mL which was correlated with cell density as determined by cell counts in Neubauer hemocytometer (cells/mL). The flasks were then incubated on an orbital rotary shaker (VWR, USA) at 250 rpm, at 26–28 °C for *Nannochloropsis* sp., and at 24–26 °C for *Isochrysis* sp., under continuous cool-white fluorescent light with an intensity of 5000 lux during the experiment. High rotary shaking speed was selected to minimize aggregation and settlement of the NPs during the exposure experiments. Control groups without addition of NPs but containing algal cells and nutrient medium were also included.

Experimental setup for α -Fe₂O₃ and γ -Fe₂O₃ NP exposure studies with marine micro-algae are shown in Fig. 1. From left to right, **a)** Stock solution of α -Fe₂O₃ (light yellow) and γ -Fe₂O₃ (orange) NPs, **b)** Culture media of *Isochrysis* sp. (light brown) and *Nannochloropsis* sp. (green), **c)** Sterile Erlenmeyer flasks (250 mL) containing 100 mL of OECD medium with varied concentration of dispersed NPs on the orbital rotary shaker, **d)** Culture media of *Nannochloropsis* sp. with α -Fe₂O₃ NPs before centrifugation, **e)** Culture media of *Nannochloropsis* sp. with α -Fe₂O₃ NPs after centrifugation (pictures of *Isochrysis* sp. culture media were not displayed).

For ICP-MS analysis, stock iron (Fe) standard solution (100 μ g mL⁻¹) was purchased from SCP Science (Champlain, NY). The algal samples were rinsed in distilled water thrice to remove NPs from the cell surfaces. Trace metal grade nitric acid (HNO₃, Fisher Scientific) was used for the dissolution of the algae samples collected after exposure to determine uptake amount (if any) at the end of the experiments according to the protocols described previously (Arslan et al. 2011). The sample solutions were analyzed by ICP-MS using a Varian 820 MS ICP-MS instrument (Varian, Australia). The process of quantifying different kinds of samples in terms of their phase contrast values was carried out in grayscale using a phase contrast microscope (Micromaster, Model 12-575-252, Fisher Scientific) equipped with a digital camera to elucidate the deposition of NPs. Algal samples (control and treatment groups) were collected at the beginning and at the end of each experiment. Images were captured by Micron Imaging software from live *Nannochloropsis* sp. and *Isochrysis* sp. with NPs in petri dishes. All images were taken in 50 μ m scale to get a clear picture of the algal cells.

3. Results and discussion

3.1. Characterization of NPs and aggregation

The size and morphology of the α -Fe₂O₃ and γ -Fe₂O₃ NPs were carried out by TEM images (Fig. 2). The TEM images (in a 100 nm scale) randomly selected from the different area of the carbon-coated copper grids showed that the α -Fe₂O₃ and γ -Fe₂O₃ NPs are in different sizes ranging from 10 to 90 nm and 10 to 80 nm, respectively. It appears that greater presences of rod-like shapes are found in the case of alpha compared to gamma iron nanoparticles. Also, the number of nearly spherical-shape NPs is dominant for gamma iron oxide and most of them have similar size. TEM analysis revealed that both nano-structured iron oxides have homogenous morphologies (Prodan et al. 2013; Cuong et al. 2014). There is a general tendency of agglomeration due to nano particle interactions (Layek et al. 2010).

The management of FT-IR spectra was realized with instrument software (Omnic User's Guide, 2006; Stengl et al., 2010; Palaniappan and Pramod, 2011). Specific wavelengths and their corresponding transmittance values for the finger print region are presented in Table 1. It was observed that each nano size Fe_2O_3 metal oxide has a specific spectrum in the finger print region. Thus, for $\alpha\text{-Fe}_2\text{O}_3$ nano-particles, two characteristic sharp bands with maxima at 480.8 and 570.9 cm^{-1} were recorded, with transmittance values of 54.5% and 20.8%, respectively. They match with the values previously reported and correspond to the Fe-O bond (Li et al. 2012; Duhan and Devi, 2010; Sahoo et al. 2010).

