Sustinere

Journal of Environment & Sustainability

Sustinere
Journal of Environment and Sustainability

EDITORIAL TEAM

Sustinere
Journal of Environment & Sustainability

MAKE A SUBMISSION

ABOUT

About

Scope

GUIDES

INFORMATION

INDEXING

PEOPLE

This edition is published in collaboration with The 5th International Conference on Environmental Engineering: Sustainable Development of Environment towards Climate Change Resilience

Editor:

Dr Ervin Nurhayati

Guest Editor:

Dr Arie Dipareza

Published: 2017-12-29

Articles

Editorial Board

PDF

Application of ecodrainage system in reducing the potential for flooding in the District of Sarnpang

Fauzan Andikha, Adhi Yuriarto (Author)

63-73

PDF

Drainage system evaluation and control of inundation in campus and housing areas of ITS, Surabaya

Eldo Fikri Alvin, Mas Agus Mardyanto (Author)

74-83

PDF

Remediation of Pb(II) and Cd(II) in Sea Water by Skeletonema costatum

Thin Soedarti, Tini Surtiningsih, Sucipto Hariyanto, Eko P. Kuncoro (Author)

84-93

PDF

Study of turbidity treatment in Karangpilang II Water Treatment Plant

Arief Rahman, Ali Masduqi (Author)

94-98

PDF

The strategy of domestic wastewater management in Kenjeran Surabaya

Daniel Wicaksono, Agus Slamet (Author)

99-108

PDF

Mitigation strategy on reduction greenhouse gas (GHG) emission of landuse sector for the province of Papua

Elvis Franklin Suebu, Joni Hermana, Rachmat Boedisantoso (Author)

109-117

PDF

The Study of urban drainage system based on spatial structure plan

Rahmat Irawan, Ellina S. Pandebesie, Ipung Fitri Purwanti (Author)

118-130

PDF

Data driven analysis using fuzzy time series for air quality management in Surabaya

Dalbet Darmawan, Mohammad Isa Irawan, Arie Dipareza Syafei (Author)

131-143

PDF

RESEARCH PAPER

Remediation of Pb(II) and Cd(II) in seawater by Skeletonema costatum

Thin Soedarti[*], Tini Surtiningsih, Sucipto Hariyanto, and Eko P. Kuncoro

Department of Biology, Faculty of Sciences and Technology, University of Airlangga, Surabaya Indonesia

Abstract. The heavy metals that polluted seawater can end up polluting fish pond too. Hence, it is necessary to remove the heavy metals before the seawater enters the fish pond. The aim of this study was to know the growth, remediation ability and the highest removal efficiency of marine diatom Skeletonema costatum in the lead (Pb(II)) and cadmium (Cd(II)) solution. This study used 3 x 4 factorial design, i.e. concentrations of cell inoculation (5000 cells mL-1; 10000 cells mL-1; and 15000 cells mL-1) and concentrations of Pb(II) and Cd(II) (0; 0.5; 1; and 2 ppm), replicated five times. During five day exposure time, the cell density was observed daily using a microscope (400X), while filtrate of media was analyzed using AAS. The result showed that S. costatum could grow in Pb(II) and Cd(II) at 2 ppm, and could remediate Pb(II) and Cd(II) at 2 ppm. The highest removal efficiency of marine diatom S. costatum in Pb(II) solution was 80.5% (5000 cells mL-1 at 2 ppm on the first day), and in Cd(II) solution was 80% (15000 cells mL-1 at 0.5 ppm on the fifth day).

Keywords: Remediation; Pb (II); Cd (II); Skeletonema costatum.

1. Introduction

Today, marine pollution becomes one of the world environmental experts' focus of attention including in Indonesia. Marine pollution occurs due to the entry of pollutants in the form of substances, organisms or energies into the marine environment so that the quality of seawater gets worse and the seawater can't function according to the allocation of its utilization (*Peraturan Pemerintah Indonesia No. 19 Tahun 1999*, 1999). The degradation of aquatic environment quality is mainly caused by waste which is containing heavy metals. Basically, a heavy metal type is divided into two, namely essential and non-essential metals. Non-essential heavy metals are heavy metals that are toxic to organisms. The hierarchical order of heavy metal toxicity is $Hg^{2+} > Cd^{2+} > Ag^{2+} > Ni^{2+} > Pb^{2+} > As^{2+} > Cr^{2+} > Sn^{2+} > Zn^{2+}$ (Waldichuck, 1974). The toxicity of heavy metals cadmium (Cd) and lead (Pb) are the second and fifth sequences.

