Decreasing Of Lead And Cadmium By *Cladosporium* and Albizia (*Paraserianthes falcataria L. Nielsen*) Mycorrhizal On Oil Sludge Phytoremediation

Abstract

Oil sludge as we know is one of hazardous petroleum waste that make a particular concern in the environment. Beside of Polyaromatic Hydrocarbon (PAH) oil sludge consist of several heavy metals such as lead and cadmium that can inhibit growth and development of plants and other organism. *Cladosporium* is one of fungi genera isolated from Tanjunguban oil sludge and known can produce bio-surfactants and tolerant in heavy metal stress. The treatment used is a combination of *Cladosporium* and Albiziamycorrhizal in two oil sludge concentration which is 30% and 35%. This study carried out for 60 days and contain of two steps. The first step is composting process by *Cladosporium* during 30 days and continued by Albizia for the phytoremediation process during 30 days. Parameters measured consist of content of lead and cadmium after 60 days treatment analyzed with Atomic Absorption Spectrophotometry (AAS) method and colonization of AMF in Albizia root. The results showed that both of lead and cadmium have a bigger decrease at 35% oil sludge concentration. *Cladosporium* can decrease lead content at 17.94% and cadmium content until 32.22% after 60 days treatment. On the comparative, colonization of AMF in Albizia root reached 100% in all treatment along the decrease of lead and cadmium.

Keywords: Albiziamycorrhizal; Cadmium; *Cladosporium*; Lead; Oil sludge

Research Article

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Introduction

In Indonesia, oil refinery industry production get up to 1.2 million barrels per day and take out 150 thousand tons of waste per year. Oil sludge is the residual of oil distilled from crude oil processing companies that make a particular concern with the environment, because it include to hazardous and toxic waste if referring to the rules of the Ministry of Environment 18 and 33 year of 2009 [1]. This regulation states that no activities are allowed to pollute the environment and that generated are hazardous and toxic must be processed before.

The problem of oil sludge existence in the environment is difficult to degrade naturally and consist of several toxic content such as Polyaromatic Hydrocarbons (PAHs) and heavy metal. Analysis in 2009 showed that Tanjunguban oily sludge contains 30 kinds of PAHs with a chain of C (Carbon) ranged from C12-C43. Concentration of Cu (91.9ppm), Pb (u.d), Zn (251.9ppm), Ni (264.2ppm), Cd (0.215ppm) and Cr (15.8ppm). Whereas, the analysis carried out in 2000, content of Total Petroleum Hydrocarbon (TPH) in Balongan oil sludge about66%, oil and grease 43.56-60%, Asphaltent 0.86%, Cd (0.14ppm), Cr (2.96ppm), Cu (3.58ppm), Ni (58.21ppm), Pb (7.90ppm), Zn (73.53 ppm) (Rossiana, Supriatun, 2010). It means that every refinery contain different characteristics depend on drilling location. From whole content of heavy metal in oil sludge, lead and cadmium are several toxic heavy metals (*Black metal*) that can disturb enzymatic function because it can easy to bind with the amine group of proteins [2].

One method to manage petroleum waste and contaminated soil is bioremediation or the process of biological waste decomposition by utilizing microorganisms refers to Minister stipulation no. 128 year of 2003. Based on our research indigenous fungi strain obtained from TanjungUban oil sludge that have high bio-surfactants production and Total Petroleum Hydrocarbon (TPH) decreasing is *Cladosporium*. *Cladosporium* discovered can produce bio-surfactants about 23.55ppm and decrease TPH about 17.92% after 15 days fermentation. Phytoremediation is a further development of bioremediation techniques that exploit the ability of various plants together with microorganisms to restore land or water bodies are polluted. The principle is based on an optimization of indigenous microorganisms and plant root system to degrade the target compound. Albizia plants (*Paraserianthes falcataria* L. Niesen) can be associated either with endomycchorhiza in extreme environments, critical nutrients, and water. So, aim of this research is to find out the ability of *Cladosporium* towards lead (Pb) and cadmium (Cd) content in oil sludge with combination using Albiziamycorrhizal as phytoremediation agent.

