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Acute toxicity of TiO₂, CuO and ZnO nanoparticles in brine shrimp, Artemia franciscana

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Abstract

The brine shrimp, Artemia spp., is widely used in ecotoxicological research as a biological index. In the present study, aquatic stability and acute toxic effects of TiO₂, CuO and ZnO nanoparticles (NPs) on Artemia franciscana were investigated. Acute exposure was conducted in sea water with different concentrations of selected nanoparticles at 24h, 48h, 72h and 96h. The mortality rate of A. francisca increased significantly with increasing concentrations and duration of exposure of all NPs. The toxicity pattern of metal oxides to A. francisscana was as follows: CuO>TiO₂>ZnO. Our results point to the fact that both TiO₂ and ZnO NPs exhibited moderate toxicity to Artemia larvae in 24h as compared with CuO, regardless of their size and concentrations.

Keywords: Acute toxicity, *Artemia franciscana*, Titanium dioxide, Copper oxide, Zinc oxide.

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Introduction

In recent years, the brine shrimp play an role aquaculture; important in approximately 10 million pounds of brine shrimp eggs are harvested each winter and sold as food for tropical fish. (Brix et al., 2003a). Artemia is a suitable organism for bioassay and toxicity studies. This species has a key role in the aquatic food chain (Ozkan et al., 2015). Nanoparticles are known for their small size (1–100 nm) and specific physical and chemical properties. These specific properties result in different characteristics (e.g. transparency, UV reflection, physical strength, etc.), which make them very useful material to use in a number of different products. As a result, many industries in the world have increased the production of nanoparticle-containing products specially in this decade (Li et al., 2008).

 NPs^1 have TiO₂ been worldwide in diverse areas including sunblock lotions, cosmetics, paints, food additives, medicines, construction materials environmental and decontamination of air, soil and water (Ozkan et al., 2015). ZnO NPs are widely used as additives in food, sunscreens and cosmetic products and in the manufacture of textiles, paint pigments, semiconductors, catalysts, polishers and water disinfection and chemotherapy (Ates et al., 2013b). CuO NPs have widespread uses in gas sensors, batteries, plastics and metallic coatings, etc. Increasing production, use

Nanoparticles can enter in the aquatic ecosystems in a highly dynamic way, which depends on the aquatic environmental conditions. Nanoparticles have been shown to both (Kasemets et al., Mortimer et al., 2010) and aggregate (Jo et al., 2012) forms. Different environmental factors (such dissolved oxygen, ionic strength, pH and natural organic matter, etc.) have been shown to influence the aggregation and dissolution of nanoparticle (Adam et al., 2015). Natural organic matter can reduce aggregation of some nanoparticles by adsorption on to their surface (Zhao et al., 2013).

The main aim of this study were (1) to determine acute toxicities of TiO₂, CuO and ZnO nanoparticles in *A. franciscana*, (2) to characterize NPs over time and (3) to define the effects of NPs based on their morphological characteristics.

Materials and methods

Nanoparticle suspensions preparation Zinc oxide (ZnO), dioxide titanium (TiO₂) and copper oxide (CuO) nanoparticles were purchased from U.S Research Nanomaterial's Inc., Houston, TX, USA. The physical properties of

and application of these nanoparticles will increase the potential of NPs discharge in aquatic ecosystems because of their small size and specific properties which may cause adverse effects to different organisms (Adam *et al.*, 2015).

^{1.} Nanoparticles

these nanoparticles are presented in Table 1.

The stock solutions of the NPs were prepared by suspending appropriate the NP powders amounts of deionized water at a stock concentration of 20% (w/v)separately. To homogenize the suspension, the contents were vortexes for 20 seconds at 2000 rpm and then exposed to ultrasound for 10 minutes for maximum dispersion. Appropriate volumes of the stock suspension were immediately transferred into the exposure containers which contained Artemia in seawater (Ates et al., 2013b).

Test organisms

Brine shrimp cysts were purchased from Bandar Imam (Iranian Fishery Research Organization, Bandar Emam) and were certified to be *A. franciscana*. Cysts were stored in the dark until used for testing. Cysts were hatched in seawater (salinity 28 g L⁻¹) at 27°C under vigorous aeration (Brix *et al.*, 2003b).

Water quality parameters

Water quality parameters (pH, dissolved oxygen, EC, TDS and salinity) were measured in each test chamber and water temperature was recorded in the physical system at the initiation of the test and every day thereafter. Water temperature was measured using a digital thermometer. Test solution pH was measured using a ColeParmer Model 5398-00 digital pH meter. Dissolved oxygen, salinity, EC

and TDS was measured using a sensefon 378 digital model.

