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Investigating uptake of water-dispersible CdSe/ZnS quantum dot nanoparticles by *Arabidopsis thaliana* plants

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ABSTRACT

Interest on the environmental impacts of engineered nanomaterials has rapidly increased over the past years because it is expected that these materials will eventually be released into the environment. The present work investigates the potential root uptake of water-dispersible CdSe/ZnS quantum dots (QDs) by the model plant species, *Arabidopsis thaliana.* Experiments revealed that *Arabidopsis* exposed to QDs that are dispersed in Hoagland's solution for 1–7 days did not internalize intact QDs. Analysis of Cd and Se concentrations in roots and leaves by inductively-coupled plasma mass spectrometry indicated that Cd and Se from QD-treated plants were not translocated into the leaves, and remained in the root system of *Arabidopsis*. Furthermore, fluorescence microscopy showed strong evidence that the QDs were generally on the outside surfaces of the roots, where the amount of QDs adsorbed is dependent on the stability of the QDs in suspension. Despite no evidence of nanoparticle internalization, the ratio of reduced glutathione levels (GSH) relative to the oxidized glutathione (GSSG) in plants decreased when plants were exposed to QD dispersions containing humic acids, suggesting that QDs caused oxidative stress on the plant at this condition.

1. Introduction

Over the past 5–10 years, there has been increasing interest with regard to the environmental implications of products of nanotechnology [1,2] as these materials are slowly penetrating the mainstream market [3,4]. Recent fate and transport studies have shown that while the aquatic environment might provide a means by which these materials can enter environment [5–7], soil will be an important sink for materials that may eventually aggregate and fall out of suspension [8–10]. Plants are considered to be one of the many organisms that will be directly impacted by nanomaterials. Recent studies have already demonstrated that nanoparticles (NPs) can penetrate different biological barriers, from mammalian cells to plant cells [11–14]. At first glance, the diameters of NPs (core: 1–100 nm) relative to the pore diameters of the plant cell wall (~3.5–5 nm) would already indicate a restriction toward the ability of NPs to penetrate the plant cell via active/passive transport [15]. Nevertheless, though typically occurring in mammalian cells, nanomaterials may still be internalized in a similar fashion by which other macromolecules have been proposed to enter plant cells [16]. Mechanisms resembling endocytosis or nonendocytic penetration allow absorption of these materials/molecules which would ordinarily be too big for the cell membrane or the cell wall [17]. Note that internalization of NPs does not, however, readily indicate immediate toxic effects to plants. Plants have physical defense structures (i.e., exclusion through cell wall, sequestration in vacuole, etc.) and chemical defenses (i.e., regulation of different plant proteins – glutathione, phytochelatins, etc.) that allow them to adapt to different types of environmental stressors [18].

Current literature points to both uptake and no uptake of NPs in different plant species (model and crop plants) [19–21]. For instance, uptake has been observed for Cu NPs in mung bean and wheat [22], ZnO in rye grass [16], mutiwalled carbon nanotubes (MWNTs) in rice [11] and in wheat [23], and CdSe/ZnS quantum dots (QDs) in annual bluegrass [24]. On the other hand, no uptake was observed for Al NPs in red kidney bean [25] and in CeO_2 NPs in maize [26]. It is interesting to note that excluding the study involving MWNTs in rice, where translocation of intact MWNTs was visualized in the roots and leaves, uptake of the NPs in these studies were only shown to be limited to the roots. Although there are few studies that report positive effects of NPs on plants (i.e.,

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TiO_2 NPs have been shown to promote photosynthesis, nitrogen metabolism, and growth of spinach [27,28]), in general, many studies report phytotoxic effects resulting from exposure to various types of NPs [20]. Phytotoxicity has been related to decreased chlorophyll and photosynthetic rate [20,27,28], poor germination rates, and hampered growth and development [16,20,22,29]. These effects are most often associated with its increased reactivity and surface area, formation of reactive oxygen species (ROS), aggregation and adsorption to cell walls, and release of toxic ions (i.e., Ag^+ from Ag NPs, Cd^{2+} and SeO_3^{2-} from CdSe QDs, Zn^{2+} from ZnO NPs, etc.) [20,30].

QDs are semiconductor nanoparticles that are being extensively developed because of their unique size-dependent optical and photophysical properties. These properties have made QDs ideal for applications such as solid-state LED lighting [31], solar energy conversion [32], biomedical imaging and cellular labeling [33], The biggest concern associated with QDs is that the most heavily studied materials are composed of toxic elements. QDs are typically manufactured as having a cadmium chalcogenide core (i.e., CdSe, CdS), with or without an outer shell of zinc chalcogenide, that are passivated by an additional layer of organic ligands. Hence, QDs structural stability is a big issue because QDs have been reported to degrade with UV light and oxygen [34], releasing toxic Cd^{2+}, Se^{2-} and/or SeO_3^{2-}.

In this study, the potential uptake of water-dispersible CdSe/ZnS QDs by a model plant species was investigated using hydroponic cultures. Our objective was to provide a quantitative and qualitative description of the uptake process using inductively-coupled plasma mass spectrometry (ICP–MS) and fluorescence microscopy to be able to demonstrate whether QDs can be taken up as intact or degraded species under hydroponic conditions. Plant stress response associated with the uptake study were also determined by analysis of reduced (GSH) and oxidized (GSSG) glutathione using liquid–chromatography mass spectrometry (LC–MS). Here, uptake of QDs and dissolved Cd^{2+} and SeO_3^{2-} were measured in *Arabidopsis thaliana* plants. Our findings suggest that while CdSe/ZnS QDs will not be readily taken up through the root systems, exposure to QDs (in the presence of humic acids, HA) can still negatively impacts the plants, as evidenced in the significant reduction in GSH-to-GSSG ratios (GSH/GSSH).

