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Effect of Plant Species and Bacterial Isolates to Development of the Plant Biomass and Total Microorganisms of Contaminated Soil

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ABSTRACT

Oil spills on the ground of tsunami areas such as Aceh, 2004, Mantawai, West Sumatra, 2010 and Japan, 2011 and a lot of accidents on the processing unit and the transport of oil can affect retardation of vegetation growth and cause soil infertility for a long time to the natural process of reproducing the stability and productivity of the land. This research aimed to determine effect of plants species and bacterial isolates to development of the total of plant biomass and microorganisms in the contaminated soil and the interaction between the two factors. The phytoremediation was conducted in pot experiment at the greenhouse and laboratory. The first factor was plant species that are *Clotalaria mucronata*, *Tithonia diversifolia*, *Impatien balsamina* L., *Hellianthus annuus* L., *Arachis hypogea* L. and *Glycine max* L. Merill. The second factor was Inceptisol soils with and without bacterial isolates of *Pseudomonas fluorescens*. The results showed that the plants species and bacterial isolates influenced significantly on shoot and root biomass and also affected the total microorganisms in the contaminated soil. There was a very significant interaction between the use of various plants with Inceptisol soil and bacterial isolates to all parameters observed. The highest total of soil microorganisms was found in Inceptisol soil with bacterial isolates planted with peanut. Peanut and garden balsam gave potential to be the plants cropped for the following experiments, including the study of root exudates in degrading contaminated soil of hydrocarbon.

Key words: Phytoremediation, plant species, bacterial isolates, hydrocarbon, inceptisol

INTRODUCTION

Decreasing of land productivity because of pollutants such as hydrocarbons relates to the activity and crop decrease agricultural and spoils the environment. Currently in Indonesia about environmental pollution cases more and more have been found. One major problem was the soil contamination caused by spills of crude oil (hydrocarbons). Hydrocarbons as organic compounds are common environmental pollutants. Unless handling it well, exploration and production of petroleum and natural disasters will have potential to pollute the environment. Phytoremediation is one of technologies that use plants and the microorganisms to reduce or minimize the concentration of hydrocarbons contaminated in soil. Some plants such as willow and *Salix capira* in sub-tropical regions can stimulate the growth of microorganisms around the roots which can degrade hydrocarbons that exist in contaminated soil (Unterbrunner *et al*., 2007; Wenzel, 2009).

Indonesia that is rich of legume plant species and some other plants that have not been utilized well such as rattlepod (*Clotalaria mucronata*) and garden balsam (*Impatien balsamina* L.). They are worth being experimented as plant species that can affect the development of microorganisms to degrade hydrocarbons in contaminated soil, besides mexican sunflower (*Tithonia diversifolia* L.), peanuts (*Arachis hypogea* L.) and soybeans (*Glycine max* L. Merril). There are several techniques how plants can be used for phytoremediation of contaminated land. They are phytostabilization, phytoextraction, phytovolatisation and phytodegradation (Salt *et al*., 1995; Wenzel *et al*., 1999). Being compared with physicochemical methods, bioremediation including phytoremediation offers an effective technology because oil degrading microorganisms around plant roots participate in the remediation of hydrocarbon contaminated soil.

In addition, the majority of products in the hydrocarbon molecule may provide a source of carbon and nitrogen for microbial growth (Van Hamme *et al*., 2003). On the other hand, strategies of coping contaminants from organic materials can be conducted with excavation and stockpiling techniques, decomposing materials of microbial contaminants in soil, aeration arrangement and provision of fertilizer and the use of plants that can degrade hydrocarbons in the contaminated soil. More specifically, Wenzel *et al*. (1999) and Unterbrunner *et al*. (2007) stated that fitodegradation hydrocarbons in the soil could be conducted in several ways, namely utilization of soil microorganisms supported by the plants. The root interactions with soil biota would increase the degradation of hydrocarbons in contaminated soil.

Various kinds of plants can be used for phytoremediation of land contaminated. Fabaceae (legumes) is one of the plant families that can be used in phytoremediation of land contaminated with pollutants from organic compounds, for instance, peanuts, soybeans, alfalfa and lupines (Gladstones *et al*., 1998). Beside that it can also be used in the Brassicaceae family, such as various types of mustard. In general, plants that can be used for the degradation of hydrocarbons must need special criteria that are tolerant of pollutants, rapid growth, high root exudates and can fix the nitrogen. In addition, organic compounds (petroleum hydrocarbons, PCBs, PAHs, TCE also TNT) contaminated soil could be remedial with Thlaspi caerulescens plants, *Alyssum murale*, *Oryza sativa* and so on (Wenzel, 2009).

