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# Investigation of the Effects of Zn and ZnO Nanoparticles on the Survival Rates of *Artemia salina*

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**Abstract**
Intensive and possible misuse of nanoscale materials is one of the biggest threats to the environment and all living things worldwide. For this reason, various control mechanisms should be investigated in use of NP. In biotreatment or toxicity studies, the most important factor affecting the researches is the selection of the organisms to be used. The aim of this study is to investigate the ecological imbalance potentials of Zinc (Zn) and Zinc Oxide (ZnO) Nanoparticles (NPs) in creatures such as fish and crustaceans, which are at the top of the food chain, as a result of the alimentary (trophic) transfer potential by using *Artemia salina* which is a primary consumer.
In this study, Zn NPs (40-60 nm) and Zn NPs (80-100 nm) and ZnO NPs (10-30 nm) were administered to *A. salina* individuals (105000 individuals in total) in 7 groups (Control, 0.2, 1.0, 5.0, 10.0, 25.0 and 50.0 ppm) with 3 repetitions . Measurements were performed at 24$^{th}$, 48$^{th}$ and 72$^{nd}$ hours and elimination values were examined at +24 hours. The survival rates of organisms after exposure to NPs were determined. According to the results of phase contrast microscopy, it was determined that the experimental organism absorbed the NPs in the environment. The survival rate of *A. salina* individuals exposed to Zn (40-60 nm), Zn (80-100 nm) and ZnO (10-30 nm) NPs was found to be between 59.67% and 13.00%; and the elimination groups were between 22.00% and 6.33%.

**Keywords:** Zn; ZnO; Nanoparticle; *Artemia salina*; Toxicity; Survival Rates

## INTRODUCTION

The nanoparticles (NPs), have crucial physical and chemical properties, have a big potentials in the aquatic and terrestrial environments to effect the life in this media (Vance et. al., 2015; Martin et. al., 2017). The researches related to environmental / ecological risk assessments of these substances have gained considerable importance. So, there is some researches on the toxic effects of the NPs in this environments and they are continuously increasing (Garner et al. 2015; Martin et. al., 2017). The production of different metal or metal oxide NPs and their contamination to ecosystem is rapidly increasing in the world (Gottschalk et al., 2009; Morales-Diaz et al. 2017). Thereby, the living / bio-organisms in these ecosystems are affected (Baysal et al., 2019). In the ecological risk assessments, the environment should be examined as much as the living organisms should be examined in detail.

To obtain the meaningful results in the toxicological researches, not only the appropriate test type but also the appropriate test organism should be selected (Rand, 1995). The selection of the organisms to be used in the bioassay or toxicity researches or experiments is one of the most important factors affecting the researches.

*A. salina* is a marine zooplanktonic organism and it is used in bioassay researches due to its ease of culture, availability, low cost and adaptation to adverse conditions in the marine environment (Madhav et al., 2017). *A. salina* is additionally used as a potential / important food source in many aquaculture systems (Léger et al., 1986). However, it is recommended by the OECD (Organization for Economic Cooperation and Development) for the especially juvenile fish feeding (OECD-210, 1992). NPs accumulated in the primary consumer organisms such as *A. salina* may easily be transferred through the alimentary (trophic) transfer potential in fish or crustaceans, which are an upper food chain.

The aim of this study is to investigate the ecological imbalance potentials of Zinc (Zn) and Zinc Oxide (ZnO) Nanoparticles (NP) on the survival rates of *Artemia salina* which is primary consumer.

## MATERIAL and METHOD

**Bioassay Organisms**

In this research, A. salina which is the primary consumer zooplankton species living sea water environments was used. Its eggs were obtained from a commercial company.

**Chemical and Nanomaterials**

Zn (40-60 nm and 80-100 nm) and ZnO (10-30 nm) were obtained from commercial companies selling SkySpring products in Turkey. All chemicals from the analytical reagent class were used without any decontamination or purification.

**Toxicity Bioassay Setup**

The toxicity immobilization tests of this study are carried out according to the guidelines of the Organization for Economic Co-operation and Development [OECD chapter 202 (zooplankton)] (OECD 2004) for each NP we acquired commercially. The control group was also prepared in the absence of targeted NPs under the same experimental conditions. A typical exposure pattern for zooplankton is shown below (Table 1). Procedures for preparation of seawater and test organisms were described in previous studies (Ates et al. 2013a; 2013b).

