American Journal of Agricultural Science
2015; 2(3): 108-120
Published online May 20, 2015 (http://www.aascit.org/journal/ajas)

AASCIT American Association for Science and Technology



Keywords
Synechococcus sp.,
Metal Stress,
Malondialdehyde (MDA),
Superoxide Dismutase (SOD)

Received: April 27, 2015
Revised: May 4, 2015
Accepted: May 5, 2015

Physiological and Biochemical Responses of *Synechococcus* sp. PCC7942 to the Stress of Barium, Chromium, Mercury and Lead

Xiangyuan Deng, Jie Cheng, Kun Gao*, Xiaoli Hu, Xiang Luo

College of Biotechnology, Jiangsu University of Science and Technology, Zhenjiang, China

Email address
gkunjn2002@126.com (K. Gao)

Citation
Xiangyuan Deng, Jie Cheng, Kun Gao, Xiaoli Hu, Xiang Luo. Physiological and Biochemical Responses of Synechococcus sp. PCC7942 to the Stress of Barium, Chromium, Mercury and Lead. *American Journal of Agricultural Science.* Vol. 2, No. 3, 2015, pp. 108-120.

Abstract
Cyanobacteria are prokaryotic algae found in oceans and freshwaters worldwide. These organisms are important primary producers in aquatic ecosystems because they can provide essential food for grazers and herbivores. In this study, physiological and biochemical responses of a freshwater cyanobacterium *Synechococcus* sp. PCC7942 to the stress of barium (Ba), chromium (Cr), mercury (Hg) and lead (Pb) were compared and evaluated using 96 h growth tests in a batch-culture system. The growth, Chl *a* concentration and soluble polysaccharides content of *Synechococcus* sp. PCC7942 were significantly inhibited by Ba, Cr and Hg, but the cyanobacterium had different responses to Pb stress (no significant at low concentration, but significant at high concentration). Moreover, remarkable physiological and biochemical responses occurred when the cyanobacterium was exposed to metal stress. And a general increase in MDA content suggests that the toxic effects of metals were probably exerted through free radical generation; and the increase in SOD activity may be viewed as an active defense against metal stress by stimulating synthesis of proteins against stress and quenching of free radicals. These observations suggest that Ba, Cr, Hg and Pb are toxic to *Synechococcus* sp. PCC7942, and their use should be moderately restricted.

1. Introduction

Because of the increasing industrial and agricultural wastewater, contamination of aquatic environments by metals has become a focus of researchers and environmentalists in recent years. Once metals are introduced into aquatic ecosystems, they can affect the growth, development, morphology, physiological and biochemical metabolism of aquatic organisms through various ways (Van Assche and Clijsters, 1990; Bidar et al., 2007). Furthermore, the non-degradability of metals, their accumulation in biota, and biomagnification along aquatic food chains (Azevedo et al., 2007) will destroy aquatic ecosystem.

Cyanobacteria are important primary producers in aquatic ecosystems because they can supply grazers and herbivores with essential food (Foster, 1964; Quinn, 1988). Elevated levels of metals exert toxic effects on the growth, development and metabolism of cyanobacteria. Toxicity mechanisms of metals include the binding to sulphydryl groups in proteins or disruption of protein structure or displacement of an essential element (Tripathi et al., 2006). In addition, metal stress would induce the uncontrolled and excessive production of reactive oxygen species (ROS) via Haber-Weiss and Fenton reactions (Stohs and Bagchi, 1995), which can cause peroxidation of lipids, inactivation of enzymes, and

damaged DNA and other constituents of cells (Mallick, 2004; Sabatini et al., 2009; Gill and Tuteja, 2010; Ahmad et al., 2010). To minimize the damaging effects of ROS, algae have evolved antioxidant defense mechanisms, including enzymes which can catalyze reactions of ROS scavenging. Among the antioxidant enzymes, superoxide dismutase (SOD) is the most important enzyme and can detoxify superoxide anions (Beyer et al., 1991; Mellado et al., 2012). Moreover, malondialdehyde (MDA) is a cytotoxic product of lipid peroxidation and an indicator of free radical production and consequent tissue damage (Janero, 1990). Polysaccharides are important class of biological polymers and major components of organism materials. Their function is usually either structure or storage related.

The unicellular cyanobacterium *Synechococcus* sp. PCC7942, ubiquitous in Chinese freshwater, was used in this study. The aim of this study is to explore the physiological responses of *Synechococcus* sp. PCC7942 to barium (Ba), chromium (Cr), mercury (Hg) and lead (Pb). The objectives are as follows: 1) to compare and evaluate the toxicity of Ba, Cr, Hg and Pb to *Synechococcus* sp. PCC7942; 2) to investigate growth characteristics of *Synechococcus* sp. PCC7942 under different metals stress; and 3) to evaluate the changes of cell contents and antioxidase activity in *Synechococcus* sp. PCC7942 under the stress of Ba, Cr, Hg and Pb, respectively.

2. Materials and Methods

2.1. Test Chemicals and Solutions

The chemicals, such as $BaCl_2 \cdot 2H_2O$, $K_2Cr_2O_7$, $HgCl_2$ and $Pb(NO_3)_2$ were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). All of the chemicals were of analytical grade. Test solutions were prepared by adding a certain amount of different metals in BG11 medium. And the following concentrations were used according to preliminary experimental results: 100, 200, 300 and 400 mg L^{-1} (Ba); 50, 100, 150 and 200 mg L^{-1} (Cr and Pb); 2, 4, 6 and 8 mg L^{-1} (Hg) for cytotoxicity tests.

2.2. Microorganism

Synechococcus sp. PCC7942 was obtained from Prof. Xiaowen Zhang (Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences). The stock culture and inoculum were grown in BG11 medium (Stanier et al., 1971). The inoculum was precultured aseptically in 500 mL Erlenmeyer flasks with 200 mL of BG11 medium. The flasks were placed in a 28 °C illuminated incubator (Jiangnan Instrument Factory, Ningbo, China) for 7 days under a 12 h light/12 h dark photoperiod and a light density of 40 μE m^{-2} s^{-1}.

2.3. Experimental Set Up

After precultivation, the algal inocula reached exponential growth phase. Up to 20 mL of the algal inoculum was collected using centrifugation (4,000 ×g, 4 °C, 15 min). The deposited algal cells were washed twice with ddH_2O, and then inoculated into growth medium.

In growth inhibition experiments, the cultures were grown in 250 mL Erlenmeyer flasks containing 100 mL of modified BG11 medium with different concentrations of the test chemicals, each in triplicate. And another set of flasks containing BG11 medium without test chemicals was prepared as control, also in triplicate. The other cultivation conditions were as described above.

2.4. Microalgal Growth Analysis

Daily microalgal cell density was determined turbidometrically at 680 nm using a spectrophotometer (UV1800, Mapada Instruments Company, Shanghai, China). The relationship between microalgal cell density (D, cells mL^{-1}) and optical density of the microalgal culture at 680 nm (OD_{680}) is shown in Eq. (1):

$$D = 1.827 \times 10^8 \, OD_{680}, \ (R = 0.9916) \quad (1)$$

2.5. Biochemical Analysis

2.5.1. Chlorophyll *a* (Chl *a*) Determination

Triplicate 5 ml of well-blended cultures were centrifuged at 4000 g for 15 min to discard the supernatants. And the pellets were homogenized with 80% (V/V) acetone for Chl *a* extraction. The mixtures were vigorously shaken using a vibrator, and then placed in a refrigerator in the dark at 4 ºC for 24 h. Then the extracted samples were centrifuged at 10,000 g for 5 min to remove the pellets. Supernatants were transferred into 1×1 cm glass cuvettes, and measured for Chl *a* at 663 nm and 645 nm using a spectrophotometer. All absorbance values were corrected using the 80% acetone as control. The concentration of Chl *a* was calculated by the following equation (Hao et al., 2007) (Eq. 2):

$$\text{Chl } a \text{ (mg } L^{-1}\text{)}: \ C_a = 12.71A_{663} - 2.59A_{645} \quad (2)$$

2.5.2. Polysaccharide Content Determination

After 48 and 96 h of test chemical exposure, triplicate samples of 10 mL well-blended cultures were centrifuged at 4,000 ×g for 15 min to discard the supernatants. The pellets were placed in 1.5 mL ddH_2O, and immediately boiled for 20 min to extract soluble polysaccharides. Then, the cellular homogenate was centrifuged at 12,000 ×g for 10 min, and the supernatant liquid was used for polysaccharide content determination.

