

Strontium effects on growth, lipid peroxidation and antioxidant enzyme activities in the marine microalga *Dicrateria inornata* (Prymnesiophyceae)

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(Received 2 July 2003, accepted 17 February 2004)

Abstract — The effects of strontium on growth, peroxidation and antioxidative enzymes have been investigated in *Dicrateria inornata*. The marine microalga grew at all treatment concentrations of strontium, but samples exposed to 512 and 2048 mg strontium l⁻¹ were inhibited by 15.9% and 51.0%, respectively, compared to controls. Among the responses to added strontium, accumulation of malondialdehyde increased significantly at 2048 mg strontium l⁻¹ and was 1.23 fold higher than in controls, and protein content increased at low external concentrations but decreased above 128 mg l⁻¹. In the case of antioxidant enzyme activities, SOD, CAT and GPX activities increased by 85.7, 19.8 and 74.4%, respectively, at 2048 mg strontium l⁻¹ compared to the control. The GSH content increased by 5.2% at 512 mg l⁻¹ and by almost 100% at 2048 mg l⁻¹ strontium compared to controls.

antioxydant / *Dicrateria inornata* / enzymes / glutathione / malondialdehyde / strontium

Résumé — Effets du strontium sur la croissance, la peroxydation des lipides et les activités d'enzyme antioxydante chez la microalgue marine *Dicrateria inornata* (Prymnesiophyceae). Les effets du strontium sur la croissance et les enzymes de peroxydation et antioxydatives ont été recherchés chez *Dicrateria inornata*. La microalgue marine se développe à toutes les concentrations de strontium, mais les échantillons exposés à 512 et 2 048 mg strontium l⁻¹ sont inhibés par, respectivement 15,9 % et 51,0 %, par comparaison aux témoins. Parmi les réponses à l'ajout de strontium, l'accumulation de malondialdéhyde augmente significativement à 2048 mg strontium l⁻¹ ; elle est 1,23 fois plus élevée que dans les témoins ; la quantité de protéine s'accroît aux basses concentrations externes mais décroît au dessus de 128 mg l⁻¹. Les activités enzymatiques antioxydantes, SOD, CAT et GPX, augmentent avec, respectivement, 85,7, 19,8 et 74,4 %, à 2 048 mg strontium l⁻¹ par comparaison avec les témoins. La quantité de GSH s'accroît de 5,2 % à 512 mg l⁻¹ et de presque 100 % à 2048 mg l⁻¹ strontium par comparaison avec les témoins.

antioxydant / *Dicrateria inornata* / enzymes / glutathione / malondialdéhyde / strontium

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Communicating editor: John Beardall

INTRODUCTION

Strontium is a minor component of seawater (Song, 2000) but is a major hazardous contaminant of wastewater and sludge produced by the nuclear industry (Bradley *et al.*, 1996). ^{90}Sr , a normal by-product of nuclear fission, can occur in the environment as a consequence of controlled or accidental release (Eisenbud & Gesell, 1997). When strontium isomorphously replaces the calcium in bones, it is more mobile than calcium, thus causing Urov's disease (Osteoarthritis Deformans Endemica) (Sergievsky, 1948). The fact that ^{90}Sr is a potentially dangerous contaminant in an environment has resulted in many investigations of both its biogeochemistry and its metabolism by plants and animals (Kevern, 1964). Therefore, strontium has been ranked as one of the most potentially hazardous heavy metals to humans and aquatic organisms.

