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Accumulation of Cu(II) and Pb(II) in Three Rhodophytes of the Genus *Gracilaria* and the Impact of the Metals on the Algal Physiology

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Abstract

The accumulation of two heavy metals, copper (Cu(II)) and lead (Pb(II)) in the tissues of three *Gracilaria* species (Rhodophyta): *G. edulis, G. manilaensis* and *G. salicornia* and the impacts of the metals on the algal maximal quantum yield (i.e. F_v/F_m), relative growth and chlorophyll (chl) a content were studied. The algae were exposed to 1 mg L^{-1} of the metals individually for 8 hrs. Results showed that the three species of *Gracilaria* reacted differently against the two metals. For every kilogram of thallus, > 1000 mg of Cu(II) and Pb(II) was collected for *G. edulis* and *G. manilaensis*. Furthermore, both algae has a Bioconcentration Factor (BCF) value of >1 for both metals. *G. salicornia*, however, collected < 1000 mg Cu(II) and Pb(II) for every kilogram of thallus, and has a BCF value of >1 for Cu(II) and < 1 for Pb(II). These values indicate that all algae are good accumulators of Cu(II) while *G. edulis* and *G. manilaensis* are good accumulators of Pb(II) but *G. salicornia*, on the other hand, is an excluder of Pb(II). There was a reduction of the algal F_v/F_m in both metals, with the highest reduction observed for *G. manilaensis* in Cu(II). Relative growth of the algae was also reduced in both metals. Cu(II) induced the synthesis of chl a in *G. edulis* and *G. salicornia* but inhibited chl a synthesis in *G. manilaensis* while Pb(II) induced the production of chl a in all algae.

Keywords

Gracilariaceae, Heavy Metals, Quantum Yield, Relative Growth, Bioconcentration Factor

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1. Introduction

The increase in industrial activities has caused many water bodies receiving loads of heavy metals that exceed the maximum permissible limit for wastewater discharge designed to protect the environment [1]. To make matter worse, the heavy metals in the wastewater can be directly discharges into coastal waters. Pollution by metal ions, including copper (Cu^{2+}) and lead (Pb^{2+}), has become a major issue due to their possible toxic impacts [2]. These metals are known to inhibit the photosynthetic process of plants and other autotrophs. Increased concentration of Cu, for instance, results in chlorosis and reduced growth of two brown seaweeds [3]. Pb exposure damaged the structure and function of photosystem II (PSII) in the aquatic plant *Spirodela polyrrhiza* [4]. The metals can become a problem because they cannot be easily degraded or destroyed. Fortunately, they can be removed from contaminated waters. Since most conventional methods are neither effective nor economical, new separation methods are required to reduce heavy metal concentrations to environmentally acceptable levels and is affordable. Bioremediation, a process which uses organisms, such as microalgae, to return the natural environment altered by pollutants to its original state has thus, the potential to contribute to the achievement of this goal [5].

The ability of macroalgae to respond to its environment,

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make them an efficient tool for bioremediation. Macroalgae like any other autotrophs require inorganic nutrients for growth. The fast-growth rate of some species of macroalgae can account for rapid nutrient removal from marine waters. Most of them are able to immobilize the metals to make them less toxic [6]. They also 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 [7]. In addition, macroalgae play a major role in marine ecosystems. As the first organism in marine food chains, they provide nutrients and energy for animals as well as provide shelter and habitat for many coastal animals.

Thus, the aim of this study is to investigate the impacts of two most highly found heavy metals pollutants in Malaysian marine ecosystem, Cu(II) and Pb(II) on three species of *Gracilaria* (Rhodophyta), *G. edulis, G. manilaensis* and *G. salicornia* in terms of their dark-adapted quantum yield, relative growth, chlorophyll (chl) a content and their ability to accumulate metals in their tissues. This study is a preliminary study on the potential use of *Gracilaria* as a bioremediator of the metals-polluted waters.

