Phytoremediation of Soil Contamination by Petroleum Hydrocarbons

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Abstract

Has conducted research on phytoremediation contaminated soil by petroleum hydrocarbons. From the research it was found that *impatiens* sp have the ability to encourage the degradation of pollutants better than *Cynodon dactylon, Cyperus rotundus,* and *Eupatorium riparium* with allowance for the constant (K) 0.05702 / day or (Kp) 0.002851 / day. At the treatment plant densities produced the best degradation rate on plant density of 20 g of 40.41% while the treatment of pollutant loads best degradation rate of 48.25% in 1% pollutants load. The results of the study plant interactions with soil bacteria, planting *impatiens* sp can increase the bacterial population and the level of degradation of pollutants, and the plant works passively in the allowance for pollutants.

Keywords: Phytoremediation, *impatiens* Sp, density, level of degradation

A. **Introduction**

Oil pollution in the soil can cause interference with the ecosystem and causes the land no longer productive. Therefore, it needs an effective remedy, fast, precise and does not disturb the environment. Many methods can be used for the remediation of oil pollution. According to Udiharto, 1992: Methods of oil pollution remediation in the environment can be done in three ways there are physics, chemistry and biology. Remediation methods in physics can be done with filtering, drowning, burning, and the use of *gelling agent.* Reduction in physics in terms of time is very effective, but often result in removal of contaminants such as combustion can briefly elimination petroleum contaminants, also can cause air pollution and waste combustion results require further processing. As well as chemical control, because in this process using materials chemicals so that there is the possibility of new contaminants that are *xenobiotik* (Clark, 1986). Another way of overcoming weaknesses in physics and chemistry is the cost of relatively expensive operation. Therefore need other methods in the response to oil pollution. Biological methods of Bioremediation and phytoremediation can be an alternative method of oil pollution prevention because it has recognized advantages in terms of operational costs are cheaper, effective and environmentally friendly because organic compounds (crude oil) through mineralization and producing the final product is stable and not toxic. Although this method requires a longer time than the reduction in physics or chemistry (Thomas et al, 1992).

Phytoremediation is a technology using a process or plant vegetation to remove and restore the land or waters that have been contaminated by pollutants (Melethia et al., 1996). Phytoremediation is an inexpensive technology compared with conventional methodology, funding for the restoration of contaminated soil 10 times cheaper or save

75-85% of funds when compared with conventional methods. Several studies to look at the effectiveness of the method have been carried out phytoremediation remedies including uranium contaminated water using sunflower plants grown in *streafoam* whose roots reach the water and can eliminate 95% of pollutants (Alice in Melethia, 1996). Other studies for hydrocarbon pollutants have been carried out by T Crossman. The study showed the concentration of the soil before it was given treatment by the method phytoremediation contains more than 100 ppm total petroleum hydrocarbons (TPH), after being given treatment TPH content found in samples of less than 10 ppm (Kelly, 1997).

From the above discussion it will be research on *contaminated soil fitoremediasi petroleum hydrocarbons* which have the following objectives:

1. Identify plants that can remove pollutants or contaminated soil to recover petroleum hydrocarbons.
2. Determine the removal efficiency of petroleum pollutants.
3. Determining the effects of plants and microorganisms synergism pendegradasi petroleum pollutants.

B. Research Methodology

This study consists of several stages of work, which is the research design diagrams shown in the scheme below.

