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Effects of foliar application of graphene oxide on cadmium uptake by lettuce

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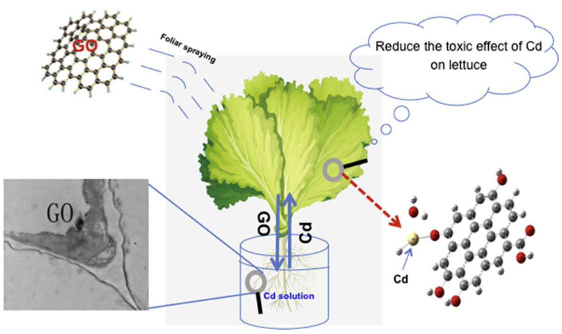
GRAPHICAL ABSTRACT



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ABSTRACT

Although graphene oxide (GO) has been widely used to enhance soil quality and crop yield, there is currently little information regarding the effects of foliar application of GO on cadmium (Cd) toxicity to plants. In this study, we investigated the response to GO in lettuce cultivated under Cd stress in hydroponic conditions. Lettuce was grown from seeds in a nutrient solution supplemented with 2 mg/L Cd and the leaves were sprayed with 0, 30, and 60 mg/L GO. The results indicated that application of 30 mg/L GO significantly increased the total length, surface area, average diameter, and hair number of lettuce roots, and effectively alleviated the negative effects of Cd on root growth. Furthermore, foliar application of 30 mg/L GO, but not 60 mg/L GO, significantly improved the quality of lettuce, including reduction in Cd accumulation in leaves and roots and increase in soluble sugar, protein, and vitamin C content. Transmission electron microscopy revealed that GO nanoparticles, which entered the leaves and were subsequently transported to the roots via the vascular system (phloem), reduced the damaging effect of Cd on cellular organelles, including the cell wall and membrane, chloroplasts, and starch granules. The effect may be attributed to the absorption of GO by lettuce cells, where it fixed Cd^{2+}, thus reducing Cd^{2+} bioavailability, or to the improvement of Cd tolerance through regulation of lettuce metabolic pathways. Gaussian simulation analysis revealed that Cd caused significant changes in the GO molecule, resulting in detachment of an epoxy group from the GO carbon ring and breakage of O—H bonds in hydroxyl groups, whereupon the oxygen freed from the O—H bond formed a new bond with Cd. Collectively, these results indicate that foliar application of 30 mg/L GO can enhance the tolerance of lettuce to Cd, promote plant growth, and improve nutritional quality.

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1. Introduction

Heavy metals, which are highly toxic to plants, animals, and humans, are major environmental pollutants worldwide. Among these metals, Cd is one of the contaminants most commonly released into soil and water, as a consequence of various industrial activities, including mining and plastic manufacturing, and agricultural practices, such as the use of Cd-containing fertilizers and pesticides (Mishra et al., 2006). Cd can be absorbed from the soil by plant roots and is subsequently transported to the stems and leaves, where it suppresses photosynthesis and respiration and reduces the uptake of water and nutrients, causing irreversible damage to cellular structures and resulting in reduced crop yields (Bedoui et al., 2008; Dias et al., 2013; He et al., 2015). For example, Romero-Puertas et al. (2002) found that Cd altered the permeability of plant cell walls and promoted protein degradation, thus negatively affecting plant growth. Furthermore, many studies have indicated that Cd-contaminated plant material can pose a threat to both animal and human health upon transmission through the food chain (Keller et al., 2015; Bayçu et al., 2017). Therefore, environmental pollution due to Cd has become a global concern, and effective measures aimed at reducing Cd toxicity to plants should accordingly be developed.

Rapid advances in carbon nanoscience and nanotechnology have provided innovative solutions to various problems in industry and agriculture. Graphene oxide (GO), a two-dimensional carbon nanomaterial, has been widely used in heterogeneous catalyses, flow reactor technology, biotechnology and biomedicine, the manufacture of polymer composites, energy storage, and environmental protection (Dreyer et al., 2014). Therefore, it is very important to assess the ecological risk and health effects of GO before its application. Some studies indicate that GO has a better biocompatibility with living organisms when compared to other nanomaterials (Hu and Zhou, 2013; Akhavan et al., 2012; Ruiz et al., 2011) and may exert positive effects on crop plants (Younes et al., 2019; Anjum et al., 2014). Accordingly, GO has been widely applied as an agent regulating plant loading with micronutrients and preventing phytotoxicity of soil contaminants to improve crop yield (Kabiri et al., 2017; Rizwan et al., 2019). However, few studies have investigated the effects of GO in plants contaminated with heavy metals, and the results are sometimes contradictory (Hu et al., 2014; Gurunathan, 2015; Cheng et al., 2016; Rizwan et al., 2019). For example, Yin et al. (2018) found that GO at 100–1500 mg/L reduced the detrimental effects of Cd^{2+} on the growth of rice buds and seeds under hydroponic conditions, which was similar to the effect of foliar spraying of zinc oxide nanoparticles on corn grown in Cd-polluted soil (Rizwan et al., 2019). At the same time, Hu et al. (2014) observed that root application of GO (0.1–10 mg/L) enhanced the toxicity of arsenic to wheat. Consistent with these results, our previous studies also indicate that GO application to wheat seedling roots increased Cd accumulation and reactive oxygen species production, resulting in cell damage and biomass reduction (Gao et al., 2019a,b). Overall, these findings suggest that the effects of GO on plants treated with heavy metals may depend on GO dosage, application route, heavy metal type, and plant species. Therefore, further studies are necessary to ascertain the safety of using GO to alleviate Cd toxicity to particular plants, including important crops.

Molecular dynamics is a numerical simulation method that can be used to analyze the physical movements of and interactions between atoms and molecules based on the classical equations of motion, which enables examination of the structure, equilibrium, and dynamic characteristics of molecular systems (You et al., 2019). In this regard, Gaussian, a computational software package for quantum chemistry that is widely used in chemistry, biochemistry, physical chemistry, chemical industry, and other fields, represents a powerful tool for the analysis of substitution effects, reaction mechanisms, potential energy surfaces, excited state energy levels, and molecular dynamics. For example, Borthakur et al. (2016) applied Gaussian 03 software to simulate the interactions of inorganic ions within the GO slipping plane and demonstrated that the effects on GO electrokinetic potential are ion-specific and depend on ion polarizability. To date, however, Gaussian has rarely been applied in environmental studies to elucidate the molecular mechanisms underlying the effects of various pollutants, including heavy metals. Therefore, in the present study, we investigated the feasibility of using Gaussian to examine the mode of interaction between GO and Cd in plants.

The specific aims of this study were to determine the effects of foliar application of GO on the Cd accumulation, cell structure, and quality of lettuce grown under Cd stress, and to examine the interaction between GO and Cd at the atomic level by molecular dynamics simulation.

2. Materials and methods

2.1. Reagents

Cd stock solution (1000 mg/L) prepared from $CdCl_2$ (99.0 % purity) was obtained from the Hengshan Company (Tianjin, China). The standard sample of Cd (II) (GBW07604[GSV-3]) was provided by the Center of National Standard Reference Material of China (Beijing, China) Deionized water was prepared using a Millipore-Q water purification system (Direct-Q 3, 5, 8; Merck Millipore, France). Plant soluble sugar, protein, and vitamin C contents were measured using specific kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). All other chemicals were of analytical grade and were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd (Tianjin, China).

2.2. Characteristics of GO

GO (thickness: 0.8–1.2 nm; purity: 96.0 %) was obtained from Jining Lite Nano Technology Co., Ltd (Jining, China). Scanning electron microscopy was performed using JSM-7500 F (JEOL, Japan). Raman spectrometer (Thermo Fischer DXR, America) was used for the Raman spectroscopy study, and the test laser wavelength was 532 nm. Samples were prepared and dispersed in absolute ethanol, and transmission electron microscopy (TEM) and zeta potentials determination were then performed on a JEM-2100 F (JEOL, Japan) or ZS-90 (Malvern, UK), respectively.

