

Influence of Interaction Between α -Fe₂O₃ Nanoparticles and Dissolved Fulvic Acid on the Physiological Responses in *Synechococcus* sp. PCC7942

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Abstract The ecotoxicity of α -Fe₂O₃ nanoparticles (NPs) and its interaction with a typical natural organic matter (NOM), fulvic acid (FA) on the physiological responses of *Synechococcus* sp. PCC7942 was studied. α -Fe₂O₃ NPs inhibited the algae growth at concentration higher than 10 mg L⁻¹ and induced oxidative stress, indicated by enhanced antioxidant enzymes activities, elevated protein and sugar content. FA could efficiently recover cell growth and reduce antioxidant enzyme activities which induced by α -Fe₂O₃ NPs, indicating the toxicity of NPs was alleviated in the presence of FA. α -Fe₂O₃ NPs could form large aggregates coating on cell surface and inhibit cell growth. FTIR spectra verified FA interacted with α -Fe₂O₃ NPs through carboxyl groups, partly replaced the binding sites of α -Fe₂O₃ NPs on algal cell walls, thus reduced NPs aggregates coating on cell surface. This favors reducing the oxidative stress caused by direct contact and increasing light availability, thus mitigate NPs toxicity.

Keywords Nanoparticles · α -Fe₂O₃ · *Synechococcus* · Fulvic acid · Antioxidant enzymes · Surface coating

Nano ferric oxide (nano-Fe₂O₃) has been widely used in magnetic storage, medicine, chemical industries and water purification (Mohapatra and Anand 2010), and is inevitably brought to the aquatic environment. Nano-Fe₂O₃, has been found to cause significant structural changes in

the epithelium and inhibit *Staphylococcus aureus* growth (Tran et al. 2010; Kalive et al. 2012). As the key primary producers, algae play an ecological important role in most aquatic ecosystem and have been used as the model organism for toxicity assessment of toxicants and nanomaterials (Ji et al. 2011). Therefore, more attention has been paid to the effect of nanoparticles (NPs) on algae. Only a few studies reported the toxicity of nano-Fe₂O₃ on aquatic organisms. Demir et al. (2015) reported the toxicity of α -Fe₂O₃ and γ -Fe₂O₃ on *Nannochloropsis* sp. and *Isochrysis* sp. appeared at a low concentration of 1 mg L⁻¹. An opposite observation of higher toxicity of α -Fe₂O₃ than γ -Fe₂O₃ to *Chlorella pyrenoidosa* was reported in Lei's work (Lei et al. 2016), with the IC₅₀ of ~71.0 and ~132.0 mg L⁻¹, respectively. Unfortunately, only morphological changes and cell growth were presented in the above works and still little is known about the physiological and biochemical changes of algae in response to nano-Fe₂O₃.

In aquatic environment, nano-Fe₂O₃ might adsorb pollutants and interact with other nanoparticles, and consequently alter their toxicity. Natural water environment always contain widely-distributed dissolved natural organic matter (NOM), which is a complex organic molecule and has aroused widespread attention due to its unique properties and high concentration (near 100 mg L⁻¹ in fresh water) (Kim et al. 2012). The interaction between NPs and NOM may result in the formation or breakage of aggregates, which is mainly determined by their surface properties (Stumm and Morgan 2012). It is reported that NOM could decrease the attachment of the NPs to the bacteria and alleviate the nanotoxicity (Fabrega et al. 2009; Li et al. 2010). However, the influence of NOM is likely to be dependent on NP-type and test-species. NOM could enhance the toxicity of nano-CuO by increasing Cu²⁺ concentration, membrane permeability and internalization (Wang et al. 2011).

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To understand the toxicity of the nanoparticles in the aquatic ecosystem to algae, it is essential to study their interaction with NOM, due to its common existence in the aquatic environment. Therefore, *Synechococcus* sp. PCC7942 was selected as the representative model for the indicative organisms in this work to study the effect of nano-Fe₂O₃ in the aquatic environment. In addition, the interaction of nano-Fe₂O₃ with a representative NOM, fulvic acid (FA) was selected for investigating the effect of its interaction with α -Fe₂O₃ NPs on the physiological responses of *Synechococcus* sp. PCC7942 (here after presented as *Synechococcus*).

Materials and Methods

The freshwater strain, *Synechococcus* sp. PCC7942, was obtained from Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. Algal cells were pre-grown in 500 ml erlenmeyer flasks containing 300 ml of BG11 medium with initial cell density of 10⁷ cells mL⁻¹. The cultures were grown at 28 ± 0.5 °C and illuminated from two sides at a light intensity of 40 μE m⁻² s⁻¹ under a dark/light cycle of 12/12 h for 48 h. Nano ferric oxide (α -Fe₂O₃, CAS: 1309-37-1, purity > 99.5%), with an average particle size of 30 nm was used to prepare nanoparticle suspensions. Fresh nano Fe₂O₃ stock suspensions (300 mg L⁻¹) were prepared by sonication for 60 min. Before the inoculation of algal cells, different concentrations of the sterilized suspension of test substances were added into the growth media to the final concentrations for α -Fe₂O₃ NPs of 0.3, 1, 3.3, 10, 30 mg L⁻¹. Measured concentrations of Fe in water were 1.84, 6.11, 19.87, 66.12, 186.77 μM respectively. Fe concentrations in medium were performed according to ISO 6351-1985 (E), using an atomic absorption spectrometer instrument (AAAnalyst 700, Perkin Elmer). Detection limits for Fe was 3 nM. The fulvic acid (CAS: 1415-93-6, purity > 90%) stock solution (FA) of 1.2 g L⁻¹ was prepared and stirring for 24 h at 25 ± 1 °C to equilibrate before use. A wide range of α -Fe₂O₃ NPs concentration (0.3–150 mg L⁻¹) was tested for the impact on *Synechococcus* growth in our previous experiment (data not shown), among which 10 mg L⁻¹ of α -Fe₂O₃ NPs was found to impose the maximum repression on cell growth of *Synechococcus*. Therefore, a range of 0.3–30 mg L⁻¹ was tested in this work for understanding the impact of α -Fe₂O₃ NPs, and 10 mg L⁻¹ of α -Fe₂O₃ NPs applied to study the effect of FA on the toxicity of α -Fe₂O₃ NPs. The common tested FA concentration ranges from 0.01 to 100 mg L⁻¹ (Palomino and Stoll 2013; Ahmad et al. 2016), thus three concentrations (10, 20, 40 mg L⁻¹) of FA were added to the medium with and without 10 mg L⁻¹ of α -Fe₂O₃ NPs. The cultures without addition of α -Fe₂O₃ NPs and FA were set as the control groups. The cultures were exposed to α -Fe₂O₃ NPs and FA

