Effect of CuO Nanoparticle on *Ceratophyllum demersum*

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**A R T I C L E  I N F O**

**ABSTRACT**

In this study, it was aimed to determine some physiological effects of CuO nanoparticle on *Ceratophyllum demersum*. The plants were collected from an uncontaminated pond in Gaziantep province. Different concentrations of CuO (0, 25, 50, 100 and 200 mg/L) were applied to the macrophytes after being acclimatized in controlled conditions. Some analyses were made on macrophytes harvested at the end of the application. The contents of protein, total carbohydrate, photosynthetic pigment, total phenolic compound, non-protein sulfhydryl groups of tissues increased with increasing CuO concentration under the influence of applied CuO concentrations. In addition, increases in hydrogen peroxide and MDA contents were also detected. As a result, it was determined that the applied CuO concentrations caused some physiological changes in *C. demersum* tissues.

**Keywords:** *Ceratophyllum demersum*, CuO, Oxidative stress, Physiological effect, Nanoparticle

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**Introduction**

Nanotechnology refers to the interdisciplinary activities that research and develop the production, design, characterization, assembly and systems of nanoscale materials. Nanotechnology designs and synthesizes artificial structures by processing known molecules with different atoms and molecules. With nanotechnology, it has become possible to produce materials that are more functional, fast, take up less space, consume less energy, are more durable, inexpensive and have extraordinary new properties (Rao et al., 2005). Advances in nanotechnology and nanotechnology-based industries and applications are growing tremendously. With the studies in the field of nanotechnology, remarkable breakthroughs are made in many different fields such as materials, nanoelectronics, energy, biotechnology, medicine and health, national security and information technologies (Siddiqui et al., 2015).

Among the different types of nanomaterials, metal and metal oxide nanoparticles differ in their small size and large surface area, as well as their various physical and chemical properties such as surface loads, shapes, conductivity, differences in melting and freezing points (Dizaj et al., 2014; Falcaro et al., 2016). Due to its unique properties, many metals, metal oxides and alloy nanoparticles are produced and used in many fields (Karlsson et al., 2008). For example, metal nanoparticles such as TiO\(_2\), Cu, Zn, Al and Ag nanoparticles are used as additives in consumer and industrial products.

In the toxicity and genotoxicity studies of metal nanoparticles, metal particles such as gold, silver, titanium and zinc are widely used. *In vivo* and *in vitro* studies conducted with these particles show that the toxic effects usually vary depending on the method, cell line and concentration used (Lanone et al., 2009). Many studies have been conducted on the environmental levels and effects of engineered nanomaterials on living organisms. However, the possible effects of these nanomaterials on ecosystems and plants are still largely unclear. Therefore, this study was carried out to determine some physiological effects of nano-CuO applications on *Ceratophyllum demersum*, an aquatic plant.
Materials and Method

**Plant Material and Application**

*C. demersum* was collected from Karapınar pond (Yavuzeli-Gaziantep, Turkey). The plants were acclimatized in 10% nutrient solution (0.88 mM K₂SO₄, 2 mM Ca (NO₃)₂, 0.25 mM KH₂PO₄, 1 mM MgSO₄, 0.11 mM KCl, 100 μM Fe-EDTA, 10 μM H₃BO₃, 5 μM MnSO₄, 10 μM ZnSO₄, 2 μM CuSO₄ and 0.2 μM (NH₄)₂MoO₄), under 25±1°C, 16 h light (120 μE m⁻² s⁻¹) and 8 h dark periods (Snijders Scientific, Netherlands). After the plants were acclimatized in the nutrient solution, they were cultured in jar (1000 mL) containing different concentrations of CuO (0, 25, 50, 100 and 200 mg/L). After 7-days of CuO treatments, the plants were harvested. They were washed three times with deionized water to remove any elements adhered to plant surface. All chemicals used in the study were prepared in analytical grade.