Soluble polysaccharide content was estimated according to anthrone sulfuric acid method (Li, 2000). All test tubes were boiled for 20 min, and absorbance was read at 625 nm after cooling. Then, the polysaccharide content was calculated according to the calibration curves, and represented as micrograms per 10^7 cells (μg × 10^7 $cell^{-1}$).

2.5.3. SOD and MDA Determination

After 48 and 96 h of test chemical exposure, 30 mL well-blended cultures were harvested by centrifugation at 4,000 ×g for 15 min at 4 °C. The harvested microalgae were

placed in 1.5 mL extraction buffer containing 0.1 M sodium phosphate buffer (pH 7.0), and immediately lysed by sonication (Scientz Biotechnology Co. Ltd, Ningbo, China) for 10 min with a repeating duty cycle of 5 s in an ice bath. The cellular homogenate was centrifuged at 12,000 ×g for 10 min at 4 °C, and the supernatant liquid was stored at -70 °C for use.

The SOD and MDA assay kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). According to manufacturer's instructions, SOD and MDA were extracted and determined from the supernatant liquid above. The results of SOD and MDA assay are given as units of enzyme activity per 10^7 cells (U × 10^7 $cell^{-1}$) and nmol per 10^9 cells (nmol × 10^9 $cell^{-1}$).

2.6. Statistical Analysis

SPSS PASW Statistics 18 software was used in all statistical analyses. The mean values, confidence intervals, and standard deviation values of the triplicates for each treatment were calculated. The effects caused by metal stress on growth characteristics and antioxidant enzymes were evaluated using one-way ANOVA at $P < 0.05$.

3. Results

3.1. Microalgal Growth Characteristics

Ba, Cr, Hg and Pb are non-essential metals for the microalgal growth. Their effects on cell density and growth characteristics of *Synechococcus* sp. PCC7942 were compared and analyzed in this study. As shown in Figure 1, Ba, Cr, Hg and Pb inhibited the growth of *Synechococcus* sp. PCC7942 in different treatments. However, negative effects of Ba, Cr, Hg and Pb treatments had great difference. Pb treatments had little inhibition up to 24 h, and then showed negative effects, especially at higher concentration. Although Ba presented a certain inhibition as its concentration increased, it nearly had no negative effects when its concentration was less than 50 mg L^{-1}. Hg and Cr treatments had great inhibitory effect on the growth of *Synechococcus* sp. PCC7942, even in 2 mg L^{-1} Hg. Visual and microscopic observation showed that the microalgal cells almost completely died after 72 h exposure with Hg and could not revive even in fresh, normal BG-11 medium.

3.2. Chlorophyll *a* Content

Figure 2 shows the changes of Chl *a* content in *Synechococcus* sp. PCC7942 with Ba, Cr, Hg and Pb treatments respectively. The changes of Chl *a* were not significant in Ba treatments, but differences between the treatments and untreated group were great. The change trend was similar to that of cell density in *Synechococcus* sp. PCC7942. In Cr treatments, high concentration (150 and 200 mg L^{-1}) inhibited the Chl *a* production and showed difference with low concentration (50 and 100 mg L^{-1}) after 72 h exposure. And there also had great difference between the treatments and controls. Chl *a* content greatly decreased in Hg treatments, particularly after 48 h exposure. And then, Chl *a* concentration was almost close to 0 at 72 and 96 h. However, Pb treatments exhibited little negative effects until 100 mg L^{-1}, and high concentration (150 and 200 mg L^{-1}) had great inhibition on the production of Chl *a*.

3.3. Soluble Polysaccharides Content

The changes of soluble polysaccharides content in *Synechococcus* sp. PCC7942 in Ba, Cr, Hg and Pb treatments are shown in Figure 3. The changes exhibited good dose-dependent relationships in Ba treatments, and soluble polysaccharides content decreased with the increase of Ba concentrations (Fig. 3A). Figure 3B illustrates the effects of Cr on soluble polysaccharides content of *Synechococcus* sp. PCC7942, which showed that Cr inhibited the production of soluble polysaccharides, especially in high concentration (150 and 200 mg L^{-1}) at 96 h exposure. There are similar change trends between Hg and Cr treatments (Figs. 3B and 3C). The soluble polysaccharides have also not been detected in high concentration (6 and 8 mg L^{-1}) at 96 h Hg exposure. However, soluble polysaccharides content improved as the concentration of Pb increased.

3.4. Lipid Peroxidation

The level of lipid peroxidation was determined in terms of MDA content. As shown in Fig. 4, an increase of cellular MDA content was observed in all treatments, but effects of Ba, Cr, Hg and Pb had great difference. At 48 h exposure with Ba, MDA content had no significant increase with the increase of Ba concentrations, but a sharp increase was observed in 300 and 400 mg L^{-1} Ba treatments at 96 h exposure (Fig. 4A). When the microalgae were exposed to Cr, the highest value of MDA content was detected at 48 h with 200 mg L^{-1} Cr, and there are rising trends with the increase of Cr concentrations at 48 and 96 h (Fig. 4B). MDA content was also induced by Hg treatments, and Fig. 4C illustrates the effects of Hg on MDA content of *Synechococcus* sp. PCC7942. In addition, Pb treatments could also enhance MDA content of *Synechococcus* sp. PCC7942 and a slight increase at 48 and 96h exposure.

3.5. Antioxidase Activity

Algae have various antioxidant enzymes to combat increased production of ROS caused by heavy metals. SOD is the most important enzyme among antioxidant enzymes, which can detoxify superoxide anions (Beyer et al., 1991; Mellado et al., 2012). In this study, the changes of SOD activity were determined and compared under different heavy metals to investigate the tolerance of *Synechococcus* sp. PCC7942 to Ba, Cr, Hg and Pb stress (Fig. 5). The SOD activity was generally improved with the increase of metal concentrations. At 48 h Ba exposure, SOD activity significantly increased from 0.087 to 0.182 U 10^7 $cell^{-1}$ when the Ba was added from 0 to 400 mg L^{-1}. The SOD activity had also remarkable increase with the increase of Ba concentration at 96 h exposure. Cr induced higher SOD activity than control at 48 and 96 h exposure. However, the SOD activity decreased

under Cr concentrations higher than 150 mg L^{-1} in the cultures at 96 h exposure. In Hg treatments, higher concentrations (>4 mg L^{-1}) caused significant increase of SOD activity with respect to control, but there were no remarkable difference in statistics (P>0.05). Changes of SOD activity were not significant at 48 h Pb exposure, but SOD activity was increased with respect to control. At 96 h exposure, SOD activity was enhanced with the increase of Pb concentrations.

4. Discussion

Although cyanobacteria are considered important primary producers in aquatic ecosystems and serve as essential energy and food sources for many microphagous grazers, information available regarding the toxicity of heavy metals on cyanobacteria is scarce in peer-reviewed literature. This study examined the effects of Ba, Cr, Hg and Pb on growth characteristics and physiological responses of unicellular cyanobacterium *Synechococcus* sp. PCC7942.

