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Original paper

TOXICITY AND BIOACCUMULATION OF LEAD IN *Chlorella* AND *Dunaliella*

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ABSTRACT

The aim of this research was to observe the effect of body size in micro algae as significant factor responsible to bioaccumulation of Pb. The research was conducted in Balai Budidaya Laut Hanura, South Lampung using micro algae Chlorella and Dunaliella, observing various Pb concentrations in culture medium and dry weight of micro algae to describe their tolerance and sensitivity levels. Pb concentration in culture medium was determined using AAS (Atomic Absorbance Spectrophotometer) and micro algae biomass observed by optical density approach. Effective concentration of Pb as growth inhibitor of Chlorella and Dunaliella was 50 and 150 µg/l. Dunaliella has greater absorption ability than Cholorella. Less bioaccumulation in Dunaliella indicated high tolerance level to Pb. Dunaliella, whose wider cell surface, has a greater bioaccumulation ability than Chlorella.

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Keywords: *Chlorella, Dunaliella*, lead (Pb), toxicity, bioaccumulation

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INTRODUCTION

In natural environments, organisms living in chronically polluted sites are exposed to low concentrations of heavy metals for long periods. In the other cases, the organism may be abruptly exposed to high levels of metals upon the outfall of a pollutant in coastal waters. The storage of metals by cellular detoxifying mechanism makes them available for assimilation by the biota and bio-magnification along the aquatic food chains. In this context, standardized bio toxicity tests are useful tools to determine the effects of pollutants on cell growth and viability. It is important to evaluate the potential impacts of pollution on aquatic ecosystems, aiming to prevent possible injuries to the biota by establishing maximum tolerable levels of toxicants (Chapman *et al*., 1996)

Lead is one of the most dangerous, important, and potential toxic metal in marine ecosystem. The toxicity levels sensitivity may vary among different micro algae (Soldo and Behra, 2000). It may affect photosynthetic, growth, and enzyme activity and even respiration of micro algae. The tolerance mechanism of micro algae is physiological exclusion of free metal ions due to reduced cell membrane permeability (Brown *et al*., 1988). Chelating to organic complexes is another possible mechanism regulating metal ions detoxification (Gerringa *et al*., 1995).

Dunaliella and *Chlorella* are unicellular, naked, green algae, and grown widely as food sources in aquaculture.

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Both are the richest algal source of β-carotene, amino acids, and glycerol (Borowitzka and Borowitzka, 1988). *Dunaliella* and *Chlorella* are slightly different in diameter of body size. *Dunaliella* has smaller diameter (5-10 μm) than *Chlorella* (15-20 μm). Various studies of several aspects of micro algae have been published (Borowitzka and Borowitzka, 1988) but the effect of body size to bioaccumulation of Pb has not been well known, especially in the both of micro algae tested.

Absorption of metal ions from aqueous system by micro algae has spent much attention for wastewater treatment, which may reduce the metal ion concentration significantly. Here, little information of the inhibition effects of lead on the growth and the long-term bioaccumulation mechanisms of lead in micro algae was evaluated. The research aim was determining the affect of body size difference to bioaccumulation of Pb at micro alga. The result of this study might be a data base of initial bioaccumulation and desorption were significant factors responsible for micro algae tolerance. It was important for both evaluation of water quality and treatment of heavy metal polluted wastewater.

MATERIALS AND METHODS

Analysis of $PbCl_2$ was used in the experiments. The micro algae *Chlorella* and *Dunaliella* were collected from Balai Budidaya Laut Hanura, South Lampung. Chemical analysis of sample was carried out in Integrated Chemical Laboratory IPB, West Java.

The culture medium consisted of 0.1 g of KNO_3, 0.001 g of Na_2HPO_4, 0.003 g of $FeCl_2$, 0.005 g of Na_2SiO_4, and 1000 ml destilled water (Muhaemin, 2005). Initial pH medium was adjusted to 7.0 using 1 N HCl solution. The medium and all experiment utensils used were sterilized at 124 ^oC for 20 min (Yan and Pan, 2002).

