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Bioaccumulation of Pb^{2+} and Its Effects on Growth, Morphology and Pigment Contents of *Spirulina* (*Arthrospira*) *platensis*

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Abstract A laboratory experiment was conducted to assess the bioaccumulation of Pb^{2+} and its effects on growth, morphology and pigment contents of *Spirulina (Arthrospira) platensis*. The specimen cultured in Zarrouk liquid medium was treated with various initial metal concentrations (0, 5, 10, 30, 50 and 100 $\mu g\,mL^{-1}$). The growth of *S. platensis* was adversely affected by Pb^{2+} at high concentrations (30, 50 and 100 $\mu g\,mL^{-1}$). However, at low concentrations (5 $\mu g\,mL^{-1}$), Pb^{2+} could stimulate its growth slightly. The pigment contents (chlorophyll α and β carotene) were decreased in a dose-dependent manner. The highest reductions (67% and 53% respectively in chlorophyll α and β carotene) were observed in 100 $\mu g\,mL^{-1}$ treatment group. The LC_{50} (96 h) of Pb^{2+} was measured as 75.34 $\mu g\,mL^{-1}$. Apart from a few cases of filament breakages at elevated concentrations (50 and 100 $\mu g\,mL^{-1}$), morphological abnormalities are not specific. Metal bioaccumulation increased with Pb^{2+} concentrations, but decreased with exposure time. The maximum accumulated amount was 188 $mg\,g^{-1}$ dry weight. The bioconcentration factor (BCF) reached to a peak at day 2, followed by a gradual reduction for all the exposure concentrations. *S. platensis* is able to tolerate considerably high Pb^{2+} concentrations. Consequently it can be used as a potential species to remove heavy metal from contaminated waters.

Key words bioaccumulation; growth; Pb^{2+}; *Spirulina* (*Arthrospira*) *platensis*

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1 Introduction

Biological degradation of heavy metals is hazardous and thus their presence in aquatic environment is of primly concern (Arias *et al*., 2002). In particular, the bioaccumulation of heavy metals in aquatic food chains is highly dangerous. Cyanobacteria, a numerous and diverse group of photosynthetic prokaryotes largely responsible for global photosynthetic productivity (Ting *et al*., 2002), are excellent models for bioaccumulation studies. During the evolution, these organisms have developed diverse strategies to maintain an equilibrated relation with heavy metal ions in the surrounding medium (Perales-Vela *et al*., 2006). The toxicity of heavy metals, however, may result in diverse effects, which depend on types of algae, features and concentrations of the metal, and the environmental conditions (Heng *et al*., 2004; Satoh *et al*., 2005; Greger *et al*., 2007).

Metal phytotoxicity can result in pigment degradation, nutrient imbalance, enhancement of antioxidant enzymes activity and induction of oxidative stress in plants and algae (Cervantes *et al*., 2001; Panda and Choudhury, 2005). In fact, growth inhibition and chlorosis are common symptoms of metal toxicity, in which photosynthesis is probably the most affected metabolic process (Ali *et al*., 2006). Alterations in morphology and ultrastructure have also been frequently reported (Choudhury and Panda, 2005). However, different organisms proved to show various sensitivities to the same metal, while the toxicity of different metals to the same organism may also vary (Fathi, 2002).

Spirulina, a filamentous cyanobacterium, is globally concerned as a multi-nutritional source of healthy food, feed and pharmaceutical products (Hirata *et al*., 2000; Liu *et al*., 2000). Due to its high economic value, the species has been cultured in mass-scale in China (Zhang, 1998; Michael, 1999) and in some other countries. *Spirulina* sp. is known to have versatile metabolic modes. It can grow either photoautotrophically, heterotrophically or mixotrophically (Chojnacka and Noworyta, 2004). The cell wall of *Spirulina* is surrounded by a porous three-dimensional macromolecular network (Chojnacka *et al*., 2005) and consists of peptydoglycan, teichuronic acid, teichoic acid, polysaccharides and proteins (Schiewer and Wong, 2000), which display carboxylic, hydroxyl and phosphate groups (Aksu, 2002; Markai *et al*., 2003). The presence of anionic and cationic sites gives algal wall amphoteric properties and, depending on the pH, the groups are either

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protonated or deprotonated (Esposito *et al*., 2002). Chojnacka and Mowoyta (2001) reported that *Spirulina platensis* was able to accumulate cadmium. Chojnacka *et al*. (2005) studied heavy metal biosorption equilibrium of *Spirulina* in the absence of metabolic processes. Rangsayatorn *et al*. (2004) reported that its metal adsorption rate was rapid. And the bioremediation potential of *Spirulina platensis* was also assessed by Chen and Pan (2005).

The present laboratory study was performed in order to assess the bioaccumulation potential of lead by the cyanobacterium *Spirulina (Arthrospira) platensis* and its effects on growth, morphology and pigment contents of the species.

