Brief Report

Comparative Toxicity Assessment of Eco-Friendly Synthesized Superparamagnetic Iron Oxide Nanoparticles (SPIONs) in Plants and Aquatic Model Organisms

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Abstract: This study aimed to evaluate the toxicity of superparamagnetic iron oxide nanoparticles (SPIONs) synthesized by biogenic (BS) and chemical (CH) routes. The nanoparticles were characterized by X-ray diffraction (XRD), X-ray spectroscopy (XPS), atomic force microscopy (AFM), vibrating-sample magnetometry (VSM-SQUID), Fourier-transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM). The toxicity of SPIONs was evaluated using Artemia salina as model aquatic organisms and Raphanus sativus and Lactuca sativa as model plants to evaluate their phytotoxicity. The results obtained from XRD, XPS, and AFM confirmed the formation of spherical nanoparticles of 41.9 ± 1.00 nm (BS route) and 19.8 ± 0.47 nm (CH route). VSM-SQUID demonstrated the superparamagnetic behavior of both nanoparticles, and FT-IR provided evidence of the differences in the surface of SPIONs, suggesting the presence of phenolic compounds on the surface of BS-SPIONs. For the assays with Artemia salina, the results demonstrated (i) nonsignificant differences of BS-SPIONs in mortality rates, and (ii) significant toxicity (p < 0.05) was observed for CH-SPIONs at 300 and 400 mg L⁻¹. The Raphanus sativus plant assay tests showed (i) BS-SPIONs and CH-SPIONs improved the root elongation of seedlings. However, BS-SPIONs demonstrated significant activity on root seedling elongation (p < 0.05) in the range of 300 mg L⁻¹ to 600 mg L⁻¹. To the best of our knowledge, this is the first report to compare the toxicity of chemically and biogenically synthesized SPIONs. In conclusion, although BS-SPIONs and CH-SPIONs present similar structures, their characteristics of magnetic saturation and surface structure are nonidentical, providing differences in their biological activity.

Keywords: superparamagnetic iron oxide nanoparticles (SPIONs); biogenic synthesis; toxicity assessment; mortality assay

1. Introduction

The synthesis of superparamagnetic iron oxide nanoparticles (SPIONs) is increasing due to their environmental applications in waste management, water treatment, and plant growth [1–6]. The main characteristics of SPIONs are their superparamagnetic behavior, which provides strong magnetic susceptibility, lack of remanent field and coercivity, and absence of hysteresis. Under an external magnetic field, these individual nanoparticles express the magnetic moment as a single domain to show magnetism. Once the external magnetic field is removed, the magnetism of the individual particles becomes zero [7].
This peculiar behavior of SPIONs has attracted the focus of many researchers for two reasons: (i) they can be easily removed from the working zone, and (ii) they provide evidence of nonmagnetic interferences with cell communications associated with biological applications. Therefore, SPIONs are widely described in agricultural applications, which makes them good candidates to be used in different fields [8–10]. In this regard, iron micronutrients are taken up by the root plants from the soil or foliar spray and transferred through vascular systems to the xylem and phloem structure [11,12]. Particularly, in the soil, nanoparticles can interact with microorganisms and compounds, which might facilitate or hamper their adsorption. Therefore, the uptake and translocation of iron micronutrients to improve the iron concentration and other chemical elements in organ plants may be enhanced by using superparamagnetic nanoparticles [6].

Potential adverse effects of SPIONs after they are released into the environment should be considered. First, there is a need for more studies to evaluate the performance, fate, and unintentional impact of nanoparticles over the long term [13,14]. Second, holistic environmental analysis such as life-cycle assessment is required to understand and measure the fate of nanoparticles. Third, consumers tend to distrust technology unfamiliar to them, making it necessary to demonstrate the benefits of nanotechnology, ensuring human and animal safety [11,13]. Generally, the fate of environmental SPIONs is constituted by soil to plant organs, and thus the food chain, representing an important task for the research of diverse ecosystems such as aquatic, herbal, and forest, among others. For example, Gaharwar et al. [15] demonstrated that iron oxide nanoparticles generated oxidative damage in cell membranes due to their size and further accumulation. Additionally, Pérez-De-Luque [11] highlighted the functionalization and coating of the nanomaterial surface, which can greatly change and alter its adsorption and accumulation. In this regard, authors have suggested that the synthesis of polymer-coated SPIONs is strictly related to their further toxicity, evidencing an interesting research gap for new approaches associated with green synthesis methods [16].