2.3. Lettuce culture

Lettuce (*Lactuca sativa* L. var. *ramosa* Hort.) seeds were provided by Shandong Shouguang Xinrun Horticulture Co., Ltd (Shandong, China). Seeds were sterilized by immersing in 5% sodium hypochlorite solution for 15 min, rinsed thoroughly with distilled water, placed in a seedling tray containing distilled water, and cultivated in an artificial climate chamber under the following conditions: a 12 h/12 h light-dark cycle, 60 % relative humidity, and temperature of 25 ± 1 °C.

2.4. Exposure experiment

When lettuce seedlings had produced two true leaves and one apical bud, three plants of a similar size and shape were selected, gently washed with deionized water to avoid root damage, and transplanted into pots containing 1/4 Hoagland nutrient solution with or without or with Cd (2.0 mg/L). Thereafter, the lettuce leaves received foliar applications of 40 mL GO at concentrations of 0, 30 or 60 mg/L prepared by homogenization for 30 min using a KQ5200DE ultrasonic cleaner (Kunshan, China), and then evenly sprayed with a watering pot until the solution was dripping from the leaves; 1/4 Hoagland nutrient solutions in pots was similarly replenished at 7-day intervals. All treatments were performed in triplicate. The lettuce plants were cultured in the artificial climate chamber under the same conditions described in Section 2.2. After 28 days of growth, plants were collected for analysis.

2.5. Physiological and biochemical analyses

2.5.1. Root morphology

Lettuce plants were washed repeatedly with distilled water and dried with filter paper. Root growth indices, namely total length, surface area, average diameter, and hair number, were analyzed using an Expression imager (Epson Electronics America Inc., Wakefield, MA, USA).

2.5.2. Cd content

Lettuce leaves were washed with deionized water. Roots were washed with 20 mmol/L Na-EDTA for 10 min to desorb Cd from the root surface and then rinsed repeatedly with deionized water. Samples were dried at 65 ± 1 °C until a constant weight had been attained, ground, and aliquots (0.25 g) of each sample were placed in plastic dissolving tanks, to which 7 mL of HNO_3 was added. After sample were digested at 115 ± 1 °C until the solution was transparent, the inner tank was removed from the acid rack, and the solution was gradually heated at 180 °C until the liquid evaporated. The resulting residue was then dissolved in 2 mL of 30 % H_2O_2 and filtered. Reagent blank and standard samples were included in every experimental replication for quality control. After adjusting the volume to 25 mL with ultrapure water, Cd content was measured by atomic absorption spectroscopy (Zeenet 700; Analytik-Jena AG, Jena, Germany) at 228.8 nm. Cd^{2+} standards were analyzed with every 10 samples. Relative standard deviation was < 2.4 %, and the percentage recovery was 94.4 %–102 %. The detection limit of atomic absorption spectroscopy for Cd was 0.05 mg/L.

2.5.3. Lettuce quality analysis

Samples of leaves or roots (0.1 g) were homogenized in 1 mL of distilled water in an ice bath, transferred into capped centrifugal tubes, and centrifuged at 8000 × g and 4 °C for 10 min. The supernatants were used to measure soluble sugar content using the anthrone method at 620 nm, protein content using a bicinchoninic acid assay at 562 nm, and vitamin C content using a double-antibody sandwich enzyme-linked immunosorbent assay at 450 nm. About 20 % of samples (roots or leaves) were analyzed twice to ensure reproducibility.

2.5.4. Transmission electron microscopy

Lettuce leaves or roots were immersed in 20 mM phosphate buffer, pH 7, cut into 1–2-mm pieces using a new scalpel, and immediately fixed in 3% glutaraldehyde solution in phosphate buffer at room temperature for 2 h, followed by staining in 2% osmium tetroxide in the same buffer at room temperature for 1 h. The samples were washed twice in 20 mM phosphate buffer (pH 7) and dehydrated in a graded acetone series (10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, and 90 %, and twice in 100 %) for 20–30 min per step. The dehydrated samples were subsequently embedded in epoxy resin (ETON 812), polymerized in an oven at 70 °C, cut into 50–60-nm sections using an ultramicrotome (EM UC6; Leica, Wetzlar, Germany), and observed and photographed under a transmission electron microscope (JEM1230; JEOL Ltd., Tokyo, Japan).

2.6. Molecular dynamics analysis

Gaussian view 6 version (32-bit Windows, V32128445015899W-5259 N; Gaussian Inc., Wallingford, CT, USA) was used to analyze the interaction between GO and Cd. The structure of GO comprising six rings was built according to the report of De Mendonça et al. (2018) and optimized via B3LYP/6-31 molecular geometry using Gaussian 16 W (G32131694014899W-5259 N; Gaussian Inc.). The generated chk format file was converted to the fch format using Gaussian 16 W and molecular surface quantitative analysis of the fch file was performed using a multifunctional wavefunction analyzer software (Beijing Kein Research Center for Natural Sciences, Beijing, China) to calculate electrostatic potential distribution on the GO surface, which was visualized using visual merchandize design. In the images thus obtained, bluer and redder areas represent more negative and more positive electrostatic potentials, respectively. The % chk = cd.chk instruction was used to name the output file, % nprocshared = 4 to call four processors for calculation, and % mem = 1GB to call 1 GB computational memory. After that, the process of Cd adsorption to GO optimized at the level of rmp2/3-21 G based on Cartesian coordinates (opt = Cartesian) was simulated using Gaussian 16 W.

2.7. Statistical analysis

All data were analyzed using SPSS 20.0 statistical software (SPSS Corporation, Chicago, IL, USA) and presented as the mean ± standard deviation. Duncan's multiple range test was used to evaluate differences between treatment groups. A P-value < 0.05 was considered to indicate statistical significance.

3. Results

3.1. Characteristics of GO

SEM analysis indicated that GO had a sheet structure with surface ripples and wrinkles (Fig. S1), suggesting that GO had a peculiar surface area with good flexibility (Wang et al., 2012). TEM images revealed that GO in the water phase looked like gauze, with obvious edges and wrinkles (Fig. S2). The zeta potential was negative (about 29 mV), showing good stability (Fig. S3). The Raman spectrum (Fig. S4) demonstrated a prominent D-band (1360 cm^{-1}) indicating structural defects caused by the attachment of hydroxyl and epoxide groups on the carbon basal plane, and a wider G-band (1580 cm^{-1}) representing the symmetry and order of the GO structure. The intensity ratio (I_D/I_G) of the D and G bands was 0.91, which was dramatically higher than that of typical graphene, indicating that the oxygen-containing functional group was connected with the carbon atom to form an sp^3 hybrid bond with disordered structure, leading to a higher degree of disorder in GO (Chen et al., 2012).

Table 1
Effects of GO foliar application on lettuce roots morphology without and with Cd.

Cd content (mg/L)	GO concentration (mg/L)	Total root length (cm)	Total root surface area (mm^2)	Average root diameter (mm)	Root hairs number (unit)
0	0	216 ± 1.7b	21.1 ± 1.4b	0.35 ± 0.006a	497 ± 6.0b
	30	235 ± 4.1a	23.3 ± 0.8a	0.36 ± 0.006a	516 ± 3.1a
	60	221 ± 4.1b	22.5 ± 0.4b	0.35 ± 0.008a	506 ± 3.5b
2	0	178 ± 7.5b*	18.7 ± 0.6b*	0.29 ± 0.009b*	238 ± 5.5c*
	30	192 ± 7.9a	20.4 ± 0.5a	0.32 ± 0.012a	291 ± 5.3a
	60	181 ± 6.9b	19.7 ± 0.7ab	0.31 ± 0.009a	264 ± 5.7b

Different letters indicated statistically significant differences ($P < 0.05$) based on Duncan tests. The * indicated a significant difference between the Cd alone and the control.