Pb (II) and Cd (II) ions are widely used for electroplating, color paint, heat insulation (factory tools), battery industry, etc. (Darmono, 1995). Heavy metals are contaminants that have harmful effects because they are considered as non-biodegradable and can be stable (Palar, 2004). Cd (II) causes rheumatic and myalgias (muscle pain) disease (Slamet, 1996). Moreover, Pb(II) causes nerve damage, the risk of cancer, anemia, birth defect, delayed growth, etc. (Ahamed & Siddiqui, 2007). Garcia-Leston et al. (2010) state that Pb(II) causes gene damage. Similarly, the US Agency for Research on Cancer classifies Pb(II) as a possible human carcinogen.

Sumiyani et al. (2005) reports that *Anadara antiquates* in Coastal Kenjeran Surabaya have been contaminated by heavy metals Pb(II) and Cd(II). From the results of several studies, the content of Pb(II) in Water Kenjeran is 1.2246 to 2.0713 mg L^{-1} (Dyah, 2007). The concentration is higher than the threshold value set by the Ministry of Environment (KEMENLH) which is the quality standard for marine life for the Pb(II) of 0.008 mg L^{-1} (KemenLH, 2004).

Organisms in marine water, particularly the fish, are one of the export commodities in Indonesia. Meanwhile, the content of Pb(II) and Cd(II) in fish and shrimp in ponds need to be considered. Hence, techniques to process polluted seawater before use for the fish pond is necessary. One such technique is remediation. This technique transforms the heavy metal into a harmless element. Bioremediation is one of the remediation techniques that uses elements of biology (microorganism) as a remediator. This technique does not cause environmental damage and death of the biota in polluted waters (Jamil, 2001).

Marine diatom, *Skeletonema sp.* can remediate Pb(II) with an efficiency of 96% in 0.9 ppm (Leonard, 2014). Besides, *Skeletonema costatum* can grow in Cd (II) at 0.224 ppm (Nassiri et al., 1997). *Skeletonema sp.* including microalgae are aquatic organisms that have the molecular mechanisms to distinguish non-essential heavy metals from heavy metals essential to growth.

Skeletonema costatum can absorb heavy metals in two ways: absorption and adsorption. Adsorption occurs because of *Skeletonema costatum* have cell walls. The cell walls of *Skeletonema costatum* consist of cellulose. Cellulose in the cell walls has functional groups such as hydroxyl which can bind with heavy metals (Gupta et al., 2000; Knauer et al.1997) or replace Zn contained in the cell wall (Fauziah, 2011). Absorption is undertaken by *Skeletonema costatum* because it produced phytochelatins. Phytochelatins bond with metal has been observed in the vacuole of *Skeletonema Costatum* (Nassiri et al., 1997). *Skeletonema costatum is* like other microalgae that also produces phytochelatins, namely peptides metallothionein class III (Mt-III) to detoxify heavy metals (Perales-Vela et al., 2006). Biosynthesis Mt-III can be induced by the presence of heavy metals such as Cd^{2+}, Ag^{2+}, Bi^{3+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Hg^{2+}, and Au^{2+} both in vivo and in-vitro(Shaw, 1989).

Based on this, it needs to know the growth and the ability of bioremediation of marine diatom *Skeletonema costatum* in lead-contaminated seawater ex-situ so that the seawater before entering into the fish pond is free of heavy metals lead. Thus, the *Skeletonema costatum* can be given to the seawater that will be used for fish ponds.

2. Materials and Methods

The materials utilized in the study were: to isolate microalgae *Skeletonema costatum* that was obtained from the BBAP (Brackish Water Aquaculture Development Centre) Situbondo. *S. Costatum* has been identified using the book "Examination of Diatom: Found on The Surface of The Sea of Java" (Cleve, 1873); XMU (i.e. KNO 3, Na2HPO4.12H2O, Na2SiO3, and FeSO47H2O) as a culture medium (Soedarti et al., 2009); Pb(NO$_3$)$_2$; distilled water; seawater; and sea soil (as a medium of treatment).

The instrumentation in study were Ponar grab, aerator 6 volts dc, ice box, hoses, light microscopy, culture bottles of 350 mL, glass beaker 500 mL, measuring cups 250 mL, tube Erlenmeyer 500 mL and 100 mL, pH indicator paper, thermometer, hand refractometer, Atomic Absorption Spectroscopy (AAS), bunsen, laminar air flow, electric stove, shaker, analytical balance, measuring pipette, tip, micropipette, vein, autoclave, 40 watt fluorescent lamp and a hand counter.

2.1. Preparation of media
Sea soil supernatant

Sea soil 1 Kg was mixed with distilled water 1000 mL, then stirred. After that, it was boiled for approximately 60 minutes. After two days, it was filtered through filter paper (two layers). The supernatant obtained was stored in the refrigerator (Soedarti et al., 2005).

