CHEMICAL ENGINEERING TRANSACTIONS
VOL. 110, 2024
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Marco Bravi, Antonio Marzocchella, Giuseppe Caputo

ISBN 979-12-81206-10-6; **ISSN** 2283-9216
DOI: 10.3303/CET24110071

Toxicity on *Artemia Salina* of Fe_3O_4 Nanoparticles Synthesized by Microwave Assisted Hydrothermal Method

Katherine N. Vasquez-Villanueva[a], Gina G. Zavaleta-Espejo[a,c,*], Fanny V. Samanamud-Moreno[b,c,d], Santos L. Chucchucan-Gonzalez[b,c], Juan J. Pedro Huaman[a], Rosmery A. Hoyos-Honorio[a], Segundo R. Jáuregui-Rosas[b,c,d]

[a]Department of Biological Sciencies, Faculty of Biological Sciencies, National University of Trujillo, Trujillo, Peru.
[b]Faculty of Physical and Mathematical Sciences, National University of Trujillo, Trujillo, Peru.
[c]Multidisciplinary Nanoscience and Nanotechnology Research Group, Faculty of Physical and Mathematical Sciences, National University of Trujillo, Trujillo, Peru.
[d]Laboratory of Nanotechnology Investigation, Faculty of Physical and Mathematical Sciences, National University of Trujillo, Trujillo, Peru.
gzavaleta@unitru.edu.pe

Nanotechnology has gained much attention, especially in medical research, but its rapid development and the widespread use of nanoproducts raise questions about the ecotoxicity of these materials. In this work we studied the toxicity induced by nano Fe_3O_4 in a model organism, *Artermia salina* (*A. salina*) in an aquatic environment. These nanoparticles were synthesized by the microwave-assisted hydrothermal method. The methodology was a completely randomized experimental design and had 8 treatments which were: filtered and autoclaved seawater, $CuSO_4$, 10 mg/L, 100 mg/L, 250 mg/L, 500 mg/L, 1000 mg/L and 10000 mg/L of Fe_3O_4, each with 3 replicates. The experimental units were composed of 100 uL of each treatment and 10 instar III nauplii in a 24-well microplate. After 24 hours, the number of dead larvae and LC_{50} were determined using the statistical program R free version. As a result, the X-ray diffractogram indicated the formation of Fe_3O_4 with a crystallite size of 10.1nm; FT-IR spectroscopy confirmed the pure phase of the material with the presence of its characteristic bands. The LC50 had a value of 5356.76 mg/L of Fe_3O_4 at 24 hours of exposure to *A. salina*. In conclusion, Fe_3O_4 nanoparticles have a toxic effect causing larval death at concentrations above 5356.76 mg/L.

1. Introduction

Nanoscience and nanotechnology represent a growing research area, which involves structures, devices, and systems with novel properties and functions due to the arrangement of the nanoparticles atoms on the 1–100 nm scale (Bayda et al., 2019). Recent research on magnetic nanoparticles shows that they are an important class of nanomaterials that have various technological applications, therefore, they become a promising alternative in the field of health (Nguyen et al., 2021). Their small size makes them bioavailable; in addition, they enhance the absorption capacity of nanomaterials by biological systems (Ezealigo et al., 2021). Furthermore, they can be used as biocatalysts for the oxidation of various phenolic compounds (Grebennikova et al., 2022) and humic acid degradation (Lee et al., 2023).
Nowadays, magnetic iron oxide nanoparticles (magnetite, Fe_3O_4 or hematite, Fe_2O_3) in the range of 2-20 nm are of scientific and technological interest due to their superparamagnetic behavior (Dukenbayev et al.,2019), they are also investigated for biomedical and biotechnology aplications (Lokesh et al., 2020) due to their unique physicochemical properties, high strength and good magnetic properties (Chen et al., 2012). Magnetite nanoparticles are widely applied due to their biocompatibility, high magnetic susceptibility, chemical stability, innocuousness, high saturation magnetisation, and inexpensiveness (Shaterabadi et al., 2018). In this sense, the synthesis of nanoparticles has been widely used in different fields, resulting in the intentional or unintentional release of nanoparticles into the aquatic environmen, where we find several species of great biological importance, including a marine crustacean and non-selective filterer: *Artemia salina* (Chen et al., 2012).