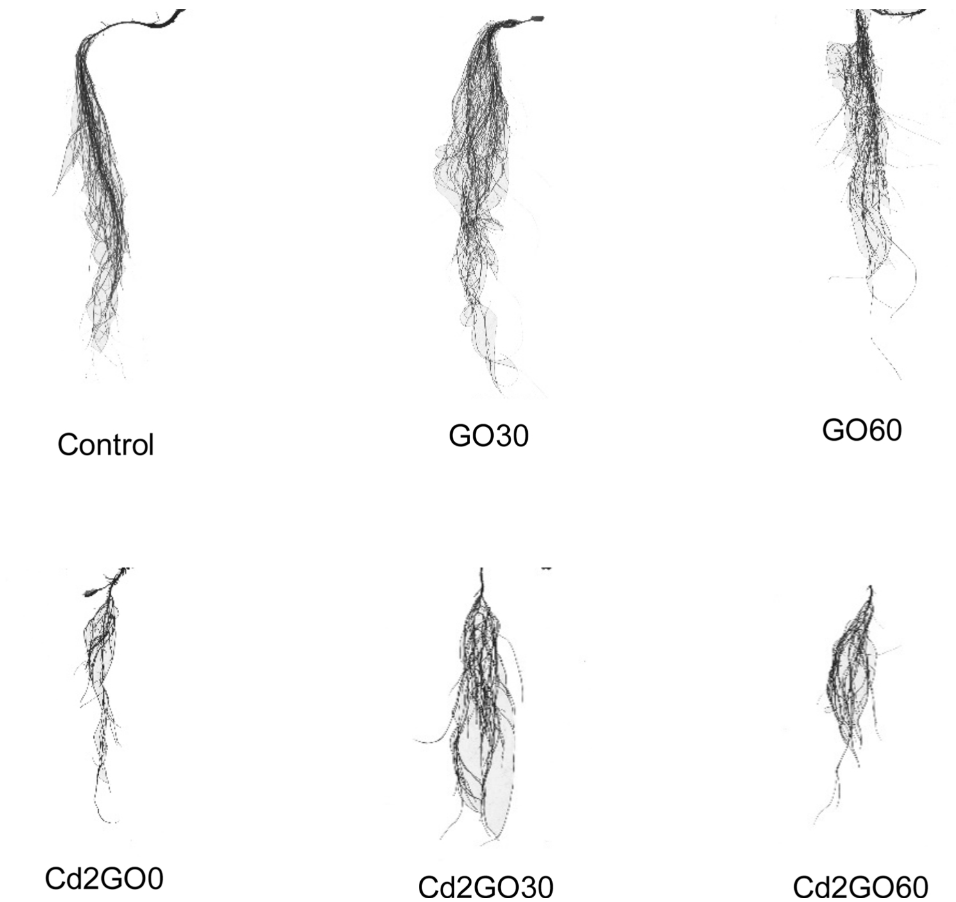


Fig. 1. Root morphology in lettuce treated with Cd and graphene oxide (GO). Control, untreated plants; GO30, plants sprayed with 30 mg/mL GO; GO60, plants sprayed with 60 mg/mL GO; Cd2GO0, plants grown in the presence of 2 mg/mL Cd; Cd2GO30, plants grown in the presence of 2 mg/mL Cd and sprayed with 30 mg/mL GO; Cd2GO60, plants grown in the presence of 2 mg/mL Cd and sprayed with 60 mg/mL GO.

3.2. Effects of the foliar application of GO on lettuce roots subjected to Cd stress

The effects of GO on the root parameters of lettuce grown with or without Cd are shown in Table 1. In the absence of Cd, the total length of lettuce roots was increased by 8.7 % after spraying with 30 mg/L GO, whereas application of 60 mg/L GO had no significant effects compared to the control. In plants treated with 30 mg/L GO and Cd, the total root length exceeded that of plants treated with Cd alone ($P < 0.05$), whereas application of 60 mg/L GO with Cd had no effect compared to Cd alone ($P > 0.05$). Changes in the total root surface area and root hair number were found to be consistent with those observed for total root length.

In the absence of Cd, foliar application of GO was found to have no significant effect on the average root diameter of lettuce plants. Compared with the control, Cd alone significantly decrease the average root diameter, whereas spraying plants with GO reversed this effect ($P < 0.05$ compared with the Cd group).

Analysis of root morphology confirmed the effects of Cd and GO on root growth (Fig. 1), by showing that foliar application of 30 mg/L GO alleviated the toxic effects of Cd on lettuce roots, whereas treatment with 60 mg/L GO resulted in only slight improvement.

3.3. Effects of the foliar application of GO on Cd uptake in lettuce

Fig. 2 shows the effects of GO application on Cd accumulation in lettuce roots and leaves. In Cd-exposed plants, spraying with 30 mg/L reduced Cd content in the roots and leaves by 19.9 % and 37.6 % ($P < 0.05$), whereas 60 mg/L GO reduced it by 6.6 % and 9.5 % ($P > 0.05$), respectively, compared to Cd alone.

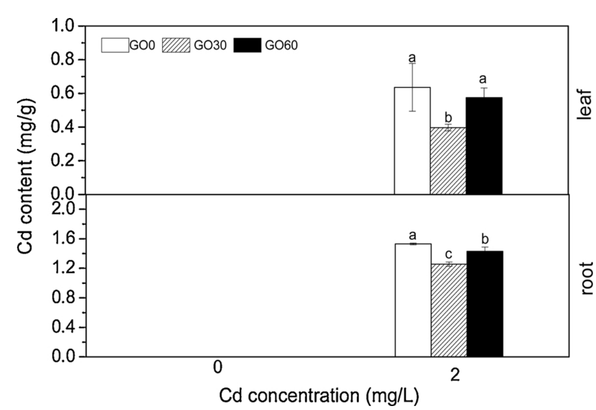


Fig. 2. Effect of graphene oxide (GO) on Cd content in lettuce. Different letters indicate statistically significant differences ($P < 0.05$) based on the Duncan test. GO0, no foliar application of GO; GO30, foliar application of 30 mg/mL GO; GO60, foliar application of 60 mg/mL GO.

3.4. Ultrastructure of lettuce cells

Analysis of cellular ultrastructure indicated that the cell walls and membranes of root cells (Fig. 3a) and chloroplasts containing normal starch granules in the leaves (Fig. 3b) were intact in the control group. However, in plants exposed to 2 mg/L Cd, we observed damage to the cell wall and disruption of the cell membrane in lettuce root cells (Fig. 3c), whereas in leaves, this treatment was found to promote leakage of the cytoplasm and disintegration of the compact chloroplast structure (Fig. 3d). However, the deposition of GO in roots (vascular organs) and leaves (chloroplasts and other organelles) following foliar application was found to alleviate the cellular damage caused by Cd