XMU media

Sea soil supernatant 15 mL was mixed with salt (KNO 3 400 mg, Na2HPO4.12H2O 40 mg, 20 mg Na2SiO3, and FeSO47H2O 14 mg) and 1000 mL of pure seawater, then stirred for approximately 10 minutes (the pH was measured from 7.8 to 8.5). Then, it was sterilized by autoclave at a temperature of 121^{0}C for 15 minutes (Soedarti et al., 2009).

Lead stock solution

Pb II concentration used in this study was 0.5; 1; and 2 ppm. Dilution concentration of 1000 ppm stock solution of equation (1).

$$V_1 N_1 = V_2 N_2 \qquad (1)$$

Description: V_1 = volume of the stock that has been known (mL); N_1 = the stock concentration that has been known (ppm); V_2 = volume of the stock that is unknown (mL); and N_2 = the stock concentration that is unknown (ppm).

Skeletonema costatum culture

Skeletonema costatum was entered into XMU media 250 mL. To make the Skeletonema costatum grow well, the culture was placed under the light of 3199 lux (fluorescent lamp 40 watts) and at a temperature of 25°C (Soedarti et al., 2005).

Stage treatment

This study used a 3 X 4 factorial design that was four kinds of varying concentrations of lead and three variations of cell inoculation. The treatment consisted of 12 treatments and within each treatment, there were five repetitions. Each treatment was inoculated

with different numbers of cells at 5 x 10^3 (S1), 10 x 10^3 (S2) and 15 x 10^3 (S3) cells mL^{-1} *Skeletonema costatum* in a total volume of 250 mL each treatment were exposed to heavy metals lead at concentrations of 0 (Control); 0.5 ppm (P1); 1 ppm (P2); and 2 ppm (P3).

Measurement of the physical condition of the culture medium

Measurements were made every 24 hours during the study (the first day to the fifth day), including room temperature (^{o}C) by using a thermometer, pH using pH indicator paper, light intensity using a lux meter and salinity using a hand refractometer.

Data of the growth and efficiency of adsorption

Observations on the growth of *Skeletonema costatum* conducted directly by counting the number of cells per mL using a hemocytometer under a microscope (400X). Sampling was taken from the first day to the fifth. Observations and data collection capabilities lead the bioremediation by *Skeletonema costatum*. Each sample on day two, three, four and five are drawn as many as ten specimens of *Skeletonema costatum* mL of growth media by centrifugation at a speed of 5000 rpm for 5 minutes, to obtain filtrate and supernatant. The filtrate was filtered through filter paper and dried in an oven. The dried filtrate was ready for destruction. From the filtrate that has been destroyed, the levels of lead were analyzed using AAS with a wavelength of heavy metals (283.3 nm) (Leonard, 2014). The supernatant also analyzed the heavy metal content to know the concentration of heavy metals remaining in the media (Yusafir et al., 2012).

The calculation of the absorbed heavy metal concentrations used Langmuir method (Yusafir et al., 2012) which calculated the efficiency of removal by the following equation (2) and (3):

$$C_s = C_0 - C_f \qquad\qquad (2)$$

$$E_p = C_s/C_0 \text{ x } 100\% \qquad\qquad (3)$$

Where, E_p = adsorption efficiency (%); C_s = Concentration of metal adsorbed (mg L^{-1}); C_0 = Concentration of metal prior to contact (mg L^{-1}); and C_f = Concentration of metal after contact (filtrate) (mg L^{-1}).

Data analysis

Data obtained were in the form of *Skeletonema costatum* cell density and the concentration of lead that was absorbed by the cells of *Skeletonema costatum*. Multivariate ANOVA (*F*-test) at α equal to 0.05 was implemented to determine the different growth and uptake of heavy metals by *Skeletonema costatum,* the combination treatment of heavy metal concentrations and the number of cell inoculation. If the different growth caused by treatment has occurred, Duncan test at α = 0.05 was applied.

3. Results
Growth of *Skeletonema* costatum

Microalgae *Skeletonema costatum* in this study has proven able to grow on media treatment that was given Pb(II) and Cd(II) until the fifth day (Figure 1 and Figure 2). Figure 1 shows that the optimum growth of *Skeletonema costatum* has occurred in the second and third day. Optimum growth on the third day of inoculation found in 5,000 cells

mL^{-1} and 10,0000 cells mL^{-1} for all treatments, except the control treatment on 10,000 cells mL^{-1} inoculation (second day). Optimum growth medium on the second day of inoculation was found at 15,000 cells mL^{-1}.

