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A removal of Zn metal concentrate using *Rhizophora apiculata* mangrove plants

Mohammad Razif, Isbir Farhan
Department of Enviromental Engineering, Institut Teknologi Sepuluh Nopember, Sukolilo Surabaya 60111, Indonesia

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**Corresponding author email:*
mohammadrazif70@gmail.com

Abstract

Pollutants from industrial waste can pollute the river water and have a negative impact in estuary ecosystem, changes in heavy metal content. One of the industries whose waste contains heavy metals (Chromium, Cyanide, Copper, Zinc, Nickel, Lead and Cadmium) is the electroplating industry. The purpose of this study is to calculate how much the efficiency of *Rhizophora apiculata* mangrove plants in Zn metal removal made from $ZnSO_4.7H_2O$ artificial solution using laboratory scale reactor with the batch system.

Plant acclimatization process has been done with Zn metal exposure test for seven days using soil media without pollutants and using tap water. After the acclimatization, preliminary test in the form of Range Finding Test (RFT) was conducted. The results of the RFT were concentrations of 100 mg /L, 200 mg /L, 300 mg /L which were feasible to be used in the main study phase. The main study phase was done by adding artificial solution into each tub of plastic reactor measuring 270 mm x 233 mm which has been filled with *Rhizophora apiculata* mangrove plant along with soil media. Samples were taken every five days within 20 days, and Zn concentration was measured using a spectrophotometer. The pH and temperature measurements were intended to monitor whether the pH and temperature of the waste water during the study were within the normal range for *Rhizophora apiculata* growth.

Based on the result of the study, the efficiency of metal removal of *Rhizophora apiculata* mangrove plant at a concentration of 100 mg/L was 89,83%, at concentration 200 mg/L the efficiency of metal removal was 84,92% and at the concentration 300 mg/L the efficiency of metal removal was 74,94%.

Keywords: Mangrove, Zn metal, *Rhizophora apiculata*, Spectrophotometer

Introduction

Mangrove forest area in Indonesia, especially in Java Island continue to degrade due to land use conversion from mangrove in the coastal area into the pond, mangrove logging for every purpose, and low public awareness of mangrove forest ecological function (Said & Smith, 1997). This condition is the result of rapid population growth, which requires land for settlements and daily needs. Thus, mangrove forests as coastal ecosystems that are eventually adjacent to residential areas will be very vulnerable to threats and pressures, so that their sustainability is very vulnerable to environmental changes (Tomlinson, 1986). The remaining products of human activities on land, such as domestic waste, agriculture, and industry are generally wasted in the river and will culminate in the estuary of the river and coast. Communities and industries have the assumption that rivers and seas are waste bins that can be used to dispose of waste in a very easy and inexpensive way. Environmental management is still seen as a burden to businessmen, and decision makers are not so easily encouraged to adopt environmental aspects in their policies. The functions and benefits of mangroves are well known for spawning, watersheds, and abrasions by waves, wind protection, marine intrusion into the land, wildlife habitat, bird migration sites and absorbing heavy metals that are harmful to life, settling mud and filtering pollutants. The ecological balance of coastal waters will be maintained if mangroves are maintained as mangroves can function as biofilters, polluting agents and polluting traps, enter a very large pollutant source and have a high tolerance for heavy metals (Macfarlane *et al*, 2003). The absorption of heavy metals by root is influenced by root system and root surface area, for example, *Rhizophora mucronata* can absorb Cd (21,342 ppm), Cu (24,431 ppm), Zn (19,546 ppm) (Handayani, 2006). *Rhizophora apiculata* is commonly found in coastal areas of Indonesian islands, including in South Sumatra (Rachmat & Fauziyah, 2015), East Java (Sudarmadji, 2004) and the majority in East Kalimantan (Warsidi & Endayani, 2017). Pollutants from industrial waste can pollute the river water and have a negative impact of changes in estuary ecosystem changes in temperature, pH, BOD and COD and heavy metal content that greatly affects the life of aquatic flora and fauna. One of the industries whose waste contains heavy metals is the electroplating industry. Electroplating or coating (plating) is one of the process of solid coating material with a metal layer using electric current through an electrolyte solution (Istiyono, 2008). The heavy metal content found in the electroplating industry waste is the Chromium VI ion, Total Chromium, Cyanide, Copper, Zinc, Nickel, Lead and Cadmium (Sumada, 2006). The concentrations of some metals such as Cr, Ni, Zn are higher than the levels that are allowed to be removed. According to East Java Governor's Regulation No. 52 of 2014 (GEJP, 2014) on the quality standards of industrial metal coating wastewater, maximum total Cr content is (0.5 mg /L), Cr^{6+} (0.1 mg /L), Cu (0.6 mg /L), Zn (1 mg /L), Ni (1 mg /L), Cd (0.05 mg /L) and Pb (0.1 mg /L). Therefore, appropriate processing is required to remove this heavy metal from waste water before the wastewater is released into the environment (Meena *et al*, 2005). *Rhizophora apiculata* mangrove species is used because there have been some previous studies that this mangrove species can remove heavy metals and is also a major species in the mangrove forest ecosystem. Zn metal is measured because it is a dangerous metal the presence of Zn metal in water of only 1 mg /L can cause hemochromatosis and gastrointestinal disease in the liver and kidneys (Li *et al*, 2010). Zn metal is of concern due to toxicity and non-biodegradability with a negative impact on fish when released into the river (Gerhardt *et al*, 2004). Therefore it is necessary to conduct a research to know the role of *Rhizophora apiculata* mangrove to exposure to metal Zn. The longer the process means the longer the exposure time. The pH in each of the reactors is decreased to a lower value. This is happened because there is a process of plant respiration, which produces CO_2 that can decrease the pH value. This situation is possible to occur because the CO_2 is released by the plant from the respiration results. The CO_2 is reused for the photosynthesis process. Thus, it will shift the balance to the right equation, which means there is a reduction of H + (acid) ions in Zn artificial waste. According to Pratama et al (2011), the adsorbed pH optimum Zn occurs at pH 6 - 7. Temperature has a great influence on the process of substance exchange (metabolism) in living things based on to Permana (2003). The temperature affects the growth of the plants due to the plant's metabolic processes. According to Hutching & Saenger (1987), the optimum temperature for mangrove growth is in the range of 26-30 ° C.