Ba, Cr, Hg and Pb are all non-essential metal for the algal growth, which have become serious pollutants in water environment because of its widespread industrial, agricultural and anthropogenic use (Atli and Canli, 2010). Elevated levels of heavy metals elicit deleterious impact on algae and cyanobacteria. Toxicity of heavy metals to algae primarily results from their binding to sulphydryl groups in proteins or disruption of protein structure or displacement of an essential element (Tripathi et al., 2006).

Ba is used for a variety of industrial purpose including paper manufacturing, fabric printing and dyeing, synthetic rubber production, and drilling fliuds. Ba has been shown to cause myocardial toxicity in humans and animal models due to modifications in membrane permeability and possible alterations in cell function (Delfino et al., 1988; Perry et al., 1989). However, there is relatively little information of the toxic effects of Ba to algae.

Cr, which is not essential for any metabolic process, is the seventh most abundant metal in the earth's crust (Rodríguez et al., 2007). Cr can easily cross the membrane through simple diffusion. Cr phytotoxicity can result in degradation of pigment status, nutrient balance, enhancement of antioxidant enzymes activity, and induction of oxidative stress in plants and algae (Wong and Chang, 1991; Rai et al., 1992; Cervantes et al., 2001; Panda and Choudhury, 2005), together with alteration of chloroplast and membrane ultrastructure (Choudhury and Panda, 2005).

Hg can replace metals ion in photosynthetic pigments to cause a decrease in photosynthesis rates (Tabaldi et al., 2007), and represents a major hazard to microorganisms at low concentrations. Aquatic plants can be affected by Hg in water at concentrations of 1 mg l^{-1} (Boening, 2000). In a study (De et al., 1985) using *Pistia stratiotes* was exposed for 2 days to mercuric chloride at concentrations between 0.05 and 20.0 mg l^{-1}. The highest dose of mercury decreased chlorophyll content, protein, RNA, dry weight, catalase and protease activity, and increased production of free amino acids.

Pb, which is also one of the main inorganic pollutants in our environment, exerts a wide range of adverse effect on growth and metabolism of algae (Piechalak et al., 2002; Tabaldi et al., 2007). Pb is strongly phytotoxic, partly because of the generation of reactive oxygen species (ROS) that react with lipids, proteins, photosynthetic pigments and nucleic acids causing lipid peroxidation, membrane damage, metabolite degradation, inactivation of enzymes and cell death (Hegedüs et al., 2001).

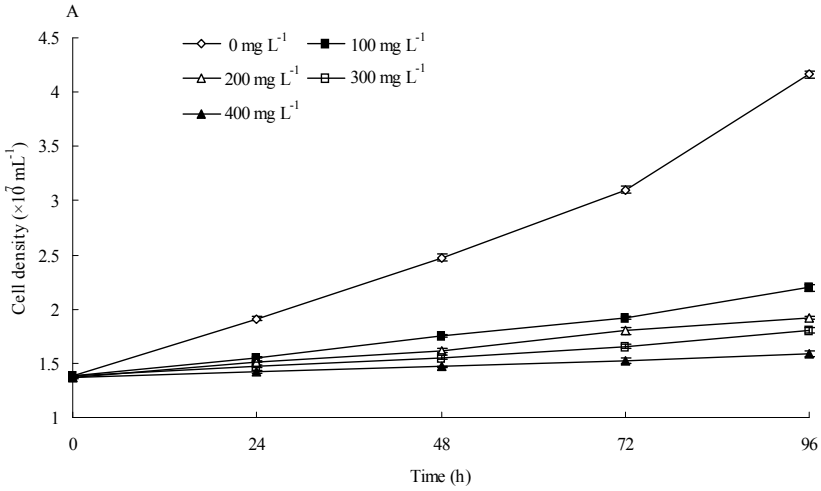
In this present study, the growth of *Synechococcus* sp. PCC7942 was inhibited by Ba, Cr, Hg and Pb, but the inhibition effects had great differences among the four metals and the effect order was Hg>Cr>Ba>Pb. The reasons may be that metals have different action mechanism for toxic effects, which have been described above. In addition, chlorosis, a common symptom of metal toxicity, occurred in Ba, Cr and Hg treatments before 48 h, which suggested that the Chl *a* formation was restrained by Ba, Cr and Hg. Some studies have also described similar results in other metal treatments (Lee and Lustigman, 1996; Sabatini et al., 2009; Wang et al., 2011). Compared with untreated group, the production of Chl *a* was not obviously inhibited by Pb at low concentrations (<100 mg L^{-1}), while that was inhibited significantly at high concentrations (>150 mg L^{-1}). Therefore, results demonstrated that Ba, Cr, Hg and Pb had great different toxic effects on growth of *Synechococcus* sp. PCC7942.

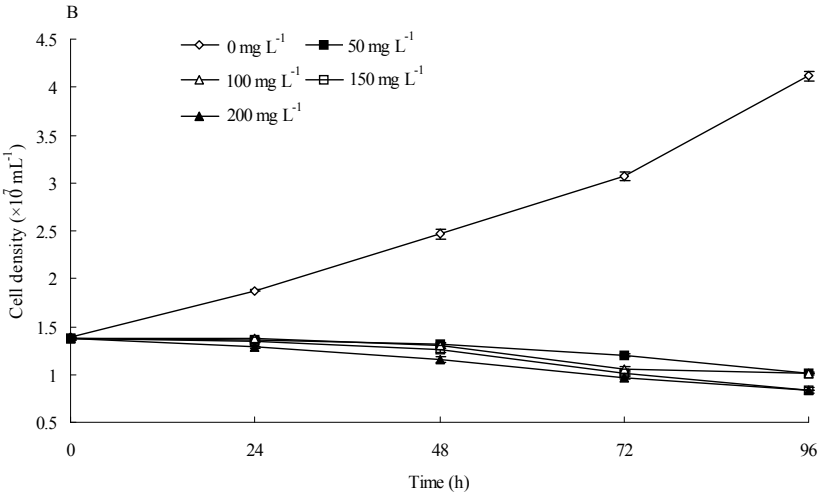
The soluble polysaccharides content decreased with the increase of Ba concentrations, and the changes exhibited good dose-dependent relationships (Fig. 3A). The changes in soluble polysaccharides content presented similar trends for the Cr and Hg treatments, which inhibited the production of soluble polysaccharides, especially in high concentration at 96 h exposure. However, soluble polysaccharides content improved as the concentration of Pb increased, and the stimulation effects were significant at 96 h exposure. The increase in polysaccharide content may be due to the increase in biosynthesis, which is a commonly observed symptom of toxicity in algae (Han et al., 2008). These results may explain that Pb block the photosynthetic electron capture in chloroplasts, and excessive energy is used for the biosynthesis of polysaccharides and other substances.

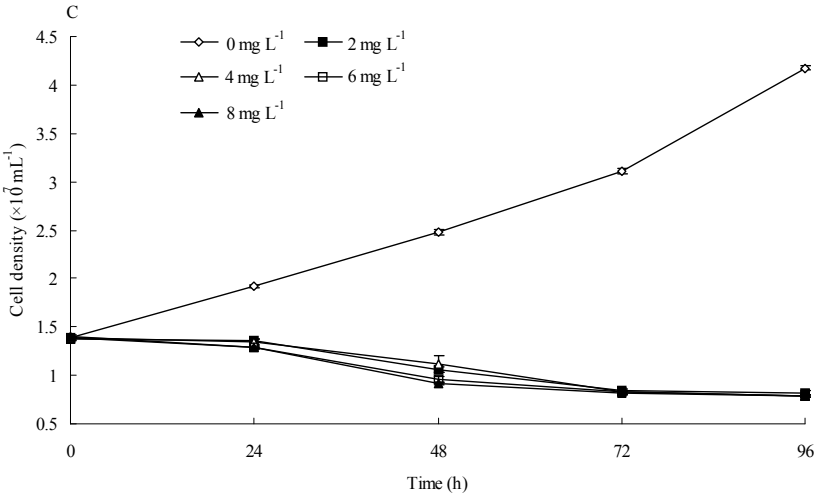
MDA content is often considered as an indicator of lipid peroxidation status. In this work, an increase of cellular MDA content was observed in all treatments, which indicates free radical generation under metal stress. It is similar to the effects of other contaminants on microalgal species (Choudhary et al., 2007; Kumar et al., 2008; Hong et al., 2009; Sabatini et al., 2009). Moreover, MDA content increased with increasing Ba, Cr, Hg and Pb concentrations, which suggest that the toxic effects of Ba, Cr, Hg and Pb are probably exerted through free radical generation. The increased MDA level is a dose-dependent effect of free radical generation.

