UPTAKE OF CdTe QUANTUM DOTS BY MAIZE SEEDLINGS: EFFECTS ON SEED GERMINANTION AND ASSOCIATED PARAMETERS

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Abstract

In this study, we prepared CdTe quantum dots using microwave synthesis method (300 W/4 min) and monitored the effect of CdTe on the germination of maize (*Zea mays* L.) at defined time intervals (24, 48, and 72 h). All experiments were standardized to a single concentration of cadmium (Cd; 100 µM) and were carried out in three independent experiments (100 caryopses per variant, darkness, temperature 23 ± 2 °C, humidity 55 ± 5%). The potential effect of solvent on the germination was also studied. Germination was 58.4 ± 6.1 % in the control variant (n = 3). Cd alone inhibited germination rate for 10 ± 1.5%; whereas, 18 ± 1.8 % and 14.8 ± 1.6 % germination rates were recorded for CdTe QDs in distilled water and tap water, respectively. The germinative energy and coefficient velocity germination were concluded as the most sensitive parameters for determining the effect of CdTe QDs on *Z. mays*.

Keywords: Cadmium toxicity, germination, oxidative stress, quantum dots synthesis, solvent, nanomedicine

1. INTRODUCTION

Development of new nanotechnologies in recent years led the present research to focus on the materials with extremely small dimensions (such as carbon or iron nanoparticles, quantum dots, QDs). Functionalized QDs could be used to visualize and detect cancer cells, and may also represent a suitable tool for personalized medicine [1]. In fact, QDs may be used for mapping of tumour cells without the necessity of biopsy. Importance of these materials is expected to grow significantly over the time. Hence, together with their development, it is necessary to monitor their potential effects on non-target organisms because of the necessity to insure their safe use and also to maintain their long-term sustainability. It has been shown that nanomaterials are able to enter the ecosystems and food chain, where interact with individual abiotic factors such as water, soil and air. These interactions may lead to changes of their surface properties [2, 3]. These changes then influence the fate of nanoparticles in the environment and thus their (bio)availability to organisms. It is assumed that semipermeability of the cell wall allows the transport of small molecules, the transport of larger molecules in the case of aggregates of nanoparticles is limited by pore (plasmodesma) size (5 - 60 nm). Subsequently, nanoparticles enter the cells, where they may interact with various organelles (including endoplasmic reticulum, Golgi apparatus, lysosomes), and eventually influence major metabolic processes. Phytotoxicity of nanoparticles has been summarized in May reviews [3]. The size of nanoparticles is the most important factor that mainly controls their transport across the cell wall and plasma membrane. Cell wall contains both macropores (approximate size: 4.0 nm) and micropores (approximate size: 0.5 nm), which limit transport of

nanoparticles across the cell wall, and also prevent larger nanoparticles to enter the plant cells. Both apoplast and symplast transport of nanoparticles have been shown in horseradish (*Armoratia rusticana*). Transport across the epidermis (rhizodermis) and cortex of roots enables translocation of nanoparticles into aerial parts. Ability of nanoparticles to release ions of metals is next very important factor that contributes to their toxicity. Released metal ions contribute to a stress reaction via participation in generation of reactive oxygen species (ROS) [4]. The elimination of ROS is closely connected with glutathione-ascorbate cycle as well as enzymes closely associated with this cycle (e.g., superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione peroxidase, GPX; **Figure 1**).

Earlier, our group has studied the effects of cadmium (Cd) on the course of stress reaction and role of thiol compounds in plants in our works [5-7]. Even with regard to a number of published works in the study of effect of nanoparticles on different plants species, this area is still open for extensive research. In the case of specific plants species such as maize (*Zea mays* L.), information about the potential effect of nanoparticles on and underlying mechanisms in plants is very limited [8].

