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The Acute Toxicity of Tin Dioxide Nanoparticles on *Chlorella vulgaris* Algae

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Abstract

Nowadays, nanotechnology and the use of its components, including nanoparticles, have successfully improved the situation of industries in advancing production goals. Among these nanoparticles, SnO_2, or tin dioxide nanoparticle, which was used in this study, can be mentioned. Tin dioxide is used in the manufacture of batteries and fuel cells, capacitors, and the negative effects of factory effluents entering rivers and other water sources will affect catalysts, and the health of living organisms. In this study, the biotoxicity of tin oxide nanoparticles on *Chlorella vulgaris* algae, which is one of the primary producers and most important levels of the food chain, was investigated. This research was conducted using the OECD acute toxicity test method (counting method for algae, method 201), and statistical probit analysis was performed in order to obtain toxicity data using the probit method. The results of exposure for *Chlorella vulgaris* in 48 and 72 hours were EC_{50} and EC_{90} equal to 6.99, 57.54, and 13.08 and 1.07 x 10^{10} mg L^{-1}, respectively. The highest growth decrease after 48 and 72 hours was observed in 5.5 mg L^{-1} SnO_2NPs. During the test period, no morphological changes were observed for any of the microorganisms, which are based on the toxicity of tin oxide nanoparticles.

Key words: Algae, *Chlorella vulgaris*, Tin oxide nanoparticle, Toxicity

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1. Introduction

Nanotoxicology is one of the new branches of science that studies and investigates the toxicity potential of micromaterials and microparticles. Breaking the solid material and turning it into small particles causes the particles to shrink and increase their overall surface area, which can reveal the emerging properties of such materials. As the particle size decreases to 0.1 nm, quantum effects also appear (Ranjbar et al., 2006). SnO_2 is an n-type semi-conducting nanoparticle whose capacity to destroy colored environmental pollutants is known (Khedmati, 2013). Metal oxide nanoparticles have recently been manufactured in industries at the engineering level with large-scale effects (Pendashteh et al., 2011). Tin oxide nanoparticles (SnO_2 NPs) are one such material that has seen widespread use in a variety of applications, including electronics, solar cells, and coatings. SnO_2 NPs can act as a photocatalyst for the degradation of pigments in colored materials. These nanoparticles can also be used in the decontamination of dye-contaminated water in textile factory effluents.

With the advancement of nanotechnology science and the increase in the use of nanoparticles, it is expected that the consequences of releasing wastewater containing nanoparticles into the environment will emerge. The first group of organisms to be affected by these events are algae, which form the first group in the food chain. *Chlorella vulgaris* is a group of green algae. The members of this group are very diverse in terms of morphological forms, reproduction methods, life cycles, and habits, and structurally they have very advanced examples (Kianmehr, 2005). *Chlorella vulgaris* is a freshwater alga that has a wide distribution and is a good species for biotoxicity tests (Auffan et al., 2011). The studies reported a range of negative impacts of SnO_2 on aquatic organisms, including reduced growth and survival, altered behavior, and changes in biochemical and physiological parameters (Ahamed et al., 2016; Chávez-Calderón et al., 2016; Park & Park, 2009; Wang et al., 2019; Zhang et al., 2019).

Bounnit et al. (2022) studied the effects of SnO_2 NPs on Picochlorum maculatum and observed that these nanoparticles had a toxic effect on algae growth. Also, it was observed that lower doses had more negative impacts than higher doses because of nanoparticle agglomeration, which resulted in a reduced effect on cell morphology and appearance. Protein production was inhibited, too (Bounnit et al., 2022). The long-term effects of SnO_2 exposure on aquatic organisms are not yet fully understood, but several studies suggest that chronic exposure to SnO_2 can have negative impacts on growth, reproduction, and survival. It is mentioned that intracellular ROS accumulation decrease of photosystem II (ϕ PSII) in algae were observed in microalgae (P. subcapitata) exposed to SnO_2 NPs.