Micro algae were grown in 1500 ml flask containing 1000 ml inoculums at 26 ^oC under light intensity of 40 μmol m^{-2} s^{-1} in a 12:12 light: dark ratio. A spectrophotometer was used to measure the optical density at 650 nm (OD650 nm) to express biomass and initial OD650 nm. Lead stock solution of 200μg/l was prepared using sterilized distilled water. Lead toxicity levels were applied at concentration of 25, 50, 100, 150, and 200 μg/l. The research was conducted in triplicate with anticipated relative standard deviations of less than 10 %.

Dry weight of micro algae was determined with a digital balance after cells filtered on a 0,22 μm membrane were dried at 103 ^oC for 2 h.

The total ambient of Pb concentration in the medium was measured using the standard analytical method recommended by Chinese EPA (Chinese EPA, 1989). Muhaemin (2005) showed that the concentration of heavy metal in culture medium and intracellular may use AAS (Atomic Absorbance Spectro-photometer). The Method relied on law Lambert-Beer, which is correlated with number of ray among concentration of samples. Regression equation ($Y = a + bX$) was used to express linear correlation among the absorbance of sample. Control consisted of 100 μg/l Pb in the medium without micro algae. All the experimental conditions were the same as those of micro algae.

RESULTS AND DISCUSSION

Results

Dunaliella has bigger biomass than *Chlorella* expressed as OD650 nm in the absence of lead added. The biomass decreased as the initial lead concentration increased (**Fig. 1**). The OD650 nm decreased the concentration of *Dunaliella*

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less than *Chlorella*. The ambient concentrations of growth of *Dunaliella* and *Chlorella* were estimated 150 and 50 µg/l. *Dunaliella* was more tolerant to lead than *Chlorella*.

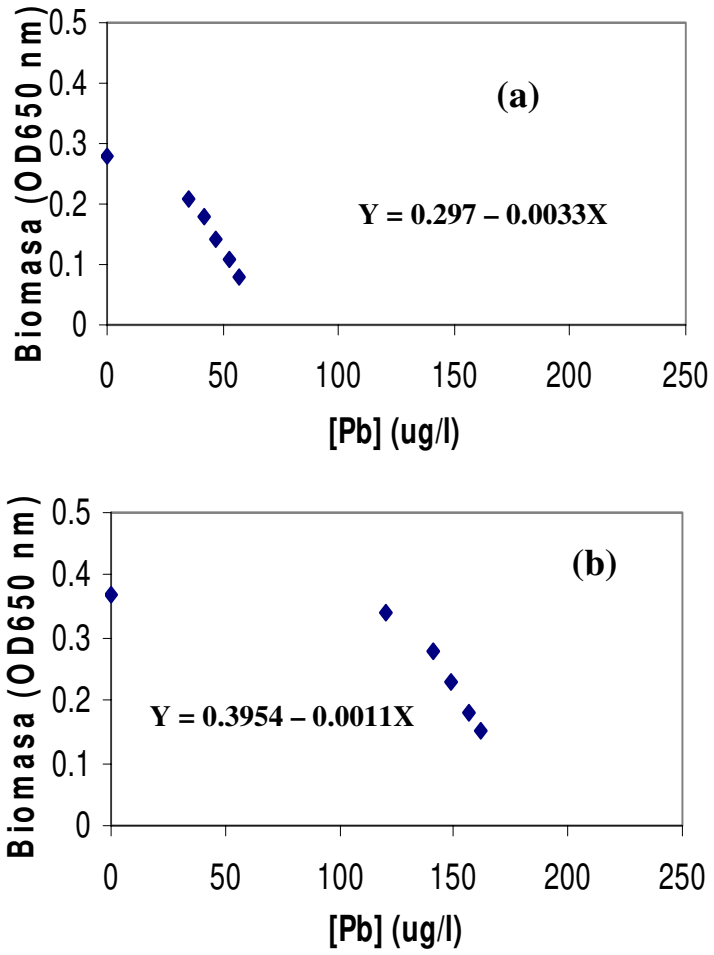

Fig. 1. Toxic effect of lead on the inhibition of micro algae growth at 96 h. (a) *Chlorella* and (b) *Dunaliella*.

