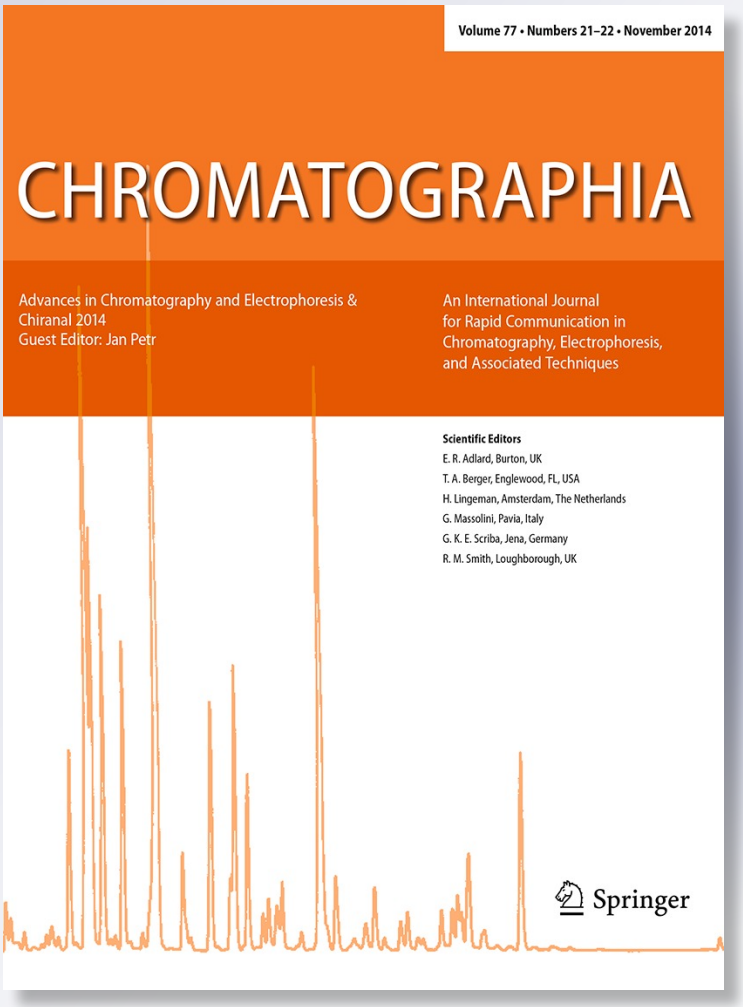
Biosynthesis of Quantum Dots (CdTe) and its Effect on Eisenia fetida *and* Escherichia coli

Marketa Kominkova, Petr Michalek, Amitava Moulick, Barbora Nemcova, Ondrej Zitka, Pavel Kopel, Miroslava Beklova, Vojtech Adam, et al.

Chromatographia
An International Journal for Rapid Communication in Chromatography, Electrophoresis and Associated Techniques

ISSN 0009-5893
Volume 77
Combined 21-22

Chromatographia (2014) 77:1441-1449
DOI 10.1007/s10337-014-2775-9



Springer

Chromatographia (2014) 77:1441–1449
DOI 10.1007/s10337-014-2775-9

ORIGINAL

Biosynthesis of Quantum Dots (CdTe) and its Effect on *Eisenia fetida* and *Escherichia coli*

Marketa Kominkova · Petr Michalek · Amitava Moulick · Barbora Nemcova · Ondrej Zitka · Pavel Kopel · Miroslava Beklova · Vojtech Adam · Rene Kizek

Received: 3 March 2014 / Revised: 15 August 2014 / Accepted: 25 September 2014 / Published online: 9 October 2014

Abstract Biosynthesis belongs to one of the new possibilities of nanoparticles preparation, whereas its main advantage is biocompatibility. In addition, the ability of obtaining the raw material for such synthesis from the soil environment is beneficial and could be useful for remediation. However, the knowledge of mechanisms that are necessary for the biosynthesis or effect on the bio-synthesizing organisms is still insufficient. In this study, we attempted to evaluate the effect of quantum dots (QDs) not only on a model organism of collembolans, but also on another soil organism—earthworm *Eisenia fetida*—and in also one widespread microorganism such as *Escherichia coli*. Primarily, we determined 28EC_{50} as 72.4 μmol L^{-1} for CdTe QDs in collembolans. Further, we studied the effect of QDs biosynthesis in *E. fetida* and *E. coli*. Using determination of QDs, low-molecular thiols and antioxidant activities, we found differences between both organisms and also between ways how they behave in the presence of Cd and/or Cd and Te. The biosynthesis in earthworms can be considered as its own protective mechanism; however, in *E. coli,* it is probably a by-product of protective mechanisms.

Keywords Biosynthesis · Quantum dots · CdTe · *Collembola* · *Eisenia fetida* · *Escherichia coli*

Published in the topical collection *Advances in Chromatography and Electrophoresis & Chiranal 2014* with guest editor Jan Petr.

M. Kominkova · P. Michalek · O. Zitka · P. Kopel · V. Adam · R. Kizek (✉)
Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech Republic
e-mail: kizek@sci.muni.cz

M. Kominkova · P. Michalek · A. Moulick · O. Zitka · P. Kopel · M. Beklova · V. Adam · R. Kizek
Central European Institute of Technology, Brno University of Technology, Technicka 3058/10, 616 00 Brno, Czech Republic

B. Nemcova · O. Zitka · M. Beklova
Department of Ecology and Diseases of Game, Fish and Bees, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Palackeho 1-3, 612 42 Brno, Czech Republic

Introduction

Quantum dots (QDs) are considered as nanomaterials, which can be synthesized using various approaches and ways [1] including electron beam irradiation, polyol-hydrolysis method, chemical precipitation method, photochemical synthesis, γ-radiation route or microwave-assisted aqueous synthesis [2, 3]. After their synthesis, these nanomaterials can be further modified by various inorganic and organic substances. Due to the fact that QDs are made of metallic substances, their potential toxic properties are discussed, whereas these depend on modifications, too [1, 4]. Negative effects of QDs have been determined in bacteria, algae, invertebrates, fishes and also in some mammals [5–7].