2. Materials and Methods

2.1. Algal Samples and Heavy Metals Treatment

Gracilaria edulis and *G. manilaensis* were collected from the coast of Kuala Muda, Kedah, Malaysia while *G. salicornia* was collected from the coast of Port Dickson, Negeri Sembilan, Malaysia. The algal samples were brought back in tanks containing seawater and further cultivated at the Marine Hatchery, Universiti Malaysia Terengganu in an open tank system. Prior to treatments, the algae were cleaned to get rid of unwanted materials or parasites.

About 5 g of the algae were treated with 1 mg L^{-1} of copper(II) nitrate ($Cu(NO_3)_2$), and lead(II) nitrate ($Pb(NO_3)_2$) for 8 hrs in aerated beakers containing filtered seawater under white light. Conditions of controls were similar to that of treated algae but with no metals added.

2.2. Determination of Algal Metals Uptake

The concentration of the metals in the thalli of the algae and treatment medium was determined using the Spectra AA 220FS atomic absorption spectrophotometer (AAS) (Varian Inc., USA) according to the manufacturer's protocol. A series of Cu(II) and Pb(II) concentrations (i.e. 1-5 mg L^{-1}) were used as standards and the concentration of the metals in the samples were determined from the standard curves obtained. Bioconcentration factor (BCF) was determined to quantify the metal's accumulation efficiency in the algae by comparing the concentration in the thalli and external medium using the formula by [8] (Equation 1):

$$BCF = C_a/C_s \quad \text{Eq (1)}$$

where, C_a and C_s are heavy metal concentrations in algal thalli (mg kg^{-1}) and in medium (µg L^{-1}), respectively.

2.3. Determination of Algal Chl a Fluorescence

Chl a fluorescence was measured in terms of the maximal quantum yield or F_v/F_m 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 maximal quantum yield was then calculated as Equation 2:

$$F_v/F_m\ [r.u.] = (F_m-F_o)/F_m \quad \text{Eq (2)}$$

2.4. Determination of Algal Relative Growth

For the relative growth (RG) experiment, treated algae were gently blotted and weighed before and after treatment. Percentage of RG was then calculated as Equation 3:

$$RG\ [\%] = (\text{Final FW (g)} \div \text{Initial FW (g)}) * 100\% \quad \text{Eq (3)}$$

2.5. Determination of Algal Chl a Content

Chl a content was determined by incubating the alga (~0.5-0.6 g) 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 [9] (Equation 4):

$$\text{Chl a (mg/L)}: (12.7*A_{664.5nm} - 2.79*A_{647nm}) \quad \text{Eq (4)}$$

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

2.6. Statistical Analyses

Values of all the parameters tested were converted to 100% of controls for better comparison unless otherwise stated. Mean values and standard deviation were determined from three replicates of each treatment. The statistically significant differences between the metals were analyzed using a one-way ANOVA followed by Tukey HSD *post-hoc* test at probability level of 0.05. The statistical software used was Daniel's XL Toolbox v. 6.53 Add-in for Microsoft Excel.

3. Results and Discussion

The algae showed a positive uptake of metals into their tissues. Cu(II) was observed to be more preferred than Pb(II) by *G. edulis* (p=0.001), *G. manilaensis* (p=0.026) and *G. salicornia* (p=0.000) to be translocated within the thalli (Table 1). Similar results were observed by [10] and [11] on several macroalgae species in which accumulation occurred in the order of Cu > Pb. This behavior can be explained by the fact that Cu is an essential micronutrient for the plants as well as algae which is required for several important metabolic and physiological processes [12]. However, at high concentrations, Cu can be toxic to the algae [13]. Comparatively, Pb is not an essential element for biological processes, but it can be easily absorbed and accumulated in different parts of the organism [14]. However, at high concentrations, Pb can exert negative impacts on the algal morphology, growth and photosynthesis [15] as also shown in this study. F_v/F_m (Fig 1A) and growth (Fig 1B) of the algae were reduced in the presence of high concentration of Pb in the thalli (Table 1). These results were observed for Cu as well.