The growth processes of micro algae during 6 d period underwent a lag, exponential, and stationary phase (**Fig. 2**). It shows that the greater growth rate of *Dunaliella* and greater sensitivity of *Chlorella* to Pb. The growth order of *Dunaliella* was also greater than *Chlorella*, with an initial lead concentration of 50 µg/l. *Chlorella* growth was inhibited strongly and its exponential growth phase was postponed if initial Pb added. The growth of *Dunaliella* was also inhibited and the lag phase was prolonged when Pb added was increased from 50 to 150 µg/l.

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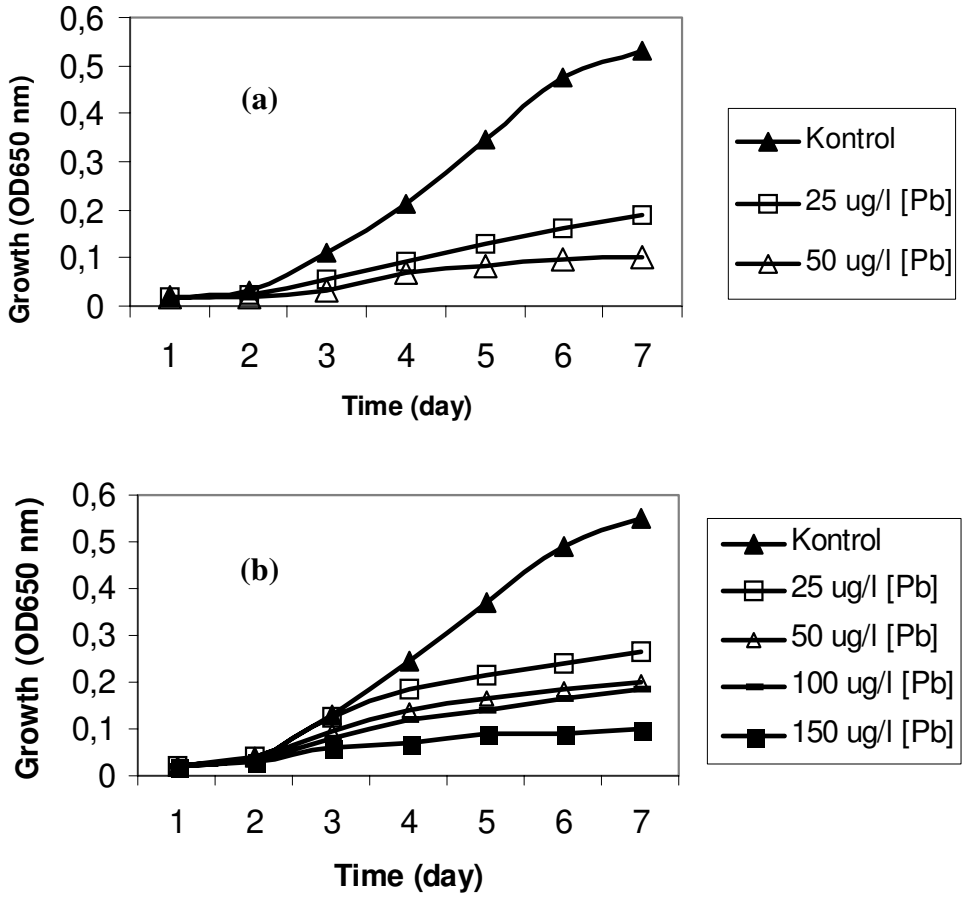

Fig. 2. Micro algae growth at different initial levels of lead. (a) *Chlorella* and (b) *Dunaliella*.

