

Toxicity effects of magnesium oxide nanoparticles: a brief report

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ABSTRACT

In this work, magnesium oxide nanoparticles were synthesized by the sol-gel route and their ecotoxicity was tested in worms of the *Eisenia andrei* species. Magnesium oxide nanoparticles were characterized by XRD, surface area via BET, TEM and SEM-FEG/EDS. The lethality test with *Eisenia andrei* earthworm species followed the recommendations of ISO 11268-1 (ISO, 2012) in a completely randomized design with six replicates for each concentration tested (1.06, 2.12, 4.24, 8.48 and 16.96 µg of NPs-MgO/kg of soil), plus the control. The concentrations were mixed to the tropical natural soils, Entisol Typic Quartzipsamments and Oxisol, with no agricultural use history. The morphological and structural analyses of the nanoparticles indicated the formation of magnesium oxide with cubic structure, constituting agglomerates of nanostructures of the order of 20 to 50 nm. The results of toxicity were submitted to analysis of variance (ANOVA One-way), followed by the Dunnett test ($p < 0.05$). Based on standardized toxicological tests it was found that NPs-MgO, at the concentrations tested, did not affect the survival of *Eisenia andrei* species in the natural soils studied.

Keywords: Terrestrial toxicity of nanoparticles, edaphic fauna, Terrestrial toxicity of magnesium oxide nanoparticles.

1. INTRODUCTION

Several metal oxides have been studied and produced at the nanoscale due to their potential for application in several segments [1]. The development of these nanomaterials has expanded the opportunities of many industrial segments, such as the industries dedicated to agriculture, pharmaceutical industries and for the chemical industries.

Combined with these opportunities, important questions about the toxicity of these nanoparticles have been made, mainly by the scientific community, considering that the nanoparticles are transformed into products containing water and can be deposited in the soils when applied by the agricultural industries and when present in effluents from other industries. The presence of nanoparticles in these media can promote negatively effects at the environment and, therefore, deserves the dedication of scientific studies regarding their toxicity [2, 3].

Magnesium oxide nanoparticles (NPs-MgO) are a good example and have been studied because they present possible applications as a catalyst, as antimicrobial agents, in the remediation of toxic water and as an additive for refractory materials [4]. It has also been used in electrochemical sensors and in biocomponents [5], as packaging additives [1] and in the addition of inks [6].

NPs-MgO can be absorbed through the skin, lungs and digestive system and to accumulate in the tissues.

However, many of its effects are still unknown [7], mainly in relation to its toxic effect. Some studies have already investigated the toxic effects of the NPs-MgO using model organisms, such as fishes, for example. The results revealed that magnesium oxide bulk particle was found to be more toxic when compared to NPs-MgO [8].

For terrestrial organisms, even today, the lack of information on the use of some nanoparticles, especially in natural soils, is a major concern. Studies on the toxicity of NPs-MgO should be systematized to better understand the effects when these nanoparticles are incorporated into the soil [9], especially when it is considered that this is a likely destination for numerous nanoparticles after their application.

Toxicological tests have been conducted in the laboratory with soil quality bioindicators to obtain information about the effects of some nanoparticles on soil organisms, such as NPs-ZnO, NPs-Ag, NPs-TiO₂ and others [10-13].

The bioassays carried out in the nanomaterial toxicity on soil studies generally use earthworms of the *Eisenia andrei* and *Eisenia fetida* species, as they are representative of the soil invertebrates [12, 14-15]. These organisms play an important trophic role in soils, with rapid behavioral and physiological reactions [16] and different mechanisms of contamination. The earthworms are organisms of the soil macrofauna belonging to the class *Oligochaeta*, with size between 4 to 200 mm [17, 18].

In Brazil, for example, there is no specific legislation to evaluate the toxic effect of nanoparticles in soils, and their potential contamination is evaluated using the normative n. 84 of IBAMA from October 15, 1996, in its Annex IV [19]. The Resolution of CONAMA n. 460/2013 provides for soil quality guiding criteria on the presence of chemicals, establishing criteria for the environmental management of these substances in the environment and the acute toxicity tests (earthquake) with earthworms as standards for the evaluation of these substances with the use of natural and artificial soils [20].