Hydrocarbon degradation in soil is influenced by several factors such as soil biota (microrganisms), temperature, water content, soil organic matter and nutrient supply (Pritchard and Costa, 1991; Bragg *et al*., 1994; Wright *et al*., 1997; Margesin *et al*., 2000; Syafruddin *et al*., 2010). Furthermore, Lin *et al*. (1999) reported that fertilizer application could increase the population growth of marsh plant growth, soil microbial populations, increasing grit and microbial respiration showed the potential to enhance biodegradation of hydrocarbons in the soil. Bacteria are microscopic single-celled organisms, which the organic objects penetrate the cells and used as food. If the amount of food and nutrition are enough, so the bacteria will rapidly multiply until the food source is finished. In general, microorganisms could remodel or recycle pollutants into simpler compounds that are not harmful to the environment and could be utilized by other organisms as a source of nutrients (Pelczar and Chan, 1988; Pathak *et al*., 2008). Bacteria have a high capability of degrading petroleum hydrocarbons soil is often used to solve the hydrocarbon pollutants (Bertrand *et al*., 1983).

Decreasing land productivity because of the pollution of soil, water and air is the main problems in improving agriculture, especially food resistance. Additionally, prone countries of disasters and the highest use of crude oil (hydrocarbons), it is necessary to find ways and patterns that are appropriate to deal with contaminated soil, particularly pollution caused by hydrocarbons. In

addition, the Inceptisol soil in coastal areas is highly vulnerable to be contaminated as described above. The main problems of improving the soil-contaminated soil were how to find the right method and not to make new problems. Beside that, the method used was a low-cost assembly technology and friendly environment. Alternatively, we used plant species and bacterial isolates to stimulate the growth of microorganisms that degrade hydrocarbons in contaminated soil. These investigation related to some research who conducted by several researcher before Simonich and Hites (1995), Walworth *et al.* (1997), Siciliano and Germida (1998), Susarla *et al.* (2002), Siciliano *et al.* (2003).

In details, the formulation of the problems that came in this study were (1) How was the influence of some plant species such as rattlepod, mexican sunflower, garden balsam, sunflower, peanut and soybean on the development of plant biomass and total microorganisms in degrading hydrocarbons and (2) How were the effects of bacterial isolates in the soil gave a positive influence on the development and total hydrocarbon degrading microorganisms in contaminated soil. So, the purpose of this study is to determine the effects of the use of plant species and Inceptisol soil with different availability of bacteria status to the development of plant biomass and total microorganisms in the contaminated soil. Besides, we want to study the interactions between these two factors to the development of plant biomass and total hydrocarbon degrading microorganisms in soil.

MATERIALS AND METHODS

Phytoremediation pot experiments was conducted in greenhouse. While soil analysis performed at the Laboratory of Soil Biology, Faculty of Agriculture, Syiah Kuala University, Banda Aceh, Indonesia. The stages of this research are soil preparation, bacterial isolates, preparation of plant species, planting, cultivating and harvesting and calculation of plant biomass and soil analysis.

Soil preparation: The experimental soil used was Inceptisol soil material mixed with crude oil 13.3 g kg^{-1}. Soil pH was 6.21 with silty clay loam texture. The soil was put in a pot experiment. Each pot experiments consisted of 5 kg soil. Characteristic of experimental soil can been seen in Table 1.

Bacterial isolates: Material of Inceptisol soil was divided into two treatments consisting of without and with bacterial isolates, respectively. Bacterial isolate used was *Pseudomonas fluorescens* (Bf.U1). The contaminated soil gave bacterial isolates amount 10 mL kg^{-1}.