Also, different biological models have been described showing that SnO_2 produced and accumulated significantly more intracellular ROS than control with the consequent cell oxidative disturbances, including lipid peroxidation and cell membrane damage (loss of integrity), an overwhelmed antioxidant defense system, reduced mitochondrial function, chromatin condensation, DNA damage, and cell death through the apoptotic pathway. It was observed that the viability of yeast Saccharomyces cerevisiae cells was reduced in a dose-dependent way when exposed to SnO_2 (Soares & Soares, 2021).

A study by Poynton et al. (2013) found that chronic exposure to SnO_2 NPs reduced the growth and production of Daphnia magna, a common freshwater invertebrate. Similarly, Yu et al. (2020) reported that chronic exposure to SnO_2 NPs causes significant damage to the gill filaments and liver tissues of *Clarias gariepinus*, a freshwater fish. Other studies have suggested that chronic exposure to SnO_2 NPs can lead to changes in biochemical and physiological parameters, indicating potential sublethal effects on aquatic organisms. For example, Lu et al. (2015) found that chronic exposure to SnO_2 NPs caused oxidative stress and apoptosis in the liver of zebrafish. Overall, the long-term effects of SnO_2 NPs exposure on aquatic organisms are likely to depend on a variety of factors, including the dose and duration of exposure, the species and life stage of the organism, and the environmental conditions in which the organism lives. The

mechanisms through which SnO_2 impacts aquatic organisms include physical interactions, such as obstruction of gill filaments in fish, and chemical interactions, such as the release of toxic ions from SnO_2 NPs.

Navaro et al. (2008) and Adams et al. (2006) studied the toxicity of silver nanoparticles and nanooxides of titanium, zinc, and silicon, respectively. In their research, Mouivand and Fallahi investigated the effect of nanosilver on the growth rate and reproduction of blue-green algae (Anabaena flosaquae) for 3 months in 2010 (Mouivand & Fallahi, 2010). A study was carried out in 2012 with the aim of determining the acute toxicity of zinc oxide nanoparticles on two algae, Scenedesmus dimorphus and *Chlorella vulgaris* (Pendashteh et al., 2011). In an investigation, freshwater algae (*P. subcapitata*) were exposed to 25 to 600 mgL^{-1} zinc oxide nanoparticles for 72 hours (Tsai et al., 2007).

Undoubtedly, SnO_2 NPs will be used in the wastewater of factories and industries during the waste production process, and considering the irreparable risks they have on water damage, especially at the initial levels of the food chain, this research related to the toxicity of tin dioxide nanoparticles on two species of algae that form the basis of the food chain was done. While the advantages of nanomaterials are numerous, their potential impacts on the environment and living organisms are not yet fully understood. In particular, the impacts of SnO_2 NPs on aquatic organisms have received relatively little attention compared to the extensive research on terrestrial organisms. This paper aims to study the impacts of SnO_2 NPs on the algae *Chlorella vulgaris*, which is at the initial levels of the food chain.

2. Materials and Methods

SnO_2 NPs with a size of 40 nm were prepared by the chemistry department of Lahijan Azad University. In the toxicity test, the working method and implementation of the steps were carried out according to the OECD method (201). *Chlorella vulgaris* algae was obtained from Anali Wetland and cultivated to make the main algae stock. Algae culture was done in the agar-agar medium on special culture plates under ultraviolet rays and then transferred to the liquid culture medium (Zinder) in a sterilized Erlenmeyer flask. Cultivation was carried out under controlled temperature conditions of 22°C, 12 hours of darkness, 12 hours of 4000 lux light, and 14 days in a germinator.