This work was carried out to contribute with information about the potential effects of NPs-MgO considering the lack of toxicity data on the edaphic fauna, particularly *Eisenia andrei* worms, considered a bioindicator organism of soil quality and model for toxicity studies with nanoparticles [21]. Although Brazilian law requires lethality tests to be used for contamination risk assessments, it is suggested that studies of toxicity with soil organisms be expanded, evaluating other parameters of ecological relevance, such as reproduction and bioaccumulation tests.

In this context, NPs-MgO were synthesized and characterized and the toxicological effects evaluated in the *Eisenia andrei* on soil. Toxicological studies of the NPs-MgO were carried out on earthworms of the *Eisenia andrei* species and on natural tropical soils.

2. MATERIALS AND METHODS

2.1 Synthesis of the NPs-MgO

Magnesium acetate Mg(CH₃COO)₂, tartaric acid (C₄H₆O₆), both from Neon, and cetyl trimethyl ammonium bromide (CTAB), from Dinâmica, were used for the production of magnesium oxide nanoparticles. The synthesis process followed the procedure already studied by MASTULI *et al.* [22].

The amount of 10.0 g of magnesium acetate was dissolved in 100.0 ml of deionized water of a 0.001 M aqueous solution of CTAB and ethanol until the solution became clear. The pH of the solution was adjusted to 5 by slowly adding a 1 M solution of tartaric acid and ethanol. The solution was stirred until a gel was formed. The gel was allowed to stand for 1 day and subsequently filtered and washed with water and ethanol. The gel was subjected to the drying process with controlled temperature of 100.0°C in a vacuum oven for 24 h. The dried material was calcined at 600.0°C with ambient atmosphere for 6 h.

2.2 Morphological and structural characterization of the NPs-MgO

In order to evaluate the physical and chemical properties of the NPs-MgO the techniques of X-Ray Diffraction (XRD), Surface Area Analysis (BET), Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM-FEG) and Energy Dispersive X-ray Spectroscopy (EDS) were used.

The x-ray spectra were obtained on a Shimadzu-type LabX XRD-6000 scanning equipment with a scanning speed of 5 degrees/min, using copper target and 2Theta range between 10.0 and 80.0 degrees.

The tests to determine the surface area by the BET procedure were performed using the Autosorb 1C automatic adsorbometer, Quantachrome, in the fisistion mode. The samples were previously dried at temperatures between 100.0 and 300.0°C under vacuum (10⁻⁷ torr) and then the nitrogen gas adsorption was meas-

ured at 77 K in relative pressure values between 0 and 1.0. The BET surface area was determined using the volume values of adsorbed gas in the range of relative pressures of 0.05 to 0.25.

Electron transmission microscopy analyses were performed using the JEM-1011 TEM equipment with maximum beam acceleration voltage of 100 kV. The samples were initially dispersed in ethanol solution and then sonicated for 10 min before being placed in the grid for analysis.

The SEM-FEG and EDS assays were performed on a scanning electron microscope of the JEOL brand, model JSM-6390 LV with Thermo branded EDS probe, model 6733A-1NES-SN.

2.3 Toxicity characterization of the NPs-MgO

The NPs-MgO toxicity evaluation experiment was conducted in the soil laboratory of the Community University of the Region of Chapecó - Unochapecó. Two soils were used: an Entisol Typic Quartzipsamments, collected in the municipality of Araranguá - SC and an Oxisol collected in the municipality of Chapecó - SC, both with no history of agricultural use and collected in the 0 - 20 cm depth. The evaluation of the physical-chemical parameters of the soils were performed and is presented in Table 1.

Table 1: Physical-chemical parameters of the typical orthodontic oxisol and entisol evaluated at depth 0-20 cm.

