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Copper-, Lead- and Mercury-Induced Changes in Maximum Quantum Yield, Chlorophyll A Content and Relative Growth of Three Malaysian Green Macroalgae

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ABSTRACT

In this study, we reported on the responses of three Malaysian green algae, *Caulerpa racemosa*, *Caulerpa lentillifera* and *Ulva reticulata* against three heavy metals, copper (Cu), lead (Pb) and mercury (Hg). Responses were determined as maximum quantum yield (F_v/F_m) for photosynthetic quantum efficiency, chlorophyll (chl) a content and relative growth measured as changes in fresh weight. The algae were exposed for 8h in two concentrations of metals, which were 1 mg/L and 2 mg/L. In general, all algae were severely affected by the presence of Hg compared to the other two metals. F_v/F_m of the algae was significantly reduced to the lowest of 18% in 1 mg/L Hg as observed in *C. racemosa* while the lowest for Cu and Pb was 29% and 41%, respectively, also observed in similar algal species. All algae showed an undetected value of F_v/F_m when concentration of Hg was increased to 2 mg/L. An increase in F_v/F_m was observed for *C. lentillifera* in 1 mg/L of Cu and Pb but the value showed a reduction when the concentration of both metals was increased to 2 mg/L. Among the algae, F_v/F_m of *C. racemosa* was severely affected by the presence of all metals particularly at 2 mg/L where it showed undetected value. An increase in the content of chl a was observed in *C. racemosa* and *C. lentillifera* for each metals at both concentrations while a decrease in the content was observed in *U. reticulata*. Algal relative growth was negatively affected by the presence of metals with Hg showed the strongest effect. However, some algae showed a positive effect of Pb on their growth.

| Heavy Metals | Green Algae | F_v/F_m | Chlorophyll a | Relative Growth |

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1. INTRODUCTION

One of the worldwide pollution concerns is the water contamination by heavy metal ions due to their possible toxic effects on aquaticorganisms. Industries contributed the most for the discharge wastes that lead to this environmental problem include mining and smelting of metalliferous compounds, surface finishing industry, energy and fuel production, fertilizer and pesticide industry, and electroplating [1]. These discharges can contaminate the marine ecosystems which are among the largest of the aquatic ecosystems. According to the 2011 Malaysia Environmental Quality Report [2], the main sources of heavy metals found in Malaysian marine waters were from oil and gas activities, coastal development activities, ports and land-based discharges. Among the metals monitored, copper (Cu) was found to be the most frequent metals found in the Malaysian marine waters, followed by lead (Pb) and mercury (Hg) [2].

The toxicity of metals and their compounds, however, largely depends on the mechanisms of uptake by the organisms through cell membranes, intracellular distribution, and binding to cellular macromolecules [3]. Thus, the effects may vary between metals.Cu, for example, is an essential component of enzymes involved in photosynthesis and respiration [4]. Elevated concentration of Cu can decrease the efficiency of photosystem II (PSII) [5] and can also lead to activation of oxidative damage [6]. Pb has no known biological function [7] but at high concentrations, it exerts adverse effects on morphology, growth and photosynthesis of some autotrophs [8]. Hg, on the other hand, is a unique metal in that it can be found in the environment in several physical and chemical forms. High levels of Hg in the form of Hg^{2+}, for example, have strong phytotoxic effects and when present in toxic concentrations can induce visible injuries and physiological disorders in cells triggering the production of reactive oxygen species (ROS) leading to cellular disruption [9].

These metals become a problem because they cannot be easily degraded or destroyed. Nevertheless, they can be removed from the contaminated water bodies. To remove these toxic ions, many techniques have been tried such as precipitation, filtration, ion exchange and membrane separation. However, not all of these methods work efficiently [10]. New separation methods are therefore, required to reduce heavy metal concentrations to environmentally acceptable levels at affordable cost. Bioremoval, the use of biological systems for the removal of metal ions from polluted waters, has the potential to contribute to the achievement of this goal [11].

The use of macroalgae to mitigate the heavy metal pollution problems in freshwater and marine ecosystem has

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been extensively studied [12]. This is due to their widedistribution, size, longevity, easy to identify andpresence at pollution site[13]. The fast-growth rate of some species of macroalgae can account for rapid nutrient removal from contaminated waters. Most of them are able to immobilize the metals to make them less toxic [14]. In addition, they have the ability to adsorb and metabolize trace metals due to their large surface:volume ratios, the presence of high-affinity, metal-binding groups on their cell surfaces, and efficient metal uptake and storage systems [15].