**Physiological Analysis**

100 mg of fresh plants were weighed for pigment determination and homogenized with 80% acetone in porcelain mortar. The final volume was then completed to 10 mL of 80% acetone and centrifuged at 3000 rpm for 5 minutes. Samples were read against acetone in a UV/VIS spectrophotometer at 662, 645 and 470 nm. pigment calculations were made according to Lichtentaler and Wellburn (1985). Protein contents of plant tissues were determined according to Lowry et al. (1951). Bovine serum albumin was used as a standard. Determination of non-protein –SH groups of the plant tissues was carried out according to Ellman (1959). Content of total phenolic compounds was determined by Ratkevicius et al. (2003). 50 μL of the supernatant of the centrifuged samples were taken at room temperature for 30 minutes by adding 3% sodium carbonate and 0.3 N Folin-Ciocalteau to a final volume of 1 mL. These samples were then read on the spectrophotometer at 765 nm. Gallic acid is used as standard. Anthron method was used to determine total carbohydrate contents (Plummer, 1998). Glucose was used as the standard. H₂O₂ content in the plant tissues was determined according to the method of Sergiev et al. (1997). The content of hydrogen peroxidases was calculated from a standard curve. Determination of malondialdehyde (MDA) of the plant tissues was carried out according to Zhou (2001).

**Data Analysis**

Statistical analysis of data was performed using SPSS 11.0. One-Way ANOVA LSD test was used to determine which group or groups were different. Pearson correlation analysis was used to determine the relationship between the data.

**Results and Discussion**

In this study, the physiological effects of different concentrations of CuO in *C. demersum* grown under controlled conditions were determined. The chl-a content of the plant is found to increase as 41.25%, 39.4%, 53.7%, and 57.3% at 25, 50, 100, and 200 mg/L CuO, compared to control, respectively (Figure 1a). Similarly, increases up to 52.6% were found in chlorophyll-b contents. In addition, there was no significant change in carotenoid contents (Figure 1a). It was determined that the chl-a and chl-b contents of *Solanum tuberosum* leaves increased the Cu nanoparticle effect (Mushtinskiy and Aminova, 2019). However, there are conflicting data in the literature regarding the effect of nanoparticles on chlorophyll content. For example, some researchers talk about an increase in pigment content (Korotkova et al., 2017), while others talk about a decrease in pigment content (Lebedev et al., 2019). The reason for this is the nanoparticle type, nanoparticle diameter, concentration, plant variety, plant development period, application environment and conditions, etc. can be caused by many reasons.

The anthocyanin content of *C. demersum* is found to increase as 6.1%, 37.1%, and 48.9% at 50, 100, and 200 mg/L CuO, compared with control, respectively, but it is decreased as 3.4% at 25 mg/L CuO compared with control (Figure 1b). Similarly, significant increases in anthocyanin content were detected in Arabidopsis plants exposed to Ag nanoparticles (Qian et al., 2013).

The protein content of *C. demersum* was measured that which change by the effect of copper oxide nanoparticles (Figure 1c). The protein content of the plant is found to increase as 21% 32%, 29.7% and 21.2% at 25, 50, 100, and 200 mg/L CuO, compared to control, respectively. There are previous studies indicating an increase in protein content with concentrations of heavy metals, as the protein content of wheat plants treated with copper is lower compared to plants that have been treated with zinc (Singh et al., 2007). These results are also similar to Manivasagaperumal et al. (2011) who found that plants treated with low zinc concentration had a high protein and amino acid content in them, and this increase may be due to oxidative stress.

It was determined that the total carbohydrate content of the plant increased up to 22.1% (Figure 1d). Various results have been published regarding the effects of nanoparticles on macromolecules of plants. It has been reported that nano CuO at 50 and 100 mg/kg did not affect carbohydrate content in green pea seeds (Ochoa et al., 2018). On the other hand, nano Cu was found to reduce the carbohydrate content of thyme leaves (Du et al., 2015). However, carbohydrate synthesis was induced in tomato plant under the influence of TiO₂ nanoparticles (Song et al., 2013).