Exposure setup

Acute toxicity was conducted according to the Organization for Economic Cooperation and Development testing guidelines (OECD 202) with organisms in each test (OECD, 2004). A total of 60 Artemia larvae were exposed to different concentrations of the NPs (In each beaker we used 20 Artemia larvae) for 24h, 48h, 72h and 96h. For ZnO NPs concentrations were 100, 120, 140, 160, 180 and 200 mgL⁻¹, for CuO the concentrations were 1, 3, 5, 7, 9 and 10 mgL⁻¹ and for TiO₂ NPs the test concentrations were 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mgL⁻¹. A control group was also set up without the test compound, using only the filtered sea water. Exposures were carried out in triplicate groups in 1.0 L beakers in 500 mL of filtered seawater.

Slight aeration was provided through the bottom of the beaker to prevent settling of NPs from the suspension. For all test groups the light regime of 16:8 h light: dark was maintained. No food was provided during the course of the exposure. Details of the experimental conditions are summarized in Table 2.

Artemia larvae were exposed to each NP solution for 24, 48, 72 and 96 h. At the end of each time healthy Artemia larvae were counted and then we could calculate the dead larvae. This step was repeated for 24, 48, 72 and 96 h exposure tests.

Table 1: Size distribution and other characteristics of nanoparticles.

Nano Particles	APS	SSA	Purity	Color
CuO	40 nm	$\sim 20 \text{ m}^2 \text{ g}^{-1}$	99%	Black
ZnO	10-30 nm	$20-60\text{m}^2\text{ g}^{-1}$	+99%	Milky white
TiO_2	20 nm	$10 - 45 \text{ m}^2 - \text{g}^{-1}$	+99%	White

SSA: Specific Surface Area, APS: Average Particle Size.

Table 2: Physico-chemical properties of the test.

Characteristics/Parameter	Range	Mean
Room temperature	29 – 31.2 (°C)	30.0
Water temperature	28 – 28.2 (°C)	28.1
Dissolved oxygen	$6.7 - 6.9 \text{ (mg L}^{-1}\text{)}$	6.8
TDS	$24.8 - 25.8 \text{ (g L}^{-1}\text{)}$	25.3
Salinity	24.8 – 25.5 (%)	25.1
EC	38800 - 39200 (mho cm ⁻¹)	39000
pН	7.8 - 8.1	7.9

After the acute toxicity morphological changes and mortality rate of the Artemia exposed to NPs were recorded under a phase contrast microscope (Nikon Eclipse 80i). Every exposed group was compared to the control group and the potential anomalies were recorded. Completely motionless Artemia were counted as dead, and the percentages of mortality the compared to control were calculated.

The LC₅₀ value and the related 95% confidence limits were calculated using the Probit Method (Zhu *et al.*, 2008; Strigul *et al.*, 2009) Significant differences between controls and treated samples were determined using the Bonferroni nonparametric post hoc tests, where p<0.05 was considered to be significantly different.

Results

No *Artemia* larvae died during toxicity tests in the control group. During all acute toxicity tests, the measured pH of

the test dispersions remained within the range of 7.8 and 8.1 and did not vary by more than 1.0 unit in any given test. The water temperature ranged from 28.0°C to 28.2°C during all acute tests. The oxygen content of the test dispersions in all acute toxicity tests was between 6.7– 6.9 mgL⁻¹. Thus, all tests met validity criteria set by the OECD guidelines. The physicochemical characteristics of the test water are presented in Table 2.

The toxicity of NPs to *Artemia* larvae increased with increasing NP concentration and duration of exposure. The concentrations that killed 50 % (LC₅₀) of *Artemia* larvae varied with the NPs as shown in Table 3. The results of the 69 acute toxicity tests (66 cases and 3 controls) performed with TiO₂, CuO and ZnO, expressed as LC₁₀, LC₅₀ and LC₉₀ values, are summarized in Tables 4 and 5. Hence, the tests met the biological validity criterion as required in the OECD guideline 202.