When algae are exposed to various abiotic stresses, ROS production increased in the algal cells. ROS are highly reactive and toxic, and can causes damage to proteins, lipids, carbohydrates and DNA (Gill and Tuteja, 2010). Algal cells produce many antioxidant defense components, such as SOD and CAT, two important enzymes in ROS scavenging, to

protect against ROS. In addition, the activity of these two enzymes may be enhanced following exposure to moderate environmental stresses (Malanga and Puntarulo, 1995; Kumar et al., 2008; Qian et al., 2008; Sabatini et al., 2009). Ba, Cr, Hg and Pb greatly enhanced SOD activity compared with untreated group. This was accounted as evidence of enhanced free radical production under metal stress. However, strong environmental stresses may cause inactivation of SOD. The suppression of SOD activity under high concentrations of Cr (>150 mg L^{-1}) indicates that antioxidant defense enzymes such as SOD might be an important site of action for Cr in microalgae. The inactivation of SOD resulted in high lipid peroxidation and low cellular inclusion content (chl *a* and soluble polysaccharides content), thereby inhibiting algal cell growth.







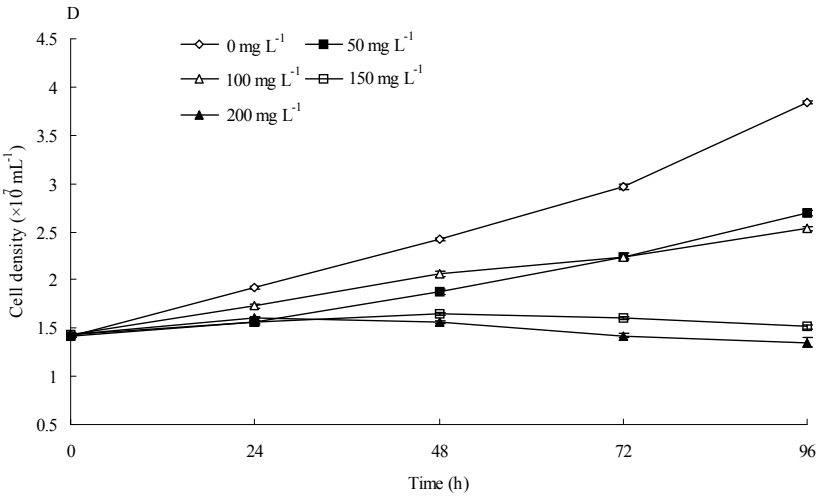
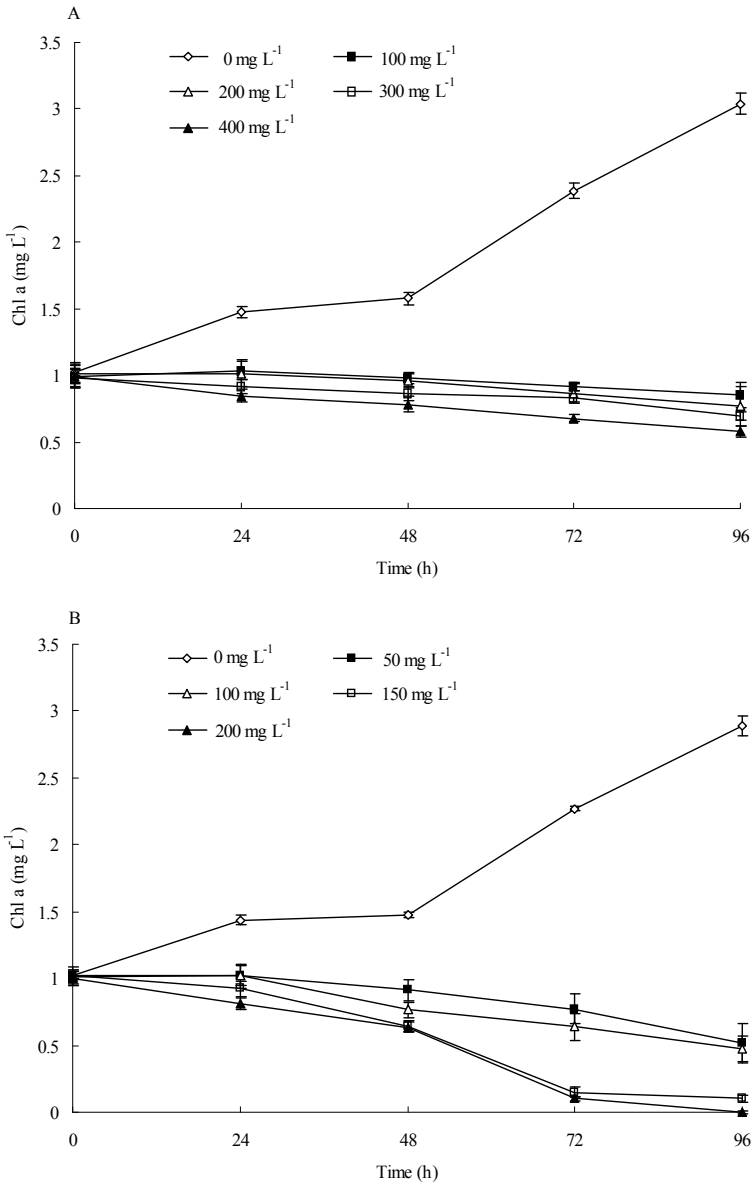


Figure 1. *Growth profiles of Synechococcus sp. PCC7942 under different Ba (A), Cr (B), Hg (C), and Pb (D) concentrations (mg L^{-1}) during 96 h exposure. The points represent the means of three replicates (n = 3); error bars represent standard deviations.*