Materials and Method

This study was conducted in Randomized Completely Design use oil sludge concentration (Q) with two levels i.e. 30% (q_1), 35% (q_2). Combination of fungi and Albiziamycorrhizal (R) with one levels i.e. *Cladosporium* sp. and Albiziamycorrizal (r_1). Soil samples were taken at the time of fungal addition (day-0) to be analyzed first, and then after 30 days treatment (day-30) soil samples were taken back to analyzed. The last analyzed soil samples and plant roots taken after the phytoremediation process (day -60).

The main parameters to be measured in this study is the content of heavy metal content Pb and Cd (ppm) in oil sludge medium and plant roots. Supporting parameters were measured consist of total plate count (TPC) of microorganisms (CFU/ml) and percentage of mycorrhizal infection (%).

Methods

Treatment of fungi: Preparation of starter medium for single culture inoculum done by mixing oil sludge 10% with soil and 15% vermicompost as nutrition, then sterilized. Starter medium is stored in bottles 300 g, then inserted the isolate of *Cladosporium* fungi. This inoculum allowed to stand for ±1 month.

Implanted inoculum into medium treatment consist of 30% and 35% oily sludge, soil and vermicompost in the pot. Composted for 1 month with *Cladosporium*inoculum.

Treatment of the plant

a. **Seedling and Mycorrhizal Inoculation:** Seedling media consist of soil and vermicompost is inserted into polybag (5x10 cm), then sterilized. Albizia seeds soaked in hot water ((±80 °C) for 1 minute. The seeds were soaked again in cool water about 24 hours, drained. Seeds are planted in media that has been added by mycorrhizal propagules (1 plants require mycorrhizal propagules about 15g).

b. **Planting and plant maintenance Albizia:** After ±2-month-old plants, Albizia moved into composting oil sludge medium 30% and 35% by *Cladosporium*. Maintenance includes watering plants regularly.

Determination

Determination of heavy metal (Pb and Cd): Determination of heavy metal content was done by as much as 0.5 gram sample was dissolved in 10 ml distilled water (HCl and Nitric Acid 3:1) in a beaker and heated until dry. The samples were given 5 ml of nitric acid and heated back aquadest until late. After a cool, added aquadest back to 50ml. Then the sample centrifuge for 10 minutes at 3000-4000rpm, supernatant taken, then measured the content of heavy metal using Atomic Absorption Spectrophotometry (AAS) [3].

Determination of mycorrhizal infection

Roots of plants aged 3 months washed until there is no soil attached. Plant roots that have been clean from the soil soaked in 10% KOH solution and heated for 30 minutes at 90 °C. Then, roots rinsed with distilled water and soaked again in HCl 1% for 5 minutes. Put the roots on the staining jar filled by carbol fuchsin and soaked for 1-2 days.

Observation of sample roots

Provided 10 pieces of plant roots that have been colored in the object glass. Add a little distilled water and covered with a cover glass. For each treatment is replicated 3 times. Observe themycorrizal infection under the light microscope.

Observation of fungal population

Soil sample about 1g from each treatment inserted into the physiological NaCl solution and dilute until six times (10^{-6}). Three last dilution (10^{-4}, 10^{-5}, 10^{-6}) were planted on Potato Dextrose Agar (PDA). Form of fungal colonies was counted after incubation during 3 days.

Result and Discussion

Heavy metal content in oil sludge medium and plant roots

Heavy metal were observed in this research consist of lead (Pb) and cadmium (Cd). Heavy metal content was observed in composting stage (before: D-0; after: D-30) and after phytoremediation process (D-60). Content and removal average of heavy metal by *Cladosporium* and Albiziamycorrhizal can be seen in Table 1.

