BIOREMEDIATION OF Pb AND Cd METAL FROM INNER AMBON BAY SEDIMENT WHICH CONTAMINATED WITH HEAVY METAL USING *Aspergillus niger*

Yusthinus T. Male[1]*, Cecilia A. Seumahu[2], Dominggus Malle[3]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Pattimura University*
[2]*Department of Biology, Faculty of Mathematics and Natural Sciences, Pattimura University*
[3]*Department of Animal Husbandry, Faculty of Agriculture, Pattimura University*

**Corresponding author, e-mail: yusmale@fmipa.unpatti.ac.id*

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ABSTRACT

Bioremediation is a method that use microorganism to extract heavy metal from contaminated waste. In this research *Aspergillus niger* was used to extract heavy metal such as Pb and Cd of marine sediment from Waiheru shore, Inner part of Ambon Bay, which was detected as significantly high heavy metal contaminated site among seven sites. Bioremediation were done using *Aspergillus niger*s to extract Pb and Cd metal from sediment, then their solubility being measured in filtrate media. Result shows that Cd metal were only detected 15 days after incubation while Pb were detected since the first day incubation. This result also showing the fluctuating solubility of Pb metal. It is suspected that this occurs due to biosorption ability of the fungi that being used which triggers metal accumulation in the cell structure. It is therefore can be concluded that *Aspergillus niger* can be used in bioremediation of sediment that are being contaminated by Pb and Cd heavy metals.

Keywords*: Ambon Bay, bioremediation, Aspergillus niger, metal, Pb, Cd.*

INTRODUCTION

Coastal towns and industries, together with power stations, have historically discharged their waste either directly into the sea, or into estuary which then quickly reaches the sea. In more recent times sewage effluent is treated to a reasonable standard before direct discharge, but most storm water drains, which are most highly polluted, discharged directly with no treatment at all. Storm water from industrial and urban areas has been identified as a source of organic and inorganic pollution (Reichelt-Brushett, 2012). High oil concentration, and metal concentration including zinc, lead, nickel, mercury, copper and chromium in sediments and organisms have been related to discharge from both industrial and urban areas.

High lead concentrations in waters around urban areas have been attributed to the combustion of lead petrol. Sadiq (1992) suggests that as the consumptions of leaded petrol products decreases, will decreases the lead concentrations. Agricultural runoff often consists of a combination of contaminants including fertilizers, pesticides (halogenated hydrocarbons) and hydrocarbon pollutants. There are several sources of trace metals in agricultural runoff. Phosphate fertilizers contain 5-10 μg/g of cadmium, and the cadmium concentration is directly correlated with the amount of total phosphorus in the fertilizer (O'Neil, 1985, Logantahan *et al.*, 1997).

Inner part of Ambon Bay (*Teluk Ambon bagian Dalam*-TAD) is the traffic lanes and port for many ships and ferry. Ambon Bay also became a big pond for effluent, plastic, organic, chemicals and many wastes from domestic and industrial activity in those area. Some of toxic heavy metals were accumulated in sediments, e.g. Cd, Pb, As, and Hg (Widowati *et al.*, 2008). River and bay sediments were proportionately higher in available mercury than elemental mercury (Male *et al.*, 2013). Cadmium (Cd) and Lead (Pb) are heavy metals that are harmful to human health so that the US-EPA (United States Environmental Protection Agency) establish maximum levels of metal in sediment for was 5 ppm (mg/L) for Cd and 15 ppm for Pb. Anthropogenic sources of Cd metal were paints, pigments, plastic stabilizers and waste from metal coating industries whereas Pb metal comes from burning leaded fuel, battery waste, insecticides and herbicides (Dixit *et al.*, 2015).

