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Comparative estimation of ^{232}Th and stable Ce (III) toxicity and detoxification pathways in freshwater alga *Chlorella vulgaris*

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ABSTRACT

The impacts of radiological and chemical toxicity from naturally occurring radionuclides are discussed in the context of protecting freshwater ecosystems from radiation exposure. The present study aimed to determine the toxicity of ^{232}Th and it stable chemical analogue Ce to the green alga *Chlorella vulgaris* Beijerinck (thermophilic strain). Parameters of the regression equation for the concentration-effect relationship and concomitant Effective concentration (50%), EC_{50}, showed that ^{232}Th was more toxic to chlorella after a 24-h exposure than Ce. However, the No-observable-effect concentration (NOEC) and Lowest-observable-effect concentration (LOEC) for ^{232}Th were approximately equal to those for Ce. NOEC, LOEC and EC_{50} for ^{232}Th were 1.6 μM, 2.2 μM and 15.4 respectively. Those for Ce were 1.8, 2.1 and 35.7 μM respectively. Consideration of the results obtained suggests differences in the main detoxification pathways of ^{232}Th and Ce (III). It was found that 0.02 mM caffeine (used as DNA metabolism disturbance reagent) has no effect on Ce toxic action, but 0.02 mM BSO (as a selective inhibitor of γ-ECS, a glutathione biosynthetic pathway enzyme) enhanced it. In contrast, 0.02 mM caffeine significantly increased the toxic action of ^{232}Th, but 0.02 mM BSO has no effect on it. The peculiarities mentioned were suggested to be caused by differences in the physicochemical properties of the elements. The combined potential detrimental effect of ^{232}Th acting both as a radiation source (α-, β- and γ-emitter) and a chemically toxic element is discussed.

1. Introduction

Thorium-232 is a primordial radionuclide occurring ubiquitously in the environment with a half-life of $1.4{\cdot}10^{10}$ years. The radionuclide is an α – (90%), β – (1%) and γ – (9%) emitter (Seaborg, 1954). Furthermore, ^{232}Th is a chemically toxic heavy metal (Riabchikov and Golbraih, 1960) and the relative importance of modes of biological action arising from chemical versus radiation exposures is poorly explored at the present time.

The activity concentration of ^{232}Th in soil varies from 20 Bq kg^{-1} dry weight for background sites (Sheppard et al., 2008) up to 140–1240 Bq kg^{-1} in areas of high level natural background radiation (Ramli et al., 2005; Singh et al., 2009). There are some regions on earth which have high background radiation levels due to enhanced levels of ^{232}Th decay series radionuclides. Examples include locations within Australia, Brazil, China, France, India and Italy (Malanca et al., 1993; Paschoa et al., 1993). Rare mineral deposits rich in thorium in the Russia Federation are located in the Altai Ridge, Ural Mountains, Timan Ridge and the Kola Peninsula (Vinogradov, 1959).

Enhanced concentrations of radionuclides from the ^{232}Th decay series in soils and rocks have resulted in pollen sterility, morphological abnormalities and increased frequency of cytogenetic damage both in somatic and generative plant cells (Nayar et al., 1970).

Concentrations of ^{232}Th in natural water sources vary over a wide range. Typically, surface and groundwater exhibit low ^{232}Th concentrations in the range 0.009–2.9 μg L^{-1} (Kochhann et al., 2009), but water contaminated by drainage from uranium and iron ore mines in southeastern Brazil exhibit levels of ^{232}Th in the range 800–1400 μg L^{-1} (3.3–5.8 Bq L^{-1}) (Veado et al., 2006). Thorium-232 concentrations in river and stream water range from 0.2 μg L^{-1} (0.001 Bq L^{-1}) (Zhang et al., 2005) up to 0.48 mg L^{-1} (2 Bq L^{-1}), and levels as high as 0.66 mg L^{-1} (2.7 Bq L^{-1}) have been measured in stagnant reservoirs (Ramli et al., 2005).

Concentration ratios for ^{232}Th for different water plant species are high and can be more than unity but tend to vary over a wide range (Sheppard et al., 2008): from hundreds for higher aquatic plant species up to several hundred thousands for phytoplankton (Marchiulionienė et al., 1986). However, the toxicity of ^{232}Th for freshwater plants is poorly characterized. Only two studies

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are recorded in the ERICA radiation effects database (Copplestone et al., 2008) for low level γ-radiation exposure from ^{232}Th as an external source to alga *Synechococcus lividus* (Conter et al., 1984, 1986), but there are no data on incorporated ^{232}Th effects on freshwater plants.