In spite of the fact that some of toxic effects are discussed, these are not so great to be an obstacle to use them to treat an organism. However, one-step-way to produce QDs with none toxic effects are still looked for. Biosynthesis sometimes also called as green synthesis fulfilling this presumption is one of the newest ways for QDs synthesis, [8–12]. Some of the organisms are capable of QDs biosynthesis such as *Escherichia coli* [10, 13–15], *Rhodobactersphaeroides* [16], *Klebsiella pneumoniae* [17], *Klebsiella aerogenes* [18], *Gluconoacetobacter xylinus* [19] and yeasts [20] including *Saccharomyces cerevisiae* [21],

Schizosaccharomyces pombe [22], *Torulopsis* species [23], *Rhodosporidium diobovatum* [12] and *Fusarium oxysporum* [24, 25]. Although these organisms can produce various types of QDs (ZnS, PdS, CdS and CdSe), CdTe is one of the most commonly biosynthesized type of QDs [26]. As s strain, recombinant *E. coli* expressing metal-binding peptides and proteins, such as phytochelatin and/or metallothionein, belong to the most popular [10, 13–15]. Besides the above mentioned organisms, plants can be also used for green synthesis of QDs, in which their extract is used as the reducing agent [27–29]. The biosynthesis has been also found in animals, of which rats [9, 30], mice [31] and earthworms [32] have been able to produce these nanoparticles [33, 34].

Green synthesis of QDs in an organism is associated with the ability of an organism to resist metals adverse effects [35]. It was found that during the biosynthesis of CdTe in organisms, the gene expression of the metal-binding polypeptides and proteins such as phytochelatin and metallothionein was enhanced [8, 10, 15]. The studies also show that oxidative stress and other negative effects on organisms exposed to the action of a heavy metal can be reduced through the biosynthesis. If QDs are not biosynthesised in a body of an organism in the presence of Cd(II), stability of lysosomal membrane may be disturbed, changes in gene expression can occur, oxidative stress may increase, growth, sexual development, cocoon production and hatchability and juvenile viability can be reduced, and the mortality may increase [36, 37]. Therefore, biosynthesis of QDs can be considered a protective mechanism against metal ions, which occur mainly in organisms living in polluted environment [38, 39]. As one of the indicator of soil pollution, collembola (*Folsomia candida*), an arthropod occurring in soils worldwide, can be employed. These organisms are used as the standard test organisms for ecotoxicological tests; earthworms may be used in this case, too [43, 44].

The aim of this study was to determine the effect of QDs on collembolans reproduction and the possibilities and influence of biosynthesis of these particles in *E. coli* and earthworm *Eisenia fetida*.

Experimental

Tested Organisms

The collembola (*Folsomia candida*) and earthworm (*Eisenia fetida andrei*) originated from cultures from the Ecotoxicological Laboratory of the University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic. *E. coli* (NCTC 13216) were obtained from the Czech Collection of Microorganisms, Faculty of Science, Masaryk University, Brno, Czech Republic.

Chemicals

Cadmium chloride ($CdCl_2$), sodium tellurite (Na_2TeO_3), sodium borohydride ($NaBH_4$) and other chemicals listed in the text were purchased from Sigma-Aldrich (St. Louis, MO, USA) and meet the specification of American Chemical Society (ACS), unless stated otherwise. The deionised water was prepared using reverse osmosis equipment Aqual 25 (Aquals.r.o., Brno, Czech Republic). The deionised water was further purified using apparatus Milli-Q Direct QUV equipped with an UV lamp from Millipore (Billerica, MA, USA). The resistance was 18 MΩ. The pH was measured using pH meter WTW inoLab (Weilheim, Germany).

Synthesis of QDs and Determination of Inhibition of Springtails' Reproduction

The microwave synthesis of QDs was performed according to Skalickova et al. [45]. The inhibition test of springtail (*Collembola*) reproduction was carried out according to Nemcova et al. [46, 47]. Nominal concentrations of 5, 10, 100, 500, 1,000 μmol L^{-1} QDs per vessel were applied into the soil. Five replicates were used for each of tested concentrations and five repetitions were made to determine the concentration of Cd(II) in springtails. The EC_{50} (median effective concentration) was determined after 28 days. Simultaneously, the control sample containing $CdCl_2$ (100 μmol L^{-1}) was also prepared.

Biosynthesis of QDs by *E. coli* and Earthworms

The strains were stored as a spore suspension in 20 % glycerol (*v*/*v*) at −20 °C. Prior to use, the strains were thawed and the glycerol was removed by washing with distilled water. The cultivation medium consisted of meat peptone 5 g L^{-1}, NaCl 5 g L^{-1}, bovine extract 1.5 g L^{-1}, yeast extract 1.5 g L^{-1} (HIMEDIA, Mumbai, India) and sterilized MilliQ water with 18 MΩ. The pH of the cultivation medium was adjusted to 7.4 before sterilization. The sterilization of the media was carried out at 121 °C for 30 min in a sterilizer (Tuttnauer 2450EL, Israel). The prepared cultivation media were inoculated with bacterial cultures into 25 mL Erlenmeyer flasks. After the inoculation, the bacterial cultures were cultivated for 24 h on a shaker at 600 rpm and 37 °C. The bacterial culture cultivated under these conditions was diluted with cultivation medium to $OD_{600} = 0.6$ and used in the following experiment: 1 mL of *E. coli* solution was diluted to 45 mL with LB medium in a Erlenmeyer flask and then 4 mL of 0.04 mol L^{-1} cadmium chloride ($CdCl_2$), 100 mg of trisodium citrate dihydrate, 1 mL of 0.04 mol L^{-1} sodium tellurite (Na_2TeO_3), 60 mg of mercaptosuccinic acid (MSA) and 50 mg sodium borohydride ($NaBH_4$) were added under constant stirring, followed by an incubation for 24 h at 37 °C on a rotary shaker (130 rpm) [26].

The earthworms, *E. fetida,* were kept for 11 days in the reference 30 % moisture soil. This humidity was achieved by addition of water containing $CdCl_2$ and Na_2TeO_3 ($CdCl_2$ and Na_2TeO_3, all the additions were at a concentration of 50 μg g^{-1} of soil). After the exposure time, the earthworms were transferred to wet filter paper and cultivated for 2 days to make clean their intestinal tract [32]. Part of the earthworms prepared in this way was subsequently used for the various tests.