After 6 d exposure culture, when initial lead concentration was 50 µg/l, *Dunaliella* and *Chlorella* removed 96% and 34% Pb (**Table 1** and **2**). *Dunaliella* has a greater ability to remove Pb from culture medium (**Fig. 2b** and **Table 2**). The greater ability of *Dunaliella* to absorb Pb from culture medium might describe the active absorption of micro algae to Pb. Pb removal by *Dunaliella* tends to be proportional with the initial Pb concentration in the culture medium.

Table 1. Biomass (OD650 nm) of *Chlorella* at given Pb concentration and day of culture

Chlorella	Day of Culture						
	0	1	2	3	4	5	6
Control	0.02	0.032	0.11	0.214	0.345	0.476	0.531
25 ug/l [Pb]	0.02	0.023	0.057	0.094	0.131	0.163	0.187
50 ug/l [Pb]	0.02	0.019	0.034	0.069	0.084	0.095	0.102
> 50 ug/l [Pb]	0.02	0	0	0	0	0	0

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Table 2. Biomass (OD650 nm) of *Dunaliella* at given Pb concentration and day of culture

Dunaliella	Day of Culture						
	0	1	2	3	4	5	6
Control	0.02	0.041	0.132	0.243	0.372	0.491	0.552
25 g/l [Pb]	0.02	0.041	0.123	0.186	0.213	0.241	0.264
50 g/l [Pb]	0.02	0.039	0.095	0.141	0.165	0.185	0.201
100 g/l [Pb]	0.02	0.036	0.082	0.121	0.142	0.165	0.184
150 g/l [Pb]	0.02	0.028	0.059	0.071	0.089	0.092	0.101
200 g/l [Pb]	0.02	0	0	0	0	0	0

Concentration of lead remained in the culture medium decreased rapidly and then slowly reduced during 6 d period of research (**Fig. 3**). The accumulation of Pb reached a maximum level after 1 d and slightly decreased after 2 d followed by a low rate at the following 6 d (**Fig. 3a** and **3b**).

