Proceedings of the 16th Tri-University International Joint Seminar and Symposium 2009
Mie University, Japan, October 19–22, 2009

TRIU09ET-13

Testing Toxicity of Lead and Cadmium in Micro Algae *Chaetoceros gracilis*

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Abstract *Chaetoceros gracilis* as a primary producer, the biggest genus of marine diatom in the ocean and the important food for the other organism like copepode and bivalvae. Increasing the number of industrial will cause much of waste especially heavy metals in the marine environment. The objective of the study are to estimate the toxicity level of cadmium and lead to micro algae *Chaetoceros gracilis*. So, this experiment can be used as the material of Water Quality Criteria.

The study were conducted by using short-term bioassay as 96 hour with growth of *Chaetoceros gracilis* as the end point, and the result shows that the estimated value of IC_{50} (Inhibition Concentration) for cadmium and lead are 1.3 mg Cd/l and 0.7 mg Pb/l respectively. And the value of LOEC (Lowest Observed Effect Concentration) for cadmium and lead are 0.56 mg Cd/l and 0.26 mg Pb/l respectively.
Key words: *Chaetoceros gracilis*, cadmium, lead, IC50, LOEC

1. Introduction

The Increase of industrial will cause high releasing of industrial waste into the marine environment. One of waste industries that release into the marine environment are heavy metals. Cadmium (Cd) and Lead (Pb) are two kinds of heavy metals that often used as the main material or additional material in industries.

Cadmium (Cd) and lead (Pb) can be tested by using micro algae *Chaetoceros gracilis.* This species was chosen because was used as food for bivalvae and copepode larvae, as the base of food web, the biggest genus of marine diatom in the ocean and sensitive indicator of environment stresses [1]. The research about toxicity testing of cadmium to *Chaetoceros gracilis* was done by (Hindarti, 1997,2008) and (Damayanti, 2008).

Increasing concentration of lead and cadmium in the *Chaetoceros gracilis* will influence to metabolism processes by inhibiting cell growth. For that reason, the research was needed to estimate the concentration of lead and cadmium that influence to cell growth of *Chaetoceros gracilis* by using short-term bioassay as 96 hour.

The aim of the research are to estimate the value of IC_{50} (Inhibition Concentration) , LOEC(Lowest Observed Effect Concentration), and NOEC (No Observed Effect Concentration) of lead and cadmium for 96 hour to cell growth of *Chaetoceros gracilis*.

2. Research Method

2.1 Location and Time Research

The research was done in February until May 2009 in the Eco toxicology and Chemistry Analysis Laboratory, Research and development Oceanography, LIPI., North Jakarta.

2.2 Parameter Measured

There are some parameter measured in testing toxicity of lead and cadmium in microalgae *C. gracilis*. The parameter measured are determination of *C. gracilis* growth cell, determination of water quality, Determination of IC50 (Range Finder test and Definitive Test), and Determination of NOEC and LOEC.

2.2.1Cultivation System of *C. gracilis*

A culture of *Chaetoceros gracilis* obtained from Marikultur Research and development Oceanography, LIPI, North Jakarta was used as test organism. Micro algae was cultured in the autoclave sea water and enrich with walne medium that consist of trace metals, vitamin, and nutrient. The micro algae were cultivated in 100 ml medium into sterile 250 ml erlenmeyer flask, at 27°C. The density cell was counted every day as one week with three replicate. The 4th days age culture was adjusted to 10^6 cell/ml so that can be used as organism test..

2.2.2Determination of Water Quality

There are four water quality parameter were measured, consist of salinity, dissolved oxygen, pH and temperature. Measuring salinity using refraktometer, dissolved oxygen using Dissolved oxygen meter, pH and temperature using pHmeter. Measuring water quality was important to certain the assuming of Complete Random Design.

2.2.3 Determination of IC_{50}

1000 mg/l cadmium and lead stock solution by diluting a certain amount of $CdCl_2$ and $Pb(NO_3)_2$ in 1000 ml of aquades

There are two stage to determine of IC50:

a. Range finder test

A range finder test was conducted for lead in a wide range of concentration as no information available in previous studies. The test was done in the three replicate.

b.Definitve Test

A definitive test was performed based on range finder test result. The five nominal concentration of lead were 0.32, 0.56, 1, 1.8,and 3.2 mg/l . Cadmium was used as reference toxicant with a series of five nominal concentration and ranged from 0.56 mg/l to 5.6 mg/l. Final toxicity test (definitive test) was done in 96-hour. The effective metal concentration required to reduce population growth by 50% when compared the growth in the control expressed as IC_{50}[2]. The ICPIN program[3] was used to calculate IC_{50} value.

