Dose-Dependent Effect of ZnO Quantum Dots for Lettuce Growth

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ABSTRACT: As the cadmium-free semiconductor quantum dots, ZnO quantum dots (ZnO QDs) have wide potential applications in agriculture. However, the effects of ZnO quantum dots on crop growth and nutritional quality have not been fully studied. In this work, the lettuce was sprayed with different concentrations of ZnO QDs from 50 to 500 mg·L\(^{-1}\) to evaluate their influence on lettuce antioxidant, biomass, and nutritional quality. The results showed that ZnO QDs existed in the lettuce in the form of Zn\(^{2+}\). Lettuce treated with 500 mg·L\(^{-1}\) ZnO QDs would produce a large amount of reactive oxygen species (ROS), which adversely affected the absorption of nutrients, soluble protein content, and chlorophyll content, thus reducing plant biomass. When the concentrations range from 50 to 200 mg·L\(^{-1}\), the antioxidant enzyme systems of lettuce were triggered to counteract the damage caused by excessive ROS. Moreover, ZnO QDs at this level promoted Ca, Mg, Fe, Mn, Zn, and B absorption and accumulation; increased soluble sugar content; and improved the lettuce biomass and nutritional quality.

1. INTRODUCTION

With the rapid development of nanotechnology, nanomaterials have been widely used in the fields of agriculture and biology.\(^\text{1,2}\) In the past few decades, plant cultivation technology has made remarkable innovation and progress. Nanomaterials were also used as fertilizers, pesticides, or plant growth regulators\(^\text{3,4}\) in agri-biotechnological applications. The influences of the type, particle size, surface charge, and surface modification of nanomaterials on plant growth also have been fully studied.\(^\text{7–10}\) With an extensive application of nanomaterials, the toxicity of nanomaterials has become an important issue. Because crops safety is the first thing to ensure human food security. The understanding of the interaction between nanomaterials and plants has become a crucial topic for studying the impact of nanomaterials on ecosystems.

Carbon-based nanomaterials (such as fullerenes, carbon nanotubes, graphene quantum dots, and carbon quantum dots) and metal or metal oxide nanomaterials (such as nCuO, nFeO\(_2\), nTiO\(_2\), nCeO\(_2\), CdS QDs, etc.) have been used to study their interaction with plants.\(^\text{11–17}\) Using CdS:Mn/ZnS quantum dots to treat Snow Pea (Pisum sativum), when the concentration was up to 40 \(\mu\)g·mL\(^{-1}\), the content of total chlorophyll decreased significantly, resulting in phytotoxicity.\(^\text{12}\) Majumdar et al.\(^\text{13}\) reported that kidney been exposed to high concentrations of CeO\(_2\) nanoparticles (NPs) (500 mg·mL\(^{-1}\)) significantly decreased the root antioxidant enzyme activities, but the soluble protein increased by 204%. Szymańska et al.\(^\text{14}\) found that high concentrations (1000 mg·mL\(^{-1}\)) of TiO\(_2\) NPs could cause toxic symptoms of Arabidopsis thaliana, increase antioxidant levels, and promote root growth. Azhar et al.\(^\text{15}\) observed that CuO NPs had an adverse effect on Arabidopsis biomass, chlorophyll content, guard cells, stomata aperture, and other organelles, and significantly increased the accumulation of reactive oxygen species (ROS) in leaves; the toxicity of CuO NPs was proved by the reduction of biomass. Rui et al.\(^\text{16}\) reported that the Fe content in peanut plants treated with Fe\(_2\)O\(_3\) NPs was higher than that in the control; the authors suggested that Fe\(_2\)O\(_3\) NPs adsorbed on sandy soil to improve the utilization of Fe; Fe\(_2\)O\(_3\) NPs enhanced the root length, plant height, and biomass of peanut plant and promoted the peanut growth by changing phytohormone content and antioxidant enzyme activity. Ag NPs induced dose-dependent toxicity by affecting the shoot and root length, biomass, photosynthesis, antioxidant enzyme activity, and the content of nutrient elements in maize.\(^\text{17}\) The relationship between different types of nanoparticles and plant species is further studied to clarify the unique effects of various nanoparticles on plant species, so as to promote the safety and risk assessment of nanomaterials.