Table 1 showed that the treatment with the addition of *Cladosporium* and Albiziamycorrhizal has a varied removal of Pb and Cd. The highest decrease percentage occurred in the media with 35% oil sludge that is 17.94% of Pb removal and 32.22% for Cd removal. Whereas, in the media of 30% oil sludge there is no removal of Pb after 60 days treatment, but Cd content in 30% oil sludge medium decreasing about 28.27% after 60 days. *Cladosporium* known as fungi that can be an efficient asbiosorben for several toxic heavy metals such as Cadmium (Cd), Copper (Cu) and other organic compound like aromatic hydrocarbon [4]. From the result we can observe that *Cladosporium* has a better ability to accumulate cadmium than lead in both of oil sludge concentration.

Table 1: Average of removal and heavy metal content of oil sludge medium. Information: (-): No removal after 60 days treatment

Treatment	Heavy metal	D-0 (ppm)	D-30 (ppm)	D-60 (ppm)	Removal (%)
Cladosporium and Albiziamycorrhizal on 30% oil sludge medium	Pb	16	24.7	20.9	-
	Cd	0.125	0.12	0.09	28.27
Cladosporium and Albiziamycorrhizal on 35% oil sludge medium	Pb	26.5	14.3	21.7	17.94
	Cd	0.12	0.12	0.08	32.22

Mechanism of heavy metal biosorption by microorganisms is a complex process, consisting of: metal transport through the cell membrane, ion exchange, and production of organic acids by microorganisms. Ion exchange occurs between ions in the cell membrane (polysaccharide) with metal ions in the form of bivalent [5,6]. The ion exchange process is done either by bacteria or fungi in heavy metal binding. In addition, the microorganisms produce organic acids (citric, oxalic, gluonnic, fumaric, lactic, and males), which allows for cheating toxic metal. These organic acids help dissolve the metal compound and irrespective of the surface. Metals are absorbed by the carboxyl group of polysaccharides contained in the cell membranes of microorganisms [5].

In this study chelating substance derived from organic acids produced by microorganisms may cause of the increase of Pb and Cd. Pb and Cd are the limited element availability for plants

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and needed in small amounts (micronutrients). However, the presence of Pb and Cd can be improved by adding a chelate to the soil material.

To find out the accumulation of Pb and Cd on roots, sample of roots was taken to analyze use AAS after phytoremediation process (D-60). The content of heavy metal of Albizia's root can be seen in Table 2.

Table 2: Accumulation of Pb and Cd on Albizia's root.

Treatment	Heavy metal	D-60 (ppm)
Cladosporium and albiziamycorrhizal on 30% oil sludge medium	Pb	1.54
	Cd	0.09
Cladosporium and albiziamycorrhizal on 35% oil sludge medium	Pb	9.51
	Cd	0.059

The highest accumulation of Pb about 9.51ppm occurred in Albizia that planted in the 35% oil sludge medium. Whereas, Cd accumulation about 0.09ppm bigger in Albizia that planted in 30% oil sludge medium composting by *Cladosporium*. Accumulation of heavy metals in rats can be influenced by endomycorrhiza. Endomycorrhiza known can bind heavy metals in the carboxyl group in the cortex of host plants, the sheath cell wall polysaccharides and the hype.

Number of fungal population

Similar growth pattern of *Cladosporium* on oil sludge 30 and 35% seen on day 15 until day 30. *Cladosporium* on both of oil sludge concentration on day 45 have an increasing number of population. But on oil sludge 35%, *Cladosporium* increase significantly and *Cladosporium* in the last day of treatment have decreased of number population. Graphic of *Cladosporium* growth pattern can be seen in Figure 1.

Based on the fungal growth observation, although the number of *Cladosporium* decrease on each day of treatment, this fungus was not inhibited to grow and tolerant on polluted condition. This is because of oil sludge compound can be a nutrition for that fungi, so *Cladosporium* is the effective fungi for bioremediation efforts. Beside of that decreasing of *Cladosporium* population influenced by the nutrition competition with Albizia that planted on day 30.