In terms of biomass, microalgae play an important role in the conversion of solar energy into chemical energy through photosynthesis. Furthermore, they have also been considered to play a promising role in environmental protection. Serious environmental pollution by heavy metals results from their increasing utilization in industrial processes and, because most heavy metals are transported into the marine environment and accumulated without decomposition, pollution of the marine environment has become an issue of major concern in recent years. In polluted aquatic ecosystems, algae can be used as biosorbents and accumulators of metals (Holan *et al.*, 1993). They are reported to accumulate metals which otherwise are toxic to biota, and have been considered to play a promising role in environmental protection. An understanding of the regulatory mechanisms of metal tolerance and the components involved in them will help in metal removal from aquatic ecosystems. Tolerance and protective mechanisms have evolved to scavenge free radicals and peroxides generated during various metabolic reactions. These protective mechanisms include antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), and antioxidant compounds such as glutathione (GSH) and carotenoids (CAR). Superoxide radicals generated in plant cells are converted to hydrogen peroxide by the action of superoxide dismutase (SOD). Accumulation of hydrogen peroxide, a strong oxidant, is prevented in the cell by catalase (CAT), which converts the hydrogen peroxide into water. Glutathione peroxidase (GPX) can also catalyze the reduction of hydrogen peroxide or organic hydroperoxides to water or alcohols, thereby protecting cells against oxidative damage. Glutathione, another component of the antioxidative defense system, has been found to increase under oxidative stress conditions (Noctor & Foyer, 1998). The responses of those antioxidant enzymes and antioxidants to metal stress varied among plant species and the metal involved (Mazhoudi *et al.*, 1997). Thus it is of great importance to study metal-induced oxidative stress in order to understand the mechanisms of metal tolerance evolved by plants.

There have been several reports on antioxidant enzymes associated with metal stress in plants (Chaoui *et al.*, 1997; Mazhoudi, *et al.*, 1997), but studies on microalgae are very few (Lee & Shin, 2003). The aim of the present study is to investigate the effects on growth, lipid peroxidation and some antioxidant enzyme activities of the marine microalga *Dicrateria inornata* (Prymnesiophyceae) in response to elevated concentrations of strontium.

MATERIALS AND METHODS

Microalgal culture

The marine microalga *Dicrateria inornata* (3009) was provided by the Institute of Oceanology, Chinese Academy of Sciences, Qingdao and maintained in sterilized artificial seawater (Lyman & Fleming, 1940) enriched with *f/2* medium (Guillard & Ryther, 1962). The algae were grown at $24 \pm 1^\circ\text{C}$ and illuminated with cool white fluorescent lamps at a photon flux of $120\text{--}125 \mu\text{mol photon m}^{-2}\cdot\text{s}^{-1}$. A 12:12 h L/D cycle was maintained in order to obtain synchronous cultures.

Experiments were conducted in 500 ml flasks that had been autoclaved at 121°C for 20 min. Strontium-bearing solutions were prepared by dissolving analytical grade strontium chloride in deionized water. Sr^{2+} solutions in the range 8 to 2048 mg l^{-1} were prepared by the dilution of a concentrated stock solution. The initial culture was removed by centrifugation and the microalgal pellet was resuspended in medium of different strontium concentrations. The experiments were carried out under the conditions described above.

The growth of microalgae was measured spectrophotometrically (VIS-7220 spectrophotometer, Suntek Science Instrument Co. Ltd) at a reference wavelength of 420 nm in a cuvette with a 1cm light path. $\text{OD}_{420\text{nm}}$ values were converted to cell counts using the liner regressions between optical density and cell counts which had been determined in preliminary experiments ($y = 50.69\text{OD}_{420\text{nm}} + 0.93$, $r = 0.999$, cell counts = $y \times 10^5$).

Antioxidative Enzymes Assay

For enzyme assays, *D. inornata* samples cultured for 15 d were centrifuged and re-suspended in 0.067 mM of pre-cooled sodium phosphate buffer (pH 7.0). After sonication for 5 min in an ice bath, the cell debris was removed by centrifugation at 12000 rpm for 20 min at 4°C . The content of lipid peroxidation products (MDA) was determined according to the methods provided by Heath & Parker (1968). Total superoxide dismutase activity (SOD, EC 1.15.1.1) was determined by the ferric cytochrome *c* method using xanthine / xanthine oxidase as the source of superoxide radicals, and a unit of activity was defined as that described in McCord & Fridovich (1969). Catalase activity (CAT, EC.1.11.1.6) was assayed spectrophotometrically with an Hitachi U-3000 spectrophotometer by measuring the decrease of absorbance at 240 nm due to H_2O_2 decomposition (Rao *et al.*, 1996). Glutathione peroxidase (GPX, EC.1.11.1.9) activity was tested in the presence of added glutathione (Thomas *et al.*, 1990). The concentration of reduced glutathione (GSH) was determined spectrophotometrically with dithionitrobenzoic acid (DTNB) at 412nm (Rijstenbil *et al.*, 1994). Protein content of homogenates was determined by reaction with Coomassie Blue dye, using bovine serum albumin as the standard, in a VIS-7220 spectrophotometer (Bradford, 1976).