Conventional nano-ZnO has been widely studied in agriculture because of its environmental protection, low cost, low toxicity, and high electron mobility.\(^\text{18–20}\) On this basis, ZnO has been developed into quantum dots material with rich surface functional groups and excellent water solubility.\(^\text{21,22}\) ZnO QDs have been widely used in the biological field.
Also, the extensive application of ZnO QDs will unavoidably cause excessive ZnO QDs to enter into crop plants. However, there were few reports on the effect of ZnO QDs in plant cultivation. In this study, we used lettuce as a model plant to analyze the effect of ZnO QDs on lettuce physiology. The element content of lettuce was measured, and the effect of ZnO QDs on the absorption and distribution of Ca, Fe, Mn, Mg, Zn, and B was analyzed. ROS production; the lipid peroxidation level; and the activity of antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) were measured. Finally, the effects of ZnO QDs on plant biomass and photosynthetic pigment contents were measured to assess the oxidative stress response induced by dose.

2. RESULTS

2.1. Characterization of ZnO QDs. As shown in Figure 1A, the transmission electron microscopy (TEM) image shows that the ZnO QDs are uniform and monodispersed. The lattice spacing of ZnO QDs is 0.25 nm, corresponding to the (001) crystal plane of wurtzite. From the size distribution diagram, it can be seen that the average size of ZnO QDs is 3.76 nm (Figure 1B). Such small and uniform nanosize makes it possible for them to enter the plant body. The photo-luminescence (PL) emission spectrum of the ZnO QD solution with the excitation from 360 to 420 nm is shown in Figure 2A. It can be seen that the emission peak of the ZnO QD solution is located at 570 nm with independent-excitation effects, which proves that ZnO QDs have stable PL emission.

Table 1. ζ-Potential (mV) of ZnO QDs at Concentrations in the Deionized Water

| ZnO QDs | 50 mg·L⁻¹ | 100 mg·L⁻¹ | 200 mg·L⁻¹ | 500 mg·L⁻¹ |
|---------|-----------|------------|------------|------------|
| ζ-potential (mV) | 40.27 ± 0.65 | 41.93 ± 1.88 | 41.36 ± 2.35 | 42.67 ± 0.83 |

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As shown in Table 1, the ζ-potential of 50–500 mg·L⁻¹ ZnO QDs ranges from 40.3 to 42.7 mV, indicating the good water stability of the further applied concentration of ZnO QDs.²⁸ In Fourier transform infrared (FTIR) spectra (Figure 2B), the stretching vibration band of Si–O can be clearly observed at 880 cm⁻¹, indicating that silane has been coated onto the ZnO QDs.²⁹ The peak at 3413 cm⁻¹ is due to the stretching vibration of the O–H bond, while the peak at 3243 cm⁻¹ corresponds to stretching of the N–H bond.³⁰ Bending vibration of C–H is observed at 1394 cm⁻¹. Due to the presence of these hydrophilic functional groups, ZnO QDs can be well dispersed in the aqueous solution.