Figure 1. Charts of *Skeletonema costatum* growth in various concentrations of Pb(II) for five days. C-5K: Control, inoculation of 5 000 cells mL^{-1}; C-10K: Control, inoculation of 10 000 cells mL^{-1}; C-15K: Control, inoculation of 15 000 cells mL^{-1}; P1-5K: Pb(II) 0.5 ppm, inoculation of 5 000 cells mL^{-1}; P1-10K: Pb(II) 0.5 ppm, inoculation of 10 000 cells mL^{-1}; P1-15K : Pb(II) 0.5 ppm, inoculation of 15 000 cells mL^{-1}; P2-5K: Pb(II) 1 ppm, inoculation of 5 000 cells mL^{-1}; P2-10K: Pb(II) 1 ppm, inoculation of 10 000 cells mL^{-1}; P2-15K : Pb(II) 1 ppm, inoculation of 15 000 cells mL^{-1}; P3-5K: Pb(II) 2 ppm, inoculation of 5 000 cells mL^{-1}; P3-10K : Pb(II) 2 ppm, inoculation of 10 000 cells mL^{-1}; P3-15K : Pb(II) 2 ppm, inoculation of 15 000 cells mL^{-1}

In the treatment (P1, P2 and P3), the highest growth was the inoculation of 15,000 cells mL^{-1} on the 2nd day, amounted to 13.30 x 10^4 cells mL^{-1} in media which was added by 0.1 ppm lead (P2). This amount was more than inoculating 5,000 cells mL^{-1} and 10,000 cells mL^{-1}, that amounted to 12.07 x 10^4 cells mL^{-1} (in Pb(II) 1 ppm (P2) on the third day) and 10.70 x 10^4 cells mL^{-1} (in Pb(II) 0.5 ppm (P1) on the third day). The result of the statistical test has shown that inoculation of 15,000 cells mL^{-1} in the treatment of P2 (the concentration of lead (Pb II) 1 ppm) on the second day was able to live as well as inoculation of 15,000 cells mL^{-1} in the control treatment on the fourth day.

Figure 2 has shown the optimum growth of *Skeletonema costatum* in the second and third day. Optimum growth on the third day of inoculation was found in 5,000 cells mL^{-1} and 10,0000 cells mL^{-1} for all treatments, except the control treatment that was on 10,000 cells mL^{-1} inoculation (second day). Optimum growth medium on the second day of inoculation was found at 15,000 cells mL^{-1} for all treatments. In the treatment (P1, P2, and P3), the highest growth was the inoculation of 15,000 cells mL^{-1} on the second day, amounted to 13.30 x 10^4 cells mL^{-1} in 0.1 ppm Cd(II) (P2). This amount was more than inoculating 5,000 cells mL^{-1} and 10,000 cells mL^{-1}, that amounted to 12.07 x 10^4 cells mL^{-1} in Cd(II) 1 ppm (P2) on the third day and 10.70 x 10^4 cells mL^{-1} in Cd(II) 0.5 ppm (P1) on the third day. The result of the statistical test has shown that inoculation of 15,000 cells

