Effects of lead(II) on the extracellular polysaccharide (EPS) production and colony formation of cultured *Microcystis aeruginosa*

Xiang-dong Bi, Shu-lin Zhang, Wei Dai, Ke-zhing Xing and Fan Yang

**ABSTRACT**

To investigate the effects of lead(II) on the production of extracellular polysaccharides (EPS), including bound extracellular polysaccharides (bEPS) and soluble extracellular polysaccharides (sEPS), and the colony formation of *Microcystis aeruginosa*, cultures of *M. aeruginosa* were exposed to four concentrations (5.0, 10.0, 20.0 and 40.0 mg/L) of lead(II) for 10 d under controlled laboratory conditions. The results showed that 5.0 and 10.0 mg/L lead(II) stimulated *M. aeruginosa* growth throughout the experiment while 20.0 and 40.0 mg/L lead(II) inhibited *M. aeruginosa* growth in the first 2 d exposure and then stimulated it. As compared to the control group, significant increases in the bEPS and sEPS production were observed in 20.0 and 40.0 mg/L lead(II) treatments (*P* < 0.05). Large colony formations were not observed throughout the experiment. However, four tested concentrations of lead(II) could significantly promote the formation of small and middle colonies after 10 d exposure (*P* < 0.05), and 40.0 mg/L lead(II) had the best stimulatory effect. Lead(II) could stimulate bEPS production, which conversely promoted colony formation, suggesting that heavy metals might be contributing to the bloom-forming of *M. aeruginosa* in natural conditions.

**Key words** | bEPS, colony formation, growth, lead(II), *Microcystis aeruginosa*, sEPS

**INTRODUCTION**

*Microcystis* blooms can result in water anoxia, secretion of algal toxins as well as several other adverse effects (Pitois et al. 2004). *Microcystis aeruginosa*, a photosynthetic prokaryote, is the dominant bloom-forming cyanobacteria in eutrophic freshwaters, and poses a great threat to freshwater aquaculture all over the world (Coles & Jones 2003). In addition, *M. aeruginosa* is detrimental to human health for its production and secretion of the potent hepatotoxins called microcystins (MCs), which have been implicated in the development of liver cancer, necrosis and even deadly intrahepatic bleeding (Falconer & Humpage 2005). *M. aeruginosa* has two physiologically different phenotypic types (Wu & Song 2008; Yang et al. 2008): a large colony under natural conditions, and single cells or a few paired cells in axenic cultures in the laboratory (Reynolds et al. 1981; Bolch & Blackburn 1996; Yang et al. 2006; El Herry et al. 2009). This phenomenon suggests a strategy in natural systems whereby colony formation of *M. aeruginosa* provides a competitive advantage over other phytoplankton species (Reynolds et al. 1981; Kearns & Hunter 2000; Wu et al. 2007; Gan et al. 2012). The intercellular spaces of the colony of *M. aeruginosa* provide buoyancy to stimulate the growth of algal cells (Zhang et al. 2011), and the colonies can effectively deter zooplankton from grazing (Yang et al. 2008). Colony formation of *Microcystis* may be stimulated by zooplankton grazing (Burkert et al. 2001; Yang et al. 2006, 2008) and environmental factors, such as nitrogen content and MCs content in culture media (Wang et al. 2010; Gan et al. 2012).

Since the early 1950s, more than 100 cyanobacterial strains, including *M. aeruginosa*, have been found producing and secreting extracellular polysaccharides (EPS). The chemical and rheological properties show that such polysaccharides are complex anionic heteropolymers, in about 80% of cases containing six to 10 different monosaccharides and in about 90% of cases containing one or more uronic acids. Owing to their carboxyl groups, uronic acids can provide binding sites to bind metal ions efficiently.