2. Materials and methods

2.1. General

Water-dispersible CdSe/ZnS QDs were from Invitrogen-Qdot® 655 ITK™ carboxyl (Eugene, OR). Prior to their use, the QDs were dialyzed in deionized (DI) water using a Spectra/Por® Biotech cellulose ester dialysis membrane (0.5–1 kDa MWCO, Spectrum Laboratories Inc.) to remove free ions and unbound ligands. $CdCl_2$ (99.99% purity) and H_2SeO_3 (98% purity) solids from Sigma–Aldrich were used in preparing the Cd^{2+} and SeO_3^{2-} solutions. Hoagland's nutrient solution, used as exposure medium, was modified and prepared to only contain major nutrients (2 M-KNO_3, 2 M-$Ca(NO_3)_2$, 2 M-$MgSO_4$ and 1 M-KH_2PO_4, pH 6), using salts from J.T. Baker. This solution was sterilized (autoclave) prior to use. Suwannee river humic acid (SRHA-II) standard, used as model HA, was from the International Humic Substances Society [35]. Concentrated HNO_3 (Aristar® Ultra) and 30% H_2O_2 used for plant digestions were from BDH and J.T. Baker, respectively. DI water used in the dialysis experiments and to prepare all solutions/dispersions was from a Barnstead NANOpure water system (18.2 MΩ/cm resistivity).

2.2. Exposure experiments

A. thaliana plants (*Columbia* accession CS907) were initially grown in soil (see Supporting information, SI). After 3–4 weeks, plants that have 7–10 leaves (rosette production stage) were harvested. Harvested seedlings were rinsed with DI water to remove soil and debris from the roots prior to exposure experiments. Seedlings were transplanted into individual 1.5 mL centrifuge tubes that contain 1/4 strength Hoagland's solution (HS); this solution has been successfully utilized in hydroponic cultivation of plants, particularly *Arabidopsis* [36]. Each transplanted seedling was supported by a rubber cap with the roots immersed in HS. After 24 h, each seedling was transferred to HS containing 5 μg/mL Cd^{2+}, 5 μg/mL SeO_3^{2-} or 5.8 nM QD (5 μg/mL in Cd^{2+}) (solution pH: Cd^{2+}, 6.0; SeO_3^{2-}, 5.3; and QD, 5.8). All exposure concentrations were standardized against Cd^{2+}; 5 μg/mL Cd^{2+} is a practical level for quantitation that has already been shown to have minimal impact on growth of *Arabidopsis* (wild-type) [37]. Since the ligands used to coat the QD were proprietary, no set-ups were prepared as control for the ligands. Uncontaminated HS was used as negative control. In separate experiments, seedlings were transferred to HS containing 10 μg/mL HA (HS + HA) to investigate the effect of HA on the uptake of Cd^{2+} and the QD (solution pH: Cd^{2+}, 5.3; and QD, 5.0). The prepared suspensions all have pH values that are considered suitable for the exposure experiments to maintain good plant condition; slightly acidic pH values are routinely used for hydroponically-grown plants to maximize nutrient availability and absorption. Exposure experiments were done for 1/3/5/7 days to demonstrate uptake based on a gradual increase in concentration of Cd/Se/QD in the plant; kinetics of uptake was not calculated in this study. For the Se experiments, 7-day exposure was no longer performed due to the poor survival of the plants. Every day until the end of the exposure period, the volume of the solutions was adjusted with uncontaminated nutrient solution to compensate for changes due to evaporation and absorption. The solutions were also vortex-mixed to gently redisperse particles in solution. For each treatment, 9–12 set-ups were prepared. All solutions were protected from light by wrapping the container with aluminum foil. Set-ups were placed in the growth chamber with 50–100 μmol/m^2 s^2 light from fluorescent bulbs, 16 h-light and 8 h-dark at 27 ± 2 °C. QDs in HS and HS + HA suspensions were also prepared as controls.

2.3. Analysis and instrumentation

2.3.1. QD characterization

QDs used were characterized based on their spectral properties (UV–Vis absorption and emission spectroscopy), structural and suspension stability (emission spectroscopy and ζ-potential measurements), size, and crystallinity (transmission electron microscopy, TEM, and selected area electron diffraction, SAED). Absorption and emission spectra were collected using a Hewlett Packard 8452A diode array spectrophotometer and Cary Eclipse (Varian, USA) fluorimeter, respectively. ζ-potential data were acquired using a Zetasizer Nano Z90 instrument (Malvern Instruments, UK). TEM, as well as SAED patterns, were collected for pristine QDs using a JEM-2010 instrument (JEOL, Japan) operated at an accelerating voltage of 200 kV. Samples for TEM were prepared by slow evaporation of a drop of aqueous solution placed onto 400 mesh carbon/formvar grids.

2.3.2. Metal analysis

The total Cd/Se concentrations were determined for the exposed plants by ICP–MS. After each exposure, 3–6 plants were recovered and roots were thoroughly rinsed with DI water (or 10 mM $CaCl_2$) to remove material that is neither adsorbed nor integrated in the plant tissues; $CaCl_2$ was used to promote displacement

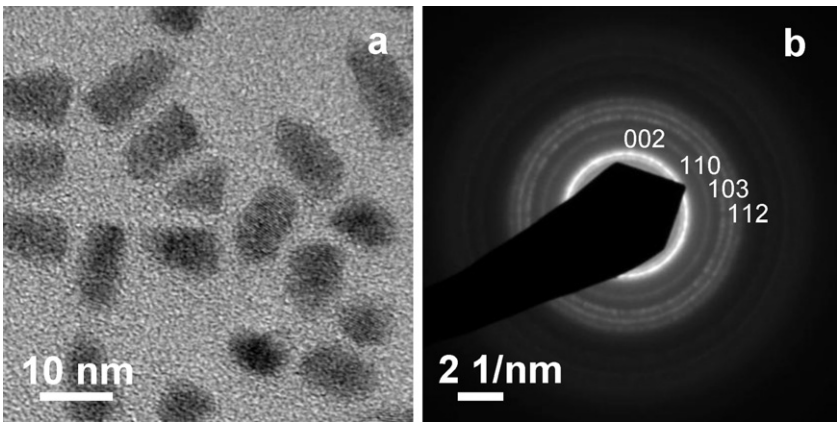

Fig. 1. (a) TEM image and (b) SAED pattern of pristine QDs used.