for 48 h before determination of physiological and biological changes. Assays were performed by triplicate.

Chlorophyll *a* content (Chl *a*) were measured spectrometrically by methanol extraction (Lichtenthaler and Wellburn 1983). The total sugar content was measured by phenol–sulfuric acid method (Kaplan et al. 1987). The protein content was determined by bicinchoninic acid method using the total protein assay kit (Nanjing Jiancheng Biology Engineering Institute, China). Algae cells of approximately 0.4 g fresh weight were resuspended in 5 ml of 0.1 M phosphate buffer (pH 7.4) and broken by sonication (Scientz Biotechnology Co. Ltd, Ningbo, China). The supernatant was collected for measurement of total soluble protein, catalase (CAT), peroxidase (POD), superoxide dismutase (SOD). The CAT and POD activities were measured by assay kits (Nanjing Jiancheng Biology Engineering Institute, China). The SOD activity was determined according to the method of Mishra (Mishra et al. 1993). The activity of SOD was defined as the quantity of SOD required to inhibit 50% the photochemical reduction of nitroblue of tetrazolium (NBT). The accuracy of measurements was as follows: 20 μg/mL for protein; CAT, POD and SOD was 0.0001, 0.0005 and 0.001 U/mL respectively.

The particle size and shape of α -Fe₂O₃ nanoparticles were characterized by Transmission electron microscopy (TEM, Tecnai G2 F30 S-TWIN, FEI Company, US). 10 mg L⁻¹ of α -Fe₂O₃ NPs was immersed in acetone solution and sonicated for 15 min at 750 W in a ultrasonic processor. The dispersed particles were then deposited onto the lacey-carbon-coated copper grid and subsequently submitted to TEM imaging. After exposure to α -Fe₂O₃ NPs for 48 h, the surface of algal cells were observed by Field emission Scanning electron microscopy (SEM, Hitachi S-4800, Japan). Aliquots of 10 mL algal suspensions under treatments with 10 mg L⁻¹ α -Fe₂O₃ NPs and with both α -Fe₂O₃ NPs (10 mg L⁻¹) and FA (10 mg·L⁻¹) were withdrawn and cells were harvested at 1000 rpm for 3 min. The pellets were then chemically fixed for ~ 24 h using 4% (w/v) glutaraldehyde. Then the samples were subjected to a dehydration procedure by graded series of ethanol before air-drying. Finally, the sample films were coated with gold and loaded for SEM analysis. Each sample was analyzed for at least 15 view fields at different magnifications. The sizes of NPs aggregates and their coverage area on cell surface were roughly estimated and compare between treatments.

Fourier transform infrared (FTIR) sample preparation was carried out as described by D'Souza et al. (2008). 2 mg of dry algal powder was sampled and mixed thoroughly with 2.5 mg of dry potassium bromide (KBr). The mixed powder was tableted to obtain a clear press disc before subjecting to FTIR scans. All IR spectra were recorded at 25 ± 1 °C in the mid infrared range (4000–400 cm⁻¹) by Nicolet IR200 FTIR Spectrometer (Thermo Scientific Instruments Groups, US).

All samples were evaluated in three replicates. Results from different treatments were treated using one way ANOVA with Tukey's multiple comparisons test. The mean values and standard deviations were measured for each treatment. Differences were determined to be significance at a probability of 5% ($p < 0.05$). IBM SPSS Statistics 20 software was used for statistical analysis.

Results and Discussion

The TEM images (Fig. 1) showed the α -Fe₂O₃ NPs in different sizes ranging from 12 to 67 nm. Nearly all the particles exhibited spherical shape. The effects of α -Fe₂O₃ NPs on the cell density of *Synechococcus* sp. PCC7942 are showed in Fig. 2a. Compared to the control group, a slight inhibition on cell growth was observed in cultures with 10 and 30 mg L⁻¹ of α -Fe₂O₃ NPs. Cultures treated with low concentration of α -Fe₂O₃ NPs (0.3, 1 and 3.3 mg L⁻¹) presented similar cell density as the control group. Similar trend was observed in

changes of Chl *a* content, with a reduction in Chl *a* content at 10 and 30 mg L⁻¹ of α -Fe₂O₃ NPs (Fig. 2b). Significant increases in total soluble sugar (Fig. 2b), protein content (Fig. 3a) and antioxidant enzyme activities (Fig. 3b–d) were observed in cultures treated with 3.3, 10 and 30 mg L⁻¹ of α -Fe₂O₃ NPs ($p < 0.05$) after 48 h exposure. Compared to the control group, the highest total soluble sugar and protein content was recorded at 30 mg L⁻¹ of α -Fe₂O₃ NPs, which was increased by 47% and 1.35 fold respectively. Activities of CAT, POD and SOD increased with the increasing α -Fe₂O₃ NPs concentrations. The maximum activities of CAT, POD and SOD were obtained at 30 mg L⁻¹ of α -Fe₂O₃ NPs after 48 h exposure (Fig. 3b–d).