2 Materials and Methods

2.1 Strains and Culture Conditions

The cyanobacterum *Spirulina platensis* strain 6-1 preserved in our laboratory at Life Science College, Ocean University of China, was used for the study. The species was cultured in Zarrouk liquid medium (Parada *et al*., 1998) adjusted to pH 7.0 at 25℃ ± 1℃. The cultures were gently stirred and illuminated with white light produced by neon tubes at 50 mmol photon m^{-2} s^{-1} with a light/dark cycle of 14:10 h. At the late exponential growth phase, the cultures were filtered through muslin cloth and the pellets were resuspended in fresh medium for use in the metal treatment experiments.

2.2 Chemicals and Analytical Methods

Analytical grade chemicals were used throughout the experiments without further purification. A stock solution of lead in 1000 μg mL^{-1} was prepared using $Pb(NO_3)_2$ with de-ionized water obtained from a Millipore Milli-Q system. A spectrophotometer (UV-2102) was used to measure optical density. Lead concentrations in the medium were measured using an Atomic Absorption Spectrophotometer (PGENERAL, TAS-986). All measurements of weight were performed using a digital balance (Sartorins, BS 210 S).

2.3 Lead Accumulation Studies

Assay of the effect of Pb^{2+} was performed in 150 mL Erlenmeyer flasks. Algal cells were inoculated into fresh 100 mL Zarrouk medium supplemented with various initial metal concentrations (5, 10, 30, 50 and 100 μg mL^{-1}). The algal cells without Pb^{2+} in the medium served as the controls. Suspensions were continuously homogenized in a rotary shaker at 100 r min^{-1} and cultured for 10 d under the conditions as described above. The initial OD (at 560 nm) was set to 0.1 and the pH was adjusted to 7 ± 0.1 with 0.1 mol L^{-1} HNO_3 or NaOH to minimize Pb^{2+} precipitation. At regular intervals (once in two days) aliquots of 5 mL from each suspension were withdrawn and algal biomass was separated by centrifugation at 10000 r min^{-1} for 10 min to determine the total dissolved Pb^{2+} concentration in the medium.

2.4 Growth of Microalgae

The growth of the algae was monitored turbidimetrically at 560 nm. At the end of the incubation period, the cultures were subjected to pigment content analysis. Chlorophyll α and β carotene were extracted in 90 % acetone and assayed with the method of Ben-Amotz and Avron (1983).

2.5 Accumulated Amount and Bioconcentration Factor (BCF)

Accumulated amount (q) of Pb^{2+} (mg g^{-1} - dry weight) was calculated using the simple concentration difference method (Volesky and Holan, 1995)

$$q = (C_0 - C_t)V / 1000 \cdot W,$$

where q is the accumulated amount (mg g^{-1}); C_0 is the initial concentration of Pb^{2+} in the medium (mgL^{-1}); C_t is Pb^{2+} concentration at a given time t (mgL^{-1}); V is the total volume of suspension (L); W is the dry weight of *Spirulina* (g).

The bioconcentration factor (BCF) was used to assess heavy metal removal ability of the species. It was calculated as the ratio between the accumulated amount, which included Pb^{2+} adhered to the cell-wall plus accumulated Pb^{2+} in the cells, and the Pb^{2+} concentration in the solutions. In order to measure the dry weight, algal cells were filtered through muslin cloth, washed 3 times with deionized water, and dried at 103 ℃ for 2 h. The linear relationships, derived as dry weight (gL^{-1}) = 0.207 + 0.960 x OD_{560} nm (r = 0.9927), was used to estimate algal dry weight at relevant OD values. All the procedures were performed under aseptic conditions and the treatments were triplicated.

3 Results

3.1 Growth

The algal growth results revealed that *Spirulina* could tolerate low Pb^{2+} concentration (5 μg mL^{-1}), resulting in 3.7 % growth stimulation at 10 d. However, the species could no longer withstand higher lead concentrations. The growth inhibitions at 10 d were 5, 40, 49 and 78 % respectively for Pb^{2+} at 10, 30, 50 and 100 μg mL^{-1} (Fig.1).

3.2 Pigment Contents

Fig.2 shows chlorophyll α and β carotene contents at different Pb^{2+} concentrations. The pigment contents were decreased in a dose-dependent manner. The highest reductions (67 % and 53 % respectively in chlorophyll *a* and β carotene) were observed at the Pb^{2+} exposure group of 100 μg mL^{-1}, whereas the reductions in the chlorophyll *a* content in the cultures exposed to Pb^{2+} at 50, 30 and 10 μg mL^{-1} were 34%, 15% and 4% respectively .The corre-