Xiang-dong Bi
Shu-lin Zhang (corresponding author)
Wei Dai
Ke-zhing Xing
Fan Yang

Key Laboratory for Aquatic Ecology and Aquaculture of Tianjin, Department of Fisheries Science, Tianjin Agricultural University, Jinjing Road 22, Tianjin 300384, China

E-mail: hy101018@163.com

doi: 10.2166/wst.2012.632
The cyanobacterial EPS can be divided into two main groups: the ones bound to the cell surface (bound extracellular polysaccharides, bEPS) and the polysaccharides released into the surrounding environment, which are soluble in the aquatic environment (soluble extracellular polysaccharides, sEPS) (De Philippis et al. 1998, 2001). bEPS, being referred to as sheath, capsule or slime, play an important role in the attachment of *M. aeruginosa* cells into the colony, as well as in protecting the cells from unfavorable environmental conditions (De Philippis & Vincenzi 2003). The sEPS can be easily recovered from liquid cultures and, due to their physicochemical properties, are suitable for a variety of industrial applications, making cyanobacteria one of the most attractive sources of new polymers (De Philippis et al. 1998, De Philippis & Vincenzi 2003). In addition, EPS, possessing large number of effective cation-chelating binding sites for metal ions, have been studied for the removal of toxic heavy metals (Chen et al. 2006) in the aquatic environment.

With the development of industry and agriculture, more and more heavy metals are released into water bodies. Algal cells can secrete polysaccharide, which combines with heavy metal and moves it away from the metabolic process by transporting it into the vacuole (Lau et al. 2005). Sharma et al. (2008) reported that Cr(VI) concentrations increased the amount of EPS produced by *Gloeocapsa calcearea* and *Nostoc punctiforme*. A significant and regular increase in the production of EPS in two isolates of *Synechocystis* sp. was observed after Cd(II) exposure (Ozturk et al. 2010).

Lead, a non-essential heavy metal, is one of the most toxic metals affecting the aquatic environment. Romera et al. (2007) compared biosorption of heavy metals using different types of algae and found experimental data fitted a Langmuir model very well according to the following sequence of the sorption values: Pb > Cd > Cu > Zn > Ni. In this study, we conducted experiments to examine the effect of lead(II) on the EPS production and colony formation of *M. aeruginosa*, which will be helpful to understand the mechanism of colony formation of *M. aeruginosa* under natural conditions.

**MATERIALS AND METHODS**

**Materials and experimental design**

*M. aeruginosa* was provided by the Institute of Hydrobiology, Chinese Academy of Sciences. The alga was grown in BG11 medium containing (g/L): NaNO₃, 1.5; K₂HPO₄, 0.4; MgSO₄·7H₂O, 0.075; CaCl₂·2H₂O, 0.036; citric acid, 0.006; iron(III) ammonium citrate, 0.006; Na₂EDTA, 0.001; Na₂CO₃, 0.02; and 1 mL trace elements solution (mg/L): H₂BO₃, 61; MnSO₄·H₂O, 169; ZnSO₄·7H₂O, 287; CuSO₄·5H₂O, 2.5; (NH₄)₆Mo₇O₂₄·4H₂O, 12.5; pH: 6.8 (Rippka et al. 1979), under 12/12 h light/dark cycle with a light density of 60 μmol/m²·s at 25 ± 1 °C. To reduce any effects related to minor differences in photon irradiance and to maintain homogeneity, the flasks were shaken slightly four times each day and rearranged randomly.

Pb(NO₃)₂ (Merck) was dissolved in distilled water to prepare the stock solution (4,000 mg/L), which was sterilized using micro pore filtration. Lead(II) was added into algal culture with the initial algae density of 3.0 × 10⁶ ind/mL to the final concentrations as follows: 0 (control), 5.0, 10.0, 20.0 and 40.0 mg/L with three replicate flasks per concentration. The experiment was run for 10 d, and samples were removed from the cultures at 2 d intervals to determine the algal cell density, EPS production and colony size.

**Effects of lead(II) on *M. aeruginosa* growth**

Algal cell density was tested using a blood counting chamber under a microscope (×40), and the cell counting of colonial *M. aeruginosa* was determined using the boiling method (Joung et al. 2006). To reduce erroneous results, samples were taken 1 cm below the water surface with movement. The relative growth rate (RGT) of algae was calculated using the following formula:

\[
\text{RGT} = \ln(NT - N_0)/({\geq} t)
\]

where \(N_0\) is the population cell density at the beginning of a time interval, \(N_T\) the cell density at the end of the time interval, and \(\geq t\) the length of the time interval in days (\(t_T - t_0\)).