Physical-chemical parameters ¹	Oxisol	Entisol Typic Quartzipsamments
MO ² (%)	2.90	0.90
CTC ³	10.47	4.92
pH (H ₂ O)	4.60	5.80
P (mg dm ⁻³)	5.80	6.70
K (mg dm ⁻³)	60.00	34.00
Ca (cmolc dm ⁻³)	1.30	2.00
Mg (cmolc dm ⁻³)	0.30	0.83
Al (cmolc dm ⁻³)	0.70	0.00
H + Al (cmolc dm ⁻³)	8.71	2.00
Cu (mg dm ⁻³)	3.50	1.50
Zn (mg dm ⁻³)	< 0.20	1.00
Fe (mg dm ⁻³)	< 5.00	72.50
Mn (mg dm ⁻³)	12.50	2.10
Clay ³ (%)	63.00	4.00

^[1]Analyzed according to Tedesco *et al.* (1995) and Embrapa (1997). ^[2]OM - Organic Matter. ^[3]CTC - Cation exchange capacity at pH 7.0.

The soils were sieved in 2 mm mesh and defaunated with 2 cycles of freezing and thawing 24/24 h. Before the beginning of the test, the chemical parameters of the natural soils were determined: pH in H₂O (ratio 1: 1 [w: v]), percentage of organic matter, cation exchange capacity (CTC), macro and micronutrients level, according to the methodology described by TEDESCO *et al.* [23].

The experimental design was completely randomized with six replicates for each concentration tested. The treatments were composed of the following concentrations of NPs-MgO applied to natural soils: 1.06; 2.12; 4.24; 8.48 and 16.96 µg NPs-MgO/kg soil plus the control treatment. These concentrations values were defined According to preliminary germination tests that have been carried out with seeds treated in the laboratory with magnesium oxide nanoparticles. For the tests, the pH of the natural soils was corrected to 6.0 ± 0.5 with addition of CaCO₃ and the humidity adjusted to 60% of the maximum water retention capacity (CRA) [24].

The lethality test with the *Eisenia andrei* earthworm species followed the recommendations of ISO 11268-1 (ISO, 2012) [25]. Each replica was composed of a circular plastic container with a mass capacity of 1 kg, which received 500 g of contaminated dry soil and 10 adult cloned earthworms weighing between 250

and 600 mg and aged between two months and one year. The tests were kept in an environment with a temperature of $(20 \pm 2)^\circ\text{C}$ and photoperiod of 12:12 h (light/dark). The moisture of the experiment was adjusted every 7 days with distilled water and the organisms were fed at the beginning of the test and every 14 days with 5 g of equine waste moistened with distilled water. After 28 days of assay assembly, adult individuals were counted for the number of survivors.

Survival data were submitted to analysis of variance (ANOVA One-way), followed by the Dunnett test ($M < \text{control}$, $p < 0.05$). The normality and homogeneity of the data were verified through the Kolmogorov-Smirnov test, when the assumptions were not fulfilled the data were transformed.

3. RESULTS AND DISCUSSIONS

3.1 Morphological and structural characterization of the NPs-MgO

Figure 1 shows images obtained with Transmission Electron Microscopy of NPs-MgO. The images confirm the formation of nanoparticles with dimensions between 20 and 50 nanometers. The formation of NPs-MgO agglomerates with varying shapes with dimensions greater than 100 nm is observed, Figure 1 (a). It is also observed the formation of regular faces in the NPs-MgO, due to its high degree of crystallinity and the great susceptibility of the nanoparticles to form large agglomerates, in some situations coalesce, possibly due to their high polarity and surface area. It is also observed the formation of regular faces in the NPs-MgO due to their high degree of crystallinity and also the great susceptibility of the nanoparticles to form large agglomerates, in some situations coalition, possibly due to their high polarity and surface area, Figure 1(b). Similar results were obtained by GANGULY *et al.* [26] and BALAKRISHNAN *et al.* [27] that studied the catalytic properties of MgO nanoparticles synthesized with dimensions of 10 nm and 26-37 nm, respectively.