The aim of this study is to determine the effects of three most highly found heavy metals pollutants in Malaysian marine ecosystem, Cu, Pb and Hg on three species of the green macroalgae in terms of their maximum quantum yield, chlorophyll a content and relative growth. This study is a preliminary study on the potential use of macroalgaeas bioremediators as well as bioindicators of the metals-polluted waters.

2. EXPERIMENTAL

2.1 Algal Materials

The threegreen algae (i.e. Chlorophyceae) studied were *Caulerpa racemosa*, *Caulerpa lentillifera* and *Ulva reticulata*. All algae were collected from the coastal area of Port Dickson, Negeri Sembilan, Malaysia and further cultivated at the Marine Hatchery, Universiti Malaysia Terengganu in an open tank system. Prior to analysis, the algae were cleaned to get rid of unwanted materials or parasites.

2.2 Heavy Metals Treatment

For the heavy metals treatment, ~5 g of the alga was treated with 1 mg/L and 2 mg/L of copper(II) nitrate ($Cu(NO_3)_2$), lead(II) nitrate ($Pb(NO_3)_2$) and mercury(II) nitrate ($Hg(NO_3)_2$) for 8 h in aerated beakers under white light. The conditions used for control were similar as above but with no additional metals (i.e. untreated alga). Each experiment was done in triplicates.

2.3 Chlorophyll a Fluorescence Determination

Chlorophyll (chl) a fluorescence was measured with a handheld chl fluorometer, AquaPen-P AP-P 100 (Photon Systems Instruments, Czech Republic). At the start of the measurement, a short, red, actinic pulse (~3000 µmol m^{-2} s^{-1} at 655 nm) was prompted for 5 s to ensure a stabilized fluorescence emission during the following F_m measurement. Then, F_o was measured with a pulsed, blue measuring light (~900 µmol m^{-2} s^{-1}, 455 nm), and F_m was determined with a saturating white light pulse (~3000 µmol m^{-2} s^{-1}). The maximum quantum yield, F_v/F_m was calculated as $(F_m-F_o)/F_m$.

2.4 Chlorophyll a Content Determination

To determine the chl a content of the alga, the alga (~0.5-0.6 g) was incubated in 5 mL of dimethylformamide (DMF) for 5 days at 4˚C in darkness. After 5 days, the absorbance of the DMF extract was measured at 664.5 and 647 nm using DMF as blank. The chl a content was measured according to a formula by Inskeep and Bloom [16]:

$$\text{Chl a (mg/L)}: 12.7*A_{664.5nm} - 2.79*A_{647nm}$$

The value of the chl a content was in the unit of mg/g fresh weight (FW) of alga.

2.5 Relative Growth Determination

Treated and control algae were gently blotted and weighed before and after treatment. Relative growth was then calculated as: Final FW (g)÷Initial FW (g).

2.6 Statistical Analysis

Values of all parameters tested were related to 100% of controls for better comparison. Mean values and standard deviation were determined from three replicates of each treatment. The statistical significance of differences among means was calculated according to a one-way ANOVA followed by Tukey's HSD post-hoc test. A probability level of $p<0.05$ was applied. All statistical analyses were done using Daniel's XL Toolbox (v. 5.08) for Microsoft Excel.

3. RESULTS & DISCUSSION

3.1 Maximum Quantum Yield, F_v/F_m

The photosynthetic efficiency of the algae was affected by the presence of metals as shown by a decrease in F_v/F_m (Figure 1.0). In general, all the algae were greatly affected by the presence of Hg with 82% F_v/F_m reduction in *C. racemosa*, 47% reduction in *C. lentillifera* and 13% reduction in *U. reticulata* at 1 mg/L (Figure 1.0a). The effect was more severe at 2 mg/L whereby all the algae showed undetected value of F_v/F_m (Figure 1.0b). In particular, F_v/F_m of *C. racemosa* was severely affected by all metals at 2 mg/L.At the physiological level, the measurement of F_v/F_m is an effective parameter to assess the photosynthetic status particularly the PSII of the alga under stress in which a reduction in this parameter indicates that the alga has been exposed to stress [17]. Measurements of F_v/F_m provide a first insight into changes of the photosynthetic apparatus upon the action of the metals [18] and can reveal the mechanisms involved in metals toxicity [19].