Table 1. Mean (±SD) of Cu(II) and Pb(II) in medium and algal biomass, and bioconcentration factor (BCF) values of treated *Gracilaria*.

Algal Species	Medium samples (μg L^{-1})		Algal samples (mg kg^{-1})		BCF	
	Cu(II)	Pb(II)	Cu(II)	Pb(II)	Cu(II)	Pb(II)
G. edulis	373 ± 47	340 ± 82	2360 ± 128	1538 ± 123	6.4	4.7
G. manilaensis	300 ± 17	987 ± 98	1841 ± 208	1083 ± 317	6.2	2.0
G. salicornia	424 ± 35	500 ± 30	783 ± 92	169 ± 45	1.8	0.3

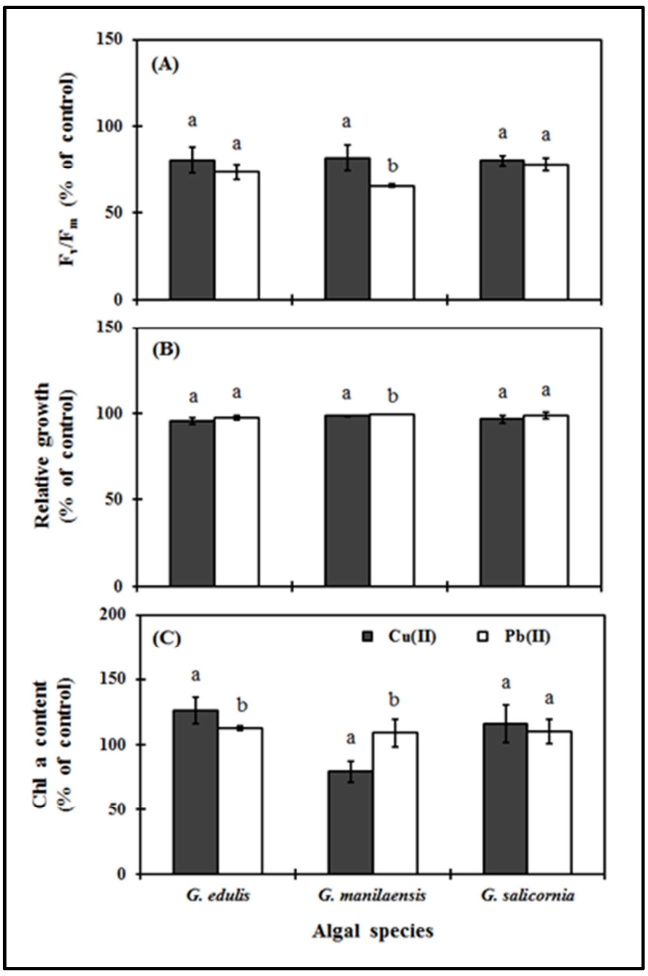

Fig. 1. F_v/F_m (*A*), relative growth (*B*) and chl a content (*C*) of the three *Gracilaria* after 8 hrs treatment with Cu(II) (*black bars*) and Pb(II) (*white bars*). Different letters above bars indicate statistically significant differences at p<0.05 between metals within similar species.

According to [16], plants that can be categorized as good metal accumulators are plants that can accumulate > 1000 mg kg^{-1} of Cu or Pb. Thus, it can be said that *G. edulis* and *G. manilaensis* are good accumulators but not for *G. salicornia* (Table 1). As reported by [17], BCF is regarded as a better indicator to classify a particular plant as a good accumulator because BCF takes into account the trace element concentrations in the solution. Furthermore, BCF value can be used to estimate a potential for phytoremediation purposes [18]. Three categories of heavy metal accumulation are proposed based on BCF [17]: < 1 excluder, > 1 metal accumulator, and, >1000 a good metal accumulator. Since *G. edulis* and *G. manilaensis* in this study has BCF value of > 1 for Cu and Pb, they can be considered as metal accumulator, thus, has the potential in bioremediation of both metals. *G. salicornia*, on the other hand, can be considered as a Cu accumulator and a Pb excluder.

