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New synthesized nanoparticles (Fe_2O_3 1A, ZnO – G18 and ZnFeO) – influence on *Daphnia magna* (*Straus, 1820*)

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Abstract

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Daphnia magna (*Straus, 1820*), a small species of water fleas with a short life cycle and high sensitivity to water pollution, is model used as a keystone species in eco-toxicological experiments in freshwater ecosystems to assess the biological effect of new synthesized nanoparticles on the environment.

Preliminary studies were conducted with *Daphnia magna* (Crustacea, Cladocera) and three types of nanoparticles: Fe_2O_3-1A, ZnO-G18 and ZnFeO. The preliminary results showed high toxicity and mortality in daphnia treated with Fe_2O_3 1A and ZnO-G18 and low toxicity and mortality of daphnia treated with ZnFeO nanoparticles. The ferrite nanoparticles (Fe_2O_3-1A) obtained by physical means were toxic to *D. magna* at concentrations of 0.5 mg L^{-1}. For ZnO-G18 a concentration of 0. 5 mg L^{-1} killed 90% of daphnia at 48 h and a concentration of 0.05 mg L^{-1} it killed 70% of daphnia. The treatment with ZnFeO nanoparticles demonstrated a weaker effect on daphnia. Survival was 100% at a concentration of 0.02 mg L^{-1} and 90% at a concentration of 0.2 mg L^{-1}.

Keywords: ecotoxicology; nanoparticles; water crustaceans

Introduction

Nanotechnology is used increasingly in various industries and moving to a large-scale production. This leads inevitably to the entry of particles into the aquatic environment. Reliable procedures need to be developed for risk assessment and for dealing effectively with potential eco-toxicological hazards. Many scientists are working in this field, conducting research on the influence of a number of types of nanoparticles on prokaryotic and eukaryotic organisms.

Many studies have shown that a toxic effect is observed after exposure to individual nanoparticles, but the effects of exposure to combined nanoparticles are unknown or poorly studied. It is important to consider that in real life we are exposed simultaneously to mixtures of nanoparticles, usually at low levels, but with possible additive, antagonistic, potentiating or synergistic actions of the resulting mixture.

Among the most widely used nanomaterials worldwide is zinc oxide (ZnO) (Klingshirn, 2007), its specific physicochemical properties determine its wide application in medicine and cosmetics, as well as in many other industries. Bondarenko et al., (2013) investigated the toxicity of ZnO, but the debate about the contribution to the overall toxicity of metal-based nanoparticles and their dissolved ions is still open. According to some authors, the toxicity of ZnO nanoparticles in aqueous solution is due to the dissolved zinc ions (Adam et al., 2015, Heinlaan et al., 2008; Bacchetta et al., 2016), while according to others, zinc ions only partially

contribute to the toxicity (Bai et al., 2010; Santo et al., 2014). Hao et al. (2013) considered zinc ions to be a minor contributor to overall toxicity compared to that due to nanoparticles. According to Poynton et al. (2011) the toxicity of ZnO is due to the combined action of ions and nanoparticles. All these opinions show that the problem is not fully understood.

Elder et al. (2009) consider that of all manufactured nanoparticles, those of metals and metal oxides are the most dangerous due to their ability to disrupt epithelial barriers. Griffitt et al. (2009) argue that these nanoparticles can become toxic after being absorbed by organisms. According to Blinova et al. (2010), nanoparticles become toxic by dissolution, with subsequent release of ions, and Poynton et al. (2011) suggested a combined effect of nanoparticles and released ions, both contributing to toxicity. Limbach et al.(2007) considered that the toxicity of nanoparticles is due to possible impurities. Claims about the role of nanoparticle size in determining their toxicity are controversial. It has long been believed that biological activity increases with decreasing particle size, but some studies have disproved this claim (Gratton et al., 2008.). Extensive studies by Santo et al. (2014) of ZnO nanoparticles on *D. magna* show that the size of the nanoparticles is a very important parameter for cytotoxicity, but not the only one. The smaller the particle, the higher the toxic effect, but dissolved ions were not the single reason for the effects as their concentration measured by Inductively Coupled Plasma Spectroscopy was below the observed effect level. According to Santos-Rasera et al (2022), toxicity depends on the size of the nanoparticles and the presence or absence of surfactants. Dissolved zinc contributes to increased toxicity, but powdered forms tend to accumulate in greater amounts than dissolved zinc. The authors did not observe any depuration of ZnO nanoparticles in the 24 h after the exposure, which they explained with strong adsorption of nano ZnO on the inner surface of the digestive tract or agglomeration of the particles blocking the digestive tube.