Table 1: Characteristics of soil experiment (Inceptisol)

Parameter	Value	Method
pH H_20	7.220	pH 1: 2.5
pH KCl	6.210	pH 1: 2.5
C-Organic (%)	2.040	Walkley and Black
N total (%)	0.180	Kjeldahl
P av (ppm)	9.230	Bray II
K (me 100 g^{-1})	0.260	NH_4OAc pH 7
KTK (me 100 g^{-1})	20.21	NH_4OAc pH 7
Texture	Silty clay loam	Pipette
	Sand 13.72%	
	Silt 56.28%	
	Clay 30.00%	

Sources: Syafruddin (2011)

Preparation of plants species: Plants used in this study were rattlepod (*Clotalaria mucronata*), mexican sunflower (*Tithonia diversifolia* L.), garden balsam (*Impatien balsamina* L.), sunflower (*Hellianthus annuus* L.), peanut (*Arachis hypogea* L.) and soybean (*Glycine max* L. Merril). The seeds were selected before being planted in the pot experiment.

Planting, cultivating and harvesting: All crops were planted with seeds planted except *Tithonia diversifolia* planted was by seedling process before. In the early planting, the plants were given nutrients in the form N (NH_4NO_3) 100 mg kg^{-1} and P (KH_2PO_4) 50 mg kg^{-1}. Every day the plants were looked after and watered, depending on the water needed, based on the Water Holding Capacity (WHC) or an average of 100 mL pot^{-1}. The plants were harvested when they had already been 3 months.

Calculation of plant biomass and soil analysis: Shoot dry weight and roots biomass were weighed at the harvest and then they were put into an oven with the temperature of 60°C for two days to obtain shoot and root biomass dry weight. Soil analysis was conducted to obtain the information about the total soil microorganisms using the Nutrient Agar. Total of microorganisms was measured in units $\times 10^4$ Colony Forming Unit (CFU) g $soil^{-1}$.

Design of experiments: This experiment was a factorial experiment with two factors. Plotted pot experiment consisted of three replicates and based on Factorial Experiments, Randomized Complete Block Design with three replications and consisting of 36 experimental units. There were two factor sunder study, the first factor was plant species, consisting of six levels: Rattlepod, mexican sunflower, garden balsam, sunflower, peanuts and soybeans. The second factor was the Inceptisol soil given bacterial isolates consisted of two levels namely soil without bacterial isolates and soil with bacterial isolates. The parameters observed in this experiment were (1) Plant biomass at the harvest as shoot and root biomass, consisting of fresh and dry weight, respectively and (2) Total of microorganisms in soil by using the method of Nutrient Agar (NA).

Statistical analysis: The effects of the use of plant species and soil types with different bacterial status on the development of hydrocarbon degrading microorganism would be evaluated by using ANOVA ($p<0.05$) two-way and proceed with the Tukey's HSD (Honestly Significant Difference) Test at level 5% to determine significant differences between treatments.

RESULTS

Effects of plants species on the biomass weight: Results of the analysis of variance of F test showed that the factors of the plant species used were very significant effects on shoot biomass fresh weight, shoot biomass dry weight, root biomass fresh weight and root biomass dry weight. The average of shoot biomass fresh weight, shoot biomass dry weight, root biomass fresh weight and root biomass dry weight of all the plants can be seen in Table 2. The highest shoot biomass fresh weight was rattlepod (*Clotalaria mucronata*) and significantly different from the other plants. The lowest shoot biomass fresh weight was sunflower, they are significantly different from the other plants. While the highest shoot biomass dry weight was garden balsam (*Impatien balsamina* L.) and it's significantly different from other plants. The lowest shoot biomass dry weight was

Table 2: Average of the shoot biomass fresh weight, shoot biomass dry weight, root biomass fresh weight and root biomass dry weight at various plant species

	Shoot biomass		Root biomass	
Plant species	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Rattlepod	35.30^{f}	14.04^{e}	8.47^{d}	3.39^{d}
Mexican sunflower	16.59^{b}	4.55^{a}	4.95^{c}	0.93^{b}
Garden balsam	32.23^{e}	13.06^{d}	11.33^{e}	4.53^{e}
Sunflower	11.02^{a}	4.39^{a}	3.30^{b}	1.89^{c}
Peanuts	30.01^{d}	11.99^{c}	12.77^{f}	5.11^{f}
Soybeans	24.50^{c}	9.81^{b}	2.01^{a}	0.80^{a}
Tukey's HSD test ($p<0.05$)	0.25	0.22	0.03	0.01

Values followed by the same letter, the same colunm is not significantly different at Tukey's HSD test ($p<0.05$)

Table 3: Average of the shoot biomass fresh weight, shoot biomass dry weight, root biomass fresh weight and root biomass dry weight at the inceptisol soil without and with bacterial isolates