**Effects of lead(II) on the EPS production of *M. aeruginosa***

An equal volume (30 mL) of algal culture was centrifuged at 10,000 g for 10 min to prepare supernatant and deposit for subsequent sEPS and bEPS determination. The resulting supernatant was precipitated overnight at 4 °C with six volumes of 95% ethanol (EtOH). Precipitated sEPS were recovered by centrifugation and the EtOH precipitation step repeated once again. After centrifugation (12,000 × g for 30 min at 4 °C), successively obtained
deposits were mixed and dissolved in distilled water, dialyzed (3,600 mol wt cutoff tubing) against distilled water at 4 °C and then concentrated to 2 mL by rotary evaporation. The content of sEPS (pg/cell) was estimated by the phenol–sulfuric method (Dubois et al. 1956).

The algal cell deposit was washed with 0.9% NaCl solution using a glass rod and the resulting suspensions were stirred with glass beads in order to detach bEPS associated with the algal cells. Cells were then removed by centrifugation at 10,000 × g for 30 min at 4 °C. The resulting supernatant was used to determine the content of bEPS (pg/cell) according to the above method.

**Effects of lead(ii) on the colony formation of *M. aeruginosa***

The types of *M. aeruginosa* colony were classified as unicell or two-cells, small colony (3–10 cells), middle colony (11–50 cells) and large colony (more than 50 cells). One milliliter of algae culture was sampled 1 cm below the medium surface and transferred to a settling chamber using a wide-mouth pipette. Fifty visual fields were randomly chosen under microscope to count different types of *M. aeruginosa* colonies. Each sampling was replicated three times and averaged. The proportions of different types of *M. aeruginosa* colony were calculated with the following formulae:

\[
P_{\text{unicell or two-cells}}(\%) = \frac{N_{\text{unicell or two-cells}}}{N_{\text{unicell or two-cells}} + N_{\text{small colony}} + N_{\text{middle colony}} + N_{\text{large colony}}} \times 100\% \\
P_{\text{small colony}}(\%) = \frac{N_{\text{small colony}}}{N_{\text{unicell or two-cells}} + N_{\text{small colony}} + N_{\text{middle colony}} + N_{\text{large colony}}} \times 100\% \\
P_{\text{middle colony}}(\%) = \frac{N_{\text{middle colony}}}{N_{\text{unicell or two-cells}} + N_{\text{small colony}} + N_{\text{middle colony}} + N_{\text{large colony}}} \times 100\% \\
P_{\text{large colony}}(\%) = \frac{N_{\text{large colony}}}{N_{\text{unicell or two-cells}} + N_{\text{small colony}} + N_{\text{middle colony}} + N_{\text{large colony}}} \times 100\%
\]

where \( P \) = proportions of different types of *M. aeruginosa* colony, \( N \) = number of different types of *M. aeruginosa* colony.

**Statistical analysis**

The results were expressed as mean ± SD. One-way analysis of variance (ANOVA, SPSS version 11.0) followed by Fisher’s LSD (Least Significant Difference) multiple range test (Meier 2006) was used to examine whether there were any significant differences among treatments. Statistical significance was established at \( P < 0.05 \).

**RESULTS**

**Effects of lead(ii) on *M. aeruginosa* growth**

The cell density and RGT of *M. aeruginosa* showed various trends in response to different concentrations of lead(ii) (Figures 1 and 2). As compared to the control group, 5.0 and 10.0 mg/L lead(ii) groups exhibited significant stimulatory effects on *M. aeruginosa* growth in a short time (\( P < 0.05 \)) while 2 d RGT reached 15.80 and 17.26%, and then returned to 8.01 and 10.08%, closing to the RGT level of control group after 4 d exposure (Figure 2). A concentration of 20.0 and 40.0 mg/L lead(ii) exhibited significant inhibitory effects on *M. aeruginosa* growth in
2 d ($P < 0.05$): RGT was 2.40 and $-1.11\%$ and rose to 18.78 and 19.97\% respectively after 4 d exposure, then returned to 9.18 and 10.08\%, which were close to the RGT level of control group after 8 d exposure (Figure 2). *M. aeruginosa* exposed to 20 and 40 mg/L lead(II) showed a marked overcompensatory growth, indicating *M. aeruginosa* has high tolerance to the toxic effects of lead(II).