A number of studies have focused on biological effects from Ce exposure of aquatic organisms. There are essential similarities between the chemistry of Ce (III) and that of Th (Cotton and Wilkinson, 1988). Th and Ce have similar electron configurations: $5f^2\ 7s^2$ and $4f^2\ 6s^2$, respectively, and their ionic radii are also of similar dimensions, specifically 0.99 and 1.03 Å (Cotton and Wilkinson, 1988). These similarities in outer electron configurations and ionic radii of the elements result in a common environmental behavior of Ce and Th (Vinogradov, 1959). Earlier investigations on the chemical properties of Th and Ce have indicated that their toxicity is caused by an ability to form water-soluble complex compounds with amino acids and organic acids, and insoluble compounds with phosphates, carbonates and oxalates. Furthermore, both elements form complex compounds with isolated DNA via phosphate groups (Riabchikov and Golbraih, 1960). It is of interest to consider whether similarities in the chemical toxicity of Th and Ce determined *in vitro* would be detected in the case of live organisms, bearing in mind that, in contrast to stable Ce, ^{232}Th is both a chemically toxic heavy metal and an α-, β- and γ-emitter. We hypothesize that due to differences in physicochemical properties the toxicity and the main detoxification pathways might differ between ^{232}Th and its stable chemical analogue Ce (III), when effects are observed *in vivo*. The aims of the present study were:

- to determine the toxicity of ^{232}Th and Ce (III) to the freshwater green alga *Chlorella vulgaris*;
- to investigate *in vivo*, the role of the glutathione pathway and DNA metabolism disturbances for this freshwater alga in response to exposures from ^{232}Th and Ce (III).

2. Materials and methods

2.1. Reagents

^{232}Th was used in the form of $Th(NO_3)_4{\cdot}4H_2O$ (pure analytical grade, Izotop, Russia). The stable isotope of Ce was used in the form of $Ce(NO_3)_3{\cdot}6H_2O$ (pure analytical grade, Reaktiv, Russia). Caffeine ($C_8H_{10}N_4O_2$) (99%, Sigma) and DL-buthionine-(S,R)-sulfoximine ($C_8H_{18}N_2O_3S$) (98%, Sigma) were used. Reagents for Tamija medium were chemically pure grade (Vekton, Russia). All solutions were prepared with distilled water.

2.2. Cells and growth conditions

Chlorella vulgaris – unicellular freshwater green alga – is a useful test object to study toxicity of water soluble compounds, metals, radionuclides and other pollutants (Shehata et al., 1999; Franklin et al., 2002; Hogan et al., 2005). A thermophilic strain of *Ch. vulgaris* Beijerinck, S-39/64688, was obtained from the algal collection of the Institute of Biology, St. Petersburg State University (Russian Federation). Chlorella cells were grown at 37 °C for 1 d in Tamija liquid medium under continuous illumination 1400 l×.

2.3. Assessment of chlorella biomass production

An increase in alga biomass production can be quantified using optical density measurements (Converti et al., 2009). Therefore, increases in chlorella biomass production were determined by optical density measurements at 670 nm with a spectrophotometer Spectrumlab SS 2107 (LEKI instruments, Finland).

2.4. Toxicant solutions

Stock solutions of toxicants were 0.77 mM ^{232}Th as thorium nitrate or 0.71 mM Ce (III) as cerium nitrate. All the concentrations tested were obtained by stock dilution with distilled water. The following concentration ranges were used: 0.001–28.013 μM ^{232}Th and 0.036–71.367 μM Ce.

2.5. General toxicity test

Before toxicity testing, the alga culture was diluted with Tamija medium to an optical density of 0.140 ± 0.005 and carefully mixed. Then, 0.25 mL of the alga suspension was inoculated in 6 mL of distilled water (control sample) or toxicant solutions (experimental sample). The suspension's optical density at the beginning of all experiments was about 0.006, corresponding to 154 000 ± 26 000 chlorella cells per mL, in each experimental and control sample. Cell numbers were determined with a hematocytometer. Alga cell culture in exponential growth phase was used for all experiments.

All experiments were conducted in a propagator where flasks with control and experimental samples were maintained at a constant temperature of 37 ± 0.5 °C, continuous illumination 1400 l× and CO_2 content in air of approximately 0.03%. Six replicates were made for each concentration of toxicant. Control and experimental samples were tested simultaneously. The experiments lasted 24 h.