	Shoot biomass		Root biomass	
Provision of bacterial isolates	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Inceptisol soil without bacterial isolates	25.44^{b}	10.14^{b}	18.80^{a}	8.080^{a}
Inceptisol soil with bacterial isolates	24.44^{a}	9.13^{a}	24.03^{b}	8.560^{b}
Tukey's HSD test ($p<0.05$)	0.13	0.11	0.01	0.003

Values followed by the same letter, the same column is not significantly different at Tukey's HSD test ($p<0.05$)

sun flower plants. The highest root biomass fresh weight was in peanut and the lowest root biomass fresh weight was soybean that is significantly different from other plants. The same trend is seen in root biomass dry weight (Table 2).

Effect of soil isolates without and with bacterial isolates on the biomass weight: Result showed that soil factors Inceptisol without and with bacterial isolation was very significant effects on shoot biomass fresh weight, shoot biomass dry weight, root biomass fresh weight and root biomass dry weight. Average of the shoot biomass fresh weight of the Inceptisols oil without and with bacterial isolates is shown in Table 3. The highest shoot biomass fresh weight was in the Inceptisol soil without bacterial isolates. The same phenomena happen on the the soil without bacterial isolates gives the best results compared the Inceptisol soil with bacterial isolates. The highest root biomass fresh weight was in the Inceptisol soil with bacterial isolates and it was significantly different from the plants without any bacterial isolates. While the same phenomena happen on the root biomass fresh weight and root dry weight of soil with bacterial isolates shows the best results compared with the Inceptisol soil without bacterial isolates (Table 3).

Interaction between plant species and inceptisol soils with bacterial isolates to the shoot fresh weight: There was a very significant interaction between the factors of using plant species and soils Inceptisol without and with isolates to the shoot biomass fresh weight. The average of shoot biomass fresh weight in the use of plant species and Inceptisol soils without and with bacterial isolates is shown in Table 4. The Inceptisol soils without bacterial isolates the highest shoot biomass fresh weight was rattlepod (*Clotalaria mucronata*) and the lowest was sunflower plants

Table 4: Average of the fresh and dry weight of shoot biomass of various plant species at Inceptisol soils without and with bacterial isolates

	Shoot fresh weight (g)		Shoot dry weight (g)	
Plant species	Soil without bacterial isolates	Soil with bacterial isolates	Soil without bacterial isolates	Soil with bacterial isolates
Crotalaria mucronata	39.35^{eb}	31.26^{ea}	15.54^{eb}	12.53^{da}
Mexican sunflowers	16.14^{ba}	17.04^{bb}	6.44^{bb}	2.65^{aa}
Impatien sp.	29.39^{da}	35.06^{fb}	11.76^{da}	14.35^{eb}
Sunflowers	13.55^{ab}	8.48^{aa}	5.41^{ab}	3.37^{ba}
Peanuts	29.37^{da}	30.65^{db}	11.75^{da}	12.23^{db}
Soybeans	24.84^{cb}	24.15^{ca}	9.94^{ca}	9.67^{ca}
Tukey's HSD test ($p<0.05$)	0.36	0.33		

Values followed by same lowercase in total of microorganism at the same row and columns are not significantly different at Tukey's HSD test ($p<0.05$), Values followed by same uppercase in total of microorganism at the same row and columns are not significantly different at Tukey's HSD test ($p<0.05$)

they are significantly different from the others, while the Inceptisol soils with bacterial isolates the shoot biomass fresh weight was *Impatien balsamina* L. (garden balsam) and the lowest one was sunflower plants they were significantly different other plants.

Interaction between plant species and soils with bacterial isolates to shoot dry weight biomass: Results of the analysis range of the F test showed that there was a very significant interaction between the use factors of plant species and Inceptisol soils without and with bacterial isolates of the shoot biomass dry weight. The average of shoot biomass dry weight to the use of plant species and Inceptisol soils without and with bacterial isolates is shown in Table 4. The Inceptisol soil without bacterial isolates showed the highest shoot biomass dry weight on rattlepod (*Clotalaria mucronata*) and the lowest one was sunflower that is significantly different from the other treatments. While on the Inceptisol soils with bacterial isolates showed the highest shoot biomass dry weight on garden balsam (*Impatien balsamina* L.) and the lowest was mexican sunflower that was different from other treatments.

