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Plant-enhanced phenanthrene and pyrene biodegradation in acidic soil

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Abstract: *A study was undertaken to assess if corn plant (Zea may L.) may be able to enhance the degradation of phenanthrene and pyrene in acidic soil inoculated with a bacterial strain (Pseudomonas putida MUB1) capable of degrading polycyclic aromatic hydrocarbons (PAHs). Planting with corn, inoculating with MUB1, or a combination of the two were found to promote the degradation of phenanthrene and pyrene in acidic soil at different rates. In the presence of corn plants, the rates of phenanthrene and pyrene removal were 41.7 and 38.8% in the first 10 days, while the rates were 58.8 and 53.6%, respectively, in the treatment which received MUB1 only. After 60 days, the corn + MUB1 treatment led to the greatest reduction in both phenanthrene and pyrene biodegradation (89 and 88.2%, respectively). In control autoclaved soil, the rates of phenanthrene and pyrene removal were 14.2 and 28.7%, respectively, while in non-autoclaved soil, the rates were 68.7 and 53.2%, respectively. These results show that corn, which was previously shown to grow well in PAH-contaminated acidic soil, also can enhance PAH degradation in such soil. Inoculation with a known PAH degrader further enhanced PAH degradation in the presence of corn.*

Key words: *Acidic soil, Biodegradation, Phenanthrene, Plant-microbe interaction, Pyrene*
PDF of full length paper is available with author ***(*chouychai@yahoo.com)***

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds comprised of multiple aromatic rings. These compounds occur in coal tar, wood preservatives, and wastes from manufacturing and petrochemical industries. Many of these compounds are toxic and/or potent carcinogens (Dean *et al*., 2001). Many higher molecular weight (MW) PAHs are also recalcitrant under conditions prevailing in the terrestrial environment, resulting in their persistence over long periods of time (Sims and Overcash, 1983). Thus, there is a need to develop the science / technology to increase the rate of degradation of persistent PAH compounds from the environment.

Many bacterial strains from different genera are known to degrade PAHs under aerobic conditions. However, some authors have found limited success while relying on microorganisms alone for PAH degradation because of the poor bioavailability and slow rate of movement of these PAHs, such as benzo(a)pyrene, dibenzo(ah)pyrene, to competent microorganisms (Huang *et al*., 2004). For this reason, improvement of bacterial remediation in contaminated soil is necessary.

Some plants such as ryegrass (Binet *et al*., 2000), *Lolium perenne* L. (Corgie *et al*., 2004), alfalfa, fescue, sudangrass and switchgrass (Reilley *et al*., 1996) have been shown to increase PAH biodegradation in soil. Likely, the presence of plant roots promoted the microbial degradation of PAH in soil (Hynes *et al*., 2004). While the precise mechanism(s) for this effect is not known, the results showed that the use of plants has promise and deserves to be investigated in efforts to increase the effectiveness of microbial degradation of pollutants in soil.

Several other studies have reported the ability of plants to enhance PAH degradation in soil. For example, tall fescue (*Festuca arondinacea*) was reported to enhance the removal of PAHs, especially those of high molecular weight (MW), from soil (pH 7.6) at rates significantly higher than mixed bacterial cultures of *Pseudomonas putida, Flavobacterium* sp and *Pseudomonas aeruginosa.* Tall fescue was also found to promote the removal of benzo(a)pyrene, dibenzo[ah]pyrene, benzo (ghi) perylene and indo (123-cd)pyrene by 5-20% while the mixed bacterial inocula could not degrade these 4 PAHs (Huang *et al*., 2004). The phenanthrene biodegradation rate in soil planted with *Sinapis alba* and inoculated with the phenanthrene-degrading *Sphingomonas yanoikuyae* is higher than treatments which received only plant or bacterial inoculum alone. Similarly, the extent of phenanthrene biodegradation in planted soil was higher than soil which received only bacterial inocula (Hynes *et al*., 2004). However, these experiments were done at neutral soil pH.