Table 3: Mortality (nercent) (of the 4	rtomia	franciscana	exposed to NPs
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Nano	Concentration				
particles	(mgL ⁻¹)	24 h	48 h	72 h	96 h
	Control	0.00	0.00	0.00	0.00
	100	0.00	0.00	11.67	15.00
	120	0.00	3.33	18.33	23.33
ZnO	140	1.67	16.67	25.00	31.67
	160	5.00	20.00	33.33	43.33
	180	8.33	23.33	43.33	53.33
	200	11.67	26.67	50.00	63.33
	Control	0.00	0.00	0.00	0.00
	1	3.33	8.33	13.33	20.00
	3	8.33	16.67	26.67	33.33
CuO	5	16.67	23.33	38.33	46.67
Cuo	7	33.33	26.67	48.33	60.00
	9	41.67	53.33	61.67	70.00
	10	50.00	63.33	71.67	83.33
	Control	0.00	0.00	0.00	0.00
	10	1.67	8.33	16.67	28.33
	20	6.67	11.67	23.33	36.67
	30	10.00	18.33	28.33	46.67
	40	13.33	21.67	33.33	51.67
TiO_2	50	16.67	30.00	40.00	56.67
	60	23.33	38.33	46.67	61.67
	70	28.33	41.67	55.00	68.33
	80	38.33	50.00	61.67	80.00
	90	45.00	53.33	68.33	85.00
	100	46.67	60.00	71.67	91.67

Table 4: Lethal concentrations (LC₅₀) of NPs on Artemia franciscana.

Nano particles	Concentrations (mgL ⁻¹)			
	24 h	48 h	72 h	96 h
CuO	11.42	8.51	6.21	4.32
ZnO	293.10	247.13	201.21	173.20
TiO_2	115.55	86.11	57.31	30.54

Table 5: The toxicity (LC $_{10},$ LC $_{50},$ LC $_{90}$ (mgL $^{-1})$ at 24h, 48h, 72h, 96h) of NPs on $\it Artemia\ ranciscana$.

Nano particles	Time	LC_{10}	LC_{50}	LC_{90}
	24h	2.83	11.42	46.15
CuO	48h	1.69	8.51	42.87
00	72h	1.01	6.21	37.99
	96h	0.72	4.32	25.85
	24h	189.19	293.10	454.07
ZnO	48h	139.38	247.13	438.16
Ziio	72h	96.86	201.21	417.97
	96h	91.05	173.20	329.48
	24h	30.27	115.55	448.28
T:O	48h	16.92	86.11	438.20
TiO_2	72h	9.33	57.31	352.00
	96h	5.48	30.54	170.21

The controls showed no mortality in 24h, 48h, 72h and 96 h. As pointed out above, the exposures were conducted in absence of feeding. experimental mortalities for the controls clearly demonstrate that deprivation from food did not induce any lethal effects on Artemia larvae even up to 96 h. In the treatments, the mortalities increasing increased with concentration and time (p < 0.05). In 24 h, the average mortality ranged from 0% (100, 120 mgL⁻¹) to 11.6% (200 mgL⁻¹) for ZnO NPs, from 33% (1 mgL^{-1}) to 50% (10 mgL^{-1}) for CuO NPs. and from 1.6% (10 mgL⁻¹) to 46.6% (100 mgL⁻¹) for TiO₂ NPs.

The lethal effects recorded for 96 h exposure were more prominent. The average mortality was about 15% in 120 mg mL⁻¹ suspensions of the ZnO NPs and increased to 63.3% in 200 mgL⁻¹ suspensions (Fig. 1). For TiO₂ NPs the average mortality was about 28.3% in 10 mg mL⁻¹ suspensions and increased to 91.6% in 100 mgL⁻¹ suspensions (Fig. 2). Likewise, the CuO NPs was 20% in 1mgL⁻¹ suspensions and increased to 83% in 10 mgL⁻¹ suspensions (Fig. 3). Our images confirmed the accumulation of the NPs inside the gut and other parts of Artemia. (Figs. 4 and 5).

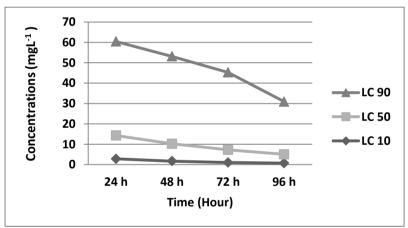


Figure 1: Acute toxicity of ZnO NPs Concentration (mgL⁻¹) in Artemia franciscana.

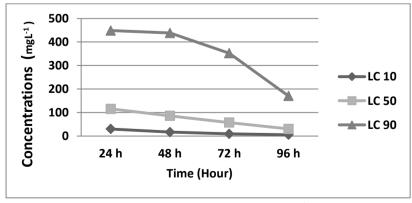


Figure 2: Acute toxicity of TiO₂ NPs Concentration (mgL⁻¹) in Artemia franciscana.

