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Short-term assessment of cadmium toxicity and uptake from different types of Cd-based Quantum Dots in the model plant *Allium cepa* L.

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ABSTRACT

We report on the toxicity and bioaccumulation of three different types of Cd-based quantum dots (QDs), dispersed in aqueous medium, for a model plant *Allium cepa* L. It is believed that encapsulation of nanoparticles should reduce their toxicity and increase their stability in different environments; in this work we studied how QD encapsulation affects their phytotoxicity. Core, core/shell, and core/shell/shell QDs (CdTe, CdTe/ZnS, and CdTe/CdS/ZnS QDs capped by 2-mercaptopropionic acid) were tested and $CdCl_2$ was used as a positive control. After 24-h and 72-h exposure, total Cd content (M_{Cd}) and bioaccumulation factors (BAFs) were determined in all parts of *A. cepa* plants (roots, bulb, shoot), and the total length of the root system was monitored as a toxicity end-point. Measurements of total Cd content versus free Cd^{2+} content (with Differential Pulse Voltammetry, DPV) in exposure media showed differences in chemical stability of the three QD types. Correspondingly, selected QDs showed different toxicity for *A. cepa* and different Cd bioaccumulation patterns. CdTe QDs were the most toxic; their effect was similar to $CdCl_2$ due to the release of free Cd^{2+}, which was confirmed by the DPV measurements. Plants exposed to CdTe QDs also bioaccumulated the most Cd among all QD exposure groups. CdTe/ZnS QDs showed no toxicity and very low bioaccumulation of Cd in *A. cepa;* the main source of measured Cd in the plants were QDs adsorbed on their roots, which was confirmed by fluorescence microscopy. On the contrary, CdTe/CdS/ZnS QD toxicity and bioaccumulation patterns were similar to those of CdTe QDs and pointed to unstable CdS/ZnS shells.

1. Introduction

Quantum dots (QDs) are fluorescent semiconductor nanocrystals which are being extensively developed because of their unique size-dependent optical and photophysical properties (Drbohlavová et al., 2009; Stanisavljevic et al., 2014). These properties have made QDs ideal for applications in various mainstream market products, i.e. light emitting devices such as computer and television screens (Hobson, 2009), and in science, mainly as labels for tagging and imaging in biological systems (Jamieson et al., 2007; Šobrová et al., 2013).

In contact with aqueous media, core Cd-based QDs (CdTe, CdSe) have been shown to leach Cd^{2+} (Xu et al., 2010), which is known for its high toxicity (Agency for Toxic Substances and Disease Registry, 2015). To prevent Cd leaching, QD core can be encapsulated with inert and stable materials (Parani et al., 2016); this has led to the recent development of core/shell QDs (CdTe/ZnS, CdTe/CdSe, CdSe/ZnTe, CdSe/CdS) and core/shell/shell QDs (CdTe/CdS/ZnS, CdSe/ZnSe/ZnS, CdSe/ZnTe/ZnS). The most common encapsulating material is ZnS, which enhances QD fluorescence efficiency (Hines and Guyot-Sionnest, 1996), makes them less prone to oxidation and photobleaching, increases their chemical stability, and extensively reduces toxicity imparted by the highly reactive core (Rzigalinski and Strobl, 2009). Several studies have demonstrated that ZnS shell encapsulation effectively reduces the release of Cd^{2+} from core Cd-based QDs in aqueous media (Derfus et al., 2004; Bakalova et al., 2005; Chan et al., 2006; Parani et al., 2016). Also, ZnS shells were found to reduce free radical generation by QDs due to air oxidation (Derfus et al., 2004). A recent cytotoxicity study also noted some advantages of multilayer-protected QDs (i.e. CdTe/CdS/ZnS QDs), which offer enhanced photostability, stability in acids, and oxidation stability compared to monolayer-

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protected QDs, both in aqueous solutions and intracellular environment (Parani et al., 2016).