### Effects of lead(II) on the bePS and sEPS production of *M. aeruginosa*

As shown in Table 1, four tested concentrations of lead(II) increased the bePS content of *M. aeruginosa* in varying degrees, and 20.0 and 40.0 mg/L lead(II) significantly increased the bePS content of *M. aeruginosa* over the whole experiment period ($P < 0.05$). As shown in Table 2, as compared to the control, the sEPS content in 5.0 and 10.0 mg/L lead-treated groups decreased significantly for the first 6 d ($P < 0.05$), and then increased to the level of control group at 8 d. Decreased sEPS might be attributed to increased EPS sliming to the cell surface from the action of active positive ions when algal cell density significantly increased in the first 6 d. sEPS in 20.0 and 40.00 mg/L lead-treated groups increased to maximum at 2 d and then decreased to minimum at 10 d.

Table 1 | Effects of various concentrations of lead(II) on bePS content of *M. aeruginosa* (pg/cell)

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>0 d</th>
<th>2 d</th>
<th>4 d</th>
<th>6 d</th>
<th>8 d</th>
<th>10 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>$0.361 \pm 0.008a$</td>
<td>$0.358 \pm 0.011a$</td>
<td>$0.355 \pm 0.009a$</td>
<td>$0.362 \pm 0.010a$</td>
<td>$0.364 \pm 0.007a$</td>
<td>$0.371 \pm 0.005a$</td>
</tr>
<tr>
<td>5.0</td>
<td>$0.363 \pm 0.008a$</td>
<td>$0.424 \pm 0.013b$</td>
<td>$0.378 \pm 0.015b$</td>
<td>$0.376 \pm 0.014a$</td>
<td>$0.375 \pm 0.012a$</td>
<td>$0.373 \pm 0.010a$</td>
</tr>
<tr>
<td>10.0</td>
<td>$0.363 \pm 0.010a$</td>
<td>$0.484 \pm 0.003c$</td>
<td>$0.416 \pm 0.005c$</td>
<td>$0.397 \pm 0.017b$</td>
<td>$0.388 \pm 0.010b$</td>
<td>$0.382 \pm 0.016a$</td>
</tr>
<tr>
<td>20.0</td>
<td>$0.364 \pm 0.010a$</td>
<td>$0.568 \pm 0.010d$</td>
<td>$0.465 \pm 0.012d$</td>
<td>$0.421 \pm 0.009c$</td>
<td>$0.408 \pm 0.005c$</td>
<td>$0.392 \pm 0.011b$</td>
</tr>
<tr>
<td>40.0</td>
<td>$0.364 \pm 0.005a$</td>
<td>$0.632 \pm 0.015e$</td>
<td>$0.509 \pm 0.008e$</td>
<td>$0.453 \pm 0.011d$</td>
<td>$0.422 \pm 0.018d$</td>
<td>$0.394 \pm 0.010b$</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD, $n = 3$. Values sharing the same letters or no letters are not significantly different ($P > 0.05$), whereas those with different letters are significantly different ($P < 0.05$).

Table 2 | Effects of various concentrations of lead(II) on sEPS content of *M. aeruginosa* (pg/cell)

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>0 d</th>
<th>2 d</th>
<th>4 d</th>
<th>6 d</th>
<th>8 d</th>
<th>10 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>$0.222 \pm 0.002a$</td>
<td>$0.219 \pm 0.003b$</td>
<td>$0.216 \pm 0.005c$</td>
<td>$0.212 \pm 0.005c$</td>
<td>$0.208 \pm 0.008a$</td>
<td>$0.204 \pm 0.006a$</td>
</tr>
<tr>
<td>5.0</td>
<td>$0.221 \pm 0.004a$</td>
<td>$0.167 \pm 0.007a$</td>
<td>$0.174 \pm 0.005a$</td>
<td>$0.184 \pm 0.004a$</td>
<td>$0.205 \pm 0.007a$</td>
<td>$0.207 \pm 0.001a$</td>
</tr>
<tr>
<td>10.0</td>
<td>$0.224 \pm 0.004a$</td>
<td>$0.178 \pm 0.007a$</td>
<td>$0.184 \pm 0.004b$</td>
<td>$0.192 \pm 0.008b$</td>
<td>$0.203 \pm 0.009a$</td>
<td>$0.210 \pm 0.010a$</td>
</tr>
<tr>
<td>20.0</td>
<td>$0.218 \pm 0.004a$</td>
<td>$0.312 \pm 0.010c$</td>
<td>$0.237 \pm 0.006d$</td>
<td>$0.229 \pm 0.006c$</td>
<td>$0.226 \pm 0.005b$</td>
<td>$0.222 \pm 0.005b$</td>
</tr>
<tr>
<td>40.0</td>
<td>$0.220 \pm 0.006a$</td>
<td>$0.368 \pm 0.018c$</td>
<td>$0.292 \pm 0.004e$</td>
<td>$0.251 \pm 0.009d$</td>
<td>$0.241 \pm 0.005b$</td>
<td>$0.227 \pm 0.007b$</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD, $n = 3$. Values sharing the same letters or no letters are not significantly different ($P > 0.05$), whereas those with different letters are significantly different ($P < 0.05$).