2.2.4 Determination of LOEC and NOEC

NOEC is the maximum concentration of the test material that produces no statically significant harmful effect on test organism compared to control in a specific test. [4]. LOEC is the lowest concentration that has a statically significant deleterious effect on test organism compared to controls in a specific test.[3].To determine NOEC and LOEC value, data obtained were analyzed by TOXSTAT program[3]

3. Result and Discussion

3.1 The growth pattern of *Chaetoceros gracilis*

Culivation system *of C. gracillis* as a part from preparing toxicity test as 7 days, the result of *C. gracilis* growth pattern can be showed in Figure 1.

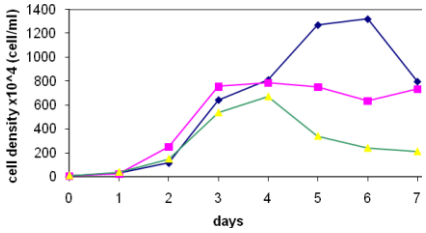

Fig 1.Growth curve of *Chaetoceros gracilis* as 7 days

Based on the fig.1, it can be seen the growth pattern of *Chaetoceros gracilis* is logaritmic. It can help us to calculate NOEC and LOEC value with transforming all data, based on the growth pattern of *C. gracilis*.

Based on the growth curve of *C. gracilis*, 0 until 1st days is an adaptation phase. In the 1st until the 2scd days is acceleration phase. In 2rd until 3rd days is an exponential phase. In 3rd until 4th is retardation phase. In 4th until 5th is stationary phase. And in the 5th until 7th days is a declining phase. For that reason, the best time to inoculate *C. gracilis* in the exponential phase at 3th and 4th days. Based on the result, *C. gracilis* was proper as organism test.

3.2 Water Quality

The result measuring water quality can be shown in the Table 1 and Table 2.

Table 1. Water Quality Test of cadmium to micro algae *Chaetoceros gracilis*

Nominal concentration (mg Cd/L)	Actual consentration (mg Cd/L)	pH	DO (mg/L)	Temperature (^{0}C)	Salinity ($^0/_{00}$)
control	0	8.13	6.21	24.1	34
0.56	0.56	8.14	6.3	24.2	34
1	0.92	8.15	6.32	24.2	34
1.8	1.7	8.19	6.3	24.2	34
3.2	3.2	8.15	6.31	24.1	34
5.6	4.9	8.17	6.37	24.2	34

Table 2. Water Quality Test of lead to micro algae *Chaetoceros gracilis*

Nominal concentration (mg Pb/L)	Actual concentration (mg Pb/l)	pH	DO (mg/L)	Temperature (^{0}C)	Salinity ($^0/_{00}$)
control	0	8.15	6.28	24.1	34
0.32	0.26	8.15	6.27	24.2	34
0.56	0.45	8.15	6.29	24.2	34
1	0.71	8.17	6.29	24.2	34
1.8	1.79	8.18	6.21	24.2	34
3.2	2.74	8.15	6.24	24.2	34

Measurement of water quality parameter include pH, salinity, temperature and dissolved oxygen carried out the beginning of the test, range as follows pH 8±0.02, salinity 34 ^{oo}%, temperature 24±0.05 ^{0}C, and DO 6±0.05 mg/l. Based on the value of measurement parameter from testing medium, it was a proper habitat for *C. gracilis* and fill the assume of Completed Random Design.

3.3 Range Finder Test

Range finder test was done to find definitive range concentration from lead. Table 3 showed the result of range finder test.

Table 3. The Inhibition of *Chaetoceros gracilis* density concerning to lead in range finder test

Toxicant	Nominal Concentration (mg/l)	Actual Concentration (mg/l)	Cell Density (x10^4 sel/ml)	I (%)	S (%)
Timbal (Pb)	kontrol	-	73.67	-	-
	0.01	-	58.91	20	-
	0.1	-	53.67	27.2	-
	1	-	30.42	58.7	-
	10	-	52.17	29.2	-
	100	-	93.92	-	27.5

I(%): Inhibition
S(%): Stimulation

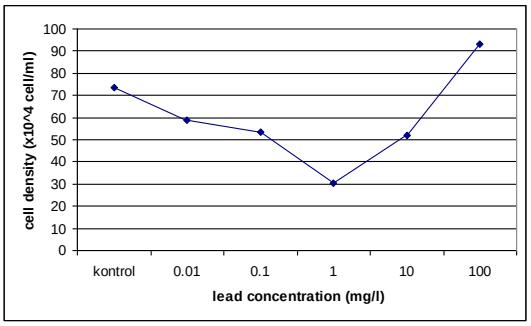

Fig 2. Graphic of cell density of *Chaetoceros gracilis* with variation of lead concentration during 96 hour in range finder test