A more complex spectrum was observed for $\gamma\text{-Fe}_2\text{O}_3$ in the same region, with small peaks at 440 and 497.3 cm^{-1} and transmittances of 47.7% and 58.8%, respectively, with a specific intense band with maxima at 562.9, 639.4, and 694.5 cm^{-1} and corresponding transmittances of 28.8%, 24.5% and 45.4%. These peaks and the band were assigned to Fe-O bond from $\gamma\text{-Fe}_2\text{O}_3$ (Guo et al. 2008; Li et al. 2012; Sahoo et al. 2010). Thus, the polymorph phase of metal oxides NPs has some influence on the positions and transmittances of specific bands from FT-IR spectra. The bands with maxima at 3416.1, 1637.5 cm^{-1} of $\alpha\text{-Fe}_2\text{O}_3$ NPs and at 3428.2, 1629.4 cm^{-1} of $\gamma\text{-Fe}_2\text{O}_3$ NPs were assigned to adsorbed water (Li et al. 2012; Kamzemzadeh and Abolghasem, 2012). Also, the sharp peaks from 799.9, 897.5 cm^{-1} of $\alpha\text{-Fe}_2\text{O}_3$ NPs and from 873.3 cm^{-1} of $\gamma\text{-Fe}_2\text{O}_3$ NPs were ascribed to O-H out of plane bond (Lopez et al. 2010). The small bands at 1440.7, 1438 cm^{-1} for $\alpha\text{-Fe}_2\text{O}_3$ and $\gamma\text{-Fe}_2\text{O}_3$ NPs, respectively, were assigned to O-C-O from carbonate, and peaks from 1384.1 cm^{-1} of $\alpha\text{-Fe}_2\text{O}_3$ and $\gamma\text{-Fe}_2\text{O}_3$ NPs to O-C-O from bicarbonate (Ates et al. 2013a). The high values of transmittances recorded at specific wavelengths for Fe-O bonds of nano-structured iron oxides proved their purity, in agreement with homogeneous morphologies determined by TEM analysis.

The measured particle sizes of $\alpha\text{-Fe}_2\text{O}_3$ and $\gamma\text{-Fe}_2\text{O}_3$ NPs with TEM and DSL in the medium without algal species are shown in Table 2. Initial sizes of $\alpha\text{-Fe}_2\text{O}_3$ and $\gamma\text{-Fe}_2\text{O}_3$ NPs immediately after ultra-sonication were found as 141.2 and 329.0 nm, respectively. This observed size difference was consisted with measured zeta potentials. We observed an increasing size with time for both NPs in the aqueous medium. As indicated in Table 2, $\alpha\text{-Fe}_2\text{O}_3$ and $\gamma\text{-Fe}_2\text{O}_3$ NPs possessed positively and negatively charged surfaces, respectively (see zeta potentials). The charge at the surface of the nanoparticle suspensions plays an important role in the particles' stability in the solvent against agglomeration as well as in determination of their interaction with biological systems (Clogston and Patri, 2011). The value of Zeta potential not only elucidates the positive or negative charge variation at the surface, but is indicative of attractive and repulsive forces on the surface. In a recently published article, we reported that particles aggregated in water indicate that the surface charge was not sufficient to prevent the aggregation of the NPs in the water even in the absence of counter ions, but aggregates are smaller compared to other NP studies consistent with the measured surface charges (Ates et al. 2013b).

3.2. Interaction of nanoparticles with the microalgae surface

The phase contrast microscopic images (with two different microscopes) of the control and treatment group are given in Fig. 3 for *Nannochloropsis* sp. cultures (the phase contrast

microscopic images of *Isochrysis* sp. were similar to *Nannochloropsis* sp. cultures. Thus, only *Nannochloropsis* sp. images were displayed). Interestingly the flocculation of the algal cells was observed with the interaction of α -Fe₂O₃ NPs that aggregated particles covered the surface of exposed algal cells. Importantly, the cell size for *Nannochloropsis* sp. in the exposure medium (for γ -Fe₂O₃ and α -Fe₂O₃) was always smaller than that in the medium of the experiment (control) without the NPs (see Figure 3 a, b). The cells sizes reduced around 50% for the 50 mg/L NP exposed group (Images for other concentrations were not displayed due to page limitation of the article). This might be due to the attachments of NP aggregates on the surface of algae that these attached aggregates could have negative impact on photosynthetic or respiratory processes of algal species (Sadiq et al. 2011). In both algal species, toxicity of α -Fe₂O₃ or γ -Fe₂O₃ was evident even at the lowest concentration tested (1 mg/L) and not significantly changed with increasing concentration, indicating no-effect concentration. Miller et al. (2012) also reported no-effect concentration for TiO₂ NPs for growth of phytoplankton species, *Isochrysis galbana*.