X-ray diffraction (XRD) is used to further characterize the crystalline characteristics of ZnO QDs. As shown in Figure 2C, the peaks can be indexed to the crystal planes of (100), (002), (101), (102), (110), (103), (112), and (202) of ZnO, which can be attributed to the diffraction of wurtzite ZnO.³¹ X-ray photoelectron spectroscopy (XPS) is used to characterize the binding state of ZnO QDs. The peaks for C 1s, N 1s, O 1s, Zn 2p, and Si 2p have been marked in Figure 2D. Among them, Zn 2p and O 1s belong to ZnO, while N 1s, C 1s, and Si 1s belong to the surface modifier APTES. High-resolution XPS further reveals the valence bond relationship in ZnO QDs (Figure S1). The peaks at 1021.6 and 1044.6 eV are from Zn 2p₁/₂ and 2p₃/₂ in ZnO QDs, respectively. The distance between the two peaks is 23.0 eV, which is consistent with the 2p₁/₂ and 2p₃/₂ peak spacings of Zn(II) in the standard manual. The binding energy position of Zn 2p₁/₂ is 1021.6 eV, which is 0.2 eV lower than the standard peak position of Zn(II) in ZnO (1021.8 eV). This might be due to the influence of surface modification.³² Because APTES is modified on the surface of quantum dots. In the high-resolution spectrum of O 1s, there are two peaks in O 1s, one at 530.2 eV and the other at 531.8 eV. The former comes from the O–Zn bond and the latter is mainly from the C–O/Si–O bond. The C 1s spectrum mainly contains C–C, C–O, C=O, and C–H bonds. Two peaks appear in spectra of N 1s. The peaks at 399.7 and 398.3 eV correspond to the N–C and N–H bonds (N 1s). These results for the XPS spectra are consistent with those for the FTIR spectra. Based on the above results, ZnO QDs are composed of C, H, O, N, and Zn elements, all of which are essential for plant growth and development. Therefore, it is meaningful to study the effect of ZnO QD on plants.

2.2. Effects of ZnO QDs on Zn Uptake by Lettuce. After 10 days of incubation in a nutrient solution without Zn²⁺, the content of Zn in lettuce was measured to evaluate the absorption, transport, and accumulation of ZnO QDs by lettuce. As shown in Figure S2, with the increase of treatment concentration, the content of Zn element in shoot and root increased significantly, and the increment of Zn element in the shoot was larger, indicating more accumulation of ZnO QDs in leaves. The results primarily indicated the dose-dependent effect of ZnO QDs for lettuce cultivation.

2.3. Effects on Lettuce Biomass and Chlorophyll Contents. Compared to the control plants, the total fresh weights of treated groups increased by 13.95, 24.44, and 8.9% under 50, 100, and 200 mg·L⁻¹ ZnO QD exposure (Figure 3A), while the total dry weights of them increased by 10.4, 19.3, and 22.1%, respectively (Figure 3B). However, when exposed to 500 mg·L⁻¹ ZnO QDs, the total fresh weight and total dry weight of treated lettuce significantly decreased by 21.47 and 33.5%, compared to those of the control, respectively (Figure 3B). However, when exposed to 500 mg·L⁻¹ ZnO QDs, the total fresh weight and total dry weight of treated lettuce significantly decreased by 21.47 and 33.5%, compared to those of the control, respectively, which indicated the negative effect of ZnO QDs at a high concentration of 500 mg·L⁻¹. In response to this phenomenon, the chlorophyll contents were measured. The contents of chlorophyll a, chlorophyll b, and total chlorophyll were increased by 18.23, 42.82, and 18.22% only under 50 mg·L⁻¹ group. Groups of 100 and 200 mg·L⁻¹ treatments did not significantly alter the chlorophyll b and total chlorophyll contents, while 500 mg·L⁻¹ ZnO QDs decreased the chlorophyll a, chlorophyll b, and total chlorophyll contents by 17.37, 31.25, and 17.38%, compared to those of the control, respectively (Figures 3C and S3). Wang et al.³³ found that 300 mg·L⁻¹ ZnO nanoparticles could reduce the chlorophyll content and biomass of A. thaliana. We speculated that the
change of lettuce biomass might be due to the effect of ZnO on the pigment contents of lettuce leaves.

