Finding Phytotoxicity Test of Lead to Mangrove Plants of *Rhizophora mucronata*

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Abstract—**Pollution of heavy metals can occurred in river and estuary area. Lead (Pb) was one type of heavy metals that was found in river and estuary area. Pb was one of the conservative heavy metals and Pb can be toxic to human being, animals and plants. The aim of this study was to determine the survival of mangrove of *Rhizhophora mucronata* against the Pb in range finding phytotoxicity test with various concentration of Pb. Pb in various of concentration were exposured to *Rhizhophora mucronata* for 7 days. Variations of Pb concentrations were 0 mg/L as control, 50 mg/L, 100 mg/L, 300 mg/L, 500 mg/L, and 700 mg/L. The physical observation was conducted during the range finding phytotoxicity test. The results showed that the *Rhizophora mucronata* was able to survive with Pb concentration of 100 mg/L. While the concentration of mortality (LC_{50}) was at a concentration of 367.58 mg Pb /L. The death effects can be caused that the plants can absorb/accumulate contaminants in their bodies. In conclusion *Rhizophora mucronata* can survive at 100 mg/L Pb concentration.**

Keywords— **Heavy Metals, Mangrove, Range Finding Test, Phytotoxicity, Pollution.**

I. INTRODUCTION

ENVIRONMENTAL pollution that attracts much attention was pollution by heavy metals. Pollution of heavy metals was one of the factors causing the issue of environmental change, especially in terms of environmental pollution by toxic heavy metal compounds. The spread of heavy metals in soil, water, or air can be through a variety of things, such as the disposal of waste directly industries, either solid or liquid waste, can also be through the air because many industries that burn away the waste and dispose of the products of combustion into the air, without any treatment [1]. Heavy metals in water that accumulate excessively can cause a negative impact on life. The entry of Pb into the water is caused by impact from human activities. Pb toxicity had an effect on human health such as central nervous system disorders, anemia, and carcinogens. Concentration of Pb in sediment at the Wonorejo region was 31.47 mg/L [2]. Pb which accumulated excessively in plants can cause an impact on leaf tissue such as chlorosis, necrosis and black spots [3]. Pb, in sediments, can also affect the life of aquatic biota such as shellfish, because shells usually live and find food in sediments.

One of the functions of mangrove ecosystems is to absorb or bind heavy metals. Mangroves have a role as a good heavy metal bioaccumulator. [4] found metal accumulation in parts of mangrove plants (roots, stems, leaves) and sediments [4]. Mangrove has a high tolerance to heavy metals. Accumulation of heavy metals occurs in the roots, stems and leaves of mangroves [5]. Before mangrove tested with concentrations of heavy metals Pb, then seen first mangrove tolerance can survive. This toxicity test is carried out so that it can be applied to the main research, so that the concentration of heavy metal Pb which is exposed to mangroves can survive.

Mangrove has a toxic response capability, such as by weakening the toxic effect through dilution (dilution), by saving a lot of water to dilute the concentration of heavy metals in their tissues so as to reduce the toxicity of these metals. Dilution with storage of water in the tissue usually occurs in the leaves and is followed by thickening of the leaves (succulence). The excretion is also a possible effort, namely by storing heavy metal toxic material in old tissue such as old leaves and bark that is easily peeled off, so as to reduce the concentration of heavy metals in the body [6].

The range finding phytotoxicity test was conducted to find out how many plants can live in a concentration of heavy metal pollutants. Plants commonly used for the range finding test are of the the same age. This mangrove has the ability to survive heavy metal exposure as it was subjected to the range finding phytotoxicity test stage. This is a sufficient age to survive heavy metals toxicity because of the sufficient nutrients, root tissue, stems and leaves found in the mangrove [7]. During the range finding phytotoxicity test, a visualization of plant development is observed. Usually high concentrations make plants wither and die, where heavy metals have a toxic effect on mangroves.