### Effects of lead(II) on the colony formation of *M. aeruginosa*

Different types of *M. aeruginosa* colony are marked in Figure 3. A large colony was not observed over the whole experiment period. As shown in Figure 4, the proportions and the size of *M. aeruginosa* colonies increased with the increasing concentrations of lead(II) and prolongation of exposure time. All tested concentrations of lead(II) could significantly promote the colony formation after 10 d exposure ($P < 0.05$), and 40.0 mg/L lead(II) had the best effect. The proportion of cells in colonies and bEPS content of *M. aeruginosa* showed similar variation trends after 10 d exposure: increasing with the concentrations of lead(II) (Figure 5).

### DISCUSSION

Heavy metals could inhibit or stimulate microalgae growth depending on their concentrations in the algal media (Calabrèse & Baldwin 1999). Furthermore, *M. aeruginosa* showed overcompensatory growth under the stress of environmental factors, such as high temperature (Qin & Li 2010) and light.
limitation (Bi et al. 2011). We found that *M. aeruginosa* exposed to 20 and 40 mg/L lead(II) showed a marked over-compensatory growth after 2 d. Consistent with our results, overcompensatory growth was also observed in *Spirulina* sp. exposed to 20.00 mg/L lead (Chen & Pan 2005), suggesting most cyanobacteria might have high tolerance to the toxic effects of lead(II).

It is believed that EPS in cyanobacteria play a major role in protecting cells from various stresses in severe habitats and promoting the aggregation of *M. aeruginosa* cells into colonies (De Philippis et al. 1998; Yang et al. 2008). When stressed by heavy metals, algal cells could increase the secretion of EPS to adsorb heavy metals; meanwhile, heavy metals are beneficial to aggregation of EPS on the cell surface by the action of active positive ions (Lau et al. 2003). Consistent with previous studies (Sharma et al. 2008; Ozturk et al. 2010), lead-induced increases in the EPS production was observed in the present study. Until now, the genetics and biochemistry of EPS biosynthesis in cyanobacteria have not been thoroughly examined and, consequently, the information available is extremely limited (Yoshimura et al. 2007; Pereira et al. 2009). *M. aeruginosa* belongs to the prokaryotes and its EPS synthesis and secretion probably follow the EPS biosynthetic pathways in bacteria. When stressed by lead(II), *M. aeruginosa* may increase the EPS production via inducing the biosynthesis-related genes of EPS, such as *capD*, *csaB*, *tagH*, *epsL* (Pereira et al. 2009; Gan et al. 2012). In addition, low concentrations of heavy metals can improve photosynthetic activities of algae, and then increase the accumulation of EPS (Wang et al. 2002; Liang & Wang 2009). The increased bEPS conversely promote the attachment of the algal cell into colonies through chelating lead(II) with the cation-chelating binding sites of bEPS. Heavy metals, such as lead, cadmium, copper and zinc, were common pollutants in surface sediments of the *Microcystis* bloom, and could be released into the overlying water via sediment resuspension (Yu & Li 2010). Meanwhile, *M. aeruginosa* would be in a continuous heavy metals stress (Lin et al. 2003) and bound to improve the level of EPS production, which might play an important role in colony formation of *M. aeruginosa* in natural conditions.
A great amount of large colonies of *M. aeruginosa* floating on or near the water surface forms *Microcystis* blooms in natural conditions. However, we did not observe large colonies throughout the experiment, and low initial cell density (3.0 × 10⁶ ind/mL), short lead exposure time, the single sampling water layer or other factors might be responsible for this phenomenon. The results showed that lead(II) could significantly promote the colony formation, and there was a good positive correlation between the proportions of *M. aeruginosa* colony and the concentration of lead(II) after 10 d exposure (y = 0.7840x + 0.8120, R² = 0.9773). This suggested lead(II) could promote the colony formation of *M. aeruginosa* via stimulating bEPS production, which might be involved in the the bloom-forming of *M. aeruginosa* in natural conditions.