Core, core/shell, and core/shell/shell Cd-based QDs could be additionally capped by several kinds of surface ligands, such as glutathione, mercaptopropionic acid, mercaptosuccinic acid, mercaptoacetic acid, cysteamine or polyethylene glycol, among which deprotonated thiols (thiolates) are most often used (Lovrić et al., 2005). Surface ligands improve QD solubility and reduce their aggregation. They can be further conjugated with targeting molecules, such as antibodies or receptor ligands, which target QDs to specific tissues or organs (Smith et al., 2008). In addition to ZnS shell, capping with mercaptopropionic acid has been shown to further reduce the release of Cd^{2+} from CdSe/ZnS QDs (Bakalova et al., 2005). However, degradation of the shell or capping material must also be considered. For example, ZnS shell reduced but not eliminated QD cytotoxicity due to air or photooxidation (Derfus et al., 2004), and CdSe/ZnS QDs could also generate free radical species (Green and Howman, 2005).

Studies of the effects of core/shell QDs (CdSe/CdZnS and CdSe/ZnS) on terrestrial plants have so far focused on their potential for uptake, translocation, and transformation in different plant organs, mainly by the detection of QD fluorescence (Navarro et al., 2012; Wang et al., 2014; Koo et al., 2015). Phytotoxicity of Cd-based QDs and/or the possibility for their bioaccumulation has only been studied in freshwater plants, such as algae (summarized in a recent review by Rocha et al. (2017), and in duckweed (Modlitbová et al., 2018).

Since the possibility of trophic transfer has long been known for elemental Cd (Croteau et al., 2005), trophic transfer of QDs has also been studied in several papers. CdSe QDs were showed to accumulate in bacteria (*Pseudomonas aeruginosa*) and consequently to biomagnify in their protozoan predator, *Tetrahymena thermophila* (Werlin et al., 2011). If carboxyl QDs were dosed into algae (*Pseudokirchneriella subcapitata*), they showed potential for biomagnification in daphnids, *Ceriodaphnia dubia* (Bouldin et al., 2008). On the contrary, carboxylated CdTe QDs were not biomagnified through dietary uptake from protozoans to rotifers (Holbrook et al., 2008). To the best of our knowledge, trophic transfer of QDs in terrestrial plants has not been studied yet; however, as mentioned in Navarro et al. (2008), transfer of QDs through food webs could be expected due to long-lasting residence in cells.

In terrestrial plant ecotoxicology, the *Alium cepa* test (Fiskesjö, 1958) has been widely used for toxicity testing of a variety of organic/inorganic pollutants due to its high sensitivity, low cost, and easy and rapid performance (Leme and Marin-Morales, 2009). Studies on the effects of Cd salts, such as $CdCl_2$ or $Cd(NO_3)_2$, to *A. cepa* have shown toxicity on macroscopic (root and shoot length) and microscopic levels (mitotic index, nuclear abnormalities, chromosomal aberrations, catalase and guaiacol peroxidase activities), genotoxicity, mutagenicity, and possibility of bioaccumulation (Seth et al., 2008; Zou et al., 2012; Arya and Mukherjee, 2014; Hemachandra and Pathiratne, 2015). Recently, *A. cepa* has also been used for the assessment of nanoparticle toxicity, e.g. Ag (Kumari et al., 2009; Cvjetko et al., 2017), TiO_2 (Klančnik et al., 2011; Pakrashi et al., 2014), ZnO (Ghodake et al., 2011; Demír et al., 2014), SiO_2 (Koce et al., 2014), CoO (Ghodake et al., 2011) and carbon nanotubes (Ghosh et al., 2015), but not yet for Cd-based QDs. To the best of our knowledge, comparative toxicity and bioaccumulation studies with all three different types of Cd-based QDs (core, core/shell and core/shell/shell) have not been conducted in aquatic or terrestrial plants; so far only core or core/shell QDs (CdTe, CdTe/CdS, CdTe/ZnS, CdTe/SiO_2, CdSe/CdZnS, CdSe/ZnS) have been compared (Xu et al., 2010; Rocha et al., 2017; Modlitbová et al., 2018). Given the hazardous potential of Cd-based QDs, it is very important to address these knowledge gaps in plant ecotoxicology.

