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# Comparative investigation of toxicity and bioaccumulation of Cd-based quantum dots and Cd salt in freshwater plant *Lemna minor* L.

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ABSTRACT

The purpose of this study was to determine the toxicity of two different sources of cadmium, i.e. $CdCl_2$ and Cd-based Quantum Dots (QDs), for freshwater model plant *Lemna minor* L. Cadmium telluride QDs were capped with two coating ligands: glutathione (GSH) or 3-mercaptopropionic acid (MPA). Growth rate inhibition and final biomass inhibition of *L. minor* after 168-h exposure were monitored as toxicity endpoints. Dose-response curves for Cd toxicity and $EC50_{168h}$ values were statistically evaluated for all sources of Cd to uncover possible differences among the toxicities of tested compounds. Total Cd content and its bioaccumulation factors (BAFs) in *L. minor* after the exposure period were also determined to distinguish Cd bioaccumulation patterns with respect to different test compounds. Laser-Induced Breakdown Spectroscopy (LIBS) with lateral resolution of 200 μm was employed in order to obtain two-dimensional maps of Cd spatial distribution in *L. minor* fronds. Our results show that GSH- and MPA-capped Cd-based QDs have similar toxicity for *L. minor*, but are significantly less toxic than $CdCl_2$. However, both sources of Cd lead to similar patterns of Cd bioaccumulation and distribution in *L. minor* fronds. Our results are in line with previous reports that the main mediators of Cd toxicity and bioaccumulation in aquatic plants are $Cd^{2+}$ ions dissolved from Cd-based QDs.

## 1. Introduction

Quantum dots (QDs) are fluorescent semiconductor nanocrystals, commonly made up by a 3–6 nm diameter core of CdS, CdSe, PbSe, CdTe or a range of other metals, and coated by an organic polymer (Chan et al., 2002). The use of QDs has been increasing because of their great potential to replace traditional organic dyes as labels for tagging and imaging in biological systems (Jamieson et al., 2007). The main advantage of QDs in comparison to organic dyes or fluorescent proteins is that QDs are brighter, more stable against photobleaching, and can be excited for multicolor emission with a single light source (Bailey et al., 2004; Resch-Genger et al., 2008). However, analogously to other classes of nanomaterials, QDs may eventually find their way into the environment. In contact with aqueous media, Cd-based QDs have been shown to leach ionic Cd (Xu et al., 2010) which has been ranked the 7th out of 275 compounds, including organic chemicals, in the 2015 Priority List of Hazardous Substances (Agency for Toxic Substances and Disease Registry, 2015). Therefore, it is of a high importance to assess the toxicity of Cd-based QDs to environmental organisms, which may come into contact with QD-containing products when they are discarded.

Quantum dot toxicity is ascribed either to the induction of reactive oxygen species (ROS) formation or to the direct release of Cd ions; in most cells, these reactions cause cellular changes culminating in DNA damage (Gomes et al., 2011). Several studies have researched toxic effects of Cd-based QDs and/or possibility for their bioaccumulation in freshwater organisms, such as microorganisms (Gomes et al., 2011); algae – *Chlamydomonas reinhardtii* Dangeard (Domingos et al., 2011) and *Phaeodactylum tricornutum* Bohlin (Xu et al., 2010); and invertebrates – *Hydra vulgaris* (Ambrosone et al., 2012), *Leptocheirus plumulosus* (Jackson et al., 2012), *Daphnia magna* (Lee et al., 2009) and *Elliptio complanata* (Gagné et al., 2008; Peyrot et al., 2009). Free Cd released from QDs was shown to bioaccumulate in algae (Domingos et al., 2011) and amphipods (Jackson et al., 2012); to alter the synthesis of metallothioneins and trigger oxidative stress and DNA damage in mussels (Gagné et al., 2008; Peyrot et al., 2009); to cause cytotoxicity in