of the carboxylate ($-CO_2^-$) terminated QD from the root surface. Roots and leaves were separated then weighed after oven drying (70 °C, 24 h). Dried tissues were digested in 4:1 concentrated HNO_3:30% H_2O_2 for at least 2 h using a hotplate. Samples were quantitatively transferred into polypropylene tubes and centrifuged (1000 × *g*, 10 min). Supernates were reconstituted (10 mL) using DI water. Extraction recoveries were 93(±3)% Cd and 83(±1)% Se. Analysis of ^{111}Cd and ^{78}Se was carried out using an X-Series 2 ICP-MS instrument (Thermo Scientific, Germany) and ICP–MS elements solution set (includes Cd and Se) from BDH Aristar® as standards. Concentrations of Cd and Se were expressed as mg Cd or Se/(kg dry weight, DW). All concentrations are reported as ±standard deviation. Significant differences in the concentrations taken up between treatments were determined based on Student's *t*-test (two-tailed) at 95% confidence level. Comparisons were made for the highest concentrations of Cd/Se detected in the roots/leaves, unless otherwise indicated. Analytical details are in SI.

2.3.3. Intact QD analysis

Live plant roots of 1–2 plants were imaged for intact QDs using a confocal fluorescence microscope. Exposed roots were rinsed with DI water, placed on a microscope slide, covered with a cover slip then sealed with wax. Prepared slides were immediately viewed to prevent the roots from drying. The QDs were excited using a diode and continuous laser (405 nm and 637 nm; Carl Zeiss, Oberkochen, Germany). Imaging was done using a Carl Zeiss CLSM 710 confocal microscope. The detector was set to collect emission from two channels: 426–527 nm (for endogenous fluorescence) and to 644–735 nm (for QD fluorescence). Emission spectra were also collected as additional confirmation for intact QDs. Only the roots were viewed under the fluorescence microscope.

2.3.4. Glutathione analysis

The levels of GSH and GSSG in 2–3 individual whole plants were determined to monitor for any exposure/uptake-related oxidative stress. Sample extraction and thiol derivatization procedure using 5,5′-dithiobis-(2-nitrobenzoic acid) was adapted from Guan et al. [38]. Derivatized samples were analyzed for GSH and GSSG by LC–MS using a C-18 Betabasic column (Thermo Hypersil-Keystone, Bellefonte, PA) for chromatographic separation and an LCQ Advantage ion trap MS for detection (Thermo Finnigan, San Jose, CA). GSH and GSSG solids used to prepare standards were from Sigma. Concentrations are reported as ±standard deviation. Analytical details are in SI.

3. Results and discussion

3.1. Characteristics of the QDs

The CdSe/ZnS QD was characterized by TEM and SAED (Fig. 1). The polymer-coated QDs, reported to have surface carboxylate groups, were rod-like with average aspect ratio, length, and diameter of 2.0(±0.3), 12(±1) nm, and 6.3(±0.7) nm, respectively. Owing to the carboxylate groups on the QD surface, its dispersions in H_2O had a negative ζ-potential that centered at −20.5 mV (pH 7.6). Similarly, QDs dispersed in HS and in HS + HA have slightly less negative ζ-potentials centered at −10.1 mV (pH 5.8) and −14.6 mV (pH 5.0), respectively. The shift to lower ζ-potential values is expected with the high ionic strength of the nutrient solutions due to screening of electrostatic repulsions. These values suggest that the QDs would be prone to agglomeration in these suspensions, which would greatly affect its availability to the plants.

The QD exhibited narrow fluorescence emission (λ_{max} = 654 nm). The emission spectra of the different dispersions collected over time are shown in Fig. 2. In all media, small shifts in emission peak positions (Table S5) were observed, indicating minimal changes in particle size during this time period. Blue shifts greater than 1.5 nm ($\lambda_{maximum\ resolution}$) were, however, observed for QDs in H_2O, indicating some QD dissolution in this medium. Loss of fluorescent species in the dispersion is also evident in the spectra. This gradual decrease is likely a result of agglomeration and sedimentation of the particles (as suggested by the low ζ-potential values) and possible band-edge emission quenching. The quantum yield of QDs was diminished in HS and in HS + HA compared to QDs in H_2O. Within 1 day, the emission intensity of QDs in HS (also lowest ζ-potential) declined the fastest. QDs in HS + HA appeared to be more stable in suspension, with a drastic decline in emission observed only after 3 days; HA has already been shown to temporarily stabilize QDs in aqueous suspension [8]. The generally lower quantum yield of the QDs in HS + HA is consistent with other reports where HA is able to quench CdSe QDs emission [8]. These results suggest that majority of the QDs in HS and HS + HA are intact during the 7-day exposure period. Moreover, these QDs could be estimated to be most available (remains suspended in solution) to the plants during the 1st day of exposure.