Furthermore, toxicity of QDs at all trophic levels depends on their physico-chemical properties (particle size, surface coating materials, and charge), environmental factors (light, pH, dissolved oxygen, ionic strength, natural organic matter, and extracellular polymeric substances), concentration, exposure time, and species studied (Hu et al., 2016; Rocha et al., 2017). Particularly non-capsulated QDs show low resistance to pH changes (Škarková et al., 2017), and sensitivity to presence of several metal ions, e.g. copper and/or zinc (Chen and Rosenzweig, 2002); moreover, QD toxicity can be affected by surface coatings and UV excitation (Derfus et al., 2004).

In this study, we focused on several major goals. The first objective was to measure the toxicity of different Cd compounds to *A. cepa* grown in an aqueous medium. We tested $CdCl_2$ as positive control (Seth et al., 2008; Arya and Mukherjee, 2014; Hemachandra and Pathiratne, 2015) and three types of Cd-based QDs (core, core/shell, and core/shell/shell – CdTe, CdTe/ZnS, and CdTe/CdS/ZnS QDs) capped by 2-mercaptopropionic acid (MPA). After 24-h and 72-h exposure, we monitored the total length of the root system as a macroscopic toxicity end-point. The second objective was to determine the total Cd content in different plant organs (roots, bulbs, and shoots) by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The third objective was to inspect *A. cepa* roots by fluorescence microscopy to determine if Cd-based QDs are adsorbed on the root surface and how this depends on the QD type. Free Cd^{2+} content in exposure media before and after the test was measured by differential pulse voltammetry (DPV) to assess differences in chemical stability among the QD types. We discussed possible hazardous effects of Cd-based QDs for *A. cepa* as well as the environmental implications of exposure to Cd-based QDs.

2. Materials and methods

2.1. Quantum dot synthesis and analysis

Reagents for QD synthesis are listed in the Supplementary Material (page S3) together with detailed descriptions of QD preparation procedures. Briefly, CdTe QDs were prepared as described in Long et al. (2013); this core-type QDs were used as precursors for the synthesis of CdTe/ZnS QDs and CdTe/CdS/ZnS QDs. CdTe/ZnS QDs were prepared as described in Liu and Yu (2010) and CdTe/CdS/ZnS QDs were synthesized according to Li et al. (2011).

Nominal hydrodynamic particle diameter was determined by dynamic light scattering (DLS) using DynaPro NanoStar (Wyatt Technology, Dernbach, Germany). Cadmium content in all QD types was measured with ICP-OES spectrometer iCAP 6500 Duo (Thermo Fisher Scientific, Waltham, USA). Fluorescence and absorbance spectra were measured with multimode microplate Tecan reader (Tecan Trading AG, Männedorf, Switzerland). CdTe QDs, which served as precursors for the other two types of QDs, were characterized as follows: their quantum yield was evaluated with Jobin Yvon FluoroLog – Quanta φ (Horiba, Kyoto, Japan), and TEM photographs were obtained with FEI Tecnai F20 (FEI, Eindhoven, Netherlands) FEG transmission electron microscope operating at 200 kV. Individual images were collected with 16k CCD camera (FEI Eagle). pH was measured with the 913 pH Meter (Metrohm AG, Herisau, Switzerland).

2.2. Preliminary growth medium test and toxicity experiments with A. cepa

We used the Stuttgart type cultivars of onion (*A. cepa*). Onion bulbs were stored at 4 °C before the experiment, and only bulbs of 1–2 g in good condition (no dry or damaged parts) were selected. Before the experiments, the bulbs were placed on top of 20 mL glass test tubes filled with MilliQ water. After 24 h, the bulbs that grew roots about 0.3–1 cm in length were used in the experiments.

Our *A. cepa* test was based on the modified methodology of Fiskesjö (1958). We conducted the preliminary growth medium test and then two series of Cd experiments. The details of the preliminary experiment are described in Supplementary Material (page S4).

In the main Cd experiments, onion bulbs with roots were placed on top of glass tubes as described previously. Glass tubes were filled with 20 mL of growth medium (MilliQ water) containing Cd compounds ($CdCl_2$, CdTe QDs, CdTe/ZnS QDs, and CdTe/CdS/ZnS QDs). In the first