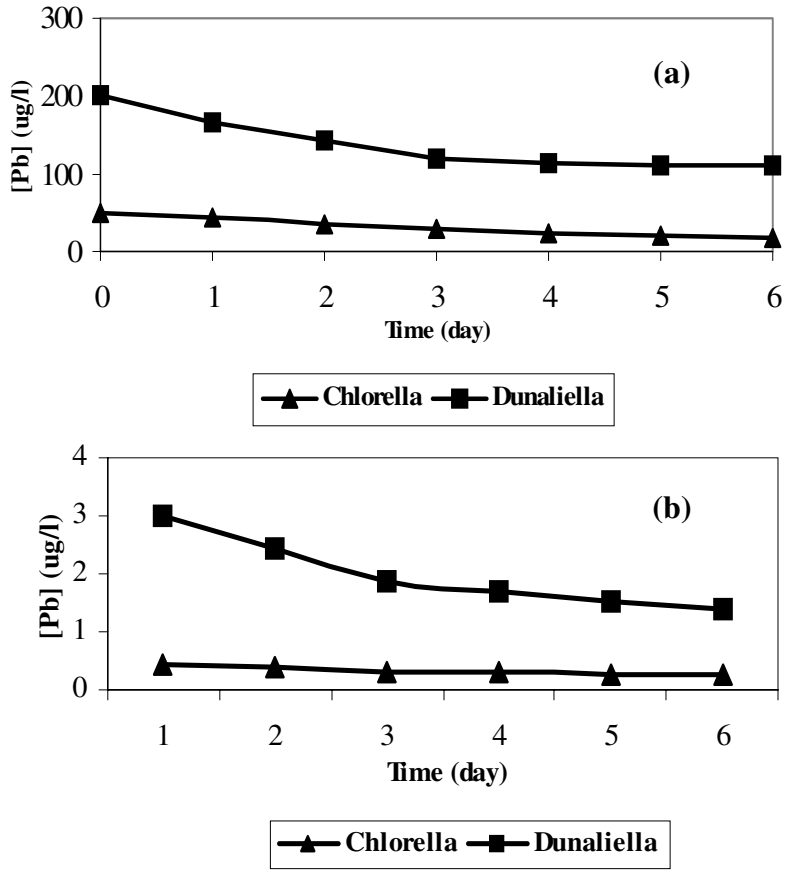

Fig. 3. Concentration of Pb. (a) in the medium (µg/l) and (b) in the dry weight of micro algae (µg/g)

Generally, the accumulation rate of Pb by *Dunaliella* is higher than in *Chlorella* at the second day culture and slightly the same in the following day. **Fig. 3a** showed the Pb removal rate by the micro algae from the culture medium. *Dunaliella* has greater ability to remove Pb from culture medium than *Chlorella*.

Desorption ability can be predicted by determining the fluctuation of Pb concentration in the dry weight of micro algae (**Fig. 3b**). **Fig. 3b** showed that *Dunaliella* desorp Pb rapidly in the first 2 d culture and slightly the same in the following day. Accumulation of Pb in *Dunaliella* was less than in *Chlorella* (**Fig. 3b**). Generally, the accumulation rate of Pb by *Dunaliella* in dry weight of body was more proportional with Pb concentration in the culture medium.

Discussion

This study indicates that the growth phase also plays an important role in determining active desorption ability of Pb by micro algae. Growth rate is a dominant factor to determine the biodegradation.

Dunaliella was more tolerant to Pb, which accumulated less than *Chlorella* especially in 1 d culture (**Fig. 1** and **2**). Large initial adsorption of Pb may cause serious damages to the algal cell. Less accumulated Pb at similar initial Pb levels did not damage *Dunaliella* because of its ability to exclude Pb. It was reported that the tolerance of *Dunaliella* to heavy metals was due to its lesser uptake of heavy metals (Gimmler *et al*., 1991). Several possible mechanisms have been suggested to describe their transport. One type of mechanism is described as molecular mimicry, whereby metals either compete for binding to multivalent ion carriers (such as Ca^{2+} channels) or after binding to low molecular weight thiols (such as cysteine), entering the cell by active transport (e.g. using amino acid transporter). In another type of mechanism, metals bound the chelating proteins (such as metalloproteins) may enter the cell by endocytosis (Muhaemin, 2005). The heavy metals can cause membrane depolarization and acidification of the cytoplasm (Corner and Schimid, 2003), and in fact, membrane injury is one important effect of metal ions that may lead to disruption of cellular homeostasis. Thus, cellular adaptations such as exudation of chelating compounds and active efflux of metal ions by primary ATPase pumps can provide some degree of metal tolerance (Rosen, 1996).

Unlike carbon based contaminants that can be completely degraded to relative harmless products, metal ions (especially Pb) can be transformed in only a limited number of ways by biological or chemical remediation processes. While such transformations are intended to limit the toxicity or solubility of a given Pb ionic species, there are usually competing processes in nature that will eventually recycle at least some of the Pb ions back into their original highly toxic state. Those can be a significant factor for the decreased of micro algal growth in the following days culture. The most common biochemical effect of lead are inhibition of the synthesis of heme (a complex of a substituted Fe^{2+} in cytoplasm) (Manahan, 2003), enzyme activity (Blake *et al*., 2001), and even photosynthetic activity (Borowitzka and Borowitzka, 1988).