The maximal quantum yield (i.e. F_v/F_m) of the algae was affected by the presence of the metals (Fig 1A). F_v/F_m of *G. edulis* and *G. salicornia* in Cu and Pb was reduced by more than 10%. Both metals however, showed a significant difference in their impact on F_v/F_m of *G. manilaensis*. The alga was more affected by Pb(II) than Cu(II) with a reduction of 34% and 18%, respectively. According to [19], F_v/F_m can be used as a sensitive indicator of plant's photosynthetic performance. A reduction in F_v/F_m is often used to indicate stress in plants which frequently occurred when plants are exposed to abiotic and biotic stresses in the light [20]. Thus, the reduction in F_v/F_m of the algae in this study may indicate that the algae were stressed by the presence of the metals.

The metals may in some ways disturb or damage the photosynthetic apparatus of the algae resulting in the decline in the quantum yield of PSII photochemistry [21]. In a study by [22], they observed that there was a strong decreased in photosynthesis-related genes including key proteins in electron transport chain (ETC) which possess iron-sulfur

clusters after exposure to high amount of Cu. Cu can displaced the iron atoms in the iron-sulfur clusters and block the electron transport. In addition, [23] stated that Cu may indirectly affect the photosynthetic apparatus through interference of Cu with calcium (Ca) ions especially at the lumen side of PSII. Pb, on the other hand, is known to accumulate in PSII and damage its secondary structure while decreasing the absorbance of visible light, inhibited energy transfer within the PSII protein–pigment complex, and reduced energy transport to chl a [4]. Additionally, Pb inhibits the electron transfer process at the PSI donor side, without recovery after the removal of the metal stress [24]. Contrastingly, in a study by [25] on a green alga, *Caulerpa lentillifera*, they observed that 1 mg L^{-1} Cu and Pb showed a positive impact on F_v/F_m of the alga where the value significantly increased to more than 25%. Toxic impacts of metals appear to be partly related to the production of reactive oxygen species (ROS) as well, which can cause oxidative damage to cells [26] including the components of photosynthetic apparatus [22, 27]. For example, activity of ascorbate peroxidase was observed to be significantly higher than catalase when *G. manilaensis* was exposed to Cu and Pb [28]. This observation showed that Cu and Pb trigger the production of high amount of H_2O_2 in chloroplasts and cytosol of the algal cells compared to that in either the mitochondria or peroxisome.

Both metals have no significant impact on the growth of the algae (Fig 1B). Cu(II), however, seemed to inhibit the growth of all the algae compared to Pb(II). A significant loss in weight, for example, was observed for *G. manilaensis* in Cu(II) with a reduction of 1.2% compared to 0.4% for Pb. According to [11], reduction in heavy metal treated algae may be due to deviation of metabolic pathways towards protection of the photosystem apparatus as a physicochemical strategy to chelate to prevent the entry of metal into the cell. Reduction in growth observed for Cu may be due to interference with cell division and/or expansion [22, 29] which can be linked to a decrease turgor and/or a change in cell wall elasticity [30]. Decreased in growth might be associated with the inhibition of mitotic index noticed with Pb metal treatment [31]. In a study by [32], they concluded that upon long-term exposure, Pb can bind non-specifically to functional groups of proteins which contain either sulfur or oxygen, inducing various impacts that may affect growth as well as photosynthesis. The reduction in plant growth during metal stress may also be due to low water potential, hindered nutrient uptake as well as accumulation of ROS [33].