Materials and Methods

Acclimatization Phase

Rhizophora apiculata was taken from mangrove cultivated in a polybag which is 90 days old, which generally begin to be planted in the mangrove area, the habitat Mangrove Wonorejo Tourism Area Surabaya. Plants Acclimatization were conducted so that *Rhizophora apiculata* plants can adjust to the conditions and media that will be used in the Range Finding Test phase and Zn metal exposure test. This acclimatization phase was carried out for seven days using unpolluted soil media and tap water. In the process of breeding mangrove by using polybag, it is always used soil media that are fertile and unpolluted. While the use of tap water during the process of acclimatization for 7 days is adjusted to the mangrove plant on polybag with a pool of water that is not waste water.

Range Finding Test Phase

Range Finding Test was conducted to find out how much the ability of plants to absorb pollutants at a certain concentration for seven days. The concentration variations used were 0 mg/L (control), 100 mg /L, 200 mg /L, 300 mg /L, 400 mg /L, and 500 mg /L. Concentrations that cause damage to the plant will not be used for the main study. Using the concentration 0 mg/L, the test is conducted as a control to see the concentration of Zn that can be dissolved into the water. If the soil and water contain Zn concentrations, then it is possible that Zn removal of these blank samples will occur during the study periods of 5.10, 15 and 20 days. If this is performed, then it is necessary to correct all removal efficiency of this study.

Main Study Phase

At this phase, the first step is to prepare artificial waste sample $ZnSO_4.7H_2O$ with concentration based on Range Finding Test result. In the main study, a long observation for 20 days is performed to determine the optimum ability of plants *Rhizophora apiculata* in absorbing the metal where the sample is taken every five days. The study was conducted by replicating three times where in each planting medium there is one *Rhizophora apiculata* plant. Therefore this research required nine treatment reactor and three control reactor with the number of plants as many as 12 reactor *Rhizophora apiculata*. This research used a plastic bucket reactor with size 270 mm x 233 mm. The reactor is filled with soil medium with the medium height is 5 - 10 cm and the water volume is as much as 8 L. The parameters measured were metal Zn, pH, and temperature. A research flow diagram shows in Fig. 1.