The tolerance of *Dunaliella* to Pb may due to its physiology. The culture conditions, such as pH, light intensity, and light-dark ratio, were more suitable for *Dunaliella* than *Chlorella*. Those caused the accumulation and desorption of Pb may vary for *Dunaliella* and *Chlorella* at the same initial Pb concentration. The amount of Pb accumulated by *Dunaliella* at 100 µg Pb/l is greater than *Chlorella* at 50 µg Pb/l. The smaller cells (*Dunaliella*) have relatively wider surface area and provide more sites for binding metals than those of larger cells (*Chlorella*). It caused accumulation of Pb in bigger cell lower than those in smaller cells (Khoshmanesh *et al*., 1997). The results supported this phenomenon when accumulation of Pb by larger cells (*Dunaliella*) was less then the smaller cells (*Chlorella*).

CONCLUSION

Dunaliella was more tolerant and *Chlorella* more sensitive to Pb. Higher initial accumulation of Pb might be responsible for the lower tolerance of micro algae to Pb. The two micro algae tested were able to desorp more Pb during the exponential phase but less in stationary growth phase. Micro algae with the smaller cell volume with the greater sensitivity to Pb are recommended for the efficient removal of Pb from wastewater or for wastewater treatment.

REFERENCES

Blake, A.D., R.M. Jones, R.C. Blake, A.R. Pavlov, I.A. Darwish, H. Yu. 2001. Antibody-based Sensors for Heavy Metal Ions. *Biosens, Bioelect*. 16:799-809

Borowitzka, M.A. and L.J. Borowitzka. 1988. *Micro algal Biotechnology*. Cambridge University Press. Cambridge.

Brown, L.N., Robinson, M.G., Hall, B.D. 1988. Mechanism of Lead (II) Tolerance in *Amphora coffeaeformis* Internal and External Binding. *Mar. Biol.* 97: 581-586

Chinese, EPA. 1989. *Standard Analytic Methods for the Examination of Water and Wastewater*. Chinese Environmental Science Pub-lisher. Beijing, China.

Chapman P.M., Allen, H.E., Godtfredsen, K. And Zi Graggen, M.N. 1996. Evaluation of Bioaccumulation Factors in Regulating Metals. *Environ. Sci. Technol*. 30: 448-452

Corner, S.D. and Schimid, S.L. 2003. Regulated Portals of Entry into the Cell. *Nature*. 422: 37-44

Gerringa, L.J.A., J.W. Rijstenbil, T.C.W. Poortvliet, J. Van Drie, M.C. Schot. 1995. Speciation of Lead (II) and Response of the Marine Diatom *Ditylum brightwellii* upon Increasing Lead (II) Concentration. *Aquat Toxicol*. 30: 77-90

Gimmler, H., Treffny, B., Kowalsky, M., and Zimmermann, U. 1991. The Resistence of *Dunaliella acidophila* Against Heavy Metals: The Importance of the Zeta Potential. *J. Plant. Physiol.* 138: 708-716

Khoshmanesh, A., Lawson, F., and Pribnce, L.G. 1997. Cell Surface Areas as a Major Parameter in the Uptake of Cadmium by Unicellular Green Microalgae. *Chem. Eng. J.* 1: 13-19

Manahan, S.E. 2003. *Toxicological Chemistry and Biochemistry*. CRC Press. Florida

Muhaemin, M. 2005. *Kemampuan Pengikatan Metaloprotein Asam Amino Methionin terhadap Pb pada Dunaliella salina.* Tesis Sekolah Pascasarjana. IPB Bogor.

Rosen, B. 1996. Bacterial Resistance to Heavy Metals and Metalloids. *J. Biol. Inorg. Chem.* 1; 273-277

Soldo, D., Behra, R. 2000. Long Term Effect of Copper on the Structure of Fresh Water Periphyton Communities and Tolerance to Copper, Zinc, Nickel, and Silver. *Aquat Toxicol*. 47: 181-189

Yan, H. and G. Pan. 2002. Toxicity and Bioaccumulation of Copper in Three Green Micro algal Species. *Chemos*. 49: 471-476