Figure 2 shows that increasing concentration of lead (II) nitrat is not always decrease the cell density of *C. gracilis.* The Lowest density cell of *C. gracilis* in 1 mg Pb/l and the highest in 100 mg Pb/l. Even based on table 3, in 100 mg Pb/l stimulation of *C. gracilis* cell growth was happened. It can be caused by the capability of lead to precipitate in the sea water, it can be showed by the following chemistry reaction [5]:

$Pb(NO_3)_2 + 2NaHCO_3 \rightarrow Pb(CO_3)\ s + 2NaNO_3 + CO_2 + H_2O$ (1)

The reaction between lead (II) nitrate and natrium hydrogen carbonate can produce lead (II) carbonate, the form of lead (II) carbonate is solid and has the capability to precipitate into the water. The capability of lead(II)nitrate to precipitate can decrease the toxicity level in the overlaying water and natrium nitrate as a nutrient for micro algae was still in the overlaying water, so can stimulate the growth cell of *C. gracilis.*

3.4 Definitive Test

Toxicity of Lead and Cadmium to Cell Growth of *C. gracilis* (Determination of IC50,NOEC and LOEC)

Water quality data shows that testing solution as an optimum condition for cell growth of *C. gracilis*. Cell density of *C. gracilis* in control cadmium solution after 96 hour is $9.74x10^5$ cell/ml and in control lead solution is $9.42x10^5$ cell/ml. it means that this testing is valid for definitive test because it suitable with the criteria from ASEAN Canada CPMS, 1995 for toxicities test in micro algae.

Response for this study is cell density of *Chaetoceros gracilis* during 96 hour with the different concentration of lead and cadmium. The result can be shown in figure 3 and figure 4.

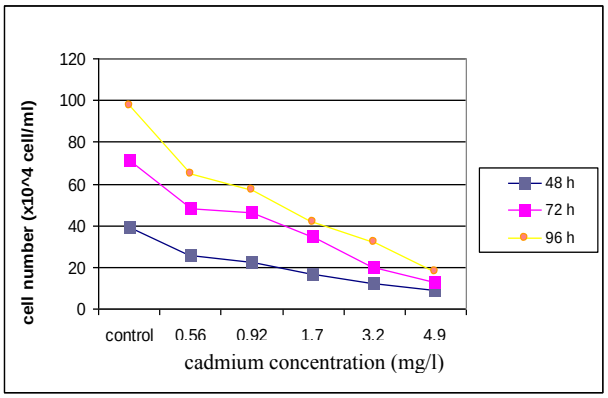

Fig 3. Graphic of cell density of *Chaetoceros gracilis* with variation of $Cd(Cl_2)$ concentration during 96 hour in definitive test

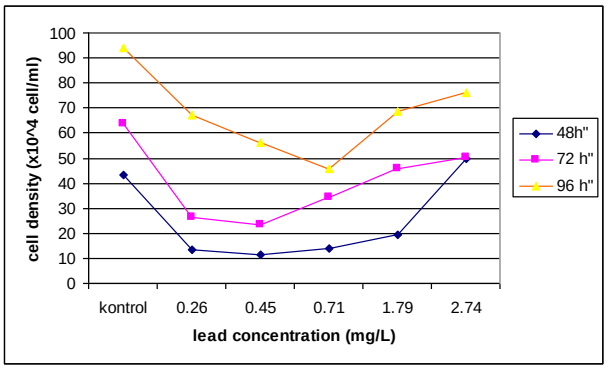

Fig 4. Graphic of cell density of *Chaetoceros gracilis* with variation of $Pb(NO_3)_2$ concentration during 96 hour in range definitive test

The development of cell density, started in 0 hour with the cell density in all concentration as 10^4 cell/ml. After observed in 48,72, and 96 hour, there are a reduction of cell density in each cadmium treatment than control. Based on the data, increasing cadmium concentration will decrease the cell growth of *Chaetoceros gracilis*. So, cadmium toxicant can inhibit the cell growth of *C. gracilis.*

Lead treatment in *C. gracilis* give the different result than cadmium treatment. In lead treatment, increasing lead concentration that be given not always decrease cell density of *C. gracillis.* In 96 hour the decreasing of cell density until concentration 0.71 mgPb/l, but in the higher concentration than 0.71 mg Pb/l will increase the cell density slowly.

The previous research about toxicity test of cadmium in micro algae was done by (Hindarti, 2008;Yap, 2004;Darmayanti, 1998) and all have the similar response (increasing cadmium concentration will decrease the cell density of micro algae). Because the result is consistent so cadmium was used as reference toxicant.