3.2. Uptake of QDs by the A. thaliana plants

Arabidopsis was chosen as a model plant because it develops, reproduces and responds to stress much the same as many crop plants [39]. It is a widely accepted model plant with a significant

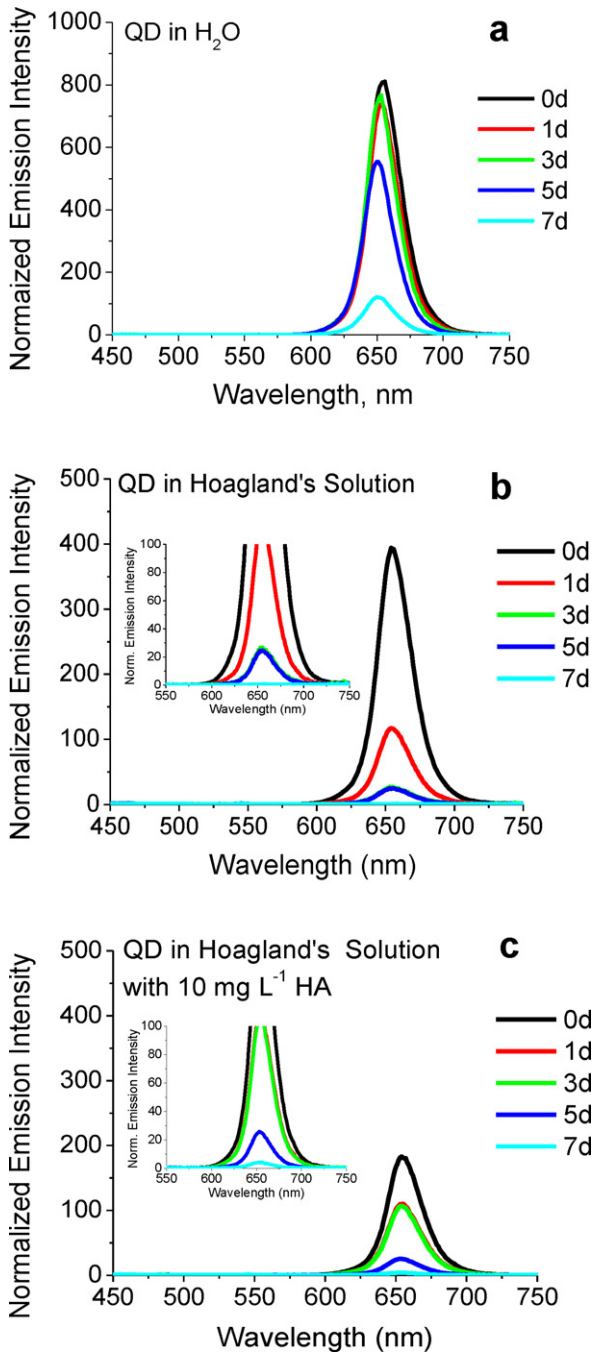

Fig. 2. Emission spectra of the QDs in (a) H_2O, (b) HS, and (c) HS + HA collected over 7 days to evaluate QD stability in suspension. Enlarged spectra are in the insets. Spectra were normalized against the intensity of the water Raman peak at 485 nm.

body of data concerning its physiology and genetics available [39]. In this work, uptake was studied specifically in hydroponic cultures to ensure that the QDs are readily available for uptake and that delivery of the QDs to the root surface is not limiting. For the different exposure treatments, the distribution of Cd and Se in the roots and leaves were plotted in Fig. 3. Uptake of the QDs by *Arabidopsis* was compared to the uptake of free Cd^{2+} and SeO_3^{2-}. SeO_3^{2-} was chosen as spiking material because weathering of CdSe QDs has been reported to release this form of Se [40]. Consistent with literature, *Arabidopsis* was able to take up Cd^{2+} and SeO_3^{2-}. Membrane transporters typically mediate entry of these ions through the hydrophobic lipid bilayer. Uptake of Cd^{2+} by plants is generally mediated by cation (Ca^{2+}/Fe^{2+}/Mn^{2+}/Zn^{2+}) transporters [41]. In the

Table 1
Calculated Cd:Se mole ratios in the roots and leaves of *Arabidopsis* plants exposed to QDs in HS and HS + HA.

Days	QD only	QD in Hoagland's Solution		QD in Hoagland's Solution with 10 mg L^{-1} HA	
		Roots	Leaves	Roots	Leaves
0	4.4 ± 1.3	–	–	–	–
1	4.4 ± 1.3	3.5 ± 0.7	–	3.4 ± 0.3	2.9 ± 1.4
3	4.4 ± 1.3	4.1 ± 2.3	–	3.2 ± 0.2	45.8 ± 71.5
5	4.4 ± 1.3	2.9 ± 0.2	–	3.1 ± 0.2	4.4 ± 3.4
7	4.4 ± 1.3	3.0 ± 0.4	–	3.2 ± 0.3	7.3 ± 13.9

Values are reported as ± standard deviation.

case of SeO_3^{2-}, there is no evidence of uptake mediated by membrane transporters [42]; SeO_4^{2-}, however, has been proposed to be taken up by SO_4^{2-} transporters [42]. Cd and Se concentrations in the roots and leaves are significantly different from the control (all time points, $p_{Cd\text{-}exposed} < 0.05$, $p_{Se\text{-}exposed} < 0.05$). The levels of Cd in the roots and leaves continuously increased from 0 to 5 days of exposure. However, 7-day plants did not to appear to internalize as much Cd. Concentrations as high as 2.70(±0.64) mg Cd/kg DW_{roots} and 0.25(±0.12) mg Cd/kg DW_{leaves} were measureable in plants exposed to Cd^{2+} (5 days). Unlike Cd-exposed plants, the levels of Se in the roots and leaves did not increase with time. The plants were wilted (not dry) even with 1-day of exposure to 5 μg/mL SeO_3^{2-}, which could have affected uptake [42]. Concentrations as high as 0.75(±0.24) mg Se/kg DW_{roots} and 0.05(±0.01) mg Se/kg DW_{leaves} were measureable in plants exposed to SeO_3^{2-} (1 day). Between Cd^{2+} and SeO_3^{2-}, more Cd^{2+} is evidently taken up compared to SeO_3^{2-}. In both Cd- and Se-exposed plants, the presence of Cd and Se in the leaves indicated some translocation of these ions from the roots. Among various plants, tolerable levels range from 5 to >100 mg Cd/kg DW [43] and from 2 to >500 mg Se/kg DW [44].