In general, there was an induction in chl a after treatment with both metals in all the algae except for *G. manilaensis* in Cu(II) (Fig 1C). In *G. edulis,* the content of chl a was higher in Cu(II) (i.e. 26% increase) compared to Pb(II) (i.e. 12% increase). In comparison, Cu(II) and Pb(II) produced contradictory impacts in *G. manilaensis*. While Cu(II) inhibits the production of chl a in this alga, Pb(II) induces the synthesis of chl a. A more or less similar effect of both metals was observed for *G. salicornia.* Stimulation of chl a in algae was also observed by [34] and [35]. As reported by [36], significant increases in chl have been found to occur in response to a range of environmental stresses and can be correlated with stress resistance. Normally, heavy metals have been known to reduce chl production. It was suggested that heavy metals could interfere with chl biosynthesis either through the direct inhibition of enzymatic steps or through the substitution of the central Mg ion [37], [38]. High concentrations of Cu, for example, may induce oxidative damage that can inhibit the enzymes involved in chl production [39]. 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 PSI since PSI was consisted entirely of chl a while chl fluorescence only probes the PSII [40].

4. Conclusion

The different metals stress responses of the algae observed indicate that different mechanisms may be employed by the algae to counterattack the impact of the metals. Reductions in chl fluorescence, relative growth as well as changes in chl a content indicate the sensitivity of *Gracilaria* photosynthetic metabolism and other cellular processes for Cu(II) and Pb(II). PSII efficiency of the algae was more sensitive towards Pb(II) than Cu(II) while the growth and chl a content was more sensitive towards Cu(II) than Pb(II). A reduction in F_v/F_m may indicate stress in the algae with damage or interference to the PSII or any other components of photosynthetic apparatus. However, the impacts on the chl a fluorescence yield do not reflect their chl a content. Both metals induce chl a synthesis as a resistance mechanism in the algae except for Cu(II) which inhibits the production of *G. manilaensis* chl a. Both metals also affects the growth of the algae but not as much as F_v/F_m or chl a content indicating that growth is not a suitable biomarker for Cu(II) and Pb(II) impacts on these three *Gracilaria*. The observed changes in F_v/F_m, relative growth and chl a content may be due to high accumulation of both metals in the thalli. BCF value of >1 indicates potential of the algae as bioremediator of Cu(II) and Pb(II) except for *G. salicornia* which is not a good candidate for bioremediation of Pb(II). Further analysis will be done in order to understand more about the underlying mechanisms that generate these results and finally to be able to find which of these algae is the best bioremediator for Cu- and Pb-polluted waters.

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References

[1] Iqbal, M. and Saeed, A. (2007). Production of an immobilized hybrid biosorbent for the sorption of Ni (II) from aqueous solution. *Process Biochemistry*, 42: 148-157.

[2] Aydin, H., Bulut, Y. and Yerlikaya, C. (2008). Removal of copper (III) from aqueous solution by adsorption onto low-cost adsorbents. *Journal of Environmental Management*, 87: 37-45.

[3] Connan, S. and Stengel, D. B. (2011). Impacts of ambient salinity and copper on brown algae: 1. Interactive impacts on photosynthesis, growth, and copper accumulation. *Aquatic Toxicology*, 104, 97-107.

[4] Qufei, L. and Fashui, H. (2009). Impacts of Pb^{2+} on the structure and function of photosystem II of *Spirodela polyrrhiza. Biological Trace Element Research*, 129: 251-260.

[5] Zayadan, B. K., Sadvakasova, A. K., Hassan, M. M. and Beisenova, A. Z. (2013). Bioremediation of heavy metal contaminated water by microalgae. *International Journal of Biology and Chemistry*, 5: 32-35.

[6] Sánchez-Rodríguez, I., Huerta-Diaz, M. A., Choumiline, E., Holguin-Quinones, O. and Zertuche-Gonzalez, J. A. (2001). Elemental concentrations in different species of seaweeds from Loreto Bay, Baja California Sur, Mexico: implications for the geochemical control of metals in algal tissues. *Environmental Pollution*, 114:145–160.

[7] Rajamani, S., Siripornadulsil, S., Falcao, V., Torres, M., Colepicolo, P. and Sayre, R. (2007). Phycoremediation of heavy metals using transgenic microalgae. *Advances in Experimental Medicine and Biology*, 616: 99-109.