Paper Received: 9 February 2024; Revised: 17 May 2024; Accepted: 20 June 2024
Please cite this article as: Vasquez-Villanueva K.N., Zavaleta-Espejo G.G., Samanamud-Moreno F.V., Chucchucan-Gonzalez S.L., Pedro Huaman J.J., Hoyos-Honorio R.A., Jauregui-Rosas S.R., 2024, Toxicity on Artemia Salina of Fe3o4 Nanoparticles Synthesized by Microwave Assisted Hydrothermal Method, Chemical Engineering Transactions, 110, 421-426 DOI:10.3303/CET24110071

However, depending on which method is used to prepare nanoparticles, there will be differences on their morphology, stability and physicochemical properties, therefore, one of the problems associated with metal and metal oxide NPs is their possible toxicity (Turan et al, 2019).
Comparative cytotoxic assays of nanostructures have been performed using cell cultures with the MTT assay and the *A. salina* test resulting in, no significant differences between the two models, therefore, the use of *A. salina* constitutes an available, economical and reliable biological model (Rajabi et al., 2015). Likewise, the toxicity of metal nanoparticles on cysts and larvae of *A. salina* was evaluated, reporting that there was larval mortality and inhibition of cyst hatching caused by silver nanoparticles (Pecoraro et al., 2020).
Shokry et al. (2021) developed nanotoxicological studies with nanocomposites, quantum dots and graphene oxide in *A. salina* presenting low toxicity. Özgür et al. (2018) carried out toxicity studies of Fe_3O_4 nanoparticles in sperm of Oncorhynchus mykiss "tilapia" obtaining oxidative damage from them directly proportional to their concentration.
A. salina plays a fundamental role in the flow of energy from food chains, filtering a large amount of water per hour. Therefore, it has significant interactions with the aquatic environment, and faces a higher risk of exposure to environmental pollutants compared to other aquatic species (Wang et al., 2017).
Lagarto et al. (2001) describe it as part of a general trial, widely used to determine the lethal effect of materials on nauplii of *A. salina*. According to Pelka et al. (2000) this test is considered a useful tool for the preliminary assessment of toxicity of fungal substances, heavy metals, insecticides and in cytotoxicity tests.
Therefore, the present research aimed to determine the toxicity induces by Fe_3O_4 nanoparticles on *A. salina* from different concentrations, these results will serve as a contribution to future research, in addition, to helping those responsible for the synthesis of magnetite in improving their production process so that they can have the expected applications..

2. Materials and methods

2.1. Synthesis of Fe_3O_4 nanoparticles

The Fe_3O_4 nanoparticles were synthesized by the microwave-assisted hydrothermal method from aqueous solutions of Fe^{3+} (0.11 mol/L) and Fe^{2+} (0.055 mol/L), to which NaOH (0.96 mol/L) was added at room temperature. The mixture was microwaved for 2 minutes in a domestic oven, then it was allowed to cool, recovering the black precipitate with a permanent magnet and washed 3 times with distilled water. Finally, the nanoparticles were dried for 12 hours on a stove at 70°C, pulverized in an Agata mortar and stored for characterization and toxicity tests.

2.2. Characterization of Fe_3O_4 nanoparticles

The structural characterization of Fe_3O_4 nanoparticles was carried up by X-ray diffraction (Rigaku diffractometer, Miniflex 600). In turn, for the chemical analysis of residual groups Fourier transform infrared spectroscopy in ATR mode (FT-IR Nicolet-iS50, Thermo Scientific) was used. The nanoparticles were also analyzed by scanning electron microscopy (TESCAN VEGA 3) and thermogravimetric analysis (TGA 5500, TA Instruments).