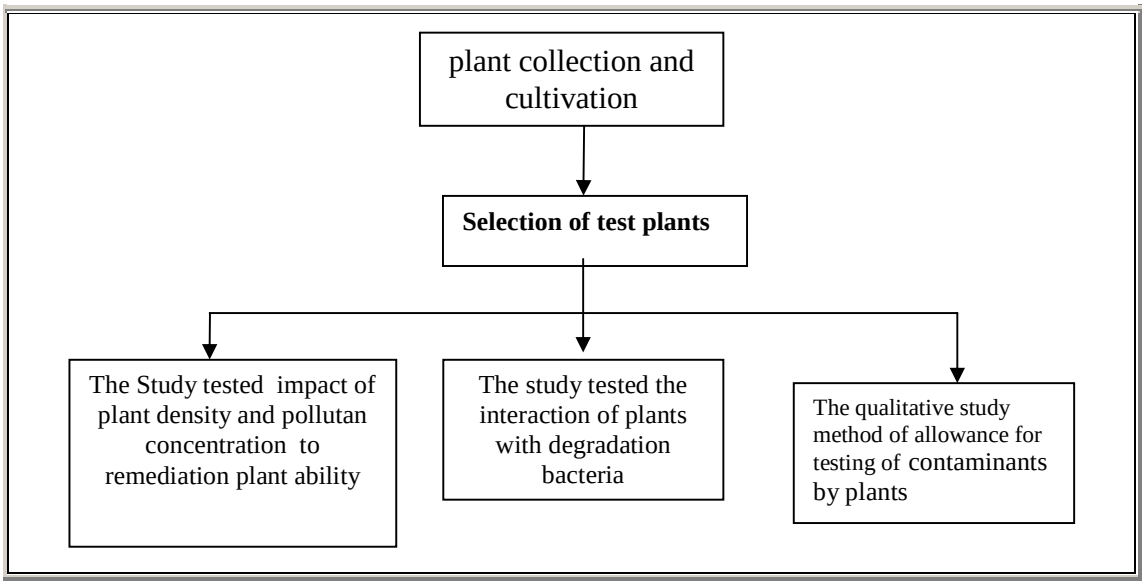


Figure.1. Research design scheme

Selection of test plants

Each stage of the test plant collection and cultivation washed with aquades to eliminate soil-borne, then the plants as much as 20g planted in plastic pots with a soil

medium that has been mixed with 2.5% *crude oil.* Determination of the concentration of contaminants was measured by determining TPH *(total petroleum hydrocarbons)* by the method of Gravimetry in the interval 0, 14 days, 28 days and 42 days. Degradation constants for each test plant is determined by a plot of Ln (Ct-Ct0) against time (t) the resulting gradient is constant degradation.

$dC/dt = Kp.\ dC / dt = Kp.\ C$……(1)

$dC/dt = K.\ dC / dt = K.\ C$……..(2)

$K = Kp.\ K$

C = Konsentrasi kontaminan C = Concentration of contaminant

t = time (days)

P= density of plants

Kp Constant degradation / mass

K= constant of degradation

(Pavlostahis et al, 1998)

Determination of test plants that have degraded the ability of the highest magnitude is determined by the constant degradation and performance of the plant.

Test plants density influence on the ability of the allowance for contaminants

Test plants grown in plastic pots with soil medium supplemented with 2.5%. W / v crude oil.. Density variations of the test plants used were: 0 g, 10 g, 20 g and 40g.. TPH method is determined by Gravimetry in the time interval 0 days, 14 days, 28 days and 42 days.

Impact Test petroleum hydrocarbon concentrations on the ability of contaminants by plants allowance.

Test plants grown in plastic pots with soil medium supplemented with crude oil. Variations crude oil concentration concentration 1%, 2.5% and 5%. TPH determined by methods Gravimetry in the interval 0 days, 14 days, 28 days and 42 days.

The study tested the interaction of plants with degradation bacteria

There are 4 design treatments to see the interaction of plants with soil microorganisms, namely: 1 pots filled with soil and with crude oil which was sterilized, 2 pots with soil conditions and crude oil without sterilization, Pot 3 soil and crude oil that

has sterilized the test plants. Pot 4 soil and crude oil without sterilized and planted crops. Crude oil used was 2.5% w / v and the density of plants is 20 g. Measured parameters are temperature, soil moisture, pH, TPH and microbial population.

Total Plate Count

One gram of soil akuades dissolved into 100 ml, then made dilutions ($10^{-4,}$ $10^{-5,}$ $10^{-6,}$ $10^{-7).}$ One ml of each dilution was taken and grown on NA medium in a petri dish, then incubated at a temperature of 30^{0} C for 48 hours. The number of bacteria per ml obtained by counting the number of colonies that grow to 30 to 300, then the number is multiplied by the dilution factor (Cappuccino and Sherman, 1987).