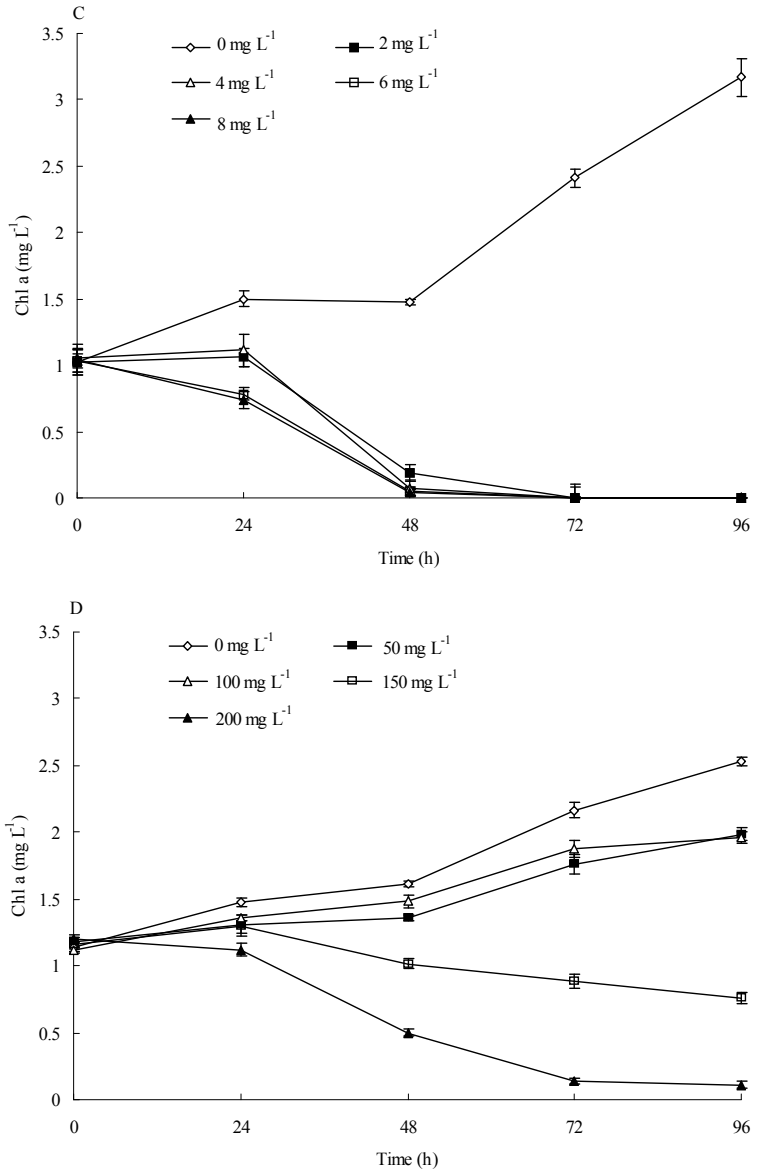
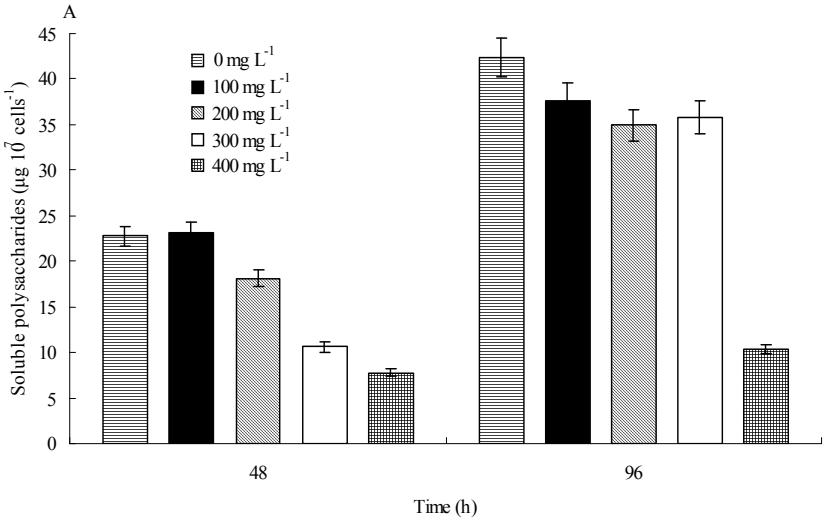


Figure 2. *Effects of Ba (A), Cr (B), Hg (C), and Pb (D) on the chlorophyll a content of Synechococcus sp. PCC7942 during 96 h of exposure. The points represent the means of three replicates (n = 3); error bars represent standard deviations.*



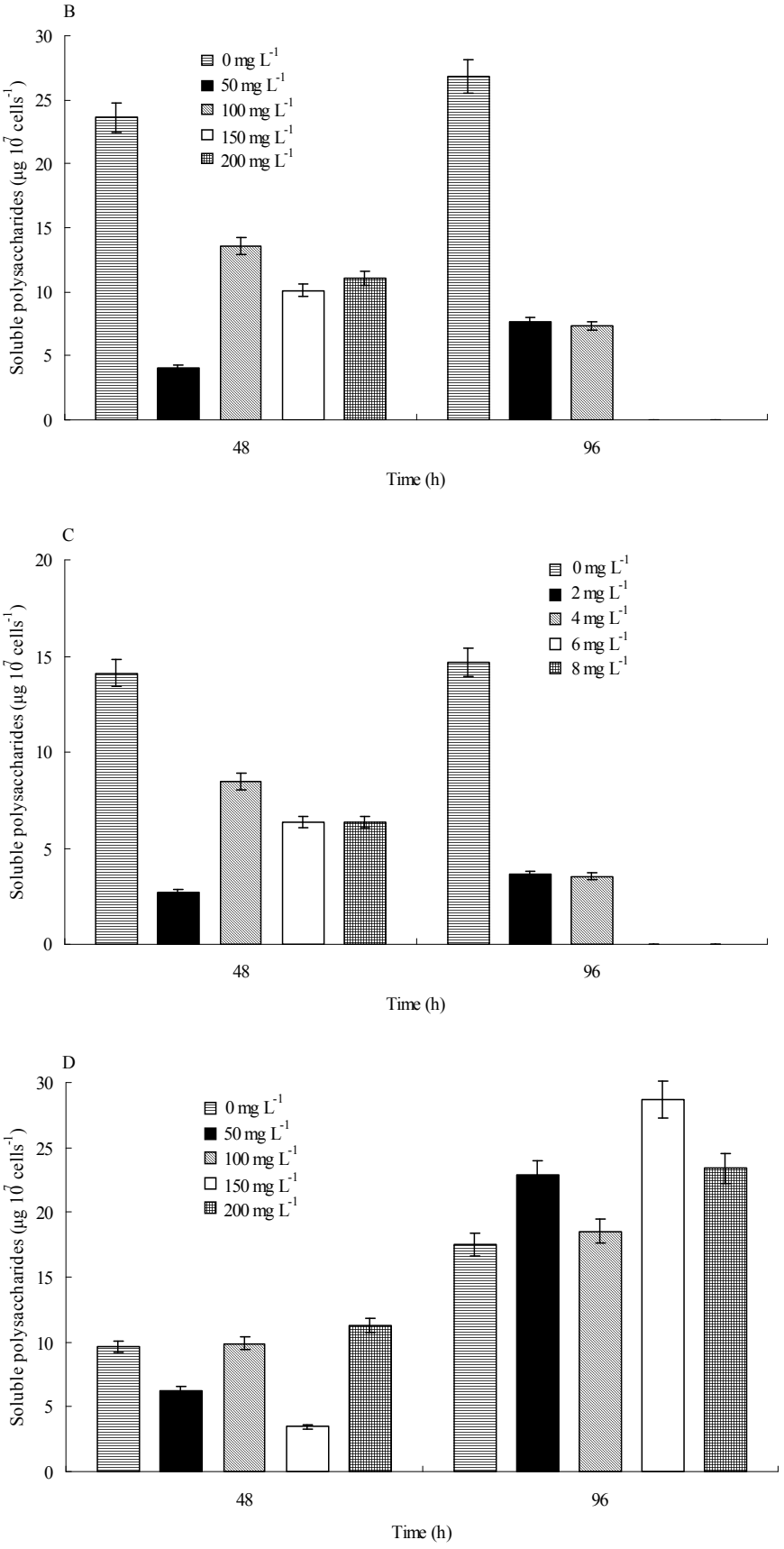
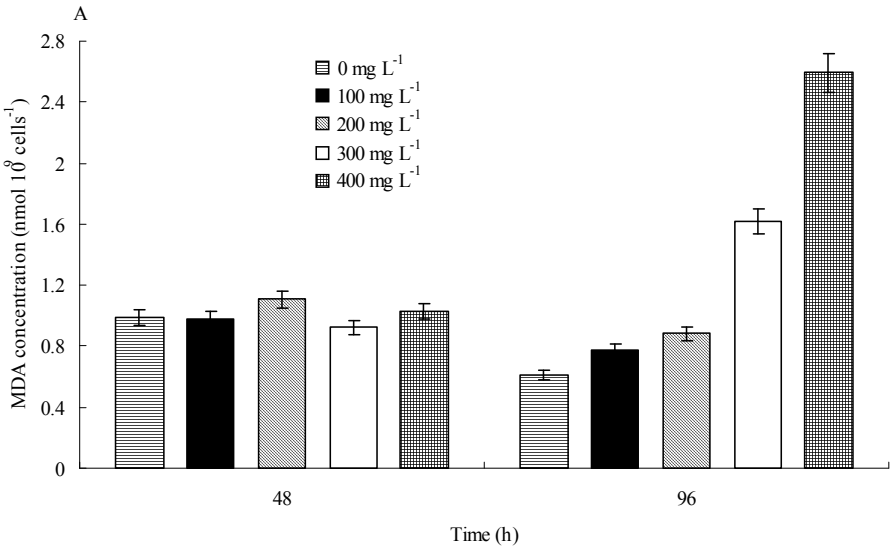
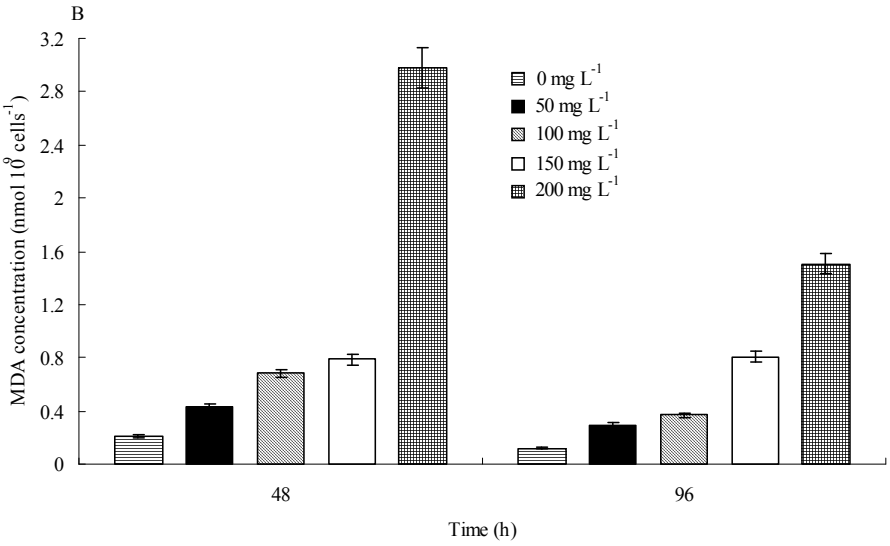
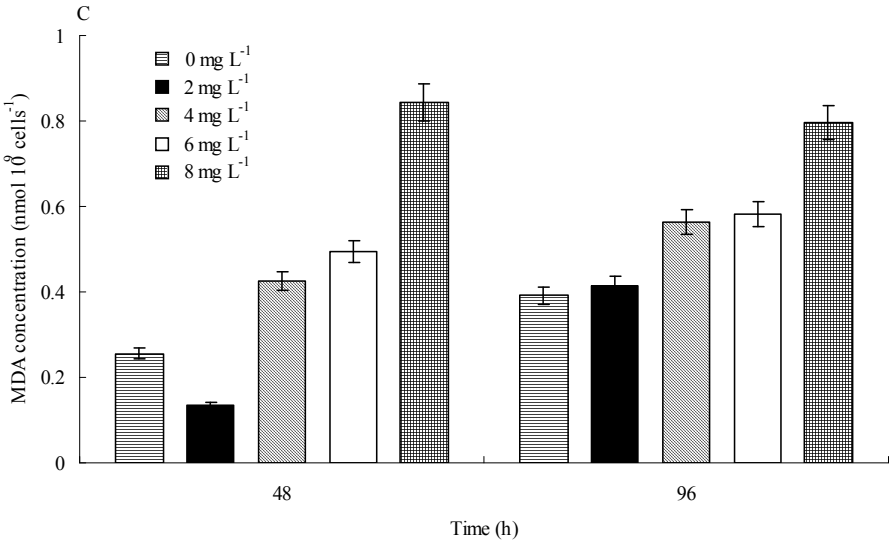