To reduce the heavy metal content in the sediment, some method can be used, including remediation. Conventionally, the remediation of heavy metal contaminated objects can be done with excavation and solidification/stabilization. Although this technique is sufficient to maintain the amount of pollutants, it does not dispose of heavy metals well so that heavy metals still present in contaminated objects (Yanga *et al*, 2009). Chemical extraction with strong acids, chelators or organic acids can also be used to extract heavy metals from contaminated objects (Peters, 1999). Fungi are organisms that can be exploited as heavy metal bioremediation agents because the fungi are capable of producing large amounts of organic acids in large quantities, the fungus is able to withstand large pH vulnerabilities and even fungi have high tolerance limits for heavy metal contamination (White *et al*., 1997; Iram *et al*., 2012).

The sampling focus was in the inner part of Ambon Bay which associated with domestic, industrial and agriculture activity. Samples of marine sediments were collected from seven sites around TAD by van Veen grab at deeper marine locations (Figure 1). Samples were stored in polyethylene bags and transported on ice to the Inorganic Chemistry Laboratory, University of Pattimura, where they were immediately frozen. Samples were dried at 60 ºC, ground then thoroughly homogenized and kept in a silica gel desiccator until analyzed. All glassware and other containers were soaked in 10% HNO_3 for 24 h and rinsed three times with deionized water prior to use. Samples were weighed into separated 100 mL beaker glass and digested using *aqua regia* (5.0 mL HNO_3:15 mL HCl) on hotplate at 140 ºC for one hour. After cooling, the solution was filtered with Whatman no.1 filter paper and diluted with deionized water to 25 mL in volumetric flask. Solution then transferred to 30 mL polypropylene vials, stored in fridge prior to analysis with AAS (*Atomic Absorption Spectroscopy*).

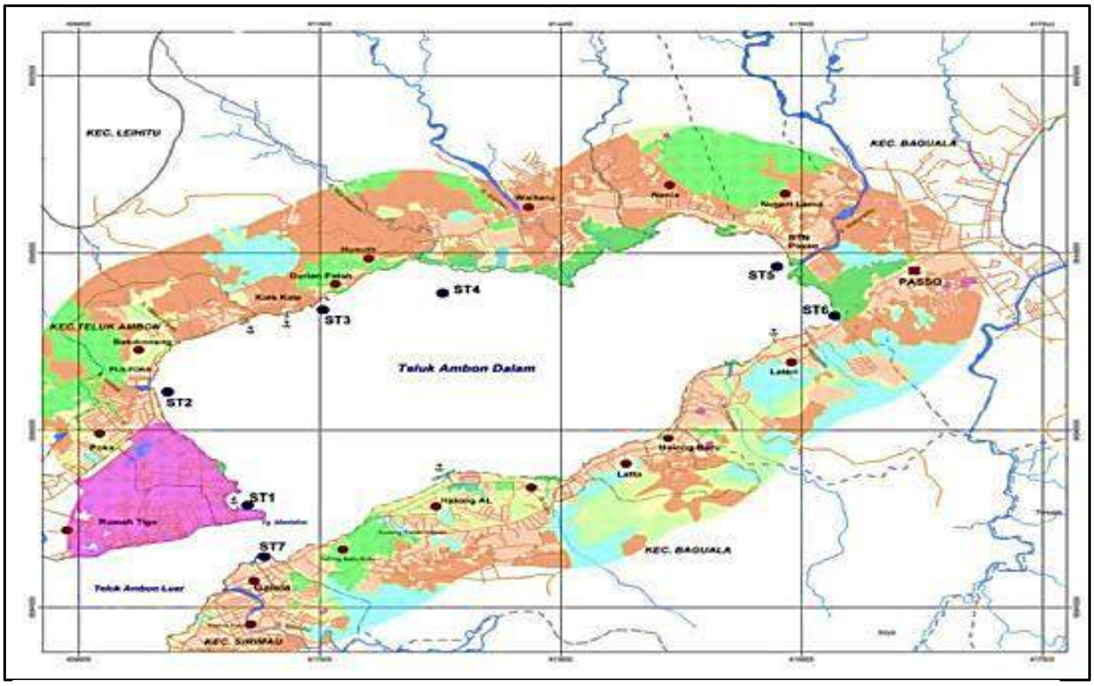

Fig. 1. The map of the inner part of Ambon Bay (TAD) with the sampling sites

Aspergillus is one of the fungi who have the ability to reduce the heavy metals concentration (Congeevaram *et al*., 2006). *Aspergillus niger* has been reported to be capable of removing lead, cadmium, copper and nickel ions in wastewater (Kapoor *et al*., 1998). She-Bardan *et al*. (2012) reported that *Aspergillus fumigates* is capable of producing oxalic and citrate acids capable of removing Pb from contaminated soils of heavy metals.