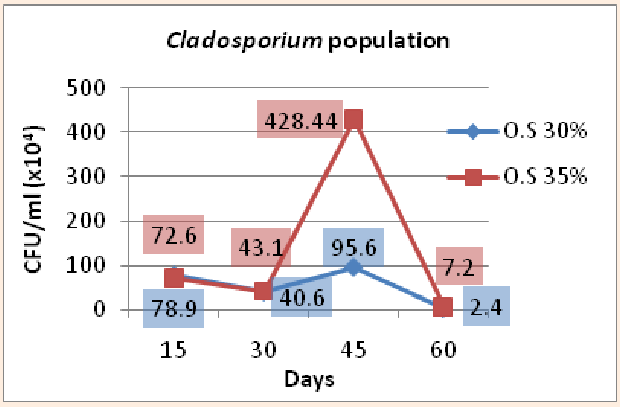

Figure 1: *Cladosporium* population during 60 days treatment

Percentage of endomychorriza infection

The percentage of endomychorriza infection at the Albizia's root and single culture of *Cladosporium* addition to both of oil sludge concentration was 100% infection. Infection of endomycorrhiza on Albizia's root can be seen in Figure 2.

It can be observed that the roots have a lot of hype and vesicle from endomychorriza. External hype from mycorrhiza can be a barrier and inhibit the translocation of heavy metal to the other organs of plants. Beside of that vesicle of endomycorrhiza can accumulate heavy metal so that the heavy metal cannot inhibit plant growth. Heavy metal in soil can also bonded by glomalin, one of glycoproteins that produced by Arbuscular Mychorriza Fungi (AMF) [7]. Mycorrhiza are able to adapt to the soil containing a high metal, soil acidity and high aluminum content have not been limiting factor for mycorrhizal fungi, but it is a problem for plant growth [8].

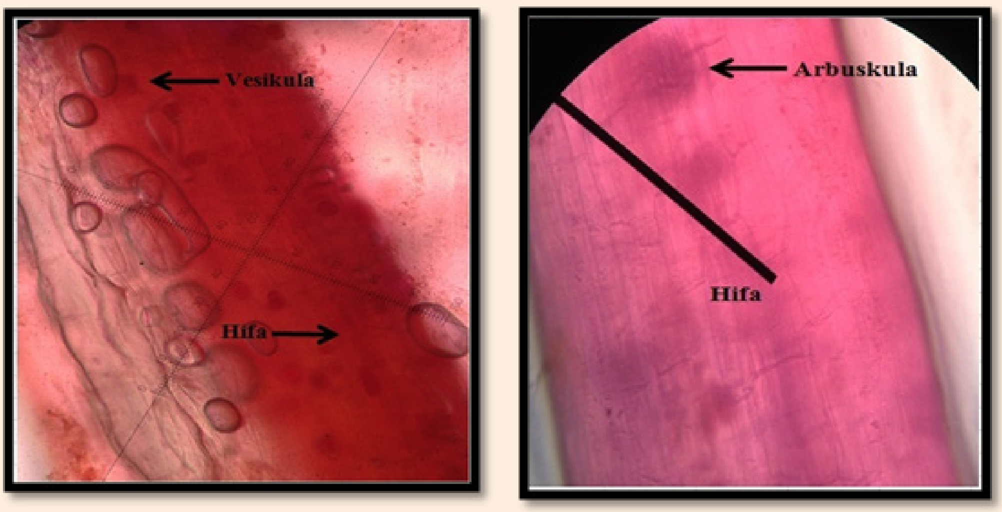

Figure 2: Infection of Endomychorriza on Albizia's root (A) Root of Albizia on oil sludge 30%; (B) Root of Albizia on oil sludge 35%.

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Conclusion

From the result of the research it can be concluded that:

- *Cladosporium* and Albiziamycorrhizacan reduce levels of Cd on oil sludge 30% (28.27%) and 35% (32.22%)
- *Cladosporium* and Albiziamycorrhiza cannot reduce levels of Pb on oil sludge 30%, but can reduce content of Pb on oil sludge 35% (17.94%).
- *Cladosporium* and Albiziamycorrhizahas a similar growth pattern on both oil sludge concentration
- Accumulation of Pb and Cd in Albizia root influenced by infection of endomychorriza.

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