2.6. The choice of modifying reagents

To investigate the role of the glutathione pathway and DNA metabolism disturbances in the freshwater alga response to exposures to ^{232}Th and Ce (III), two modifying reagents were chosen: DL-buthionine-(S,R)-sulfoximine (BSO) and caffeine. DL-buthionine-(S,R)-sulfoximine (BSO) is a selective inhibitor of γ-glutamylcysteine synthetase (γ-ECS), a glutathione biosynthetic pathway enzyme (Griffith and Meister, 1979). BSO was used as a tool to determine the depletion of glutathione and to investigate the role of phytochelatin (PCs) biosynthesis in detoxification of heavy metals (Grill et al., 1987; Reese and Wagner, 1987; Gussarson et al., 1996). Caffeine sensitizes cells to the lethal and mutagenic effects of DNA-damaging reagents due to DNA metabolism disturbances (Kaufmann et al., 2003) such as: binding to damaged regions of DNA (Lehmann, 1972; Downes et al., 1990); interference with DNA repair enzymes (Selby and Sancar, 1990) and with the supply of purine nucleotides needed for repair (Waldren and Patterson, 1979). Caffeine also affects cell cycle function and perturbs key cell cycle regulatory proteins (Bode and Dong, 2007).

2.7. The choice of modifying reagent concentrations

Preliminary experiments demonstrated no significant decrease in chlorella biomass production after 24 h exposure to BSO concentrations ranging from 0.02 up to 0.3 mM. A concentration of 0.02 mM BSO has been shown to inhibit glutathione synthesis *in vitro* (Griffith and Meister, 1979) and was therefore used to study the role of the glutathione pathway in detoxification of ^{232}Th and Ce.

Similarly no significant decrease in chlorella biomass production after 24 h exposure to caffeine concentrations ranging from 0.02 up to 1.3 mM was observed. Therefore, 0.02 mM caffeine (the same as for BSO) was selected to estimate the role of DNA metabolism disturbances in the response of chlorella to exposures to ^{232}Th or Ce.

2.8. Toxicity test procedure modified by BSO or caffeine

Experiments with BSO and caffeine were carried out under the same conditions as a general toxicity test. Thus, every flask contained 0.25 mL of the alga suspension (with an optical density 0.140 ± 0.005) and 6 mL of one of following solutions:

- distilled water (control sample 1).
- 0.02 mM BSO or 0.02 mM caffeine (control sample 2).
- 0.02 mM BSO + metal toxicant or 0.02 mM caffeine + metal toxicant (experimental sample).

The absence of any significant difference between the optical density of control sample 1 and sample 2 was monitored for each experiment.

2.9. Toxicity assessment

^{232}Th and Ce toxicity (%) was calculated as the optical density ratio:

$$D_r = D_{exp.}/D_{cont.} \cdot 100, \quad (1)$$

where $D_{exp.}$ is the mean value of the optical density of the experimental sample; $D_{cont.}$ is the mean value of the optical density for chlorella suspension after 24 h growth in distilled water (control sample 1) or in 0.02 mM BSO or 0.02 mM caffeine (control sample 2).

2.10. Data statistical analysis

Statistical analyses were performed with the SPSS program (Version 16; Tabachnick and Fidell, 2006). The significance of the differences between the optical density of the control and that of the experimental samples were established using the One-Way ANOVA Post Hoc multiple comparisons analysis (Dunnett's test). To calculate an effective concentration resulting in a 50% decrease in chlorella biomass production (EC_{50}), probit analysis was used.

Regression analyses were applied to identify the shapes of relationships between biological effect levels and metal toxicant concentration in the growth medium. To test the significance of the coefficient of determination (R^2) whether decreasing or increasing, the Hayek criterion (Gofman, 1990) was applied:

$$H = \sqrt{\frac{\mu\left(R^2_{np'} - R^2_{np''}\right)}{1 - R^2_{np'}}}, \quad R^2_{np'} > R^2_{np''} \quad (2)$$

where $R^2_{np'}$ and $R^2_{np''}$ corresponded to regression models with np' and np'' parameters, $\mu = N - np' - 1$ – degrees of freedom of the regression model constructed on the whole set of np′ explanatory variables. H-statistics follow the Student distribution.

3. Results

3.1. Toxicity of ^{232}Th and Ce to Chlorella vulgaris

Risk management procedures for environmental toxicants commonly rely on effects assessments based on the determination of threshold values, e.g. no observed effect concentrations (NOECs) and estimations of the concentration–response relationships for a single substance (Cassee et al., 1998). To compare the toxicity of ^{232}Th and its stable chemical analogue Ce (III) to chlorella, both criteria were used. Statistically significant changes in alga biomass production compared with the control under ^{232}Th treatment (Table 1, Fig. 1) were not observed in a range of concentrations from 0.001 up to 1.60 μM. Thus the no-observable-effects concentration (NOEC) was 1.6 μM. The lowest-observable-effect ^{232}Th concentration (LOEC) causing a statistically significant ($p < 0.05$) toxic effect was 2.2 μM. The EC_{50} value for ^{232}Th was 15.4 μM.