It is known that heavy metals could seriously affect the photosynthetic apparatus by irreversibly binding the components of photosynthetic electron transport chain. For example, Cu and Pb are able to substitute Mg in the centre

of chl molecule leading to termination of photosynthesis activity by forming nonfluorescent inactive metals-substituted chl [20]. In addition, Cu can reduce or inactivate the rate of photosynthetic electron transport of algae either by destructing the photosynthetic carbon reduction cycle [21] by modifying the structure of oxygen-evolving complex of PSII [22] or inhibiting electron transfer within PSII [23]. Pb, on the other hand, can decrease photosynthetic rate by distorting chloroplast ultrastructure, obstructing electron transport, and inhibiting activities of Calvin cycle enzymes [24]. An increase in Hg content inhibits electron transfer from Q_A^- to Q_B, resulting in a significant increase in the proportion of the Q_B-non-reducing PSII reaction centres [25]. Lu *et al.*[25] also suggested that PSII reaction centres were the sites for Hg-induced damage. This suggestion was further supported by a study of Kukarskikh *et al.*[26] which observed an increase in the steady-state level of P700 photo-oxidation indicating a disturbance in electron transfers between photosystems as well as an increase in fraction of closed reaction centres leading to reduction in non-photochemical quenching process. Toxic effects of metals appear to be partly related to the production of ROS as well, which can cause oxidative damage to cells [27].

An interesting response towards Cu and Pb toxicity was shown by *C. lentillifera*whereby the value of F_v/F_mrose to 128% and 166% in 1 mg/L Cu and Pb, respectively (Figure 1.0a).The value, however, had lessen when concentrations of the metals was increased to 2 mg/L (Figure 1.0b). An increase in F_v/F_m of this alga may indicate that the alga was tolerant to high concentration of both metals, particularly towards Pb.The alga may have triggered some sort of mechanism that plays a role in metal homeostasis by balancing between the metal requirements and oxidative damage that may be caused by high accumulation of the metals [28]. The increase in F_v/F_m also coincides with the increase in chl a content (Figure 2.0) and growth (Figure 3.0).

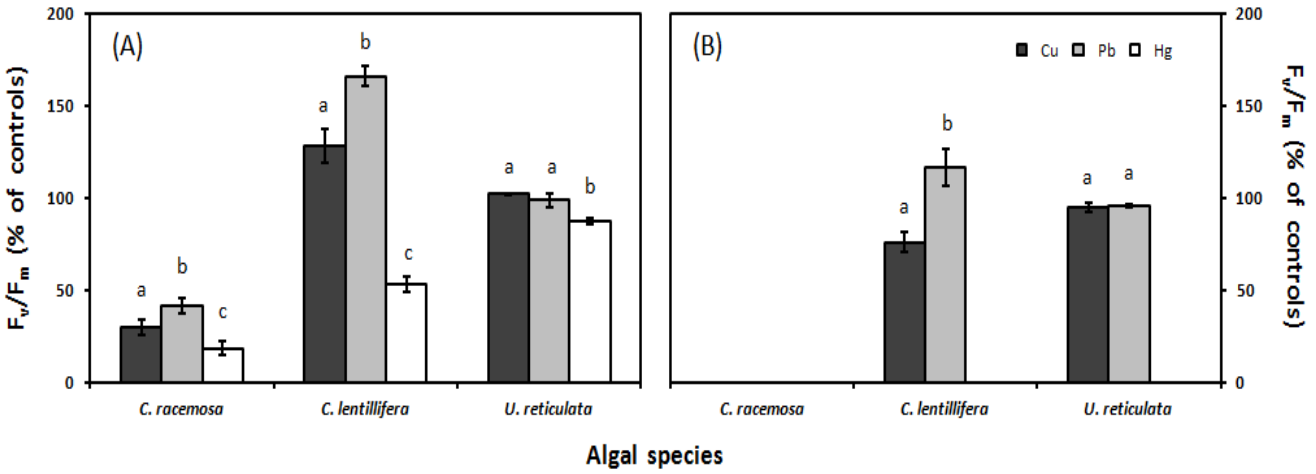

Fig. 1. Maximal quantum yield (F_v/F_m) of the algae after 8h treatment with 1 mg/L (*A*) and 2 mg/L (*B*) of copper (Cu, *black bars*), lead (Pb, *grey bars*) and mercury (Hg, *white bars*). Data are mean±SD values. Different letters above bars indicate statistically significant difference at p<0.05 between metals within similar algal species.