2.4. Analysis of Oxidative Stress. Confocal laser scanning microscopy (CLSM) of dichlorofluorescein (DCF) fluorescence was used to characterize the formation of ROS in the lettuce leaves. As depicted in Figures 4 and 5, no significantly increased fluorescence intensity was observed with the treatment of 50 and 100 mg·L⁻¹ ZnO QDs compared to the control. The fluorescence intensity increased obviously at 200 mg·L⁻¹ and reached the peak at 500 mg·L⁻¹ (Figure 4D,E). Differently, the fluorescence intensity of treated root showed that ROS generation increased significantly only at 500 mg·L⁻¹ (Figure 5E), which resulted in less accumulation of ZnO QDs in roots than in leaves (Figure S2).

In addition, the measurement of MDA content was used to analyze the stress induced by ZnO QDs. In Figure 6A, the shoot MDA content of lettuce exposed to 50 mg·L⁻¹ ZnO QDs was not significantly different from that of the control. With an increase of the concentration, the MDA contents of root and shoot both increased significantly. When the concentration reached 500 mg·L⁻¹, the MDA content also reaches the maximum, and the MDA contents of the shoot and root increased by 26.68 and 41.91% compared with that of the control, respectively.

The antioxidant enzymes were measured to study the antioxidant activity of ZnO QD-treated lettuce. From Figure 6B, compared with the control group, groups of 50, 100, and 200 mg·L⁻¹ remarkably increased the CAT activity of the lettuce shoot, while that of the 500 mg·L⁻¹ group significantly decreased. Only groups of 200 and 500 mg·L⁻¹ showed a significant increase in CAT activity compared to other groups. As can be seen in Figure 6C, all treated groups except the group of 500 mg·L⁻¹ significantly improved the SOD activity of lettuce. When the concentration was up to 500 mg·L⁻¹, the activity of root SOD obviously decreased. Peroxidase (POD) can catalyze H₂O₂ to react with acids and amines to reduce the damage to the cell membrane and stabilize the selective permeability of the membrane. It can be seen from Figure 6D that the effect of ZnO QDs on POD activity increased first and then decreased with an increase of ZnO QD concentration. When the exposure concentration reached 500 mg·L⁻¹, the POD activity decreased both in the shoot and root compared to that in the control (Figure 6D). Results above demonstrated that an increased concentration of ZnO QDs would improve the antioxidant activity of lettuce by enhancing its CAT, SOD, and POD activities. However, when the treated concentration is up to 500 mg·L⁻¹, the enhanced antioxidant activity induced by ZnO QDs would be ineffective.

Figure 5. ROS production in lettuce root after exposure to ZnO QDs. (A) Control Deionized water, (B–E) ZnO QD exposure of 50, 100, 200, and 500 mg·L⁻¹, respectively.

Figure 6. Lipid peroxidation. (A) MDA and the activity of antioxidant enzymes (B) CAT, (C) SOD, and (D) POD in lettuce shoot and roots exposed to different concentrations of ZnO QDs. Different letters indicate that ZnO QDs with different concentrations have significant differences at the P < 0.05 level. The letters in the figure that follows have the same meaning.
2.5. Effect on Shoot Soluble Protein and Soluble Sugar Contents. As shown in Figure 7A, the total soluble protein content of lettuce reached its maximum at 100 mg·L⁻¹ ZnO QD exposure, which was about 50.86% higher than that of the control. This may due to the stress response of the plant that promotes protein synthesis. When the ZnO QD concentration was up to 500 mg·L⁻¹, the soluble protein content decreased by 31.1%. The total soluble sugar contents of treated groups with concentration less than 500 mg·L⁻¹ all increased by 11.87, 31.51, and 33.81% compared to the control group, respectively. However, the total soluble sugar content of the 500 mg·L⁻¹ group significantly decreased by 33.62% (Figure 7B). Results above demonstrated the high toxicity of 500 mg·L⁻¹ ZnO QDs for lettuce growth.