Statistical analysis

The differences between the control and treated samples were analyzed by one-way ANOVA, taking $P < 0.05$ as significant according to Turkey' s multiple range tests. Each experiment had three replicates. The mean values \pm S.D. are reported in Figures.

RESULTS

Effects on growth

It can be seen in Fig. 1 that *D. inornata* could grow under all experimental conditions. The inhibition of growth was not significant at strontium concentrations lower than 128 mg l⁻¹ whereas at 512 mg strontium l⁻¹ and above, a significant reduction in the number of microalgal cells was apparent. At the end of the experiment, the number of cells in the control was in the range of 2.51×10^6 cells ml⁻¹, while at 2048 mg strontium l⁻¹ it was 1.23×10^6 cells ml⁻¹. Growth was inhibited by 15.9% and 51.0%, respectively, when compared to the control, at strontium treatment concentrations of 512 and 2048 mg l⁻¹. The cell size was approximately 20.89 μ m in diameter for both control and strontium-treated samples.

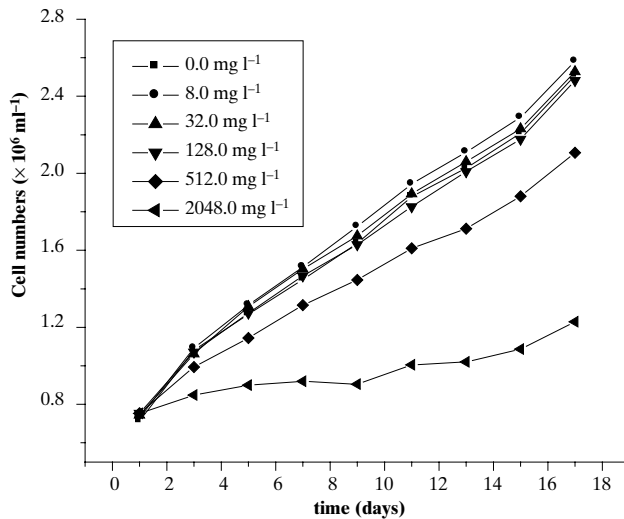


Fig. 1. Growth curves of *D. inornata* supplemented with 0 - 2048 mg strontium l⁻¹. Algae were cultured at $24 \pm 1^\circ\text{C}$, $120\text{-}125 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ and 12:12 h L/D cycle.

Effects on content of lipid peroxidation products and soluble protein

The MDA content significantly decreased at strontium concentrations of 8, 32, 128 and 512 mg l⁻¹ whereas at 2048 mg strontium l⁻¹, MDA significantly increased to 123.0% of the control value (Fig. 2). Protein content remained relatively similar at concentrations up to 512 mg l⁻¹ whereas at 2048 mg l⁻¹ there was a significant (49.7%) decrease (Fig. 3)

Effects on the activities of antioxidant enzymes

The activities of SOD, CAT and GPX at 2048 mg strontium l⁻¹ were significant higher than those of the controls, increasing by 85.7, 19.8 and 74.4%, respectively (Fig. 4). A decrease in SOD and CAT activities are evident at lower external concentrations whereas GPX activity was not significantly affected by concentrations lower than 2048 mg l⁻¹.