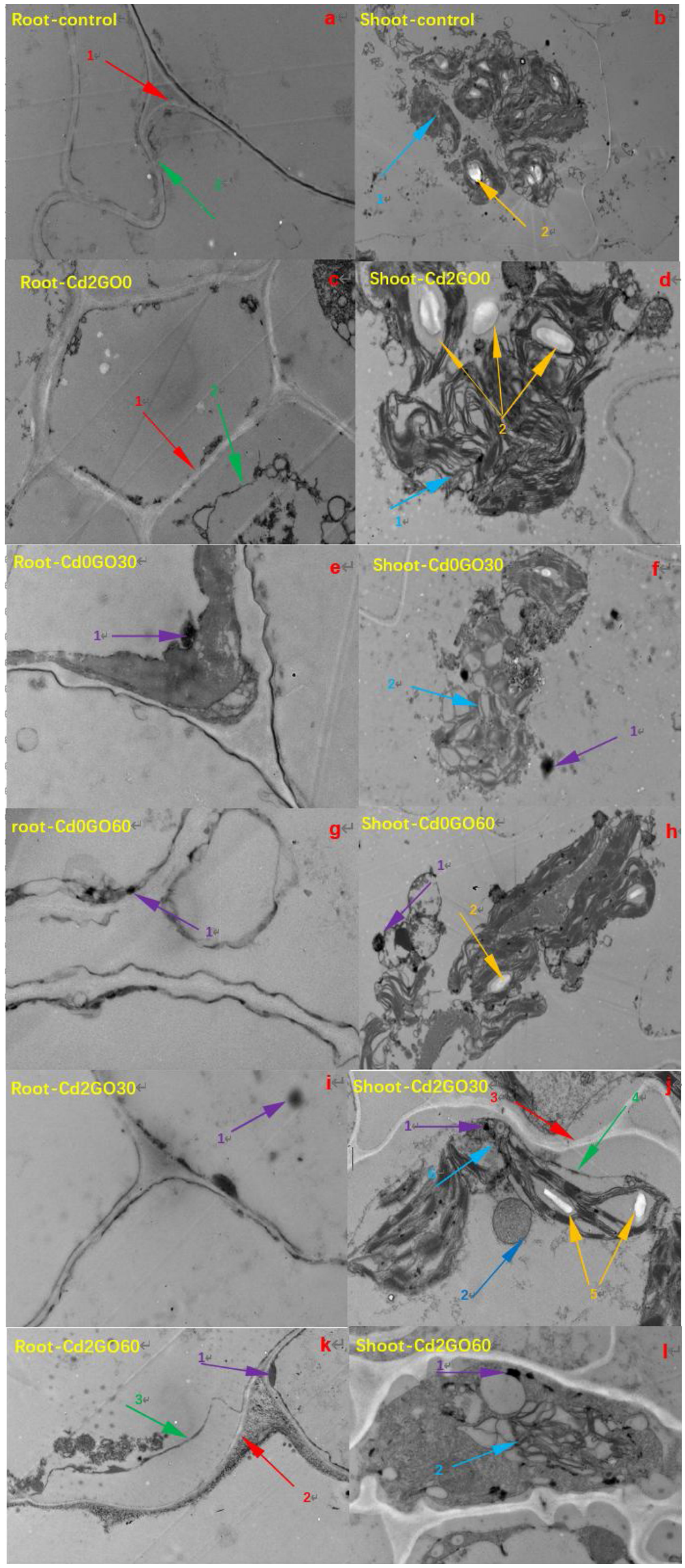


Fig. 3. Transmission electron microscopy images of lettuce cells. Root (a, c, e, g, i, k) and shoot (b, d, f, h, j, l) cells of control plants (a, b) and plants grown with 2 mg/mL Cd (c, d), sprayed with 30 mg/mL graphene oxide (GO) (e, f), sprayed with 60 mg/mL GO (g, h), grown with Cd and sprayed with 30 mg/mL GO (i, j), grown with Cd and sprayed with 60 mg/mL GO (k, l). Arrows in different colors indicate the following: green, cell membrane; red, cell wall; light blue, chloroplasts; navy blue, nucleus; purple, GO nanoparticles; yellow, starch granules.

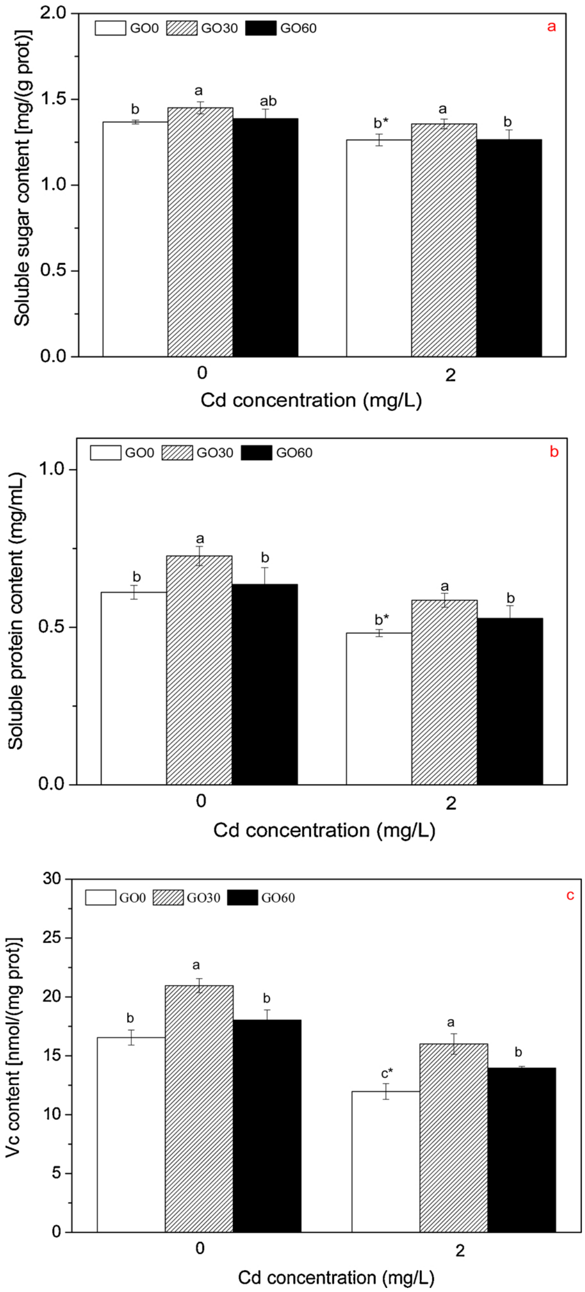


Fig. 4. Effects of graphene oxide (GO) spraying on (a) soluble sugar, (b) soluble protein, and (c) vitamin C content in lettuce treated with Cd. Different letters indicate statistically significant differences ($P < 0.05$) based on the Duncan test; the asterisk indicates significant difference between the Cd and control groups.

(Fig. 3c–i). Specifically, in plants treated with Cd plus 30 mg/L GO, we observed that the ellipsoidal shape of chloroplasts, compact chloroplast structure, and some irregular starch granules in leaves were relatively intact (Fig. 3i, j), whereas in plants treated with Cd plus 60 mg/L GO, although the integrity of root cell walls and membranes was preserved (Fig. 3k), chloroplast structure still showed evidence of slight damage (Fig. 3l).

3.5. Effect of foliar application of GO on the quality of lettuce under Cd stress

Treatment of lettuce plants with Cd alone resulted in a significant reduction in soluble sugar and protein content in lettuce leaves, However, foliar application of 30 mg/L GO to plants grown in Cd-supplemented medium significantly increased soluble sugar and protein content ($P < 0.05$), whereas that 60 mg/L, GO caused only a slight increase in soluble protein (Fig. 4a, b) compared to treatment with Cd alone.

Treatment with Cd alone caused a 27.7 % reduction in the vitamin C content of lettuce leaves compared to control ($P < 0.05$), whereas foliar application of 30 mg/L GO alone significantly increased it ($P < 0.05$) (Fig. 4c).When lettuce was exposed to Cd together with 30 and 60 mg/L GO, vitamin C content increased by 33.7 % and 16.7 %, respectively, compared to treatment with Cd alone (both $P < 0.05$; Fig. 4c).

