Original Article

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JPHS

Toxicity of nanoZnO in Daphnia magna fed with ZnO containing Chlorella vulgaris and Scenedesmus dimorphus algae

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Abstract

Nano ZnO is currently used in the rubber, electronics, electrical appliances, enamel, cosmetics and medical industry. Whereas most studies have used the ecological toxicity of nanoparticles , the toxic effects of nanoparticles in diet is not extensively explored. Because the algae are at the base of the food chain, any change in their density, biomass and population, would affect the food chain in aquatic ecosystems. Daphnia magna is widely used in environmental pollution control measures, especially in nanoparticle toxicity tests for its short reproduction time and high sensitivity. In this study, Chlorella vulgaris and Scenedesmus dimorphus algae were contaminated with nano ZnO. Five treatment concentrations and three replications at any concentration were compared between test and control groups and the results were analyzed by Probit method. LC_{50} of Chlorella vulgaris and Scenedesmus dimorphus algae fed by nano ZnO containing Daphnia magna was obtained at 1.78 and 2.59 mg/l levels after 96 hours respectively. Moreover the NOEC (No Observed Effect concentration) and LOEC (lowest observed effect concentration) were calculated as 0.17 and 0.25 mg/l for Scenedesmus dimorphus algae and Chlorella respectively. Our results showed that Daphnia magna is more sensitive to a diet comprising nano ZnO fed Scenedesmus dimorphus algae as compared to Scenedesmus dimorphus environmentally contaminated with nano ZnO.

Keywords: Nano ZnO, Scenedesmus Dimorphus, Chlorella Vulgaris, Daphnia Magna, NOEC, LOEC

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Introduction

Nanotechnology is a fast growing science and nano products developed on a daily basis are continuing their impact on human lifestyle. Limited studies have dealt with the potential dangerous aspects of nanoproducts on ecosystem. One major reason for this scarcity is difficulty of identifying the effects of nanoparticles using currently available test methods. Nanoparticles can enter environment and transmit through air, soil and underground water, potentially affecting the health of organisms living in these environments. In addition, nanoparticles can enter the food chain by micro-organisms such as bacteria. Presence of nanoparticles in living organisms may have a potentially destructive effects leading to irreversible damage to them.While producing and irregular use of these nano materials can ultimately lead to exposure of ecosystems to these substances there is no comprehensive information about the rate of this exposure (Monica et al., 2009).

Among identified features of nanoparticles, mobility via water, absorption by plants, presence in plant tissue and soil, and suspension in the air can be mentioned (Ferry et al., 2009). Because of small sizes, nanoparticles can pass many obstacles and filters. Use of nanoparticles in commercial scale has the potential negative consequences (Nanotechnology., 2006). These particles can easily be transmitted through the wind and because of having large and active surface area, they can stick to heavy metals, chemical and organic materials there by causing soil and surface water pollution. It is well documented that toxic effects of nanoparticles can dramatically increases by reduction of their size and increasing their concentration (Oberdorster et al., 1996. Yang et al., 2005, Lee et al., 2008). Nanoparticles are usually not soluble in aqueous environments; rather they mostly appear in suspended form. It is important to note that the mobility of nanoparticles in aquatic environments results in the fact that a higher number of marine organisms including algae and fish are exposed to the potential irreparable damages associated with their toxic activity (Corredor et al., 2009).

Among the most widely used nanoparticles, nano ZnO (n-ZnO), has been widely used in rubber, electronics and electrical industries, and in developing cosmetics products, and medical treatments moreover. N-ZnO can lead to generation of free radicals in skin cells, damaging DNA, and altering the structure of proteins, which can potentially lead to promotion of cancer disease (Environmental Protection Agency Nanotechnology, 2007)

There is a limited ecological toxicity dealing with the toxicity of nanoparticles on phytoplankton.This phenomenon showed that surface structure and matrix of cell wall can function as a surface for growth of nanoparticle (Hund rinke., 2006). In addition, some other investigators have identified some forms of stable nano crystals in marine phytoplankton in contact with cadmium. Similar studies dealing with bacteria are very limited. However, antibacterial effects of dioxid titanium and silver nanoparticles are well documented (Duran et al., 2007) .It can be therefore expected that these substances have a potential toxicity to other bacteria as well. Oberdorster et al. (2006) have investigated the relationship between the size of silica nanoparticles Chlorella kessleri algae and their toxic potentials. The study found an inverse relationship between size and toxicity of silica nanoparticles in this species. (Fujiwara et al., 2008)

While numerous studies has been carried out on the toxic effects of n-ZnO on living animals, studies regarding transmission of this substance from a surface of food chain to another is limited. Given this limitation, the present study was focused on the impact of n-ZnO on algae Scenedesmus dimorphus and Chlorella vulgaris as two producers of Daphnia magna.