mL^{-1} in 2 ppm Cd(II), on the second day was able to live as well as inoculation of 15,000 cells mL^{-1} in the control treatment on the second day.
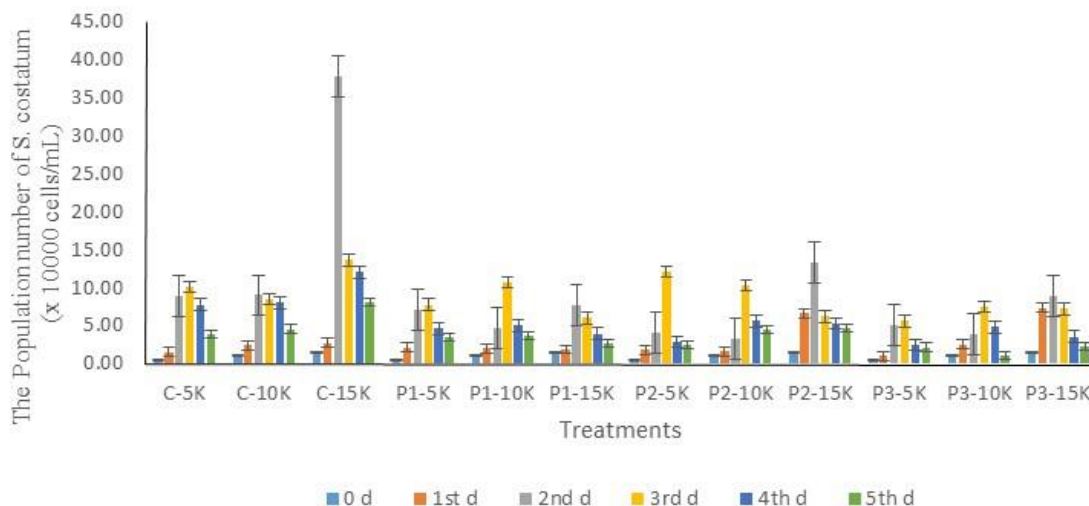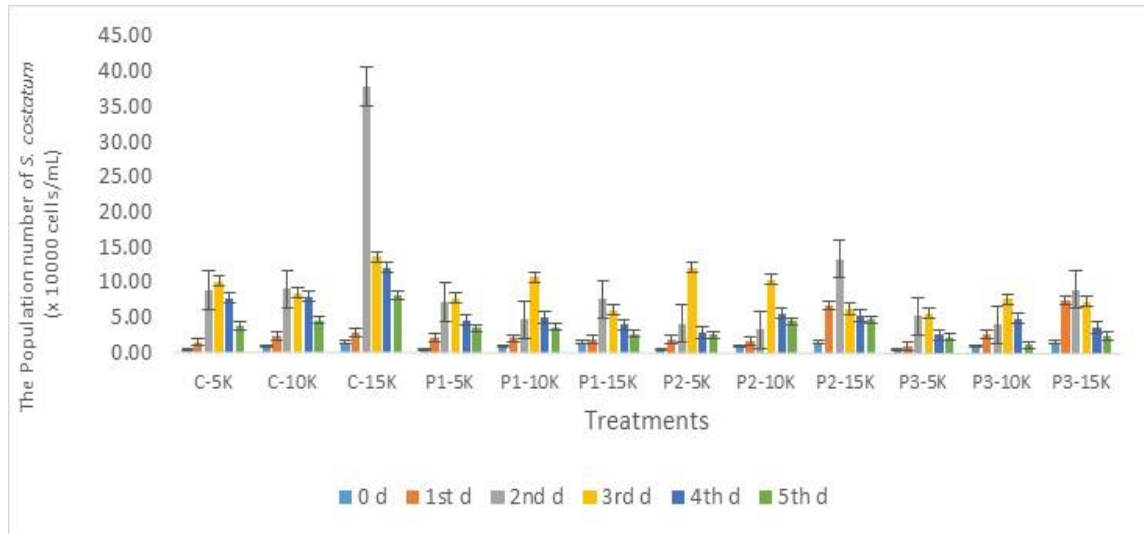

Figure 2. Charts of *Skeletonema costatum* growth in various concentrations of Cd(II) for five days. C-5K: Control, inoculation of 5 000 cells mL^{-1}; C-10K: Control, inoculation of 10 000 cells mL^{-1}; C-15K: Control, inoculation of 15 000 cells mL^{-1}; P1-5K: Cd(II) 0.5 ppm, inoculation of 5 000 cells mL^{-1}; P1-10K: Cd(II) 0.5 ppm, inoculation of 10 000 cells mL^{-1}; P1-15K : Cd(II) 0.5 ppm, inoculation of 15 000 cells/mL; P2-5K: Cd(II) 1 ppm, inoculation of 5 000 cells mL^{-1}; P2-10K: Cd(II) 1 ppm, inoculation of 10 000 cells mL^{-1}; P2-15K : Cd(II) 1 ppm, inoculation of 15 000 cells mL^{-1}; P3-5K: Cd(II) 2 ppm, inoculation of 5 000 cells mL^{-1}; P3-10K : Cd(II) 2 ppm, inoculation of 10 000 cells mL^{-1}; P3-15K : Cd(II) 2 ppm, inoculation of 15 000 cells mL^{-1}

Removal Efficiency

Skeletonema costatum bioremediation capabilities could be seen from the value of the removal efficiency (%) (Table 1). Table 1 has shown that inoculation of *S. costatum* 5 x 10^4 cells mL^{-1} in media of lead 2 ppm on the first day had the highest rate of removal efficiency of 80,5 %.