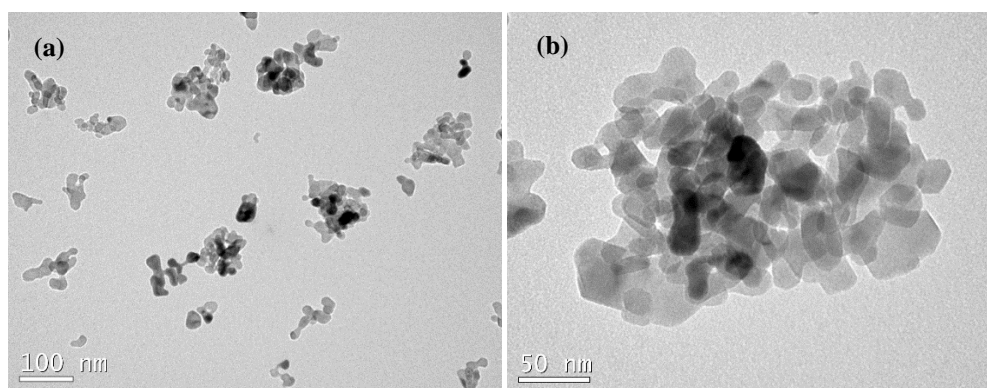


Figure 1: Images obtained by Transmission Electron Microscopy for the magnesium oxide nanoparticles. (a) agglomerates with varying shapes with dimensions greater than 100 nm and (b) NPs-MgO with regular faces.

Figure 2 shows images obtained by Scanning Electron Microscopy (SEM-FEG) of the NPs-MgO structures. The images show clusters with flat and regular faces, typical of structures with a high degree of crystallinity, Figure 2(a). With magnification of 10000 times it is possible to observe the formation of agglomerates of fibrous structures of NPs-MgO with micrometric dimensions, Figure 2(b).

The Energy Dispersive Spectroscopy by x-ray fluorescence (EDS) results show that the structures formed by the nanoparticles are composed of oxygen and magnesium, a strong indicator of the formation of magnesium oxide (NPs-MgO) nanoparticles, Figure 3. Peaks evident in the spectrum at 0.5 keV correspond to the electronic transitions of the oxygen elements and at 1.25 keV the transitions of the elements of magnesium. The peak at 2.1 keV corresponds to the transitions of the carbon element present in the conductive tape used for the fixation and electrical contact of the sample in the stub.

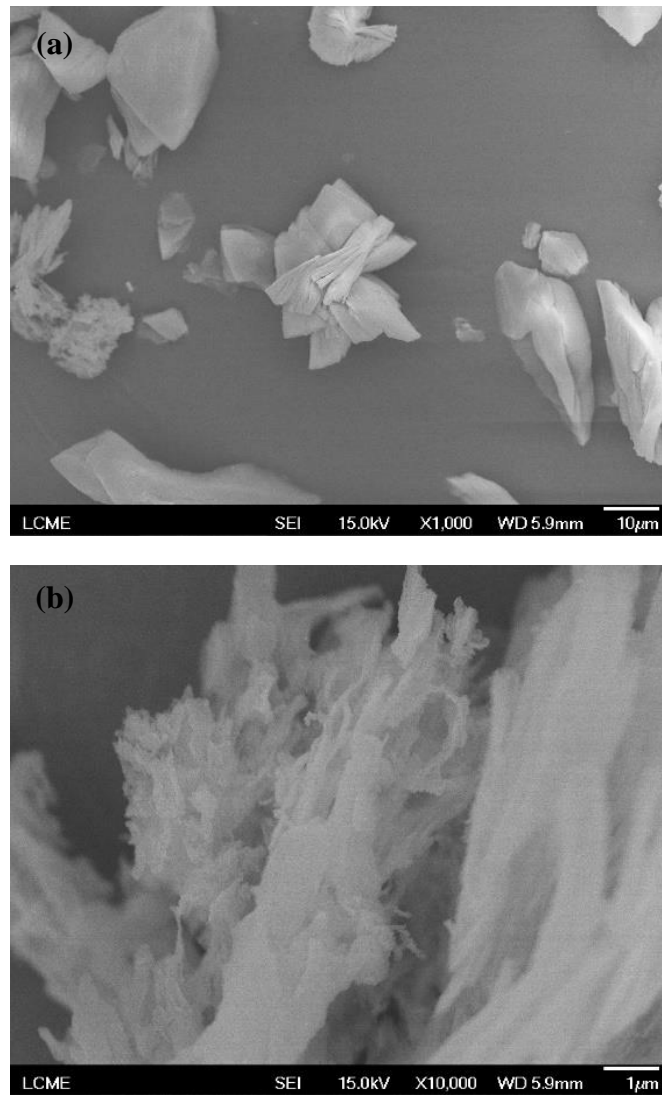


Figure 2: Images obtained by Scanning Electron Microscopy (TEM-FEG) of the structures obtained with NPs-MgO. Magnification of (a) 1000 times and (b) 10000 times.