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algae (Xu et al., 2010); and to generate ROS in daphnids (Lee et al., 2009). Although studies on the distribution of Cd-based QDs in plants (core/shell QDs: CdSe/CdZnS and CdSe/ZnS) by detection of their fluorescence in different parts of plants have also been conducted (Navarro et al., 2012; Wang et al., 2014; Koo et al., 2015), they focused on the potential of core/shell QDs for uptake, translocation, or transformation, but not on the evaluation of toxicity values. To the best of our knowledge, no toxicity studies with QDs have been conducted in aquatic plants, except in green algae (Xu et al., 2010; Domingos et al., 2011). Given the hazardous potential of QDs, it is very important to address these knowledge gaps in plant ecotoxicology.

The free floating macrophyte *Lemna minor* L. (Lemnoideae, Araceae) is a common aquatic ecotoxicity test organism: it is a bioindicator species for the detection and monitoring of metal pollution (Garnczarska and Ratajczak, 2000) and also metal bioaccumulator. *L. minor* has already been used as a model organism in toxicity and bioaccumulation studies of several types of nanoparticles (NPs), e.g. Ag (Gubbins et al., 2011; Jiang et al., 2012; Oukarroum et al., 2013; Üçüncü et al., 2014), CuO (Shi et al., 2011; Perreault et al., 2014), $C_{60}$ (Santos et al., 2013), $Al_2O_3$ (Juhel et al., 2011), $TiO_2$ (Song et al., 2012; Li et al., 2013), and ZnO (Hu et al., 2013) NPs, but not yet Cd-based QDs. The effects of $Cd^{2+}$ on *L. minor* have been studied with the use of Cd salts: $CdCl_2$ (Razinger et al., 2008; Tkalec et al., 2008; Balen et al., 2011) and less typically $CdSO_4$ (Drost et al., 2007) or $Cd(NO_3)_2$ (Kwan and Smith, 1991). These studies have shown that Cd accumulates in *L. minor* and causes adverse effects, such as decreased growth, reduced levels of photosynthetic pigments, impaired chloroplast ultrastructure, increased activities of antioxidant enzymes, increased lipid peroxidation and decreased chlorophyll and protein contents (Razinger et al., 2008; Tkalec et al., 2008; Balen et al., 2011). As Cd is known to leach from Cd-based QDs (Xu et al., 2010) and dissolved ions are the main mediators of nanoparticle toxicity to organisms (Ivask et al., 2015), similar Cd bioaccumulation and toxicity as in the case of Cd can be expected upon exposure of *L. minor* to Cd-based QDs.

In this study we focused on several major goals. The first objective was to measure the toxic effects of different Cd compounds in *L. minor,* i.e. Cd salt and two types of Cd-based QDs (QDs capped by glutathione, GSH-QDs, or by 3-mercaptopropionic acid, MPA-QDs), where $CdCl_2$ served as a positive control for Cd toxicity to *L. minor*. After 168-h exposure two toxicity endpoints were monitored in *L. minor* : growth rate inhibition and final biomass inhibition according to the OECD 221 norm (OECD, 2002). The second objective was to determine the total content of Cd in plants and to distinguish how different sources of Cd are accumulated in *L. minor* fronds by using a conventional method for metal detection, ICP-OES. In this part, $CdCl_2$ served as a reference compound to test the hypothesis that *L. minor* bioaccumulates Cd ions that leach from Cd-based QDs (Xu et al., 2010). The third objective was to demonstrate that Laser-Induced Breakdown Spectroscopy (LIBS) is a useful tool for mapping the elemental distribution in *L. minor* fronds, as well as to monitor whether there are differences in the bioaccumulation patterns of Cd salt or Cd-based QDs. In the recent years, LIBS has been developed as an alternative and fast method for investigation of spatial distribution of elements; its applicability in plant samples has been summarized in three extensive reviews (Kaiser et al., 2012; Pořízka et al., 2012; Santos et al., 2012). However, LIBS has so far been used only for the detection of nano Ag in root tissues of *Vicia faba* L. (Krajcarová et. al, 2017), therefore further studies of its applicability for the detection of NPs in plants are needed. The fourth objective was to inspect *L. minor* fronds by transmission electron microscopy (TEM) to determine if QDs are able to penetrate cell walls and become accumulated in plant tissue or they are only adsorbed on frond surface. We discuss possible hazardous potential of QDs in aquatic environment for *L. minor* as a bioindicator species. We also show that LIBS technique is a successful alternative to conventional analytical methods due to the ability to map large areas of samples in short time with sufficient resolution.