The NOEC and LOEC (1.8 and 2.1 μM, respectively) for Ce were similar to those for ^{232}Th. In contrast, EC_{50} value for Ce was 35.8 μM, much higher than for ^{232}Th (Table 1, Fig. 2).

The goodness-of-fit and suitability of the models applied in determining the relationship between D_r-values and concentrations of ^{232}Th or Ce were compared using several statistical tests presented in Table 2. All chosen models were able to fit the data satisfactorily ($p_F < 0.001$). The improvement in data fitting with the linear and non-linear models was supported by the Hayek criteria. The Hayek criteria value showed that the R^2 calculated for the exponential model was significantly higher than that for the linear and quadratic models ($p < 0.05$). In addition exponential model yielded a lower value of residual sum of squares than the linear and quadratic models. Thus, the exponential model provides the best result in describing the empirical dependencies between ^{232}Th (Fig. 3) or Ce (Fig. 4) concentrations and *Chlorella* biomass growth data set.

Regression equations for these models after linear transformation were as follows:

$$\mathrm{LnY}_1 = 98.99 - 0.05[^{232}\mathrm{Th}] \quad (3)$$

$$\mathrm{LnY}_2 = 86.40 - 0.02[\mathrm{Ce}] \quad (4)$$

where Y_1 – D_r-values (form Eq. (1)) for ^{232}Th toxic effect estimation; Y_2 – D_r-values for Ce toxic effect estimation; [^{232}Th] and [Ce] – concentrations of ^{232}Th or Ce, respectively.

The value of the regression coefficients in Eqs. (3) and (4) indicate that increasing the ^{232}Th concentration by 1 μM results in a decrease in the *Chlorella* biomass production by 0.05%. Increasing the Ce exposure by a similar concentration leads to a decrease of 0.02% in biomass.

3.2. Modification by BSO toxic effects of ^{232}Th and Ce

The NOEC and LOEC for ^{232}Th in the presence of BSO were 1.1 and 1.3 μM, respectively, which were both lower than measured when *Chlorella* was exposed to ^{232}Th alone. The EC_{50} values, in contrast, were not significantly different (Table 1). There were no substantial changes in the regression equation describing the relationship between the ^{232}Th concentrations and *Chlorella* biomass growth in the presence of BSO compared with the equation characterizing response for the exposure by ^{232}Th alone (Fig. 3):

$$\mathrm{LnY}_3 = 95.81 - 0.05[^{232}\mathrm{Th}] \quad (5)$$

where Y_3 – D_r-values when ^{232}Th and BSO were both present.

By comparison, NOEC, LOEC, and EC_{50} calculated for Ce in the case of simultaneous exposures with BSO were significantly ($p < 0.05$) lower than those obtained for Ce exposure (Fig. 2, Table 1) in isolation. Furthermore, the value of the regression coefficient in the equation indicated that BSO increases the toxic effect of Ce (Fig. 4):

$$\mathrm{LnY}_4 = 79.08 - 0.03[\mathrm{Ce}] \quad (6)$$

where Y_4 – D_r-values for Ce and BSO were both present.

3.3. Modification by caffeine toxic effects of ^{232}Th and Ce

The LOEC of ^{232}Th where the exposure occurred in the presence of caffeine was found to be about four times lower than in the case of simultaneous treatment with BSO and seven times lower than the LOEC for ^{232}Th exposures in isolation (Table 1). The EC_{50} calculated for the combined exposure to ^{232}Th and caffeine was significantly ($p < 0.05$) lower than that obtained for ^{232}Th alone. The

Table 1
NOEC, LOEC, EC_{50} of ^{232}Th and Ce separate and combined with 0.02 mM BSO or 0.02 mM caffeine 24 h exposure on *Chlorella vulgaris*.

Experiment variant	NOEC (μM)	LOEC (μM)	EC_{50} (μM)	95% confidence limits for EC_{50}
^{232}Th	1.6	2.2	15.4	13.9–17.1
^{232}Th + BSO	1.1	1.3	17.0	14.8–19.6
^{232}Th + caffeine	0.2	0.3	2.9	2.2–3.9
Ce	1.8	2.1	35.8	26.7–39.5
Ce + BSO	0.7	1.0	17.7	12.7–25.9
Ce + caffeine	1.8	2.1	30.0	21.9–44.7