For plants exposed to dispersions of QDs, 2.35(±0.97) and 0.38(±0.28) mg/kg DW_{roots} of Cd and Se, respectively, were found (7 days); this is the highest concentration found in the roots of plants exposed to QDs in HS. Concentrations of Cd and Se found in the roots were significantly different from the control (all time points, $p < 0.05$). As seen in Fig. 3, compared to Cd^{2+} and SeO_3^{2-} ions, the uptake pattern of Cd and Se species are very similar, suggesting that these may be QDs. Indeed, the calculated Cd:Se mole ratios, which provides an additional means for verifying intact QDs, were not significantly different from the Cd:Se mole ratios of the pristine QDs (Table 1, all time points, $p > 0.05$). Based on the mass of Cd found in the roots (corrected for Cd measurable in the control roots) and the initial mass of Cd (from the QD) in the exposure solution, 7–11% of the QDs could be estimated to be present in the roots of plants during the 1–7 days exposure period. Compared to the Cd-exposed plants, it is noticeable that the levels of Cd and Se did not show a trend of increasing or decreasing with time. This variability could be related to the availability of the QDs in the nutrient solution. As seen from the ζ-potential and emission spectra of the control solutions, the QDs would be most available during the 1st day of the exposure period – when QDs are in suspension. Cd and Se measurable in the leaves of QD-exposed plants were not significantly different from the controls ($p > 0.05$) indicating that QDs were not translocated from the roots. Only 0.015(±0.009) mg Cd/kg DW_{leaves} was present while Se was not detected. In a similar study, Lin et al. [16] reported that exposure of rye grass to dispersions of ZnO for 12 days also did not show any translocation.

Examination of the root tissues using fluorescence microscopy revealed that most, if not all, of the QDs can only be found adsorbed onto the root cell wall (Fig. 4). Majority of the QDs were present on the zone of cellular maturation, where several root hairs are present. Root hairs are generally known to help in the

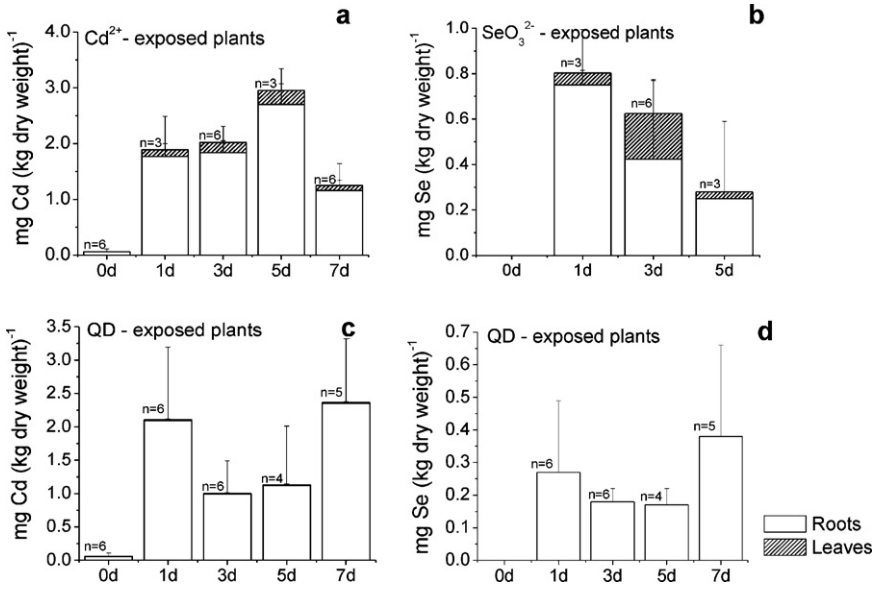

Fig. 3. Distribution of Cd and Se in the roots and leaves of plants exposed to (a) Cd^{2+}, (b) SeO_3^{2-}, and (c and d) QDs in HS. Error bars correspond to standard deviation. Levels of Cd/Se found in the roots and the leaves are significantly different from the control.

absorption of water and nutrients [18]. The relatively high surface area of the roots hairs could promote the adsorption of the QDs. Based on Fig. 4, the adsorbed QDs were also visibly aggregated, which is expected from the ζ-potential values of the QDs in the nutrient solution. Note that the plant roots were water-rinsed to remove weakly adsorbed QDs. A *z*-stack of 2-dimensional images of the roots, confirms that the QDs were not internalized (Fig. S1). The emission spectra collected for the fluorescent particles were consistent with the spectra obtained for pristine QDs (Fig. S2) indicating that QDs found were the same particles. No emission that could suggest presence of the QDs was detected in the control (un-exposed) plants. These images indicate that majority of the Cd and Se detected in the roots by the ICP–MS were only on the outside. Moreover, when $CaCl_2$ was used for rinsing, the concentrations of Cd and Se in the roots remained the same (Fig. S3). This strong adsorption of the QDs onto the roots indicates that $CaCl_2$ cannot easily disrupt the interaction between the root surface and the QDs. All these observations are consistent with the study by Whiteside et al. [24], where carboxylate-terminated CdSe/ZnS QDs were not taken up by annual bluegrass. Uptake of QDs was observed only when the NPs are conjugated to organic nitrogen substrates like glycine, arginine and chitosan. Given that the QDs are terminated