Before starting the exposure operation, range finding was done to determine the required concentrations of tin dioxide nanoparticles. Experimental concentrations of 0.01, 0.1, 5, and 10 mg L^{-1} of nanoparticles were prepared in Zinder culture medium (Table 1), and $5\times10_3$ cells of algae were added to each of the treated test tubes. After doing the range finding results, the main concentrations were calculated through logarithmic base, and their values were 0.01, 0.08, 0.66, 5.50, 50, and 100 mg L^{-1}. All treatments were done with three replicates. Three control tubes without nanoparticles were also examined as control samples. The amount of 5×103 cells from the main stock of *Chlorella vulgaris* algae (according to the standard method of the OECD) was added to the test tubes, and the samples were kept in the germinator for 24, 48, and 72 hours under a temperature of 22 ± 2 degrees Celsius and 12 hours of light and darkness. After 24 hours, the number of cells in the algae treatments was counted under a Nikon light microscope (model N-180M, Japan) by an improved Thoma-ruled hemocytometer consisting of nine 1 x 1 mm (1 mm2) squares. After counting and recording the data, the average number of cells in the top and bottom squares was calculated, and then the number of cells was calculated using the following equation:

Cell density per ml= all cells counted in the large square$\times10^4$ (1)

The specific growth rate (μ), inhibition percentage (%I), and doubling rate (G) were obtained using the following equations (Fogg et al., 1987):

$$\mu = \frac{(\ln X0 - \ln X1)}{t0 - t1} \qquad (2)$$

In equation (2), μ is the rate of specific growth, X=0 is the number of cells at time t0, and X=1 is the average number of cells at time t1.

$$G = \ln 2\mu^{-1} \qquad (3)$$

$$\%I = \mu_c - \mu_t / \mu_c \qquad (4)$$

In equation (4), μt, and μc are defined as the growth rates in the treatment mean value of μ in the control, respectively.

Table 1. The composition of Zinder culture medium (Sakamoto et al., 2019)

Reagents	Zinder (g L^{-1})
NH_4Cl	0.5
KH_2PO_4	0.4
$MgCl_2.6H_2O$	0.1
$CaCl_2.6H_2O$	0.15
$NaSO_4$	1.12
Sodium lactate	2.24
Rezasurin	0.0001
$FeSO_4$	0.73
Solution vitamins	1 ml L^{-1}
Trace metal solution	10 ml L^{-1}
Sodium bicarbonate	1.0

After determining the algae cell number, 24-, 48-, and 72-hour levels of EC_{10}, EC_{50}, and EC_{90} were derived from the Probit analysis table, and NOEC was calculated (Equation 5) (Finny, 1971). To determine the significance of differences among treatments in various concentrations of algae cells and control samples, a one-way ANOVA was used. The Tukey's test was used to identify differences between each level of treatment.

$$NOEC = EC50/10 \quad (5)$$

3. Results

3.1 Cell Count Test (cell ml^{-1}) for *Chlorella vulgaris* algae

The comparison of the control with each treatment revealed that an obvious reduction in algae cells was observed after 48 and 72 hours, but a difference was observed after 24 hours of 0.08 mg L^{-1} treatment (Fig. 1). This reduction in cell density during 48 and 72 hours, especially at 5.5 and 100 mg L^{-1} concentrations, indicates the acute toxicity of SnO_2 NPs and its effect on *Chlorella* algae growth. The results showed that growth-inhibitory effects and a reduction in cell density usually appear after 24 hours. The highest decrease occurred at 5.5 and 100 mg L^{-1} with the control (Fig. 1). Based on the ANOVA test, a significant difference was observed between the control and 5.5 and 100 mg L^{-1} exposures ($p<0.05$).