3.6. Mechanism of interaction between Cd and GO

3.6.1. GO surface electrostatic potential

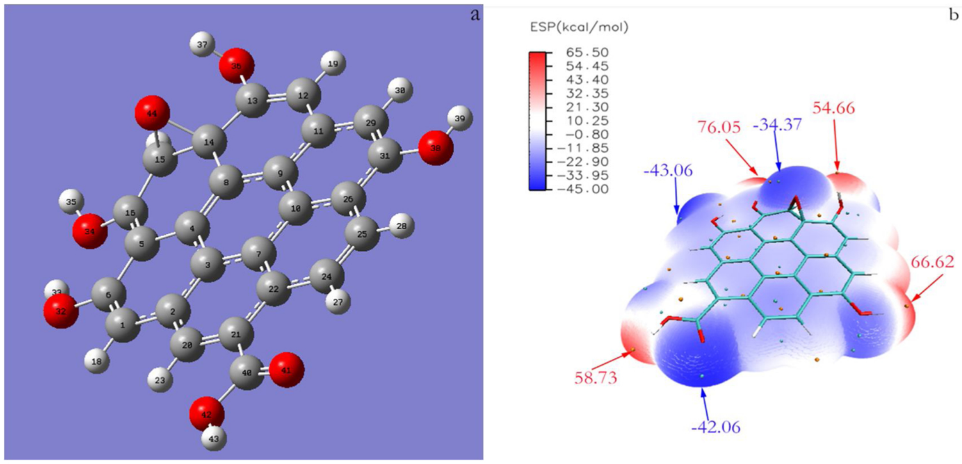
The optimized structure obtained for GO is shown in Fig. 5a. Analysis of the surface distribution of electrostatic potential indicated that the GO surface was predominantly characterized by a negative charge (Fig. 5b). The maximum electrostatic potentials of hydrogen and oxygen atoms in the hydroxyl group were +70.05 and -43.06 kcal/mol, respectively, and in the carboxyl group they were 58.73 and -42.06 kcal/mol, respectively. We also identified a zone with weak negative potential in the central π region of the benzene ring. Quantitative assessment of the electrostatic potential according to surface area confirmed that the molecular surface of GO had a primarily negative electrostatic potential (-20–0 kcal/mol) (Fig. 5c).

3.6.2. Structural changes in GO caused by Cd adsorption

Fig. 6 shows alterations in the GO structure induced by Cd adsorption. Simulation analysis indicated that the relative positions and structures of Cd and GO underwent changes in response to Cd adsorption on the GO surface. The epoxy ring on the GO surface was broken, an oxygen (44) was dissociated from the surface, a hydroxyl bond (O36-H37) was disrupted, and a free oxygen (36) formed a linkage with Cd (45), whereas a free hydrogen (46) bonded with oxygen (44). The distance between Cd (45) and oxygen (36) decreased from 4.58 to 2.16 Å and that between Cd (45) and oxygen (44) increased from 1.62 to 2.26 Å, whereas the distance between hydrogen (37) and oxygen (36) increased from 0.95 to 1.71 Å and that between hydrogen (46) and oxygen (44) decreased from 1.64 to 0.98 Å. Subsequent to Cd adsorption, the bond angle of the non-participating portion of GO changed slightly, whereas that of the participating portion altered significantly. Thus, the bond structure changed from C(13)-O(36)-H(37) to C(13)-O(36)-Cd(45), and the bond angle increased from 112.85° to 118.88°, respectively. Oxygen (44) and the carbon ring were not linked. Furthermore, Cd adsorption changed the dihedral angle of C(8)-C(14)-C(13)-O(36) from 175.77° to -176.78°. The structures C(14)-C(13)-O(36)-H(37) and C(9)-C(13)-O(36)-H(37) became C(14)-C(13)-O(36)-Cd(45) and C(9)-C(13)-O(36)-Cd(45), with the corresponding angles changing from -130.20° and 39.44° to 54.92° and -28.99°, respectively. The newly formed dihedral angle of C(13)-O(36)-Cd(45)-H(47) was -78.44°.

4. Discussion

In this study, we investigated Cd accumulation and toxicity in lettuce sprayed with the GO nanoparticles. The results indicated that foliar application of 30 mg/L GO significantly stimulated root growth, including the total length, surface area, and average diameter of roots, as well as the number of root hair, irrespective of Cd exposure, which is consistent with previous findings (Lahiani et al., 2015; Younes et al., 2019). However, when GO was applied at a higher concentration (60 mg/L), its effect on root growth was weakened. These results indicate that at low dosages, GO sheets can induce cell division and proliferation and stimulate root growth (Ruiz et al., 2011), whereas at



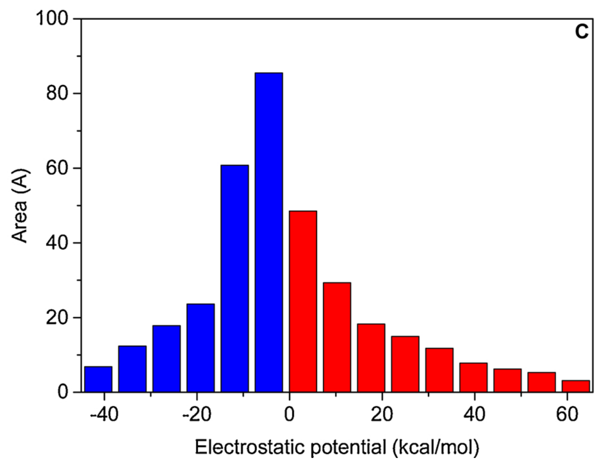


Fig. 5. Optimized structure and surface electrostatic potential of graphene oxide (GO). (a) The optimized GO structure (red, oxygen; light grey, hydrogen; dark grey, carbon). (b) Surface distribution of the electrostatic potential; blue and red indicate negative and positive charges, respectively. (c) Molecular surface areas at different electrostatic potential intervals.

high concentration, GO nanoparticles may aggregate and accumulate in the epidermis and intercellular space, which reduces their transportation from leaves to roots and adsorption of Cd (Su et al., 2017). Wang et al. (2013) also observed that transportation of Fe_2O_3 nanoparticles in plants decreased at the increase of sprayed Fe_2O_3 nano-aerosol concentration from 1×10^6 to 5×10^6 nanoparticles/cm^3.

Importantly, we found that foliar application of GO at a concentration of 30 mg/L significantly reduced Cd uptake and accumulation in lettuce roots and leaves, application of 60 mg/L GO caused only slight decreases. These observations are consistent with the findings of previous studies investigating the effects of nanomaterials on Cd accumulation in plants. For example, Lian et al. (2019) found that foliar exposure to TiO_2 nanoparticles could significantly reduce Cd contents in corn buds (by 15.2 %–17.8 %), thereby attenuating the toxic effects of Cd. Furthermore, it has been observed that with the addition of Si, Zn, Fe, and hydroxyapatite nanoparticles to soil can effectively reduce Cd accumulation in plants by decreasing its bioavailability (Rizwan et al., 2012; Cui et al., 2017; Yu et al., 2016; Yang et al., 2020). Rizwan et al. (2012) also observed that foliar application of Si nanoparticles significantly reduced Cd accumulation in wheat grain through a “dilution effect” (Adrees et al., 2015). Therefore, we speculate that the foliar application of GO may reduce the Cd^{2+} content in lettuce by enhancing the production of plant biomass (Rizwan et al., 2019). Another possible reason behind positive effects of GO may be that GO can positively modulate the metabolic processes in lettuce so that its Cd tolerance is improved (Lian et al., 2019). In addition, GO has a strong absorbance capacity for Cd^{2+} and can accumulate it in the interlayer space (Yin et al., 2018), thus preventing Cd^{2+} transport (Tan et al., 2016) and reducing its bioavailability. However, He et al. (2019) found that in rice, GO applied to roots enhanced Cd uptake from soil by converting the inorganic form of Cd, which is not readily absorbed by plants, into a more absorbable form. Similarly, we have previously observed that when GO is supplied to lettuce via the roots, it promotes a significant increase in Cd accumulation (Gao et al., 2019a). Accordingly, we believe that the route of application and species-specific differences are critical factors in defining the effects of nanoparticles on the uptake of heavy metals by plants.