The toxic effects of ZnO nanoparticles on aquatic invertebrates including crustaceans can be attributed to the release of free metal ions and formation of free oxygen species from the nanopartcles (Senthamarai and Malaikozhundan, 2022). Oxidative stress serves as a central mechanism of toxicity leading to ZnO-NP-induced damage.

According to Bordin et al. (2024) nanoparticles are a valuable resource ready for further exploration as nanotechnology advances, making research essential to explore experimental scenarios that closely mimic real-world environmental conditions. Therefore, it is necessary to gain knowledge about the environmental transformations that nanoparticles undergo, to identify the most sensitive species and to determine ecologically relevant concentrations. All of this, in conjunction with the assessment of potential environmental risks associated with the release of nanoparticles into aquatic systems, represents a challenge in the field of eco-toxicological research (Bordin et al., 2024).

Fe_2O_3 nanoparticles are widely used in biomedical applications, such as cell labeling, drugs, tissue repair, in vitro bioseparation, hyperthermia, etc., due to their low toxicity (Pankhurst et al.,2003; Laurent et al., 2008). Gökçe et al, (2020) tested $CuFe_2O_4$, $CoFe_2O_4$, and $NiFe_2O_4$ nanoparticles for short-term bioassays, (96 h) on water flea in the laboratory (16:8 photoperiod). The most toxic to Daphnia were copper-ferrite nanoparticles at a concentration of 1.455 mg L^{-1}. The less toxic were cobalt-ferrite nanoparticles at a concentration of 39.834 mg L^{-1}. Alhadlaq et al, (2015) prove the toxic effect of zinc ferrite nanoparticles on 3 different human cell lines in concentrations of 10-40 mg L^{-1}. Foca-nici et al. (2010) prove the toxicity of zinc ferrite nanoparticles on Sunflower root cells by determining the cellular division rate and the percentage of chromosomal aberration. There are some investigations of Iron oxide nanoparticles that affect their morphology and function (Gökçe et al, (2020); Magro et al, (2018), but only one on Magnetic-zeolite composites (Zarria-Romero & Ramos-Guivar, 2024). Brunner et al, (2006) reported toxicity of iron oxide nanoparticles on human mesothelioma and a rodent fibroblast cell line, but Gupta&Gupta (2005) reported the cytotoxicity suppression of surface modified magnetic nanoparticles.

Our investigation is focused on the toxicity effects of three kinds of physically obtained metal oxide nanoparticles: ZnO, Fe_2O_3 and ZnFeO using an acute test on *Daphnia magna*.

Materials and Methods

The experiments were carried out with a laboratory culture of *Daphnia magna*. An acute *Daphnia* toxicity test was performed, and the exposure experiments were conducted in accordance with the OECD protocol (Tests No. 202). Two concentrations of the substances with 3 repetitions each and the corresponding controls were used. They were monitored for 24 and 48 hours at 20.5±0.5 °C with 16 hours of light. Each vessel contained 10 *D. magna* neonates in 100 mL of standing water, without feeding for 48 h. Oxygen, pH and mortality in the controls were checked to ensure the validity of the tests. The immobilization of the daphnids was monitored in each dish, at 24 and 48 hours. Animals unable to swim within 15 seconds of gently stirring the test vessel were considered immobile, while those, whose heartbeats had stopped were considered dead. New synthesized nanoparticles were kindly provided by Dr. Peter Georgiev, Faculty of Physics.

The nanoparticles were between 0–50 nm in size considered by us to have a similar effect according to Santo et al. (2014). The concentrations used in the preliminary tests were:

- 0. 2 mg L^{-1} and 0.02 mg L^{-1} for ZnFeO;
- 0.5 mg L^{-1} and 0.05 mg L^{-1} for Fe_2O_3-1A
- 0.5 mg L^{-1} and 0.05 mg L^{-1} for ZnO-G18.