Antimicrobial testing.

The results of extraction are used as anti-microbial substances.. One ml of test bacteria grown on NA medium. Paper discs ditetesi 1 ml extracts of plants and plant roots on the medium that has been inoculated NA bacteria. Incubation at room temperature for 24-48 hours and then calculated the diameter of bacterial growth inhibitory power (Cappuccino and Sherman, 1987).

The qualitative study method of allowance for testing of contaminants by plants

Content extraction test plants

Part of the test plants will be tested abortion is part of leaves, stems and roots. 5 g plant organs tested from plants grown in soil media that does not contain crude oil and crude oil containing for extraction. The results tested with gas extraction kromatograpi.

Test volatilisasi

Test plant leaves wrapped in a plastic bag for 24 hours. Plant transpiration fluid test results from the plastic bags were collected and then tested with gas chromatography.

Methods Gravimetry

Five grams of soil extracted by using n-hexane in Soxlet for 4 hours.. Extracted and then inserted into the evaporator to remove the solvent. Oil obtained was weighed to determine the amount of oil contained. Degradrasi level of oil can be measured with the formula

$$\text{The level of oil degradation} = \frac{\text{initial weight - The weight of oil specified time}}{\text{Initial oil weight}} \times 100\%$$

Gas chromatography analysis

Petroleum chemical analysis carried out by using gas chromatography is a technique that serves to separate compounds that have the motion of the gas phase and through the columns of a silent phase. Results of gas chromatography is a form of chromatogram peaks showed that compounds different. To determine the type of compound needed each biomarker peaks. Gas chromatography analysis of oil samples to be carried out to see the degradation of oil or not. Shimadzu. Gas chromatography analysis will be performed at the chemistry department of Education University of Indonesia with the type of gas chromatography-QP5050A Shimadzu GCMS.

C. Research Results

Collection of test plants

These plants is *Cynodon dactylon, Cyperus rotundus, Eupatorium riparium* and *impatiens* sp. The four plants collected from several places in the city of Bandung and its surroundings from the forest park Ir H Juanda and Lembang area. The plant is collected and planted back into the pot plants. The fourth test plants allowed to grow for one week as a potted plant in the stage adaptation.

Selection of test plants

Selection of test plants is a step in the process of contaminated soil phytoremediation pollutants to identify plants that have the greatest ability in pollutants from the soil aside. From the observations it was found that of the four plants test plants which have the highest capability in the provision of hydrocarbon pollutants from the soil is I *mpatien* sp (Tabel.1). The highest allowance is determined the ability of the allowance for a constant value of hydrocarbon pollutants, the constant *impatiens* sp allowance has the largest hydrocarbon pollutants is 0.05702 / day or 0.002851 / day for each gram of plant *impatiens sp.*

Table.1. Allowance for a constant value of hydrocarbon pollutants

Species name	Allowance constants ($K_{1)}$	Constant Allowance / g plant ($K_{p)}$	The correlation coefficient (r)	The coefficient of determinant ($r^{2)}$
Cynodon dactylon	0.029522	0.001476	0.952475	0.907209
Cyperus rotundus	0.022626	0.001131	0.969305	0.939552
Eupatorium riparium	0.053523	0.0002676	0.952282	0.906842
Impatiens sp	0.05702	0.002851	0.960682	0.92291

The influence of plant density Against Pollutants Degradation Rate

Research results obtained from measurements of degradation rates in Table .2.

Table.2. The influence of plant density on the level of oil degradation

Plant density (g)	**The level of degradation (%)**			
	0 days	14 Days	28 Days	42 Days
0	0	7.73	12:45	16.61
10	0	13.89	14.99	26.26
20	0	24.27	34.69	40.40
40	0	22:28	31.90	37.33

. The highest level of the resulting degradation in the plant density of 20 g with 40.4% degradation level. The level of degradation increases with plant density increase, but the density of 40 g decreased the degradation rate was caused by a factor of competition.