The second toxicant that used was lead. The research was done by Yap, 2004; Henaldi, 1998 and Rivkin,1979. All the previous research have the different range concentration. In this case, there is a phenomena after the lead concentration 0.71 mg/l will increase the cell density of *C. gracilis*. It happened twice, that are in range finder test and definitive test. There are some factor why it happened. First, the lead is easy to precipitate in the salt water. This case was simulated and it was evidenced by visual observed that in lead concentration as 10 mg/l precipitate less than 100 mg/l. Perhaps with that reason, the two previous research using lead concentration less than 1 mg/l. Second, there are a phycochelatin in the micro algae which the function as heavy metals detoxcificant. Bajguz (2004) said that maximal phycochelatin in the *Chlorella vulgaris* will increase with the increasing of lead.

Toxicant	IC50	NOEC	LOEC
Cadmiun (mg/l)	1.3	<0.56	0.56
Lead (mg/l)	0.7	<0.26	0.26

Table 4. IC50,NOEC, and LOEC value in testing toxicity of lead and cadmium in micro algae *C. gracilis*

Calculating IC50 based on the linier interpolation method and in this research was used ICIPIN program. Value of IC50-96 hour from toxicant cadmium and lead to *C. gracilis* are 1.3 mg Cd/l and 0.7 mgPb/l. It means that in concentration cadmium as 1.3 mg/l and lead 0.7 mg/l can inhibit the cell density of *C. gracilis* as 50%.

LOEC and NOEC value are calculated by TOXSTAT program. To use the TOXSTAT program, enter the data and then transform the data to logarithmic. Shapiro wilk and Bartlet test was done to determine normality and homogeneity of variance, if this test pass, parametric analysis involves an ANOVA and Dunnets t-test. LOEC 0.56 mg/l for cadmium and 0.26 mg/l for lead mean that 0.56 mg Cd/l and 0.26 Pb/l are the lowest concentration that can reduce population growth of *C. gracilis*. NOEC value between control and LOEC.

Both of the heavy metals have the capability to inhibit cell density because using the heavy metals ion by organism in the cultivation system by two stage; passive system and active system [6]. Passive absorption happened when the heavy metals had interaction with cell wall and active absorption happened when the heavy metals had been transported pass the cell membrane to cytoplasm. After the heavy metals ion pass the cell membrane, the enzyme and cell organelles be the aim of heavy metals ion. It cause in cells structural will lost much of carbohydrates, decreasing vacuole number, strained the cell wall and the significant influence is disturbing of chloroplast.[7]

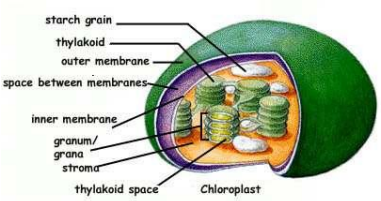

Fig 9. Cell organeles of chloroplast [8]

Over cadmium and lead will influence to chloroplast, that are in the structure and metabolism process. It will cause tylakoid membrane degradation and the tylakoid as a part of chloroplast that accept sun light. So, the degradation of tylakoid membrane will inhibit the chemistry reaction of photosynthesis. It will impact to ATP and NADPH forming as the output from photosynthesis. The Inhibition of ATP and NADPH forming will inhibit micro algae activity like respiration, cell metabolism and cell reproduction. If the energy don't have the capability to support the basic necessary for organism, so it will be dead. This reason that cause the degradation of cell density of *C. gracilis* after adding heavy metals.

4. Conclusion

The result of test toxicity of lead and cadmium in micro algae *C.gracilllis* shows that the response that given as the response from heavy metals treatment not from the other influence like the parameter of water quality.

The effective concentration of lead and cadmium to reduce the

population growth of *C. gracillis as* 50% for 96 h are 0.7 mgPb/l(IC50 0.7 mg/l) and 1.3 mgCd/l (IC50 1.3 mg/l). The lowest concentration of lead and cadmium that can reduce the population growth of *C. gracillis* are 0.26 mg Pb/l (LOEC 0.26 mg/l) and o.56 mg Cd/l (LOEC 0.56 mg/l). The highest concentration of lead and cadmium that have not significant to *C.gracillis* are less than 0.26 mg Pb/l (NOEC 0.26 mg/l) and 0.56 mg Cd/l (NOEC 0.56 mg/l)

5. Acknowledgment

Thanks are due to Research and Development Oceanography, LIPI for expensed this research, Miss. Dwi Hindarti for helping my research and reviewing my paper ,Mr Kaswadji for lending me some reference and reviewing my paper and Miss Sri Endah Agustina as lecture supervisor of Tri University in Bogor Agricultural University.

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