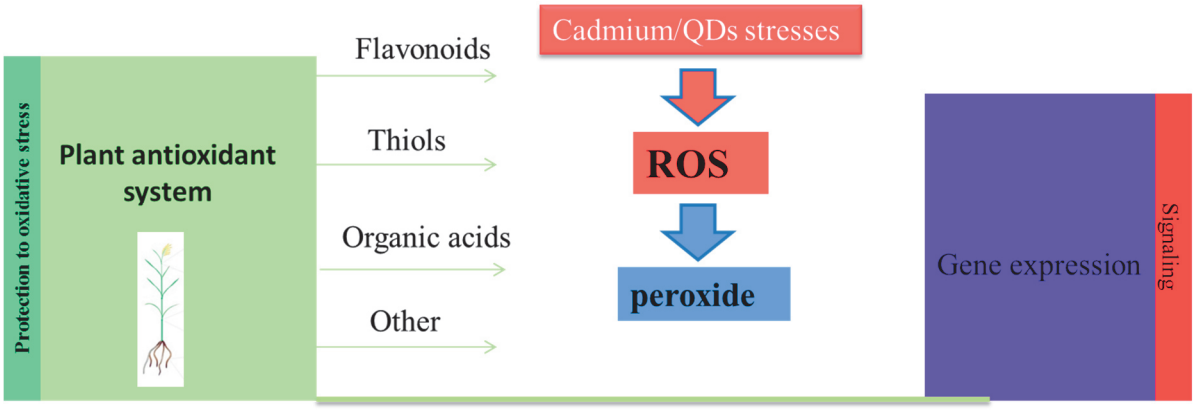

Figure 1 A simplified scheme of biological effects of cadmium ions (and probably also quantum dots) on homeostasis in plants. Cadmium ions are able to induce generation reactive oxygen species (ROS) including both radical and non-radical forms. Plant antioxidant system focuses on the protection of plant cells against oxidative stress and participates on maintaining of redox balance inside cells. It consists of non-enzymatic and enzymatic "antioxidants". Non-enzymatic antioxidants include flavonoids and generally polyphenols, thiols, and organic acids, lipid soluble compounds (α-tocopherol, β-carotene, lycopene) and also both water soluble antioxidant metabolites namely ascorbate and glutathione; whereas, superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) belong to the list of enzymatic antioxidants. The highlighted above antioxidant system is closely connected with cell signalling and subsequently with gene expression

2. METHODS

Chemicals procurements, and nanoparticle synthesis and characterization

All reagents for syntheses, standards, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) in ACS purity, unless noted otherwise. Solutions were prepared using Milli-Q water (Millipore) as solvent. The size and zeta potential of QDs were determined measured by Dynamic light scattering (Zetasizer Nano ZS90, Malvern instruments, Malvern, UK). QDs were further characterized within the meaning of their optical properties (fluorescence and absorbance) using multifunctional reader Infinite 200 (Tecan, Männedorf, Switzerland). To evaluate the electrochemical behavior of QDs was employed potentiostat 910 PSTATmini (Metrohm, Switzerland). Measurements in electrochemical cells were done in a volume of 1.0 ml; where the electrolyte was 0.2 M acetate buffer (pH 5). Parameters of voltammetry were as follows: initial potential –1.2 V;

end potential –0.3 V; potential step 3 mV; and potential deposition –1.0 V. QDs were synthetized according to [9, 10]. Briefly, a microwave system made by Samsung was used for the preparation of CdTe QDs. The system can operate at 2450 MHz frequency and work at 0–1000 W power. Cadmium acetate dihydrate $Cd(OAc)_2 \cdot 3H_2O$ (0.266 g) was dissolved in ACS water (50 ml). MSA (1.0 ml; 3.0 g /50 ml) was slowly added to the stirred solution. White precipitate was formed, which disappeared after addition of 1.8 ml of 1M NH_4OH (pH = 9.88) and 1.5 ml Na_2TeO_3 (0.2215 g / 50 ml) and 40 mg $NaBH_4$. The obtained solution was stirred for 2 h. Volume was adjusted to 100 ml with addition of water and after that it was heated in vials filled with 2.0 ml of the solution in microwave oven (300 W, 120 °C, 4 min).