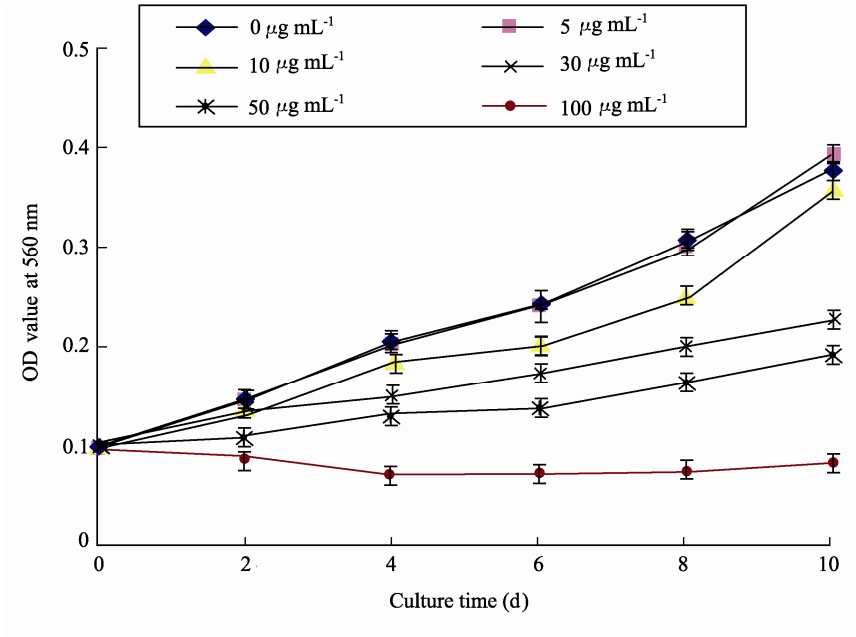

Fig.1 The effect of different concentrations of Pb^{2+} (μg mL^{-1}) on growth of *S. platensis* (S_{6-1}) cultured in Zarrouk medium (pH 7.0) under an illumination intensity of 50 mmol photon m^{-2} s^{-1} at 25℃ ± 1℃ (mean ± SD, n = 3).

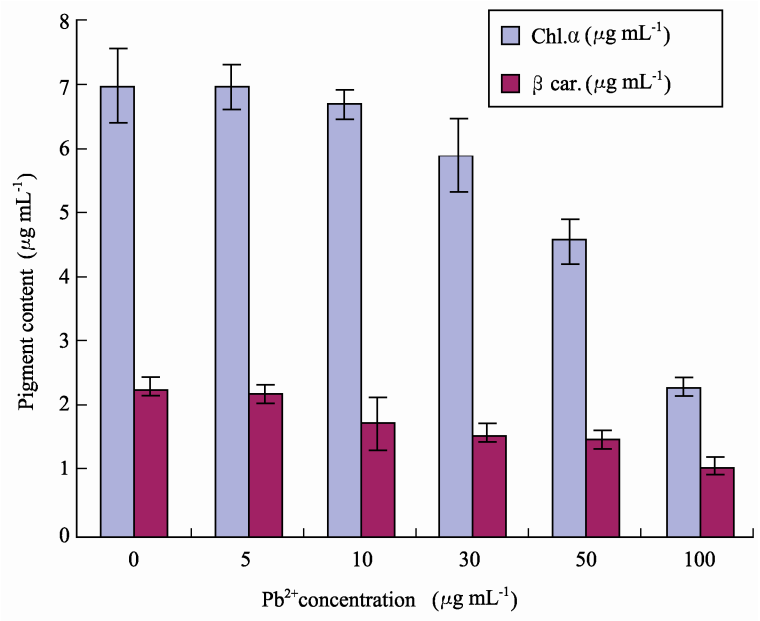

Fig.2 The effect of different concentrations of Pb^{2+} (μg mL^{-1}) on pigment content (μg mL^{-1}) of *S. platensis* (S_{6-1}) cultured in Zarrouk medium (pH 7.0) under an illumination intensity of 50 mmol photon m^{-2} s^{-1} at 25℃ ± 1℃ (mean ± SD, n = 3, Chl. α – chlorophyll α; β car. –β carotene).

sponding reductions in β carotene content were 34%, 31% and 23%.

3.3 Toxicity

The toxicity of Pb^{2+} expressed as lethal concentration (LC_{50}) was calculated as 75.34 μg mL^{-1} with 95% confidence limits of 97.05 and 58.47 μg mL^{-1}, indicating a considerable tolerance of *S. platensis* to Pb^{2+} toxicity. Microscopic observations revealed a few crumbled and severely damaged filaments (Fig.3) at elevated Pb^{2+} concentrations. However, the symptoms were not specific and could be attributed to the increased Pb^{2+} concentration in the medium.

3.4 Accumulated Amount and Bioconcentration Factor

Accumulated amounts of Pb^{2+} at different concentrations are shown in Table 1. Metal accumulation increased with metal concentrations in the medium and decreased with exposure time. However, the Pb^{2+} concentration in the medium remained almost unchanged after 2 d of incubation (Table 2).