The influence of burden on the Degradation of Pollutants

From the research it was found that the burden of pollutants tehadap influence the level of pollutant degradation. In table 3 can be explained that the increased burden of pollutants decreased degradasinya level. i. This is due to the effects of inhibitors of petroleum hydrocarbon pollutants on bacterial biodegradation activity.

Table.3. The influence of pollutant loads to the level of oil degradation

Pollutant Load (%)	**The level of degradation (%)**			
	0 days	14 Days	28 Days	42 Days
1 1	0	34.89	46.91	48.24
2,5	0	24.28	38.65	40.40
5	0	24.70	35.93	40.23

Study the interaction of plants and bacteria pendegradasi pollutants

In testing the interaction of plants and bacteria found pendegradasi pollutants that positive interactions between plants and bacteria in the petroleum pollutants degrade Seen in Table .4.

Table.4. The influence of plants and bacteria to the level of oil degradation

Treatment	**The level of degradation (%)**			
	0 days	14 Days	28 Days	42 Days
IS	0	5.67	7.92	22:53
I-ST	0	13.89	14.99	26.26
I-NS	0	24.28	34.69	40.41
I-NST	0	34.66	49.50	50.09

Note: IS: Land sterilized without plants + 2.5% Pollutants
Polutan I-ST: Soil Plant sterilized + 20 g + 2.5% Pollutants
Polutan I-NS: non-sterilized Soil without plants + 2.5% Pollutants
Polutan I-NST: non-sterilized Soil Plants + 20 g = 2.5% Pollutants

From the above table can be seen that plants interact positively in increasing the level of degradation of pollutants, it is because the plant has a passive role of providing a good environment for bacteria pendegradasi on *rhizozpher* area for bacteria to grow so that the population increases and can cause degradation rate increases as well.

Plants can also play an active role in the provision of pollutants by absorbing pollutants in the soil and degraded or accumulated in the plants. Organs extracted from plants found there was an accumulation test a small portion of pollutants in the plant compound and the above test fewer compounds and no pollutants are released through *transpiration* of plants *(volatilisasi).* In table 5 can be seen that the tendency of small plants due to play an active role only a small fraction of compounds in 36 types of pollutants that can accumulate in plants, this could happen due to that the plants have mechanisms to inhibit the toxic pollutant compounds for these plants to enter the plant .

From the results of this study found that the passive role of plants in the interacting with the bacteria can be seen also on the test results with anti-bacterial compounds extracted was found that the extraction does not provide power to inhibit bacterial growth, but instead pushed for increasing the growth of bacteria, look at the growth of bacteria around the disk more than others (Fig. 1).

Table.5. Pollutant content of the compounds tested in the plant organs

No	Biological compounds Pollutants			
	Roots	Stem	Leaf	Volatile
1	$C_{11} H_{24}$	$C_{13} H_{28}$	$C_{11} H_{24}$ $C_{11} H_{24}$	

	Octane 2,4,6-trimethyl	Undecana,2,5-dimethyl-(CAS) 2,5-Dimethylundecana$$	Octane 2,4,6-trimethyl	
2	$C_{11} H_{24}$ Octane 2,4,6-trimethyl-(CAS	$C_{13} H_{28}$ Dodecana, 6-methyl-$$- 6-methyldodecana $$	$C_{11} H_{24}$ Octane 2,4,6-trimethyl-(CAS)	
3	$C_{15} H_{32}$ Dodecana, 2,7,10 Trimethyl-(CAS)	$C_{15} H_{32}$ Dodecana, 2,6,10 Trimethyl-(CAS) farnesane $ $ Farnesane $ $ 2,6,10 trimethyldodecane		
4	$C_{15} H_{32}$ Dodecana,2,7,10 trimethyl-			
5	$C_{15} H_{32}$ Dodecana,2,6,10 trimethyl-(CAS) farnesane $$ Farnesane$$2,6,10 trimethyldodecane			
6	$C_{12} H_{10} N_4 O_3$ Benz0(g)pteridine,2,4(3H,1OH)-dione,8hydroxy			
7	$C_{27} H_{56}$ Heptacosane (CAS) n-heptacosane$$			
8	$C_{30} H_{62}$ Tetracosane 2,6,10,15,19,23 hexamethyl-(CAS) squalane$$robane$$squalan$$cosbi			