Fluorescence Measurement by Camera and Absorption and Fluorescence Spectra

The fluorescence was analysed by Carestream In vivo Xtreme Imaging System (Rochester, USA) under the following conditions: exposition time 10 s, binning 2 × 2 pixels, f-Stop 1.1, field of view 15 × 15, excitation 410 nm, emission 510 nm.

The absorption and fluorescence spectra were measured by multifunctional microplate reader Tecan Infinite 200 PRO (TECAN, Switzerland). The absorption scan was measured within the range from 230 to 800 nm per 5 nm steps. The detector gain was set to 100. For the fluorescence spectra measurement, 300 and/or 650 nm was used as excitation wavelengths and the fluorescence scan was measured within the range from 230 to 850 nm. The detector gain was set to 100, too. The extracts were placed in UV-transparent 96 well microplate with flat bottom by CoStar (Corning, USA). In each well, 100 μL of sample was pipetted. All measurements were performed at 30 °C controlled by Tecan Infinite 200 PRO (TECAN, Switzerland).

Determination of Cd(II) and of Reduced and Oxidized Glutathione Ratio (GSH/GSSG)

The determination of Cd(II) in tissues and cells was carried out using the method of differential pulse voltammetry, as described previously [45]. The determination of GSH/GSSG in tissues and cells was carried out using the high-performance liquid chromatography with electrochemical detection (HPLC-ED), as described previously [48].

Determination of Antioxidant Activity

The determination of antioxidant activity in the tissues and cells was performed using the automatic spectrophotometer, as described previously [49].

Determination of Metallothionein and Expression of MT Gene

Metallothioneins in tissues and cells were determined using differential pulse voltammetry Brdicka reaction, as previously described [50].

Expression of MT Gene

Earthworms were transferred to the sieve and rinsed with milliQ water to remove any residues coming from cultivation media. These worms were then moved to the cellulose, where they were kept to dry. One earthworm was used as the sample, which was cut into pieces and put into the tube with RNA later (Ambion, USA). These pieces were ground in a mortar with liquid nitrogen. Further, 1–10 mg of the sample was used for the isolation of RNA according to the manufacturer's instructions (MagNA, Roche, Switzerland). The mRNA was converted to DNA using PrimeScript One Step RT-PCR Kit Ver.2 (TaKaRa, Japan) according to the manufacturer's instructions. The obtained DNA mixed with loading buffer was pipetted into the wells and run in 2 % agarose gel in 1 × TAE buffer with ethidium bromide for 90 min, 90 V. The bands were visualized with a UV transilluminator (Transilluminator Multiband TFX-35.MC, Torcy, France, excitation: 312 nm). The intensities of the bands on agarose gel were measured using Carestream Molecular Imaging Software Xtreme Edition (Rochester, USA).

Descriptive Statistics

Mathematical analysis of the data and their graphical interpretation were realized by Microsoft Excel®, Microsoft Word® and Microsoft PowerPoint®. Results are expressed as mean ± standard deviation (SD) unless noted otherwise.

Results and Discussion

Collembolans

In spite of the fact that QD using has been growing, their effect on organisms remains not fully understood [51]. Therefore, we focused on studying of the influence of QDs and their biosynthesis on bacterial and invertebrate organisms. In this case, we selected a typical soil test organism, collembolans (*Folsomia candida*), to evaluate the effect of QDs (Fig. 1aa). These organisms were kept on artificial soil, where different concentrations of cadmium in the form of CdTe QDs (0, 5, 10, 100, 500 and 1,000 μmol L^{-1}) were applied. After the end of the reproductive test, the collembolans together with a pattern of land were sampled for determination of cadmium and for acquisition of fluorescence micrographs. For the purpose of obtaining the fluorescence micrographs of the collembolans, the test control group was prepared simultaneously. The control collembolans were kept in the presence of 100 μmol L^{-1} cadmium. From the results obtained we can observe the