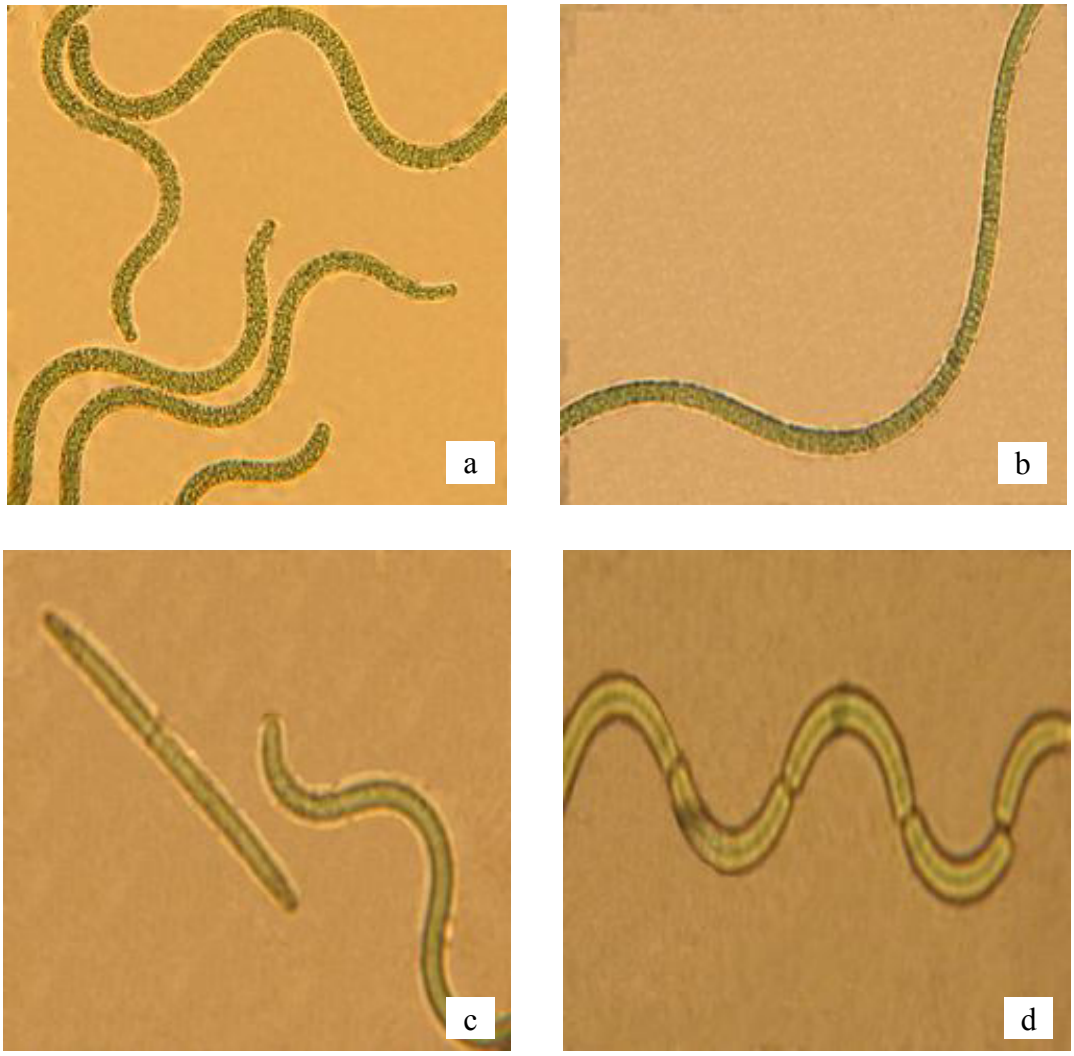

Fig.3 Light micrographs (10× 30) of *S. platensis* ($S_{6\text{-}1}$) filaments (a) control cells (b), (c) and (d) loosing the spiral shape, straightening and breaking the filament respectively, in response to Pb^{2+} toxicity.

Table 1 The changes in accumulated amount of Pb^{2+} (μg mg^{-1}) in *S. platensis* ($S_{6\text{-}1}$) cultured in Zarrouk medium (pH 7.0) under an illumination intensity of 50 mmol photon m^{-2} s^{-1} at 25℃ ± 1℃†

Culture time (d)	Accumulated amount of Pb^{2+} (μg mg^{-1}) ††				
	Treatment (μg mL^{-1})				
	5	10	30	50	100
2	5.32 ± 0.27	9.58 ± 1.05	35.88 ± 2.68	67.80 ± 2.71	188.32 ± 5.13
4	4.35 ± 0.57	8.60 ± 0.97	26.79 ± 2.30	57.05 ± 3.48	156.30 ± 4.25
6	3.98 ± 0.79	8.28 ± 0.82	24.57 ± 2.45	53.93 ± 2.99	152.21 ± 5.06
8	3.18 ± 0.83	7.15 ± 0.99	22.44 ± 3.12	45.10 ± 3.96	154.48 ± 2.45
10	2.66 ± 0.35	6.92 ± 0.61	19.32 ± 1.88	45.05 ± 2.00	145.07 ± 4.77

Note: † Mean±SD, n = 3. ††Accumulated amount refers to the Pb^{2+} bound in the cell wall plus accumulated Pb^{2+} in the cells.

Table 2 The changes in Pb^{2+} concentration (μg mL^{-1}) in the medium†

Initial Pb^{2+} Con. (μg mL^{-1})	Residual metal concentration in the medium (μg mL^{-1}) ± SD				
	Culture time (d)				
	2	4	6	8	10
5	3.27 ± 0.11	3.34 ± 0.05	3.36 ± 0.11	3.43 ± 0.12	3.44 ± 0.07
10	6.50 ± 0.11	6.74 ± 0.16	6.68 ± 0.11	6.79 ± 0.01	6.83 ± 0.08
30	18.77 ± 0.75	21.40 ± 0.95	21.55 ± 0.83	21.05 ± 1.31	21.79 ± 0.95
50	29.12 ± 1.53	32.20 ± 1.27	33.39 ± 1.74	31.95 ± 0.87	32.75 ± 1.18
100	42.75 ± 1.63	44.70 ± 1.90	44.70 ± 0.94	44.85 ± 1.10	44.45 ± 1.44

Note: † Mean±SD, n = 3.