The phenolic content of the plant is found to increase as 11.2%, 23.1%, 28.6% and 46.9% at 25, 50, 100, and 200 mg/L CuO, compared to control, respectively (Figure 1e). Elzaawely et al. (2007) found that the total phenolic content in copper-treated plants increased significantly compared to untreated Alpinia plants. The non-protein SH groups content of the plant is found to increase as 11.6%, 18.6%, 25.3% and 24.7% at 25, 50, 100, and 200 mg/L CuO, compared to control, respectively (Figure 1f). Increased amounts of non-protein sulfhydryl groups were found in Cu-sensitive strains of *Silene cucubalus* (De Vos et al., 1992). In conclusion, plants under stress may have increased production of stress metabolites such as phenolic compounds and non-protein sulfhydryl groups compared to plants without stress.
Table 1. Correlation analysis of the biochemical parameters obtained after CuO applications

<table>
<thead>
<tr>
<th></th>
<th>Chl-a</th>
<th>Chl-b</th>
<th>Car</th>
<th>Anth</th>
<th>Pro</th>
<th>TC</th>
<th>SH</th>
<th>TPC</th>
<th>H₂O₂</th>
<th>MDA</th>
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<tr>
<td>Chl-a</td>
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<td></td>
<td></td>
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<tr>
<td>Chl-b</td>
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<td></td>
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<tr>
<td>Car</td>
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<td></td>
<td></td>
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<tr>
<td>Anth</td>
<td>0.457</td>
<td>0.302</td>
<td>0.237</td>
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<tr>
<td>Pro</td>
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<td>0.517*</td>
<td>0.295</td>
<td>0.312</td>
<td>1</td>
<td>1</td>
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<tr>
<td>TC</td>
<td>0.468</td>
<td>0.218</td>
<td>0.227</td>
<td>0.870**</td>
<td>0.426</td>
<td>1</td>
<td>1</td>
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<td>SH</td>
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<td>0.310</td>
<td>0.246</td>
<td>0.569**</td>
<td>0.695**</td>
<td>0.694**</td>
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<td>TPC</td>
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<td>0.406</td>
<td>0.251</td>
<td>0.813**</td>
<td>0.440</td>
<td>0.949**</td>
<td>0.718**</td>
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<tr>
<td>H₂O₂</td>
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<td>0.468</td>
<td>0.074</td>
<td>0.512</td>
<td>0.733**</td>
<td>0.700**</td>
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<td>MDA</td>
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<td>0.908**</td>
<td>0.774**</td>
<td>0.852**</td>
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Car: Carotenoid; Anth: Anthocyanin; Pro: Protein; TC: Total Carbohydrate; Non-protein SH groups: SH; TPC: Total phenolic content; H₂O₂: Hydrogen peroxide; MDA: Malondialdehyde
There is an increase in reactive oxygen species (ROS) as a result of toxicity caused by nanoparticles (Melegari et al., 2013; Mohammed et al., 2021). As the amount of ROS in the cell increases, the negative effects of oxidative stress also increase (Sevidnik et al., 2017; Mohammed et al., 2020; Pehlivan et al., 2021). The $\text{H}_2\text{O}_2$ content of the plant are found to increase as 24.5%, 34.2%, 31.9% and 38.5% at 25, 50, 100, and 200 mg/L CuO, compared to control, respectively (Figure 1g). MDA content, which is an important peroxidation product of polysaturated fatty acids, increased due to oxidative stress caused by high CuO concentrations. Based on the findings the MDA content of the plant are found to increase as 20.9%, 38.2%, 129.4% and 126.6% at 25, 50, 100, and 200 mg/L CuO, compared to control, respectively (Figure 1h). Similar to our findings, it has been shown in many previous studies that nano-Cu application causes an increase in the amount of $\text{H}_2\text{O}_2$ and MDA in plant tissues as a result of oxidative stress (Shaw and Hossain 2013; Nair and Chung 2015; Chung et al., 2019).

Nanotechnology is a recent area having many prospective to effect on the environment and plants. As a result, it was observed that CuO concentrations caused an increase in the content of protein, total carbohydrate, photosynthetic pigment, total phenolic compound, non-protein sulfhydryl groups of plant tissues. Also, the amounts of MDA and $\text{H}_2\text{O}_2$ in C. demersum tissues showed an increase under the effect of CuO, and was observed high concentrations of applied CuO cause oxidative stress. Depending on correlation analysis, relationships between oxidative stress parameters ($\text{H}_2\text{O}_2$ and MDA) and other analyzed parameters (Table 1) showed that these changes may be related to oxidative stress triggered by CuO.

References
Lebedev SV, Gavrish IA, Galaktionova LV, Korotkova AM, Sizova EA. 2019. Assessment of the toxicity of silicon nanooxide in relation to various components of the agroecosystem under the conditions of the model experiment. Environmental Geochemistry and Health, 41: 769-782.