The results of Pb and Cd concentrations in sediments are show that from the seven sampling sites, the sediments in the Waiheru marine waters contain the highest levels of Pb and Cd metals. This result is supported by sediment type analysis where sediment on Waiheru marine waters is dominated by silt and clay which has high affinity to heavy metals (Male *et al*., 2017).

The availability of heavy metals in sediments is closely related to the nature and size of the sediments. Sediments containing clay and organic quantities will tend to accumulate higher metals, since they have binding properties (Arifin *et al*., 2006). The size of sediment particles (grain size) is one of the factors that influence concentration and heavy metal adsorption process in sediment. The affinity of heavy metals is generally greater in finer sediments, so that heavy metal concentrations are larger on the surface of sediments having smaller particle sizes (Parera, 2004). The highest concentrations of heavy metals are found in sediments in the form of mud, clay, muddy sand or mixtures of the three compared to pure sand (Schaule and Patterson, 1981).The purpose of this study was to investigate the separation of heavy metals from contaminated sediments with bioremediation (bioleaching) methods using *Aspergillus niger* fungi.

METHODOLOGY

Experimental Details

Standard Curve of Pb and Cd

From stock solution of Pb and Cd (1.000 ppm), 10 mL of each solution were transferred 100 mL volumetric flasks and diluted with deionized water. From the new stock solutions (100 ppm), 10 mL of each solution were transferred 100 mL volumetric flasks and diluted with deionized water to make 10 ppm stock solution. To obtain working solutions with each concentration was 0.1; 0.2; 0.3; 0.4 and 0.5 ppm respectively, 1 mL, 2 mL, 3 mL, 4 mL and 5 mL from 10 ppm stock solution were transferred into single 100 mL volumetric flasks and diluted with deionized water prior to analyzed with AAS.

Preparation of microorganisms

The microbes used are *Aspergillus niger* from the moldy copra that has been provided from the Microbiology Laboratory, F-MIPA Unpatti. Potato Dextrose Agar (PDA) was used as a growth medium for *Aspergillus niger* fungi. Some of 100 g of potatoes that have been peeled and washed and then diced then boiled with 500 mL deionized water and filtered. Then add 10 g dextrose and the aqueous mixed medium added to the volume of 500 mL. The medium is then fed into a 500 mL erlenmeyer and capped with cotton, then sterilized using an autoclave at 121° C with 1 atm pressure for 15 minutes.

C. Bioleaching of Pb and Cd Metals from Sediments

After *aspergillus niger* inoculant was growth for 7 days, inoculant was added in spore form into 250 mL liquid medium Potato Dextrose Broth (PDB) as nutrient and 2 g fine sediment samples. Medium contains samples was transferred into a 500 mL erlenmeyer flask and incubated at 37 °C for 0, 5, 10 and 15 days. After the bioleaching process was finish, the sample were centrifuged at 2200 rpm for 10 minutes then filtered to separate the filtrate and residue prior to filtrate was analyzed with AAS.

RESULTS AND DISSCUSION

Bioleaching of Pb Metal

The concentration of Pb metal as product of bioleaching process are shown in Figure 2. Based on the Figure 2 it can be seen that from the 1st day until the 5th day, there was an increase of dissolved lead metal concentration (Pb). At 1^{st} day, concentration of lead metal (Pb) was 6.825 mg/kg and on the 5^{th} day became to 36.775 mg/kg. In the bioleaching process, the concentration tends to increase linier to bioleaching period. The effectiveness of the bioleaching process depends on the ability of microorganisms and the mineral and chemical composition of the extracted metal (Zhang *et al*., 2008).