**Table 1.** Bioassay design of organisms exposed to metal and metal oxide nanoparticles

| Groups | Control | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 |
|---|---|---|---|---|---|---|---|
| NP* (ppm) | 0 | 0.2 | 1 | 5 | 10 | 25 | 50 |
| *A. salina* ** | 15.000 | 15.000 | 15.000 | 15.000 | 15.000 | 15.000 | 15.000 |
| Repetition | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

**A separate experimental setup was established fororganism. It shows the mg / L ratio of the metal and metal oxide nanoparticle concentration.*

*** It was initiated by placing 30-35 A. salina organisms in an average of 1 mL in each group in a plastic container with a total internal volume of 500 mL at the beginning of the experiment.*

Measurements were performed at 24th, 48th and 72nd hours and elimination values were examined at +24 hours. The survival rates of organisms after exposure to NPs were determined.

**Phase Contrast Microscopic Analysis**

Phase contrast images were taken using a phase-contrast microscope equipped with a digital camera (Micromaster, Model 12-575-252, Fisher Scientific) to obtain gray-scale images in order to clarify metal accumulation in the zooplankton group examined at the end of the exposure process. Images were obtained from living organisms in a special slide using Micron Imaging software.

**Zooplankton Count**

Briefly, the counting strategy is as the following. 100 mL solution containing incubated zooplankton was placed in a clean glass beaker. 1.0 mL of this stock was transferred into 100 mL through continuous mixing and diluted with water to 100 mL (100-fold dilution). Then, 0.1 mL of this diluted solution was removed via a straw while stirring and counted under the light. *D. magna* samples were placed in a petri dish and counted (Zhu et al., 2010). The number of zooplanktons was visually determined in this volume. Once the required number of zooplankton for the experiment was set, the organisms were exposed to NP at the concentration ratios indicated in Table 1. above.

**Statistical Analysis**

All experiments were independently repeated thrice and the data were recorded as means with standard deviation. Tukey's multiple comparisons and one-way analysis of variables (ANOVA SPSS / 24.0 software) were used to find significant differences between the groups. P-value was taken as <0.05 in all data analyses.

## RESULTS and DISCUSION

Firstly, the characterization analyses were carried out for every NPS in the another research. And in characterization analysis; it was found that the majority of NPs form a round or spherical structure in the TEM results; that metal and metal oxide NPs have the feature of growing in aqueous medium in the DSL results; that NPs show a positive (+) surface load in the Zeta potential results; that the crystallinity of ZnO (10-30 nm) and Zn (40-60 nm and 80-100 nm) are proved by the sharp appearances of all peaks and their calculated average crystallite dimensions are 21.33 nm, 52.54 nm and 89.50 nm in the XRD results; that the average size of the particles of NPs are within the range of nanometres having crystalline nature in the SEM results.