3.2 Chlorophyll A Content

Several trends in the effect of metals on chl a content were observed (Figure 2.0). At 1 mg/L, all metals induced the production of chl a in *C. racemosa* and *C. lentillifera* but inhibited the production of chl a in *U. reticulata* (Figure 2.0a). Increasing the amount of metals to 2 mg/L had a negative effect on the chl a of *C. racemosa* and *C. lentillifera* but did not seemed to have an effect on chl a of *U. reticulata* (Figure 2.0b). Shakya *et al.*[6] and Rekha *et al.* [29] stated that heavy metals have an ability toreduce chl production by inhibiting its biosynthesis. This effect is accomplished through the interaction of the metal with functional sulfhydryl (-SH-) groups of the enzymes in the chl biosynthetic pathway [29]. High concentrations of Cu may also induce oxidative damage that can alter the cell membrane properties thereby demonstrating the inhibitory effect on the enzymes involved in chl production [7]. Cu, Pb and Hg which have the ability to substitute magnesium ion (Mg^{2+}) at the centre of chl molecule, is an important damage mechanism because it prevents the process of light harvesting which directly affects photosynthesis [20, 30]. This may also explains the reduction in F_v/F_m observed for the algae as shown in Figure 1.0.

The stimulation of chl awas also observed by Knauer *et al.*[31] and Janssen and Heijrick [32] at low concentration of metals. In contrast, Bossuyt and Janssen [5] observed a significant increase in chl aat higher concentrations of metals in a freshwater green alga *Pseudokirchneriella subcapitata*. Soto *et al.*[33] on the other hand, reported that the chl a is increased at lower concentration of Cu but decrease when the concentration is increased. This was also

observed in our study with *C. racemosa* and *C. lentillifera*. In a study by Han *et al.*[34], an increase in chl a was observed in the green alga *Ulva armoricana* exposed to 100 µg/L Cu with no reduction in F_v/F_m but a decrease in relative growth rate. This observation supports the idea that there was an exchange between energetic resources being used for pigment biosynthesis and growth. Similar responses were also shown by *C. lentillifera* in our study with 1 mg/L of Cu. More generally, significant increases in chl have been found to occur in response to a range of environmental stresses and are associated with stress resistance [35]. The results obtained in this study between F_v/F_m and chl a content also showed that chl a content of the algae did not reflect the fluorescence yield. The disparity observed may be due to chl a affected was mostly the component of photosystem I (PSI) since PSI was consisted entirely of chl a while chl fluorescence only probes the PSII [34].

3.3 Algal Relative Growth

The growth of the algae was stunted in the presence of Hg at both concentrations (Figure 3.0). According to Mor *et al.* [36] and Zhou *et al.* [37], the reduction in growth by Hg could be due to blocking of cell division or elongation. Furthermore, Israr *et al.*[38] reported that extra energy from metabolism may be needed by the cells to cope with the high accumulation of Hg in the cells, leading to reduction in biomass. In addition, Hg can trigger oxidative stress that was responsible for the disturbances that lead to reduction in cell growth [39]. Presence of Cu also inhibited the growth of the algae and this effect was higher at 2 mg/L (Figure 3.0b) than at 1 mg/L (Figure 3.0a). Contrastingly, Pb induced the growth of *C. lentillifera* at both concentrations and *C. racemosa* at 2 mg/L while inhibited the growth of *C. racemosa* at 1 mg/L and *U. reticulata* at 2 mg/L. One mg/L of Pb, however, had no effect on growth of *U. reticulata*.As reported by Fernandes and Henriques [40], effect of Cu^{2+} on growth of plants and algae has been attributed to a massive failure of many cellular processes. Cu-induced interference with cell division and/or expansion has been proposed as a possible reason for the observed reduction in growth [41] which may be linked to a decrease turgor and/or a change in cell wall elasticity due to Cu toxicity [42]. Pb, on the other hand, can disturb microtubule organization in meristematic cells of plants [43]. The reduction in plant growth during stress may also be due to low water potential, hampered nutrient uptake and secondary stress such as oxidative stress [44]. Scheidegger *et al.*[45] observed that there was no inhibitory effect of Pb on growth of *Chlamydomonas reinhardtii* for short-term exposure but upon long-term exposure, Pb inhibit almost 100% of the algal growth. They concluded that upon long-term exposure, Pb binds non-specifically to functional groups of proteins containing sulphur or oxygen, inducing various effects that may affect growth as well as photosynthesis.