Dead plants can be calculated by the concentration of death. Median lethal concentration (LC_{50}) is the concentration that causes the death of 50% of the test organism, which can be seen in the graph at a certain time of observation, for example LC_{50} 48 hours, LC_{50} 96 hours until the life time of the test biota [8]. According to [9] LC_{50} values of Pb toxicity on Frangipani *(Oreochromis niloticus)* were < 1000 mg/L. *Avicennia marina* was 403.44 mg/L, *Rhizophora mucronata* was 709.7 mg/L and *Sonneratia alba* was 801.75 mg/L [9]. Toxicity of Pb on *A. marina* has a stronger potency than on other mangrove plants.

The aim of the research was to determine the survival of mangrove of *Rhizhophora mucronata* against the Pb in range finding phytotoxicity test with various concentration of Pb.

II. METHOD

A. Mangrove Preparation

The were many spesies of mangrove, however the mangrove species of this study was *Rhizophora mucronata* due to *Rhizophora mucronata* grew well at estuary area. The age of *Rhizophora mucronata* was 3 - 4 months, it took from Wonorejo Forest mangroves. After that, *Rhizophora* *mucronata* was took from polybags, cleaned the root, then planted in a propagation reactor. Previously, the *Rhizophora mucronata* that had been cleaned, acclimatized for 7 days until new shoots appeared.

B. Determination of PM Concentration

The artifial Pb solutioan was made from pro analysis powder of $Pb(NO_3)_2$ (Meck, USA). The Pb stock solution was made with concentration of 1000 mg/L. The 1000 mg/L stock solution was prepared by dissolving 1.6 g Pb into 1 L of distilled water [10]. The variations of Pb concentration were obtained by diluting the Pb stock solution. The formula for dilluting was written below.

$$C_1 x V_1 = C_2 x V_2 \quad (1)$$

Explanation

C_1 : Pb concentration in stock solution
V_1 : Volume of stock solution
C_2 : Pb concentration in reactor
V_2 : Volume solution in reaction

C. Preparation of Artificial Salinity

Saline water was made from pro analysis NaCl (Merck, USA). Salinity of 20.000 mg/L was prepared from 20 g powder of NaCl that was dilluted with 1 L of aquades.

D. Range Finding Phytotoxicity Test

Preliminary tests were carried out to determine the critical range which was the basis for determining the concentration used in the basic test or the actual toxicity test, namely the concentration that could cause the greatest mortality approaching 50% and the smallest death approaching 50%. The percentage of solution in this preliminary test is in accordance with USEPA, namely 5 variations in exposure and control concentrations [11].

Range finding phytotoxicity test was carried out using a plastic reactor with a diameter of 28 cm and 32 cm high. There were two layers of media. The bottom layer was sand layer as a medium for planting mangroves with a height of 15 cm. The second layer was saline water with high of 5 cm. The salinity water was $20^0/_{00}$ due to the mangrove species of *Rhizophora mucronata* can survive the conditions of the saline water [12]. Figure 1 showed the design of a reactor in range finding phytotoxicity test.

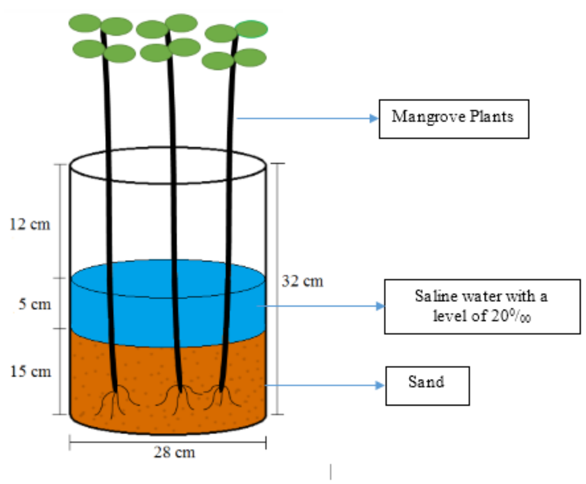


Figure 1. Range Finding Test reactor design.