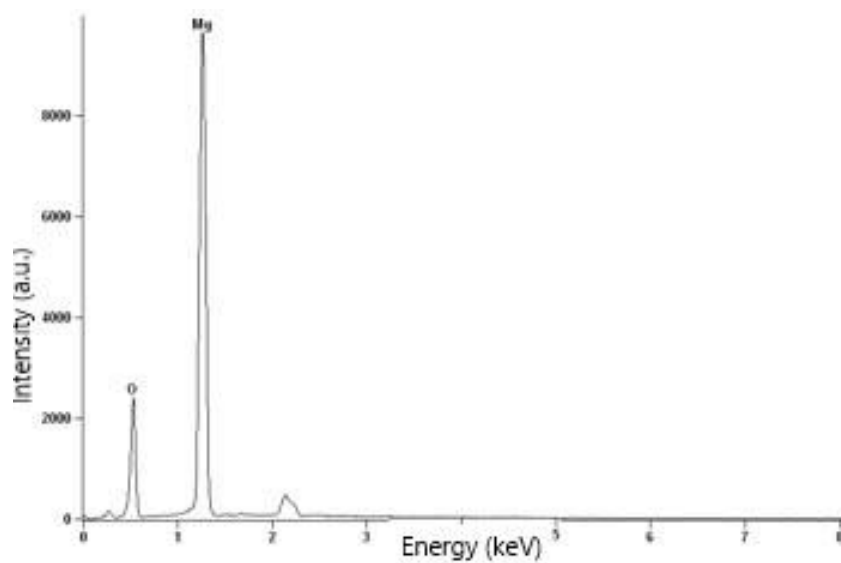


Figure 3: EDS spectrum obtained for structures consisting of magnesium oxide (NPs-MgO) nanoparticles.

Figure 4 shows the x-ray diffraction (XRD) diffractogram obtained for the NPs-MgO. Comparing the diffractogram with the JCPDS No. 87-0653 [27] confirms the formation of magnesium oxide with high crystallinity.

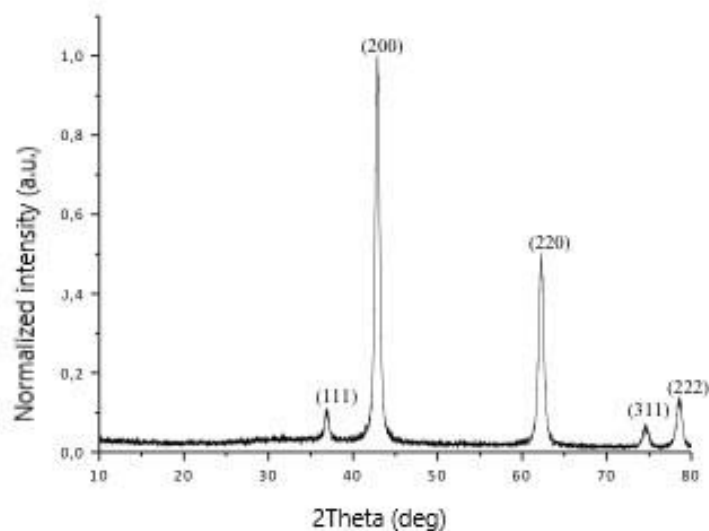


Figure 4: X-ray diffraction (XRD) diffractogram obtained for NPs-MgO. Comparing the diffractogram with the JCPDS No. 87-0653 [27] confirms the formation of NPs-MgO.

The diffraction peaks indexed in (111), (200), (220), (311) and (222) correspond to the cubic structure magnesium oxide and the crystallite size, calculated by the method and Scherrer, presented the mean value of 15.45 nm, indicating that the crystallites correspond to the structures of the NPs-MgO.