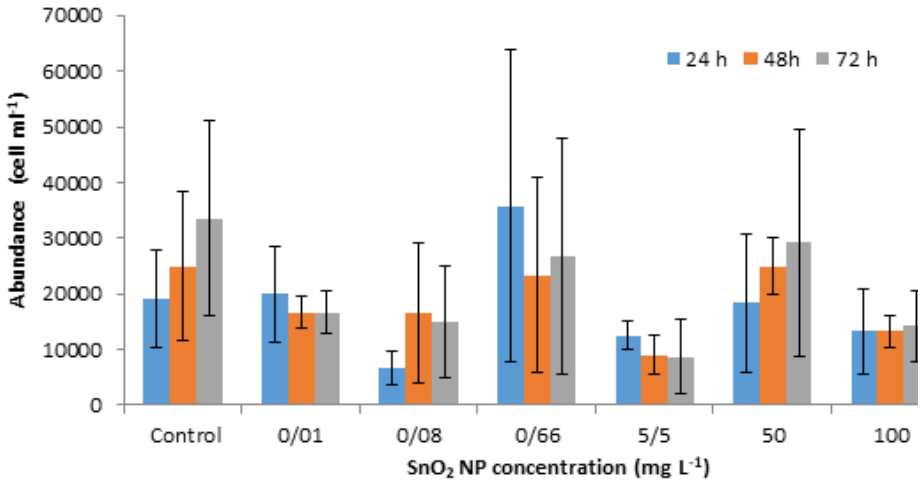

Fig. 1. Number of *Chlorella vulgaris* algae exposed to SnO_2 NPs

As shown in Fig. 2, the maximum growth of *Chlorella* algae has occurred in 48 hours at a concentration of 0.66 mg L^{-1}, which has decreased in 72 hours.

The lowest rate of cell division in the concentration of 5.5 mg L^{-1} has occurred in the first 48 hours of exposure, and the highest rate of cell division in the first 48 hours is in the concentration of 0.66 (Fig. 3).

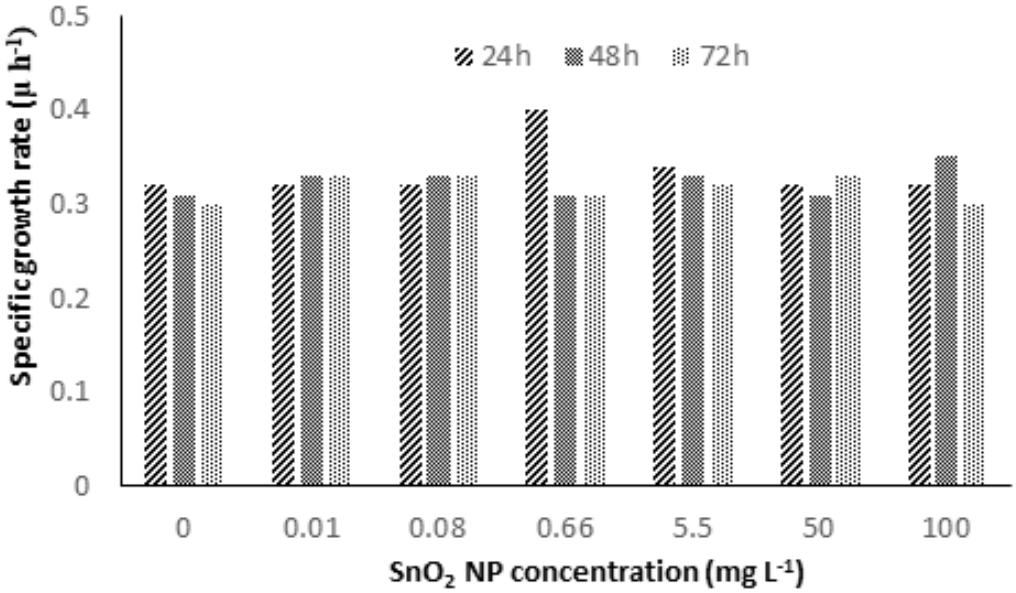

Fig. 2. Specific Growth Rate (μ) of *Chlorella vulgaris* algae after exposure to SnO_2 NPs