The newly synthesized nanoparticles were provided in different amounts, which determined the tested concentrations. Graphs and statistics were processed using Excel 2013 functions.

Results

1. In our studies, we treated *D. magna* with Fe_2O_3-1A – nanoparticles. At the higher concentration of 0.5 mg L^{-1} we found 60% survival of daphnia at 24 hours, and at 48 hours 100% mortality was observed. The lethal time (LT50) for 50% of the population at given concentration was assessed at 28h. At the lower concentration used – 0.05 mg L^{-1}, up to 48 hours, 60% survival of daphnia was demonstrated (Fig. 1). Fig. 2 shows a picture of a dead *Daphnia* encrusted with

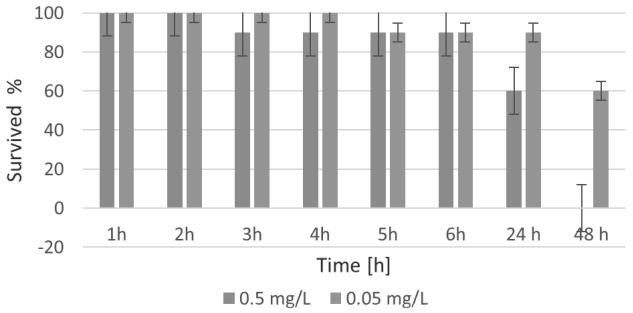

Fig. 1. Survival of *D. magna*, treated with Fe_2O_3-1A nanoparticles in acute 48h test

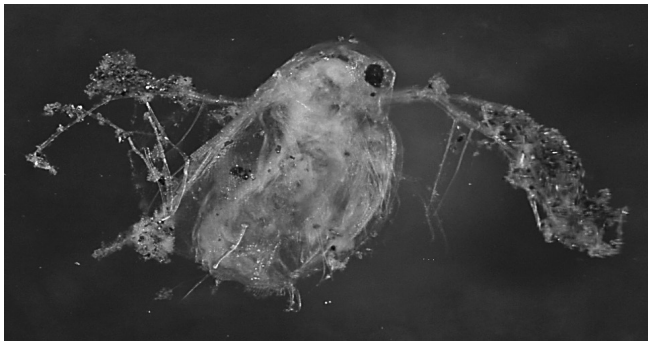

Fig. 2. Dead *D. magna*, treated with Fe_2O_3-1A nanoparticles
(*Photo*: E. Nenova)

magnetic nanoparticles from our experiments, in agreement with Baumann et al. (2014), and Hu et al. (2012). Accumulated ferrite particles can be seen on the surface of the body and the antennae, as well as in the intestine.

2. The studies conducted with the second type of nanoparticles – ZnO-G18 showed that a concentration of 0.5 mg L^{-1} was also highly toxic to daphnia – only 10% survived at 48 hours. At the lower concentration – 0.05 mg L^{-1} LC_{50} was established at 24 hours for *Daphnia magna*. The 48-hour long test showed that 30% of daphnia survived the concentration of 0.005 mg/ L^{-1} and at 48 hours 70% of daphnia were dead (Fig.3). The LC_{50} value at the 48 hours of ZnO-G18 nanoparticles was found to be 0.03 mg L^{-1}.

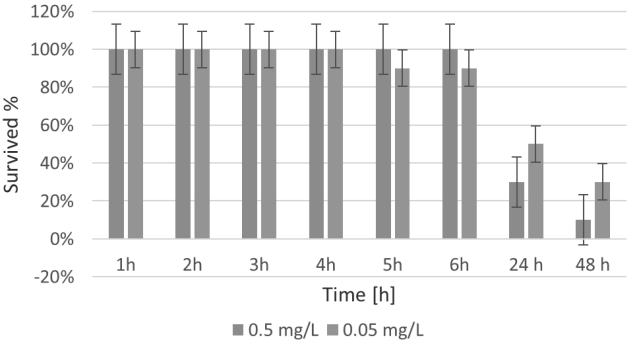

Fig. 3. Survival of *D. magna*, treated with ZnO-G18 nanoparticles

3. Other studies were also conducted with a ZnFeO nanoparticles. The results showed 100% daphnia survival at concentrations of 0.02 mg L^{-1} and 90% daphnia survival at 0.2 mg L^{-1} (Fig.4).