Analysis of the surface area results via BET indicated that the surface area of the NPs-MgO structures has an average surface area of 118.7 m²/g, a pore volume of 0.3174 cm³/g and a mean pore diameter of 10.69 nm. Although it has been obtained agglomerates constituted by NPs-MgO the value of the surface area for these nanostructures can be considered elevated. The agglomerates are formed by NPs-MgO, but the surface area is associated with the characteristics of the agglomerates, as well as the volume and dimensions of their pores. Thus, the high surface area is due to the structure of the porous agglomerates, which are formed by NPs-MgO with high crystallinity, according to the results of the XRD.

3.2 Toxicity characterization of the NPs-MgO

The lethality test of *Eisenia andrei* complied with the validation criteria in accordance with ISO 11268-1 (ISO, 2012). The lethality rate did not exceed 10 % of the total control individuals (mean 98 % survival for the Oxisol and 100 % survival for the Entisol) and, for both soils, the coefficient of variation was < 30 % (CV = 1.38 % Oxisol; and 0 % Entisol). The soil worm survival was not affected in any of the concentrations evaluated for the natural soils tested ($p > 0.05$), Figure 5.

The results demonstrate that the use of NPs-MgO, with different concentrations tested in soils, does not cause lethality in the worms of the *Eisenia andrei* species. There are no other studies evaluating the toxicological potential of the NPs-MgO in earthworms and other soil fauna organisms, mainly in natural soils, but it is possible to compare these results with results obtained for other types of nanoparticles.

In addition, other nanoparticles dispersed in natural soils, such as zinc oxide (ZnO), studies have shown that the number of living specimens of *Eisenia andrei* and *Eisenia fetida* were not affected either [28-29,12]. However, with silver nanoparticles (NPs-Ag), the work of Kwak and An (2016), it was observed the mortality of *Eisenia Andrei* juvenile worms when in soil contaminated with concentrations of 500 µg of NPs-Ag/kg of soil, but considered too much concentration in practical applications. These studies also evaluated the transfer in the food chain of NPs-Ag in *Lobelia sokamensi*, fed with dead worms contaminated at concentrations of 50, 200 and 500 µg of NPs-Ag/kg of soil. With the lowest concentration the negative effects were insignificant, but with the highest concentration the locomotion was inhibited.

Although no terrestrial ecotoxicology research data, aquatic toxicity studies with the NPs-MgO in *Danio rerio zebrafish* [7] and in the freshwater snail of the species *Radix luteola* with the concentration of NPs-

MgO of 50, 100, 200 and 400 mg/l. Ali *et al.* (2016) demonstrated that these organisms are sensitive to the presence of these nanoparticles at the concentrations tested: 0, 10, 20, 40, 80, 120 and 200 $\mu\text{g/ml}$ [30].

In the concentrations tested in this work it is verified that the magnesium oxide nanoparticles are not harmful to earthworms of the *Eisenia andrei* species. These results are strong indicators that NPs-MgO is not toxic to the terrestrial environment, but it is suggested that similar studies continue for higher soil concentrations.

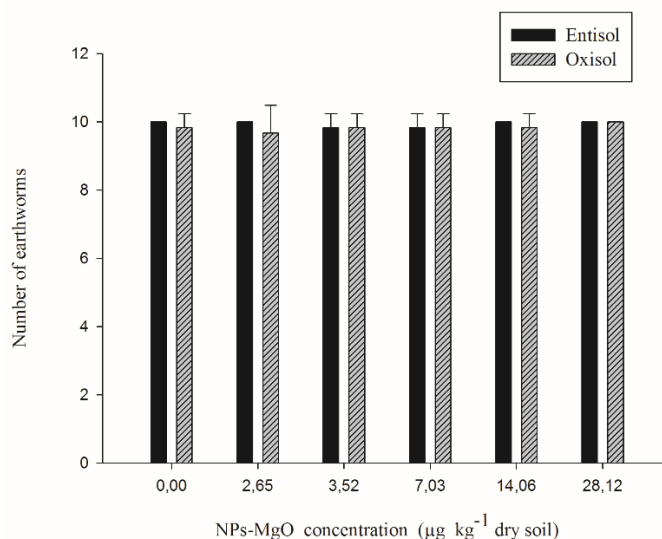


Figure 5: Average live individuals of *Eisenia andrei* earthworms in soils contaminated with increasing concentrations of Magnesium Oxide nanoparticles (NPs-MgO). (⌈) Standard deviation (n = 6).