Table 1. Pb(II) Removal Efficiency of *Skeletonema costatum*

Treatment of cells number	Days to	Adsorption efficiency (%)			
		C (Lead 0 ppm)	P1 (Lead 0,5 ppm)	P2(Lead 1 ppm)	P3 (Lead 2 ppm)
S1 (5 000 cells mL-1)	1st	0 ± 0.00a	71.33 ± 4.16r	76.67 ± 1.15s	80.50 ± 0.50t
	2nd	0 ± 0.00a	62.00 ± 2.00op	61.00 ± 1.00op	71.50 ± 0.50r
	3rd	0 ± 0.00a	50.00 ± 2.00l	55.33 ± 2.52m	65.00 ± 1.00pq
	4th	0 ± 0.00a	40.67 ± 1.15jk	40.00 ± 2.00jk	62.00 ± 1.00op
	5th	0 ± 0.00a	37.33 ± 2.31ij	37.67 ± 2.52ij	55.33 ± 4.91m
S2 (10 000 cells mL-1)	1st	0 ± 0.00a	60.67 ± 1.15m	56.33 ± 1.52mn	68.17 ± 1.26qr
	2nd	0 ± 0.00a	49.33 ± 1.15l	48.00 ± 2.00l	62.00 ± 0.50op
	3rd	0 ± 0.00a	40.00 ± 2.00jk	40.33 ± 0.57jk	58.33 ± 0.76mno
	4th	0 ± 0.00a	29.33 ± 3.05efg	34.33 ± 2.08hi	55.83 ± 0.76m
	5th	0 ± 0.00a	26.67 ± 2.31def	30.67 ± 0.58fgh	50.33 ± 2.56l
S3 (15 000 cells mL-1)	1st	0 ± 0.00a	48.00 ± 5.29l	34.00 ± 2.64hi	60.00 ± 1.00op
	2nd	0 ± 0.00a	42.67 ± 5.03k	29.00 ± 1.00efg	55.33 ± 0.58m
	3rd	0 ± 0.00a	32.00 ± 2.00gh	25.67 ± 0.58de	51.17 ± 1.61l
	4th	0 ± 0.00a	24.00 ± 4.00cd	21.00 ± 1.00bc	50.17 ± 2.84l
	5th	0 ± 0.00a	20.00 ± 4.00b	19.00 ± 1.73b	47.67 ± 2.56l

Information: The number followed by the same letter are not significantly different
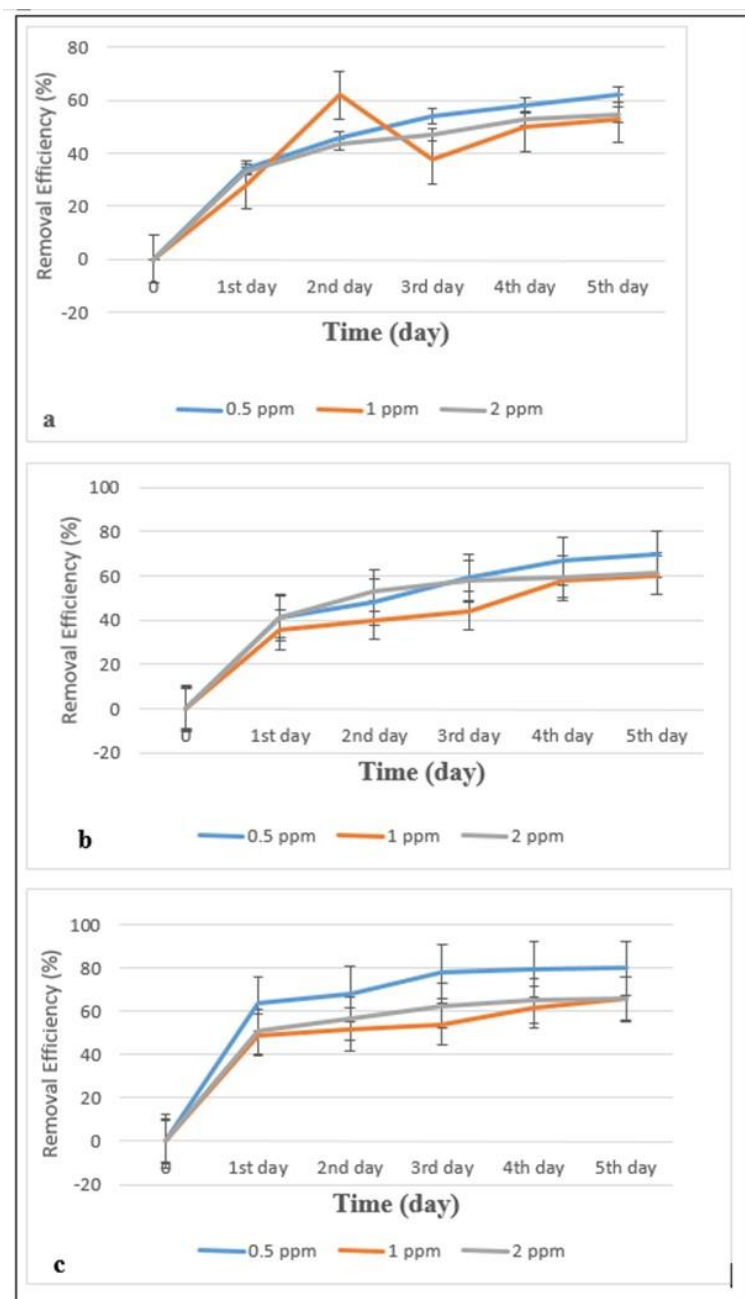
Figure 3. Charts of removal efficiency in various concentrations of Cd(II) for five days: (a) inoculation of 5 000 cells mL^{-1}; (b) inoculation of 10 000 cells mL^{-1}; (c) inoculation of 15 000 cells mL^{-1}.