Total of *Rhizophora mucronata* was 3 plants in 1 reactor with variations of Pb concentration of 0 mg/L as control, 50 mg/L, 100 mg/L, 300 mg/L, 500 mg/L and 700 mg/L. This range finding phytotoxicity test was carried out for 7 days. The addition of nutrients was not carried out during perliminary phytotoxicity tests due to the application of nutrient fertilizers could alleviate the phytotoxicity process [13]. The physical observations on *Rhizophora mucronata* was conducted during the test every day.

E. Determination of Fresh and Dry Weight

Fresh weight measurements were carried out after the range finding phytotoxicity test completed. Each plant of *Rhizophora mucronata* was washed to remove sand and was dried using a tissue to reduce moisture. *Rhizophora mucronata* subsequently separated between the roots, stems, and leaves to weigh the wet weight. All mangrove plants were dried in Memmert (Germany) oven at 105 °C for 24 hours and then measured dry weight [12].

After drying, the plants were put into the desiccator for 1 hour, then the plants were weighed with an analytical balance (Sartorius AG Gontigen BP 2215 made, United States). Calculation of sometimes water water calculated by equations [14]:

$$\text{Water content} = BB - BK \quad (2)$$

Where

BB : Wet weight
BK : Dry weight

F. Statistical Analysis

Analysis of statistics were conducted using Analysis of Variants (ANOVA.) The ANOVA test includes parametric tests. As with other parametric tests, before the test was conducted, the data sample must be checked. The data sample must fulfill the assumptions of the test [15].

In this study, wet and dry weights were tested by ANOVA to determine whether there was a significant difference. The difference in the level of precision in relation to sampling errors (sampling error), was a range where the exact population value is predicted. This range was often expressed using percentage points, for example 1% or 5%. If the Sig value was <0.05 then the data is significant, if the Sig value was >0.05 then the data was not significant. A value of 5% was used for research that does not require high accuracy while for p <1%; it was used for research that requires greater accuracy usually used in the field of health related to human life [16].

III. RESULTS AND DISCUSSION

A. Physical Observation on Rhizophora mucronata

Table 1 showed the condition of the mangrove *Rhizophora mucronata* after the range finding phytotoxicity test had been completed. Based on the figure in Table 2, it showed that the leaves were brownish yellow and the stem becomes soft and dry compared to other concentrations at Pb concentration of 300 mg/L, 500 mg/L, and 700 mg/L. *Rhizophora mucronata* can grow well at the control until exposure to 100 mg/L of Pb concentration. However, at Pb concentration of 100 mg/L, *Rhizophora mucronata* leaves begin to show signs of toxicity. The leaves at this concentration begin to yellow colored.

Table 1.
Physical Observation During the *Range Finding Test*.

Pb Concentration	Day 0	Day 2	Day 7
0 mg/L			
50 mg/L			
100 mg/L			
300 mg/L			
500 mg/L			
700 mg/L			

Table 2.
Physical Condition of Mangroves after a *Range Finding Test*

Pb Concentration	Physical condition of mangroves	Pb Concentration	Physical condition of mangroves
0 mg/L		300 mg/L	
50 mg/L		500 mg/L	
100 mg/L		700 mg/L	

Table 3.
Percentage FW and DW Mangrove Rhizophora mucronata

ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	10.656	1	10.656	6.228	.067[a]
	Residual	6.844	4	1.711		
	Total	17.500	5			