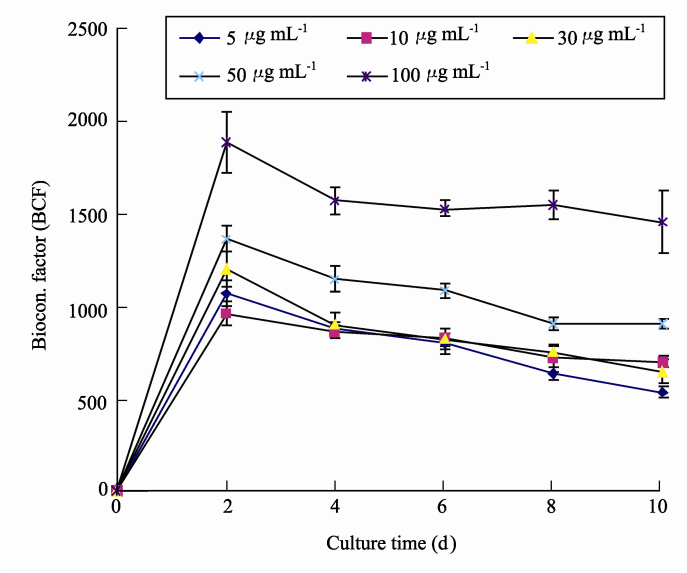

Fig.4 The changes in Bioconcentration Factor (BCF) of Pb^{2+} for *S. platensis* ($S_{6\text{-}1}$) cultured in Zarrouk medium (pH 7.0) under an illumination intensity of 50 mmol photon m^{-2} s^{-1} at 25 ℃ ± 1 ℃ (mean ± SD, n = 3).

Fig.4 shows the changes in the bioconcentration factor of Pb^{2+}. The BCF for all treatments reached a peak at 2 d (1064, 958, 1196, 1356 and 1883 respectively for 5, 10, 30, 50 and 100 μg mL^{-1} of Pb^{2+}). The BCF also decreased with incubation time as shown in the accumulated amount.

4 Discussion

Growth inhibition in microalgae is well known for metal toxicity and found to be related to the amount of metal bound to the algal cell surface in some cases, to the amount of intracellular metal (Franklin *et al*., 2000, 2001; Ma *et al*., 2003) and to the chemical nature of the metal (Tripathi and Gaur, 2006). The growth of *Spirulina* was severely inhibited by Pb^{2+} at high concentrations. This is in agreement with the results of Lamaia *et al*. (2005), who reported that the relative growth of *Cladophora fracta* decreased significantly when metal concentrations (Pb^{2+} and Cd^{2+}) were increased. The dose-dependent manner of growth inhibition as observed in the present study, was also reported by Fargasova (1999), Fathi *et al*. (2000) and Fathi (2002). Several reasons could contribute to the growth inhibition, including a possible degradation of indole acetic acid (IAA) in the algal tissues. In spite of the stimulatation of this hormone to the plant growth and multiplication, the presence of Pb^{2+} in the growth medium could induce the activity of the peroxidase enzyme that involves the degradation of IAA. Stimulation of chlorophyllase activity, disorderness of membrane system and inactivation of electron transport functions in the photosystem could also be involved in the growth inhibition. We observed slight growth stimulation effects at 5 μg mL^{-1} of Pb^{2+}, which is in agreement with the views of El-Enany and Issa (2000), who reported a significant stimulation of growth in *Nostoc linckia* and *Nostoc rivularis* at low waste treatments containing 0.4 and 1.6 μg mL^{-1} of Cd^{2+} and Zn^{2+} respectively. Substitution of heavy metals for Zn^{2+} in some metabolloenzymes *in vitro* and *in vivo* could be the possible reason for growth promotion at low metal concentrations (El-Sheekh *et al*., 2003).

Heavy metals can cause membrane depolarization and acidification of the cytoplasm (Cardozo *et al*., 2002; Conner and Schimid, 2003). In fact, membrane injury is one important effect of metal ions that may lead to disruption of cellular homeostasis. A chain of metabolic events, beginning with the respiration and photosynthesis and continuing with uptake and assimilation of nutrients, dilution of intracellular level of the heavy metals, *etc*., is known to be important in keeping the balance of cellular homeostasis (Tripathi and Gaur, 2006). Some metals (Pb^{2+} and Cd^{2+}) at higher concentrations can destroy the chloroplasts (Lamaia *et al*., 2005) and thylakoid membranes (Heng *et al*., 2004). Morphological changes, particularly, membrane injuries were proved to be common in cyanobacterial response to metal toxicity (Rangsayatorn *et al*., 2002). In fact, metals are capable of binding to thylakoid membranes, deteriorating their routing functions (Heng *et al*., 2004), which could eventually decrease the pigment biosynthesis. Atri and Rai (2003) reported that the biosynthesis of carotenoid was affected by heavy metals. Among the physiological mechanisms suggested for tolerance or resistance to and accumulation capability of heavy metals by algae are: metallic complex formation with metabolites produced and/or exuded by algae (Lombardi and Vieira, 2000); adsorption, immobilization, and precipitation of toxic metals in extracellular and cellular compartments such as cell walls, phosphate-rich granules, lipid bodies, vacuoles, nucleus, and physodes (Amado Filho *et al*., 1999; Andrade *et al*., 2002); metal binding in specific organic-metallic compounds such as metalloproteins and phytochelatins (Lombardi and Vieira, 2000).