The common toxicity mechanism of the NPs involves the generation of (reactive oxygen species) ROS, leading to oxidative damage to protein and other macromolecules, and final cellular organelles injury (Wang et al. 2011). Enhancement of CAT, POD and SOD activities is the most important way to scavenge ROS. The increase of protein content (Deng et al. 2012), sugar content (Mohamed 2008)

Fig. 1 TEM analysis of ferric oxides nanoparticle dispersion

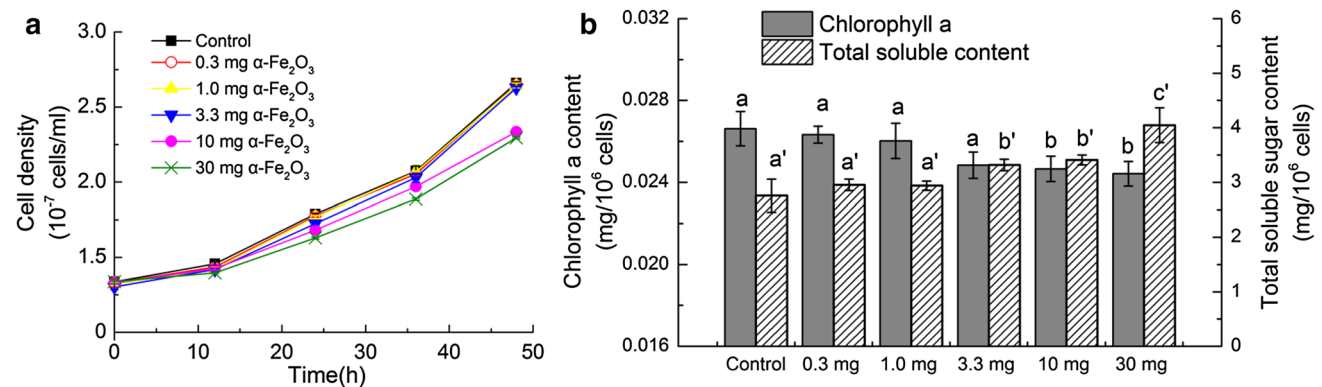
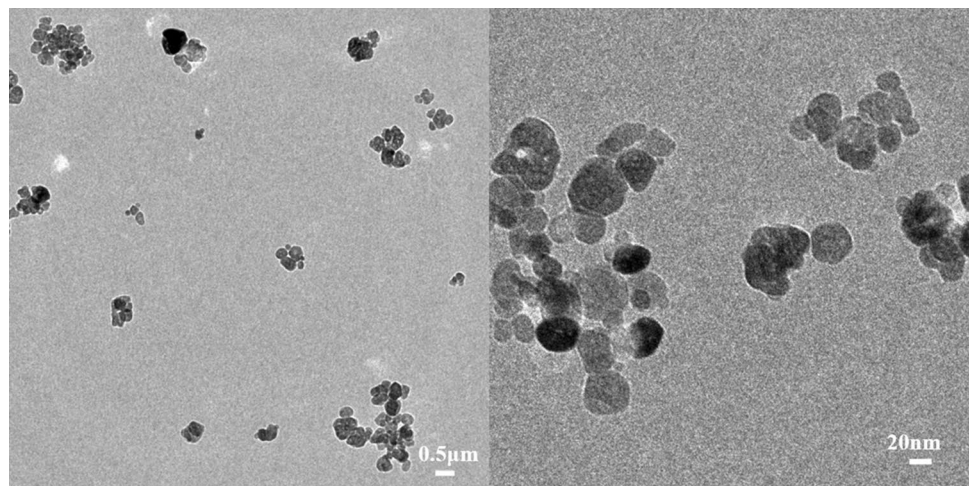


Fig. 2 The effects of α -Fe₂O₃ NPs on the **a** cell density, **b** chlorophyll *a* and total soluble sugar content of *Synechococcus* sp. PCC7942 for 48 h exposure. Different letters above the bars indicate significant differences ($p < 0.05$, $n = 3$) between the treatments

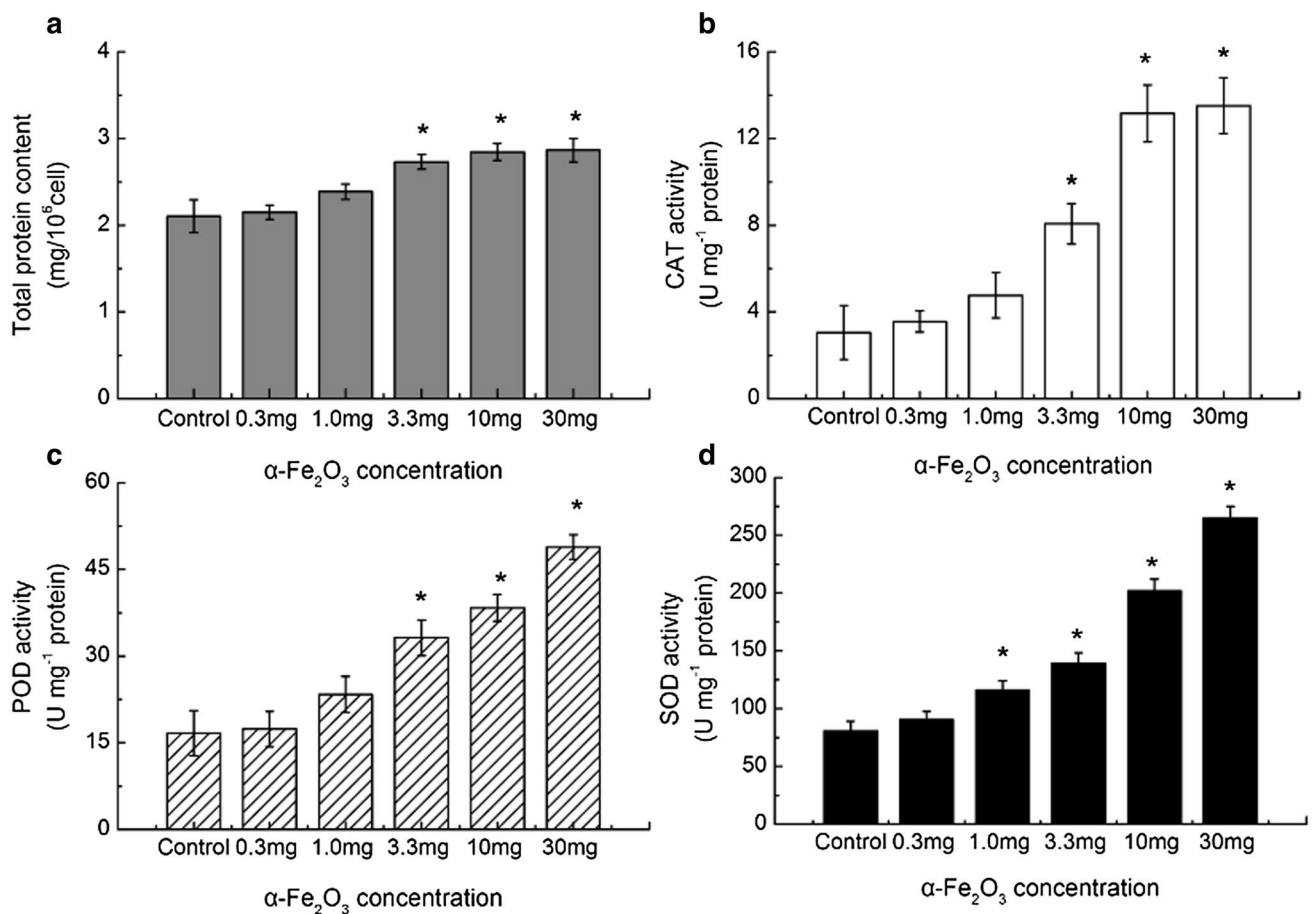


Fig. 3 Effects of α -Fe₂O₃ NPs on **a** total soluble protein content, **b** CAT, **c** POD and **d** SOD activities of *Synechococcus* sp. PCC7942 for 48 h exposure. Asterisks indicate the statistically significance differences ($p < 0.05$) between treatments

is usually considered as an active defense mechanism to prevent algae cells from damaging by abiotic stress, via protecting against oxidative stress and scavenging the free radicals in response to nanoparticles. Our results showed that 3.3 mg L⁻¹ of Fe₂O₃ NPs was able to stimulate protein, soluble sugar accumulation, as well as the antioxidant enzymes activities, while the inhibiting concentration for cell growth was 10 mg L⁻¹, suggesting that α -Fe₂O₃ NPs would induce toxicity to algae when its concentration reaches 10 mg L⁻¹ in the aquatic environment. Above results also indicated that antioxidant enzymes, along with accumulation of protein and sugar might serve as the key barrier to scavenge the burst of ROS generation induced by α -Fe₂O₃ NPs even at low concentration (3.3 mg L⁻¹). However, this defense response was limited and insufficient to diminish the toxic effect induced by increasing concentration of nanoparticles (> 10 mg L⁻¹), thus inhibition on cell growth was observed under higher concentration of α -Fe₂O₃ NPs (> 10 mg L⁻¹) treatments. Under such condition, excessive ROS which was not depleted promptly would induce nanotoxicity to algae.

As shown in Fig. 4a, the addition of FA at all the tested concentrations increased the cell density of *Synechococcus* sp. PCC7942 treated with 10 mg L⁻¹ of α -Fe₂O₃ NPs. Treatments with different concentrations of FA showed negligible differences in cell density, comparing with the normal growth cultures without nanoparticles and FA. Similarly, the Chl *a* content (Fig. 4b) in cultures treated with 10 mg L⁻¹ of α -Fe₂O₃ NPs was restored to normal level (without α -Fe₂O₃ NPs) when exposed to FA. Insignificant differences in the total soluble sugar (Fig. 4b) and protein content (Fig. 5a) among untreated cultures and the cultures exposed to both α -Fe₂O₃ NPs and different concentration of FA were observed. Cultures with α -Fe₂O₃ NPs alone (10 mg L⁻¹) produced significant higher soluble sugar, compared to the group with FA. As shown in Fig. 5b–d, the activities of CAT, POD and SOD in all the treatments with FA decreased to the same level as in the untreated group. There was no significant differences in the activities of antioxidant enzymes of the treatments among different FA concentrations.

After addition of FA, nanoparticle-treated algal cells also exhibited unrepressed growth behavior as untreated algal

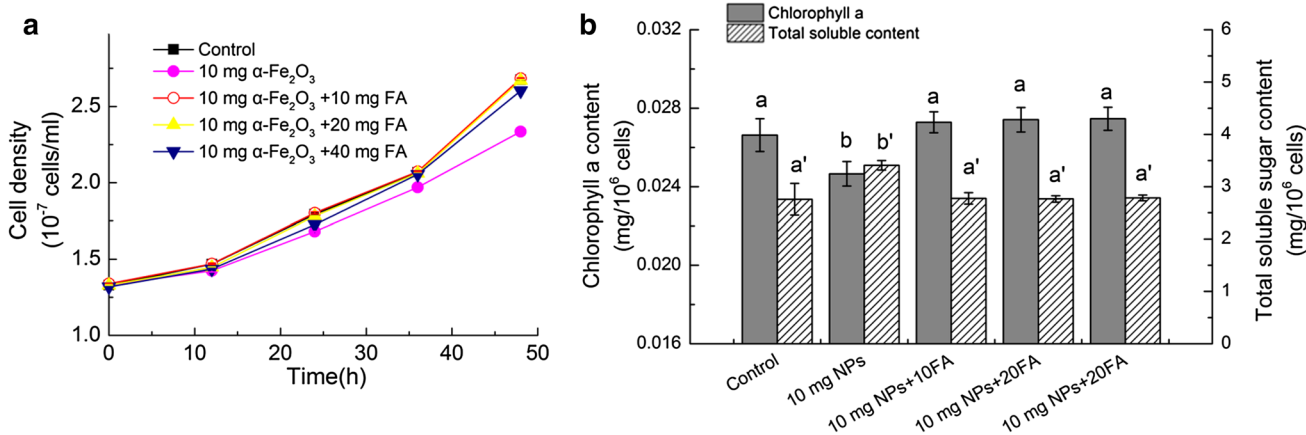


Fig. 4 The effects of FA on the **a** cell density, **b** chlorophyll *a* and total soluble sugar content of *Synechococcus* sp. PCC7942 under treatments with 10 mg L⁻¹ α-Fe₂O₃ NPs for 48 h exposure. Different letters indicate the statistically significance differences (*p* < 0.05) between treatments

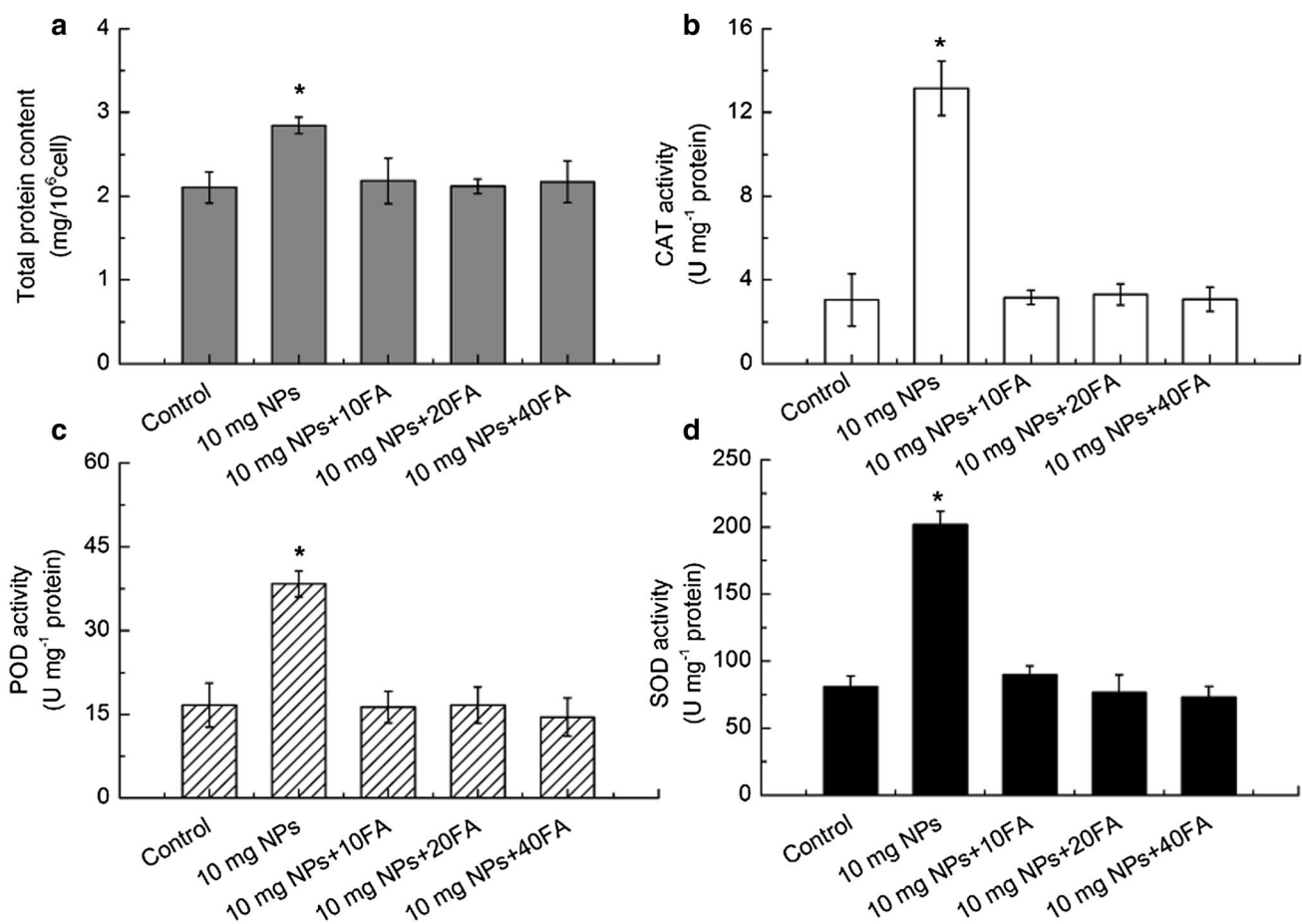


Fig. 5 Effects different concentrations of FA (10, 20 and 40 mg L⁻¹) on **(a)** total soluble protein content, **(b)** CAT, **(c)** POD and **(d)** SOD activities of *Synechococcus* sp. PCC7942 exposed to 10 mg L⁻¹ of α-Fe₂O₃

NPs. Asterisks indicate the statistically significance differences (*p* < 0.05) between treatments

cells (Fig. 4a). Meanwhile, the content of biochemical component such as Chl *a* (Fig. 4b), total soluble sugar (Fig. 4b) and total soluble protein (Fig. 5a) returned to the basal level as the normal cultures. Therefore, FA was supposed to alleviate nanotoxicity of α -Fe₂O₃ NPs to *Synechococcus* sp. PCC7942 in this study. NOM might act as an antioxidant and react with reactive oxygen species (Fabrega et al. 2009). FA is found to inhibit ROS (O²⁻, ·OH, ¹O₂) generation by WS₂, MoS₂ and CdS NPs, especially almost completely

represses ¹O₂ generation (Maurer-Jones et al. 2013). Significant reduction in the activity of antioxidant enzymes in cultures under FA treatment was observed (Fig. 5b–d). This might be attributed to the reduction of ROS generation under FA treatments, possibly due to the direct reaction with ROS, which in turn reduced the activity of scavenging enzymes.