Table 1 has shown that the removal efficiency of lead on the first day (after one day or 24 hours) was the highest number. Adsorption ability of lead of *S. costatum* at inoculation treatment of 5000 cells mL^{-1} in the lead 2 ppm on the fifth day was not different from inoculation treatment P3 of 15 000 cells mL^{-1} in the lead 2 ppm.

Figure 3 has shown that the highest removal efficiency in Cd (II) was 80% (at 15 000 cells mL^{-1} in Cd(II) 0.5 ppm on the fifth day) (Figure 3.c). The removal efficiency in 1 ppm Cd (II) at 5000 cells mL^{-} on the second day (after two days or after 48 hours) was the highest number. After that, removal efficiency was decreasing (Figure 3.a).

The adsorbed Pb(II) and Cd(II) amount have shown the ability of *S. costatum* in doing bioremediation (Table 2 and Figure 3). This was due to the cell wall of *S. costatum* that have functional groups such as hydroxyl which can bind heavy metals (Gupta et al., 2000; Knauer et al.1997). The functional groups could bind metal ions due to the reaction between the negative charge functional groups contained within the cell wall with the positive charge of the metal ion of Pb and Cd.

Naturally, the removal of metal ions consists of two mechanisms involving the passive uptake and active uptake. The passive uptake is known as biosorption. This process occurs when the heavy metal ions bound to the cell wall in two ways. The first, exchange of monovalent and divalent ions, such as Na, Mg, and Ca, on the cell wall with heavy metal ions; and the second, Complex formation between heavy metal ions with functional groups such as carbonyl, amino, thiol, hydroxyl, phosphate, and hydroxy-carboxyl located on the cell wall. The bonding process of heavy metal ions on the surface of these cells can occur in dead cells and living cells (Suhendrayatna, 2012).

The *S. costatum* growth in the treatments was lower than the control. Although, *S. costatum* could live in Pb(II) and Cd(II) (Fig. 1 and 2). This was because the Pb(II) and Cd(II) concentrations were not yet toxic. Besides, *S. costatum* is one of the high-protein phytoplankton of 37% (Erlina et al., 2004). This protein is composed of a carboxyl group (-COOH) which can bind well with heavy metal ions (Sembiring et al., 2009). Thus, *S. costatum* could adsorb heavy metal ions. Therefore, if *S. costatum* had more carboxyl group, the adsorption increased. Thus, *S. costatum* could be given to a seawater that would be used for fish ponds.

4. Conclusions

Based on the results of the research, we have concluded that (1) *Skeletonema costatum* could grow in lead exposure until 2 ppm in lead [Pb II] and cadmium (II) solution, and (2) the bioremediation ability of *S. costatum* in lead and cadmium (II) contaminated seawater was 2 ppm, and (3) the removal efficiency of Pb(II) was highest at 80.50 % (at 5 000 cells/mL inoculation in 1 ppm on the first day) and the removal efficiency of Cd(II) was highest at 80% (at 15 000 cells/mL inoculation in 0.5 ppm fifth day).

Acknowledgement

Reference

Ahamed, M., & Siddiqui, M. K. J. (2007). Environmental lead toxicity and nutritional factors. *Clinical Nutrition (Edinburgh, Scotland)*, *26*(4), 400–8. http://doi.org/10.1016/j.clnu.2007.03.010

Cleve, P. (1873). *Examination of Diatom: Found on The Surface of The Sea of Java*. Stockholm: P.A. Norstedt & Soner.

Darmono. (1995). *Logam Berat dalam Sistem Biologi Makhluk Hidup*. Jakarta: UII Press.

Dyah. (2007). *Kadar logam Timbal (Pb) dan Kadmium (Cd) pada air laut dan kerang darah*. Universitas Surabaya, Surabaya.

Erlina, A., Amini, S., & Endrawati, H. (2004). *Kajian Nutritif Phytoplankton Pakan Alami pada Sistem Kultivasi Massal*. Jepara: BBPBAP Jepara.