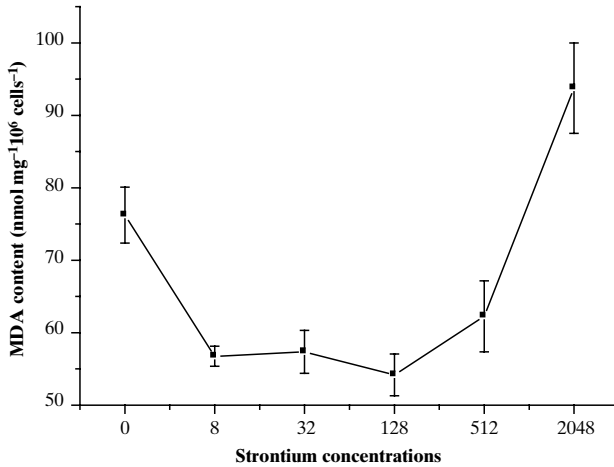


Fig. 2. Level of lipid peroxidation products (MDA) measured as thiobarbituric acid reactive substances in *D. inornata* exposed to different concentrations of strontium. Data are the means \pm SD of three replicates.

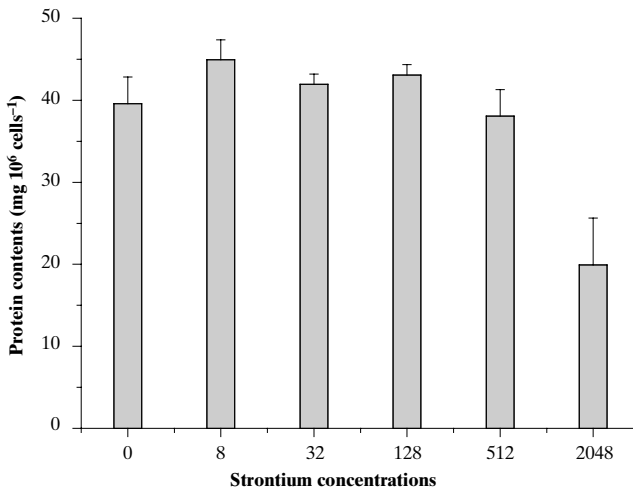


Fig. 3. Soluble protein contents of *D. inornata* supplemented with 0-2048 mg strontium l⁻¹. Data are the means \pm SD of three replicates.

Effects on GSH content

The GSH content was dependent on the strontium concentration. A concentration of 2048 mg l⁻¹ significantly increased (99.9%) GSH compared to the control, while 512 mg l⁻¹ only slightly increased (5.2%) GSH content (Fig. 5). The content of GSH at lower strontium concentrations did not differ from that of the control.