An increase in the number of cells and cell division of a green alga,*Chlorella vulgaris* in the presence of low concentration of Pb was observed by Falkowska *et al*. [46]. They suggested that the ability of the alga to adapt to the low level Pb was due to protective characteristic of gibberelic acid, a phytohormone that plays an important role in growth and metabolism of plants and algae. However, this hormone fails to minimize the toxicity effect at high concentrations of metals. This may explain why there is an increase in growth for all algae studied at 1 mg/L but a reduction in growth at 2 mg/L as shown by *U. reticulata*.

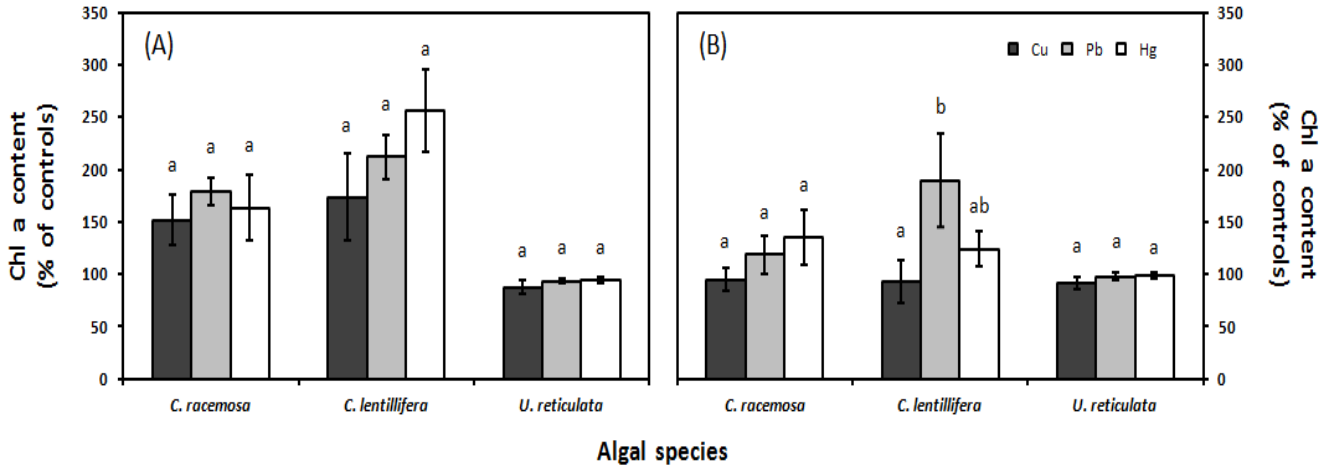

Fig. 2. Chlorophyll (chl) a content of the algae after 8h treatment with 1 mg/L (*A*) and 2 mg/L (*B*) of copper (Cu, *black bars*), lead (Pb, *grey bars*) and mercury (Hg, *white bars*). Data are mean±SD values. Different letters above bars indicate statistically significant difference at $p<0.05$ between metals within similar species and treatments.