Plant exposure conditions and assays

For the germination protocol, maize (*Zea mays* L. cv. Silien) caryopses were rinsed three times with deionised water and placed into plastic boxes with filter paper and tap water (100 ml). The experiments were performed from 01. 04. 2016 to 30. 07. 2016. *Z. mays* caryopses were kept in darkness at temperature 23 ± 2 °C, humidity 55 ± 5 % in three independent triplicates. All the experiments were standardized to a single concentration of Cd (100 µM). Samples were collected in 24, 48, and 72 h time intervals, and were carefully washed three times with distilled water. The germination rate of 100 caryopses per variant was evaluated. All variants were photo-documented and the parameters were determined and calculated for all experimental variants including control.

3. RESULTS

For the purpose of our preliminary pilot experiment, nanoparticles (CdTe quantum dots) were prepared using microwave synthesis protocol. Final concentration of Cd was 100 µM and the prepared CdTe QDs were designated as a batch 1550. **Figure 2a** shows typical appearance of the solution under the white light and the appearance of the solution after irradiation with UV light (300 nm); distinctive red fluorescence was well evident. **Figure 2b** shows a typical recording of absorption spectrum of CdTe QDs, batch 1550, maximum at 594 nm, which probably corresponds to modification with mercaptoethanol. Increase in absorbance at wavelengths near to UV (about 300 nm) was also well evident. Fluorescence properties of CdTe QDs, batch 1550, were also studied. These analyses helped in the determination of excitation and emission spectra (λ_{exit} = 250 nm; λ_{em} = 580 nm), see in **Figure 2c.**

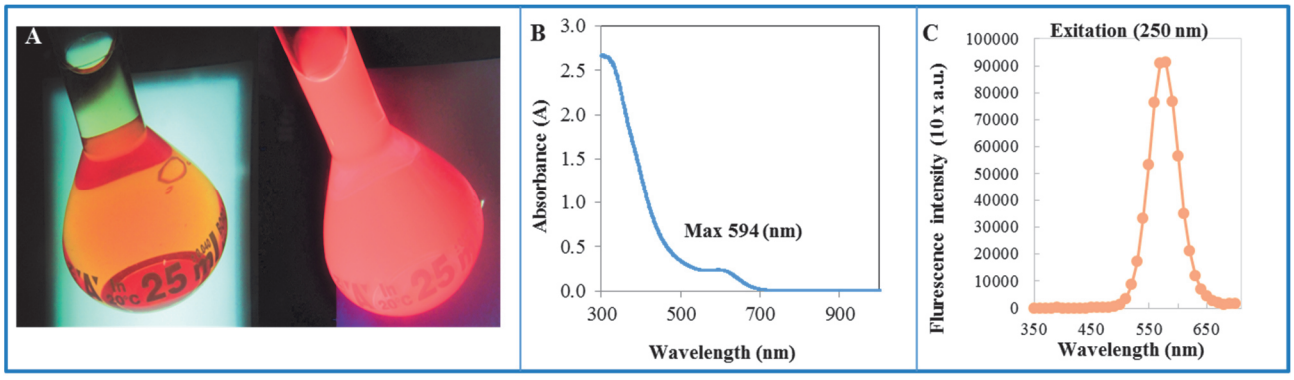

Figure 2 Microwave synthesis of CdTe QDs (concentration of cadmium: 840 µM). (**A**) appearance of solution under the white light and under UV (300 nm). (**B**) typical recording of absorption spectrum of CdTe QDs, batch 1550. (**C**) typical recording of fluorescence spectrum of prepared CdTe QD. *For details, see Material and Methods section, batch 1550*

The prepared CdTe QDs, batch 1550, were applied in experiment that focused their effect on a process of germination of *Z. mays*. *Z. mays* is an excellent model plant system in experiments focused on stress reaction. In common experiments monitoring germination parameters, tap water is used as a typical solvent since it

contains common ions. Due to this fact, this study also monitored the effect of tap water on germination of *Z. mays*. Applied concentration of Cd was standardized to a single concentration of 100 µM. Dilution of the CdTe QDs, batch 1550, with distilled water maintained their fluorescence properties. On the contrary, tap water gradually quenched their fluorescence. The prepared working solutions (100 ml) were applied in experiments, where 100 caryopses were used per variant. The differences in germination rate after 72 h were as follows: 58.4 ± 6.1 % for distilled water, 48 ± 5.1 % for Cd in the form of Cd $(NO_3)_2$, 40 ± 4.3 % for CdTe QDs in distilled water, and 44 ± 4.7 % for CdTe QDs in tap water. No differences between roots and aerial parts of experimental plants were observed. Obtained data are summarized in **Figure 3**.