4. CONCLUSIONS

In this work, NPs-MgO were obtained with dimensions in the order of 20-50 nm and with high crystallinity. The agglomerates were formed with a surface area of 118 m^2/g and with a pore volume of 0.3174 cm^3/g and with dimensions in the order of 10.69 nm.

The tested concentrations of NPs-MgO added to the oxisol and entisol had no effect on the survival of the *Eisenia andrei* earthworms. However, it is recommended to carry out further studies evaluating other ecological studies in tropical soils, as well as the use of other fauna organisms in different concentrations, for a better understanding of the response of the activity of this nanoparticle in the environment.

5. ACKNOWLEDGE

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6. BIBLIOGRAPHY

- [1] ZHAO, J., MU, F., QIN, L., *et al.* Synthesis and characterization of MgO / ZnO composite nanosheets for biosensor. *Materials Chemistry and Physics*, v. 166, p. 176–181, 2015.
- [2] MAYNARD, A. D., AITKEN, R. J., BUTZ, T., *et al.* Safe handling of nanotechnology. *Nature*, n. 444, p. 267-269, 2006.
- [3] REN, C., HU, X. and ZHOU, Q. Influence of environmental factors on nanotoxicity and knowledge gaps thereof. *NanoImpact*, n. 2, p. 82-92, 2016.
- [4] PATEL, M. K., *et al.* Biocompatible nanostructured magnesium oxide-chitosan platform for genosensing application. *Biosensors and Bioelectronics*, v. 45, p. 181–188, 2013.
- [5] PATEL, M. K., *et al.* Nanostructured magnesium oxide biosensing platform for cholera detection. *Applied Physics Letters*, v. 102, n. 14, p. 144106, 2013.
- [6] LI, M., *et al.* Electrochemical biosensor based on one-dimensional MgO nanostructures for the simultaneous determination of ascorbic acid, dopamine, and uric acid. *Sensors & Actuators: B. Chemical*, v. 204, p. 629–636, 2014.
- [7] GHOBADIAN, M., NABIUNI, M., PARIVAR, K., *et al.* Toxic effects of magnesium oxide nanoparticles on early developmental and larval stages of zebrafish (*Danio rerio*). *Ecotoxicology and Environmental Safety*, n. 122, p. 260-267, 2015.
- [8] THOMAS, J., VIJAYAKUMAR, S., THANIGAIVEL, S., *et al.* Toxicity of magnesium oxide nano parti-