## 2. Materials and methods

### 2.1. Quantum dot synthesis and analysis

Two types of QDs were synthesized – GSH-QDs and MPA-QDs. The methods of preparation were based upon extensive instructions available in Lišková et al. (2011) and Řezáčová et al. (2015). Quantum dot properties were analyzed as follows: the nominal average particle size was determined by FEI Tecnai F20 electron microscope (Thermo Fisher Scientific, Waltham, Massachusetts, USA); zeta potential was measured with Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) and Cd content in QDs was measured by ICP-OES spectrometer iCAP 6500 Duo (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

### 2.2. Toxicity experiment with L. minor

#### 2.2.1. Experimental setup

Laboratory stock culture of *L. minor* was used. Seven days before testing, sufficient colonies were transferred aseptically into fresh sterile modified Steinberg medium (ISO, 2005) and cultured under the test conditions. No contaminating organisms (such as algae) were present.

*Lemna* test was performed according to the OECD (Organization for Economic Co-operation and Development) Test No. 221: *Lemna* sp. Growth Inhibition Test using Steinberg medium (OECD, 2002). Toxicity tests were carried out in 200 mL beakers filled with 150 mL solution which consisted of the dilution series of test compounds in the Steinberg medium. Presence of EDTA in Steinberg medium was proven not to influence the metal uptake (Drost et al., 2007). *L. minor* plants were exposed to three Cd-containing compounds: GSH-QDs, MPA-QDs and $CdCl_2{\cdot}2.5H_2O$ (hereafter referred to as $CdCl_2$). For both GSH-QDs and MPA-QDs, the nominal test concentrations were 0, 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 7.5, 10, and 15 mg compound/L; for $CdCl_2$, the nominal test concentrations were 0, 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 7.5, and 10 mg compound/L.

One exposure group consisted of seven test replicates (beakers) for each test compound at each exposure concentration. There was one control group for each test compound. Before the exposure, the beakers were inoculated with four plants with three fronds each, which resulted in 12 fronds per beaker. The test was carried out for seven days (168 h) at the temperature of 24 ± 2 °C and light intensity of 8000 lx. pH of the Steinberg medium was 6.8 ± 0.1. The test was considered valid if the number of fronds in controls had grown eightfold.

Immediately after the exposure, the number of all *L. minor* fronds (both healthy and necrotic) was counted in each vessel as “all fronds” and “green fronds” (fronds without any evidence of necrosis or damage). The number of “green fronds” was used for the calculation of *L. minor* growth rate (μ) and growth rate inhibition (% $I_r$) for six replicates of each exposure group. Then each exposure group was divided into three parts for various analyses. Three replicates per exposure group were dedicated to the assessment of Cd bioaccumulation, another three replicates to the assessment of final biomass inhibition, and one replicate to the investigation of Cd spatial distribution in fronds and QD adsorption on the plant surface.

Plants from the seventh replicate of each exposure group were thoroughly washed in deionized water. Three or four fronds per beaker were used for LIBS spatial distribution mapping experiments and two fronds per beaker for TEM analyses. For LIBS measurements, fronds were carefully dried, molded and glued by epoxide glue onto a glass slide. For TEM analyses, frond cross-sections were prepared and photographed.

#### 2.2.2. Toxicity parameters

After the exposure period, the number of *L. minor* fronds in each beaker was counted. For all the tests, the *L. minor* growth rate μ was used as the first toxicity endpoint. The plant growth rate was calculated on the basis of frond numbers as: