Front. Environ. Sci. Eng. 2014, 8(3): 427–432
DOI 10.1007/s11783-013-0566-4

RESEARCH ARTICLE

Cadmium and lead toxicity and bioaccumulation in *Microcystis aeruginosa*

Piotr RZYMSKI (✉)[1], Barbara PONIEDZIALEK[1], Przemysław NIEDZIELSKI[2], Piotr TABACZEWSKI[1], Krzysztof WIKTOROWICZ[1]

1 Department of Biology and Environmental Protection, Poznan University of Medical Sciences, Poznan 61-848, Poland
2 Faculty of Chemistry, Department of Water and Soil Analysis, Adam Mickiewicz University, Poznan 61-614, Poland

Abstract The growth of human population leads to intensification of agriculture and promotes, through eutrophication, development of cyanobacteria. One of the most widespread and bloom-forming species in freshwater is toxic *Microcystis aeruginosa* (*M. aeruginosa*). Combustion of fossil fuels and metallurgical processes are the main sources of heavy metals contamination in surface water including cadmium (Cd) and lead (Pb). The following study was conducted in order to determine the effect of 1–20 mg·L^{-1} of Cd and Pb on photochemistry (using flow cytometry) and growth (based on chlorophyll concentration) of *M. aeruginosa* as well as to estimate levels of metal bioaccumulation. We have found that 1–10 mg·L^{-1} of Cd and 1–5 mg·L^{-1} of Pb induced continuous enhancement of chlorophyll fluorescence during 24 h of incubation. No significant degradation of chlorophyll was observed in these samples. At higher concentrations of 20 mg·L^{-1} of Cd and 10–20 mg·L^{-1} of Pb chlorophyll level significantly decreased and its fluorescence was quenched. *M. aeruginosa* demonstrated high capability of Cd and Pb bioaccumulation, proportionally to initial metal concentration. In samples with initial concentration of 20 mg·L^{-1} of Cd and Pb bioaccumulation of 87.3% and 90.1% was observed, respectively. Our study demonstrates that *M. aeruginosa* can potentially survive in highly metals polluted environments, be a primary source of toxic metals in the food chain and consequently contribute to enhanced toxicity of heavy metals to living organisms including human.

Keywords *Microcystis aeruginosa*, heavy metals, bioaccumulation, chlorophyll, flow cytometry

Received December 19, 2012; accepted August 1, 2013

E-mail: rzymskipiotr@ump.edu.pl

1 Introduction

Microcystis aeruginosa (*M. aeruginosa*) is one of the most widespread cyanobacteria with the high capability to proliferate in different types of freshwater. Stimulated by high nutrients availability (particularly nitrates and phosphates) and sunny, calm weather, it can grow to high densities and form surface blooms in lakes and slow flowing rivers [1]. The relentless growth of human population forces higher demands for food, consumption and consequently, inter alia, more intense, fertilizer-based agriculture – the well known cause of eutrophication. As a result the number of cyanobacteria blooms with *M. aeruginosa* predomination appears to be increasing in freshwater in many countries including China [2]. These events have been associated recently with toxic cyanobacteria compounds contamination and have raised serious health concerns. *M. aeruginosa* is among the main producers of microcystins (MCs) – a group of hepatotoxins involved in animal and human poisoning [3,4]. MCs contamination of drinking water has been recognized as a major risk factor for primary liver and colon failure and cancer in China [5–7].

It has been reported that cyanobacteria including *M. aeruginosa* can develop in heavy metals polluted freshwater [8–10]. Elements such as cadmium (Cd) and lead (Pb), released in large quantities into air, soil and water from combustion of fossil fuels and industrial metallurgical processes, are not considered to have specific metabolic role in majority of living organisms [11,12]. Mechanisms of Cd and Pb toxicity and resistance are variable, depending on the organism. Potential effects in microorganisms include severe inhibition of growth, photosynthesis and nitrogen fixation [13]. So far the mechanism of heavy metals action on the cyanobacteria cells has not been well enough explored [14]. It is known that toxic

metals tend to bioaccumulate and biomagnify in the food chain posing a risk of human adverse effects including poisoning due to the consumption of aquatic livestock [11,12]. For this reason, we undertook to investigate if cyanobacteria (using *M. aeruginosa* as a model system) can grow in relevant concentrations of heavy metals and accumulate them to some extent.

The following study was conducted in order to determine the effect of Cd and Pb on photochemistry (using flow cytometry) and growth (based on chlorophyll concentration) of *M. aeruginosa* as well as to estimate levels of their bioaccumulation. We demonstrate here that bloom-forming cyanobacteria species can potentially occur in highly polluted environments and that it can be a primary source of toxic metals for higher trophic levels in the food chain including humans.

2 Material and methods

2.1 Cyanobacteria culture

M. aeruginosa was obtained from Culture Collection of the Centre of Algology in Trebon, Czech Republic. The culture was maintained in plastic flasks (250 mL) containing sterile BG-11 medium (pH = 7.0) prepared as described by Rippka et al. [15]. The culture was maintained at 25°C with constant light provided by cool-white fluorescent bulbs at 60 μmol·m^{-1}·s^{-1}. Every two weeks cultures were transferred to the new flasks under sterile conditions. Purity of cultures was systematically verified with the light microscope.

2.2 Experimental design

Purified crystal cadmium (II) nitrate $Cd(NO_3)_2 \cdot 4H_2O$ (Sigma-Aldrich, Germany) and lead (II) nitrate $Pb(NO_3)_2$ (Sigma-Aldrich, Germany) served as a source of metals. *M. aeruginosa* biomass was centrifuged (10 min, 3.000 r·min^{-1}, g = 1670) and dissolved (6.5 mg wet weight per 1 mL) in distilled, UV-sterilized water (pH = 7.0) in 250 mL plastic flasks (Sarsted, Germany). Final volume of each sample was 150 mL. The initial cell density (estimated using Bürker chamber) was 8.5×10^6 cells·mL^{-1}. Metals were added to reach final concentrations of 1, 5, 10 and 20 mg·L^{-1} (±0.2 mg·L^{-1}) - confirmed with atomic absorption spectrometry (see "Analyses of Cd and Pb bioaccumulation").

Range of metal concentrations was based on recent investigations of metal biosorption by cyanobacteria [16–18]. Experiments were carried out at 25°C and constant light provided by cool-white fluorescent bulbs at 60 μmol·m^{-1}·s^{-1} for 24 h. Control samples were constituted of cells incubated under the same conditions without an addition of Cd or Pb.

2.3 Analyses of chlorophyll a fluorescence

Flow cytometer with 15 mW argon laser (FACScan, Becton Dickinson, USA) was used in order to measure the emission of chlorophyll *a* fluorescence. The excitation wavelength was 488 nm. The specific chlorophyll signal was measured at its maximum emission wavelength of 669 nm (FL-3 channel). Cells population was gated with side scatter (SSC) and forward scatter (FSC). Figure 1 demonstrates a typical image of 2-D graph with linear scale of homogenous *M. aeruginosa* population gated and separated from debris for further fluorescence analyses. Ten thousand gated events were acquired. Since flow cytometer requires low volumes of liquid for analyses, fluorescence was recorded after 3, 6, 12 and 24 h of incubation in the same sample. Fluorescence values were given in Mean Fluorescence Intensity units (MFI). 50 repetitions of each incubation for Cd and Pb incubations were conducted.

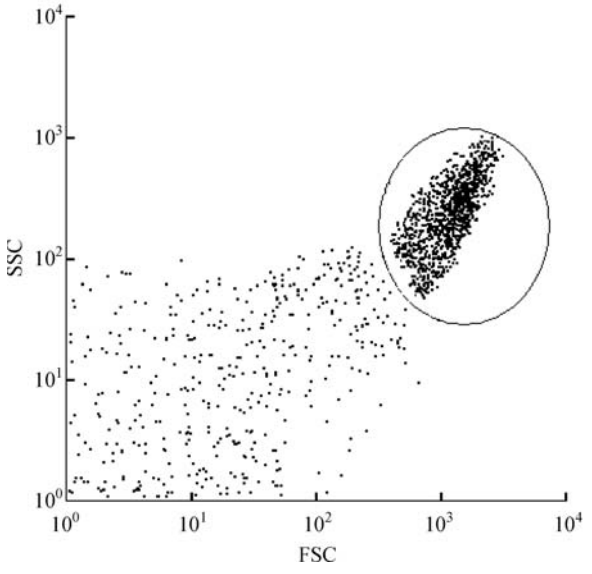

Fig. 1 Typical 2-D graph of *Microcystis aeruginosa* population gated for further fluorescence analyses. FSC- forward light scatter; SSC- side light scatter

2.4 Analyses of chlorophyll a concentration

Chlorophyll *a* concentration was determined using method proposed by Lawton et al. which is based on ethanol extraction [19]. After 24 h of incubation samples were filtered using GF/C filters (Whatman, UK). Absorbance was recorded using LambdaBio (Perkin Elmer, USA) at 750 nm and 650 nm before (turbidity correction) and after addition of 1 mol·L^{-1} HCL. Results were calculated and expressed in mg·m^{-3} of chlorophyll *a*. Thirty repetitions of each incubation for Cd and Pb incubations were conducted.

2.5 Analyses of Cd and Pb bioaccumulation

After 24 h of incubation samples were filtered three times using GF/C filters (Whatman, UK) in order to separate the biomass from the solution and acidified with HNO_3 (Merck, Germany). Filtrate prepared in this manner was subjected to atomic absorption spectrometry (AAS) analyses performed using flame fast sequential atomic absorption spectrometer SpectrAA 220 FS (Varian, Australia) equipped in HCL lamps (Varian). Wavelength of 228.8 nm and 217.0 nm, slit 0.5 nm and 1.0 nm for Cd and Pb, respectively; lamp current 5 mA were applied. Limits of detection were 0.05 $mg \cdot L^{-1}$ for Cd and 0.02 $mg \cdot L^{-1}$ for Pb. Ten repetitions of each incubation for Cd and Pb incubations were conducted.

2.6 Statistical methods

The results were analyzed with Statistica 10.0 software (StatSoft, USA). Mann–Whitney U rank test was used to compare control and Cd/Pb exposed samples. To compare more than two groups Kruskal–Wallis one-way analysis of variance with Dunn post-hoc test was used. $P < 0.05$ was considered as statistically significant.

3 Results

3.1 Chlorophyll a fluorescence

In the control samples, not exposed to Cd and Pb the fluorescence remained at the similar, comparable level of emission for every time interval the measurements were conducted. No significant differences were noted (Kruskal–Wallis, $p > 0.005$) (Fig. 2).

Cells incubated with Cd and Pb demonstrated graded enhancement or quenching of chlorophyll fluorescence over incubation time (Fig. 2). The most significant changes were observed after 24 h of incubation. Compared to control the emission in samples exposed to 1, 5 and 10 $mg \cdot L^{-1}$ of Cd increased by 15.3%, 21.6% and 38.8% after 24 h of incubation, respectively. However the first signs of enhancement were noted after 3 h of incubation (increased by 1.5%, 4.8% and 12.5% compared to control, respectively) and progressed onwards. This was demonstrated by significant changes of fluorescence emission between each of time intervals for 1, 5 and 10 $mg \cdot L^{-1}$ of Cd (Kruskal–Wallis, $p < 0.001$). Similar observations were obtained in cells exposed to 1 and 5 $mg \cdot L^{-1}$ Pb. The enhancement of fluorescence emission when compared to control was 10.9% and 25.8% after 24 h of incubation, respectively. Slight increase in emission was also observed after 3 h (increased by 2.9% and 9.9% compared to control, respectively) and progressed onwards. This was demonstrated by significant changes of fluorescence values between each of time intervals for 1 and 5 $mg \cdot L^{-1}$ of Pb (Kruskal–Wallis, $p < 0.001$). In samples incubated with 20 $mg \cdot L^{-1}$ of Cd fluorescence quenching was observed from the first time interval onwards and decreased by 12.5% compared to control. After 24 h of incubation the emission was decreased by 36.8%. Statistical analyses revealed significant changes in fluorescence emission between subsequent time intervals (Kruskal–Wallis, $p < 0.001$). Similar observations were obtained for 10

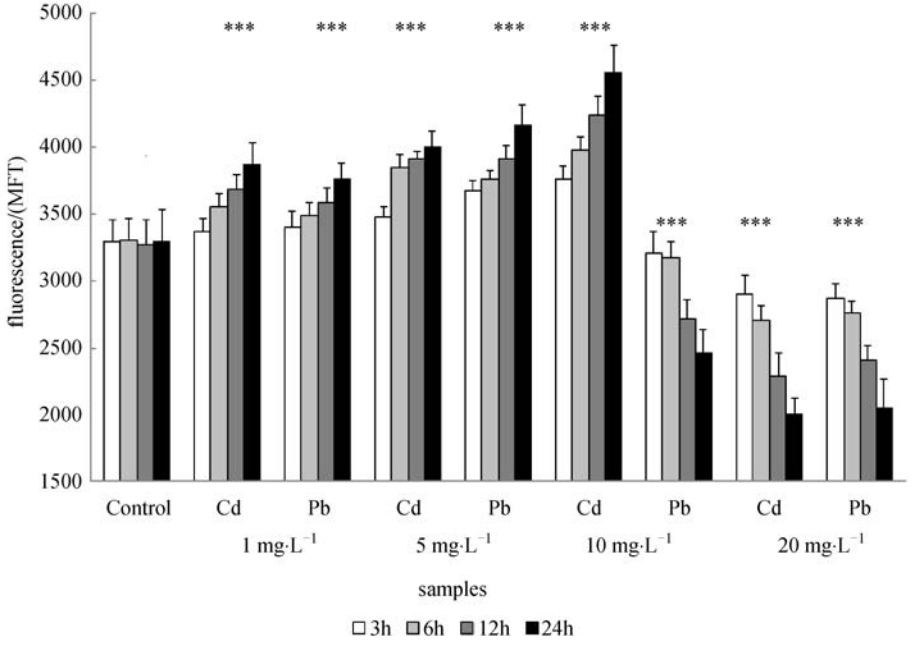

Fig. 2 Mean fluorescence emission in metal-affected and unaffected samples during 24 h of incubation. Asterisks represent Kruskal–Wallis comparison between different time intervals in each sample (*** –$p < 0.001$)

and 20 mg·L^{-1} of Pb - fluorescence quenching was observed from the first time interval onwards and decreased by 2.9% and 12.1%, respectively. After 24 h of incubation the emission was decreased by 24.7% and 35.2%, respectively. For both samples changes in fluorescence emission between subsequent time intervals were statistically relevant (Kruskal–Wallis, $p < 0.001$).

3.2 Chlorophyll *a* concentration

Chlorophyll concentration in *M. aeruginosa* cells was found to be highly stable. Compared to 24 h control there were no significant changes in chlorophyll concentration in samples incubated with 1, 5 and 10 mg·L^{-1} of Cd, and 1, 5 mg·L^{-1} Pb. In samples exposed to 20 mg·L^{-1} of Cd chlorophyll concentration decreased by 32.5% when compared to control (Mann–Whitney, $p < 0.001$). Similarly, statistically significant decrease of chlorophyll concentration was also observed for 10 mg·L^{-1} of Pb (Mann–Whitney, $p < 0.001$) with 39.4% decrease as compared to control samples. Cells exposed to 20 mg·L^{-1} of Pb demonstrated high degree of chlorophyll concentration decrease of 54% as compared to control samples, statistically significant (Mann–Whitney, $p < 0.001$).

M. aeruginosa chlorophyll levels were affected more by Pb than Cd (Fig. 3). Chlorophyll concentration in samples exposed to 10 and 20 mg·L^{-1} of Pb was significantly lower than in respective Cd exposed samples (Mann–Whitney, $p < 0.01$ and $p < 0.001$, respectively).

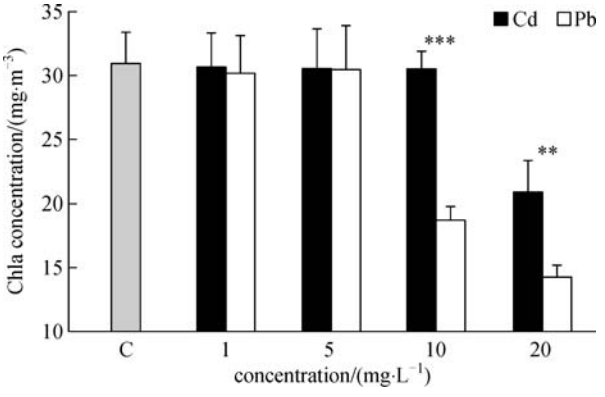

Fig. 3 Mean chlorophyll concentrations in metal-affected and unaffected samples after 24 h of incubation. Error bars represent standard deviation. Asterisks represent Mann–Whitney U comparison between respective Cd and Pb samples (** – $p < 0.01$; *** – $p < 0.001$)

3.3 Metals bioaccumulation

No traceable amounts of Cd and Pb were detected in all of the reagents used for biomass culturing, samples preparation and preservation. $Cd(NO_3)_2 \cdot 4H_2O$ was Pb-free and $Pb(NO_3)_2$ was free of Cd.

M. aeruginosa biomass demonstrated high ratio of Cd and Pb bioaccumulation after 24 h of incubation. The lowest rate was observed in samples with initial 1 mg·L^{-1} concentration of Pb - 59.9% of metal was removed from extracellular environment. The highest bioaccumulation was noted for samples with initial 20 mg·L^{-1} of Pb – the removal of 90.1% of metal from extracellular environment was observed. The comparison of bioaccumulation ratio between samples with initial 10 and 20 mg·L^{-1} concentration of Cd and Pb revealed no statistically significant differences (Mann–Whitney $p > 0.05$). However, the bioaccumulation ratio of Cd was significantly higher than Pb in samples with initial concentration of 1 and 5 mg·L^{-1} (Mann–Whitney, $p < 0.01$).

The higher the initial concentration of metal was used the greater the bioaccumulation was observed, especially for Pb (Table 1).

Table 1 Bioaccumulation of cadmium and lead in *M. aeruginosa* after 24 h of incubation

metal	initial concentration /(mg·L^{-1})	bioaccumulated/%	U-Mann–Whitney Test
Cd	1	78.7	$p < 0.05$
Pb		59.9	
Cd	5	83.6	$p < 0.05$
Pb		74.2	
Cd	10	83.2	$p < 0.05$
Pb		88.1	
Cd	20	87.3	$p < 0.05$
Pb		90.1	

4 Discussion

Our results suggest that Cd and Pb can induce toxic effects in *M. aeruginosa*. However level of toxicity manifestation varied depending on studied metal concentration. We have shown that low concentration of Cd or Pb (1–5 mg·L^{-1}) can gradually increase emission of chlorophyll fluorescence during 24 h incubation while higher metal concentration (10–20 mg·L^{-1}) can induce graded decrease of emission. According to the model proposed by Butler [20], light energy absorbed by chlorophyll molecules of supramolecular structure Photosystem II (PSII) can be used to drive photosynthesis, dissipated as a heat or re-emitted as a fluorescence. These processes occur in the strong association with each other. In photosynthetic cells grown under optimal and not disturbed conditions, relations in ratio of these processes do not undergo any significant changes while emission of fluorescence usually does not exceed 0.6%–3.0% of absorbed light energy [21,22]. However if efficiency of one of above processes is disturbed it will be reflected in respective changes of their ratios [23]. Recent study by Dudkowiak et al. demonstrated that occurrence of

heavy metals in the environment does not induce significant changes in heat dissipation in cyanobacteria [24]. Thus we believe analyses of chlorophyll fluorescence using flow cytometry can be an indirect information of photosynthetic activity. This method allows the fluorescence emission to be measured in a rapid automated way and requires low sample volumes. It is therefore increasingly used not only in medical and veterinary diagnostics but also in environmental sciences [25,26].

Fluorescence quenching observed in cells exposed to high concentration of metals is most likely an effect of impaired absorption of light as a consequence of thylakoid damage or/and degradation of chlorophyll. This suggestion is supported by analyses of chlorophyll concentration which significantly decreased when cells were exposed to 10 and 20 $mg \cdot L^{-1}$ of both, Cd and Pb. Similar results of fluorescence quenching resulting from toxic metals were obtained by Babu et al. in *Arthospira platensis* [27] and Poniedziałek et al. in *Cylindrospermopsis raciborskii* and *Aphanizomenon flos-aque* [28].

The increase in fluorescence emission, observed in cells exposed to lower concentrations of metals can be explained by gradual release of chlorophyll molecules from lipid-protein structures followed by lower rate of electron transfer from the reaction center Chl (P680) to the primary electron acceptor of PS II (QA). Consequently, the higher rate of absorbed light was re-emitted and the activity of photosynthesis decreased. The toxic effects of 1–5 $mg \cdot L^{-1}$ of Cd or Pb were not reflected in changes of chlorophyll concentration. Therefore, we argue that flow cytometric analyses of pigments can be more sensitive methods in identification of early toxic effect in PSII.

Decrease of chlorophyll content observed in samples exposed to the highest studied metals concentrations was an indicator of cell growth inhibition. Based on our results of chlorophyll fluorescence and concentration it can be concluded that *M. aeruginosa* has a greater tolerance toward Cd than Pb. Potentially this cyanobacteria species can occur in freshwater polluted with heavy metals. Only copper has been shown to be extremely toxic to this species and was proposed as one of method of cyanobacteria blooms treatment [29,30].

Our study shows that *M. aeruginosa* can uptake large quantities of Cd and Pb from extracellular environment. This process is more efficient at higher metal concentrations. This on the other hand can be explained by impairment of desorption processes due to the metals toxicity eventually resulting in both, Cd and Pb accumulation within the cell. At lower concentrations cells can effectively remove metals and decrease their toxic effects. Field studies conducted by Zeng et al. indicated that *M. aeruginosa* bioconcentration factor for Cd can be 10 times higher than for Pb, Ni, Zn, Cu or Cr [31]. This along with our experimental study strongly suggests that heavy metal polluted and eutrophic freshwater can not only favor the development of toxic *M. aeruginosa* strains but due to high metal bioaccumulation its biomass can be a primary source of metal in the food chain.

5 Conclusions

M. aeruginosa demonstrates relatively high Cd and Pb tolerance. Its growth is not inhibited by the concentrations in the 1–5 $mg \cdot L^{-1}$ range although sensitive methods (i.e. flow cytometry) can detect early toxicity effects such as impairment of the PSII and photochemical reactions

At the same time *M. aeruginosa* biomass can accumulate large quantities of both, Cd and Pb. As globally occurring primary producer (particularly in eutrophic ecosystems) this species can serve as a major component of the food web and potential source of toxic metals for the aquatic organisms.

It is therefore suggested that the occurrence of *M. aeruginosa* in the surface water in industrial areas can lead to multiple environmental and health hazards (including the release of toxins and incorporation of toxic metals in the food chain) and therefore its blooms should be prevented.

Acknowledgements This project was partially supported by funds from the Young Scientists Project of Poznan University of Medical Sciences (No. 502-14-04402503-50656).

References

1. Straub C, Quillardet P, Vergalli J, de Marsac N T, Humbert J F. A day in the life of *Microcystis aeruginosa* strain PCC 7806 as revealed by a transcriptomic analysis. PLoS ONE, 2011, 6(1): e16208
2. Wu Z X, Gan N Q, Song L R. Genetic diversity: geographical distribution and toxin profiles of *Microcystis* strains (Cyanobacteria) in China. Journal of Integrative Plant Biology, 2007, 49(3): 262–269
3. Pearson L, Mihali T, Moffitt M, Kellmann R, Neilan B. On the chemistry, toxicology and genetics of the cyanobacterial toxins, microcystin, nodularin, saxitoxin and cylindrospermopsin. Marine Drugs, 2010, 8(5): 1650–1680
4. Rzymski P, Poniedziałek B, Karczewski J. Gastroenteritis and liver carcinogenesis induced by cyanobacterial toxins. Gastroenterologia Polska, 2011, 18(4): 159–162
5. Yu S Z. Primary prevention of hepatocellular carcinoma. Journal of Gastroenterology and Hepatology, 1995, 10(6): 674–682
6. Yu S Z, Chen G, Zhi X L, Li J. Primary liver cancer: natural toxins and prevention in China. The Journal of Toxicological Sciences, 1998, 23(SupplementII): 143–147
7. Zhou L, Yu H, Chen K. Relationship between microcystin in drinking water and colorectal cancer. Biomedical and Environmental Sciences, 2002, 15(2): 166–171
8. Li J, Peng F, Ding D, Zhang S, Li D, Zhang T. Characteristics of the phytoplankton community and bioaccumulation of heavy metals during algal blooms in Xiangjiang River (Hunan, China). Science

China Life Sciences, 2011, 54(10): 931–938
9. Huang Y, Zou L, Zhang S, Xie S. Comparison of bacterioplankton communities in three heavily polluted streams in China. Biomedical and Environmental Sciences, 2011, 24(2): 140–145
10. Zeng J, Yang L, Chen X, Chuai X, Wu Q L. Spatial distribution and seasonal variation of heavy metals in water and sediments of Taihu Lake. Polish Journal of Environmental Studies, 2012, 21(5): 1489–1496
11. Järup L. Hazards of heavy metal contamination. British Medical Bulletin, 2003, 68(1): 167–182
12. Šmirjákova S, Ondrašovičová O, Kašková A, Lakticova K. The effect of cadmium and lead pollution on human and animal health. Folia Veterinaria, 2005, 49(Suppl 3): 31–32
13. Trevors J T, Stratton G W, Gadd G M. Cadmium transport, resistance, and toxicity in bacteria, algae, and fungi. Canadian Journal of Microbiology, 1986, 32(6): 447–464
14. Küpper H, Kroneck P M. Heavy metal uptake by plants and cyanobacteria. Metal Ions in Biological Systems, 2005, 44: 97–144
15. Rippka R, Deruelles J, Waterbury J B, Herdman M, Stanier R Y. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology, 1979, 111(1): 1–61
16. Cain A, Vannela R, Woo L K. Cyanobacteria as a biosorbent for mercuric ion. Bioresource Technology, 2008, 99(14): 6578–6586
17. Raungsomboon S, Chidthaisong A, Bunnag B, Inthorn D, Harvey N W. Removal of lead (Pb^{2+}) by the cyanobacterium *Gloeocapsa* sp. Bioresource Technology, 2008, 99(13): 5650–5658
18. Chakraborty N, Banerjee A, Pal R. Accumulation of lead by free and immobilized cyanobacteria with special reference to accumulation factor and recovery. Bioresource Technology, 2011, 102(5): 4191–4195
19. Lawton I, Marsalek B, Padisák J, Chorus I. Determination of Cyanobacteria in the laboratory. In: Chorus I, Bartman J, editors. Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management. London: E & FN Spon, 1999, 347–367
20. Butler W L. Energy distribution in the photochemical apparatus of photosynthesis. Annual Review of Plant Physiology, 1978, 29(1): 345–378
21. Krause G H, Weis E. Chlorophyll fluorescence and photosynthesis: the basics. Annual Review of Plant Physiology and Plant Molecular Biology, 1991, 42(1): 313–349
22. Hall D O, Rao K K. Photosynthesis. Cambridge: Cambridge University Press, 1999
23. Murata N, Takahashi S, Nishiyama Y, Allakhverdiev S I. Photoinhibition of photosystem II under environmental stress. Biochimica et Biophysica Acta, 2007, 1767(6): 414–421
24. Dudkowiak A, Olejarz B, Łukasiewicz J, Banaszek J, Sikora J, Wiktorowicz K. Heavy metals effect on cyanobacteria *Synechocystis aquatilis* study using absorption, fluorescence, flow cytometry, and photothermal measurement. International Journal of Thermophysics, 2011, 32(4): 762–773
25. Dubelaar G B J, Jonker R R. Flow cytometry as a tool for the study of phytoplankton. Scientia Marina, 2000, 64(2): 135–156
26. Rzymski P, Langowska A, Fliszkiewicz M, Poniedziałek B, Karczewski J, Wiktorowicz K. Flow cytometry as an estimation tool for honey bee sperm viability. Theriogenology, 2012, 77(8): 1642–1647
27. Babu N G, Sarma P A, Attitalla I H, Murthy S D S. Effect of heavy metal ions on the photosynthetic electron transport and energy transfer in the thylakoid membrane of the cyanobacterium, *Spirulina platensis*. Academic Journal of Plant Sciences, 2010, 3(1): 46–49
28. Poniedziałek B, Rzymski P, Kokociński M, Burchardt L, Wiktorowicz K. Changes of *Cylindrospermopsis raciborskii* and *Aphanizomenon flos-aquae* chlorophyll fluorescence under the influence of lead. Ochrona Środowiskai Zasobów Naturalnych, 2011, 58: 513–519 (in Polish)
29. Zeng J, Yang L, Wang W X. High sensitivity of cyanobacterium *Microcystis aeruginosa* to copper and the prediction of copper toxicity. Environmental Toxicology and Chemistry, 2010, 29(10): 2260–2268
30. Wang Z, Li J, Zhao J, Xing B. Toxicity and internalization of CuO nanoparticles to prokaryotic alga *Microcystis aeruginosa* as affected by dissolved organic matter. Environmental Science & Technology, 2011, 45(14): 6032–6040
31. Zeng J, Zhao D, Ji Y, Wu Q. Comparison of heavy metal accumulation by a bloom-forming cyanobacterium, *Microcystis aeruginosa*. Chinese Science Bulletin, 2012, 57(28–29): 3790–3797