Metal ions are adsorbed first to the cell surface by in-

teractions with metal-functional groups such as carboxyl, phosphate, hydroxyl, amino, sulphur, sulphide, thilo, *etc*., and then penetrate the cell membrane and enter into the cells (Wang and Chen, 2006). Complexation, ion exchange, adsorption, inorganic microprecipitation, oxidation and/or reduction have all been proposed to explain the metal uptake process (Liu *et al*., 2002). In the case of cyanobacteria, ion exchange is known to be the major mechanism (Chojnacka *et al*., 2005). We observed a rapid metal removal during the first two days of incubation, which could be the energy-independent metal ion exchange stage as reported by Sloof *et al*. (1995) for cadmium uptake by living *Selenastrum capricornutum*. The continued slow removal from day 2 onwards could be the physiological active uptake stage. In general, accumulated amounts of Pb^{2+} proved to be high. Lamaia *et al*. (2005) reported that *C. fracta* could accumulate 61400 mg g^{-1} of Pb^{2+} when exposed to 80 μg mL^{-1}. *Anabaena flosaquae* was able to accumulate 70 mg g^{-1} of Pb^{2+} at the exposure of 1μg mL^{-1} (Heng *et al*., 2004). The maximum accumulated amount of Pb^{2+} in the present study was 188 mg g^{-1} at the exposure of 100μg mL^{-1}. It could be suggested that the deviations among the results may be due to the species differences under consideration, culture conditions and exposure time as mentioned by Inthorn *et al*. (2002). In fact, metal accumulation by algae is influenced by a number of abiotic (*e.g*., pH, chelating agents, redox potential, temperature, light) and biotic (*e.g*., cellular activity, algal biomass concentrations, extra cellular products) factors (Fathi and Omair, 2006). BCF is frequently used as a reliable parameter to assess the metal-accumulating ability of plants (Raskin *et al*., 1994). The accumulated amounts and BCF in the present study were shown to be increased with metal concentrations in the medium, but decreased with exposure time. This dose-dependent accumulation pattern is in agreement with Pawlik (2000), who studied Pb^{2+} uptake by *Stichococcus bacillaris*, and with Lamaia *et al*. (2005), who studied Pb^{2+} and Cd^{2+} accumulation by C*ladophora fracta*. As documented by Yan and Pan (2002), a possible reason for the decreased accumulation amounts and BCF with exposure time may be the gradual recovery of growth. In addition, many cyanobacteria can produce extra-cellular ligands in response to metal stress (Moffett and Brand, 1996) and these ligands dilute the metal toxicity, resulting in reduced accumulation amounts and BCF as incubation continued.

From the view of phytoremediation, a good accumulator should possess a BCF of more than 1 000 (Zayed *et al*., 1998). Based on this criterion, *Spirulina platensis* seems a good accumulator of metals as it showed the highest BCF value of 1883 for Pb^{2+}. However, the practical viability must be confirmed by further field studies. Furthermore, our results proved that the species has a considerably high (75.34 μg mL^{-1} of Pb^{2+}) lethal concentration (LC_{50}), indicating the potential capability of *Spirulina platensis* to be cultured even in Pb^{2+} contaminated waters.

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References

Aksu, Z., 2002. Determination of the equilibrium, kinetic and thermodynamic parameters of the batch biosorption of nickel (II) ions onto *Chlorella vulgaris*. *Proc. Biochem*., **38**: 89- 99.

Ali, N. A., D. Dewez, O. Didur, and R. Popovic, 2006. Inhibition of photosystem II photochemistry by Cr is caused by alteration of both D1 protein and oxygen evolving complex. *Photosynth. Res*., **89**: 81-87.

Amado Filho, G. M., L. R. Andrade, C. S. Karez, M. Farina, and W. C. Pfeiffer, 1999. Brown algae species as biomonitors of Zn and Cd at Sepetiba bay, Rio de Janeiro, Brazil. *Mar. Environ. Res*., **48**: 213-224.

Andrade, L. R., M. Farina, and G. M. Amado Filho, 2002. Role of cell walls of *Padina gymnospora* (Phaeophyta) in Cd accumulation. *Phycologia*, **41**(1): 39-48.

Arias, M., M. T. Barral, and J. C. Mejuto, 2002. Enhancement of copper and cadmium adsorption on kaolin by the presence of humic acids. *Chemosphere*, **48**: 1081-1088.

Atri, N., and L. C. Rai, 2003. Differential responses of three cyanobacteria to UV-B and Cd. *J. Microbiol. Biotech*., **13**: 544-551.