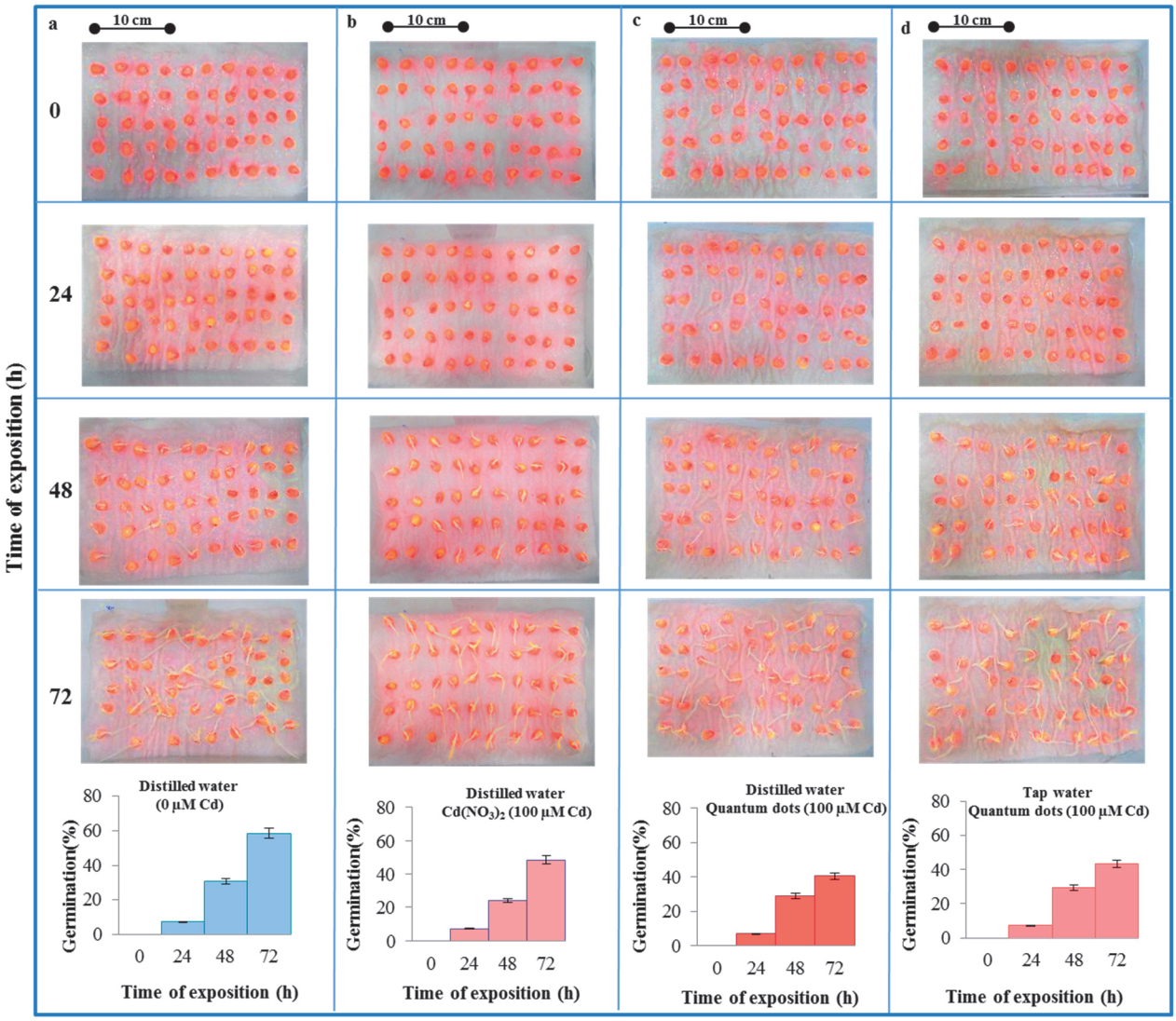

Figure 3 Study of effect of CdTe QDs (batch 1550) on germination of maize (*Zea mays* L. cv. Silien) in a time-dependent manner (0, 24, 48, 72 h). Distilled water was used as control (**a**), Cd $(NO_3)_2$ in distilled water as sample 1 (**b**), CdTe QDs (batch 1550), in distilled water as sample 2 (**c**), and CdTe QDs (batch 1550), in tap water as sample 3 (**d**). All experiments were standardized to a single concentration of cadmium (Cd; 100 µM) and were carried out in three independent experiments with 100 caryopses per each variant and experiment

In order to better illustrate the effect of solvent on germination, values for solvents were subtracted from individual experimental variants (**Figure 4**). In the case of Cd $(NO_3)_2$ and CdTe QDs in tap water, very slight stimulant effect we observed on germination after 24 h of treatment (**Figure 4a, c**). After 48 h of treatment,

germination difference (GD) was 6.8 ± 0.3 % for Cd $(NO_3)_2$ compared to CdTe QDs GD (2 ± 0.1 % and/or 1.4 ± 0.07 %). This effect was probably caused by nitrate ions, which are transported together with water inside the cells. After 72 h, minimal GD (18 ± 0.9 %) was determined for CdTe QDs in distilled water; in the case of tap water, this value was 14.8 ± 0.7 % and for Cd $(NO_3)_2$ 10 ± 0.5 %: For details, **Figure 4** may be consulted.

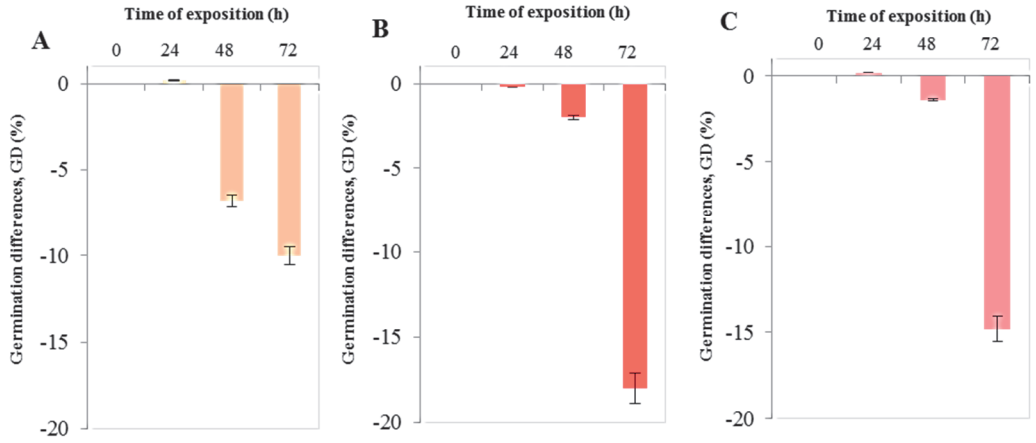