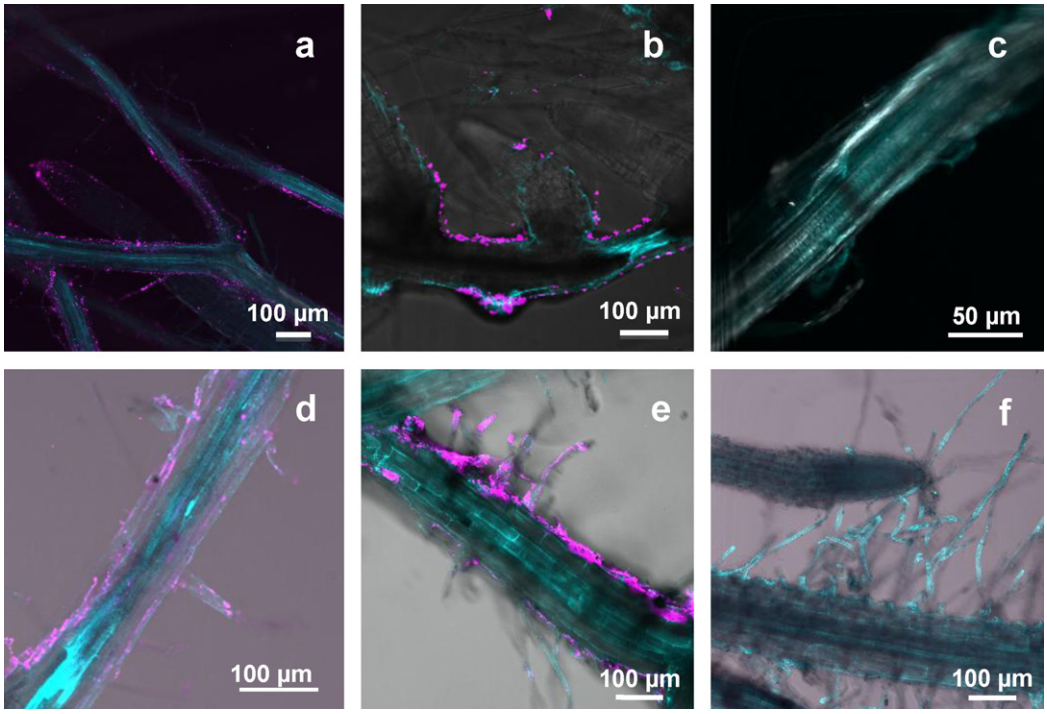

Fig. 4. Superposition of fluorescence and light microscopy images of roots from plants exposed to QD suspensions in HS for (a) 1 day, (b) 7 days, and HS + HA for (d) 1 day and (e) 7 days. Images of unexposed plants in (c) HS and (f) HS + HA are also provided for comparison. QD emission is shown in pink. Endogenous emission is shown in blue green.

with polar $-CO_2^-$ groups, entry through the hydrophobic lipid bilayer of the cell membrane was likely not favored (unless specific transporters that could interact with the surface ligands and allow passage of agglomerated QDs were present). Hence, the QDs were strongly adsorbed onto the polar/charged root surfaces. Aside from van der Waals interaction, cross-linking between the $-CO_2^-$ groups on the roots and the QDs is also a likely mechanism for QD adsorption [45]. Based on these results, it is apparent that over the 7-day exposure period, the plant cell remained impermeable to QDs; QDs were neither endocytosed or transported passively/actively through the plant roots. Zhang et al. [46] have estimated that the rate of NP uptake in mammalian cells reaches a maximum at an optimal radius of 25 nm. Aside from the barrier imposed by the plant cell wall, QD agglomerates may also have been too big for endocytosis to occur. Agglomeration and adsorption onto cells are consistent with studies involving other NPs, such as QDs [14], TiO_2 [23], and CeO_2 [23,26]. For the fluorescence microscopy studies, the leaves were no longer viewed under the fluorescence microscope due to the very low levels of Cd and negligible levels of Se.

3.3. Influence of HA in the uptake of QDs

HAs are one of the most important groups of organic acids present in soil and in water that are derived from natural organic matter. Previous studies on the fate and transport of QDs have reported the direct involvement of HAs on their stabilization/destabilization in water [8,47]. HAs can modify QD surfaces via mechanisms involving overcoating, coordinative and electrostatic interactions [8]. These surface-modified QDs are likely to be the materials that organisms encounter. Hence, for this study, it was important to investigate uptake of QDs by *Arabidopsis* plants in the presence of HAs. Herein, SRHA-II, an aquatic HA, was used. Varying effects of HAs on the availability of nutrients have been reported. HA and other organic acids have been shown to increase nutrient uptake by increasing the availability of trace minerals in soil [48] and by increasing cell membrane permeability [49]. In other reports, HA was found to decrease the bioavailability (and toxicity) of metal ions by forming of metal–humate complexes that reduces free-ion concentration in solution, and concomitant nutrient uptake [50]. The distribution of Cd and Se in the roots and leaves for the uptake of QDs in the presence of 10 μg/mL HA was plotted in Fig. 5. Uptake of the QDs by *Arabidopsis* in HA was compared to the uptake of free Cd^{2+}. Contrary to what was expected, during the exposure time frame, uptake of Cd^{2+} by *Arabidopsis* was not significantly enhanced in the presence of HA. Cd concentrations, as high as 2.63(±0.57) mg/kg DW_{roots} (5 days) and 0.27(±0.05) mg/kg DW_{leaves} (5 days) were measurable in plants exposed to Cd^{2+} in the presence of HA. These concentrations are not significantly different from the highest concentrations detected in the roots and leaves of plants exposed to Cd^{2+} (5 days) in the absence of HA ($p > 0.05$). Nonetheless, Cd concentration in the roots and leaves were significantly different from the control (plants in HS + HA only) (all time points, $p < 0.05$). The levels of Cd in the roots and leaves also continuously increased from 0 to 5 days of exposure. Cd concentrations higher than the background were also present in the leaves, indicating internalization and translocation of these ions from the roots.

For plants exposed to dispersions of QDs in the presence of HA, Cd and Se concentrations as high as 3.25(±0.91) and 0.69(±0.20) mg/kg DW_{roots}, respectively, were found (3 days). However, these levels are not significantly different from the highest concentrations detected in the roots and leaves of plants exposed to QD in the absence of HA (7 days, $p > 0.05$). Nonetheless, Cd and Se concentrations in the roots were significantly different from the control (plants in HS + HA only) (all time points, $p < 0.05$). Consistent with plants exposed to QDs in the absence of HA, the levels of Cd and Se did not show a trend of increasing or decreasing with time, which could also be related to the availability of the QDs in the nutrient solution. Similarities in the uptake pattern of Cd and Se species are also apparent in Fig. 5, suggesting the Cd- and Se-species found in the roots may be QDs. The calculated Cd:Se mole ratios were also close to the expected ratios for the pristine QDs (Table 1). Based on the mass of Cd in the roots and the initial mass of Cd in the exposure solution, 17–25% of the QDs could be estimated in the roots of plants during the 1–7 days exposure period. Fluorescence microscopy images also revealed that the QDs were aggregated and adsorbed onto root surfaces (Fig. 4d–e). Hence, it appears that HA facilitated increased adsorption of QDs onto the roots. In very rare cases, emission at the QD channel (644–735 nm) was observable within a cell (Fig. S4). While the QDs were all observed to be mostly on the root surfaces, uptake of the QDs is still feasible through damaged roots. In a recent article by Al-Salim et al. [21], QDs were shown to be internalized by *Arabidopsis* plant roots that were severed. However, in this study, the detected fluorescence cannot be entirely attributed to intact QD since it was also observable in some control roots exposed in HS + HA and was also accompanied by strong autofluorescence (426–527 nm). Fluorescence may come from phenolics, terpenes, and other unsaturated organic compounds that plants produce as part of their defense mechanism, though for this study the form of species is not known [18]. While no actual uptake was evident based on these images, Cd and Se were detectable (above the background) in the leaves of plants exposed to QD in the presence of HA (Fig. 5), indicating some internalization of these ions. Unlike in plants exposed to QDs without HA, 0.074(±0.05) mg Cd/kg DW_{leaves} and 0.018(±0.01) mg Se/kg DW_{leaves} were measurable in plants exposed to QDs after 1 day. These species are more likely to be free Cd^{2+} and Se^{2-} (or SeO_3^{2-}) given the large variations (see standard deviations) in the Cd:Se mole ratios in the leaves compared to those in the roots (Table 1). These ions may have been potentially released from QDs suspended in HS + HA and taken up by the plants. As seen from the Cd^{2+} and SeO_3^{2-} control experiments, these ions have the ability to translocate (roots-to-leaves). Again, the release of free Cd^{2+} and Se^{2-} (or SeO_3^{2-}) in different QD suspensions have been suggested in several studies [34,40]. However, for this CdSe/ZnS QD, the exact mechanism of its degradation in HA is not fully understood. Separation and quantification of free Cd^{2+}, Se^{2-}, and/or SeO_3^{2-} ions from intact QDs in plant matrix remain a challenge at this time, and therefore was not performed in this study.

3.4. Particle-induced oxidative stress response

Though internalization of intact NPs was not observed, NPs may still have an effect on exposed plants. Several studies using cell-cultures [14,51], microorganisms [52], and aquatic organisms [12,53] have reported QD cytotoxicity caused by oxidative stress. QD toxicity could be induced by the release of the core ions and surface coatings, formation of ROS and/or by intact QDs themselves. In this study, plant oxidative stress was evaluated based on the changes in the levels of GSH and GSSG in the plants. GSH is an essential component of the cellular antioxidative defense mechanism, which keeps the levels of ROS under control [54,55]. Under normal conditions, GSH/GSSG ratios are high, where 90% total glutathione is GSH and <10% is GSSG. In response to stress, this ratio decreases due to the oxidation of GSH during the ROS detoxification process [54,56]. For the different exposure experiments, the GSH/GSSG ratios were plotted in Fig. 6. As expected, GSH was depleted in response to the uptake of heavy metals by plants. The GSH/GSSG ratios in plants exposed to 5 μg/mL Cd^{2+} were lower than the control (control: 85(±21), Cd^{2+}: 37(±28)). This ratio is even lower for plants exposed to the same concentration of SeO_3^{2-} (2(±2)). Between Cd^{2+} and SeO_3^{2-}, the measured GSH/GSSG ratios suggest

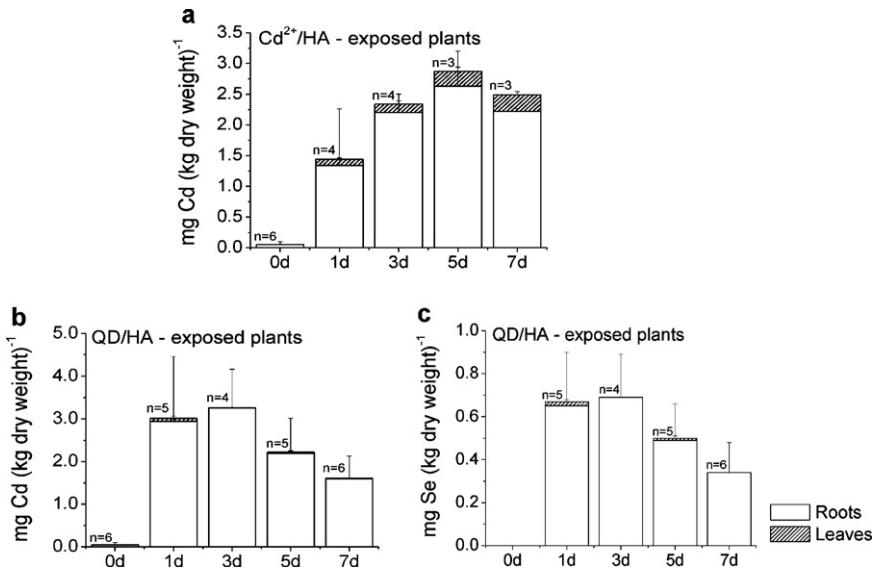

Fig. 5. Distribution of Cd and Se in the roots and leaves of plants exposed to (a) Cd^{2+}, and (b and c) QDs in HS + HA. Each bar graph section represents the concentration of Cd/Se in the roots and in the leaves. Levels of Cd/Se found in the roots and the leaves are significantly different from the control.

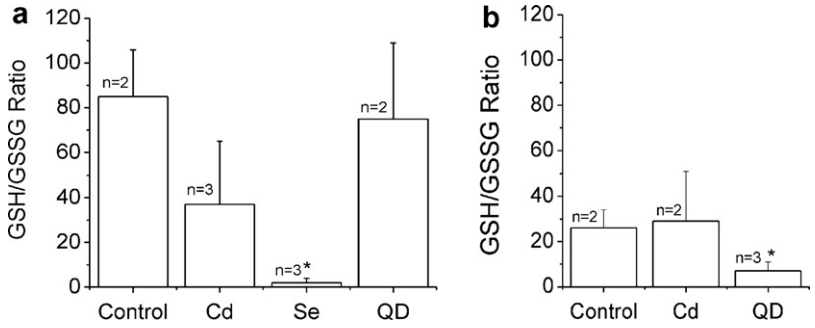

Fig. 6. Ratio of GSH to GSSG in *Arabidopsis* plants in response to Cd^{2+}, SeO_3^{2-}, and QD exposure in (a) HS and (b) HS + HA. Exposure time: control, Cd, and QD = 7 days, Se = 5 days. Asterisks (*) denote significant differences in ratios compared to the controls; Student's *t*-test (two-tailed) at 95% confidence level.

that the plants were more disturbed with the uptake of SeO_3^{2-}. This effect could be related to the different reactions/processes that take place involving GSH in the presence of these ions. Between Cd^{2+} and SeO_3^{2-} ions, ROS generation is more direct for SeO_3^{2-} than for Cd^{2+} [57,58]. It has been reported that GSH itself participates in the formation of ROS from SeO_3^{2-} [57]. The reaction between GSH and SeO_3^{2-} produces GSSG and superoxide anions ($O_2^{\bullet -}$), resulting in decreased GSH/GSSG ratio. On the other hand, while ROS is not directly generated from Cd^{2+}, GSH in Cd-exposed plants could be depleted with the formation of GS_2—Cd^{2+} complexes through the GSH-cysteine residues [59,60], and the Cd-induced synthesis of phytochelatins from GSH [61,62]. GSH/GSSG ratios in the plants were generally lower in 10 μg/mL HA. The addition of HA to the HS had no significant effect on the GSH/GSSG ratios of Cd^{2+} exposed plants (control: 26(±8), Cd^{2+}: 29(±22)). For plants exposed to dispersions of QDs, GSH/GSSG ratios were different with (6(±4)) and without HA (75(±34)). Toxic effects were generally promoted in the presence of HA based on the significantly lower GSH/GSSG ratios (QD vs. QD in HS + HA, $p < 0.05$). In the absence of HA, the GSH/GSSG ratio was not significantly different from the control. While other studies have pointed out the occurrence of membrane peroxidation upon contact of NPs to cells [16], mere adsorption of QDs onto the roots (Fig. 4a and b) did not result in apparent oxidative stress. Microscopy images revealed that the plant roots were still healthy and not visually different from the control. The significantly different GSH/GSSG ratios in the presence of HA could be related to the intense cellular autofluorescence noted in the HA set-ups (Fig. S4). Compared to all the other treatments, decreased GSH/GSSG ratios were only observed in Se-exposed plants. Since results from ICP–MS and fluorescence microscopy analysis do not suggest uptake of intact QDs, the shift to lower GSH/GSSG ratios could be due to uptake of free Cd^{2+}, and/or SeO_3^{2-} leached from QD surface deterioration. It is possible that the dissolution of Cd^{2+}, and/or SeO_3^{2-} from the intact QD was not measurable by the fluorimeter used ($\lambda_{\text{maximum resolution}} = 1.5$ nm). Hence, the presence of leached Cd^{2+}, and/or SeO_3^{2-} still needs to be experimentally verified using techniques that can separate these ions from intact QDs. The release/formation of ROS from the QDs (aside from those derived from Cd^{2+} and SeO_3^{2-}) is also possible. The type and concentration of ROS generated also needs to be confirmed. For a better understanding of the QD-induced oxidative stress, research concerning the dynamics of the interaction between QDs, HA and plant roots is necessary.

4. Conclusions

The present study suggests that the polymer-coated (carboxylate-terminated) CdSe/ZnS QDs are not internalized and translocated by *A. thaliana* as intact QDs within 7 days of exposure. Unlike its constituent ions, the QDs are not evidently taken up and are generally adsorbed onto the plant root surfaces. For these hydroponically-exposed plants, the amount of adsorbed

QDs appeared to be dependent on the stability of the QD in suspension. Despite no outstanding evidence of QD uptake, root exposure to QDs in the presence of HA could still negatively impact plants as revealed in the changes in GSH/GSSG ratios. Whether or not oxidative stress is due to the uptake of intact QDs or dissolved ions is still inconclusive as there is no significant evidence that show internalization of intact QDs or dissolution of ions. From an environmental standpoint, without taking into consideration the long-term stability of QDs and other NPs in these systems and in soil, studies that investigate exposure of plants to NPs over longer periods may be irrelevant. As recently reported, sorption of QDs onto soil will greatly influence the kinetics of QD desorption and degradation [63] which will dictate the type and form of QD species that is bioavailable for plant uptake. Nevertheless, on the basis of these results and other studies, uptake of available QDs might still be limited unless the QDs are surface modified in a manner that can be recognized by specific plant receptors that promote uptake [24,64].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.12.012.

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