Materials and Methods

In this study, neonates with less than 24 hours old were used according to the recommendations of OECD[1] (OECD Guideline for testing of Chemicals. 2002,)

In order to bearing 24-hour-old neonates, female Daphnia containing eggs were isolated. The female Daphnia were then fed with yeast extracts and Scenedesmus dimorphus algae. When the eggs hatched, neonates of less than 24 hours old were used in toxicity testing.

During the test period, pH and temperature was controlled at=7-8 and 22±2 °C, respectively. After finding the appropriate ranges, concentration treatments were calculated in logarithmic scale.

Scenedesmus dimorphus, Chlorella vulgaris algae were exposed to 0, 5, 10, 50 and 100 mg/l of n-ZnO for three days. Afterwards, in separate treatments these algae were used in nutrition for Daphnia the loosing rate of Daphnia fed with n-ZnO containing algae, in comparison with controls, was recorded in every 24 hours. Then LC10, LC50, and LC90 values for Daphnia feeding with n-ZnO containing algae were calculated using Probit analysis. A significant level of 0.05 was considered in all tests.

Results

After 96 hours, the daily surveys, with were recorded and analyzed using probit method. R2 values obtained from regression analysis, are shown in Table 1.Lethal doses of each alga contaminated with n-ZnO was calculated at different times.

Table 1: Regression Curve of nano ZnO based on the concentration algae for Daphnia

		24	48	72	96
	h(time)				
Sc	Line Equation	y=2.056x+1.921	y=0.883x+4.861	y=2.532x+4.362	y=2.532x+4.362
	R^2	0.927	0.989	0.971	0.971
Ch	Line Equation	y=3.682x+0.179	y=4.356x+1.387	y= 3.125x+3.369	y=2.898x+3.802
	R^2	0.913	0.998	0.986	0.998

Toxicity of Chlorellan-ZnO containing algae on Daphnia magna

During test period, mortality rate of Daphnia due to being feed with n-ZnO containing Chlorella valgae was recorded (Figure1). The results of acute toxicity of n-ZnO containing chlorella algae on Daphnia magnabeing fed , was calculated based on lethal concentration (LC) (Table 2). As seen, after 96-h, a LC10of 0.93, LC50of 2.59 and LC90of 7.17 mg/liter were recorded for n-ZnO algae. Figure 2 comoares the lethal doses on different days. In this test, a NOEC of 0.25 mg/l and a MAC of 0.25 mg/l were calculated.

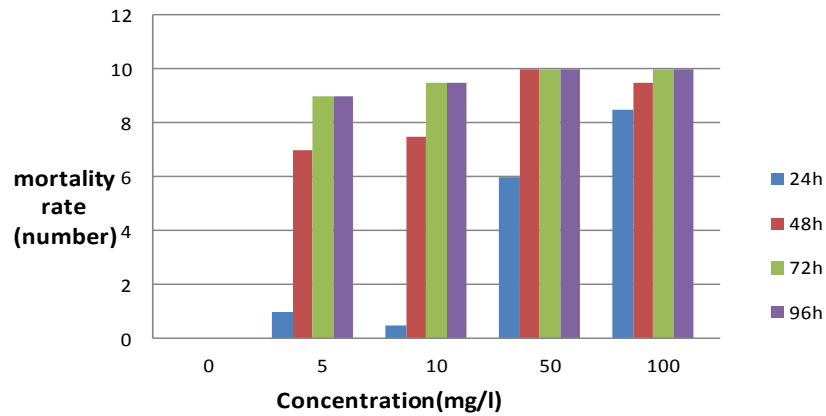1. *Organization for Economic Cooperation and Development*