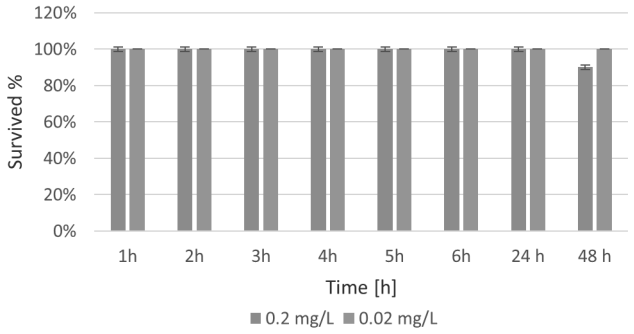

Fig. 4. Survival of *D. magna*, treated with ZnFeO nanoparticles

Discussion

Our studies show that at a concentration of 0.02 mg L^{-1} ZnFeO nanoparticles daphnia remained 100% alive at 24th

hour. At a concentration of 0.2 mg L^{-1} of the same nanoparticles after 48 hours there were 90% survivors. i.e. the LC 50 is not reached within the concentrations used. This means that research should continue with higher doses of the substance.

Our assumptions about the lower toxic action of the ZnFeO nanoparticles on daphnia are due to the lack of dissolution of ions in the water environment or obtaining an alloy during processing at high temperatures. Further research on these nanoparticles at lower concentrations and their effect on *D.magna* is needed. For Fe_2O_3-1A, the results showed that a concentration of 0.5 mg L^{-1} caused 100% mortality of daphnia at 48 hours, and at 0.05 mg L^{-1} the mortality was 40%. This could be due to the interaction of magnetic nanoparticles with the tissue of daphnia. For ZnO-G18 at 48 h a concentration of 0. 5 mg L^{-1} killed 90% of daphnia and at a concentration of 0.05 mg L^{-1} it killed 70% of daphnia. Iron and zinc nanoparticles alone were more toxic than alloyed ZnFeO, and magnetic nanoparticles were more toxic than ZnO. In subsequent studies, concentrations of eluted ions should be measured. Our results are very close to those reported by Bondarenko et al. (2013), who established that ZnO nanoparticles LC50 was 2.3 mg L^{-1} for crustaceans. Blinova et al. (2010) established L(E)C_{50} values for both bulk and nano ZnO (1.1–16 mg Zn/l). The concentrations of nanoparticles reported by Blinova are higher because natural water containing organic materials was used. Our tests were conducted in drinking tap water and it is possible that this is the reason for the higher toxicity of nanoparticles. There is clear negative effect of metal ions released from nanoparticles and from the nanoparticles themselves on aquatic organisms. Research should be centered on organic materials that could minimize their toxic effect. In the next experiments we will use more concentrations, more repetitions and broader concentration range.

Conclusion

The ferrite nanoparticles (Fe_2O_3-1A) obtained by physical means were toxic to *D. magna* and at concentrations of 0.5 mg L^{-1} they resulted in 100% mortality of *Daphnia*.

The LC_{50} value at the 48 hours of ZnO-G18 nanoparticles will be determined with broader concentration range from 0.020 mg L^{-1} till 20.0 mg L^{-1}.

The treatment with ZnFeO – nanoparticles demonstrated weaker effect on daphnia, survival was 100% at a concentration of 0.02 mg L^{-1} and 90% at a concentration of 0.2 mg L^{-1}.

It is important to consider that in real life we are exposed simultaneously to mixtures of different nanoparticles, usually for long periods of time, and at low levels, with possible additive, antagonistic or synergistic effects of the resulting mixture. In this study, we hypothesize that the coexistence of the two nanoparticles (Fe_2O_3-1A and ZnO-G18) at different concentrations in the environment may affect the life and behavior of *D. magna.* In the next study both nanoparticles will be tested together to check their synergistic or antagonistic effects on *Daphnia magna*.

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