Interaction between plant species and soils bacterial isolation to the root fresh weight: The results of the analysis range of the F test showed that there was a very significant interaction between the usage factors of plant species and Inceptisol soils without and with isolates of the root fresh weight. The average of root biomass fresh weight on the use of plant sand Inceptisol soils without and with bacterial isolates is shown in Table 5. Inceptisol soil without bacterial isolates the highest root fresh weight was garden balsam (*Impatien balsamina* L.) and the lowest is soybeans were significantly different from others. While on the Inceptisol soil with bacterial isolates, the highest root biomass the lowest was soybeans were significantly different from others (Table 5).

Interaction between plant species and soil with bacterial isolates to the root dry weight: The results of the analysis range of the F test showed that there was a very significant interaction between the use factors of plant species and Inceptisol soils without and with bacterial isolates of the root biomass dry weight. The average of the root biomass dry weight on the use of plant species and Inceptisols oils without and with bacterial isolates is shown in Table 5. The Inceptisol soil

Table 5: Average of the fresh and dry weight of root biomass at various plant species in the inceptisol soils without and with bacterial isolates

	Root fresh weight (g)		Root dry weight (g)	
Plant species	Soil without bacterial isolates	Soil with bacterial isolates	Soil without bacterial isolates	Soil with bacterial isolates
Crotalaria mucronata	5.98^{da}	10.95^{eb}	2.39^{ca}	4.38^{eb}
Mexican sunflowers	3.94^{ba}	5.97^{cb}	1.57^{bb}	0.28^{ca}
Impatiens sp.	12.24^{fb}	10.41^{da}	4.89^{fb}	4.16^{da}
Sunflowers	4.35^{cb}	2.26^{ba}	2.88^{db}	0.89^{ba}
Peanuts	9.26^{ea}	16.29^{fb}	3.69^{ea}	6.52^{fb}
Soybeans	1.83^{aa}	2.18^{ab}	0.73^{aa}	0.87^{ab}
Tukey's HSD test ($p<0.05$)	0.04		0.01	

Values followed by same lowercase in total of microorganism at the same row and columns are not significantly different at Tukey's HSD test ($p<0.05$), Values followed by same uppercase in total of microorganism at the same row and columns are not significantly different at Tukey's HSD test ($p<0.05$)

Table 6: Average of the total of microorganisms in the soil at various plant species

Plant species	Microorganisms total ($\times 10^4$ CFU g soil^{-1})
Crotalaria mucronata	2476.67^{a}
Mexican sunflowers	2829.17^{b}
Impatien sp.	3355.00^{c}
Sunflowers	2818.33^{b}
Peanuts	6083.33^{e}
Soybeans	4669.17^{d}
Tukey's HSD test ($p<0.05$)	157.82

Values followed by same letter in the same columns are not significantly different at Tukey's HSD test ($p<0.05$)

without bacterial isolates showed the highest root biomass dry weight on garden balsam and the lowest was soybeans that are significantly different from the others, while on the Inceptisol soil with bacterial isolates showed the highest root biomass dry weight on peanuts and the lowest was soybeans are significantly different from others.

Effects of plant species to the total on soil microorganisms: Result shows that the factor of using plant species affects a very real impact to the total content of microorganisms in the soil. The average content of microorganisms in the soil can be seen in Table 6. The highest total of microorganisms was peanut plants and significantly different from the others. While the result of the lowest one was rattlepod that was significantly different from the others.

Effects of inceptisol soil without and with bacterial isolates on the total of soil microorganisms: F test results on an analysis of variance showed that the factor of using plant species affects a very significant impact to the total content of microorganismsin the soil. The average content of microorganisms in the soil can be seen in Table 7. The highest total of microorganisms was in the Inceptisol soil with bacterial isolates that was significantly different from the others. While the result of the lowest one was in Inceptisol soil without bacterial isolates that was significantly different from others.