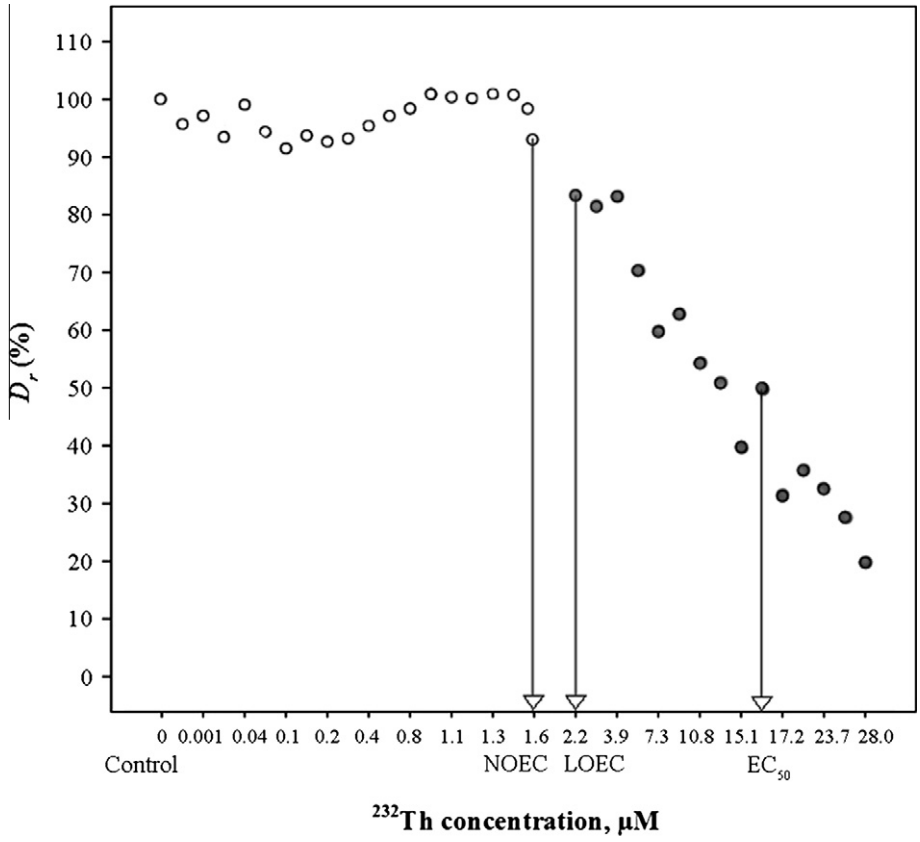

Fig. 1. Plot of decrease in *Chlorella vulgaris* biomass production mean value (*Dr*, *Y*-axis) after 24 h ^{232}Th exposure. Open circles: no significant differences from control ($p > 0.05$); closed circles: difference with control is significant at $p < 0.05$. NOEC – no-observable-effects concentration. LOEC – lowest-observable-effect concentration. EC_{50} values were calculated by probit analysis.

regression equation shows a substantial increase in ^{232}Th toxicity in the presence of caffeine (0.02 mM) (Fig. 3):

$$\mathrm{Ln}Y_5 = 90.14 - 0.27[^{232}\mathrm{Th}] \quad (7)$$

where Y_5 – D_r-values for ^{232}Th and caffeine combined effect estimation.

In contrast, LOEC, EC_{50} and the parameters of the regression equation for Ce in the presence of caffeine were not significantly ($p > 0.05$) different from those for Ce alone (Table 1, Fig. 4).

$$\mathrm{Ln}Y_6 = 90.00 - 0.02[\mathrm{Ce}] \quad (8)$$

where Y_6 – D_r-values for Ce and caffeine combined effect estimation.

4. Discussion

Progress has recently been made in understanding Ce toxicity pathways to different plant and animal species. Cerium compounds were shown to be oxidant toxic for mammals and plants (Kawagoe et al., 2005). Oxidative stress induced by exposure to Ce caused a production of antioxidants such as metallothioneins (MT) and glutathione (GSH) in mouse liver (Kawagoe et al., 2005). Cerium results in a significant increase in malondialdehyde content and decrease in superoxide dismutase (SOD) and catalase activities in *Drosophila melanogaster* (Huang et al., 2010). In plants (*Lemna minor* L.) pretreated with rare earth elements (including Ce) decreases in ascorbate and glutathione redox state and chlorophyll content and increases in lipid peroxidation (LPO) and reactive oxygen species production levels occur (Ippolito et al., 2010).