**CONCLUSIONS**

*M. aeruginosa* has high tolerance to the toxic effects of lead(II). Lead(II) could stimulate bEPS production, which conversely promoted colony formation, suggesting that heavy metals might be contributing to the bloom-forming of *M. aeruginosa* in natural conditions.

**ACKNOWLEDGEMENTS**

The financial support provided by National Natural Science Foundation of China (Grant No. 31170442) and Key Natural Science Foundation Grant of Tianjin (Grant No. 10JCYJJC18000) is gratefully acknowledged.

**REFERENCES**


Pereira, S., Zille, A., Micheletti, E., Moradas-Ferreira, P., De Philippis, R. & Tamagnini, P. 2009 Complexity of cyanobacterial exopolysaccharides composition, structures, inducing factors...
and putative genes involved in their biosynthesis and assembly. 

Pitois, S., Jackson, M. H. & Wood, B. J. 2001 Sources of the 
eturophtication problems associated with toxic algae: an 

Qin, H. J. & Li, D. H. 2013 Over-compensatory growth of 
Microcystis aeruginosa after high temperature stress. 
Environ. Sci. 31 (7), 1504–1508.

the annual cycle of the blue-green alga Microcystis 
293, 419–477.

Rippka, R. J., Deruelles, J., Waterbury, J. B., Herdman, M. & Stanier, 
R. 1979 Generic assignments, strain histories and properties of 

Romera, E., González, F., Ballester, A., Blázquez, M. L. & Muñoz, J. 
A. 2007 Comparative study of biosorption of heavy metals using 

Sharma, M., Kaushik, A., Somvir Bala, K. & Kamra, A. 2008 
Sequestration of chromium by exopolysaccharides of Nostoc 
and Gloeocapsa from dilute aqueous solutions. J. Hazard. 
Mater. 157, 315–318.

Urrutia, M. M. 1997 General bacterial sorption processes. In: 
Biosorbents for Metal Ions (J. Wase & C. Forster, eds). Taylor 

on photosynthetic system of Anabaena azotica Ley. 

Wang, W., Liu, Y. & Zhou, Y. 2010 Combined effects of nitrogen 
content in media and Ochromonas sp. grazing on colony 
formation of cultured Microcystis aeruginosa. J. Limnol. 69 
(2), 193–198.

Wu, Z. X. & Song, L. R. 2008 Physiological comparison between 
colonial and unicellular forms of Microcystis aeruginosa Kütz 
(Cyanobacteria). Phycologia 47, 98–104.

Wu, Z. X., Gan, N. Q., Huang, Q. & Song, L. R. 2007 Response of 
Microcystis to copper stress – do phenotypes of Microcystis 
make a difference in stress tolerance? Environ. Pollut. 147, 
324–330.

Yang, Z., Kong, F. X., Shi, X. L. & Cao, H. S. 2006 Morphological 
response of Microcystis aeruginosa to grazing by different 

Yang, Z., Kong, F. X., Shi, X. L., Zhang, M., Xing, P. & Cao, H. S. 
2008 Changes in the morphology and polysaccharide content 
of Microcystis aeruginosa (Cyanobacteria) during flagellate 

Yoshimura, H., Okamoto, S., Tsumuraya, Y. & Ohnori, M. 2007 
Group 3 sigma factor gene, sigJ, a key regulator of desiccation 
tolerance, regulates the synthesis of extracellular 
 polysaccharide in cyanobacterium Anabaena sp. strain 
PCC7120. DNA Res. 14, 13–24.

Yu, S. & Li, H. B. 2010 Perspectives on the release of heavy metals via 

of colony intercellular space in the Cyanobacteria bloom-

First received 28 July 2012; accepted in revised form 26 September 2012