Transmission electron microscopy (TEM) revealed that both α -Fe₂O₃ and γ -Fe₂O₃ NPs were adhering to the surfaces of phytoplankton cells as aggregates (Fig. 3d). Both NPs were toxic to the tested algal species, while α -Fe₂O₃ showed less toxicity than γ -Fe₂O₃ NPs for both algal species, which could be due to the difference in NPs' periodical vacancies, crystal structure and opacity in suspension. A comparative analysis of growth data of the two algal species exposed to Fe₂O₃ NPs revealed that *Isochrysis* sp. is more sensitive than *Nannochloropsis* sp. Our results indicated that different species behaved differently in susceptibility to studied NPs.

There are several factors like composition, concentration, size, and ion dissolution play roles in toxic behavior of NPs in aquatic organisms. The opacity of NPs suspension can also play a role in a growth inhibitory effect by decreasing the light intensity (Sadiq et al. 2011). In our study, the opacity of γ -Fe₂O₃ was greater than that of α -Fe₂O₃ NPs suspension and lower growth rate in the γ -Fe₂O₃ exposure experiment also supported this growth inhibitory effect. Navarro et al. (2008) discussed physical restraint as one of the indirect mechanisms of NPs toxicity toward algae. In their opinion, accumulation of NPs on the algal surface causes a shading effect inhibiting photosynthetic activity. Our ICP-MS results showed no detectable Fe level in algae for all concentrations studied. This also supported the findings of Sadiq et al. 2011 that there is no evidence for NPs uptake into the cells of the phytoplankton and the electron microscopy images demonstrated that the cell surfaces were covered with the particles. In addition, our ICP-MS results to determine Fe concentrations taken in the algal species indicated that increasing initial concentration of Fe₂O₃ NPs has no effect on uptake into both of the algal species.

4. Conclusions

To our knowledge this is the first study reporting the interaction of alpha and gamma Fe₂O₃ NPs and marine phytoplankton species such as *Nannochloropsis* sp. and *Isochrysis* sp. Both Fe₂O₃ NPs exhibited toxicity, though γ -Fe₂O₃ was remarkably more toxic to the studied algae species. Significant suppression of population growth occurred due to covering the surface of the algae species with Fe₂O₃ oxide NPs. This could play a pivotal role in

nanotoxicity to the algal species. In both algal species, toxicity of α -Fe₂O₃ or γ -Fe₂O₃ was evident at the lowest concentration tested and not significantly changed with increasing concentration, indicating no-effect concentration.

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Fig. 1.
Experimental setup for α -Fe₂O₃ and γ -Fe₂O₃ NPs exposure studies with micro-algae

Fig. 2.
TEM analysis of α -Fe₂O₃ (a) and γ -Fe₂O₃ (b) nanoparticle dispersion

Fig. 3.

a) Image of *Nannochloropsis* sp. control group, b) Image of *Nannochloropsis* sp. exposed groups (at the presence of α -Fe₂O₃), Arrow marks indicating the cell size for *Nannochloropsis* sp. in the media, c) Phase contrast microscopic images showing *Nannochloropsis* sp. control group, d) Phase contrast microscopic images showing agglomerates of NPs clumped with algal cells of *Nannochloropsis* sp. after interaction. Arrow mark indicating the interaction of α -Fe₂O₃ NPs that aggregates of particles covered the surface of the exposed algal cells.

Table 1

Specific wavelengths and their corresponding transmittance values for FT-IR spectra of nano size α -Fe₂O₃ and γ -Fe₂O₃

Alpha - Fe ₂ O ₃		Gamma- Fe ₂ O ₃	
cm ⁻¹	% T	cm ⁻¹	% T
1637.5	82.6	1629.4	92.4
1600.2	81.6	-	-
1440.7	81.4	1438	91.1
1384.1	82.8	1384.1	95.6
1339.2	86.8	1118.5	95.7
1122.6	88.4	873.3	94.9
897.5	73.3	684.5	45.4
799.9	82.8	639.4	24.5
570.9	20.8	562.9	28.8
480.8	54.5	497.3	58.5
412	77.4	440	47.7

Table 2Descriptive properties of the α -Fe₂O₃ and γ -Fe₂O₃ nanoparticles

Nanoparticles	TEM (nm)	DLS (nm)	Zeta Potential (mV)	Appearance
Alpha Fe ₂ O ₃	10–90	141.2	30.4	Orange
Gamma Fe ₂ O ₃	10–80	329.0	–13.2	Light yellow