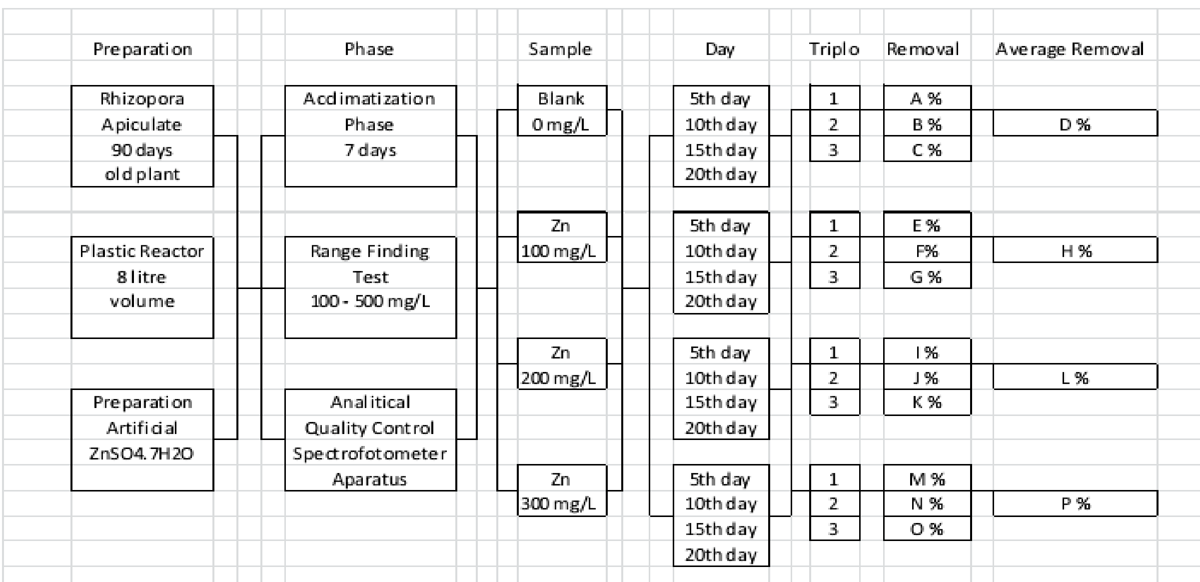

Fig. – 1: Flow diagram of research

Result and Discussion

Acclimatization Phase

Acclimatization stage is an adjustment step of an organism to adapt to a new environment. Plant acclimatization is conducted so that *Rhizophora apiculata* plants can adjust to the condition and media that will be used in Range Finding Test phase and main study phase. The acclimation process has been done by placing *Rhizophora apiculata* plants on the reactor used in the main study phase. This phase is carried out for seven days using unpolluted soil media and using tap water. In the acclimation process, it turns out that *Rhizophora apiculata* plants can live well in a state of death and not wither. Plants that have passed this acclimatization stage were used in the Range Finding Test and the main study phase.

Range Finding Test Phase

Range Finding Test (RFT) was conducted to find out how much concentration of waste water that can be received by *Rhizophora apiculata* plants in absorbing Zn metal pollutants. This phase was conducted by making variations of concentration of Zn metal pollutants then tested those pollutants on *Rhizophora apiculata* plant. Plants used in the RFT were the result of the previous plants acclimatized. Variations of Zn metal concentration used in this RFT stage are 0 mg/L (control), 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, and 500 mg/L. The RFT used reactor in the form of the bucket with size 270 mm x 233 mm. The volume of wastewater used is 8 L, with medium soil with a height of 9 cm. This phase was conducted for seven days. From the observation for seven days, it can be observed that *Rhizophora apiculata* plants were able to live well with concentrations of 100 mg/L, 200 mg/L and also 300 mg/L characterized by leaves that do not wither and not yellowing. While at concentrations of 400 mg /L and 500 mg /L, *Rhizophora apiculata* plants experience death, leaves yellow, and there were holes in the leaves and spots - brown spots.

Main Study Phase

In this main study phase, Zn metal analysis was conducted to determine the ability of *Rhizophora apiculata* mangrove plants set aside the waste water concentration when the plants were exposed to the artificial waste of Zn metal. Wastewater samples were taken every five days in a 20-days time interval. The observation length was to determine the optimum ability of *Rhizophora apiculata* mangrove plants in absorbing Zn metal pollutants which will affect the percentage of Zn removal from waste water. The artificial waste water of Zn metal used, consist of various concentrations starting from blanks, 100 mg /L, 200 mg /L and 300 mg /L in accordance with RFT. All of the concentrations were conducted as much as three repetitions (triplo). Different treatment was applied for each repetition:

a. the first repetition used 100% water corresponding to the growth of mangrove (brackish water),
b. the second repetition used 75% water in accordance with the growth of mangroves (brackish water) + 25% concentration of artificial waste water of Zn metal,
c. the third repetition used 50% water corresponding to the growth of mangroves (brackish water) + 50% artificial metal waste water Zn.