Table 7: Average of the total of microorganism in inceptisol soil with and without bacterial isolates

Provision of bacterial isolates	Microorganism total ($\times 10^4$ CFU g soil^{-1})
Inceptisol soil without bacterial isolates	2610.56 [a]
Inceptisol soil with bacterial isolates	4800.00 [b]
Tukey's HSD test ($p<0.05$)	46.85

Values followed by same letter in the same columns are not significantly different at Tukey's HSD test ($p<0.05$)

Table 8: Average of the total of microorganism at various plant species without and with bacterial isolates

	Total of microorganism ($\times 10^4$ CFU g soil^{-1})	
Plant species	Inceptisol soil without bacterial isolate	Inceptisol soil with bacterial isolates
Crotalaria mucronata	8180.00^{db}	6680^{aa}
Mexican sunflowers	7965.00^{ca}	9010^{bb}
Impatiens sp.	6010.00^{ba}	14120^{db}
Sunflowers	3100.00^{aa}	13810^{cb}
Peanuts	10860.00^{ea}	25640^{fb}
Soybeans	10875.00^{ea}	17140^{eb}
Tukey's HSD test ($p<0.05$)	33.49	

Values followed by same lowercase in total of microorganism at the same row and columns are not significantly different at Tukey's HSD test ($p<0.05$), values followed by same uppercase in total of microorganism at the same row and columns are not significantly different at Tukey's HSD test ($p<0.05$)

Interaction between plant species and bacterial isolates to the total of microorganisms: Results on analysis of variance showed that the interaction of factors of using plant species and Inceptisol soil with bacterial isolates gives very real effects to the total content of microorganisms in the soil. The average content of microorganisms in the soil can be seen in Table 8. The Inceptisol soil without bacterial isolates was the lowest total of microorganisms was sunflowers and the highest was soybeans that were significantly different from other plant but in the Inceptisol soil with bacterial isolates was the lowest total of microorganisms was in rattlepod and the highest was peanuts that were significantly different from other.

DISCUSSION

The use of plant species and soil with bacterial isolates showed very significant effects to plant biomass. Shoot and root biomass fresh and dry weight shows very variable results (Table 2 and 3). The results showed that rattlepod and garden balsam truly have higher value plant biomass. While for the fresh and dry weight of roots shows the same trend which the peanut plants in the soil with bacterial isolates have the highest values. The roots have function as elements to transfer carbons from the atmosphere into the soil and rhizosphere as compound containing carbons. Root exudates contribute significantly to carbon stocks that can be used on the bottom and the top of soil surface (Wenzel *et al.*, 1999). The availability and carbon content are the factors that greatly affect the plant species, especially in reducing the high pH, nutrient mobilization and the growth of soil microorganisms. Increasing CO_2 stimulates plant growth generally.

Unterbrunner *et al.* (2007) stated that the degradation of hydrocarbons in the soil increased with the use of plant species and the application of phosphorous optimally. Microorganisms consumed hydrocarbons as an energy source that could degrade hydrocarbons in the soil. The total of microorganisms was very high in the soil inoculated with bacterial isolates (Table 7) and there was interaction with the use of plant species (Table 8). It proved that the plant spurred growth and

stimulated microorganisms in the soil. The same symptoms boccurred in peanut crops grown on soil with bacterial isolates in this study (Table 6). Degradation of organic pollutants, one of which was hydrocarbons by using plants has been widely described by several investigators in *in situ* and *ex situ* treatment of pot phytoremediation. The successful result has been reported by several investigators (Baker *et al*., 1991; Chaney, 1983; Salt *et al*., 1995; Macek *et al*., 2000). With the results and success that they got, naturally phytoremediation becomes the primary choice for the remediation of soil contaminated with hydrocarbons. This harmony can be seen from biomass of peanut plants especially on the parameters of fresh weight and dry weight of roots (Table 2 and 3).

The success of phytoremediation can not be separated from the high or the consolidation of several important factors of the plants. Garden balsam and peanuts in this study had high shoot and root weight (Table 4 and 5). Not only the length of roots but also root activity could help to support the availability of microbial consortium phytoremediation (rhizodegradation) in the root zone (Wenzel, 2009). Beside that other important aspects are very necessary as the availability of water, nutrients and other factors should be available adequately. Therefore, the presence of interactions between plant species and fertilizer use and soil with a positive bacterial isolates will reduce the toxicity caused by pollutants in the soil both organic and inorganic pollutants (Curl and Truelove, 1986; Marschner, 1995; Brix *et al*., 1996; Uren, 2001; Jones *et al*., 2004). In general, it has become an important note that the suitability of the bacteria in this case, the use of *Pseudomonas fluorescens* bacteria supported by a synergy of plants in controlling the toxicity caused by pollutants. Some of the success of bacteria such as *Pseudomonas putida* was reported by Donnelly *et al*. (1994) and *Pseudomonas fluorescens* by Pathak *et al*. (2008) respectively. Plant resilience of adapting in contaminated soil such as peanuts, garden balsam, soybeans, rattlepod and the capacity of beneficial microorganisms would be very useful in phytoremediation technology. It is also as like the research of Burd *et al*. (2000) and Belimov *et al*. (2005). Additionally, favorable interaction between phytoremediation plants with bacteria has been widely demonstrated both in success overcome the organic contaminants and inorganic contaminants. There were even some plants such as *Brassica narfus* growing on Cd contaminated soil could increase the length of the root, shoot and roots biomass because of the support given to the bacteria in contaminated soil (Wu *et al*., 2006).