a. Predictors: (Constant), Weight Loss

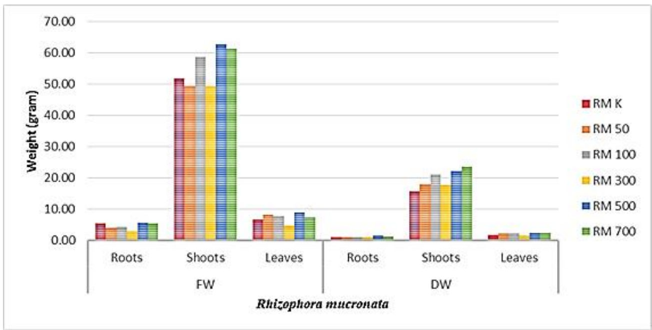
b. Dependent Variable: Concentration

Metal ions do not fully accumulate in plants because metal ions can move from the growing media through the evaporation process, where they form ionic bonds with oxygen forming new ions. Plants that changed their leaf color to yellow indicate that those plants have high salinity toxicity. High salinity can cause stress for mangroves. Salinity affects the presence of heavy metal concentrations in the water. Decreasing salinity in water can lead to increased toxicity of heavy metals and greater accumulation rates [17]. This condition occurs when high temperature affect the oxygen levels in the growing media. In higher temperature, oxygen levels decrease. Oxygen reactes with water in the growing media and binds to metal ions [18].

The decrease in growth in plants could be caused by the plants exposure to Pb over the course of time, caused by the inhibition of the synthesis of chlorophyll was in high levels. Chlorosis can occur if heavy metals inhibit the action of enzymes that catalyze chlorophyll synthesis. Beside that, necrosis could cause the death of cells, tissues, or plant organs [19].

B. Fresh and Dry Weight of Rhizophora mucronata

Figure 2 showed the average fresh and dry weight during the range finding phytotoxicity test. The biggest weight loss occurred at the root of *Rhizophora mucronata* at the control root of 4.36 g. The decreasing in fresh weight to dry weight on the stem occurred at a 500 mg/L Pb concentration, it reached 40.53 g. Meanwhile the greatest weight loss in leaves was 6.56 g at 500 mg/L Pb concentration.



RM K = *Rhizophora mucronata* control
RM 50 = *Rhizophora mucronata* with Pb 50 mg/L
RM 100 = *Rhizophora mucronata* with Pb 100 mg/L
RM 300 = *Rhizophora mucronata* with Pb 300 mg/L
RM 500 = *Rhizophora mucronata* with Pb 500 mg/L
RM 700 = *Rhizophora mucronata* with Pb 700 mg/L

Figure 2. FW and DW After the Range Finding Test were Complete..

Figure 3 showed the percentage of weight of *Rhizophora mucronata* during the range finding phytotoxicity test. After being observed for 7 days, a slight decreasing occurred at 700 mg/L Pb concentration in the stem, 37.74 grams or about 61.6% compared to the control (69.6%). Measurement of fresh and dry weight in mangrove plants was intended to see the water content that was stranded by plants and the decreasing of water content. Water was need by plant parts to grow. Leaves produced food for plants through photosynthesis. Water reached the leaves through the stem. Water was absorbed by plants. Water absorbed by roots was transported through the stem. Minerals from the soil dissolve in water so that they were also transported through the stem. Water and minerals were transported by xylem cells [20].

The statistical analysis results decribed in Table 3. It was not significant different of decreasing percentage in fresh and dry weight of *Rhizophora mucronata* with a value of $\alpha > 0.05$. Mangrove plants need moisture for the growth process. Lack of water can cause the plant to become stunted, its development becomes abnormal. Deficiencies that occur continuously during the growth period can cause the plant to die. The first toxicity signs occured on leaves. This toxicity can be caused by the absorption of water that cannot compensate for the speed of evaporation of water from plants. If the transpiration process was high and water absorption cannot compensate, then the plant become temporary wilting. However, plants get permanent wilting when the water conditions in the soil reached stabil condition [21].

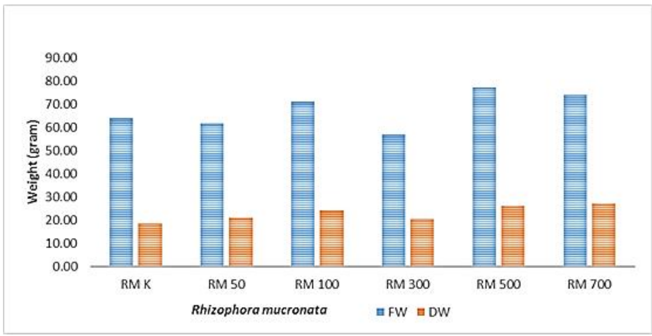


Figure 3. FW and DW of whole *Rhizophora mucronata* at the end of range finding phytotoxicity test.