Figure 4 Changes in the germination of maize (*Zea mays* L., cv. Silien) after 0, 24, 48, and 72 h for different forms of cadmium and solvents (A) Differences were related to control (distilled water). For other experimental conditions, see **Figure 3** and Material and Methods section

It was possible to determine individual parameters for germination after 72 h of treatment. Details about the calculation of these parameters are highlighted herein. Caryopses were considered germinated when they exhibited radicle extension > 3 mm. Every 24 h after the washing, number of germinating caryopses were recorded daily during the course of the experiment to determine individual germination parameters. Number of germinated caryopses was recorded 4 days after the beginning. The Final Germination Percentage (FGP) was calculated according to (1):

$$FGP = \frac{Ng}{Nt} \text{x} 100 \quad (1)$$

The Germination Index (GI) was calculated as described in [11] using following (2) formula:

$$GI = \frac{no\ of\ germinated\ seed}{counts\ of\ germinated\ seeds} \quad (2)$$

Coefficient of Velocity of Germination (CVG, 3) determined by:

$$CV = \frac{\sum Ni}{\sum NiTi} \text{x} 100 \quad (3)$$

Mean Daily Germination (MDG), which is index of daily germination, was calculated from (4):

$$MDG = \frac{FGP}{d} \quad (4)$$

Where FGP is final germination percentage and d is number of days to the maximum of final germination, see in [11]. Mean Germination Time (MGT) was calculated according to [11].