SEM images (Fig. 6) showed the attachment of α -Fe₂O₃ nanoparticles onto the cell walls of *Synechococcus* sp. PCC7942 after interaction. Intact and spherical cells were

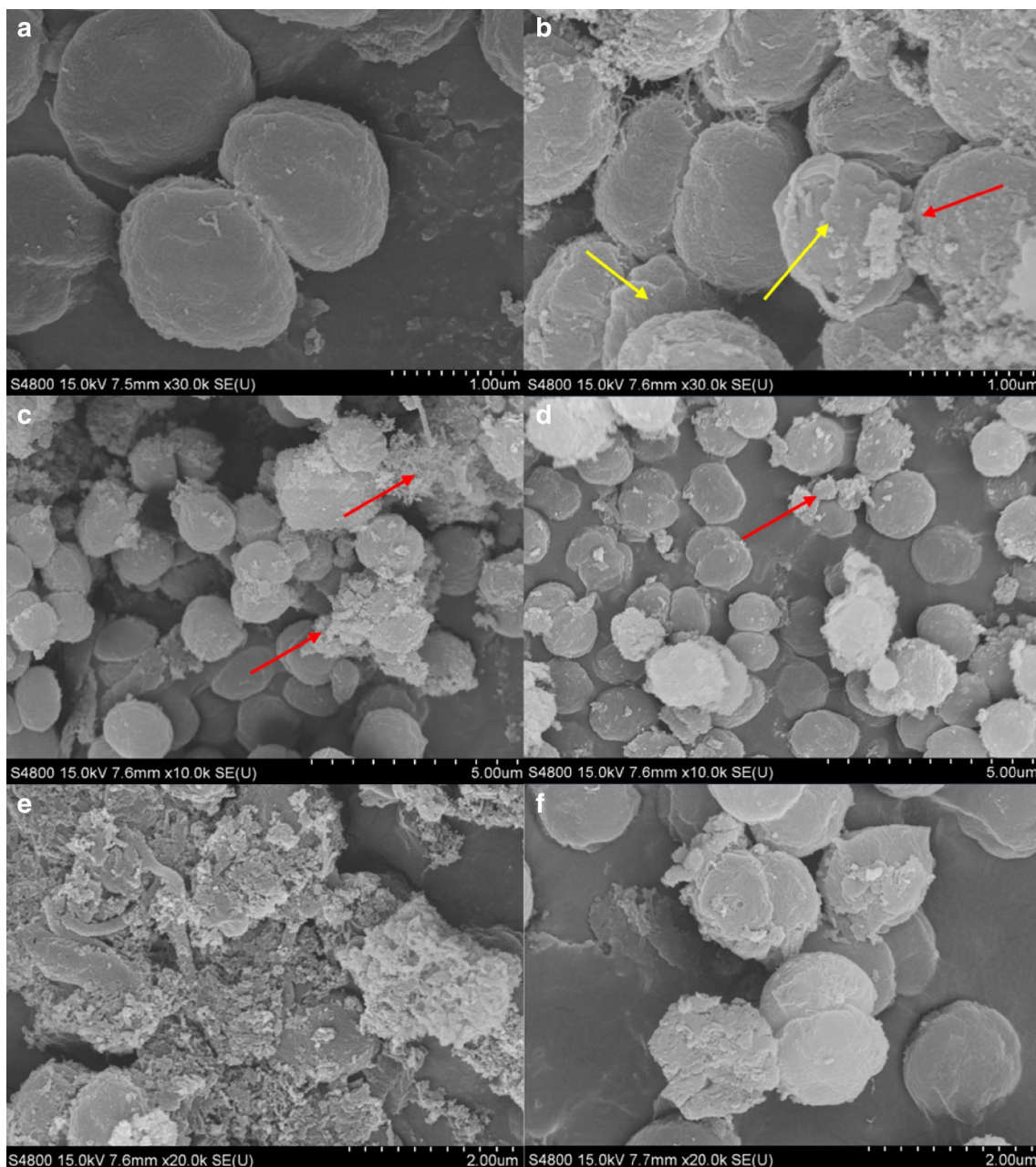


Fig. 6 Scanning electron micrographs of **a** control, **(b, c, e)** *Synechococcus* cells exposed to 10 mg L⁻¹ α -Fe₂O₃ NPs particles. **d, f** SEM images of algal cells treated with 10 mg L⁻¹ of α -Fe₂O₃ NPs

particles + FA (40 mg L⁻¹). The red arrows indicate the α -Fe₂O₃ NPs aggregates adhered to the cell walls, while the yellow arrows indicate the distorted cells under α -Fe₂O₃ NPs treatment

observed under non-treated condition (Fig. 6a). Winkled and abnormal cells can be seen clearly after incubation of algal cells in α -Fe₂O₃ NPs suspension (Fig. 6c). Large aggregates of nanoparticles wrapping the algal cells were observed under α -Fe₂O₃ NPs treatment (Fig. 6b). The aggregates of nanoparticles were smaller and entrapped less surface on the cell walls in the presence of FA (Fig. 6d, f), where cells maintained their normal spherical shapes as the in the control condition.