2.6. Effects on the Absorption of Nutrient Elements. To explore the effect of ZnO QDs on plant nutrient absorption, we measured the content of macronutrients (Ca, Mg) and micronutrients (Zn, Fe, Mn, B). Compared to the control group, 50−200 mg·L⁻¹ ZnO QD exposure significantly increased the accumulation of Ca, Fe, and Mn from root and shoot. As for the lettuce shoot (Figure 8A), compared to the control group, at the exposure of 500 mg·L⁻¹ in the shoot, Ca and Mn contents were decreased by 11.87, 33.81% and 33.62% compared to the control group, respectively. Mg contents were increased by 14.32 and 15.24% at 100 and 200 mg·L⁻¹, respectively. The Zn content in the shoot increased with an increase of ZnO concentration and reached the maximum at 500 mg·L⁻¹, demonstrating the absorption of ZnO QDs. In the lettuce root (Figure 8B), relative to the control group, the content of Mg, Mn, and B in the root decreased significantly when the concentration was 500 mg·L⁻¹. Exposure of 200 and 500 mg·L⁻¹ significantly increased the contents of Zn by 34.56 and 47.22%, respectively.

3. DISCUSSION

3.1. Oxidative Stress Response and Lipid Peroxidation. ROS in plants can play the role of signal, but excessive ROS will oxidize proteins and lipids, and ultimately affect plant growth. The existence of nanomaterials will produce oxidative stress on the plant, thus affecting the absorption of nutrients and water. Investigations have shown that nanoparticles can produce stress, generating excess ROS, which directly affects the enzyme activity in plants. The accumulation of MDA is the result of ROS on membrane lipid damage in plants. In this study, high concentrations (500 mg·L⁻¹) of ZnO QDs could significantly increase the content of ROS and MDA, which indicated that high concentrations of ZnO QDs induced stress in lettuce, resulting in membrane lipid peroxidation, which caused serious damage to lettuce. Similar results have been found in previous studies on nanoparticles.
SOD, CAT, and POD are important enzymes involved in antioxidant defense systems. Generally, when plants are under external stress, a large number of active oxygen in vivo will be produced. These antioxidant enzymes are very essential for the removal of excessive O$_2^-$ and H$_2$O$_2$. Studies have shown that nanomaterials can induce oxidative stress and change the activity of antioxidant enzymes. In this study, we found that the activity of antioxidant enzymes in the shoot was more sensitive than that in the root when lettuce was treated with ZnO QDs. Also, at low concentrations of ZnO QDs, SOD, POD, and CAT activities increased significantly in a dose-dependent manner. When the concentration reached up to 500 mg L$^{-1}$, the activities of antioxidant enzymes were significantly decreased, except for the CAT activity of root, demonstrating the stress that lettuce could not bare at 500 mg L$^{-1}$ concentration of ZnO QDs. Similar results have been reported in other nanomaterials.

3.2. Analysis of Nutritional Quality. Hayes et al. reported that nanoparticles could affect the uptake of essential elements needed for plant growth and balanced nutrition. The accumulation of nutrient elements is an effective method to test how nanomaterials affect the nutritional quality of plants. Essential nutrients elements such as Ca, Zn, Fe, Mn, and B and Mg play important roles in the growth of plants. The increased content of nutrient elements in leaves can improve the nutritional quality of the edible tissues. In this work, lettuce being exposed to the lower concentrations from 50 to 200 mg L$^{-1}$ increased the accumulation of essential elements, such as Ca, Fe, Mn, and Zn, but the content of Ca, Fe, Mn, and B decreased when treated with high concentrations (500 mg L$^{-1}$). The content of nutrients can directly increase the chlorophyll content in leaves. For example, the enzyme catalyzing chlorophyll synthesis needs the activation of Fe, while the photosynthesis of water for photosynthesis needs Mn participation, thereby increasing the photosynthetic rate and biomass of leaves.