Conventional methods have been widely developed to produce SPIONs, such as thermal decomposition, sonochemical synthesis, coprecipitation, reduction of iron salts with sodium borohydride, hydrothermal-based processes, and hydrolysis, among others [7,17,18]. However, these methods have several limitations such as low production yield and difficulties in operating conditions, generating hazardous wastes that could present important environmental risks. In this context, synthesis methods of green SPIONs have emerged as an interesting option due to their intrinsically organic layer acting as a coating agent. Biogenic SPIONs (BS-SPIONs) are a practical alternative based on the use of plant extract, which has several advantages over chemical and physical methods. Plant extract containing flavonoids, polyphenols, sugars, and aromatic compounds, among others, can reduce iron, leading to the formation of nanoparticles [19]. The main advantages of biogenic SPIONs are related to low-cost production, environmental friendliness, and high biological activity [7]. Interestingly, it has been evidenced that the green synthesis of SPIONs can improve nanoparticle dispersibility and chemical stability, enhance biocompatibility, and reduce toxic effects, enhancing nanoparticles coated with organic layers. Remarkably, the organic layer contributed to avoiding undesired agglomeration and maintaining the colloidal stability of nanoparticles to increase their efficiency in diluted systems. Therefore, this study focused on investigating the toxicity of two types of manufactured SPIONs synthesized by chemical and green routes. The toxicity comparison was carried out on two model organisms to represent how aquatic and soil ecosystems behave once SPIONs are applied. To the best of our knowledge, this is the first report to compare the physicochemical properties of chemically and biogenically synthesized SPIONs and their impact on the ecotoxicity of model organisms such as brine shrimp *Artemia salina*, *Raphanus sativus*, and *Lactuca sativa*. 
2. Materials and Methods

2.1. Green Synthesis and Coprecipitation of SPIONs

For the synthesis of SPIONs, FeCl$_3$·6H$_2$O and FeCl$_2$·4H$_2$O salts were dissolved in distilled water on precipitated glass (250 mL) at a final concentration of 40 mM and 20 mM (2:1 M ratio), respectively. Synthesis of BS-SPIONs was performed using *Galega officinalis* leaf extract according to Manosalva et al. [20]. Briefly, 10 g of young and healthy leaves was placed in a 250 mL Erlenmeyer flask with 100 mL of deionized water at 80 °C for 5 min. After this time, the leaf extract was cooled to environmental temperature and filtered through Whatman N° 1 filter paper. The obtained leaf extract was diluted to 10% v/v with deionized water and stored at 4 °C. Then, both iron salt solutions were added simultaneously to the *G. officinalis* extract to obtain a final proportion of Fe$^{3+}$ and Fe$^{2+}$ at a 2:1 M ratio. The final solution was stirred at 25 °C and adjusted to pH 11 with 1 M NaOH until the formation of BS-SPIONs. Coprecipitation synthesis was modified from Petcharoen and Sirivat [21]. NaOH solution (50 mL at 0.7 M) was poured into a burette and added dropwise to the iron salt solution (FeCl$_3$·6H$_2$O/FeCl$_2$·4H$_2$O: 40 mM/20 mM) with vigorous stirring until the formation of a black suspension. Afterward, the magnetic nanoparticles were washed with ethyl alcohol five times and then with deionized water to remove the alcohol residue. A neodymium N52 magnet was used to isolate the nanoparticles. Then, the nanoparticles were freeze-dried at $-70$ °C ± 2 °C for 48 h and stored at room temperature for forwarding analysis.

2.2. Characterization of SPIONs

Characterization of SPIONs was carried out according to the recommendations of Samrat et al. [7]. X-ray diffraction (XRD) was carried out to determine the crystallographic structure of the magnetic nanoparticles. The samples were analyzed in a STADI-P (Stoe, Darmstadt, Germany) diffractometer operating at 50 kV and 40 mA, using Mo K$_\alpha_1$ ($\lambda = 0.7093$ Å). The X-ray photons were collected using a Mythen 1K detector (Dectris®, Baden, Switzerland). XRD data were recorded in the 2$\theta$ range from 5.0$^\circ$ to 64.265$^\circ$, with step sizes of 0.015º and a counting time of 100 s at each 0.785$^\circ$. X-ray photoelectron spectroscopy (XPS) was used to determine the elemental composition of the nanoparticles. XPS analysis was performed using an XPS Thermo K-alpha spectrometer (Waltham, MA, USA). The instrument utilized a 72 W monochromated Al K-alpha$^+$ source (E = 1486.6 eV) energy-adjusted to 3000 eV, medium current, with a spot size of 400 µm and depth of 10 nm. The magnetic properties of nanoparticles were obtained using a vibrating-sample magnetometer (VSM-SQUID) Quantum Design (Quantum Design, Inc., San Diego, CA, USA), employing isothermal measurements as a function of the applied magnetic field of $-30$ to 30 kOe (M × H) with powder samples.