[8] Sekabira, K., Oryem Origa, H., Basamba, T. A., Mutumba, G. and Kakudidi, E. (2010). Application of algae in biomonitoring and phytoextraction of heavy metals contamination in urban stream water. *International Journal of Environmental Science and Technology*, 8: 115-128.

[9] Inskeep, W. P. and Bloom, P. R. (1985). Extinction coefficients of chlorophyll *a* and *b* in *N,N*-dimethylformamide and 80% acetone. *Plant Physiology*, 77: 483-485.

[10] Baumann, H. A., Morrison, L. and Stengel, D. B. (2009). Metal accumulation and toxicity measured by PAM-Chlorophyll fluorescence in seven species of marine macroalgae. *Ecotoxicology and Environmental Safety*, 72: 1063–1075.

[11] dos Santos, R. W., Schmidt, E. C., de L Felix, M. R., Polo, L. K., Kreusch, M., Pereira, D. T., Costa, G. B., Simioni, C., Chow, F., Ramlov, F., Maraschin, M., and Bouzon, Z. L. (2014) Bioabsorption of cadmium, copper and lead by the red macroalga Gelidium floridanum: Physiological responses and ultrastructure features. *Ecotoxicology and Environmental Safety*, 105: 80–89.

[12] Yruela, I. (2005) Copper in plants. *Brazilian Journal of Plant Physiology*, 17: 145-146.

[13] Machado, M. D., Lopes, A. R. and Soares. E. V. (2015). Responses of the alga *Pseudokirchneriella subcapitata* to long-term exposure to metal stress. *Journal of Hazardous Materials*, 296: 82-92.

[14] Sharma, P. and Dubey, R. S. (2005) Lead toxicity in plants. *Brazilian Journal of Plant Physiology*, 17: 35-52.

[15] Pawlik-Skowronska, B. (2002). Correlations between toxic Pb impacts and production of Pb-induced thiol peptides in the microalga *Stichococcus bacillaris*. *Environmental Pollution*, 119: 119–227.

[16] Baker, A. J. M. and Brooks, R. R. (1989). Terrestrial higher plants which hyper accumulate metallic elements - Review of their distribution, ecology and phytochemistry. *Biorecovery,* 1: 81-126.

[17] Zayed, A., Gowthaman, S. and Terry, N. (1998). Phytoaccumulation of trace elements by wetland plants: I. Duckweed. *Journal of Environmental Quality*, 27: 715-721.

[18] Nazir, A., Malik, R. N., Ajaib, M., Khan, N. and Siddiqui, M. F. (2011). Hyperaccumulators of heavy metals of industrial areas of Islamabad and Rawalpindi. *Pakistan Journal of Botany*, 43: 1925-1933.

[19] Maxwell, K. and Johnson, G. N. (2000). Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany*, 51: 659-668.

[20] Baker, N. R. (2008). Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. *Annual Review of Plant Biology*, 59: 89-113.

[21] Lichtenthaler, H. K., Buschmann, C. and Knapp, M. (2005). How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio R_{fd} of leaves with the PAM fluorometer. *Photosynthetica*, 43: 379-393.

[22] Wei, Y., Zhu, N., Lavoie, M., Wang, J., Qian, H. and Fu, Z. (2014). Copper toxicity to *Phaeodactylum tricornutum*: a survey of the sensitivity of various toxicity endpoints at the physiological, biochemical, molecular and structural levels. *Biometals*, 27: 527-537.

[23] Cambrollé, J., Mateos-Naranjo, E., Redondo-Gómez, S., Luque, T. and Figueroa, M. E. (2011). Growth, reproductive and photosynthetic responses to copper in the yellow-horned poppy, Glaucium flavum Crantz. *Environmental and Experimental Botany*, 71: 57–64.