2.3. Obtaining the biological material

The *A. salina* cysts were purchased from an aquarium located in the Florencia de Mora district, Trujillo. In addition, seawater was collected from the resort of Huanchaco-Trujillo, which was filtered and autoclaved before use. To obtain decapsulated cysts, 1g of cysts were incubated in a fish tank with 1 L of seawater at 26 °C, with a continuous light regime and strong aeration. Thus, after 48 hours the larvae of Instar III were obtained**.**

2.4. Preparation of the different concentrations of NP's Fe_3O_4

From the nanoparticles in solid state, 30 mg were weighed and diluted in 3mL of seawater. This matrix solution, at a concentration of 10 000 mg/L, was immediately sonicated for 1 hour. Then, the remaining concentrations were prepared by diluting part of the matrix solution in saline medium. In this way, the concentrations of 10000, 1 000, 500, 250, 100 and 10 mg/L were obtained.

2.5. Toxicity assay

The protocol of Pino and Jorge Lazo was followed (Pino & Jorge, 2010). For this, a microplate of 24 wells was used, where 100 µL of the prepared concentrations were placed, as well as the positive control: $CuSO_4$, and the negative control: Filtered and autoclaved seawater. After 24 hours, was determined the number of dead larvae in each well of the microplate. The larva was considered dead if constant movements of the appendages were not observed for 10 seconds, as well as the speed of the swim, which must have been in all directions.

2.6. Experimental design

The fully randomized experimental design had 8 treatments which were: Group A: filtered and autoclaved seawater, Group B: $CuSO_4$, Group C: 10 mg/L, Group D: 100 mg/L, Group E: 250 mg/L, Group F: 500 mg/L, Group G: 1 000 mg/L and Group H: 10000 mg/L of Fe_3O_4, each with 3 repetitions.

2.7. Statistical analysis

Statistical analysis was determined after 24 hours, where the number of dead larvae and LC_{50} were determined using the statistical program R free version.

2. Results and discussion

From the X-ray diffractogram, it is found that all peaks correspond to the Fe_3O_4 magnetite with cubic structure type inverse spinel without the presence of secondary phases, within the detection limit of the equipment used. Taking as reference the peak corresponding to the planes (511), using the Scherrer´s equation, the average crystallite size was obtained as 10.1nm (Figure 1a). The infrared spectrum corroborated the presence of the magnetite phase due to the presence of the typical band around 551cm^{-1} (Figure 1b) corresponding to the metal-oxygen bond. Also, a weak band at around 3500 cm^{-1} indicates that presence of water adsorbed, which is confirmed in the thermogram (Figure 2a) where a weight loss of about 7% is clearly observed, higher than reported for Fe_3O_4 nanoparticles obtained by co-precipitation (Khashan, et al. 2017), as well as a total weight loss of less than 10%. Figure 2b shows a high degree of agglomeration of the nanoparticles.