Mangrove plants have the ability to absorb metals but in varying amounts and depending on the type of the mangrove. The absorption of heavy metals is determined by the type of the tissue and the given treatment (Knox, 2000). Therefore, the most decisive factor is the type/species of the plants which in this study, *Rhizophora apiculata* plants were used. A number of plants are shown to have hyper accumulation properties, which are capable of accumulating certain metallic elements with high concentrations in the root and crown tissues, thus being hyper accumulators (Hidayati, 2005). The hyper accumulator properties can be used for heavy metals absorbed by plant roots to be stored, processed or removed when harvested. Plants are able to absorb heavy metals and translocate them into plant parts from roots (Hidayati, 2005) to leaves (Panjaitan *et al*, 2009). The following is the result of Zn artificial metal waste exposure content toward *Rhizophora apiculata* for five days, ten days, 15 days and 20 days in Table 1.

Table 1 shows the effect of Zn concentration in the wastewater by the time function from day 5 to day 20 (ie the longer time decreases, it effects the Zn concentration in waste water). Thus the longer time from day 5 to day 20, it increases the efficiency of Zn removal in the waste water as shown in Table 2.

The results above show that at first repetition, at the concentration of 100 mg /L, 200 mg /L and 300 mg /L for the blank sample yields 0 mg/L of Zn metal waste because the blank sample is only filled with water in accordance with mangrove growth (brackish water) without Zn waste. While in the second and third repetitions at the concentrations of 100 mg/L, 200 mg/L and 300 mg/L there is a significant reduction of each concentration, especially at the exposure of day 5. In the Table 2, it can be seen that the more water added according to the growth of mangroves, the more reduction of Zn artificial waste is. The ability to absorb artificial waste of Zn metal in *Rhizophora apiculata* mangrove plants decrease daily which can be seen in the Table 2. After knowing the ability to absorb mangrove in the form of concentration, the average removal efficiency of each mangrove on the fifth, 10th, 15th, and 20th day is in the form of percentage as shown in Table 3.

Table – 1: Zn Concentration obtain in water

Sample	Zn Concentration (mg/L) obtain in water			
	5th day	10th day	15th day	20th day
Blank (1)	0	0	0	0
Blank (2)	0	0	0	0
Blank (3)	0	0	0	0
100 mg/L (1)	0	0	0	0
100 mg/L (2)	16.83	10.17	6.83	3.50
100 mg/L (3)	36.83	26.83	20.17	16.83
200 mg/L (1)	0	0	0	0
200 mg/L (2)	40.17	30.17	23.50	20.17
200 mg/L (3)	80.17	63.50	50.17	40.17
300 mg/L (1)	0	0	0	0
300 mg/L (2)	63.50	50.17	40.17	35.50
300 mg/L (3)	136.83	126.83	120.17	116.83

Table – 2: Removal Efficiency Zn

Sample	Removal Efficiency Zn (%)			
	5th day	10th day	15th day	20th day
Blank (1)	0	0	0	0
Blank (2)	0	0	0	0
Blank (3)	0	0	0	0
100 mg/L (1)	100	100	100	100
100 mg/L (2)	83.17	89.83	93.17	96.50
100 mg/L (3)	63.17	73.17	79.83	83.17
200 mg/L (1)	100	100	100	100
200 mg/L (2)	79.92	84.92	88.25	89.92
200 mg/L (3)	59.92	68.25	74.92	79.92
300 mg/L (1)	100	100	100	100
300 mg/L (2)	78.83	83.28	86.61	88.83
300 mg/L (3)	54.39	57.72	59.94	61.06

Table – 3: Average Removal Efficiency Zn

Sample	Average Removal Efficiency Zn (%)			
	5th day	10th day	15th day	20th day
Blank	0	0	0	0
100 mg/L	73.17	81.50	86.50	89.83
200 mg/L	69.92	76.58	81.58	84.92
300 mg/L	66.61	70.50	73.28	74.94