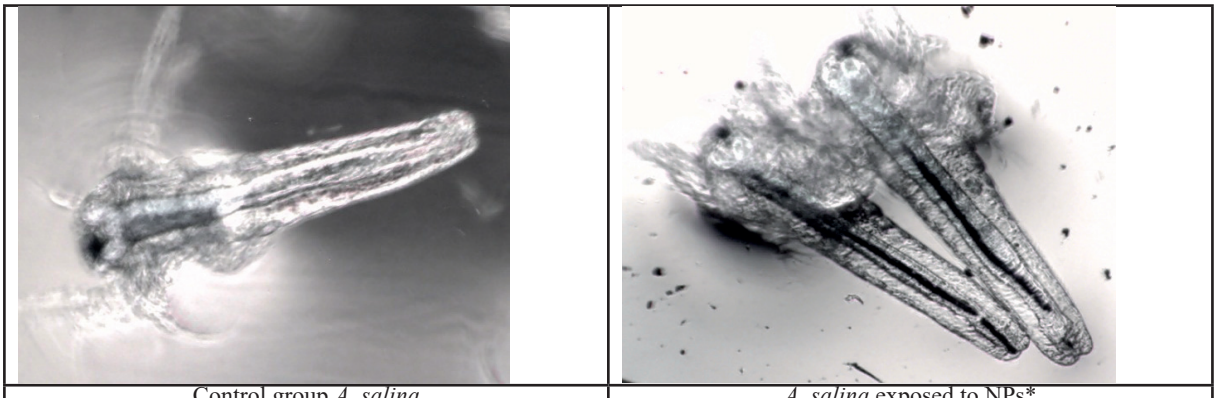
**Toxicity Bioassay Results**

In the toxicity bioassay analysis, results of phase contrast microscop and survival rates of *A. salina* were investigated.

**Results of Phase Contrast Microscopic Analysis**

Phase contrast images of zooplanktons control group and exposed to NPs (50 µg / mL, 72 h) are given in Figure 1. When the results of phase contrast microscopic are examined; *A. salina* organisms seem to have received the NPs in the medium. However, according to accumulation results of ICP-MS analysis, the accumulation rates of the NPs in the organism body increase parallelly with the increasing of NPs concentrations.

It is clear that *A. salina* organisms, which are zooplanktonic, receive NPs from environment in which live and accumulate the nanoparticles in their tissues. Because generally these kinds of organisms are fed by filtration, they take all the particles in the micro or macro size present in the aqueous medium to the body as food (Gophen and Geller, 1984). When NPs accumulation increases in the tissues of organism being in the first or second steps of the food chain, this situation may be dangerous for the upper consumers feeding these kinds of organisms.



Control group *A. salina* | *A. salina* exposed to NPs*

**Only images of one NP group is shown because of the similarity between the images of the zooplanktons exposed to all NPs*

**Figure 1.** Zooplanktons exposed to NPs (50 µg / mL, 72 h) and the control group

**Results of Survival Rates of A. salina**

When the survival rates of *A. salina* individuals exposed to Zn (40-60 nm) NPs were evaluated, it was found that the highest survival rate (59.67%) was at the beginning time of the control group and the lowest survival rate (13.00%) was at the 48$^{th}$ h of 50 ppm concentration (Figure 2.). When the elimination groups were also evaluated, the highest survival rate (22.00%) was found in the 0.2 ppm concentration and the lowest survival rate (12.66%) was determined in the 50 ppm concentration. The highest survival rates were obtained at the beginning times of all trial groups. These results showed a decrease in parallel with the increase in the concentrations. In each experimental groups in general, while the values obtained at 24 hours after the initial values were relatively high, with a fluctuation with increasing exposure times, it has been determined that this was a decrease trend. However, the values in the 5 and 25 ppm concentrations were significantly increased in the 48$^{th}$ h compared to the 24$^{th}$ h ($P<0,05$). In the elimination values, it was observed that there was a fluctuation in the survival rates of the experimental groups, but a significant increase was obtained in the concentration of 0.2 ppm. On the other hands, this fluctuation in the other experimental groups was determined as a decrease in parallel with the increase in the concentrations.

When the survival rates of *A. salina* individuals exposed to Zn (80-100 nm) NPs were evaluated, it was found that the highest survival rate (31.33) was at the beginning time of the concentration of 50 ppm and the lowest survival rate (15.00%) was at the 48$^{th}$ h of 0.2 ppm concentration (Figure 2.). When the elimination groups were also evaluated, the highest survival rate (16.22%) was found in the 25 ppm concentration and the lowest survival rate (9.11%) was determined in the 10 ppm concentration. When the survival rates were examined in all groups, the highest survival rates were obtained from the 50 ppm concentration. Although evaluated in general terms, the survival rates obtained increased in parallel with the increase in concentrations. In each group, there was an irregular fluctuation in the survival rates obtained according to exposure periods. In the elimination values, it was determined that 25 and 50 ppm concentrations showed similar survival rates with the control group and the highest rates were also obtained from these groups ($P<0,05$).. In the values of other groups, it was determined that there was a fluctuation in the direction of decrease-increase.