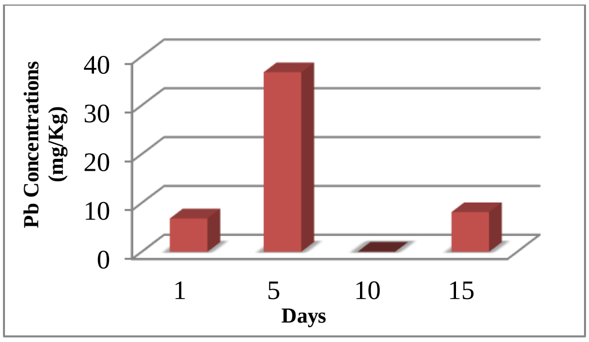

Fig. 2. The result of bioleaching Pb Metal

Concentration of Pb metal at the 1^{st} day until the 10th day greatly decreased. This differs considerably from the concentration on the 5th day. According to Iskandar *et al.* (2011), *Aspergillus niger* tend to bioacumulate Pb up to 54 mg/g of mycelium. In this study, Pb detection was not detected in mycelium but most likely that

Pb was accumulated in the cell structure so that the amount of dissolved Pb decreased.

This results also shows that fluctuations in Pb concentration occur because the process was done using non-sterile sediments so it possible for contaminated with other microorganisms which influence the biosorption and bioleaching processes (Sabra *et al*., 2012). Factors affecting the bioleaching process was nutrition, oxygen demand, pH and temperature as well as types of microorganisms that can increase or inhibit the bioleaching process (Kurniawan *et al*., 2011).

Bioleaching of Cd Metal

Concentration of Cd metal which extracted from Waiheru marine sediments is shown in Figure 3. Concentration of Cd metal at 15th days was 0.0249 ppm while at 0, 5th and 10th days was not detected. This happens because fungi can accumulate heavy metals. Fazli *et al*. (2015) have reported that *Aspergillus versicolor* and *Aspergillus fumigates* were able to accumulate Cd in cell structures with amounts greater than 7.0 mg and 5.0 mg respectively per 1 g of mycelium. In addition, another factor that is suspected to be influential is also the concentration of Cd weight metal which is found also very low compared to other heavy metals so that during bioleaching process, citric acid produced from *Aspergillus niger* is more easily dissolved other metals contained.

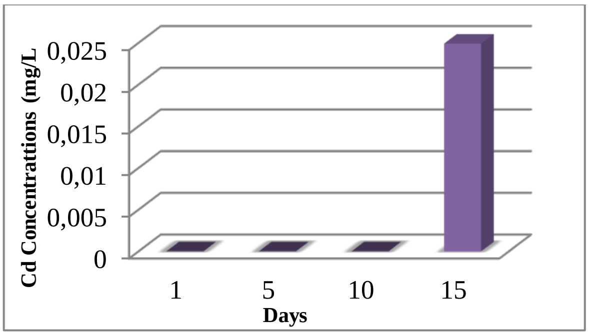

Fig. 3. The result of bioleaching Cd Metal

Aspergillus niger fungi, organic acids produced greatly affect the solubility of heavy metals. She-Bardan *et al*. (2012) also observed that *Aspergillus fumigates* was able to release 88% Pb through a two-stage process in the presence of citric acid production as the dominant acid. Zeng *et al.* (2015) even uses *Aspergillus niger* to perform heavy metal bioleaching in contaminated sediments and found that Cd metal is capable of removing with a 99.5% extraction efficiency through a two-stage process. This occurs because in the two-stage process, organic gluconic acid and succinate are produced in greater quantities than the one-stage process.

CONCLUSION

Aspergillus niger can be used to extract Pb and Cd heavy metals in marine contaminated sediment. Fluctuations in extract concentrations are due to the ability of fungal accumulation in cell structure and the use of non-sterile sediments that allow biosorption and bioleaching by other microorganisms.

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