Ben-Amotz, A., and M. Avron, 1983. On the factors which determine massive (3-carotene accumulation in the halotolerant alga *Dunaliella bardawil*. *Plant Physiol*., **72**: 593-597.

Cardozo, K. H. M., M. A. L. Oliveira, M. F. M. Tavares, P. Colepicolo, and E. Pinto, 2002. Daily oscillation of fatty acids and malondialdehyde in the dinoflagellate *Lingulodinium polyedrum*. *Biol. Rhythm Res*., **33**: 371-381.

Cervantes, C., J. Campos-Garcıa, S. Devars, F. Gutierrez-Corona, H. Loza-Tavera, J. C. Torres-Guzman, *et al*., 2001. Interactions of chromium with microorganisms and plants. *FEMS Microbiol. Rev*., **25**: 335-347.

Chen, H., and S. Pan, 2005. Bioremediation potential of *Spirulina*: toxicity and biosorption studies of lead. *J. Zhejiang Univ. Sci*., **6B** (3): 171-174.

Chojnacka, K., A. Chojnacki, and H. Go′recka, 2005. Biosorption of Cr^{3+}, Cd^{2+} and Cu^{2+} ions by blue–green algae *Spirulina sp*.: kinetics, equilibrium and the mechanism of the process. *Chemosphere*, **59**: 75-84.

Chojnacka, K., and A. Mowoyta, 2001. Mechanism of heavy metal ion biosorption by a blue-green alga *Spirulina* species. *Inzynieria Chemiczne Procesowa Poland*, **22**: 331-336,

Chojnacka, K., and A. Noworyta, 2004. Evaluation of *Spirulina* sp. growth in photoautotrophic, heterotrophic and mixotrophic cultures. *Enzyme Microb. Technol*., **34** (5): 461-465.

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

Conner, S. D., and S. L. Schimid, 2003. Regulated portals of entry into the cell. *Nature*, **422**: 37-44.

El-Enany, A.E., and A. A. Issa, 2000. Cyanobacteria as a biosorbent of heavy metals in sewage water. *Environ. Toxicol. Phar.*, **8**: 95-101.

El-Sheekh, M. M., M. E. El-Naggar, Osman, and E. El-Mazaly, 2003. Effect of cobalt on growth, pigments and the photosynthetic electron transport in *Monoraphdium minutum* and *Nitzchia perminuta*. *Braz. J. Plant Physiol*., **15** (3): 159-166.

Esposito, A., F. Paganelli, and F. Veglio, 2002. pH related equilibria models for biosorption in single metal systems. *Chem. Eng. Sci*., **57**: 307-313.

Fargasova, A., 1999. The green alga *Scenedesmus quadricauda,* a subject for the study of inhibitory effects of Cd, Cu, Zn, Pb and Fe. *Biologia*, **54**: 393-398.

Fathi, A. A., 2002. Toxicological response of the green alga *Scenedesmus bijuga* to mercury and lead. *Folia Microbiol*., **47**: 667-671.

Fathi, A. A., and M. A. Al-Omair, 2006. Effects of pH on toxicity of cadmium, cobalt and copper to *Scenedesmus bijuga*. *Protistology*, **4** (3): 221-226.

Fathi, A. A., F. T. Zaki, and A. A. Fathy, 2000. Bioaccumulation of some heavy metals and their influence on the metabolism of *Scenedesmus bijuga* and *Anabaena spiroides*. *Egypt. J. Biotechnol*., **7**: 293-307.

Franklin, N. M., M. S. Adams, J. L. Stauber, and R. P. Lim, 2001. Development of a rapid enzyme inhibition bioassay with marine and freshwater microalgae using flowcytometry. *Arch. Environ. Contam. Toxicol*., **40**: 469-480.

Franklin, N. M., J. L. Stauber, S. J. Markich, and R. P. Lim, 2000. pH-dependent toxicity of copper and uranium to a tropical freshwater alga (*Chlorella* sp.). *Aquat. Toxicol*., **48**: 275-289.

Greger, M., T. Malm, and L. Kautsky, 2007. Heavy metal transfer from composted macroalgae to crops. *Europ. J. Agronomy*, **26**: 257-265.

Heng, L. Y., K. Jusoh, C. H. M. Ling, and M. Idris, 2004. Toxicity of single and combinations of lead and cadmium to the cyanobacteria *Anabaena flos-aquae*. *Bull. Environ. Contam. Toxicol*., **72**: 373-379.

Hirata, T., M. Tanaka, M. Ooike, T. Tsunomura, and M. Sakaguchi, 2000. Antioxidant activities of phycocyanin prepared from *Spirulina platensis*. *J. Appli. Phycol*., **12**: 435-439.

Inthorn, D., N. Sidtitoon, S. Silapanuntakul, and A. Incharoensakdi, 2002. Sorption of mercury, cadmium and lead by microalgae. *Science Asia*, **28**: 253-261.

Lamaia, C., M. Kruatrachuea, P. Pokethitiyooka, E. S. Upathamb, and V. Soonthornsarathoola, 2005. Toxicity and accumulation of lead and cadmium in the filamentous green alga C*ladophora fracta* (O.F. Muller ex Vahl) Kutzing: A laboratory study. *Science Asia*, **31**: 121-127.