Figure 3. *Effects of Ba (A), Cr (B), Hg (C), and Pb (D) on the cellular soluble polysaccharides content of Synechococcus sp. PCC7942 after 48 and 96 h of exposure. The points represent the means of three replicates (n = 3); error bars represent standard deviations.*


A
MDA concentration (nmol 10^9 cells^{-1})
0 mg L^{-1}
100 mg L^{-1}
200 mg L^{-1}
300 mg L^{-1}
400 mg L^{-1}
0
0.4
0.8
1.2
1.6
2
2.4
2.8
48
96
Time (h)


B
MDA concentration (nmol 10^9 cells^{-1})
0 mg L^{-1}
50 mg L^{-1}
100 mg L^{-1}
150 mg L^{-1}
200 mg L^{-1}
0
0.4
0.8
1.2
1.6
2
2.4
2.8
3.2
48
96
Time (h)


C
MDA concentration (nmol 10^9 cells^{-1})
0 mg L^{-1}
2 mg L^{-1}
4 mg L^{-1}
6 mg L^{-1}
8 mg L^{-1}
0
0.2
0.4
0.6
0.8
1
48
96
Time (h)

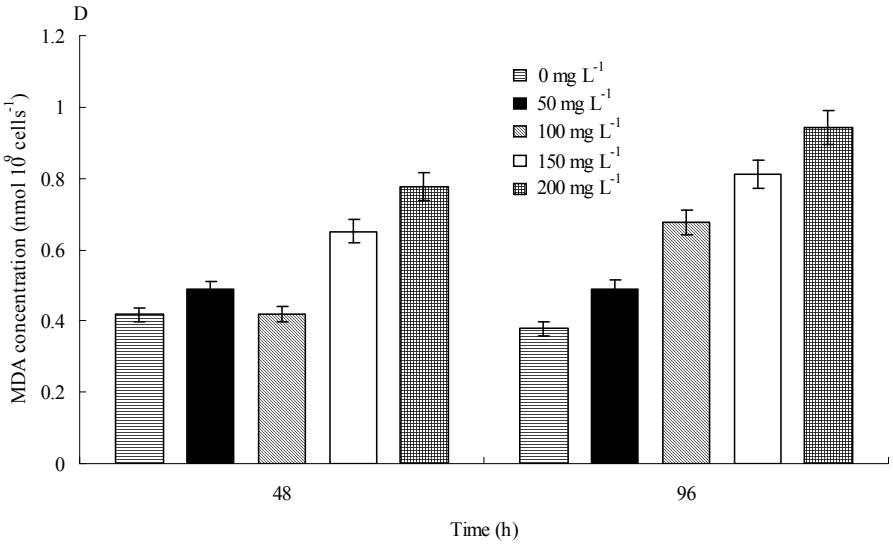
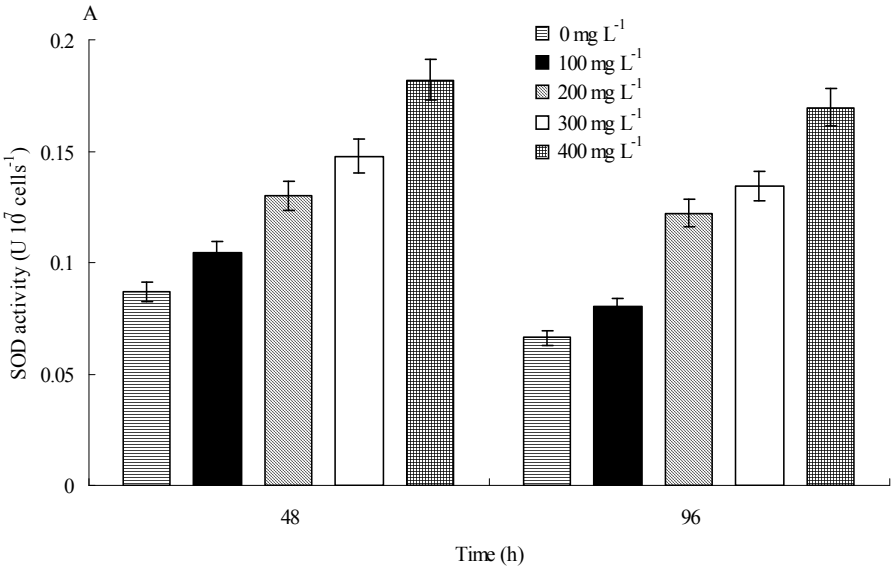
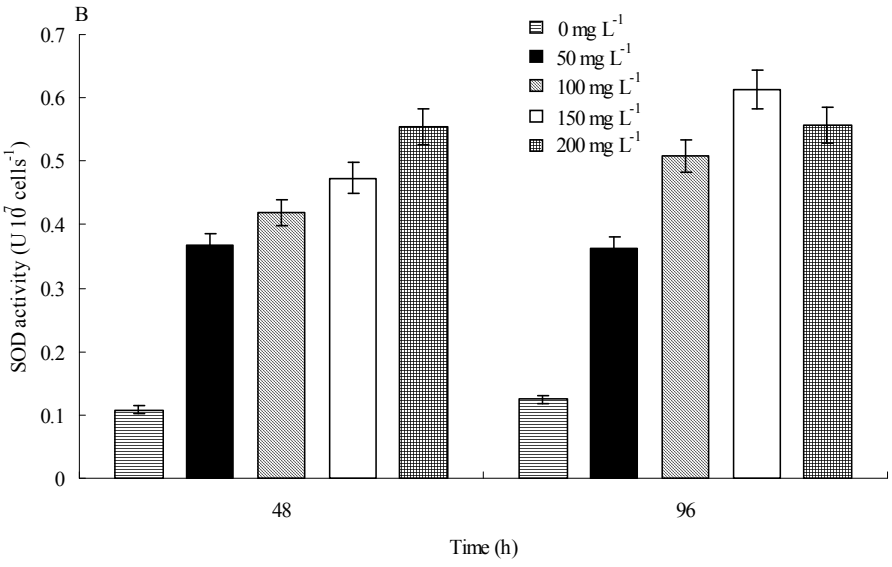