It can be explained in the Table 3 that the removal efficiency at concentration of 100 mg/L from day five to day twenty that is 73.17%, 81.50%, 86.50% and 89.83%. While at the concentration of 200 mg/L the removal efficiencies obtained respectively are 69.92%, 76.58%, 81.58% and 84.92%. At concentration of 300 mg/L the removal efficiencies obtained consecutively are 66.61%, 70.50%, 73.28% and 74.94%. The greatest removal efficiencies were found at concentrations of 100 mg /L on the 20th day while the concentrations of 200 mg/L and 300 mg/L were no greater than 100 mg/L of removal efficiency. As it shown in Table 2 and 3, the removal efficiency for sample blanks are always zero % during the study period of 5.10,15 and 20 days. While for the sample concentration of 100 mg/L, 200 mg/L, and 300 mg/L, it increased the removal efficiency to 5.10, 15 and 20 days. Sample concentrations of 100 mg/L were higher in removal efficiency compared to concentrations of 200 mg/L and 300 mg/L. This means that the lower the Zn concentration is occurred, the higher the removal efficiency that can be obtained by the use of *Rhizophora apiculate*. Plants that can absorb/accumulate pollutants in the body are called accumulator plants. While plants that have the ability to absorb as much as 100 mg/L are considered as hyper accumulator plant (Widowati, 2008). Therefore, the *Rhizophora apiculata* mangrove plant is a potential plant as a hyper accumulator. As with other aquatic plants used in phytoremediation (Tangahu, 2015). The process of absorption of Zn heavy metals carried out by roots is called rhizo-filtration. The roots of the *Rhizophora* plant can absorb heavy metals more concentrated from water and surrounding soil (Haryati, 2012). Plants secrete organic compounds and enzymes through roots called root exudates, so the area of the rhizosphere is an excellent environment for the growth of microbes in the soil. The microbes will speed up the rhizo-filtration process. Metals in the form of metal ions are soluble in fat and are capable of penetrating the cell membranes so that the metal ions will accumulate in cells and tissues. Metals can enter the cell and bind to the enzyme as a catalyst so that chemical reactions in the cells will be disrupted. Disorders may occur in epidermal tissue, sponges, and palisades. The damage can be characterized by necrosis and chlorosis in plants (Erakhrumen, 2014). In an effort to prevent metal toxicity against cells and tissues, mangrove plants have detoxification mechanisms, for example by hoarding metals in certain organs such as roots (Priyanto & Prayitno, 2004). The presence of metal accumulation is a localization effort conducted by mangrove plants by collecting in one organ (Heriyanto & Subiandono, 2011). In a plant cell, metal passes through plasmalemma, cytoplasm, and vacuole, where the metal will be localized/accumulated in vacuoles. Mangrove plants have an influence in the prevention of other toxic materials by weakening the toxic effects through dilution, i.e. by storing lots of water to dilute the concentrations of heavy metals in their tissues, thereby reducing the toxicity of those metals (Palar, 1994). This is also directly proportional to the research results that mangrove plants can absorb metals significantly on the 5th day and the percentage of dilution causes this in the addition of nutrient water in the two mangrove plants where the greater the percentage of nutrient water, the absorption of the metal through dilution becomes greater. Mangrove is one of the plants that can adapt well to the water environment, even brackish water or salt. The ability of various mangrove species to adapt to wet environments are different. The dominating species are *Avicennia sp.*, *Sonneratia griffithii* and *Rhizophora*, all species can live, but the dominating one is *Rhizophora*. Plants grown in water will be disturbed by toxic chemicals in waste. The effect of pollutants on mangrove plants may differ depending on the type of pollutant, its concentration and the duration of the pollutants are located (Palar, 1994). The Large and extensive root system of mangrove plants can retain and stabilize soil sediments, preventing the spread of contaminated material to a wider area and allow for the spread of contaminants physically. The above is directly proportional to the results of Zn metal exposure studies obtained that the concentration and duration of exposure also affect the removal efficiency of Zn metal, that the higher the concentration the mangrove's ability decreases. And the longer the exposure time the mangrove's ability to exposure to Zn metal decreases. Mangrove is used for 20 days in Zn artificially exposed artificial waste starting from the first day until the day to 20 with the same conditions and treatment.