In the present study, we found that GO nanoparticles sprayed on lettuce leaves could subsequently be detected in the cells of both leaves and roots, including the cell walls, mitochondria, and chloroplasts, as well as in the intercellular spaces, indicating that in lettuce, GO can be transported from the leaves to roots. In this regard, previous studies have shown that GO nanoparticles that are smaller than the cell wall pores in the leaves, can readily enter leaf cells and be transported to the other organs and back via the vascular system (Zhao et al., 2015; Chichiricco and Poma, 2015; Su et al., 2019). Similar to our study, Hu et al. (2014) revealed, using TEM and Raman spectroscopy, significant deposition of GO in leaves and roots of wheat seedling treated with GO and GO plus arsenic. In peppers and eggplants sprayed with graphene, it was localized in the chloroplasts and could be transported into epidermal cells and the underlying palisade tissue, indicating a role of the stomata and endocytic vesicles in graphene absorption (Younes et al.,

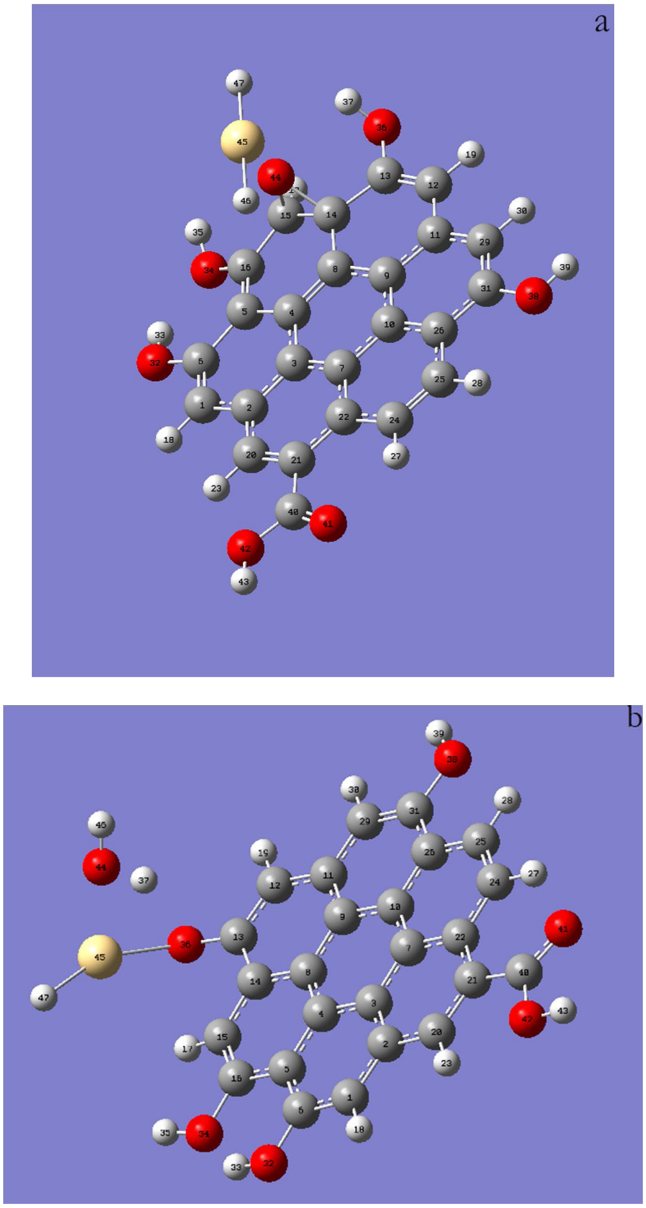


Fig. 6. Atom cluster models of graphene oxide (GO) before (a) and after (b) simulation of Cd adsorption. Red, oxygen; light grey, hydrogen; dark grey, carbon; yellow, Cd.

2019; Liu et al., 2009; Chichiriccò and Poma, 2015). Chen et al. (2019) also observed in TEM images that reduced GO could be transferred from the roots to leaves in pea. In contrast, Zhao et al. (2015) and Chen et al. (2017) found that although GO was readily taken up by plant roots, its translocation to the aboveground organs was comparatively limited. Furthermore, it has been shown that 30-nm TiO_2 nanoparticles do not accumulate in corn root cells with a diameter of 6.6 nm (Asli and Neumann, 2009). Thus, the size of GO nanoparticles appears to be an important factor with respect to their effective translocation within plants.

Our findings indicate that the exposure of lettuce plants to Cd promoted significant leakage of the root cell cytoplasm and morphological changes in leaf cell chloroplasts, as well as structural damage and a reduction in the size of starch grains. However concomitant foliar application of GO alleviated these detrimental effects of Cd on lettuce, which may be attributed to the decreased bioavailability of Cd. Our results are in agreement with the results of the study by Lian et al. (2019), who observed that foliar application of ZnO nanoparticles significantly decreased the uptake and migration of Cd and ZnO in maize, suggesting that nanoparticles may activate plant antioxidant defenses and stimulate energy metabolism to oppose the Cd-induced stress and cell damage. Li et al. (2013) have also demonstrated that spraying lettuce with silicon and/or cerium sols hindered Cd uptake, increased Cd fixation on cell walls, and reduced Cd accumulation in the shoots and roots. In contrast, Hu et al. (2014) found that root application of GO exacerbated the detrimental effects of As on the cell wall and plasma membrane and enhanced electrolyte leakage from the cell, thereby confirming that the specific effects of GO can be dependent on the application route.

Given that soluble sugars are readily hydrated, their increase in response to stress can directly reduce osmotic potential, promoting water retention and preventing dehydration and further damage to plant cells and tissues (Ma et al., 2009). Soluble proteins play an important role in controlling plant growth and floral organ morphogenesis (Kučerová et al., 2019), and their content and composition are inevitably affected by plant response to stress. In the present study, we found that foliar application of GO increased soluble sugar and protein contents in lettuce leaves and alleviated the negative effects of Cd. These observations are consistent with the findings of Younes et al. (2019), who showed that the presence of GO nanosheets in chloroplasts promoted carbon fixation, induced sugar metabolism, and increased fructose, sucrose, and starch content in the GO-treated leaves of peppers and eggplants, consequently enhancing plant yield. Similarly, it has been shown that strawberries sprayed with selenium nanoparticles have higher critical osmotic pressure, which could be attributed to increases in the contents of total soluble carbohydrates and free proline (Zahedi et al., 2019).

Vitamin C is an antioxidant and inhibitor of N-nitrosamine formation (Okafor and Nwogbo, 2005), and its content in agricultural plants is considered an important nutritional index (Konstantopoulou et al., 2010), given that 90 % of the vitamin C required by humans is derived from leafy vegetables (Lee and Kader, 2000). In the present study, we found that application of GO at the concentration of 30 mg/L increased the vitamin C content in lettuce leaves regardless of Cd treatment, indicating that foliar application of GO can enhance the nutritional quality of Cd-exposed lettuce.