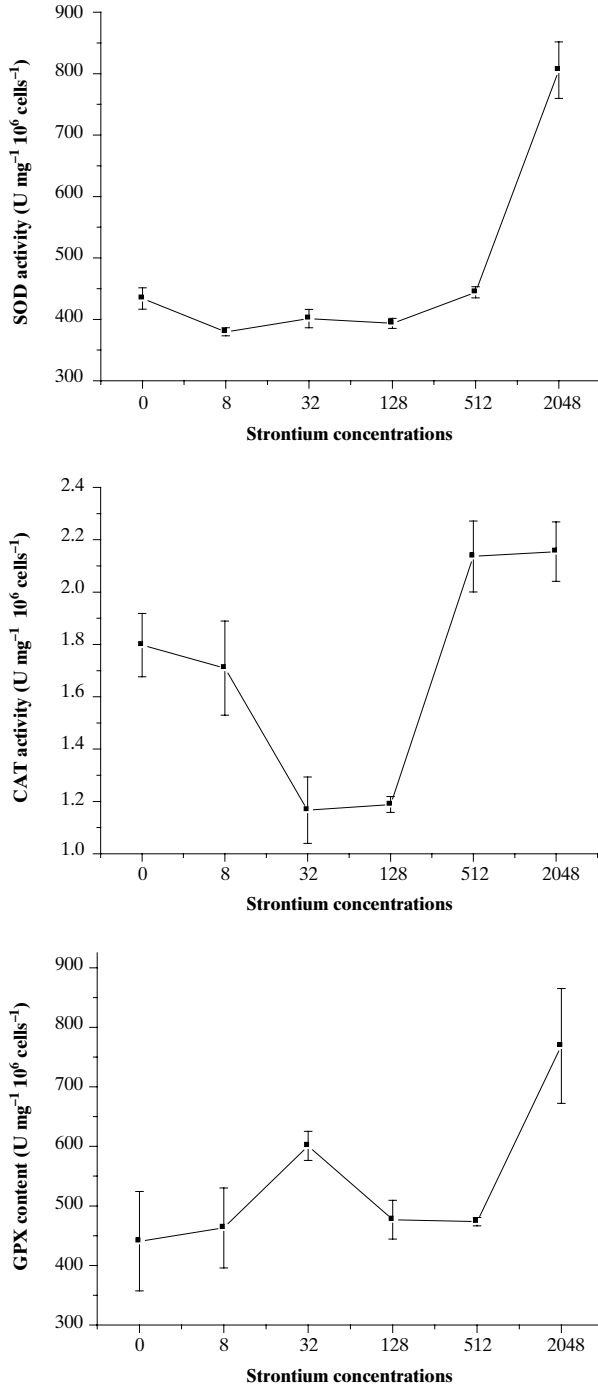


Fig. 4. Total activities of SOD, CAT and GPX of *D. inornata* supplemented with 0-2048 mg strontium l⁻¹. Data are the means \pm SD of three replicates.

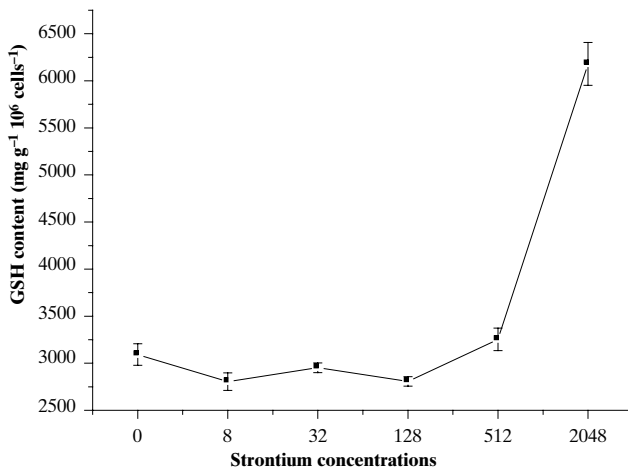


Fig. 5. GSH content of *D. inornata* supplemented with 0-2048 mg strontium l⁻¹. Data are the means \pm SD of three replicates.

DISCUSSION

The concentration of strontium in natural seawater is about 8 mg l⁻¹ (Song, 2000) and normally 7.9 mg l⁻¹ in artificial seawater (Lyman & Fleming, 1940). In our previous experiments (data not shown), saturated SrCl₂ (sediment was present in the culture) was used to determine the effect on growth. It was found that the growth and cell counts were decreased without causing death. The strontium concentrations of 0, 8, 32, 128, 512 and 2048 mg l⁻¹ used here were chosen to study the influence of strontium concentration on growth, lipid peroxidation and antioxidant enzyme activities.

It is well known that reactive oxygen species (ROS) are produced in cells when exposed to environmental stresses, e.g., exposure to high light intensities, UV radiation, metals. Increasing levels of ROS can lead to severe cellular injury or death. Therefore, the induction of antioxidant enzymes is an important protective mechanism to minimize cell oxidative damage in polluted environments. Several antioxidant enzymes and antioxidant substances are involved in the protective mechanisms adapted by plants to scavenge free radicals and peroxides (Allen, 1995). The antioxidant enzymes are important components in preventing the oxidative stress in plants because the activity of one or more of these enzymes is generally increased when exposed to stressful conditions and those elevated activities correlated to increased stress tolerance (Allen, 1995; Mazhoudi *et al.*, 1997).