- cles in two fresh water fishes tilapia (*Oreochromis mossambicus*) and zebra fish (*Danio rerio*). *International Journal of Pharmacy and Pharmaceutical Science*, n. 6, v.2, p. 487-490, 2014.
- [9] BOUR, A.; MOUCHET, F.; SILVESTRE, J., *et al.* Environmentally relevant approaches to assess nanoparticles ecotoxicity: A review. *Journal of Hazardous Materials*, n. 283, p. 764-777, 2015.
- [10] CAÑAS, J.E., QI, B., LI, S., *et al.* Acute and reproductive toxicity of nano-sized metal oxides (ZnO and TiO₂) to earthworms (*Eisenia fetida*). *Journal Environmental Monitoring*, n. 13, p. 3351-3357, 2011.
- [11] HEGGELUND, L.R., DIEZ-ORTIZ, M., LOFTS, S., *et al.* Soil pH effects on the comparative toxicity of dissolved zinc, non-nano and nano ZnO to the earthworm *Eisenia fetida*. *Nanotoxicology*, n. 8, v. 5, p. 559-572, 2013.
- [12] ROMERO-FREIRE, A., LOFTS, S., PEINADO, F. J. M., *et al.* Effects of aging and soil properties on zinc oxide nanoparticle availability and its ecotoxicological effects to the earthworm *Eisenia andrei*. *Environmental Toxicology and Chemistry*, v. 36, n. 1, p. 137-146, 2017.
- [13] DAS, P., BARUA, S., SARKAR, S., *et al.* Mechanism of toxicity and transformation of silver nanoparticles: inclusive assessment in earthworm-microbe-soil-plant system. *Geoderma*, n. 314, p.73-84, 2018.
- [14] CANESI, L. and PROCHÁZKOVÁ, P. The invertebrate immune system as a model for investigating the environmental impact of nanoparticles. In: Boraschi, D., Duschl, A. (eds). *Nanoparticles and the immune system, safety and effects*. Oxford: Academic Press. p. 91-112, 2014.
- [15] KWAK, J. I., AN, Y. J. Ecotoxicological Effects of Nanomaterials on Earthworms: A Review. *Human and Ecological Risk Assessment*, v. 21, n. 6, p. 1566-1575, 2015.
- [16] BROWN, G. G., DOMÍNGUEZ, J. Uso das minhocas como bioindicadoras ambientais: princípios e práticas - o 3º encontro latino americano de ecologia e taxonomia de oligoquetas (elaetao). *Acta Zoológica Mexicana*, n. 2, p. 1-18, 2010.
- [17] RUPPERT, E. E., FOX, R. S., BARNES, R.D. *Zoologia dos Invertebrados: uma abordagem funcional-evolutiva*. 7ª ed. São Paulo: Roca. p. 1145, 2005.
- [18] MADIGAN, M. T.; MARTINKO, J. M.; DUNLAP, P. V.; *et al.* *Microbiologia de Brock*. 12.ª ed. Porto Alegre: Artmed. p. 1160, 2010.
- [19] BRASIL. Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – IBAMA. Portaria Normativa IBAMA nº 84, de 15 de outubro de 1996.
- [20] BRASIL. Conselho Nacional do Meio Ambiente – CONAMA. Resolução CONAMA n. 460 de 30 de dezembro de 2013.
- [21] YADAV, S. Potentiality of Earthworms as Bioremediating Agent for Nanoparticles. *Nanoscience and Plant–Soil Systems. SOILBIOL*, v. 48. p. 259-278, 2017.
- [22] MASTULI, M.S., ANSARI, N.S., NAWAWI, M.A., *et al.* Effects of cationic surfactant in sol-gel synthesis of nano sized magnesium oxide. *Procedia*, n. 3, p. 93-98, 2012.
- [23] TEDESCO, M. J., GIANELLO, C., BISSANI, C. A., *et al.* *Análise de solo, plantas e outros materiais*. 2ª ed. Porto Alegre: Universidade Federal do Rio Grande do Sul. p. 174, 1995.
- [24] ISO - International Organization for Standardization. Soil quality – Determination of dry matter and water content on a mass basis - Gravimetric method. ISO 11465. Geneva, Switzerland., 1993.
- [25] ISO - International Organization for Standardization. Soil quality - Effect of pollutants on earthworms - Part 1: Determination of acute toxicity to *Eisenia fetida*/*Eisenia andrei*. ISO 11268-1:2012. Geneva, Switzerland., 2012.
- [26] GANGULY, A., TRINH, P., RAMANUJACHARY, K.V., *et al.* Reverse micellar based synthesis of ultrafine MgO nanoparticles (8–10nm): Characterization and catalytic properties. *Journal of Colloid and Interface Science*, v. 353, n. 1, p. 137-142, 2011.
- [27] BALAKRISHNAN, G., VELAVAN, R., BATOO, K.M. *et al.* Microstructure, optical and photocatalytic properties of MgO nanoparticles. *Results in Physics*. n. 16, p.103013, 2020.
- [28] GARCÍA-GÓMEZ, C., BABIN, M., OBRADOR, A., *et al.* Toxicity of ZnO Nanoparticles, ZnO Bulk, and ZnCl₂ on Earthworms in a Spiked Natural Soil and Toxicological Effects of Leachates on Aquatic Organisms. *Archives of Environmental Contamination and Toxicology*, n. 67 p. 465-473, 2014.
- [29] KWAK, J. I. and NA, Y. J.T. Trophic transfer of silver nanoparticles from earthworms disrupts the locomotion of springtails (*Collembola*). *Journal of Hazardous Materials*, n. 315, p. 110-116, 2016.
- [30] ALI, D., ALI, H. and ALARIFI, S. Eco-toxic efficacy of nano-sized magnesium oxide in freshwater



snail *Radix leuteola* L. Fresenius *Environmental Bulletin*, n. 25, v. 4, p. 1234-1242, 2016.

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