C. Letal Concentration

The percentage of *Rhizophora mucronata* death in the preliminary test can be shown in Figure 4. Based on data, the high number of *Rhizophora mucronata* death occurred at high concentrations. According to [11] the toxicity test results can be accepted and the requirements for the success of the test during the observation of the control concentration still live upper than 90% of the test biota at the end of test [11].

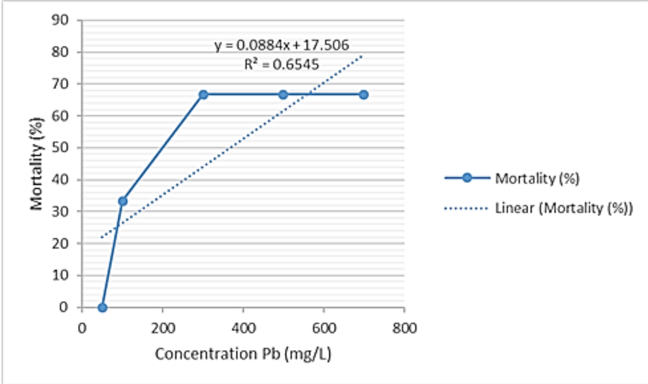


Figure 4. LC_{50} value of Pb Heavy Metal to Mangrove *Rhizophora mucronata*.

The LC_{50} value was obtained from the equation of the line that y = 50 and the value of x = 367.58 mg/L, so that the LC_{50} of Pb toxicity on *Rhizophora mucronata* was 367.58 mg/L. The effect of death was caused by ability of plants to absorb/accumulate pollutants in their bodies, it was called accumulators. If the ability to absorb as much as 100 mg/L was considered a hyperaccumulator plant (Widowati, 2008). So that *Rhizophora mucronata* indicated that plant has a potential plants as hyperaccumulators [22].

The process of Pb absorption was carried out by the root area, it was called rhizofiltration. Plants released organic compounds and enzymes through the roots, it was called root exudates.

The rhizosphere was a very good environment for microbial growth in the soil. These microbe can accelerate the rhizofiltration process. Metals in the form of metal ions can dissolve in fat and can penetrate cell membranes, so that metal ions can accumulate in cells and tissues. The metal can enter the cell and bind to the enzyme as a catalyst, so that the chemical reactions in the cell can be disrupted. Disorders can occur in epidermal tissue, sponges and palisade. The damage can be characterized by necrosis and chlorosis in plants [23]. In an effort to prevent metal poisoning from cells and tissues, mangroves have a detoxification mechanism, for example by hoarding metals in certain organs such as roots [24]. The mangrove accumulates heavy metals within its cells [25]. In plant cells, the heavy metal passes the plasmalemma, cytoplasm, and vacuole, where the metal is localized/accumulated in the vacuole.

IV. CONCLUSION

Based on the physical observation range finding phytotocixity Test over the course of seven days, *Rhizophora mucronata* showed toxicity symptoms at exposure Pb levels of 300 mg/L, 500 mg/L. Meanwhile, *Rhizophora mucronata* died at concentration Pb of 700 mg/L. It showed that *Rhizophora mucronata* could not survive at concentration Pb of 700 mg/L. *Rhizophora mucronata* leaves change color from yellow to brown and the stem leaves become soft and the roots tend to become black at these levels of heavy metal. Physiological conditions of *Rhizophora mucronata* control show that the plants were still fresh and had no symptoms of toxicity. The LC_{50} value of Pb toxicity on *Rhizophora mucronata* was 367.58 mg/L. In conclusion *Rhizophora mucronata* can survive at 100 mg/L Pb concentration although the leaves begin to turn yellow on the last day of the toxicity test.

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