There are a few published data on the biological effects of thorium, whereas studies on the bioaccumulation of this element are more numerous. Only two experimental studies were found where biochemical and cytogenetic alterations in the silver catfish exposed to different Th concentrations were investigated (Correa et al., 2008; Kochhann et al., 2009). Chronic Th exposure caused alterations in the oxidative parameters of silver catfish gills, which were correlated with Th accumulation. For example, SOD activity decreased and the LPO increased in fish exposed to 242.4 and 747.2 μg L^{-1} Th (i.e. 1.04 and 3.22 μM). In addition, levels of glutathione-S-transferase (an enzyme catalyzing glutathione-

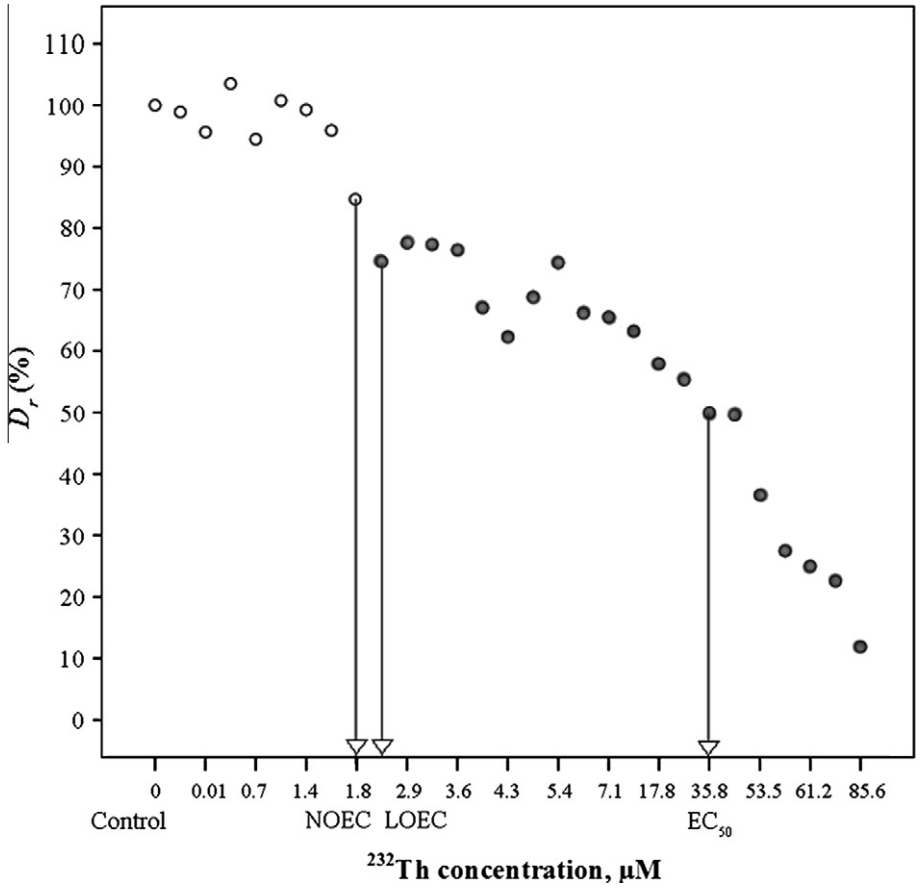

Fig. 2. Plot of decrease in *Chlorella vulgaris* biomass production mean value (*Dr*, *Y*-axis) after 24 h Ce exposure. Open circles: no significant differences from control ($p > 0.05$); closed circles: difference with control is significant at $p < 0.05$. NOEC – no-observable-effects concentration. LOEC – lowest-observable-effect concentration. EC_{50} values were calculated by probit analysis.

Table 2
Approximation quality indexes of experimental data obtained in toxicity tests with use of various model.

Test	*N*	Model	np	R^2	SS_{res}	H*
Assessment of ^{232}Th toxicity	37	Linear	2	0.93	1528	–
		Quadratic	3	0.96	865	5.12[c]
		Exponential	2	0.97	0.22	6.93[c]
Assessment of Ce toxicity	26	Linear	2	0.82	3078	–
		Quadratic	3	0.85	2620	2.19[a]
		Exponential	2	0.93	0.55	6.27[c]
Combined exposure ^{232}Th with BSO	17	Linear	2	0.95	702	–
		Quadratic	3	0.98	270	4.74[c]
		Exponential	2	0.99	0.07	8.00[c]
Combined exposure Ce with BSO	19	Linear	2	0.67	4758	–
		Quadratic	3	0.82	2662	3.76[b]
		Exponential	2	0.87	0.70	5.26[c]
Combined exposure ^{232}Th with caffeine	16	Linear	2	0.79	1863	–
		Quadratic	3	0.89	1018	3.57[c]
		Exponential	2	0.91	0.44	4.47[c]
Combined exposure Ce with caffeine	18	Linear	2	0.74	2845	–
		Quadratic	3	0.84	1800	3.16[b]
		Exponential	2	0.87	0.37	4.12[c]

Note: a model consider to be better than the linear one at [a]$p < 0.05$; [b]$p < 0.01$, [c]$p < 0.001$; *Hayek criteria value, calculated for exponential and quadratic model comparisons.

dependent reduction of H_2O_2 (Noctor and Foyer, 1998)) decreased in the gills of fish exposed to 747.2 μg L^{-1} Th.