Figure 4. *Effects of Ba (A), Cr (B), Hg (C), and Pb (D) on intracellular malondialdehyde (MDA) content of Synechococcus sp. PCC7942 after 48 and 96 h of exposure. Points represent means of three replicates (n = 3); error bars represent standard deviations.*





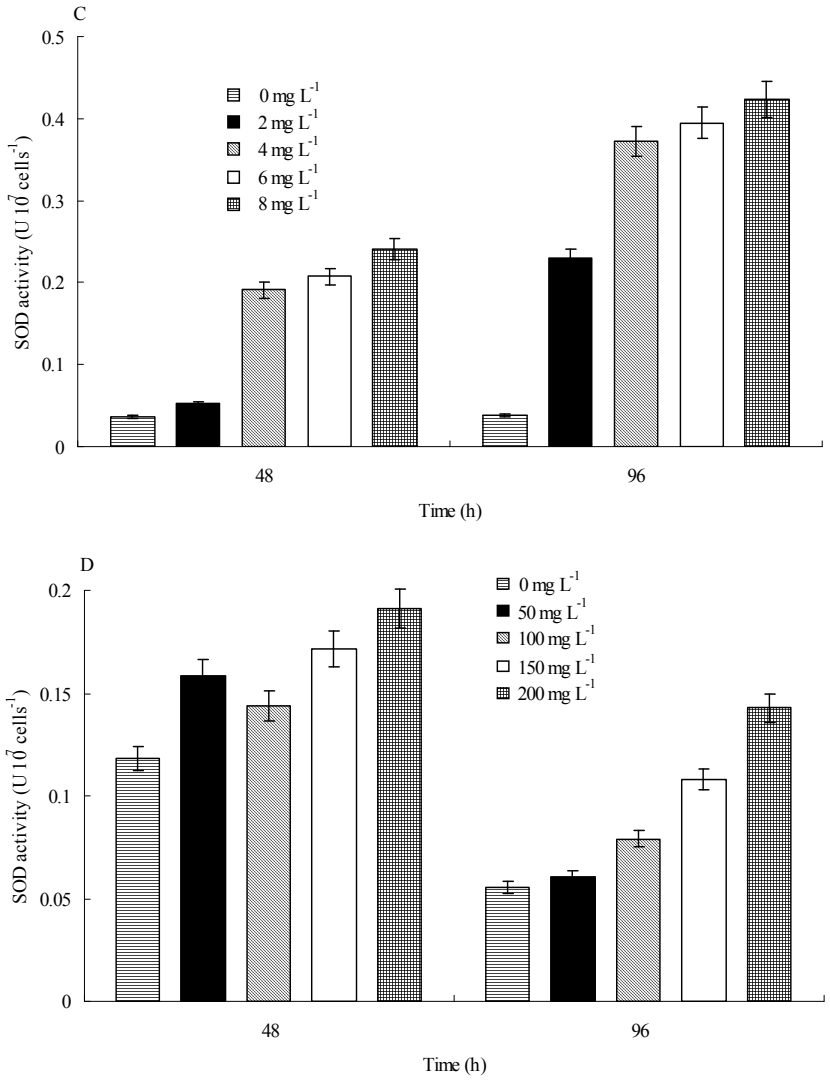


Figure 5. *Effects of Ba (A), Cr (B), Hg (C), and Pb (D) on the superoxide dismutase (SOD) activity of Synechococcus sp. PCC7942 after 48 and 96 h of exposure. Points represent means of three replicates (n = 3); error bars represent standard deviations.*

5. Conclusion

Cyanobacteria are generally considered as important primary producers in aquatic ecosystems. The freshwater cyanobacterium *Synechococcus* sp. PCC7942 was used to compare and evaluate the toxicity of Ba, Cr, Hg and Pb in this study. The growth, Chl *a* concentration and soluble polysaccharides content of *Synechococcus* sp. PCC7942 were significantly inhibited by Ba, Cr and Hg, but there are differences in Pb treatments (no significant at low concentration, but significant at high concentration). The observed growth inhibition of metals provides sufficient energy for the induction of protective measurements such as the synthesis of antioxidants. In addition, rapid and remarkable physiological responses occurred when the cyanobacterium was exposed to metal stress. General increase of MDA level suggests that the toxic effects of metals were probably exerted through free radical generation. And the increase of SOD activity may be viewed as an active defense against metal stress by stimulating the synthesis of proteins against stress and quenching of free radicals.

Acknowledgments

This manuscript was supported by the National Natural Science Foundation of China (No. 31200381), China Postdoctoral Science Foundation funded project (No. 2013M531370, 2014T70532), Key Laboratory for Ecological Environment in Coastal Areas, State Oceanic Administration (201209), and Jiangsu Planned Projects for Postdoctoral Research Funds (No. 1201063C).

References

[1] Ahmad, P., Jaleel, C.A., Salem, M.A., Nabi, G., Sharma, S., 2010. Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. Crit. Rev. Biotechnol. 30, 161-175.

[2] Atli, G., Canli, M., 2010. Response of antioxidant system of freshwater fish *Oreochromis niloticus* to acute and chronic metal (Cd, Cu, Cr, Zn, Fe) exposures. Ecotoxicol. Environ. Saf. 73, 1884-1889.

[3] Azevedo, M.M., Carvalho, A., Pascoal, C., Rodrigues, F., Cassio, F., 2007. Responses of antioxidant defenses to Cu and Zn stress in two aquatic fungi. Sci. Total Environ. 377, 233-243.

[4] Beyer, W., Imlay, J., Fridovich, I., 1991. Superoxide dismutases. Prog. Nucleic. Acid Res. Mol. Biol. 40, 221-253.