Figure 1: Mortality rate of Daphnia fed algae chlorella contains nano ZnO

Table 2: Lethol dose of chlorella algae containing nano ZnO on Daphnia magna

Time(h) LC(mg/l)	24	48	72	96
LC_{10}	7.33	0.05	0.55	0.55
LC_{50}	34.08	1.43	1.78	1.78
LC_{90}	158.37	40.62	5.72	5.72
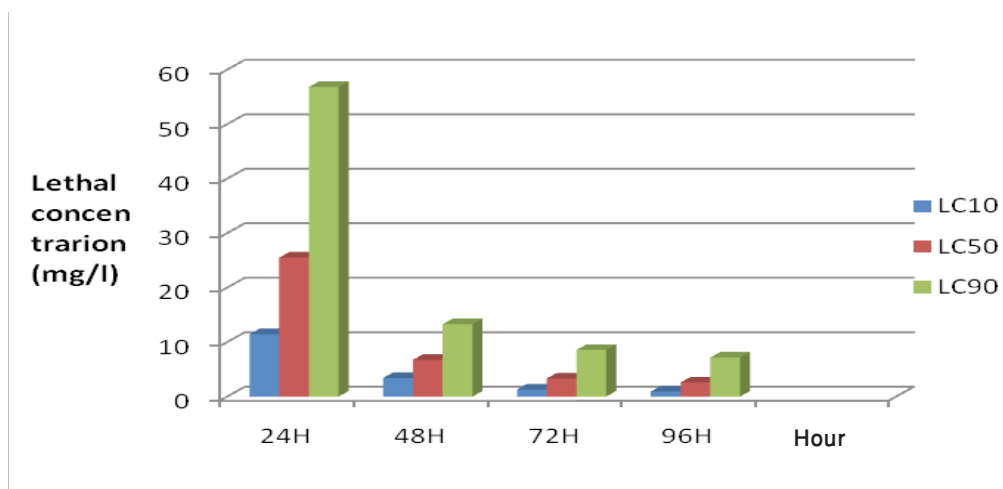
Figure 2: Comparison of lethal doses Chlorella algae containing nano ZnO on Daphnia during 96 hours

Toxicity of algae Scenedesmus dimorphus containing n-ZnO

Figure 3 shows the mortality rate of of Daphnia due to being fed by n-ZnO containing algae Scenedesmus dimorphus As concentration of n-ZnO and exposure time increased, the mortality rate of Daphnia increased as well. Toxicity of n-ZnO Scenedesmus dimorphus was calculated terms of lethal doses per day. LC50 of these n-ZnO containing algae was calculated after 96h to be 1.78 mg/l. As seen in Table 3 after 72 hours, the sensitivity of Daphnia magna to n-ZnO containing algae was remained unchanged. In addition, in this test, a NOEC of 0.17 mg/l and a MAC of 0.17 mg/l was calculated.
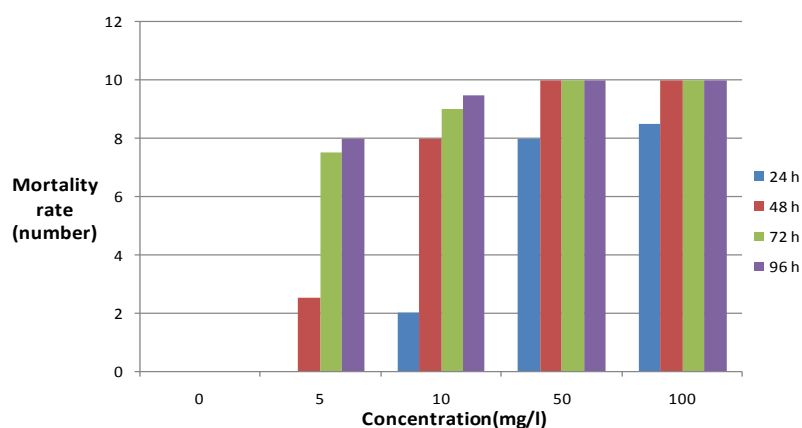

Figure 3. **Daphnia magna mortality due to be fed by Scenedesmus dimorphus containing nano ZnO**

Table 3: Lethal dose of algae Scenedesmus dimorphus containing nano ZnO on Daphnia magna