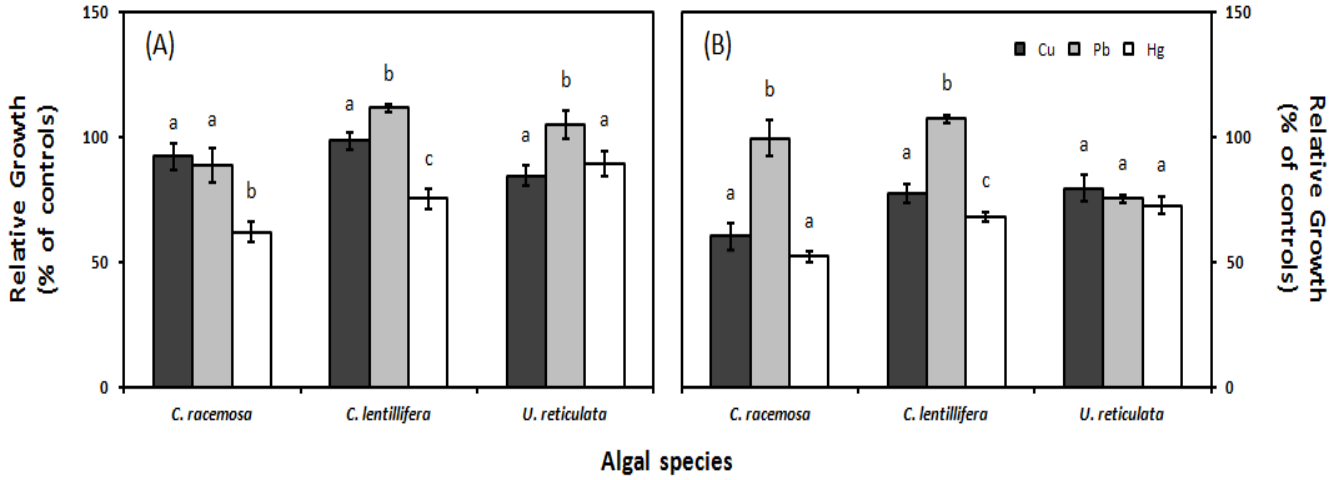

Fig. 3. Relative growth of the algae after 8h treatment with 1 mg/L (*A*) and 2 mg/L (*B*) of copper (Cu, *black bars*), lead (Pb, *grey bars*) and mercury (Hg, *white bars*). Data are mean±SD values. Different letters above bars indicate statistically significant difference at $p<0.05$ between metals within similar species and treatments.

4. CONCLUSION

The heavy metals investigated in this study demonstrate a range of different stress effects on the algae. The responses towards the metals toxicity also differ between different algal classes as well as within similar genus but different species suggesting that different mechanisms were triggered by the algae to overcome the metals toxicity effect. Decreases in chl a fluorescence and content as well as relative growth indicate the sensitivity of the algal photosynthetic as well as metabolism processes for the three metals. All the algae were more sensitive towards Hg than Cu and Pb. The parameter F_v/F_m can be used as a more useful biomarker for metals toxicity effect than chl a content and growth. *C. lentillifera* showed a promising candidate as bioremediator as well as bioindicator among the algae studied especially for Pb contamination since it shows positive responses even though at high concentrations. However, further analyses will be done in order to understand more about the underlying mechanisms that generate these results.

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REFERENCES

[1] J. Wang, and C. Chen, Biotech. Adv., 27 (2009), 195-226.
[2] Department of Environment, Malaysia (DOE), Malaysia Environmental Quality Report 2011, 2011, 87 pp.
[3] D. Beyersmann, and A. Hartwig, Arch. Toxicol., 82 (2008), 493-512.
[4] L.R. Andrade, M. Farina, and G.M. Amado Filho,Ecotoxicol. Environ. Safe.,58 (2004),117–125.
[5] B.T.A. Bossuyt, and C.R. Janssen, Aquat. Toxicol., 68 (2004),61-74.
[6] K. Shakya, M.K. Chettri, and T. Sawidis,Arch. Environ. Contam. Toxicol., 54 (2008), 412-21.
[7] C. Lamai, M. Kruatrachue, P. Pokethitiyook, E.S. Upatham,and V. Soonthornsarathool,Sci. Asia,31 (2005), 121–127.
[8] B. Pawlik-Skowronska,Environ. Pollut.,119 (2002),119–227.
[9] R. Azevedo, and E. Rodriguez,J. Bot., vol. 2012, Article ID 848614, 6 pages. doi:10.1155/2012/848614.
[10] R. Ofer, A. Yerachmiel, and Y. Shmuel, Water Environ. Res. 75 (2003), 246-251.
[11] S. Klimmek, H.J. Stan, A. Wilke, G. Bunke, and R. Buchholz,Environ. Sci. Technol., 35 (2001), 4283-4288.
[12] P. Ostapczuk, M. Burow, K. May, C. Mohl, M. Froning, B. Subenbach, E. Waidmann, and H. Emons, Chemosphere, 34 (1997),2049-2058.
[13] A.A. Al-Homaidan, A. Al-Ghanayem, andA.H. Alkhalifa, Intern. J. Wat. Res. Ar. Environ.,1 (2011),10-15.
[14] I. Sánchez-Rodríguez, M.A. Huerta-Diaz, E. Choumiline, O. Holguin-Quinones, and J.A. Zertuche-Gonzalez,Environ. Pollut., 114 (2001),145–160.
[15] S. Rajamani, S. Siripornadulsil, V. Falcao, M. Torres, P. Colepicolo and R. Sayre, Adv. Exp. Med. Biol. 616 (2007), 99-109.
[16] W.P. Inskeep, and P.R. Bloom,Plant Physiol., 77 (1985), 483-485.
[17] K. Maxwell, and G.N. Johnson, J. Exp. Bot., 51 (2000), 659-668.
[18] K. Sbihi, O. Cherifi, A. El gharmali, B. Oudra, and F. Aziz,J. Mater. Environ. Sci.,3 (2012), 497-506.
[19] A.J. Miao, W.X. Wang, and P. Juneau,Environ. Toxicol. Chem., 24 (2005), 2603-2611.
[20] H. Küpper, I. Sétlík, M. Spiller, F.C. Küpper, and O. Prásīl, J. Phycol., 38 (2002), 429-441.
[21] E. Mateos-Naranjo, S. Redondo-Gomez, J. Cambrolle, and M.E. Figueroa,Mar. Environ. Res.,66 (2008), 459–465.
[22] I. Yruela, M. Alfonso, M. Baron, and R. Picorel,Physiol. Plant., 110 (2000), 551–557.
[23] S. Connan, and D.B. Stengel, Aquat. Toxicol., 104 (2011), 97-107.
[24] R.S. Sengar, M. Gautam, R.S.Sengar, S.K. Garg, K. Sengar, and R. Chaudhary, Rev. Environ. Contam. Toxicol., 196 (2008), 73-93.
[25] C.M. Lu, C.W. Chau, and J.H. Zhang, Chemosphere,41 (2000), 191-196.