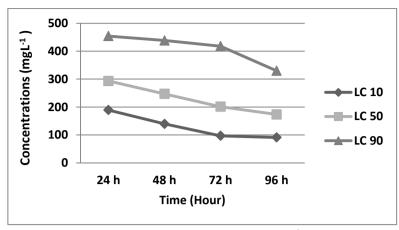


Figure 3: Acute toxicity of CuO NPs Concentration (mgL⁻¹) in Artemia franciscana.

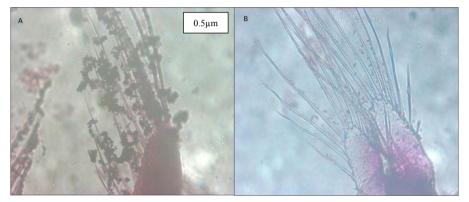


Figure 4: (A) Tail section of the brain shrimp *Artemia fransicana* in control group; (B) NPs stick to tail of brain shrimp *Artemia fransicana* (Scale 1mm: 0.5 µm).

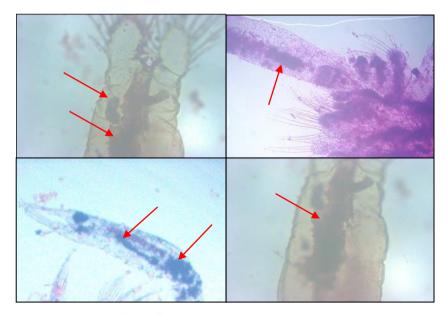


Figure 5: NPs in Artemia gut mark with arrows.

Discussion

Our results point to the fact that both TiO₂ and ZnO NPs exhibited moderate toxicity to Artemia larvae in 24h as compared with CuO regardless of their size and concentration.

The toxicity pattern of metal oxides to *Artemia francisscana* was (Cu>TiO₂>ZnO). In the present study CuO NPs was found to have a 24h LC₅₀ of 11.42 mgL⁻¹, which was over 26 times higher than that of ZnO NPs and 10 times higher than TiO₂ NPs.

The publications on the marine crustaceans are mostly related to the genus Artemia, and more specifically to the anostracan Artemia salina (Ates et al., 2013a) and A. franciscana (Minetto et al., 2014). However, the overall results are quite representative, because thev come from immobilization bioassays, biomarker measures and bioaccumulation evaluation, performed with both adults and nauplii and diversifying the exposure scenario. In both the publications, the authors could verify the toxic effects of the nTiO2 in the overall concentration range of 0.5- 100 mg L^{-1} (Ates et al., 2013a; Minetto et al., 2014).

Ozkan *et al.* (2015) assessed the toxicity effects of Tio₂ and AgTiO₂ NPs in *A. salina* and studied morphological changes in this species. In this study, aquatic stability and toxic effects of TiO₂ and AgTiO₂ nanoparticles (NPs) were investigated in *A. salina* nauplii. *n*AgTiO₂ was found to be more toxic to nauplii compared to *n*TiO₂. The mortality rate in nauplii increased

significantly with increasing concentrations and duration of exposure. TiO₂ elimination ranged between 27.8 % and 96.5 % at 50 and 1 mgL⁻¹ TiO₂ exposure to nauplii, respectively (Ozkan *et al.*, 2015).

al.(2013b).Ates studied comparative evaluation of the impacts of Zn and ZnO nanoparticles on brine shrimp (A. salina) larvae and the suspensions of the NPs did not exhibit any significant acute toxicity within 24 h, mortalities increased remarkably in 96 h and escalated with increasing concentration of NP suspension to 42% for Zn NPs (40–60 nm) (LC_{50 ~ 100} mgL⁻ 1) and to about 34% for ZnO NPs (10– $(LC_{50}>100$ mgL^{-1}). 30nm) suspensions of Zn NPs were more toxic to Artemia than those of ZnO NPs under comparable regimes (Ates et al., 2013b).

The LC₅₀ 96 h Of TiO₂ in Ozkan et al.(2015) research was 18.77 mgL⁻¹ (Ozkan et al., 2015). Our results show higher LC₅₀ 96 h (30.54 mgL⁻¹), but lower LC₅₀ 96 h concentration compared with the results of Ates et al. (2013a) who reported LC₅₀ 96 h of above 100 mgL⁻¹. The difference in toxicity thresholds may be related to differences in particle size, preparation methods. or test designs inconsistent test conditions such as pH, photoperiod and dissolved oxygen (DO). Artemia were unable to eliminate the ingested particles, which was thought to be due to the formation of massive particles in the guts and other parts of body, and had adverse effects

on *Artemia* such as on movement, swimming speed, feeding and etc. Based on the experimental results in this paper, it can be concluded that:

- The NPs used in this research (CuO, ZnO and TiO₂) may have acute dosedependent eco-toxicological effects on *A. franciscana*.
- NPs with different compositions exhibited different toxicities in *A*. *franciscana*. The CuO was observed to be most toxic among the tested compounds, while ZnO had least toxicity.
- The studied NPs can be ranked in the following order according to the *A. franciscana* acute toxicity: CuO>TiO₂>ZnO.
- Toxicity of NPs may be the result of the effects from NPs itself, dissolution products, and NPs agglomerates that develop during the experiment.
- The results of this study indicated that the potential eco-toxicity and environmental health effects of NPs should be given due attention.