Pathak *et al*. (2008) stated the bacteria were for the growth in the number of cells needed for oil or crude oil as a source of nutrients and energy sources for metabolic processes in cells. The number of bacteria showed that there was a lot of available oil or crude oil for growth. Atlas and Bartha (1987) noted that microorganisms that could utilize hydrocarbons as a source of nutrients were very dependent on the chemical nature from the components of hydrocarbon mixture and certain environmental conditions. Microorganisms could live and grow well in the neighborhood of petroleum wells and the process products such as kerosene, gasoline and diesel. The growth of microorganisms could be characterized by an increase in population and activity of microorganisms which might result in changing conditions in the surrounding environment.

In these results showed microorganisms that could live and play a role in the decomposition of hydrocarbons are bacteria. There was related to research by Chator and Somerville (1978) and Van Hamme *et al*. (2003). High total microorganisms in the soil could improve the ability for degrading hydrocarbons metabolism and breeding supported by the plants, especially peanuts. Indications were seen on the interactions between plants and soil use in this study. As one of hydrocarbon oclastic bacteria, *Peusodomonas fluorescens* had the best function in the degradation of

hydrocarbons, because the bacteria used hydrocarbons from oil as a source of nutrients for growth and energy (Ghazali *et al*., 2004; De Oteyza *et al*., 2006; Sun *et al*., 2005; Gerdes *et al*., 2005). In addition, the total degradation of petroleum could be done by using bacteria such as *Pseudomonas fluorescens*. Microorganisms were able to describe the components of petroleum because of its ability to oxidize hydrocarbons and make hydrocarbons as electron donors. These microorganisms participated to clean up oil spills with oil oxidizes into CO_2 gas (Hadi, 2003). The ability of cells to resume growth of microorganisms of degrading petroleum was completely dependent on an adequate supply of oxygen and nitrogen as a nutrient source. Beside that the activity of hydrocarbon degrading microorganisms were also influenced by environmental conditions such as temperature and pH. The unsuitable environmental conditions caused microbes not active to work in degrading petroleum. For example, the addition of inorganic nutrients such as phosphorus and nitrogen to the oil spill area supported by the use of plants increased the speed of bioremediation significantly (Hadi, 2003; Unterbrunner *et al*., 2007; Syafruddin *et al*., 2010).

The use of pot phytoremediation experiments showed very significant effects on shoot biomass fresh weight, shoot biomass dry weight, root biomass fresh weight and root biomass dry weight and development of the total microorganisms in the contaminated soil. The use of Inceptisol soil with bacterial isolates of *Pseudomonas fluorescens* showed very significant effects, not only to shoot and root biomass but also development of the total microorganisms in the contaminated soil. There were very significant interactions between the uses of various plant species with Inceptisol soil with bacterial isolates to the all parameters observed. The highest total of microorganisms was found in the combined treatment of peanut plants with Inceptisol soil with bacterial isolates. Peanut and garden balsam had potential to be come the plants that would be intercropped in subsequent experiments, including in searching the function of root exudates in soil polluted hydrocarbon degradation. Additionally, future reseach also will be conducted to investigate degradation of hydrocarbon in the Inceptisol contaminated soil.

CONCLUSION

Plants species and bacterial isolates influenced significantly on shoot and root biomass and also affected the total microorganisms in the contaminated soil. There was a very significant interaction between the use of various plants with Inceptisol soil and bacterial isolates to the plants biomass and the degradation of hydrocarbon. The highest total of soil microorganisms was found in Inceptisol soil with bacterial isolates planted with peanut.

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