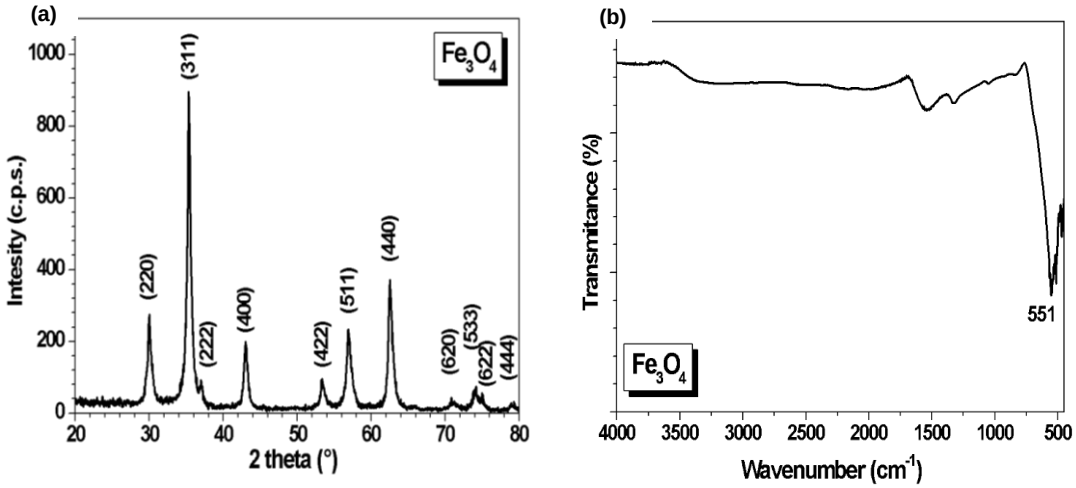

Figure 1: (a) X-ray pattern and (b) FTIR spectrum of Fe_3O_4 nanoparticles

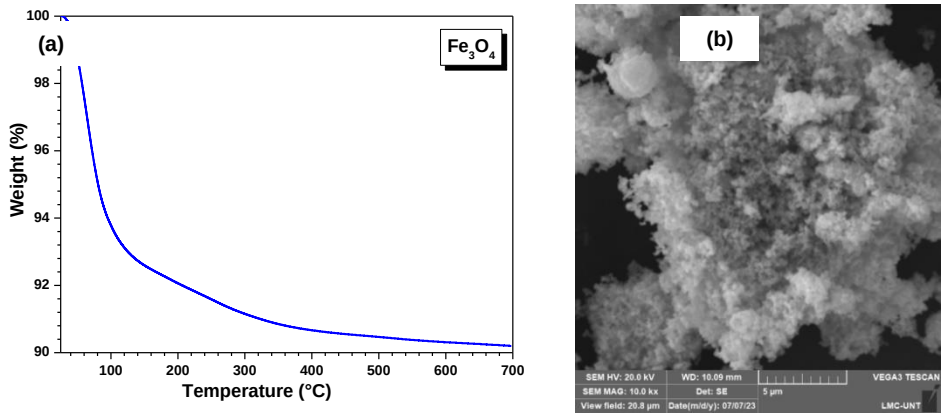

Figure 2: (a) Thermogram and (b) SEM image of Fe_3O_4 nanoparticles

Larvae of stage III of *A. salina* were analyzed after 24 hours of exposure to the 8 treatments (Table 1). In the negative control (Filtered and autoclaved seawater), the mortality of each repetition was lower compared to the positive control ($CuSO_4$), where the mortality was 100%. Likewise, a higher percentage of mortality in the treatment of 10000 mg/L was observed with respect to the treatments of 1000, 500, 250, 100 and 10 mg/L. Table 2 shows the data obtained in the R program, where LC_{50} of the nauplii of the III stage of *A. salina* was calculated. As shown, the value was 5 356.76 mg/L which indicates that, from this concentration, a mortality of 50% will be obtained in the population. In Figure 3, the larvae ingest the nanoparticles (as observed inside the abdominal cavity) leading to a higher concentration, and consequently to a greater accumulation of these, since *A. salina* is a non-selective filterer (Zhu et al., 2017). The accumulated nanoparticles could induce oxidative stress and damage to the tissues of the nauplii (Demarchi et al., 2020).

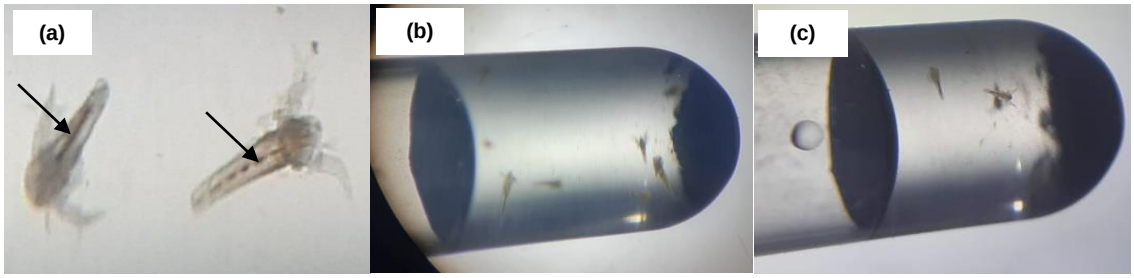

Figure 3: Observation of (a) NPs in the abdominal cavity of the nauplii of Artemia salina, (b) Mortality of nauplii at concentrations 10 ppm and (c) 10 000 ppm of ferrite NPs