[26] G.L. Kukarskikh, E.E. Graevskaia, T.E. Krendeleva, K.N. Timofeedv, and A.B. Rubin, Biofizika, 48 (2003), 853-859.
[27] I. Szivák, R. Behra, and L. Sigg,J. Phycol., 45 (2009), 427-435.
[28] S. Shcolnick, and N. Keren,*Plant Physiol.*,141 (2006),805-810.
[29] P.S. Rekha, and S.A. Mastan,J. Herb. Med. Toxicol., 5 (2011), 47-50.
[30] Z. Krupa, and T. Baszynski, Acta Physiol. Plant., 17 (1995), 177-190.
[31] K. Knauer, R. Behra, and L. Sigg,Environ. Toxicol. Chem.,16 (1997), 220- 229.
[32] C. Janssen, and D. Heijrick,Environ. Contam. Toxicol., 178 (2003), 23-52.
[33] P. Soto, H. Gaete, and M.E. Hidalgo,Lat. Amer. J. Aquat. Res.,39 (2011), 280-285.
[34] T. Han, S.H. Kang, J.S. Park, and H.K. Lee,Aquat. Toxicol., 86 (2008), 176-194.
[35] X. Zhang, E.H. Ervin, and R.E. Schmidt,Hortscience, 130 (2005), 836–841.
[36] I.R. Mor, S.J. Gokani, and S.V. Chanda,J. Plant Nutr.,25 (2002), 843-860.
[37] Z.S. Zhou, K. Guo, A.A. Elbaz, and Z.M. Yang,Environ. Exp. Bot.,65 (2009), 27–34.
[38] M. Israr, S. Sahi, R. Datta, and D. Sarkar, Chemosphere,65 (2006), 591–598.
[39] A. Elbaz, Y.Y. Wei, Q. Meng, Q. Zheng, and Y.Z. Yang, Ecotoxicol., 19 (2010), 1285–1293.
[40] J.C. Fernandes, and F.S. Henriques, Bot. Rev., 57 (1991), 246-273.
[41] W.S. Jiang, D.H. Liu, and W.Q. Hou, Bioresource Tech., 76 (2001), 9-13.
[42] M.T. Brown, and J.E. Newman, Aquat. Toxicol., 64 (2003), 201-203.
[43] S.O. Eun, H.S. Youn, and Y. Lee, Physiol. Planta., 110 (2000), 357-365.
[44] R. John, P. Ahmad, K. Gadgil, and S. Sharm,Intern. J. Plant Prod., 3 (2009), 65-76.
[45] C. Scheidegger, R. Behra, and L. Sigg, Aquat. Toxicol., 101 (2011), 423-429.
[46] M. Falkowska, A. Pietryczuk, A. Piotrowska, A. Bajguz, A. Grygoruk, and R. Szerpak, Polish J. Environ. Stud. 20 (2011), 53-59.