Consideration of the abovementioned studies leads to the tentative suggestion that there are similarities between the response of living organisms to Ce and Th exposures. Evidently, however, there are also some differences between the main detoxification pathways of ^{232}Th and Ce. The present study results showed that NOEC and LOEC for ^{232}Th were similar to those for Ce. Nonetheless, NOEC is not a unique measure of substance toxicity (Chapman et al., 1996) and may only be appropriate for regulatory use in a range

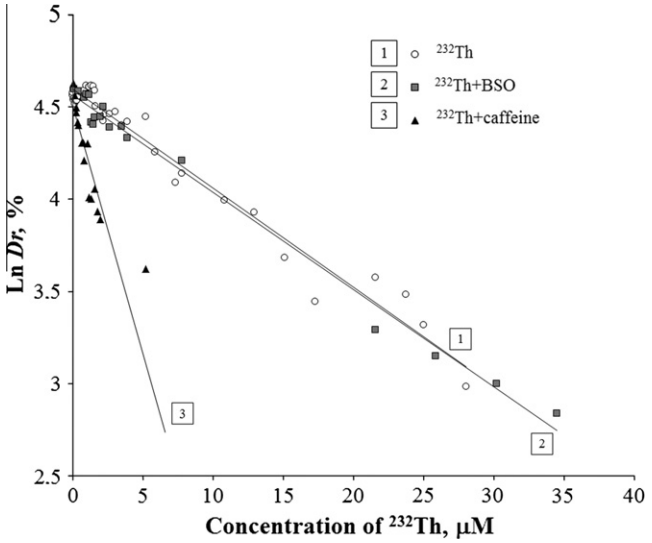

Fig. 3. Plot of linear-transformed exponential model showing relationship between *Chlorella vulgaris* biomass production and ^{232}Th concentration in case of ^{232}Th separate action (1), in presence of BSO (2), in presence of caffeine (3).

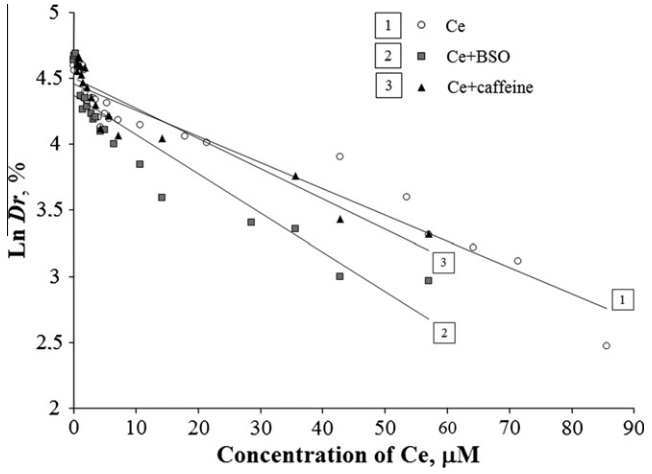

Fig. 4. Plot of linear-transformed exponential model showing relationship between *Chlorella vulgaris* biomass production and Ce concentration in case of Ce separate action (1), in presence of BSO (2), in presence of caffeine (3).

characterized by low toxicant concentrations. To assess chemical compound toxicity over a wider range of concentrations it is necessary to determine parameters that correspond to the concentration–response relationship and EC_{50}. Such estimations in our study, demonstrated that ^{232}Th is more toxic to *Ch. vulgaris* than stable Ce. In addition, differences in the alga response to ^{232}Th and Ce treatments in combination with BSO or caffeine were detected. ^{232}Th toxicity over a range of effective concentrations (i.e. higher than LOEC) does not change in the presence of 0.02 mM BSO whereas the toxic effect of Ce increased. Overall, these data mean that setting a benchmark for ^{232}Th based only on it chemical toxicity without considering radiological properties is not the most protective strategy for the environment.