Usage of MNPs depends largely on the synthesis method, optimal conditions and election agents to modify their surface. Several chemical and physical methods are employed to synthesize these nanoparticles such as chemical reduction, electrochemical synthesis, laser ablation method, mechanical milling, microwave-assisted synthesis or polyol synthesis. Depending on which one is used for the preparation of nanoparticles, there will be differences on their morphology, stability and physicochemical properties (AlNadhari et al., 2021).
The possible cytotoxic effects of free iron are thought to be due to its ability, like other transition metal ions, to generate reactive oxygen species (ROS) in conjunction with oxygen (Pisanic et al., 2009) which can cause peroxidation of lipid membranes, alter mitochondrial function (Peng et al., 2018), affect other cellular functions such as the ability to proliferate or activate pro-inflammatory processes (Zhang et al., 2017) and damage other organic cellular constituents such as proteins or DNA, which can lead to new toxic effects (García-Torra et al., 2021).

Table 1: Values obtained at 24 hours of exposure to the different concentrations of ferrite nanoparticles, $CuSO_4$ (Positive Control) and filtered and autoclaved seawater (Negative Control) with nauplii of Artemia salina hatched at 48 hours

Treatments	1		2		3		Agreggate	
	Living	Dead	Living	Dead	Living	Dead	Living	Dead
Filtered and autoclaved seawater	8	2	6	4	10	0	8±2	3.20±2.00
$CuSO_4$	0	10	0	10	1	9	0.33±0.58	5.98±0.58
10 mg/L	6	4	6	4	8	2	6.67±1.15	3.56±1.15
100 mg/L	6	4	6	4	7	3	6.33±0.58	3.58±0.58
250 mg/L	6	4	6	4	5	5	5.67±0.58	3.85±0.58
500 mg/L	6	4	6	4	6	4	6.00±0.00	3.60±0.00
1000 mg/L	5	5	4	6	6	4	5.00±1.00	4.20±1.00
10000 mg/L	4	6	4	6	5	5	4.33±0.58	4.38±0.58

The action of ROS is in direct relation to physicochemical characterization such as: the shape, type of synthesis, size, type of material, purity, surface area, electrical charge, structural characteristics, dose, route of administration, concentration in the target organ, metabolism and duration of action which determine the toxicity of nanoparticles (Malhotra et al., 2020).

Wang *et al.* (2017) measured the toxicity of α-Fe_2O_3 in *Artemia* nauplii stating that they were relatively resistant to heavy metals and could tolerate wider ranges. The values found for the LC_{50} of the instar II and III were 177.424 and 235.495 mg/L, respectively; also, Zhu et al. (2017) report an LC_{50} for Fe_3O_4 nanoparticles of 241 mg/L for III instar larvae. Unlike the value obtained in the present research, which was 5 356.76 mg/L.

Table 2: Calculation of the LC_{50}, at 24 hours of exposure to the different concentrations of ferrite nanoparticles, with nauplii of Artemia salina hatched at 48 hours using the statistical program R, free version

LC	Fit	Lwr	Vpr	Df	Chisq
10.5	5356.76	-1176.62	11890.09	1	4.69

3. Conclusion

The nanoparticles of Fe_3O_4, synthesized by the simple microwave assisted hydrothermal method, have a toxic effect in concentrations greater than 5356.76 mg/L, thus causing the death of 50% of the larval population of *Artemia salina*. The results indicate that the method used for the synthesis of Fe_3O_4 produces toxicity at high concentrations with respect to other investigations, these nanoparticles would be used for future applications, becoming an eco-friendly and sustainable alternative.