Temperature Analysis

Temperature analysis was carried out in the reactor on the main study phase after the RFT conducted for 20 days and the results were taken on five days' interval. Temperature measurements were made using a thermometer tool. The aim of temperature measurement was to determine the temperature of Zn artificial waste in each reactor. The environmental factors of all reactor temperatures ranged from 29 ºC - 31 º C for 20 days on the *Rhizophora apiculata* plant. On the 5th day, the plant temperature is 30 º C, on the 10th day the temperature is 29 º C while on the 15th and 20th day the plant's temperature was 30 º C. Reactors placed outside the laboratory affected the amount of sunlight exposure resulting in high water temperatures for each reactor. These temperature fluctuations can be affected by the ambient temperature at the site. Temperature measurement in this study was aimed to determine the temperature changes in the artificial wastewater for 20 days. The results of the temperature measurements performed on *Rhizophora apiculata* plants were still within the range of plant temperature which growth can grow well and optimum.

pH Analysis

The pH measurements were carried out in the reactor on the main study phase after the RFT was conducted for 20 days and the results were taken on five days' interval. The pH measurements were measured using pH meters toward water sample taken in the laboratory. The result of pH analysis showed that pH in Zn artificial waste water fluctuated in the range of 7.76-8.89 on *Rhizophora apiculata* plants. This is directly proportional to the actual conditions in the electroplating industry waste that the pH in the industry is 6. The pH analysis results were obtained with a pH range of 7.85-8.99, although not optimum but the *Rhizophora apiculata* plants can grow well and absorb the waste artificially well within 20 days.

Applications for waste water

Wastewater from domestic, industrial and irrigation activities discharged into rivers are characteristically very diverse and fluctuating (Razif et al., 2015a and Razif et al., 2015b). Those waste water also have environmental impacts (Razif & Persada, 2015c; Razif & Persada, 2015d; Razif & Persada, 2016). In general, wastewater can be distinguished into those that are containing organic materials, inorganic materials, and a mixture of organic and inorganic materials. For wastewater containing organic materials the waste treatment process generally uses physical-chemical processes, biological processes with decomposing microbes, and a combination of physical-chemical processes and biological processes with decomposing microbes (Mahatyanta & Razif, 2016). As for waste water containing inorganic materials, they generally use physical-chemical process or biological processes with plants. Application of mangrove plants is used as a method of wastewater treatment where mangrove plants serve as absorbent media of materials contained in waste water. Mangrove can serve to absorb inorganic material so that it can be used as heavy metal bio-indicator (Wittig, 1993). *Rhizophora apiculata* plants application has been used in this research to see how substantial the influence of waste water discharge and Zn concentration on the calculation of land area and number of plants needed as shown in Figure 2 and Figure 3.

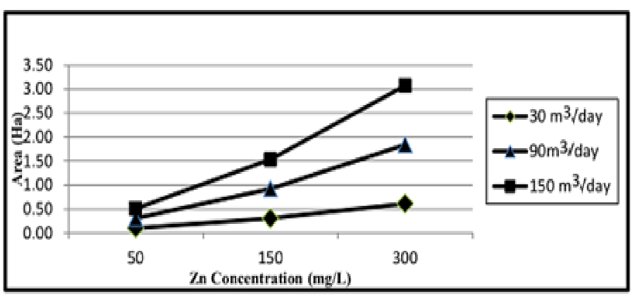

Fig. – 2: The required land area (Ha) of some wastewater discharge (m^3/day) and Zn concentration

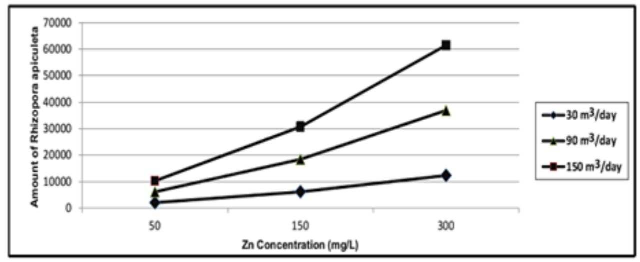

Fig. – 3: The amount of *Rhizophora apiculata* required from some wastewater discharge (m^3/day) and Zn concentration (mg/L)

Figure 2 and Figure 3 show that the higher the Zn concentration and also the higher the discharge of waste water the greater the area of land and the number of plants required. The wastewater discharge data in Figures 2 and Figure 3 are obtained from the discharge of electroplating industrial waste in the Clean River Program Implementation Report (BEJP, 1998).. If the industrial location is not in beach border, then the environmental conditions that need to be considered are the concentration of salt in the wastewater. It must always be prepared in a brackish condition in accordance with the habitat of *Rhizophora apiculeta*. This can be done with the addition of salt NaCl in industrial wastewater with wetland technology.

Conclusion

Based on the research, it can be concluded that the efficiency of removal of *Rhizophora apiculata* mangrove to artificial waste of Zn metal was at a concentration of 100 mg / L is 89,83%. While at concentration 200 mg / L the efficiency removal was equal to 84,92%. Then at a concentration of 300 mg / L the efficiency removal was equal to 74.94%.

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