As mentioned above, BSO is a selective inhibitor of γ-ECS, a glutathione biosynthetic pathway enzyme (Griffith and Meister, 1979). Numerous physiological functions have been attributed to glutathione in plants (Noctor et al., 1998), among them defense against oxidative stress, action as an important redox buffer and regulation of gene expression. Moreover, the principal pathway for plants to sequester heavy metals involves the formation of complexes with cysteine-rich peptides, called phytochelatins (PCs) (Noctor et al., 1998). An inhibition of additional PCs synthesis by BSO has been shown (Grill et al., 1987). Moreover, PCs were detected in chlorella cells (Huang et al., 2009). Taking into account the points considered above, we suggest that under our experimental conditions the glutathione pathway (including PCs component) is important for stable Ce detoxification. Further evidence for this contention comes from the studies of Kawagoe (2005) and Ippolito (2010) where Ce was able to induce oxidative stress and thus to produce antioxidants such as metallothionein (MT) and glutathione (GSH). Th exposure also causes alterations in the oxidative parameters of cells and glutathione-S-transferase level (Kochhann et al., 2009), but there are no data on the influence of this radionuclide on MT or GSH biosynthesis. Our findings suggest that the role of glutathione pathway in the detoxification of ^{232}Th is vanishingly small. In contrast to BSO, caffeine, as a reagent causing DNA metabolism disturbance considerably enhanced the toxicity of ^{232}Th in chlorella. The toxic effect of Ce, on the other hand, appears not to be altered by the presence of caffeine.

These data suggest that DNA metabolism is an important process for cells to survive after exposure to low concentrations of Th. In contrast, stable Ce detoxification via the GSH-dependent pathway is of greater importance. The peculiarities of alga response to Th and Ce exposure may be caused by differences in physicochemical properties of the elements. Furthermore, ionizing radiation energy causes water molecules ionization and free radicals formation that may enhance the adverse effect of Th and its daughter decay products on chlorella cells. In addition, thorium ions adsorbed onto cell walls could be considered as emitters with high linear energy transfer. For example, linear energy transfer for the principal radioactive emissions from the parent radionuclide ^{232}Th is equal to 129 keV μm^{-1}. A track length of ^{232}Th α-particle with average energy of 4 MeV is equal to 31 μm (Il'in et al., 1996) whereas *Ch. vulgaris* cell's diameter varies during life cycle from 1.5 up to 13.5 μm (Franklin et al., 2002). Taking into account that 34 eV is an average energy needed to form a pair of ions (Il'in et al., 1996), we have calculated that each ^{232}Th α-particle can form 120 000 pair of ions until the particle energy would be completely lost. All these events could result in primary DNA damage, which is repaired under normal condition but possibly partially is not restored when DNA metabolism has been disturbed with caffeine.

The NOEC, LOEC and EC_{50} (1.6 μM L^{-1}, 2.2 μM L^{-1} and 15.4 μM L^{-1}) correspond to ^{232}Th activity concentrations in water of 1.5 Bq L^{-1}, 2.1 Bq L^{-1} and 14.4 Bq L^{-1}, respectively. These activity concentrations can be used to calculate radiation dose rates to phytoplankton, through the application of appropriate exposure-dosimetric models such as those provided within the ERICA Tool (Brown et al., 2008). The dose calculations for this particular tool are derived through the implementation of concentration factors and dose conversion factors and results in (weighted) absorbed doses corresponding to 140 μGy h^{-1} for the NOEC and 1.3×10^3 μGy h^{-1} for the EC_{50}. Radiation studies specifically on microalgae are limited, but our data corresponds to estimation (Andersson et al., 2008) of dose rate relevant for protection of freshwater algae populations (100 μGy h^{-1}). Phytoplankton are considered (UNSCEAR, 1996) to be relatively radioresistant compared to other groups of plants and animals and from this one might contend that a 10 μGy h^{-1} (Andersson et al., 2009) dose rate criteria is overly conservative when applied specifically for this group of organisms.

Finally, the dose-rates considered within this study lie within a range that is of interest from an environmental protection perspective, especially in relation to the setting of benchmarks and further illuminate the ongoing debate (Mathews et al., 2009) concerning the chemical versus radiological toxicity of long lived naturally occurring radionuclides.

5. Conclusions

1. Based on EC_{50} and regression equation parameters, ^{232}Th was more toxic to *Ch. vulgaris* after a 24-h exposure than its non-radioactive chemical analogue Ce. In contrast NOEC and LOEC values for ^{232}Th were similar to those for Ce.
2. The main detoxification pathways of Ce (III) in *Ch. vulgaris* differ from those for ^{232}Th. The presence of 0.02 mM caffeine (DNA metabolism disturbance reagent) has no effect on Ce toxicity, but 0.02 mM BSO (selective inhibitor of γ-ECS, a glutathione biosynthetic pathway enzyme) enhances a negative response. In contrast, 0.02 mM caffeine significantly increased ^{232}Th toxicity, whereas the addition of 0.02 mM BSO to ^{232}Th caused no substantial difference to the alga response.

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