Acknowledgments

This research was possible by to the financial support of the National University of Trujillo through Canon founds of the PIC 02-2014 Project. The authors gratefully acknowledge to Mg. Gabriela Barraza for the facilities for FTIR measurements, Dr. Hernán Alvarado for SEM .

References

AlNadhari, S., Al-Enazi, N. M., Alshehrei, F., & Ameen, F., 2021, A review on biogenic synthesis of metal nanoparticles using marine algae and its applications. *Environmental Research*, *194*, 110672.

Bayda S, Adeel M, Tuccinardi T, Cordani M, Rizzolio F., 2019, The History of Nanoscience and Nanotechnology: From Chemical-Physical Applications to Nanomedicine. Molecules;25(1):112.

Chen, X., Zhu, X., Li, R., Yao, H., Lu, Z., Yang, X., 2012, Photosynthetic toxicity and oxidative damage induced by nano Fe_3O_4 on *Chlorella vulgaris* in aquatic environment, Open Journal of Ecology 2, 21–28.

Demarchi, C., Mota, L., Niedźwiecka, A., Ślawska-Waniewsk. A., Lewińska, S., Magro, J., Fossá, J., Martello, R., Rodrigues, C., 2020, Nanoecotoxicology study of the response of magnetic O-Carboxymethylchitosan loaded silver nanoparticles on *Artemia salina*, Environ Toxicol and Pharmacol., 74, 103298.

Dukenbayev, K., Korolkov, I., Tishkevich, D., Kozlovskiy, A., Trukhanov, S., Gorin, Y., Shumskaya, E., Kaniukov, E., Vinnik, D., Zdorovets, M., Anisovich, M., Trukhanov, A., Tosi, D., Molardi, C., 2019, Fe_3O_4 Nanoparticles for Complex Targeted Delivery and Boron Neutron Capture Therapy, Nanomaterials Basel, 9, 494.

Ezealigo, U., Ezealig, B., Aisida, S., Ezema, F., 2021, Iron oxide nanoparticles in biological systems: Antibacterial and toxicology perspective, JCIS Open, 4, 100027.

García-Torra, V., Cano, A., Espina, M., Ettcheto, M., Camins, A., Barroso, E., Vazquez-Carrera, M., García, M., Sánchez-López, M., and E. Souto., 2021, State of the Art on Toxicological Mechanisms of Metal and Metal Oxide Nanoparticles and Strategies to Reduce Toxicological Risk, Toxics, 9, 195.

Grebennikova O.V., Sulman A.M., Matveeva V.G., 2022, Use of Magnetic Nanoparticles Fe_3O_4 in the Synthesis of Biocatalysts Based on Horseradish Root Peroxidase, Chemical Engineering Transactions, 94, 673-678

Khashan S.,S. Dagher, N. Tit, A. Alazzam, I. Obaidat, 2017, Novel method for synthesis of Fe_3O_4@TiO_2 core/shell nanoparticles. Surf. Coat. Technol. 322, 92–98.

Lagarto, P., Silva, Y.R., Guerra., Iglesias, B., 2001, Comparative study of the assay of *Artemia salina* L. and the estimate of the medium lethal dose (LD_{50} value) in mice, to determine oral acute toxicity of plant extracts, Phytomedicine, 8, 395–400.

Lee, Y. K., Yeap, S. P., Sum, J. Y., Bakar, A. H. A., Tan, S. L., Jawad, Z. A., 2023, Immobilization of NanoFe_3O_4 onto Fabric Material through *in situ* Co-precipitation as a Flexible Catalyst for Humic Acid Degradation, Chemical Engineering Transactions, 106, 1009–1014.

Lokesh, S., Mohd, A., Rozan, M., Dilla, D., 2020, Magnetite (Fe_3O_4) Nanoparticles in Biomedical Application: From Synthesis to Surface Functionalisation, Magnetochemistry 6, 68.