The hypothetical active sites involved in the interaction between algal cells and α -Fe₂O₃ NPs in the presence of FA were determined by FTIR analysis. Typical bands of FTIR spectra such as –OH of carbohydrates and/or –NH of proteins (3400 cm⁻¹), –CH, –CH₃ and –CH₂ stretching vibrations (2960, 2925 and 2851 cm⁻¹) were marked on Fig. 7. Typical bands for α -Fe₂O₃ NPs are ranged in the region of 536–570 and 460–480 cm⁻¹ (Demir et al. 2015), which are attributed to the Fe–O vibration. After the algal cells exposed to α -Fe₂O₃ NPs, a clear band at 454 cm⁻¹ appeared, which indicates the typical peak of α -Fe₂O₃ nano-powders, suggesting the direct adsorption of α -Fe₂O₃ NPs to algal cells. Interestingly, this peak disappeared after FA treatment, implying the interaction of FA with α -Fe₂O₃ NPs might more or less remove surface coating formed by α -Fe₂O₃ NPs on algal cell wall. The transmittance at band 1384 cm⁻¹, which is indicative of stretching vibrations of C–O or COO⁻ groups, decreased after exposure to α -Fe₂O₃ NPs indicated the possible interaction with carboxyl groups on cell surface. The typical FTIR peak of algal samples at 1384 cm⁻¹ lowered, indicating the interacted substance, FA could bind to the α -Fe₂O₃ NPs directly and partly replace the surface coatings of nanoparticles (Zhou et al. 2016). The peak at 1076 cm⁻¹ is corresponded to C–O stretching

vibration in carbohydrate derivatives. Zhou (2016) observed the disappearance of the 1076 cm⁻¹ peak after the algal exopolymeric substances (EPS) coated by NPs, indicating the interaction of the direct involvement of carboxylic groups in the EPS-NPs interaction. The absorbance peak area (in Fig. 7) which was calculated by integration lowered after the exposure of nano–algal mixture to FA, suggesting the direct involvement of carboxylic groups of FA in the interaction of FA-NPs-cells. However, it's difficult to clarify whether this interaction be dominantly FA-NPs or FA-cells based on current data. It is deduced that the involvement of carboxylic groups in the interaction of FA-NPs-cells, which might assist the desorption of NPs from algal cells.

SEM (Fig. 6) and FTIR (Fig. 7) results provided direct evidence for surface coating by nanoparticles. Repression on growth rates in algal tests may be also caused by direct adhesion of α -Fe₂O₃ NPs to the cell surfaces, resulting in the occurrence of shading and agglomeration, which might cause insufficient illumination in the cultures and nutrition absorption, and finally lead to slowing down cellular division and deteriorating the algal viability (Schwab et al. 2011). The pH value at the point of zero charge (pH_{PZC}) of Fe₂O₃ NPs is usually around 7–8.5 (Baalousha 2009; Palomino and Stoll 2013; Ahmad et al. 2016), indicating Fe₂O₃ NPs is zero-valent charged or negatively charged in culture medium (Berg et al. 2009; Auffan et al. 2011). In our preliminary experiment, the pH was found to drop slightly from 7.08 to 6.77 when the FA concentration increased from 0 to 40 mg L⁻¹ in the presence of 10 mg L⁻¹ α -Fe₂O₃ NPs. Since the above pH fluctuation locates in the range of reported pH_{PZC} of Fe₂O₃ NPs (7–8.5), changes in the surface charge of NPs are supposed to be insignificant under such condition. Although the pH_{PZC} values could be shifted to lower

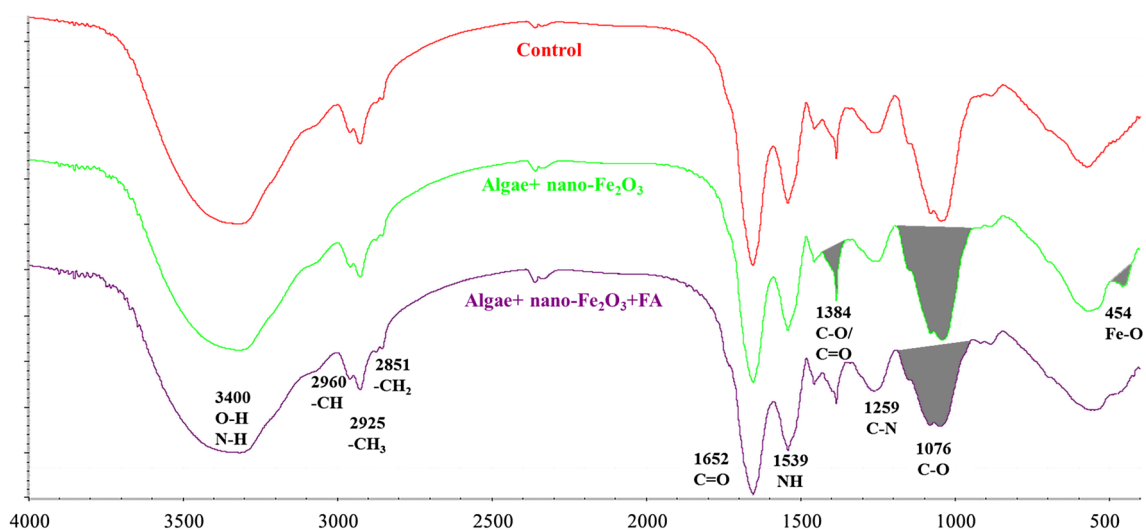


Fig. 7 FTIR spectra of *Synechococcus* sp. PCC7942 incubated in 10 mg L⁻¹ of α -Fe₂O₃ NPs and 10 mg L⁻¹ of FA. The grey shaded area on the spectra represent significant different peaks between treatments and control