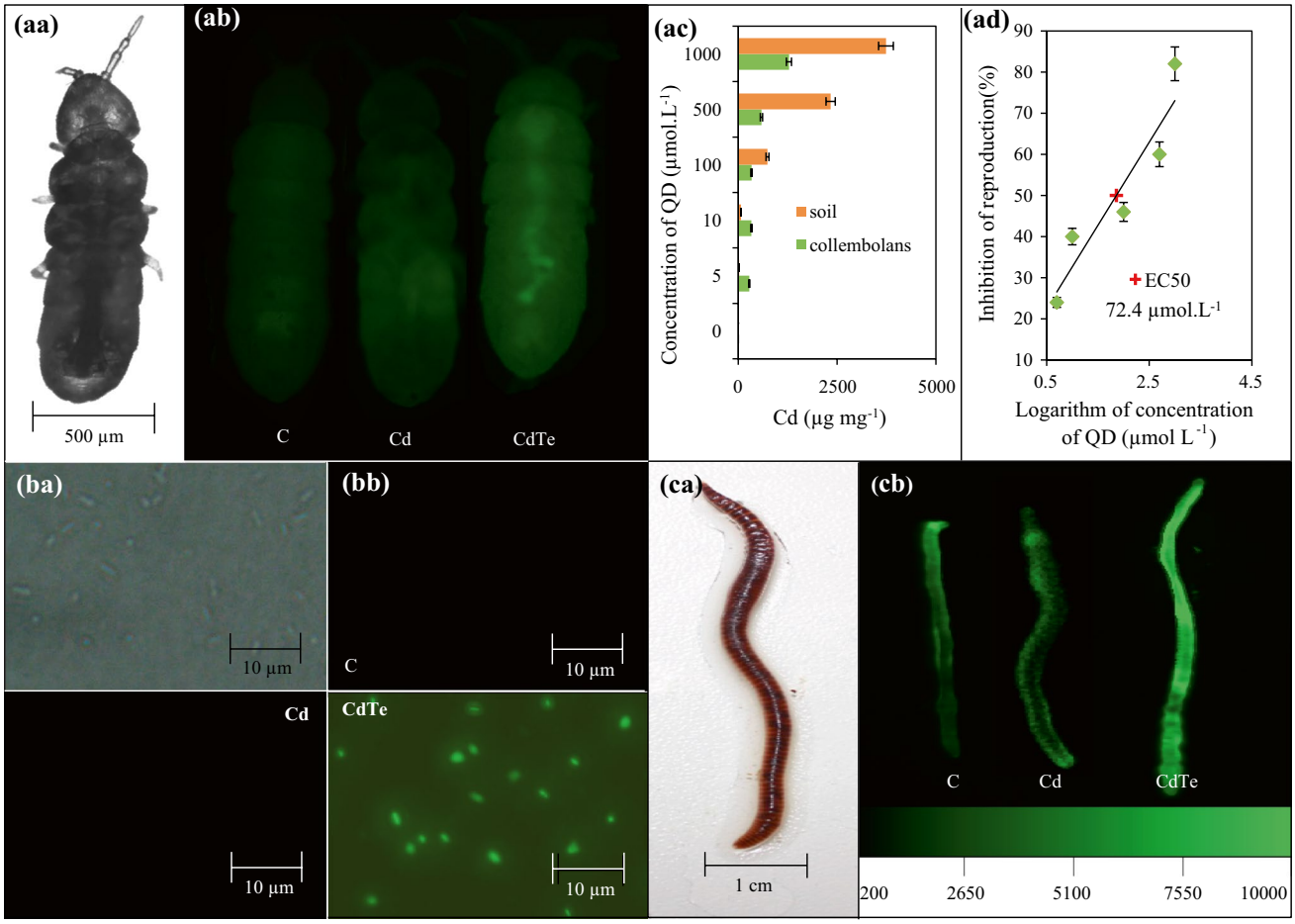


Fig. 1 (**aa**) Photos of springtail (*Folsomia candida*) used in the tests, (**ab**) microscopic fluorescence photos (excitation 460–495 nm, emission 510–550 nm), (**ac**) concentration of cadmium, (**ac**) EC_{50}. (**ba**) Photos of *E. coli*, **(bb)** microscopic fluorescence photos (excitation 460–495 nm, emission 510–550 nm). (**ca**) Photos of earthworms (*E. fetida*) used in tests, (**cb**) In vivo imaging fluorescence photos (excitation 410 nm, emission 510 nm). In figures **ab**, **bb** and **cb** there are shown: (*C*) control organism, (*Cd*) organism living in the environment of cadmium compounds and (*CdTe*) organism living in the environment of cadmium and tellurium compounds

increase in fluorescence in the intestine area of the collembolans exposed to 100 µmol L^{-1} QDs (Fig. 1ab). Intestine is an area, where the detoxification of heavy metals takes place in the test organism through intestinal exfoliation [52]. This phenomenon was not observed in intestines of control collembolans. Therefore, the absorption of QDs in the test organism was confirmed.

Further, the confirmation of entering of QDs into the body was performed by determination of cadmium in collembolans and soil (Fig. 1ac). When 5 µmol L^{-1} concentration of QDs was applied, 280 µg mg^{-1} of cadmium was found in the body of collembolans, whereas only a trace concentration of cadmium was found in the soil. For the applied concentrations of 10 and 100 µmol L^{-1} of QDs, an equal quantity of cadmium in the body of collembolans (335 µg mg^{-1}) was determined, but the content of cadmium for the applied concentration of 100 µmol L^{-1} was ten times higher compared to the values found in the soil exposed to 10 µmol L^{-1}. These results imply that the collembolans were able to extract certain amount of CdTe QDs from soil and keep them in the body.

According to the reproductive tests, $28EC_{50}$ was set at 72.4 µmol L^{-1} (Fig. 1ad) and no significant effect on mortality was found. This result corresponds to 2.9 µg g^{-1} after conversion, which is several fold lower when compared to EC_{50} values for cadmium mentioned in the literature ranging from 40 to 850 µg g^{-1} [53–56]. These values reveal that CdTe QDs are more toxic to the organism than Cd(II) alone. This effect was also observed in cell lines by Chen et al., who associated this effect to an increased local concentration of Cd(II) in QDs [57]. Another option, why cadmium ions are less toxic to the organisms, may be a biosynthesis, which is apparently not involved in the defence mechanism against application of QDs.

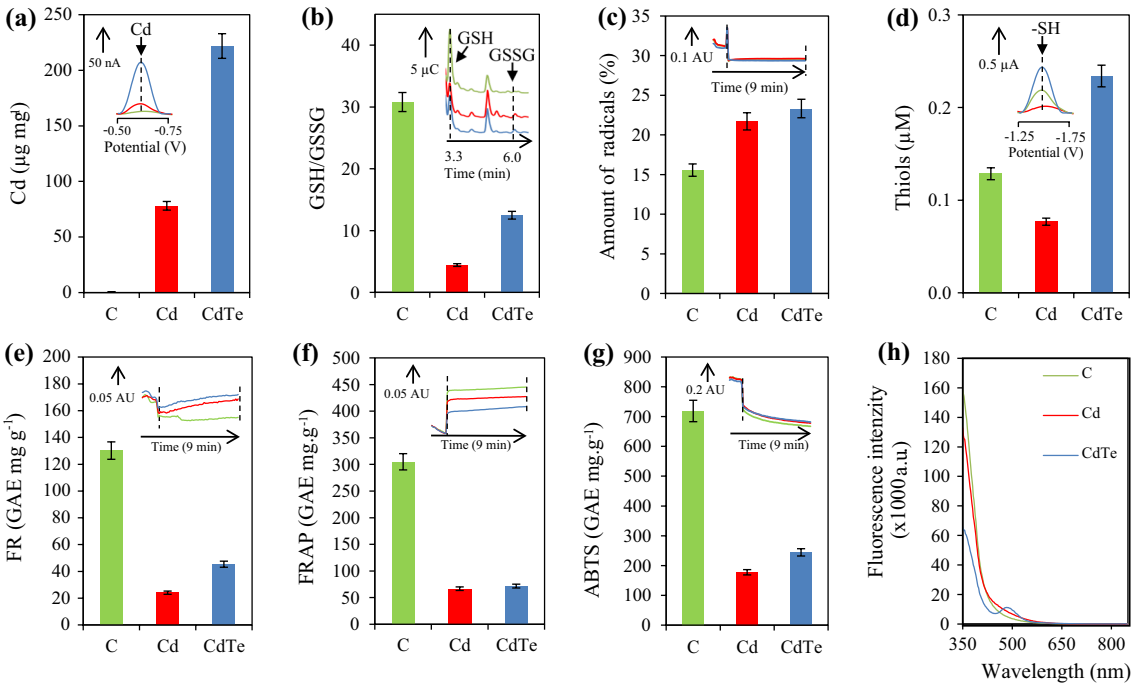


Fig. 2 Determination of cadmium concentrations and antioxidant activity of *E. coli*: control (C) *green*, living in the environment of cadmium compounds (Cd) *red*, and living in the environment of cadmium and tellurium compounds (CdTe) *blue*. Parameters from the analysis of samples: (**a**) determined concentration of cadmium, (**b**) GSH/GSSG ratio, (**c**) DPPH, (**d**) thiol compounds, (**e**) free radical, (**f**) FRAP, (**g**) ABTS, (**h**) fluorescence (excitation 300 nm, emission scan 350–800 nm). Inserted *curves* represent the results of measurements, *black dashed line* identifies areas for evaluation

Biosynthesis

It was mentioned in the "Introduction" that biosynthesis is considered as new way to hot synthetize QDs, however, the potential of this technology is still not fully understood. Microorganisms can be especially used for biosynthesis of quantum dots. *E. coli* is one of numerous microorganism, whose biosynthetic capabilities for various kind of QDs have been described [13–15]. The mechanism of biosynthesis is not yet fully understood, but it is assumed that these microorganisms utilize bacteria-secreted proteins for QDs biosynthesis [10, 20, 26]. However, some published studies show on the regulation of QDs through the glutathione metabolic pathway [58]. It is expected that invertebrates and even vertebrates can synthesize QDs through the path of glutathione [32]. Therefore, two model organisms, microorganisms *E. coli* (Fig. 1ba) and earthworms *E. fetida* (Fig. 1ca), were selected to study the synthesis of CdTe QDs. The three groups of organisms were marked throughout the study as follows: a control group that contained no supplement (C), the group with the addition of $CdCl_2$ (Cd) and the group with $CdCl_2$ and Na_2TeO_3 (CdTe).

Escherichia coli

The photo of *E. coli* in LB medium (Fig. 1bb), taken by a fluorescence microscope, shows that no fluorescence was detected compared to CdTe in C and Cd groups. These micrographs confirm the presence of QDs. Similarly, in the case of earthworms, an increased fluorescence and thereby the presence of biosynthesised QDs in CdTe group were confirmed (Fig. 1cb). Further, an analysis of fluorescence of the medium, in which *E. coli* was grown, was carried out (Fig. 2h). There is an apparent change in the emission spectrum with a maximum at 480 nm for CdTe group. Based on the increased fluorescence and changes of emission spectrum for CdTe experimental group only and not for C and Cd groups we can assume that the biosynthesis of CdTe QDs occurred, which is in good agreement with the previously published studies [20, 34]. The Cd(II) concentration in *E. coli* (Fig. 2a) was almost three times higher in CdTe group compared to Cd group. These results confirmed that although QDs were not able to penetrate the cell wall without suitable modification, biosynthesized dots were aggregated on the membrane of *E. coli* [26]. This is also shown in micrographs (Fig. 1bb), where higher fluorescence is detected.

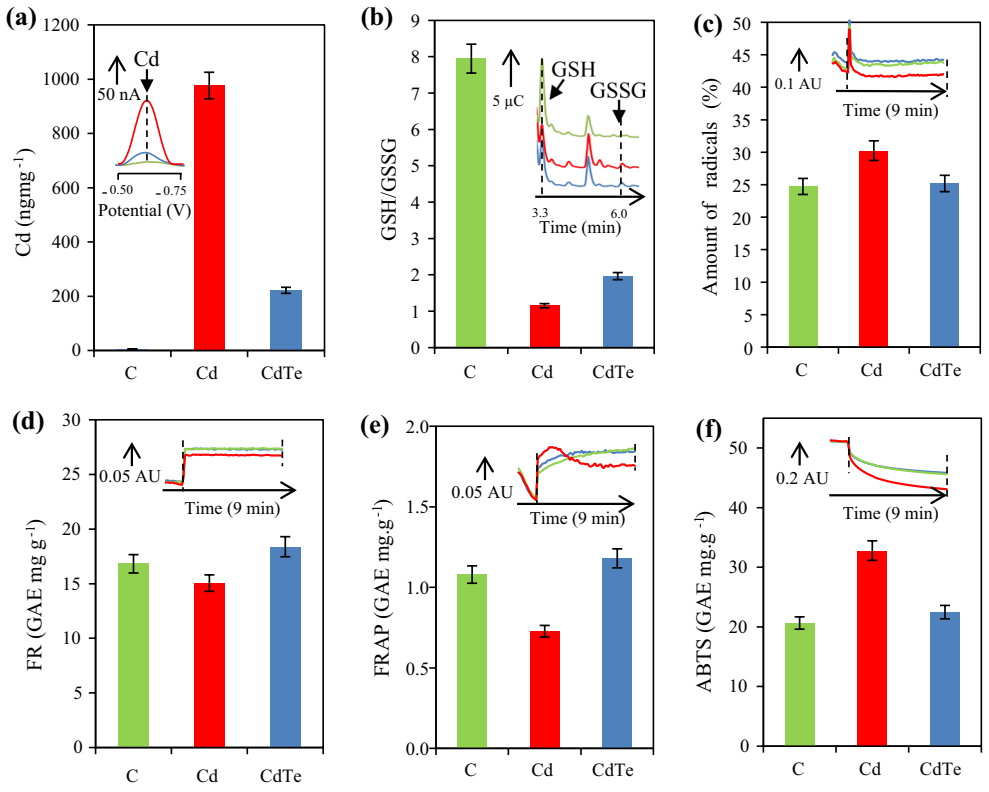


Fig. 3 Determination of cadmium concentrations and antioxidant activity of earthworm: control (C) *green*, living in the environment of cadmium compounds (Cd) *red*, and living in the environment of cadmium and tellurium compounds (CdTe) *blue*. Parameters of the analysis of samples: (**a**) determined concentration of cadmium, (**b**) GSH/GSSG ratio, (**c**) DPPH, (**d**) free radical, (**e**) FRAP, (**f**) ABTS. Inserted *curves* represent the results of measurements, *black dashed line* identifies areas for evaluation

The metabolic changes in *E. coli* was evaluated using indicators of oxidative stress as the ratio of reduced and oxidized glutathione (GSH/GSSG) and antioxidant activity tests (DPPH, Free Radical, FRAP, ABTS). GSH/GSSG ratio was approximately 2 times lower in CdTe group compared to Cd group; however, it was more than 6 times lower compared to the control (Fig. 2b). Moreover, the antioxidant activity tests showed that both groups of Cd and CdTe had a similar influence on *E. coli* (Fig. 2c–g). The analysis of the content of thiol compounds (Fig. 2c), which are involved in the main detoxification mechanisms [59], revealed a significant difference between control and other experimental groups. It clearly follows from the results obtained that, protective mechanisms, where thiol compounds are included in, were exhausted in Cd group, while the CdTe group is still able to cope with heavy metals. In the case of group of CdTe, where QDs were created, unlike in the other two groups, no significant negative influence was observed, when compared to Cd alone. In more complex organisms, we expect the involvement of other protective mechanisms, which were tested in the following experiment on earthworms.

Earthworms

The ability of QDs biosynthesis was further evaluated in earthworms *E. fetida*. The earthworms living in conditions of C, Cd and CdTe were washed with distilled water prior to analysis and then killed with chloroform. The cadmium accumulated from the environment was determined (Fig. 3a). Determined content was approximately 4 times lower in the earthworm living in CdTe environment than that in the area with Cd(II) only. Even the parameters of oxidative stress and antioxidant activity suggest that the earthworms were not so negatively affected in CdTe environment compared to the earthworms kept in Cd(II) environment only. The GSH/GSSG ratio (Fig. 3b) of Cd and CdTe groups was significantly reduced compared to the control. On the other hand, this parameter had 2× higher ratio of glutathione in CdTe compared to Cd. Besides low-molecular mass thiols, according to the tests (DPPH, Free Radical, FRAP, ABTS) showed in Fig. 3c–f, the antioxidant activity is even comparable in CdTe group to the control one. C earthworms showed significant differences compared to the other two experimental groups. A similar effect of reduction of the oxidative stress caused by Cd(II) after

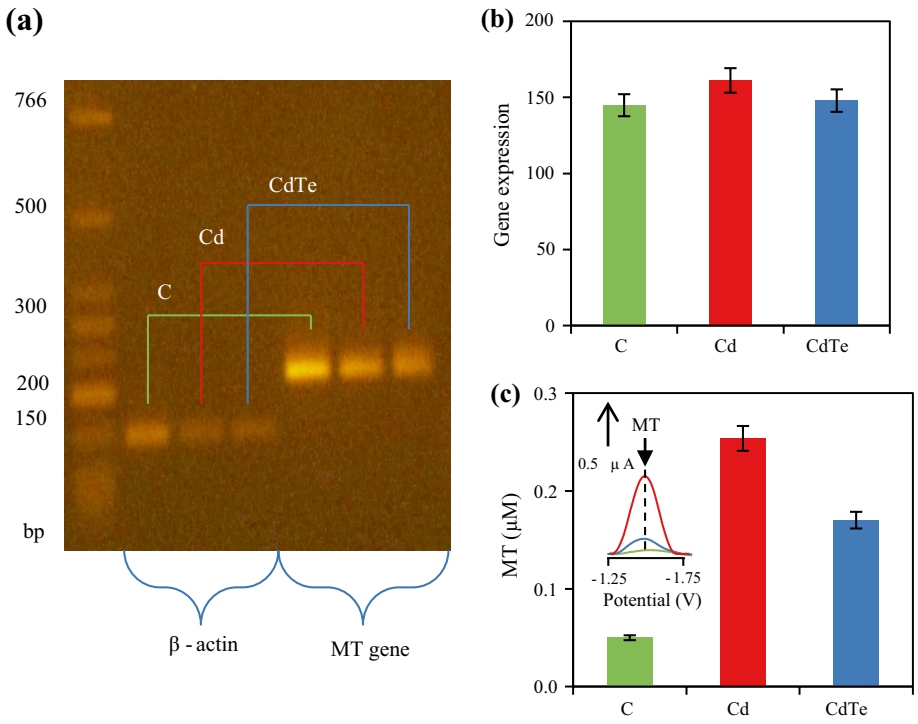


Fig. 4 Determination of the effect of cadmium and biosynthesis on the expression of metallothionein in earthworms: control(C) *green*, living in the environment of cadmium compounds (Cd) *red*, and living in the environment of cadmium and tellurium compounds (CdTe) *blue*. (**a**) The bands in gel showing the expression of two genes (control β-actin and reference MT gene) using reverse transcription PCR, (**b**) normalized band intensities of MT gene expression, (**c**) concentration of MT measured using differential pulse voltammetry Brdicka reaction

Te(-II) addition was observed in rats [9, 30] and mice [31]. All these results are confirmed by the expression of metallothionein gene (Fig. 4a, b) and its concentration (Fig. 4c), which is partly responsible for the ability of organisms to cope with heavy metals [37]. Its concentration is increased in Cd group, and the gene expression of the protein is also higher in this group compared to others (Fig. 4a, b). The results obtained from the evaluation of earthworms show different trends than that for *E. coli*. For this reason, it was necessary to evaluate the differences in both tested organism.

Comparison of the Effects on Test Organisms

The values of Cd(II) concentration, the GSH/GSSG ratio and antioxidant activity values were converted to percentages, where the highest value represents 100 %. The values determined in earthworms were subtracted from the values of *E. coli*; thus, we obtained differences comparing the influence of the biosynthesis in the selected test organism (Fig. 5). The most significant differences were observed after treatment with Cd(II). The *E. coli* accumulated more Cd in CdTe group than the earthworms. This effect was significantly different due to the different styles of biosynthesis, when earthworms create CdTe within a body and *E. coli* bio-synthesized dots extracellularly. However, this would show the opposite trend of Cd(II) accumulation than it was found. After the formation of QDs, other processes occur in the organisms. One of these processes is probably

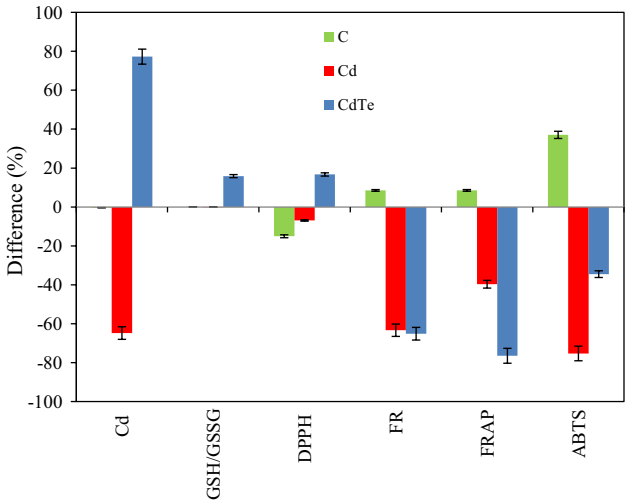


Fig. 5 Expression of differences of the influence of biosynthesis on the tested organisms. The percentages of values for each group were indicated in the *graph*. The relevant groups of earthworms were subtracted from *E. coli*

the aggregation of QDs on the membrane of *E. coli*, and this leads to uptake of QDs from the medium [26] and provides a higher concentration of Cd(II) in CdTe group compared to Cd group. However, QDs created by earthworms are synthesized in choragogen cells, which are on the surface of its intestine. Reduction of concentration of Cd(II) after the biosynthesis in the earthworm can be caused by the partial elimination of CdTe. Glutathione, one of thiol

compounds, which is responsible for detoxification of heavy metals [59], shows similar trend in both of the test organisms and can be assumed that the mechanism of protection run similarly during the biosynthesis in both organisms. In addition, significant differences were observed in the antioxidant activity. The results suggest that the mechanisms playing a role in the antioxidant activity of the tested organisms are activated by distinctly different ways. In earthworms, no significant differences between the control and CdTe group were observed, whereas the differences were found to be significant in *E. coli*.

Conclusion

CdTe QDs belong to the toxic QDs group due to their cadmium content. Compared to the cadmium itself, however, these QDs are more toxic to organisms than cadmium ions, which was confirmed by the value of $28EC_{50}$, which is a value much lower than that of cadmium itself. This may not be the case if these QDs are the product of protective mechanisms against heavy metals. There are different mechanisms, by which the microorganisms and soil organisms can biosynthesize QDs using the same substance. *E. coli* acts probably only as sources of substances that allow the synthesis of QDs. QDs, therefore, are the by-product of protective mechanisms and the biosynthesis in earthworms is very likely its own protective mechanism, as it is clear from our results.

Acknowledgments Financial support from CEITEC CZ.1.05/1.1.00/02.0068 is highly acknowledged. Special thanks are dedicated to Iva Blazkova and Dagmar Chudobova for perfect technical assistance.