Results are summarized in **Figure 5**. Control (distilled water) exhibited maximal germinative energy (GE) about 24.1, the lowest mean germination time (MGT; 2.55) and the highest value of velocity of germination CVG (13.65). All parameters were significantly decreased in the case of CdTe QDs in distilled water; whereas, tap water showed protective effect. Value of GE was decreased (compared to the control - distilled water) in the case of Cd $(NO_3)_2$ (for -6.5), for -12.8 for CdTe QDs in distilled water, and for -12.2 for CdTe QDs in. Values of MGT were significantly increased compared to the control, for 0.11/0.02/0.01 in individual variants. Values of germination index GI were decreased in all variants in comparison with the control - for -0.16/-0.02 and -0.01 in individual variants. Similarly, values of CVG were significantly decreased compared to the control - for -4.85/-7.35, and -6.95 in individual variants.

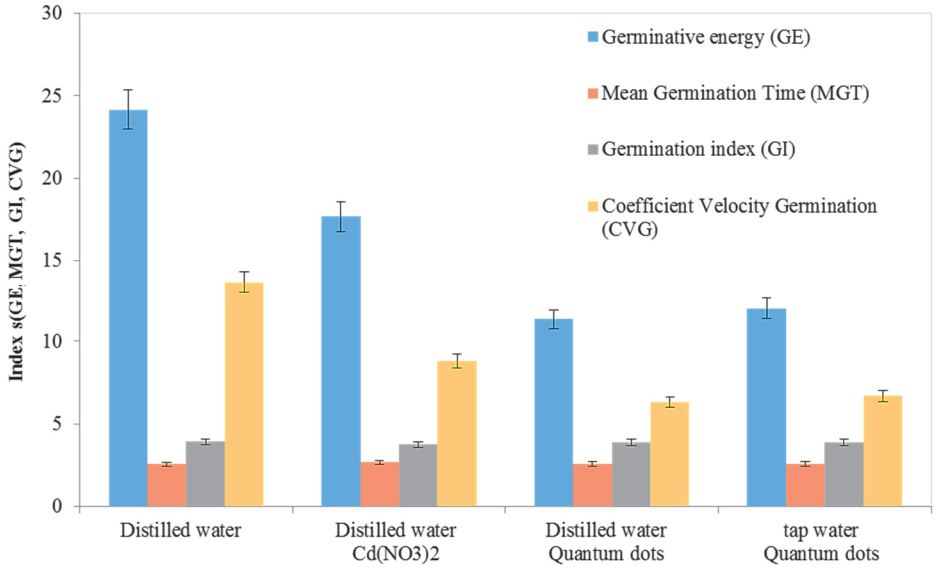

Figure 5 Summary indexes (Germination energy, Mean germination time, Germination index and coefficient velocity germination) after 72 h for different forms of cadmium. For other experimental conditions, see **Figure 3** and Material and Methods section

4. CONCLUSION

The work enlightened for the first time the information about the potential effect of CdTe QDs on the germination of *Z. mays* caryopses. Despite the fact that *Z. mays* is widely distributed crop used in human nutrition, nutrition of animals, and also in industry, our knowledge about effect of nanoparticles on germination processes is still very limited [8, 12, 13]. This study unveiled the ability of CdTe QDs to affect germination of *Z. mays* caryopses under defined experimental conditions. The germinative energy and coefficient velocity germination were concluded as the most sensitive parameters for determining the effect of CdTe QDs on crop plants such as *Z. mays*.

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