Nanoparticles were also analyzed using an atomic force microscope (AFM), model AFM/SPM Series 5500 dynamics (Agilent Technologies: Santa Clara, CA, USA) of 4 nm thickness, 125 µm in length, 30 µm frequency of 320 kHz resonance, and a force constant of 42 N/m. Fourier-transform infrared (FT-IR) spectroscopy is widely used to identify the functional groups of synthesized nanoparticles [22]. The analysis was performed using ATR-FTIR Cary 630 (Agilent Technologies, Santa Clara, CA, USA) in the range of 4000–600 cm$^{-1}$.

2.3. Ecotoxicity Tests

2.3.1. Artemia salina Test

Brine shrimp *Artemia salina* cysts were purchased from PRODAC® and maintained under constant light exposure at 37 °C in saline solution (3.2 g NaCl$_2$ /100 mL of distilled water). The toxicity test was carried out according to Gambardella et al. [23], where 10 decapsulated *A. salina* cysts were placed in 10 mL of saline solution amended with 50, 100, 200, 300, or 400 mg L$^{-1}$ of BS-SPIONs or CH-SPIONs in triplicate. Saline solution without nanoparticles was used as the control. The experiment was performed at 20 °C for
48 h. After this time, live individuals were counted, and the mortality (%) was calculated by Equation (1):

\[
\text{Mortality percentage (\%)} = \left(\frac{\text{DeA} \times 100}{M}\right)
\]

where \(\text{DeA}\) is the average amount of dead \(A.\ salina\) per SPION concentration, and \(M\) is the total number of \(A.\ salina\) added.

### 2.3.2. \textit{Raphanus sativus} and \textit{Lactuca sativa} Tests

For the phytotoxicity test, \textit{Raphanus sativus} and \textit{Lactuca sativa} seeds were selected according to the methodology proposed by Rede et al. [24] with modifications. The disinfection procedure was carried out according to Fincheira et al. [25] using sodium hypochlorite. The seed germination tests were carried out in Petri dishes (9 \( \times \) 50 mm) containing 15 test seeds exposed directly to filter paper impregnated with 3 mL of each SPION suspension (BS-SPIONs or CH-SPIONs) at different concentrations (0, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 mg L\(^{-1}\)). Petri dishes were incubated for 72 h at 25 °C exposed to a 16/8 (light/dark) photoperiod. After this period, germination rate was determined, and root elongation of germinated seeds was measured using Sigmascan Pro 5.0 image analysis software. All treatments and controls were performed in triplicate.

### 2.4. Statistical Analysis

A one-way analysis of variance (ANOVA) with a significance level of 95% was used to determine the statistical differences between treatments in \(A.\ salina\). In the seedling root elongation and germination tests, BS-SPION and CH-SPION concentrations were analyzed using the LSD test with a significance level of 95% in the JMP software.

### 3. Results and Discussion

The production of SPIONs is expected to increase exponentially in the coming years due to their environmental and agricultural applications [26]. Therefore, it is crucial to evaluate and understand their potential adverse effects on ecotoxicology. In this work, we assessed the (i) structural differences between CH-SPIONs and BS-SPIONs, and (ii) the toxicological effect of manufactured BS-SPIONs and CH-SPIONs on three model organisms: \(A.\ salina\), \(R.\ sativus\), and \(L.\ sativa\).

#### 3.1. Characterization of SPIONs

The results of BS-SPIONs and CH-SPIONs (Figure 1a,b) showed diffraction peaks characteristic of SPIONs (JCPDS File No.: 19-0629) at (111), (220), (311), (400), (422), (511), and (440), which represent angles in 2\(\theta\) of 18.65°, 30.54°, 35.94°, 43.28°, 53.24°, 56.56°, 61.54°, 69.01°, and 71.78°, respectively. To determine the crystallite diameter, the Scherrer equation was employed,

\[
D_{hkl} = \frac{0.9λ}{β_{hkl} \cos θ}
\]

where \(λ\) is the wavelength (\(λ = 0.7093 \, \text{Å, MoKα}\)) as the source), \(β\) is the peak width at half-height of the signal, and \(θ\) is the diffraction angle. The equations indicate a grain size of 5.25 and 1.81 nm for BS-SPIONs and CH-SPIONs, respectively, suggesting a smaller size for CH-SPIONs. These results indicate that both BS-SPIONs and CH-SPIONs correspond mainly to the magnetite phase [27–29]. However, the CH-SPION peak (110) (Figure 1b) is related to the characteristic maghemite nanoparticles, which can be related to a slight Fe\(^{2+}\) deficiency in magnetite, creating defects in the crystal lattice due to Fe\(^{3+}\) vacancies [30]. In the XRD pattern of SPIONs, sharp and intense peaks were observed, indicating a degree of high crystallinity for both types. According to the Scherrer equation, XRD peaks broaden when the crystallites become smaller, which may indicate smaller crystallites for CH-SPIONs than BS-SPIONs [31]. XPS analysis was carried out to verify the phase composition of the synthesized nanoparticles. As shown in Figure 1, the survey spectrum indicates Fe 2p, C 1s, and N 1s. Detailed Fe 2p spectra for the nanoparticles are shown in Figure 1c,d. The fitting of the Fe 2p region indicated both Fe\(^{2+}\) and Fe\(^{3+}\) are characteristic satellites for Fe\(_2\)O\(_4\) compounds, but the result showed slight differences in the Fe\(^{2+}\) ratio between CH-SPIONs and BS-SPIONs. Through the deconvolution of this spectrum, it is possible to obtain contributions from
the Fe II and Fe III species; thus, it is possible to quantify these species and determine the \( \text{Fe}^{3+} / \text{Fe}^{2+} \) ratio. This ratio can be used as an indication of the quality of the synthesis, where values close to 2 indicate that there is only a magnetite phase in the sample, and values greater than 2 indicate the possible oxidation of magnetite to maghemite, even though the magnetite phase was obtained in the present work. Figure 1e,f shows the hysteresis loops of BS-SPIONs and CH-SPIONs. At 27 \(^\circ\)C, it was possible to observe a superparamagnetic behavior, as no coercivity or remanence in BS-SPIONs and CH-SPIONs was observed. For BS-SPIONs, the magnetization saturation was 67 emu g\(^{-1}\), whereas CH-SPIONs presented a magnetization saturation of 26 emu g\(^{-1}\). VSM-SQUID analysis demonstrated that for both SPIONs, no coercivity or remanence was observed, confirming the superparamagnetic behavior of the nanoparticles [22]. The magnitude of saturation magnetization was higher for BS-SPIONs (67 emu g\(^{-1}\)) than CH-SPIONs (26 emu g\(^{-1}\)). This value is higher than the values of saturation magnetization previously reported for SPIONs synthesized using plant extracts [32–35].

According to SEM analysis, BS-SPIONs and CH-SPIONs showed a spherical shape, which was also confirmed by AFM analysis (Figure 2a,b). Phase-contrast images from AFM analysis were obtained from different regions of the samples, and the average diameter was...
evaluated using WSxM 5.0 software (Madrid, Spain). The data indicated an average diameter of 41.97 ± 1.00 nm and 19.78 ± 0.47 nm for BS-SPIONs and CH-SPIONs. Figure 2c,d evidences high polydispersion in BS-SPIONs, unlike CH-SPIONs, which present a monodisperse size distribution, which is in concordance with other authors [22,36]. The heterogeneous size distribution of BS-SPIONs can be attributed to different reducing agents on the G. officinalis aqueous extract, such as flavonoids, xanthophylls, anthocyanins, and phenolic acids, which could be involved in the nanoparticles [37,38]. Meanwhile, the wide size can be attributed to the excess of G. officinalis aqueous extract [39]. This insight is different from coprecipitation methods, where a specific reducing agent could be involved in the biogenic synthesis reaction.

![Figure 2](image-url)

**Figure 2.** SEM images for morphological structure of (a) BS-SPIONs and (b) CH-SPIONs. AFM phase-contrast image and size distribution of (c) BS-SPIONs and (d) CH-SPIONs. (e) FT-IR spectrum of BS-SPIONs (--) and CH-SPIONs (–).
FT-IR measurements were carried out to determine the surface chemical composition (Figure 2e) and functional groups of SPIONs and their possible surface interactions. Peaks related to 3350 cm\(^{-1}\) were observed in SPIONs, which evidence the O-H bonds from hydroxyl bonds on the surface. In addition, a 2680 cm\(^{-1}\) peak associated with C-H sp\(^3\) bonds was observed in both samples of SPIOns. Nonetheless, 1650 cm\(^{-1}\) (amide C=O) and 860 cm\(^{-1}\) (aromatic C-H) peaks were observed only in BS-SPIONs, which reveals differences in the composition and electron distribution of the surface of BS-SPIONs compared to CH-SPIONs. This difference can be attributed to the presence of a specific capping agent given by the plant extract. The evidence on the surface structure related to 3350 cm\(^{-1}\) was associated with the alcohol O-H bonds of the hydroxyl groups on the magnetite surface [40]. The 2680 cm\(^{-1}\) peak corresponds to carboxylic acid C-H sp\(^3\) [41]. These results agree with the FT-IR analysis of SPIONs synthesized by Marimón-Bolívar and González [36]. Nonetheless, 1650 cm\(^{-1}\) (amide C=O) and 860 cm\(^{-1}\) (aromatic C-H) are peaks exclusively from BS-SPIONs, attributed to the aromatic rings of protein residues from the plant extract.

3.2. Artemia salina Ecotoxicity Test

*A. salina* is a marine invertebrate widely used for practical ecotoxicity tests, recognized as a model organism because of its ease in culturing, ready availability, low cost, and adaptability to adverse conditions [42,43]. *A. salina* can ingest particles smaller than 50 µm; therefore, its continuous ingestion of SPIONs may cause damage to its tissues [44]. This damage is mainly attributed to an increase in ROS production by SPIONs, which affect the mitochondrial structure [44]. Our findings suggest that iron ion release could be a crucial factor associated with the organism metabolism, since it can directly interact with the aqueous medium, as Abdelsalam et al. [10] describes on their application. Figure 3a shows the results of the *A. salina* mortality percentage in the presence of both SPIONs. No significant differences were observed in BS-SPION treatments, whereas in the range of 300–400 mg L\(^{-1}\) of CH-SPIONs, significant differences were observed. The control treatment without exposure to SPIONs showed 13.3% *A. salina* mortality. It was noted that *A. salina* mortality correlated directly with the CH-SPION concentration (Figure 3a). Along with the mortality values, CH-SPIONs exhibited the highest mortality rate (37%) at 400 mg L\(^{-1}\), higher than the control. Raguraman and Suthindhiran [45] reported higher mortality rates (40% to 70%) in *A. salina* exposed to chemically synthesized Fe\(_3\)O\(_4\) nanoparticles (10 to 250 mg L\(^{-1}\)) for 48 h. These results suggest that the capping agent of BS-SPIONs gives significant compatibility with biological systems. This capping agent may consist of aromatic compounds and protein residues from the plant extract [46]. In this sense, CH-SPIONs present a strictly increasing mortality rate, unlike the exposure of *A. salina* to BS-SPIONs, where no significant differences were observed. Recently, NPs systems have reported hormesis as a biphasic dose–response generated by almost all biological systems due to their interaction with the various physical or chemical stimuli [47]. This study suggests hormetic behavior on mortality rates due to the differences in capping structure; additionally, the maghemite structure (presented in CH-SPIONs) indicates a significant presence of Fe\(^{3+}\) ions, which increases the activity of reactive oxygen species (ROS) [23]. Furthermore, Kumar et al., (2017) [48] reported that the ionic interactions of NPs are highly toxic beyond a threshold concentration level, concentrations that were not determined in this study, suggesting a broad concentration range of iron oxide nanoparticles (SPIOns).

3.3. Phytotoxicity Seedling Test

*R. sativus* and *L. sativa* are model plant species routinely used in phytotoxicity tests [49]. The results showed no significant differences in the germination of *L. sativis* and *R. sativus* seeds exposed to BS-SPION (73%) and CH-SPION (76%) treatments compared with the control (86%) (p < 0.05). Figure 3b,c shows that *R. sativus* and *L. sativa* seedlings exhibited an increase in root length when exposed to BS-SPIONs and CH-SPIONs. The stimulation produced by BS-SPIONs on *R. sativus* occurred at concentrations above 600 mg L\(^{-1}\) with a
maximum root length of 700 mg L$^{-1}$ (25.9% longer than control). Additionally, the root length of *L. sativa* was stimulated at intermediate concentrations (200 to 500 mg L$^{-1}$) of BS-SPIONs, with a maximum root length of 300 mg L$^{-1}$ (68.7% longer than the control). *R. sativus* exposed to CH-SPIONs showed the highest root length at 100 mg L$^{-1}$ (32.6% longer than the control), and at concentrations above 300 mg L$^{-1}$, the root length was similar to the control. This could be attributed to *R. sativus* seedlings taking most of the iron ions released by CH-SPIONs at low concentrations. Nonetheless, as the IONP concentration increases, the ions released disrupt the biochemical functions of the seedlings. This was corroborated by Saquib et al. [50], suggesting that concentrations of IONPs above 500 mg L$^{-1}$ suppress the esterase level in the protoplast, causing damage to the cell membrane and decreasing the root elongation of the seedlings.

![Figure 3](image)

Figure 3. Toxicity behavior of SPIONs on model organisms: (a) mortality percentage of *Artemia salina* exposed to BS-SPIONs and CH-SPIONs; (b) root length of *Raphanus sativus*; (c) *Lactuca sativa* germinated seeds exposed to CH-SPIONs and BS-SPIONs.

*L. sativa* exposed to CH-SPIONs showed a root length higher than the control at all concentrations tested, with a maximum root length of 900 mg L$^{-1}$ (57.0% longer than the control). Our results showed the nonphytotoxic behavior on radish (*R. sativus*) and lettuce (*L. sativa*) seedling root length, regardless of the SPION synthesis pathway. Root elongation of seedlings treated with CH-SPIONs was relatively lower than seedlings treated with BS-SPIONs. Thus, BS-SPIONs evidenced a better uptake in *L. sativa* seedlings. The iron requirements for plant growth are well known [51–53]. Iron is one of the essential nutrients for plant growth and is the cofactor of various enzymes that accelerate plant growth [51]. Like iron nanoparticles, metal oxide nanoparticles can bind with carrier proteins or organic chemicals and be absorbed by plant tissues [53]. Intracellular iron homeostasis is also essential, since phytoferritin plays a crucial role; this protein stores iron in its nanocage in the form of iron metals such as ferritin. During this process, phytoferritin is degraded...
and releases iron, promoting hydroxyl ions that destroy the protein layer of the seed [54]. Several authors have studied the effect of different oxide metals on different plants and evaluated the toxicity levels regarding the NP concentration. In this sense, Liu et al. [55] investigated the effects of FeOx NPs on the root elongation of lettuce and determined that root elongation was inhibited by 12% at a concentration of 50 mg L\(^{-1}\). On the other hand, Martínez-Fernández et al. [56] studied the effect of nanozero valent Fe nanoparticles and maghemite NPs (Y-Fe\(_2\)O\(_3\)) on the root length status of tomato seedlings (Solanum lycopersicum), and their results evidenced an inhibition by 40% on the root length with the treatment of 100 mg L\(^{-1}\) NPs.

These results reveal a gap in the toxic behavior of SPIONs on plants, where authors have described the activity on root elongation at concentrations above 500 mg L\(^{-1}\) oxide NPs as not positive (Saquib et al., 2016; Zuverza-mena et al., 2016) [50,57]. Therefore, BS-SPIONs present better uptake activity due to aromatic rings and protein residues from the plant extract, suggesting greater compatibility with radish seedlings due to capping agents.

4. Conclusions

Although BS-SPIONs and CH-SPIONs present similar structures, their characteristics of magnetic saturation and surface structure are nonidentical, providing differences in their biological activity. Our findings showed a different toxicological behavior when using SPIONs synthesized by two methods. In this sense, our research showed BS-SPIONs as an alternative material for biological systems due to their nontoxic behavior compared to CH-SPIONs. We hope that this approach inspires further critical efforts to develop nanotechnological products based on low environmental toxicity. Highlighting our study, the strengths are related to the use of SPIONs with a wide range of concentration exposures, as well as the use of model aquatic and plant organisms. Nonetheless, we found limitations associated with the lack of data on the foliar applications of SPIONs and the need for toxicity tests of SPIONs with a long exposure time. Therefore, further directions should consider (i) the environmental footprint analysis of SPION, (ii) comparative studies between SPIONs and conventional fertilizers, and (iii) the effects of SPIONs on the microbiome field.

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