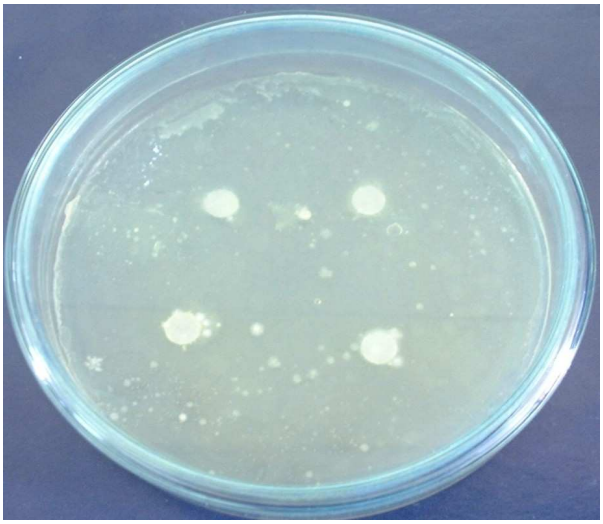


Figure.2. Test results of anti-bacterial properties of plant roots extract *impatiens sp*

It also proved more than the calculated number of colonies of bacteria that occurs growth of bacteria colonies per unit time and can be seen in table 6 Plants provide a positive effect on the growth of bacteria with the evidence that bacterial colonies growing in soil samples overgrown with bacteria than the soil that is not overgrown with bacteria. In the 28-day time decrease the number of colonies of bacteria, it can be caused by several factors such as competition occurs between the bacteria and caused the degradation of pollutants from one compound to the compounds of the bacteria that degrade so that the main products lose a source of food and die so that the resulting reduction in bacterial colonies .

Table.6. The influence of plants against bacterial population growth

Treatment	**Bacterial Population**			
	0 days	14 Days	28 Days	42 Days
IS	249000	1810000	390000	2660000
I-ST	249000	1965000	740000	4320000
I-NS	1900000	60500000	12000000	17900000
I-NST	1900000	212000000	19000000	20700000

Note: IS: Land sterilized without plants + 2.5% Pollutants
Polutan I-ST: Soil Plant sterilized + 20 g + 2.5% Pollutants
Polutan I-NS: non-sterilized Soil without plants + 2.5% Pollutants
Polutan I-NST: non-sterilized Soil Plants + 20 g = 2.5% Pollutants

Gas Chromatography analysis results.

To see the degradation of pollutants in addition to using the method of analysis was also performed Gravimetry extracted soil samples by using gas chromatography (GC-MS). GC results of the analysis done by comparing the GC profile of the curve results from the control soil extract samples (before treatment) to extract the ground after being given treatment. Extract the profile curve of each soil sample can be seen in figure 3.

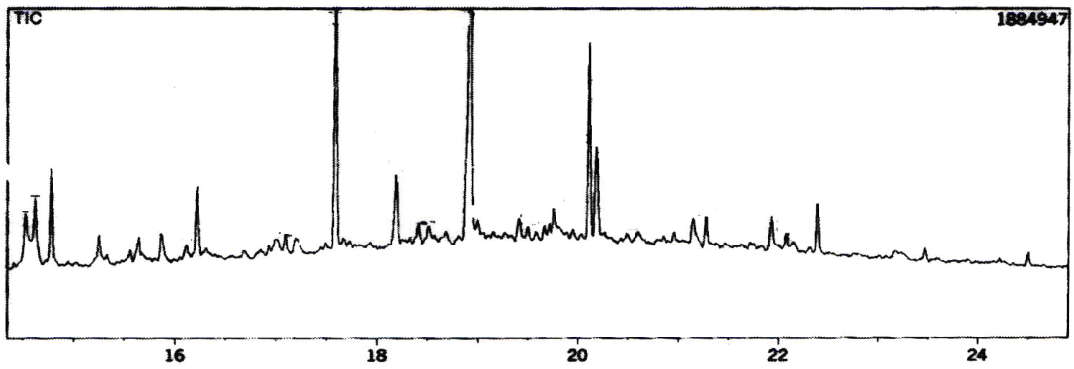
The results compare the GC profile of the curve can be seen that there was a shift towards the left curve, this shows that there is degradation of the pollutant compounds are long-chain hydrocarbons to form hydrocarbons shorter. Profiles above is a profile comparison to before treatment ($t_{0)}$ compared with the profile curve after treatment ($t_{48).}$