[5] Bidar, G., Garcon, G., Pruvot, C., Dewaele, D., Cazier, F., Douay, F., Shirali, P., 2007. Behavior of *Trifolium repens* and *Lolium perenne* growing in a heavy metal contaminated field: Plant metal concentration and phytotoxicity. Environ. Pollut. 147, 546-553.

[6] Boening, D.W., 2000. Ecological effects, transport, and fate of mercury: a general review. Chemosphere 40, 1335-1351.

[7] Cervantes, C., Campos-García, J., Devars, S., Gutiérrez-Corona, F., Loza-Tavera, H., Torres-Guzmán, J.C., Moreno-Sánchez, R., 2001. Interactions of chromium with microorganisms and plants. FEMS Microbiol. Rev. 25, 335-347.

[8] Choudhary, M., Jetley, U.K., Abash Khan, M., Zutshi, S., Fatma, T., 2007. Effect of heavy metal stress on proline, malondialdehyde, and superoxide dismutase activity in the cyanobacterium *Spirulina platensis*-S5. Ecotoxicol. Environ. Saf. 66, 204-209.

[9] Choudhury, S., Panda, S., 2005. Toxic effects, oxidative stress and ultrastructural changes in moss *Taxithelium nepalense* (Schwaegr.) Broth under chromium and lead phytotoxicity. Water Air Soil Poll. 167, 73-90.

[10] De, A.K., Sen, A.K., Modak, D.P., Jana, S., 1985. Studies of toxic effects of Hg(II) on *Pistia stratiotes*. Water Air Soil Poll. 24, 351-360.

[11] Delfino, G., Amerini, S., Mugelli, A., 1988. Barium cardiotoxicity: Relationship between ultrastructural damage and mechanical effects. Toxicol. In Vitro 2, 49-55.

[12] Foster, M.S., 1964. Microscopic algal food of *Littorina planaxis* Philippi and *Littorina scutulata* Gould (Gastropoda: Prosobranchia). Veliger 7, 149-152.

[13] Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48, 909-930.

[14] Han, T., Kang, S.H., Park, J.S., Lee, H.K., Brown, M.T., 2008. Physiological responses of *Ulva pertusa* and *U. armoricana* to copper exposure. Aquat. Toxicol. 86, 176-184.

[15] Hao, J., Kang, Z., Yu, Y., 2007. Experimental techniques of plant physiology. Chemical Industry Press, Beijing, pp. 68-72.

[16] Hegedüs, A., Erdei, S., Horváth, G., 2001. Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. Plant Sci. 160, 1085-1093.

[17] Hong, Y., Hu, H.Y., Xie, X., Sakoda, A., Sagehashi, M., Li, F.M., 2009. Gramine-induced growth inhibition, oxidative damage and antioxidant responses in freshwater cyanobacterium *Microcystis aeruginosa*. Aquat. Toxicol. 91, 262-269.

[18] Janero, D.R., 1990. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic. Biol. Med. 9, 515-540.

[19] Kumar, S., Habib, K., Fatma, T., 2008. Endosulfan induced biochemical changes in nitrogen-fixing cyanobacteria. Sci. Total Environ. 403, 130-138.

[20] Lee, L.H., Lustigman, B., 1996. Effect of barium and nickel on the growth of *Anacystis nidulans*. B. Environ. Contam. Tox. 56, 985-992.

[21] Li, H.S., 2000. Principles and techniques of plant physiological biochemical experiment. Higher Education Press, Beijing, pp. 194-197.

[22] Malanga, G., Puntarulo, S., 1995. Oxidative stress and antioxidant content in *Chlorella vulgaris* after exposure to ultraviolet-B radiation. Physiol. Plant 94, 672-679.

[23] Mallick, N., 2004. Copper-induced oxidative stress in the chlorophycean microalga *Chlorella vulgaris*: response of the antioxidant system. J. Plant Physiol. 161, 591-597.

[24] Mellado, M., Contreras, R.A., Gonzalez, A., Dennett, G., Moenne, A., 2012. Copper-induced synthesis of ascorbate, glutathione and phytochelatins in the marine alga *Ulva compressa* (Chlorophyta). Plant Physiol. Biochem. 51, 102-108.

[25] Panda, S.K., Choudhury, S., 2005. Changes in nitrate reductase activity and oxidative stress response in the moss *Polytrichum commune* subjected to chromium, copper and zinc phytotoxicity. Braz. J. Plant Physiol. 17, 191-197.

[26] Perry, H.M., Jr., Kopp, S.J., Perry, E.F., Erlanger, M.W., 1989. Hypertension and associated cardiovascular abnormalities induced by chronic barium feeding. J. Toxicol. Environ. Health 28, 373-388.

[27] Piechalak, A., Tomaszewska, B., Baralkiewicz, D., Malecka, A., 2002. Accumulation and detoxification of lead ions in legumes. Phytochemistry 60, 153-162.

[28] Qian, H., Chen, W., Sheng, G.D., Xu, X., Liu, W., Fu, Z., 2008. Effects of glufosinate on antioxidant enzymes, subcellular structure, and gene expression in the unicellular green alga *Chlorella vulgaris*. Aquat. Toxicol. 88, 301-307.

[29] Quinn, G.P., 1988. Ecology of intertidal pulmonate limpet *Siphonaria diemenesis* Quoy et Gaimard. I. Population dynamics and availability of food. J. Exp. Mar. Biol. Ecol. 117, 115-136.

[30] Rai, U.N., Tripathi, R.D., Kumar, N., 1992. Bioaccumulation of chromium and toxicity on growth, photosynthetic pigments, photosynthesis, in vivo nitrate reductase activity and protein content in a chlorococcalean green alga *Glaucocystis nostochinearum* Itzigsohn. Chemosphere 25, 1721-1732.

[31] Rodríguez, M.C., Barsanti, L., Passarelli, V., Evangelista, V., Conforti, V., Gualtieri, P., 2007. Effects of chromium on photosynthetic and photoreceptive apparatus of the alga *Chlamydomonas reinhardtii*. Environ. Res. 105, 234-239.

[32] Sabatini, S.E., Juarez, A.B., Eppis, M.R., Bianchi, L., Luquet, C.M., Rios de Molina Mdel, C., 2009. Oxidative stress and antioxidant defenses in two green microalgae exposed to copper. Ecotoxicol. Environ. Saf. 72, 1200-1206.

[33] Stanier, R.Y., Kunisawa, R., Mandel, M., Cohen-Bazire, G., 1971. Purification and properties of unicellular blue-green algae (order *Chroococcales*). Bacteriol. Rev. 35, 171-205.

[34] Stohs, S.J., Bagchi, D., 1995. Oxidative mechanisms in the toxicity of metal ions. Free Radic. Biol. Med. 18, 321-336.

[35] Tabaldi, L.A., Ruppenthal, R., Cargnelutti, D., Morsch, V.M., Pereira, L.B., Schetinger, M.R.C., 2007. Effects of metal elements on acid phosphatase activity in cucumber (*Cucumis sativus* L.) seedlings. Environ. Exp. Bot. 59, 43-48.

[36] Tripathi, B.N., Mehta, S.K., Amar, A., Gaur, J.P., 2006. Oxidative stress in *Scenedesmus* sp. during short- and long-term exposure to Cu^{2+} and Zn^{2+}. Chemosphere 62, 538-544.

[37] Van Assche, F., Clijsters, H., 1990. Effects of metals on enzyme activity in plants. Plant Cell Environ. 13, 195-206.

[38] Wang, Y., Jin, H., Deng, S., Chen, Y., Yu, Y., 2011. Effects of neodymium on growth and physiological characteristics of *Microcystis aeruginosa*. J. Rare Earth. 29, 388-395.

[39] Wong, P.K., Chang, L., 1991. Effects of copper, chromium and nickel on growth, photosynthesis and chlorophyll a synthesis of *Chlorella pyrenoidosa* 251. Environ. Pollut. 72, 127-139.