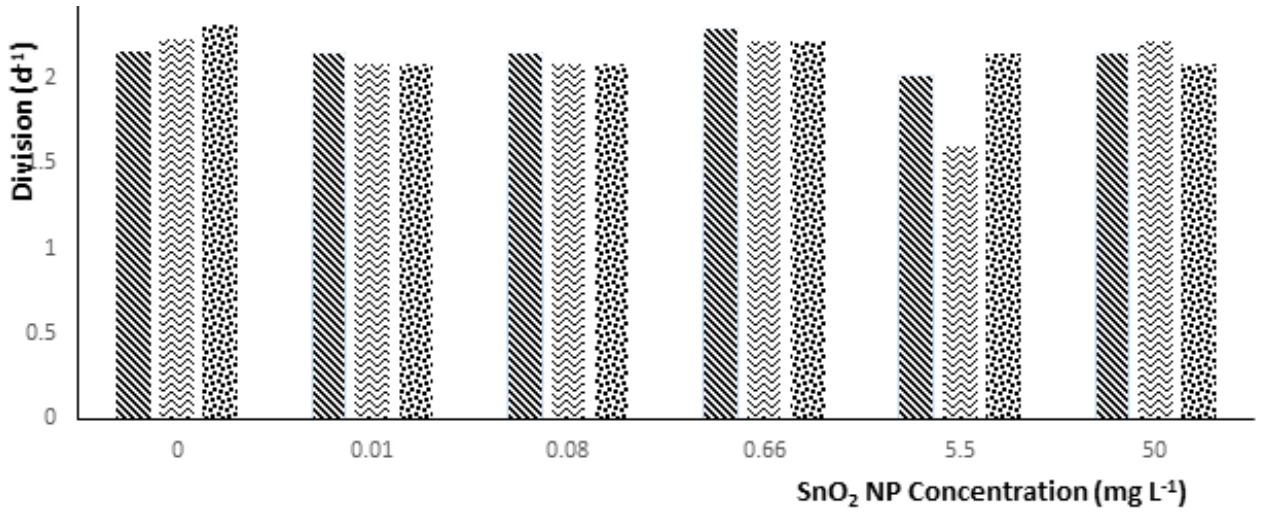

Fig. 3. Division (G) of *Chlorella vulgaris* algae after exposure to SnO_2 NPs

4. Discussion

Table 2 shows the results of the effect of SnO_2 NPs on *Chlorella vulgaris*. In general, according to the amounts of lethal concentrations in Table 2, the toxicity of SnO_2 NPs on the mentioned algae seems low. But, in the case of *Pseudokirchneriella subcapitata* microalgae, in a comparative study, the toxicity of five nanoparticles, including Al_2O_3, In_2O_3, Mn_3O_4, SiO_2, and SnO_2 NPs, was evaluated through the OECD growth inhibition method, and based on the results, SnO_2 NPs was the most toxic, with the following decreasing order: $SnO_2 > Al_2O_3 > Mn_3O_4 > SiO_2 > In_2O_3$ (Sosa et al., 2019).

According to Table 2, 72-hour EC_{90} has the highest amount. The large number of EC_{90} indicates the low toxicity of SnO_2 NPs on the proliferation of *Chlorella* algae cells. A study conducted by Gong et al. (2011) on the toxicity of nickel oxide on *Chlorella vulgaris* algae indicated the 72-hour EC50 was 32.28 mg L^{-1}. The results showed that the biotoxicity of nanoparticles and the bioavailability of nickel oxide (NiO) to marine algae are reduced by accumulation. In the present study, the 72-hour EC_{50} was 131.82 mg L^{-1}, which indicated it is less toxic compared to nickel oxide. In another study, the effect of aluminum nanoparticles on *Dunaliella* algae was tested, and 72-hour values were $EC_{10}=1.66\times10^{-3}$, EC_{50} = 0.162, EC_{50} = 31.15, and EC_{90} = 162 mg L^{-1}. The amount of cell density and growth inhibition between the algae of the control treatment and the studied concentrations had a significant difference ($P<0.05$). Aluminum oxide nanoparticles had a significant effect on the shape and the cell topography, which has caused the swelling and enlargement of Dunaliella algae cells (Ayattallahzadeh Shirazi et al., 2013).