Figure 3. Profile curves of gas chromatography

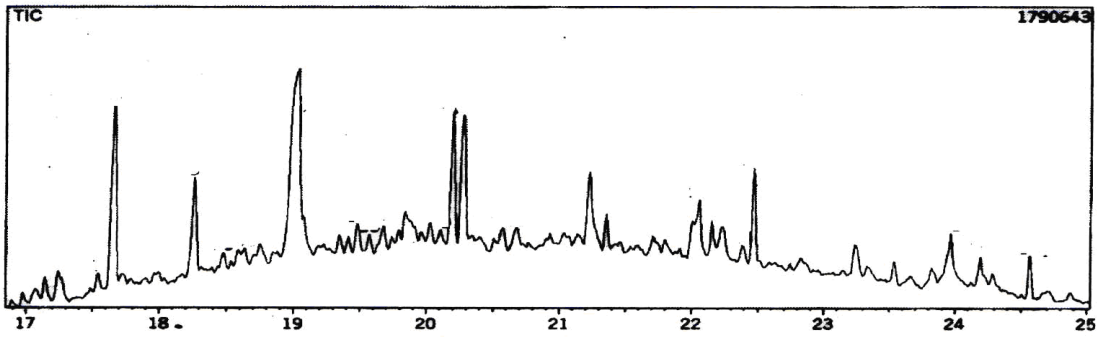
Conclusion

From the above results several conclusions can be made as follows:

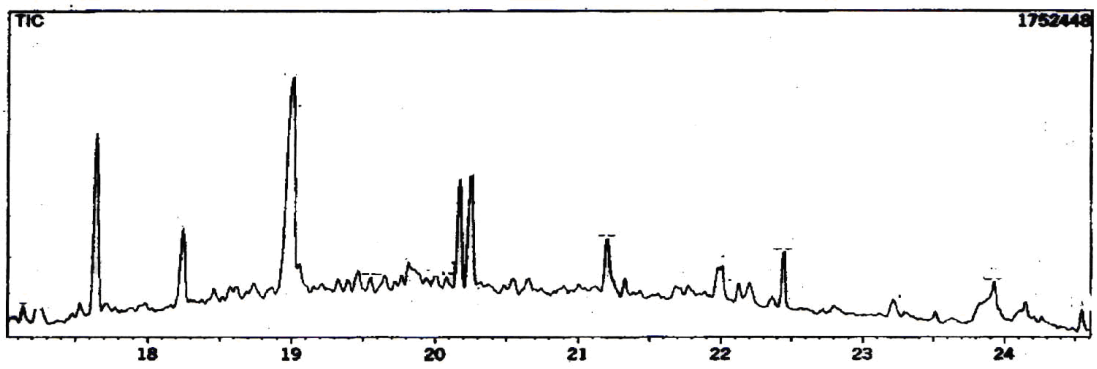
1. *impatiens* plants have the highest allowance hydrocarbon pollutants compared with the plant *Cynodon dactylon, Cyperus rotundus, Eupatorium riparium* with allowance for the kinetics 0.05702 / day or 0.002851 / day for each gram of plant.
2. Plant density effect on increasing the level of degradation of hydrocarbon pollutants.
3. Plant density of 20 g has a degradation rate of pollutants is highest in the amount of 40.41% on day 48.
4. The higher burden of pollutants decreased degradasinya level.
5. Plant *impatiens* sp works passively in the process of remedies hydrocarbon contaminated soil.
6. *impatiens* plants may encourage greater bacterial population in the *rhizosphere* region
7. Hydrocarbon pollutants degradation occurs that can be seen by the shift in the profile curve of the GC-MS



Control Treatment



I-NS Treatment



I-NST Treatment