pH in the presence of high NOM (humic acid) concentration ($> 50 \text{ mg L}^{-1}$) (Baalousha 2009), the Fe_2O_3 NPs are inferred still negatively charged in an alkaline cell growth medium. FA was found to promote stabilizing and disaggregating Fe_2O_3 NPs when they are neutral or negative charged at pH equal to or higher than pH_{PZC} (Palomino and Stoll 2013). However, the adsorbed FA could promote the aggregation of $\alpha\text{-Fe}_2\text{O}_3$ NPs when the NPs surface is positively charged at a lower pH than pH_{PZC} (Palomino and Stoll 2013). The electrostatic force between $\alpha\text{-Fe}_2\text{O}_3$ NPs and algal cells acts as the driven force for enhancing the adsorption of nanoparticles to algal cell walls and promote the formation of large aggregates on cell surface (Lin et al. 2015), leading to physical disruption of cell membrane or reduction reactions on cell surface that induce oxidative stress (Li et al. 2010).

SEM and FTIR results also indicate partial removal of the surface coating of NPs from cell surface and less direct contact between NPs and algal cells in the presence of FA. NOM could influence the stabilizing potency to nanoparticles (Van Hoecke et al. 2011) and induced disaggregation of $\alpha\text{-Fe}_2\text{O}_3$ NPs (Baalousha 2009). It has been demonstrated NOM is readily adsorb to colloidal particles and forms a surface coating on colloids and NPs, which enhances their stability via electrosteric stabilization mechanism (Baalousha 2009; Chen et al. 2011). Therefore, the presence of negatively charged FA, might disaggregate the $\alpha\text{-Fe}_2\text{O}_3$ NPs aggregates by increasing the negative charge of nanoparticles, thus enhances the electrostatic repulsion between nanoparticles and algae cells (Chen et al. 2011). This resulted in the limiting direct contact between NPs and algal cells and preventing the encapsulation of NPs on cell surface, which benefits in alleviating the shading inhibition effect on cell growth (Van Hoecke et al. 2011). In addition, limiting the adhesion of $\alpha\text{-Fe}_2\text{O}_3$ NPs might alleviate the direct physical injury to the algal cells (Lei et al. 2016). Moreover, the cellular uptake and intracellular ROS generation is related to close contact with the nanoparticles (Li et al. 2010). Limitation in direct contact between algal cells and NPs would significantly reduce the production of intracellular ROS, as indicated by the lower antioxidant enzymes activities in the presence of FA (Fig. 5b–d), therefore mitigate the nanotoxicity of $\alpha\text{-Fe}_2\text{O}_3$ NPs.

The aggregation and stability of Fe_2O_3 NPs usually depends on concentration of NPs itself, particle sizes, the time of exposure, ionic strength, concentration of natural organic matter (NOM), and pH etc (Dickson et al. 2012; Farooq et al. 2013). Ahmad (2016) reported that aggregation formed at lower Fe_2O_3 NPs concentration ($< 30 \text{ mg L}^{-1}$) but disaggregation took place with increasing Fe_2O_3 NPs concentration ($50\text{--}200 \text{ mg L}^{-1}$), possibly due to the increasing electrostatic repulsive interaction between NPs. In this study, Fe_2O_3 NPs tended to form aggregate at the tested concentration of 10 mg L^{-1} . When the NPs enter the aquatic

environment, the condition is far more complicated than in lab condition. Any changes in such a system with respect to pH, ionic strength, pH and types of biomacromolecules may destabilize the situation. Increasing ionic strength increases aggregation of most NPs (Keller et al. 2010; El Badawy et al. 2012). NPs are found to be stabilized and their aggregation is limited at realistic NOM concentrations ($1\text{--}30 \text{ mg}$ of carbon L^{-1}), while flocculation occurs at higher NOM concentrations (Maurer-Jones et al. 2013). Other biomolecules in aquatic environment, such as extracellular polymeric substance (bacterial secretion containing polysaccharides and proteins), cause an increase in nanoparticle aggregation rate (Nason et al. 2012). A sharp shift of pH from neutral or alkaline to acidic environment will cause FA adsorb on positive charged NPs at a lower pH than pH_{PZC} and promote aggregation of $\alpha\text{-Fe}_2\text{O}_3$ NPs (Palomino and Stoll 2013), leading to cellular surface coating and inducing ROS generation. Therefore, the aggregation and stability of NPs is a integration result of multiple environmental factors. Those factors should be taken into account when analyze the realistic fate of $\alpha\text{-Fe}_2\text{O}_3$ NPs and its interaction with FA.

In summary, high concentration of $\alpha\text{-Fe}_2\text{O}_3$ NPs (10 and 30 mg L^{-1}) could induce nanotoxicity to *Synechococcus* sp. PCC7942. Formation of large aggregates by $\alpha\text{-Fe}_2\text{O}_3$ NPs on cell walls induced oxidative stress to cells and limited cellular light availability, resulting in repression of cell growth. A representative NOM, FA was found to effectively restore the cell growth and alleviate oxidative stress imposed by $\alpha\text{-Fe}_2\text{O}_3$ NPs by reducing the ROS generation. The partial desorption of NPs from cell walls in the presence of FA leads to the removal of surface coating formed by NPs aggregates, avoiding direct physical injury and alleviating the inhibition of shading effect on cell growth. As a consequence, limitation of direct contact between $\alpha\text{-Fe}_2\text{O}_3$ NPs and algal walls was proposed as the main contribution to mitigating the toxicity. FA could be used as a special detoxification substance of alleviating toxicity of $\alpha\text{-Fe}_2\text{O}_3$ NPs, and possibly other nanoparticles.

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