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References

Adam, N., Vakurov, A., Knapen, D. and Blust, R., 2015. The chronic toxicity of CuO nanoparticles and

copper salt to *Daphnia magna*. *Journal of Hazardous Materials*, 283, 416-422.

- Ates, M., Daniels, J., Arslan, Z. and Farah, I.O., 2013a. Effects of aqueous suspensions of titanium dioxide nanoparticles on *Artemia salina*: assessment of nanoparticle aggregation, accumulation, and toxicity. *Environmental Monitoring and Assessment*, 185(4), 3339-3348.
- Ates, M., Daniels, J., Arslan, Z., Farah, I.O. and Rivera, H.F., 2013b. Comparative evaluation of impact of Zn and ZnO nanoparticles on brine shrimp (*Artemia salina*) larvae: effects of particle size and solubility on toxicity. *Environmental Science: Processes and Impacts*, 15(1), 225-233.
- Brix, K.V., Cardwell, R.D. and Adams, W.J., 2003a. Chronic toxicity of arsenic to the Great Salt Lake brine shrimp, Artemia franciscana. Ecotoxicology and Environmental Safety, 54(2), 169-175.
- Brix, K.V., Cardwell, R.D. and Adams, W.J., 2003b. Chronic toxicity of arsenic to the Great Salt Lake brine shrimp, Artemia franciscana. Ecotoxicology and Environmental Safety, 54(2), 169-175.
- Jo, H.J., Choi, J.W., Lee, S.H. and Hong, S.W., 2012. Acute toxicity of Ag and CuO nanoparticle suspensions against *Daphnia magna*: The importance of their dissolved fraction varying with preparation

- methods. *Journal of Hazardous Materials*, 227–228, 301-308.
- **Kasemets, K., Ivask, A., Dubourguier, H.C. and Kahru, A., 2009.** Toxicity of nanoparticles of ZnO, CuO and TiO₂ to yeast Saccharomyces cerevisiae. *Toxicology in Vitro*, 23(6), 1116-1122.
- Li, S.Q., Zhu, R.R., Zhu, H., Xue, M., Sun, X.Y., Yao, S.D. and Wang, S.L., 2008. Nanotoxicity of TiO₂ nanoparticles to erythrocyte in vitro. *Food and Chemical Toxicology*, 46, 3626-3631.
- Minetto, D., Libralato, G. and Volpi Ghirardini, A., 2014. Ecotoxicity of engineered TiO₂ nanoparticles to saltwater organisms: An overview. *Environment International*, 66, 18-27.
- Mortimer, M., Kasemets, K. and Kahru, A., 2010. Toxicity of ZnO and CuO nanoparticles to ciliated protozoa *Tetrahymena thermophila*. *Toxicology*, 269(2–3), 182-189.
- Organisation for Economic Cooperation and Development (OECD), 2004. Guideline for the testing of chemicals.

- Ozkan, Y., Altinok, I., Ilhan, H. and Sokmen, M., 2015. Determination of TiO₂ and AgTiO₂ Nanoparticles in *Artemia salina*: Toxicity, morphological changes, uptake and depuration. *Bulletin of Environmental Contamination and Toxicology*, 96(1), 36-42.
- Strigul, N., Vaccari, L., Galdun, C., Wazne, M., Liu, X., Christodoulatos, C. and Jasinkiewicz, K., 2009. Acute toxicity of boron, titanium dioxide, and aluminum nanoparticles to Daphnia magna and Vibrio fischeri. Desalination, 248(1–3), 771-782.
- **Zhao, J., Wang, Z., Dai, Y. and Xing, B., 2013.** Mitigation of CuO nanoparticle-induced bacterial membrane damage by dissolved organic matter. *Water Research*, 47(12), 4169-4178.
- Zhu, X., Zhu, L., Chen, Y. and Tian, S., 2008. Acute toxicities of six manufactured nanomaterial suspensions to *Daphnia magna*. *Journal of Nanoparticle Research*, 11(1), 67-75.