GO can interact with Cu, Cd, Zn, and other heavy metal ions via complexation, ion exchange, and electrostatic attraction (Lv et al., 2016). In the present study, we examined the mechanisms underlying the interaction between GO and Cd based on simulation analysis using the Gaussian software. The results revealed that the surface of GO had predominantly a negative electrostatic potential, which is consistent with the findings of Zhao et al. (2011), who showed that the central layer of GO provided a large number of negatively charged ion exchange sites that could attract positively charged metal ions. The GO surface is characterized by a number of oxygen-containing functional groups, including carboxyl, hydroxyl, and epoxy groups, and our simulation experiments revealed the roles played by hydroxyl and epoxy groups in Cd adsorption (the role of carboxyl groups will be addressed in our next study). Subsequent to Cd adsorption by GO, local bond lengths, as well as bond and dihedral angles, in the GO molecule underwent certain changes, indicating that Cd caused alterations in the local GO structure. Changes in bond angles and atomic distances reflect those in the vibration frequency of Cd adsorbed by GO, whereas changes in dihedral angles correspond to those in surface potential energy (Walrafen, 2004). However, further studies are needed for comprehensive understanding of the mechanisms underlying the interaction between GO and Cd, as well as their relevance to the quality of leafy vegetables.

5. Conclusions

In this study, we found that spraying lettuce leaves with graphene oxide at the concentration of 30 mg/L could significantly stimulate lettuce root growth, reduce the bioaccumulation of Cd in the roots and leaves, attenuate Cd-related cell damage, and improve lettuce quality, including increased content of soluble sugars, proteins, and vitamin C. Gaussian simulation analysis revealed that Cd adsorption by GO resulted in the disruption of the O—H bond of hydroxyl groups on the GO surface and formation of a bond between Cd and the oxygen of surface epoxy groups. Collectively, our findings indicate that foliar application of 30 mg/L GO may reduce the risk of Cd entering the human body via transmission through the food chain.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Minling Gao: Funding acquisition, Writing - original draft, Writing - review & editing. **Yalei Xu:** Investigation, Methodology, Writing - original draft. **Xipeng Chang:** Methodology, Software. **Youming Dong:** Methodology, Software. **Zhengguo Song:** Project administration, Resources, Writing - original draft, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2020.122859.

References

Adrees, M., Ali, S., Rizwan, M., Zia-ur-Rehman, M., Ibrahim, M., Abbas, F., Farida, M., Qayyumc, M.F., Irshada, M.K., 2015. Mechanisms of silicon-mediated alleviation of heavy metal toxicity in plants: a review. Ecotoxicol. Environ. Saf. 119, 186–197.

Akhavan, O., Ghaderi, E., Akhavan, A., 2012. Size-dependent genotoxicity of graphene nanoplatelets in human stem cells. Biomaterials 33, 8017–8025.

Anjum, N.A., Singh, N., Singh, M.K., Sayeed, I., Duarte, A.C., Pereira, E., Ahmad, I., 2014. Single-bilayer graphene oxide sheet impacts and underlying potential mechanism assessment in germinating faba bean (*Vicia faba* L.). Sci. Total Environ. 472, 834–841.

Asli, S., Neumann, P.M., 2009. Colloidal suspensions of clay or titanium dioxide nanoparticles can inhibit leaf growth and transpiration via physical effects on root water transport. Plant Cell Environ. 32, 577–584.

Bayçu, G., Gevrek-Kürüm, N., Moustaka, J., Csatari, I., Rognes, S.E., Moustakas, M., 2017. Cadmium-zinc accumulation and photosystem II responses of *Noccaea caerulescens* to Cd and Zn exposure. Environ. Sci. Pollut. Res. 24, 2840–2850.

Bedoui, K., Bekri-Abbes, I., Srasra, E., 2008. Removal of cadmium (II) from aqueous solution using pure smectite and lewatite S 100: the effect of time and metal concentration. Desalination 223 (1-3), 269–273.

Borthakur, P., Boruah, P.K., Hussain, N., Sharma, B., Das, M.R., Matić, S., Řeha, D., Minofar, B., 2016. Experimental and molecular dynamics simulation study of specific ion effect on the graphene oxide surface and investigation of the influence on reactive extraction of model dye molecule at water–organic interface. J. Phys. Chem. C 120 (26), 14088–14100.

Chen, D., Feng, H.B., Li, J.H., 2012. Graphene oxide: preparation, functionalization, and electrochemical applications. Chem. Rev. 112 (11), 6027–6053.

Chen, L.Y., Wang, C.L., Li, H.L., Qu, X.L., Yang, S.T., Chang, X.L., 2017. Bioaccumulation and toxicity of ^{13}C-skeleton labeled graphene oxide in wheat. Environ. Sci. Technol. 51, 10146–10153.

Chen, L.Y., Wang, C.L., Yang, S.T., Guan, X., Zhang, Q.Q., Shi, M.Y., Yang, S.T., Chen, C.Y., Chang, X.L., 2019. Chemical reduction of graphene enhances in vivo translocation and photosynthetic inhibition in pea plants. Environ. Sci. Nano 6, 1077–1088.

Cheng, F., Liu, Y.F., Lu, G.Y., Zhang, X.K., Xie, L.L., Yuan, C.F., Xu, B.B., 2016. Graphene oxide modulates root growth of *Brassica napus* L. and regulates ABA and IAA concentration. J. Plant Physiol. 193, 57–63.

Chichiricco, G., Poma, A., 2015. Penetration and toxicity of nanomaterials in higher plants. Nanomaterials 5 (2), 851–873.

Cui, J.H., Liu, T.X., Li, F.B., Yi, J.C., Liu, C.P., Yu, H.Y., 2017. Silica nanoparticles alleviate cadmium toxicity in rice cells: mechanisms and size effects. Environ. Pollut. 228, 363–369.

De Mendonça, J.P.A., Lima, A.H., Roldao, J.C., Martins, Jd.S., Junqueira, G.M.A., Quirino, W.G., Sato, F., 2018. The role of sulfate in the chemical synthesis of graphene oxide. Mater. Chem. Phys. 215, 203–210.

Dias, M.C., Monteiro, C., Moutinho-Pereira, J., Correia, C., Goncalves, B., Santos, C., 2013. Cadmium toxicity affects photosynthesis and plant growth at different levels. Acta Physiol. Plant. 35 (4), 1281–1289.

Dreyer, D.R., Todd, A.D., Bielawski, C.W., 2014. Harnessing the chemistry of GO. Chem. Soc. Rev. 43 (15), 5288–5301.

Gao, M.L., Yang, Y.J., Song, Z.G., 2019a. Effects of graphene oxide on cadmium uptake and photosynthesis performance in wheat seedlings. Ecotox. Environ. Safe. 173, 165–173.

Gao, M.L., Yang, Y.J., Song, Z.G., 2019b. Toxicity of cadmium to wheat seedling roots in the presence of graphene oxide. Chemosphere 223, 9–16.

Gurunathan, S., 2015. Cytotoxicity of graphene oxide nanoparticles on plant growth promoting *rhizobacteria*. J. Ind. Eng. Chem. 32, 282–291.

He, S.Y., He, Z.L., Yang, X.E., Stoffella, P.J., Baligar, V.C., 2015. Soil biogeochemistry, plant physiology, and phytoremediation of cadmium-contaminated soils. In: In: Sparks, D.L. (Ed.), Adv. Agron. Vol 134. pp. 135–225.

He, Y.J., Qian, L.C., Zhou, K., Hu, R.R., Huang, M.R., Wang, M., Zhao, G.K., Liu, Y.L., Xu, Z.P., Zhu, H.W., 2019. Graphene oxide promoted cadmium uptake by rice in soil. ACS Sustain. Chem. Eng. (7), 10283–10292 2019.

Hu, X.G., Kang, J., Lu, K.C., Zhou, R.R., Mu, L., Zhou, Q.X., 2014. Graphene oxide amplifies the phytotoxicity of arsenic in wheat. Sci. Rep. 4, 6122.

Hu, X.G., Zhou, Q.X., 2013. Health and ecosystem risks of graphene. Chem. Rev. 113 (5), 3815–3835.

Kabiri, S., Degryse, F., Tran, D.N., Da Silva, R.C., McLaughlin, M.J., Losic, D., 2017. Graphene oxide: a new carrier for slow release of plant micronutrients. ACS Appl. Mater. Interfaces 9 (49), 43325–43335.