When the survival rates of *A. salina* individuals exposed to ZnO (10-30 nm) NPs were evaluated, it was found that the highest survival rate (41.00%) was at the beginning time of the 50 ppm concentration and the lowest survival rate (15.15%) was at the 72$^{nd}$ h of 5 ppm concentration (Figure 2.). When the elimination groups were also evaluated, the highest survival rate (16.00%) was found in the control group and the lowest survival rate (6.33%) was determined in the 5 ppm concentration. When the survival rates were evaluated in the all experimental groups, the highest survival rates were generally obtained in the control group except the beginning value of 50 ppm concentration. With the applied concentration, a significant decrease was observed in the survival rates of the groups and the survival rates increase in the groups parallelly increasing of the concentrations. In each concentration group, it was observed that there was roughly a decrease in parallel with the increase in exposure time from the initial survival rates except 1 ppm. At 1 ppm concentration, the survival rate obtained at 48$^{th}$ h was higher than 24$^{th}$ h ($P<0,05$). In the elimination values, it was observed that there was a fluctuation in survival rates of the groups; a decresase direction from the control group to the concentration up to 5 ppm and a relative increase in the higher concentrations.

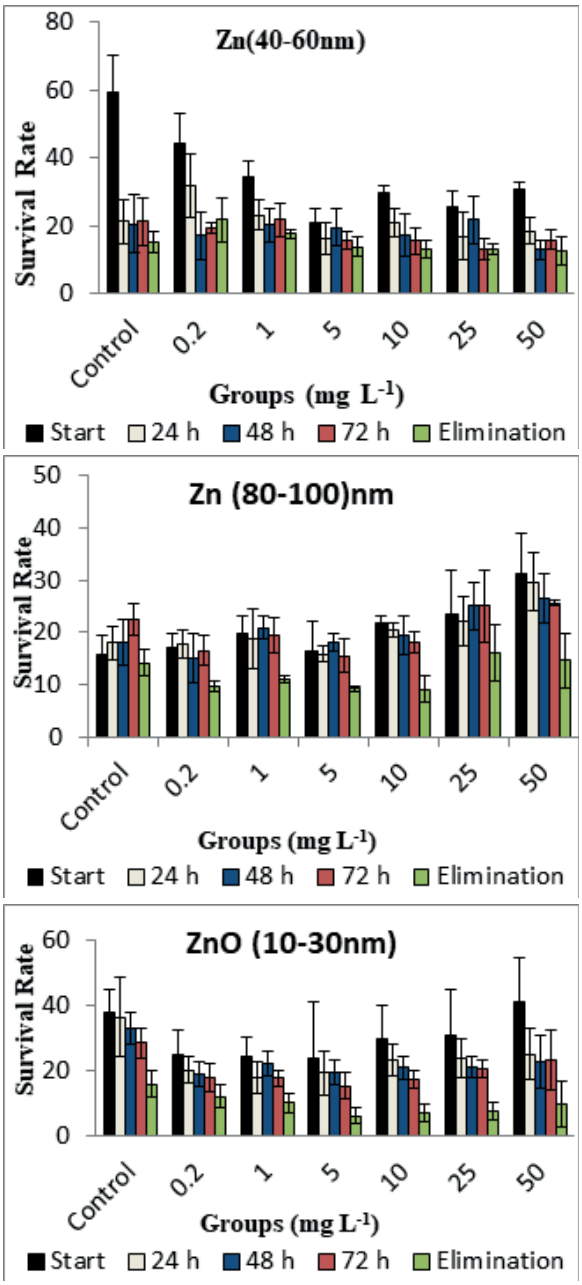



**Figure 2.** The Survival rates of *A. salina* exposed to Zn (40-60 nm), Zn (80-100 nm) and ZnO (10-30 nm) NPs

NPs have intensive production because of their extremely utility. So, they have negative effect potential in the organisms in the ecosystems as directly or indirectly. However, metal and metal oxides NPs have different toxic effects on the organisims. Since the problems issues with the potential impact of nanomaterials on human health and the environment have just emerged, our knowledge of the environmental and human health effects of nanotechnology is still very poor (Arslan et al., 2011; Primera-Pedrozo et al., 2012). With the increasing usage of these materials, NPs have become a major problem for the environment. For this reason, various control mechanisms should be developed for usage of NPs.

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