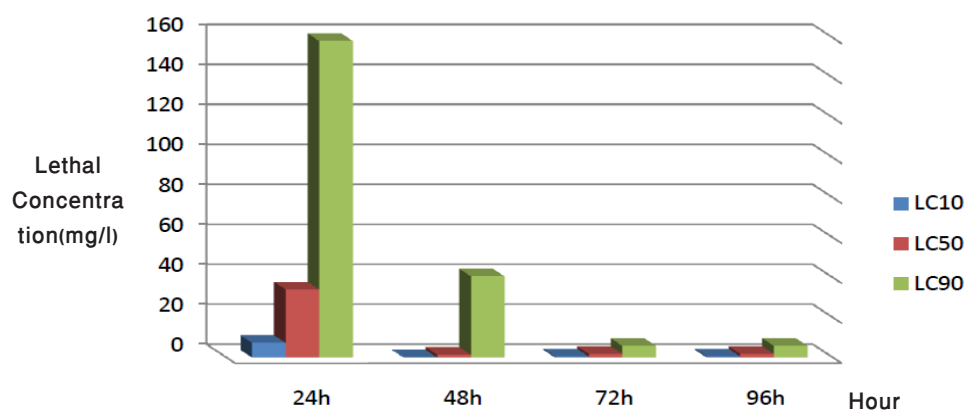| Time(h) LC(mg/l)	24	48	72	96
LC_{10}	7.33	0.05	0.55	0.55
LC_{50}	34.08	1.43	1.78	1.78
LC_{90}	158.37	40.62	5.72	5.72

Figure 4: Comparison of lethal concentrations of algae Scenedesmus dimorphus containing nano ZnO

Discussion

During feeding experiments of Daphnia with Scenedesmus dimorphus, Chlorella vulgaris containing ZnO (algae were exposed to n-ZnO after 72h) at 96 h LC50 silica nanoparticles was calculated to be 2.5 mg/l for algae chlorella and 1.78 mg/l for Scenedesmus dimorphus. On the basis of these data, it could be concluded that n-ZnO content of Scenedesmus dimorphus alga was higher than that of chlorellaIn addition, Scenedesmus dimorphus alga showed higher sensitivity as compared with Daphnia magna. Figure 3 shows that, after 48 h, the LC10, LC50, and LC90 increased with a high rate. Daphnia death rates at different concentrations and times is shown in Figure 4 and represents a higher death rate for Daphnia when compared with diagram in Figure 3.

In Figure 4, after 96 h in concentration of 5 mg/l, which was the lowest test dose, a 100% mortality rate was observed. However, the toxicity effect of Chlorella algae after 96 h in concentration of 10 mg/l , was relatively lower. Figure 5 shows LC50 of n-ZnO containing Scenedesmus dimorphus algae and Chlorella on Daphnia after 96 hours.
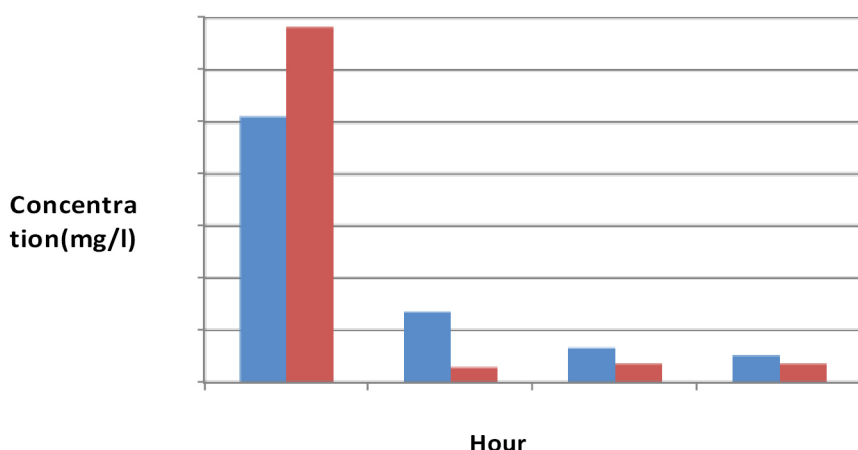
Figure 5: Compared toxicity of algae containing nano ZnO Daphnia magna.

As seen, Chlorella algae, shows a lower LC50 Daphnia than Scenedesmus dimorphus algae containing n-ZnO after 24h. This observation implies that, Chlorella algae is more toxic as compared with Scenedesmus dimorphus algae; however as time passed, the toxicity of Scenedesmus dimorphus algae containing n-ZnO in Daphnia magna is higher. This indicates a higher effectiveness of Daphnia magna as preferred food compared with Scenedesmus dimorphus algae.Limited studies have been conducted about transmission of the toxicity of nanoparticles through diet.Federici et al. showed that toxicity of nano TiO2 through diet in trout can result in intestinal damage. Moreover some biochemical evidences have indicated the presence of oxidative stress in intestinal epithelial cells (Federici et al., 2007). It seems that the physicochemical behavior of nanoparticles affects their absorption potentials to many surfaces, including water, algae, biological membranes, soil and the outer surface of living creatures.

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