Malhotra, N., Lee, J., Liman, R., Ruallo, M., Villaflores, O., Ger, T., Hsiao, C., 2020, Potential Toxicity of Iron Oxide Magnetic Nanoparticles: A Review, Molecules, 25, 3159.

Nguyen, M.D., Tran, H.-V., Xu, S., Lee, T.R. 2021, Fe_3O_4 Nanoparticles: Structures, Synthesis, Magnetic Properties, Surface Functionalization, and Emerging Applications. Appl. Sci., 11, 11301.

Özgür, M., Ulu, A., Balcıoglu, S., Özcan, I., Köytepe, S., Ates, B., 2018, The Toxicity Assessment of Iron Oxide (Fe_3O_4) Nanoparticles on Physical and Biochemical Quality of Rainbow Trout Spermatozoon, Toxics, 6, 62.

Pecoraro, R., Scalisi, E.M., Messina, G., Fragalà, G., Ignoto, S., Salvaggio, A., Zimbone, M., Impellizzeri, G., Brundo, M., 2020, *Artemia salina*: A microcrustacean to assess engineered nanoparticles toxicity, Microsc Res Tech., 1–6.

Pelka, M.; Danzl, C.; Distler, W., Petschelt, A., 2000, A new screening test for toxicity testing of dental materials, Journal of Dentistry, 8, 341-345.

Peng, Q., Huo, D., Li, H., Zhang, B., Li, Y., Liang, A., Wang, H., Yu, Q., Li, M., 2018, ROS-independent toxicity of Fe_3O_4 nanoparticles to yeast cells: Involvement of mitochondrial dysfunction, Chem Biol Interact, 287, 20–26.

Pino, O., Jorge, F., 2010, Artemia Bioassay: Useful Working Tool for Ecotoxicologists and Chemists of Natural Products, Rev. Protección Veg., 22, 34–43.

Pisanic, T.R., Jin, S., and Shubayev, V.I., 2009, Iron oxide magnetic nanoparticle nanotoxicity: incidence and mechanisms, In Nanotoxicity, S.C. Sahu, and D.A. Casciano, eds. (John Wiley & Sons, Ltd) 397-425.

Rajabi, S., Ramazani, A., Hamidi, M., Naji, T., 2015, *Artemia salina* as a model organism in toxicity assessment of nanoparticles", DARU Journal of Pharmaceutical, 23:20

Shaterabadi, Z., Nabiyouni, G., Soleymani, M., 2018, Optimal size for heating efficiency of superparamagnetic dextran-coated magnetite nanoparticles for application in magnetic fluid hyperthermia, Physica C: Superconductivity and its applications, 549, 84–87.

Shokry, A., Khalil, M., Ibrahim, H., Soliman, M., Ebrahim, S., 2021, Acute toxicity assessment of polyaniline/Ag nanoparticles/graphene oxide quantum dots on *Cypridopsis vidua* and *Artemia salina*, Scientific Reports, 11, 5336.

Turan, N. B., Erkan, H. S., Engin, G. O., & Bilgili, M. S., 2019. Nanoparticles in the aquatic environment: Usage, properties, transformation and toxicity—A review. *Process safety and environmental protection*, *130*, 238-249

Wang, C., Huali., J., Zhu, L., Zhang, H., Wang, Y., 2017, Toxicity of α-Fe_2O_3 nanoparticles to *Artemia salina* cysts and three stages of larvae, Sci. Total Environ., 598, 847–855.

Zhang, L., Xie, H., Liu, Y., and J. Wang., 2017, Research on Nanotoxicity of an Iron Oxide Nanoparticles and Potential Application, Toxicol Open Access, 3, 1000130.

Zhu, S., Xue, M., Luo, F., Chen, W., Zhu, B., Wang, G., 2017, Developmental toxicity of Fe_3O_4 nanoparticles on cysts and three larval stages of *Artemia salina*, Environmental Pollution, 230, 683–691.