Chlorophyll is the pigment that plants use to absorb light energy for photosynthesis. The increase of chlorophyll content of plants is conducive to the light absorption efficiency of photosynthesis and the formation of carbohydrates. The impact of nanomaterials on photosynthesis has also attracted more and more attention. The soluble protein content is closely related to photosynthetic pigments, which can directly affect the photosynthesis capacity. In this work, soluble protein content was increased by 6.12 and 50.86% at 50 and 100 mg L$^{-1}$, respectively. Also, the contents of chlorophyll were significantly increased at 50 mg L$^{-1}$. Hu et al. also reported a similar effect, they found that the treatment of lettuce with nTiO$_2$ increased the content of soluble protein and chlorophyll. However, a significant decrease in chlorophyll content was observed when exposed to 500 mg L$^{-1}$ ZnO QDs. It might be due to an increase of MDA content, which damaged the chloroplast membrane, resulting in a decrease in chlorophyll content. The results are consistent with the previous findings of Sturikova et al. When lettuce was treated with low-concentration zinc oxide QDs, the soluble protein and soluble sugar content increased significantly, indicating that the nutritional quality of lettuce has been improved, with potential applications in agriculture. At the same time, the accumulation of soluble protein and soluble sugar in plants is conducive to inducing antioxidation and improving plant resistance. Both soluble protein and carbohydrate content maintain a certain degree of metabolic balance in the organism, and when the plant is subjected to external stress, this balance will fluctuate. High-concentration ZnO treatment (500 mg L$^{-1}$) can significantly reduce the content of soluble proteins, which may be due to the damage of cell membrane lipid, thus inhibiting protein synthesis.

4. EXPERIMENTAL SECTION

4.1. Synthesis of ZnO Quantum Dots. Zinc acetate dihydrate (Zn (CH$_3$COO)$_2$·2H$_2$O), ethanol absolute (CH$_3$CH$_2$OH, 99.7%), 3-aminopropyl triethoxysilane (APTES), and potassium hydroxide (KOH) were purchased from McLean. All chemicals were of analytical grade and used without further purification.

Water-soluble APTES-coated ZnO QDs were synthesized by a simple sol–gel method. In brief, 16.7 mmol zinc acetate dihydrate was dissolved in 50 mL of ethanol solution followed by vigorous stirring and refluxed for 2 h at 78 °C. When zinc acetate was completely dissolved, the above solution was placed in a cold bath at room temperature. Meanwhile, 23.4 mmol KOH was dissolved in 13.5 mL of ethanol with sonication. The KOH solution was dropped into zinc acetate dihydrate solution under intense stirring at room temperature until it became colorless and transparent. APTES (800 µL) was mixed with 2 mL of deionized water and then poured into the above-mentioned ZnO QD solution; APTES was hydrolyzed in water and reacted with ZnO QDs to form silane-modified ZnO QDs. After the mixture was centrifuged, a white precipitate was obtained. The precipitate was then washed three times with ethanol to remove unreacted impurities. Finally, the precipitated ZnO QD powder obtained by heating in an oven at 60 °C for 12 h.

4.2. Characterization of ZnO Quantum Dots. To obtain the structure and morphology of the samples, high-resolution transmission electron microscopy (HRTEM, JEOL-2010) images were collected. The powder X-ray diffraction (XRD) patterns were measured in the range of 2θ from 10 to 80°. The infrared spectrum was recorded by a Nicolet 6700. Fourier transform infrared (FTIR) spectra were collected at a wavenumber of 500–4000 cm$^{-1}$, and the chemical composition of the sample was analyzed with KBr as a reference. Photoluminescence (PL) spectra were recorded with a Hitachi FL 7000 fluorescence spectrophotometer. X-ray photoelectron spectroscopy (XPS) spectra were obtained using an X-ray photoelectron spectrometer (AXIS ULTRA DLD, Kratos).

4.3. Plant Growth and Treatment. Lettuce seeds were seeded in the sponge blocks of hydroponics, and when the third true leaf of the seedling appeared, they were transferred to planting cups for the deep solution flow culture. After 2 weeks, different concentrations of ZnO QD water solutions were sprayed by foliar spraying. ZnO QD solutions with different concentrations (0, 50, 100, 200, 500 mg L$^{-1}$) were prepared in deionized water and then sprayed on the leaves once every 3 days until harvesting. Each lettuce was sprayed with 4 mL of ZnO QD water solutions on average 5 times during the treatment.