The effects of higher strontium concentration on growth of microalgae observed under our experimental conditions could be associated with the increase in lipid peroxidation, measured as MDA production. Compared to the control, MDA concentration was increased significantly at 2048 mg strontium l⁻¹ indicating a rise in lipid peroxidation for the treatment (Fig. 2). In the meantime, the growth was inhibited at this concentration. Membrane destabilization is generally attributed to lipid peroxidation resulting from an increased production of active

oxygen species (AOS) (Mead *et al.*, 1982) and lipid peroxidation can be initiated by redox activating metal ions themselves (Chaoui *et al.*, 1997). The increase in lipid peroxidation observed in our work is considered as an indicator of increased oxidative damage caused by strontium in cells. Based on the present work, we conclude that at 2048 mg l⁻¹ strontium causes oxidative damage, as evidenced by the increased lipid peroxidation in *D. inornata*.

The strontium at the greatest dose (2048 mg l⁻¹) caused a similar increase in antioxidant (GSH) and antioxidant enzymes (SOD, CAT and GPX) compared to the control, which was taken as circumstantial evidence for enhanced production of free radicals under strontium stress. Such increase in the activity of these enzymes had been reported with a variety of heavy metals applied for treatments (Van Assche & Clijsters, 1990) and the extent of increase varied with metal, metal concentration, the enzyme tested and plant species. However, detailed information concerning activation of the antioxidant system for the detoxification of metal ions in marine algae is scarce, although algae are recommended as biological monitors for heavy metal pollution (Whitton *et al.*, 1989). With respect to algae, SOD activity can be induced by both Cu exposure in the diatom *Ditylum brightwellii* and Cd exposure in *Tetraselmis gracilis* (Rijistenbil *et al.*, 1994; Okamoto *et al.*, 1996). It was found in the present study that microalgal cells, after being treated by higher strontium concentration, showed an increase in the level of SOD activity. It is well known that CAT plays an important role in reducing oxidative stress by catalyzing the oxidation of H₂O₂ (Weckx & Clijsters, 1996). Thus the increase in the activities of this enzyme by strontium suggests increased production of H₂O₂ and the markedly elevated the level of CAT, GPX and SOD following strontium treatment at highest concentration indicated the protective role of these enzymes against strontium-induced oxidative stress. Lee *et al.* (2003) also found catalase activity increased slightly when cells of the microalga *Nannochloropsis oculata* were exposed to cadmium stress.

Apart from these enzymes, some antioxidants like GSH may also play a role in inducing resistance to metals by protecting labile macromolecules against attack by free radicals, which are formed during various metabolic reactions and lead to oxidative stress (Alscher, 1988). In the present study, the level of antioxidant (GSH) exhibited a varied response to the strontium treatments, which was dependent on the metal concentration. Earlier Tukendorf & Rauser (1990) reported an increase in GSH content in maize, following treatment with Cd, due to activation of GSH synthesizing enzymes. A change in activity of enzymes involved in GSH biosynthesis is known to be a result of metal stress in plants (Scheller *et al.*, 1987), but the depletion of GSH content with increasing concentration of copper was also observed in some algae (Nagalakshimi & Prasad, 2001). Thus, the observed relation between strontium induced oxidative stress (lipid peroxidation) and antioxidative capacity (antioxidant enzymes, GSH) in *D. inornata* cell suggests that the tolerance capacity of the plants to the metal depends on the balance between the factors favoring oxidative stress and the factors reducing oxidative stress.

To our knowledge this is the first report using microalgae to study antioxidant systems under strontium stress. In conclusion, *D. inornata* under strontium stress will show induction of antioxidant enzymes, which are important protective mechanisms to minimize cell oxidative damage in polluted environments and allow the *D. inornata* to tolerate a high concentration of strontium.

Acknowledgements. We are indebted to Mr Zhonghao Xia and Mrs Shuijuan Wang for their skillful technical assistance. Special thanks go to Dr. M.T. Brown for his kind advice. This research was supported by funds from the Natural Science Foundation of Jiangsu Province (No. 95021301).

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