In another test to determine the toxicity of nanozinc oxide on *Chlorella* and *Scenedesmus* algae, it was found that this nanoparticle has a decreasing effect on the growth rate of two species of algae, and a decrease in the number of *Scenedesmus* algae was observed in all concentrations after 72 hours (Meulenkamp, 1998). Mouivand et al. (2011) investigated the effect of nanosilver on the growth rate and reproduction of blue-green algae (*Anabena flosaquae*) for 3 months in 2009. The results showed that the effective concentration of nanosilver was in the range of 0.005 to 0.050 mg L^{-1} in blue-green algae, and the maximum reduction in the growth of these algae was obtained at a concentration of 0.05 mg L^{-1} nanosilver. According to the results, the highest concentration of exposure to nanosilver had a negative effect on the growth and reproduction of Anabaena algae.

In a study conducted with the aim of determining the acute toxicity of zinc oxide nanoparticles on *Chlorella vulgaris* algae, 48-hour EC_{50} values were 2.830 mg L^{-1} when compared with the results of this study, showing that zinc oxide nanoparticles were somehow more toxic than SnO_2 (Pendashteh et al., 2011). Freshwater algae species (P. subcapitata) were exposed to 25 to 600 mg L^{-1} zinc oxide nanoparticles for 72 hours. The comparison of toxicity effects between ZnC_{l2} and ZnO (in powder form) and nano ZnO (in aqueous form) was done, and the EC_{50} value was calculated as 60 mg L^{-1} (Tsai et al., 2007).

Also, in research on determining the size-dependent toxicity of silver nanoparticles on *Chlorella* algae, extreme changes in the chlorophyll content of the algae and an increase in lipid peroxidation were observed. The target algae were exposed to zero to 10 mg L^{-1} of silver nanoparticles for 24 hours (Oukarroum et al., 2010). Metal oxides of nanoparticles can have different toxicities, and the toxicity of nanoparticles is related to the nature of their constituent elements in addition to their nanostructure and surface-to-mass ratio (Aruoja et al., 2009).

Table 2. Toxicity values of SnO2 NPs for *Chlorella vulgaris* (mg L-1)

	24h	48h	72h
EC_{10}	1.60×10^{2}	8.50×10^{2}	1.66×10^{6}
EC_{50}	16.98	6.99	131.82
EC_{90}	18.19×10^{3}	57.54	1.07×10^{10}

Since the growth process and catalytic chemical reactions take place on the surface, a certain amount of material on a nanometer scale is much more active than the same material in bulk. These characteristics may have negative effects on health and the environment and lead to the high toxicity of nanoparticles. Based on the results, many metal oxides were tested on D. magna and Secenedesmus sp. species, and it was observed that zinc oxide, silver oxide, and nickel oxide have potential toxicity and have caused disturbances in the reproduction, growth, and natural life processes of the mentioned species. But SnO_2 NPs, which is one of the metal oxides, does not have toxic properties that are evident from its high growth inhibitory concentrations (EC90), which are 1.07x1010 in 72 hours for *Chlorella*. In a study that examined the toxic effects of zinc oxide on the algae *Chlorella* sp., the 72-hour EC50 was 0.01 mg L^{-1} for *Chlorella* (Pendashteh et al., 2011). These results show that compared to SnO_2 NPs, zinc oxide has very toxic effects on this algae, and again, *Chlorella* algae shows more sensitivity to zinc oxide nanoparticles.

Since algae are the food of daphnia, daphnia are the food of fish, and fish are the food of humans, it is necessary to ensure the health of the primary levels of the food chain. Because the use of SnO_2 NPs is more limited than other nanoparticles in terms of the volume of its application and disposal in aquatic ecosystems, there is not much concern at the first and second levels of the food chain. Nevertheless, investigating and conducting toxicological tests of SnO_2 NPs on other species at different levels of the food chain, investigating the long-term effects (chronic toxicity) of SnO_2 NPs on *Chlorella vulgaris* species and other living organisms, and conducting experiments related to the impact of the toxicity of different sizes of SnO_2 NPs on different living organisms, including microscopic algae, can be effective measures to understand the effects of this nanoparticle.

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