Liu, R. X., H. X. Tang, and W. X. Lao, 2002. Advances in biosorption mechanism and equilibrium modeling for heavy metals on biomaterials. *Prog. Chem*., **14**: 87-92 (in Chinese).

Liu, Y., X. Lizhi, N. Cheng, L. Lin, and C. Zhang, 2000. Inhibitory effect of phycocyanin from *Spirulina platensis* on the growth of human leukemia K562 cells. *J. Appl. Phycol*., **12**: 125-130.

Lombardi, A. T., and A. A. H. Vieira, 2000. Copper complexation by cyanophyta and chlorophyta exudates. *Phycologia*, **39** (2): 118-125.

Ma, M., W. Zhu, Z. Wang, and G. J. Witkaamp, 2003. Accumulation, assimilation and growth inhibition of copper on freshwater alga (*Scenedesmus subspicatus* 86.81 SAG) in the presence of EDTA and fulvic acid. *Aquat. Toxicol*., **63**: 221-228.

Markai, S., Y. Andres, G. Montavon, and B. Grambow, 2003. Study of the interaction between europium(III) and *Bacillus subtilis*: fixation sites, biosorption modelling and reversibility. *J. Colloid Interface Sci*., **262**: 351-361.

Michael, A. B., 1999. Commercial production of microalgae: ponds, tanks, tubes and fermenters. *J. Biotech*., **70**: 313- 321.

Moffett, J. W., and L. E. Brand, 1996. Production of strong extracellular Cu chelators by marine cyanobacteria in response to Cu stress. *Limnol. Oceanog*., **41:** 388-395.

Panda, S. K., and S. Choudhury, 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.

Parada, J. L., G. Z. de Caire, M. C. Z. de Mule, and M. M. S. de Cano, 1998. Lactic acid bacteria growth promoters from *Spirulina platensis*. *Int. J. Food Microbiol*., **45**: 225-228.

Pawlik, B., 2000. Relationships between acid-soluble thiol peptides and accumulated Pb in the green alga *Stichococcus bacillaris*. *Aquat. Toxicol*., **50**: 221-30.

Perales-Vela, H. V., J. Pen˜a-Castro, and R. O. Can˜izares-Villanueva, 2006. Heavy metal detoxification in eukaryotic microalgae. *Chemosphere*, **64**: 1-10.

Rangsayatorn, N., E. Upatham, S. M. Kruatrachue, P. Pokethitiyook, and G. R. Lanza, 2002. Phytoremediation, Potential of *Spirulina* (*Arthrospira*) *platensis*: biosorption and toxicity studies of cadmium. *Environ. Pollut*., **119**: 45-53.

Rangsayatorn, N., P. Pokethitiyook, E. S. Upatham, and G. R. Lanze, 2004. Cadmium biosorption by cells of *Spirulina platensis* TISTR 8217 immobilized in alginate and silica gel. *Environ. Int*., **30** (1): 57-63.

Raskin, I., P. B. A. N. Kumar, S. Dushenkov, and D. Salt, 1994. Bioconcentration of heavy metals by plants. *Curr. Opin. Biotechnol*., **5**: 285-290.

Satoha, A., L. Q. Vudikariab, N. Kuranoa, and S. Miyachia, 2005. Evaluation of the sensitivity of marine microalgal strains to the heavy metals, Cu, As, Sb, Pb and Cd. *Environ. Intern*., **31**: 713-722.

Schiewer, S., and M. H. Wong, 2000. Ionic strength effects in biosorption of metals by marine algae. *Chemosphere*, **41**: 271-282.

Sloof, J. E., A. Viragh, and B. Van Der Veer, 1995. Kinetics of cadmium uptake by green algae. *Water Air Soil Poll*., **83**: 105-122.

Ting, C. S., G. Rocap, J. King, and S. W. Chisholm, 2002. Cyanobacterial photosynthesis in the oceans: the origins and significance of divergent light harvesting strategies. *Trends Microbiol*., **10**: 134-142.

Tripathi, B. N. and J. P. Gaur, 2006. Physiological behavior of *Scenedesmus* sp. during exposure to elevated levels of Cu and Zn and after withdrawal of metal stress. *Protoplasma*, **229**: 1-9.

Volesky, B., and Z. R. Holan, 1995. Biosorption of heavy metals. *Biotechnol. Prog*., **11**: 235-250.

Yan, H., and G. Pan, 2002. Toxicity and bioaccumulation of copper in three microalgal species. *Chemosphere*, **49**: 471-476.

Wang, J., and C. Chen, 2006. Biosorption of heavy metals by *Saccharomyces cerevisiae*. *Biotechnol. Adv*., **24**: 427-451.

Zayed, A., S. Gowthman, and N. Terry, 1998. Phytoaccumulation of trace elements by wetland plants: I. Duckweed. *J. Environ. Qual*., **27**: 715-721.

Zhang, X. C., 1998. Large-scale cultivation of *Spirulina* in China; Today and tomorrow. *Biosys. Study*, **1**: 66-73.