Keller, C., Rizwan, M., Davidian, J.C., Pokrovsky, O.S., Bovet, N., Chaurand, P., Meunier, J.D., 2015. Effect of silicon on wheat seedlings (*Triticum turgidum* L.) grown in hydroponics and exposed to 0 to 30 mM Cu. Planta 241, 847–860.

Konstantopoulou, E., Kapotis, G., Salachas, G., Petropoulos, S.A., Karapanos, I.C., Passam, H.C., 2010. Nutritional quality of greenhouse lettuce at harvest and after storage in relation to N application and cultivation season. Sci. Hortic. 125, 931–935.

Kučerová, K., Henselova, M., Slovakova, L., Hensel, K., 2019. Effects of plasma activated water on wheat: germination, growth parameters, photosynthetic pigments, soluble protein content, and antioxidant enzymes activity. Plasma Process. Polym. 16 (3).

Lahiani, M.H., Chen, J., Irin, F., Puretzky, A.A., Green, M.J., Khodakovskaya, M.V., 2015. Interaction of carbon nanohorns with plants: uptake and biological effects. Carbon 81, 607–619.

Lee, S.K., Kader, A.A., 2000. Preharvest and postharvest factors influencing Vc content of horticultural crops. Postharvest Biol. Technol. 20, 207–220.

Li, S.Y., Zhang, S.R., Ding, X.D., Liao, X.D., Wang, R.P., 2013. Spraying silicon and/or cerium sols favorably mediated enhancement of Cd/Pb tolerance in lettuce grown in combined Cd/Pb contaminated soil. Procedia Environ. Sci. 18, 68–77.

Lian, J.P., Zhao, L.F., Wu, J.N., Xiong, H.X., Bao, Y.Y., Zeb, A., Tang, J.C., Liu, W.T., 2019. Foliar spray of TiO_2 nanoparticles prevails over root application in reducing Cd accumulation and mitigating Cd-induced phytotoxicity in maize (*Zea mays L.*). Chemosphere 239, 124794.

Liu, J., Sun, Y.D., Drubin, D.G., Oster, G.F., 2009. The mechanochemistry of endocytosis. PLoS Biol. 7 (9), 16.

Lv, X.H., Chen, B.Y., Zhu, X.S., 2016. Water environmental behavior and biological toxicity of graphene oxide. China Environ. Sci. 36 (11), 3348–3359.

Ma, Y.Y., Zhang, Y.L., Lu, J., Shao, H.B., 2009. Roles of plant soluble sugars and their responses to plant cold stress. Afr. J. Biotechnol. 8 (10), 2004–2010.

Mishra, S., Srivastava, S., Tripathi, R.D., Govindarajan, R., Kuriakose, S.V., Prasad, M.N.V., 2006. Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L. Plant Physiol. Biochem. 44 (1), 25–37.

Okafor, P.N., Nwogbo, E., 2005. Determination of nitrate, nitrite, N-nitrosamines, cyanide and ascorbic acid contents of fruit juices marketed in Nigeria. Afr. J. Biotechnol. 4, 1105–1108.

Rizwan, M., Meunier, J.D., Hélène, M., Keller, C., 2012. Effect of silicon on reducing cadmium toxicity in durum wheat (*Triticum turgidum* L. cv. *Claudio* W.) grown in a soil with aged contamination. J. Hazard. Mater. 209–210, 326–334.

Rizwan, M., Ali, S., Rehman, M.Z.U., Adrees, M., Arshad, M., Qayyum, M.F., Ali, L., Hussain, A., Chatha, S.A.S., Imran, M., 2019. Alleviation of cadmium accumulation in maize (*Zea mays* L.) by foliar spray of zinc oxide nanoparticles and biochar to contaminated soil. Environ. Pollut. 248, 358–367.

Romero-Puertas, M.C., Palma, J.M., Gomez, M., Del, Rio.L.A., Sandalio, L.M., 2002. Cadmium causes the oxidative modification of proteins in pea plants. Plant Cell Environ. 25 (5), 677–686.

Ruiz, O.N., Fernando, K.A.S., Wang, B.J., Brown, N.A., Luo, P.G., McNamara, N.D., Vangsness, M., Sun, Y.P., Bunker, C.E., 2011. GO: a nonspecific enhancer of cellular growth. ACS Nano 5 (10), 8100–8107.

Su, Y.M., Ashworth, V., Kim, C., Adeleye, A.S., Rolshausen, P., Roper, C., White, J., Jassby, D., 2019. Delivery, uptake, fate, and transport of engineered nanoparticles in plants: a critical review and data analysis. Environ. Sci.-Nano 6 (8), 2311–2331.

Tan, P., Hu, Y.Y., Bi, Q., 2016. Competitive adsorption of Cu^{2+}, Cd^{2+} and Ni^{2+} from an aqueous solution on graphene oxide membranes. Colloids Surface A. 509, 56–64.

Walrafen, G.E., 2004. Effects of equilibrium h-bond distance and angle changes on raman intensities from water. J. Chem. Phys. 120 (10), 4868–4876.

Wang, H.B., Maiyalagan, T., Wang, X., 2012. Review on recent progress in nitrogen-doped graphene: synthesis, characterization, and its potential applications. ACS Catal. 2 (5), 781–794.

Wang, W.N., Tarafdar, J.C., Biswas, P., 2013. Nanoparticle synthesis and delivery by an

aerosol route for watermelon plant foliar uptake. J. Nanopart. Res. 15 (1).
Yang, L.Q., Liu, B.L., Lu, Y.Y., Lu, F.Y., Wu, X.Y., You, W.H., Huang, B., 2020. Bioavailability of cadmium to celery (*Apium graveolens* L.) grown in acidic and Cd-contaminated greenhouse soil as affected by the application of hydroxyapatite with different particle sizes. Chemosphere 240, 124916.
Yin, L.Y., Wang, Z., Wang, S.G., Xu, W.Y., Bao, H.F., 2018. Effects of graphene oxide and/or Cd^{2+} on Seed germination, seedling growth, and uptake to Cd^{2+} in solution culture. Water Air Soil Pollut. 229, 151.
You, X.F., He, M., Cao, X.Q., Wang, P., Wang, J.X., Li, L., 2019. Molecular dynamics simulations of removal of nonylphenol pollutants by graphene oxide: experimental study and modelling. Appl. Surf. Sci. 475, 621–626.
Younes, N.A., Dawood, M.F.A., Wardany, A.A., 2019. Biosafety assessment of graphene nanosheets on leaf ultrastructure, physiological and yield traits of *Capsicum annuum* L. and *Solanum melongena* L. Chemosphere 228, 318–327.
Yu, H.Y., Ding, X.D., Li, F.B., Wang, X.Q., Zhang, S.R., Yi, J.C., Liu, C.P., Xu, X.H., Wang, Q., 2016. The availabilities of arsenic and cadmium in rice paddy fields from a mining area: the role of soil extractable and plant silicon. Environ. Pollut. 215, 258–265.
Zahedi, S.M., Abdelrahman, M., Hosseini, M.S., Hoveizeh, N.F., Tran, L.P., 2019. Alleviation of the effect of salinity on growth and yield of strawberry by foliar spray of selenium-nanoparticles. Environ. Pollut. 253, 246–258.
Zhao, G.X., Ren, X.M., 2011. Removal of Pb (II) ions from aqueous solutions on few-layered graphene oxide nanosheets. Dalton Trans. 40, 10945–10952.
Zhao, S.Q., Wang, Q.Q., Zhao, Y.L., Rui, Q., Wang, D.Y., 2015. Toxicity and translocation of graphene oxide in *Arabidopsis thaliana*. Environ. Toxicol. Phar. 39, 145–156.