Fauziah. (2011). *Efektivitas Penyerapan Logam Kromium (Cr IV) dan Kadmium (Cd) oleh Scendesmus dimorphus*. Universitas Islam Negeri Syarif Hidayatullah.

Garcia-Leston J, Mendez J, Pasaro E, L. B. (2010). Genotoxic effects of lead: an updated review. *Environmental International*, *36*, 623–636. http://doi.org/doi: 10.1016/j.envint.2010.04.011

Gupta, R., Ahuya, P., Khan, S., Saxena, R. K., & Mohapatara, H. (2000). Microbial Biosorbent: Meeting Challenges of Heavy Metal Pollution in Aqueous Solution. *Current Science*, *78*, 967–973.

Jamil, K. (2001). *Bioindicators and Biomarkers of Environmental Pollution and Risk Assessment*. Enfield: Science publishers inc.

KemenLH. Lampiran III: Baku Mutu Air Laut untuk Biota in Keputusan Menteri Lingkungan Hidup No. 51 Tahun 2004 Tentang Baku Mutu Air Laut (2004). Indonesia. Retrieved from http://www.ppk-kp3k.kkp.go.id/

Knauer, K., Behra, R., & Sigg, L. (1997). Adsorption and uptake of copper by the green alga Scenedesmus subspicatus (Chlorophyta). *Journal of Phycology*, *33*(4), 596–601.

Leonard, R. (2014). *Studi Perbandingan Kemampuan Skeletonema sp. dan Chaetoceros sp. Sebagai Agen Bioremediasi (Fito-Akumulasi) Terhadap Logam Timbal (Pb)*. Universitas Airlangga.

Nassiri, Y., Mansot, J. L., Wéry, J., Ginsburger-Vogel, T., & Amiard, J. C. (1997). Ultrastructural and electron energy loss spectroscopy studies of sequestration mechanisms of Cd and Cu in the marine diatom Skeletonema costatum. *Archives of Environmental Contamination and Toxicology*, *33*(2), 147–155. http://doi.org/10.1007/s002449900236

Palar, H. (2004). *Pencemaran dan Toksikologi Logam Berat*. Yogyakarta: PT Rineka Cipta.

Perales-Vela, H. V., Pena-Castro, J., & Canizares-Villanueva, R. O. (2006). Heavy metal detoxification in eukaryotic microalgae. *Chemosphere*, *64*, 1–10.

Peraturan Pemerintah Indonesia No. 19 Tahun 1999 (1999). Retrieved from http://rulebook-jica.ekon.go.id/ english/27.PP_NUMBER 7 OF 1999.eng.htm

Sembiring, Z., Buhani, & Suharso. (2009). *Isoterm Adsorpsi Ion Pb(II), Cu (II), dan Cd(II) pada Biomassa Nannochloropsis sp. yang Dienkapsulasi Akuagel Silika*. Universitas Lampung.

Shaw, A. (1989). *Heavy Metal Tolerance in Plants: Evolutionary aspects*. Boca Raton: FL:

CRC Press Inc.

Slamet, J. S. (1996). *Kesehatan Lingkungan*. Yogyakarta: Gadjah Mada University Press.

Soedarti, T., Indira, P., & Surtiningsih, T. (2009). Studi Perbandingan Pertumbuhan Beberapa Jenis Diatom Laut dalam Berbagai Media. In *Seminar Nasional Biologi*. Malang: UIN Malang.

Soedarti, T., Mahendra, & Surtiningsih, T. (2005). Kultur Diatom Laut Coscinodiscus sp Dalam Berbagai Media. In *Konggres Ikatan Sarjana Oseanologi Indonesia*. Surabaya: Universitas Hang Tuah.

Suhendrayatna. (2012). Heavy Metal Bioremoval by Microorganisms in A Literature Study. Retrieved May 3, 2012, from http://www.istecs.org/Publication/Japan/010211_suhendrayatna.pdf/2011

Sumiyani, R., S, S., & M, A. (2005). Kadar logam Cd, Cr, Cu, Hg, dan Pb dalam air sungai, air laut, Pantai Kenjeran Surabaya. In *Simposium Nasional ke- 2 Hasil Penelitian dan Pengembangan Bidang Kesehatan, 7-8 Desember 2005*.

Waldichuck, M. (1974). Some Biological Concern in Metals Pollution. In V. John & W. B. Vernberg (Eds.), *Pollution and physiology of marine organisms*. London: Academic Press.

Yusafir, H., Emma, S., & Paulina, T. (2012). *Biosorpsi Campuran Logam Pb2+ dan Zn2+ oleh Chaetoceros calcitrans*. Universitas Hasanuddin.