[24] Bernardini, A., Salvatori, E., Guerrini, V., Fusaro, L., Canepan, S. and Manes, F. (2016) Impacts of high Zn and Pb concentrations on *Phragmites australis* (Cav.) Trin. Ex. Steudel: photosynthetic performance and metal accumulation capacity under controlled conditions. *International Journal of Phytoremediation*, 18: 16-24.

[25] Hazlina, A. Z. and Luqman, A. B. (2013). Copper-, lead- and mercury-induced changes in maximum quantum yield, chlorophyll a content and relative growth of three Malaysian green macroalgae. *Malaysian Journal of Fundamental and Applied Sciences*, 9: 16-21.

[26] Szivák, I., Behra, R. and Sigg, L. (2009). Metal-induced reactive oxygen species production in *Chlamydomonas reinhardtii* (Chlorophyceae). *Journal of Phycology*, 45: 427-435.

[27] Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiology*, 141: 391-396.

[28] Hazlina, A. Z. and Nor Shuhanija, S. (2013). Physiological and biochemical responses of a Malaysian red alga, *Gracilaria manilaensis* treated with copper, lead and mercury. *Journal of Environmental Research and Development*, 7: 1246-1253.

[29] Jiang, W. S., Liu, D. H. and Hou, W. Q. (2001). Hyperaccumulation of cadmium by roots, bulbs and shoots of garlic (*Allium sativum* L.). *Bioresource Technology*, 76: 9-13.

[30] Brown, M. T. and Newman, J. E. (2003). Physiological responses of *Gracilariopsis longissima* (S. G. Gmelin) Steentoft, L. M. Irvine and Farnham (Rhodophyceae) to sub-lethal copper concentrations. *Aquatic Toxicology*, 64: 201–213.

[31] Malar, S., Vikram, S. S., Favas, P. J. C., and Perumal, V. (2014). Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [*Eichhornia crassipes* (Mart.)]. Botanical Studies, 55: 54.

[32] Scheidegger, C., Behra, R. and Sigg, L. (2011). Phytochelation formation kinetics and toxic impacts in the freshwater alga *Chlamydomonas reinhardtii* upon short- and long-term exposure to lead(III). *Aquatic Toxicology*, 101: 423-429.

[33] John, R., Ahmad, P., Gadgil, K. and Sharm, S. (2009). Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. *International Journal of Plant Production*, 3: 65-76.

[34] Janssen, C. and Heijrick, D. (2003). Algal toxicity testing for environmental risk assessment of metals: physiological and ecological considerations. *Environmental Contamination and Toxicology*, 178: 23-52.

[35] Bossuyt, B. T. A. and Janssen, C. R. (2004). Long-term acclimation of *Pseudokirchneriella subcapitata* (Korshikov) Hindak to different copper concentrations: changes in tolerance and physiology. *Aquatic Toxicology*, 68: 61-74.

[36] Zhang, X., Ervin, E. H. and Schmidt, R. E. (2005). The role of leaf pigment and antioxidant levels in UV-B resistance of dark- and light-green Kentucky bluegrass cultivars. *Journal of the American Society for Horticultural Science*, 130: 836–841.

[37] Cenkci, S., Cigerci, I. H., YildIz, M., Özay, C., Bozdag, A. and Terzi, H. (2010). Lead contamination reduces chlorophyll biosynthesis and genomic template stability in *Brassica rapa* L. *Environmental and Experimental Botany*, 67: 467–473.

[38] Pourraut, B., Shahid, M., Dumat, C., Winterton, P. and Pinelli, E. (2011). Lead uptake, toxicity and detoxification in plants. *Reviews of Environmental Contamination and Toxicology*, 213: 113–136.

[39] Shakya, K., Chettri, M. K. and Sawidis, T. (2008). Impact of heavy metals (copper, zinc and lead) on the chlorophyll content of some mosses. *Environmental Contamination and Toxicology*, 54: 412-421.

[40] Han, T., Kang, S. H., Park, J. S. and Lee, H. K. (2008). Physiological responses of *Ulva pertusa* and *U. armoricana* to copper exposure. *Aquatic Toxicology*, 86: 176-194.