4.4. Growth Parameters and Photosynthetic Pigment Measurements. After harvesting, plants were thoroughly washed with tap water and then rinsed three times with deionized water to remove dust and residual ZnO QDs. Then, the shoot and root tissues were separated and weighed on a balance. Then, the shoot and root tissues were dried in an oven at 70 °C for 2 days to determine their dry weight (DW). Meanwhile, fresh leaf tissues (0.5 g) were soaked in a 50 mL...
centrifuge tube containing 25 mL of absolute ethanol and extracted for about 24 h until the leaves turned white. Then, the absorbance of the supernatant was measured at 470, 646, and 663 nm by a UV–vis spectrophotometer.

4.5. Physiological Parameters. The physiological parameters were determined by a UV–vis spectrophotometer. The activity of superoxide dismutase (SOD) was determined by the photoreduction method of nitrogen blue tetrazole, and the peroxidase (POD) activity was measured by the guaiacol method. The determination of catalase (CAT) activity was estimated using the method following Aebi. The content of malondialdehyde (MDA) was evaluated by a thiobarbituric acid (TBA) reaction. The soluble protein content was analyzed using the dyeing method with Coomassie brilliant blue G-250. The content of soluble sugar was estimated by anthrone colorimetry.

4.6. Imaging of ROS after ZnO QD Exposure. 2′,7′-Dichlorofluorescein diacetate (DCFH-DA) was used to qualitatively evaluate ROS in plants. DCFH-DA can be hydrolyzed by esterase to produce DCFH. However, DCFH does not penetrate the cell membrane, so the probe is easy to accumulate in the cell. DCFH can be oxidized with cellular ROS to form fluorescent DCF. The green fluorescence intensity is directly proportional to the level of ROS. Leaf and root slices were immersed in pH 7.0 phosphate buffered saline (PBS) buffer containing 10 μM DCFH-DA, incubated for 30 min, and then washed three times continuously to remove excess DCFH-DA. Finally, the fluorescence image was observed under a confocal laser scanning microscope (CLSM) with an excitation wavelength of 488 nm and an emission wavelength of 530 nm.

4.7. Elemental Content Analysis. Lettuce shoot and root were thoroughly washed three times with deionized water to remove dust and residual ZnO QDs. Then, the shoot and root tissues were dried in an oven at 70 °C for 2 days. The dry sample was pulverized into powder and 300 mg were put into centrifugal tubes. The contents of Ca, Mg, Zn, B, Fe, and Mn were successfully determined by flame atomic absorption spectrometry.

4.8. Statistical Analysis. Statistical analysis was performed using IBM SPSS (version 22, SPSS, Inc.). The mean values were compared using analysis of variance (ANOVA), and the results were reported as mean ± standard deviation (SD). The P value below 0.05 indicates a statistically significant difference.

5. CONCLUSIONS
In the present work, foliar application of ZnO QDs could influence the antioxidant status of lettuce, thereby affecting the changes of antioxidant enzyme activity, chlorophyll content, soluble protein, and biomass. ZnO QD exposure at low levels (50, 100, 200 mg·L⁻¹) significantly improved the lettuce biomass and nutritional quality because ZnO QDs promote the uptake and translocation of nutrients. However, when lettuce was exposed at 500 mg·L⁻¹, increased ROS fluorescent intensity and MDA content and decreased CAT, POD, and SOD activities were observed. In this study, the preliminary relationship between ZnO QDs and lettuce growth was understood. Nevertheless, additional work should be done to explore the potential mechanism of ZnO QD impact on plants for agricultural and biotechnology applications.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c00205.

Experimental details of lettuce cultivated in no Zn²⁺ nutrient solution, XPS results for Zn QDs, and Zn content and photosynthetic pigments of treated lettuce (PDF)

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Notes
The authors declare no competing financial interest.

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