References

1. Landsiedel R, Fabian E, Ma-Hock L, Wohlleben W, Wiench K, Oesch F, van Ravenzwaay B (2012) Arch Toxicol 86:1021–1060
2. Geszke-Moritz M, Moritz M (2013) Mater Sci Eng Mater Biol Appl 33:1008–1021
3. Valizadeh A, Mikaeili H, Samiei M, Farkhani SM, Zarghami N, Kouhi M, Akbarzadeh A, Davaran S (2012) Nanoscale Res Lett 7:480
4. Tsoi KM, Dai Q, Alman BA, Chan WC (2013) Acc Chem Res 46:662–671
5. Rivera Gil P, Oberdörster G, Elder A, Puntes V, Parak WJ (2010) ACS Nano 4:5527–5531
6. VartiainenTiina J, Pöhler T, Sirola K, Pylkkänen L, Alenius H, Hokkinen J, Tapper U, Lahtinen P, Kapanen A, Putkisto K, Hiekkataipale P, Eronen P, Ruokolainen J, Laukkanen A (2011) Cellulose 18:775–786
7. Elsaesser A, Howard CV (2012) Adv Drug Deliv Rev 64:129–137
8. Syed A, Ahmad A (2013) Spectrochim Acta Part a-Mol and Biomol Spectrosc 106:41–47
9. Trabelsi H, Azzouz I, Sakly M, Abdelmelek H (2013) Int J Nanomed 8:1121–1128
10. Monras JP, Diaz V, Bravo D, Montes RA, Chasteen TG, Osorio-Roman IO, Vasquez CC, Perez-Donoso JM (2012) PLoS One 7:1–10
11. Dhillon GS, Brar SK, Kaur S, Verma M (2012) Crit Rev Biotechnol 32:49–73
12. Seshadri S, Saranya K, Kowshik M (2011) Biotechnol Prog 27:1464–1469
13. Kang SH, Bozhilov KN, Myung NV, Mulchandani A, Chen W (2008) Angew Chem-Int Ed 47:5186–5189
14. Mi CC, Wang YY, Zhang JP, Huang HQ, Xu LR, Wang S, Fang XX, Fang J, Mao CB, Xu SK (2011) J Biotechnol 153:125–132
15. Park TJ, Lee SY, Heo NS, Seo TS (2010) Angew Chem-Int Ed 49:7019–7024
16. Bai HJ, Zhang ZM, Gong J (2006) Biotechnol Lett 28:1135–1139
17. Smith PR, Holmes JD, Richardson DJ, Russell DA, Sodeau JR (1998) J Chem Soc-Faraday Trans 94:1235–1241
18. Holmes JD, Smith PR, Evansgowing R, Richardson DJ, Russell DA, Sodeau JR (1995) Arch Microbiol 163:143–147
19. Li X, Chen SY, Hu WL, Shi SK, Shen W, Zhang X, Wang HP (2009) Carbohydr Polym 76:509–512
20. Bao HF, Hao N, Yang YX, Zhao DY (2010) Nano Res 3:481–489
21. Cui R, Liu HH, Xie HY, Zhang ZL, Yang YR, Pang DW, Xie ZX, Chen BB, Hu B, Shen P (2009) Adv Funct Mater 19:2359–2364
22. Kowshik M, Deshmukh N, Vogel W, Urban J, Kulkarni SK, Paknikar KM (2002) Biotechnol Bioeng 78:583–588
23. Kowshik M, Vogel W, Urban J, Kulkarni SK, Paknikar KM (2002) Adv Mater 14:815–818
24. Ahmad A, Mukherjee P, Mandal D, Senapati S, Khan MI, Kumar R, Sastry M (2002) J Am Chem Soc 124:12108–12109
25. Kumar SA, Ansary AA, Ahmad A, Khan MI (2007) J Biomed Nanotechnol 3:190–194
26. Bao HF, Lu ZS, Cui XQ, Qiao Y, Guo J, Anderson JM, Li CM (2010) Acta Biomater 6:3534–3541
27. Khan M, Adil SF, Tahir MN, Tremel W, Alkhathlan HZ, Al-Warthan A, Siddiqui MRH (2013) Int J Nanomed 8:1507–1516
28. Kharissova OV, Dias HVR, Kharisov BI, Perez BO, Perez VMJ (2013) Tr Biotechnol 31:240–248
29. Apte M, Girme G, Nair R, Bankar A, Kumar AR, Zinjarde S (2013) Mater Lett 95:149–152
30. Trabelsi H, Azzouz I, Ferchichi S, Tebourbi O, Sakly M, Abdelmelek H (2013) Int J Nanomed 8:3447–3453
31. Jamba L, Nehru B, Bansal MP (2000) J Trace Elem Exp Med 13:299–304
32. Sturzenbaum SR, Hockner M, Panneerselvam A, Levitt J, Bouillard JS, Taniguchi S, Dailey LA, Khanbeigi RA, Rosca EV, Thanou M, Suhling K, Zayats AV, Green M (2013) Nat Nanotechnol 8:57–60
33. Prozorov T, Bazylinski DA, Mallapragada SK, Prozorov R (2013) Mater Sci Eng R-Rep 74:133–172
34. Tilley RD, Cheong S (2013) Nat Nanotechnol 8:6–7
35. Narayanan KB, Sakthivel N (2010) Adv Coll Interface Sci 156:1–13
36. Zaltauskaite J, Sodiene I (2010) Ekologija 56:10–16
37. Spurgeon DJ, Svendsen C, Kille P, Morgan AJ, Weeks JM (2004) Ecotoxicol Environ Saf 57:54–64
38. Semple KT, Morriss AWJ, Paton GI (2003) Eur J Soil Sci 54:809–818
39. Nakamori T, Kaneko N (2013) Environ Pollut 176:165–170
40. Fountain MT, Hopkin SP (2005) *Folsomia candida* (Collembola): A "standard" soil arthropod. Annu Rev Entomol 50:201–222
41. Ardestani MM, Verweij RA, van Gestel CAM (2013) J Hazard Mater 261:405–413
42. de Boer ME, Ellers J, van Gestel CAM, den Dunnen JT, van Straalen NM, Roelofs D (2013) Ecotoxicology 22:619–631
43. Liu F, Zhu PF, Sheng W, Xue JP (2013) J Mater Cycl Waste Manag 15:482–488

44. Maboeta MS, Reinecke SA, Reinecke AJ (2004) Environ Res 96:95–101
45. Skalickova S, Zitka O, Nejdl L, Krizkova S, Sochor J, Janu L, Ryvolova M, Hynek D, Zidkova J, Zidek V, Adam V, Kizek R (2013) Chromatographia 76:345–353
46. Nemcova B, Bednarova I, Mikulaskova H, Beklova M (2012) Neuro endocrinol lett 33(Suppl 3):173–178
47. Nemcova B, Mikulaskova H, Bednarova I, Beklova M, Pikula J (2013) Neuro Endocrinol Lett 34:5–10
48. Zitka O, Skalickova S, Gumulec J, Masarik M, Adam V, Hubalek J, Trnkova L, Kruseova J, Eckschlager T, Kizek R (2012) Oncol Lett 4:1247–1253
49. Chudobova D, Dobes J, Nejdl L, Maskova D, Rodrigo MAM, Nedecky BR, Krystofova O, Kynicky J, Konecna M, Pohanka M, Hubalek J, Zehnalek J, Klejdus B, Kizek R, Adam V (2013) Int J Electrochem Sci 8:4422–4440
50. Pohanka M, Hynek D, Kracmarova A, Kruseova J, Ruttkay-Nedecky B, Sochor J, Adam V, Hubalek J, Masarik M, Eckschlager T, Kizek R (2012) Int J Electrochem Sci 7:11978–11992
51. Sun HZ, Zhang F, Wei HT, Yang B (2013) J Mater Chem B 1:6485–6494
52. Fountain MT, Hopkin SP (2001) Ecotoxicol Environ Saf 48:275–286
53. van Gestel CAM, Mol S (2003) Pedobiologia 47:387–395
54. Crouau Y, Chenon P, Gisclard C (1999) Appl Soil Ecol 12:103–111
55. Crommentuijn T, Brils J, Vanstraalen NM (1993) Ecotoxicol Environ Saf 26:216–227
56. Sandifer RD, Hopkin SP (1996) Chemosphere 33:2475–2486
57. Chen N, He Y, Su YY, Li XM, Huang Q, Wang HF, Zhang XZ, Tai RZ, Fan CH (2012) Biomaterials 33:1238–1244
58. Li Y, Cui R, Zhang P, Chen BB, Tian ZQ, Li L, Hu B, Pang DW, Xie ZX (2013) ACS Nano 7:2240–2248
59. Paradiso A, Berardino R, de Pinto MC, di Toppi LS, Storelli MM, Tommasi F, De Gara L (2008) Plant Cell Physiol 49:362–374