The growth of plant and inoculated bacteria may be affected by a number of factors. For example, soil pH has an important effect on the distribution and growth of plants and soil microorganisms (Neale *et al*., 1997). The soil in areas along the coastal plains of Southeast Asia, especially in Vietnam, Indonesia, Malaysia, and Thailand is mainly acidic with a high sulfur content (Bronswijk *et al*., 1995; Husson *et al*., 2000; Shamshuddin *et al*., 2004). The pH of this soil type is typically lower than 3.5. This low pH phenomenon is believed to arise from oxidized pyrite being deposited at the time when this area was the sediment before land formation (Shamshuddin *et al*., 2004). Soil pH was found to be an important factor affecting seed germination rate at pH values ranging from 4.7-7.7 and this effect was dependent on plant species (Perez Fernandez *et al*., 2006). Low pH values have the potential to leach toxic metals, such as lead and aluminum, from soils and this can retard plant growth. For example, the root length of cocoa plant (*Theobroma cacao* L.) has been shown to be decreased by aluminum toxicity in acidic soil (Saygideger and Dogan, 2005; Shamshuddin *et al*., 2004).

In Thailand, some parts of the eastern provinces (Rayong and Chonburi Provinces) with acidic soil rich in sulfate content are known to be contaminated with PAH compounds as a result of the petrochemical industries and sea transportation of crude or refined oil products in this area (Boonyatumanond *et al*., 2006). The acidity may pose additional challenges in efforts to remediate PAH contaminants from soil. In the literature, degradation of PAHs within rhizosphere has been described in pot studies using soil with pH values ranging from 5.4-10.4 (Binet *et al*., 2000; Chen *et al*., 2003; Huang *et al*., 2004; Johnson *et al*., 2004; Joner *et al*., 2001; Liste and Alexander, 2000; Merkl *et al*., 2006; Reilley *et al*., 1996; Xu *et al*., 2006). In other studies involving the inoculation of PAH degrading bacteria, the soils used had pH values ranging from 5.2-8.3 (Errampalli *et al*., 1998; Kastner *et al*., 1998; Ley *et al*., 2005; Providenti *et al*., 1995; Weir *et al*., 1995). It is not known how effectively a bacterial inoculum can degrade PAH compounds in acidic soil, nor is it known if PAH degradation in such soil can be enhanced by plants.

In this study, the ability of a PAH-degrading bacterial inoculum, in the presence or absence of an acid-tolerant plant, to degrade two model PAH compounds in acidic soil was assessed in laboratory-scale pots. The plant used was corn which has been shown to be tolerant of PAH contaminants in acidic soil (Chouychai *et al*., 2007). The bacterium used was *Pseudomonas putida* MUB1 which was isolated from oil-contaminated soil in Rayong Province, Thailand. This strain was shown to degrade phenanthrene, but not pyrene.

Materials and Methods

Soil analysis and preparation: Soil used in this experiment was collected from a forest area in Trat Province in eastern Thailand. This soil had no previous history of PAH contamination. The soil was kept at room temperature (28-31°C) in black plastic bag until use. The soil was air-dried at room temperature (28-31°C) for at least 24 hr to constant weight before use. This soil was previously characterized as being fairly acidic soil (pH 3.3), with a low organic content (0.99% w/w) and an electrical conductivity of 0.01 dS m^{-1} (Chouychai *et al.,* 2007). The soil contained 0.049% nitrogen, 12 ppm phosphorus, and 22 ppm potassium.

One kg of air-dried soil was added to a 17.8 cm diameter plastic pot in triplicate. Phenanthrene (Carlo Erba Reagent, solid, 99.5%, code number 449984 6C116288L) and pyrene (Fluka Chemie GmbH, CH-9471, Buches, Switzerland, solid, 97.0% purity by GC) were weighed individually and dissolved in acetone. Each PAH solution was transferred to a glass sprayer and spiked to a final concentration of 100 mg kg^{-1} dried soil for phenanthrene or pyrene. After thorough mixing, the spiked soil was air-dried at room temperature (28-30°C) for more than 24 hr or until the smell of acetone had disappeared. One set of soil was autoclaved at 121°C for 15 min before spiking.

Assessing PAH degradation by *Pseudomonas putida* MUB1 and strain maintenance: *Pseudomonas putida* MUB1 was previously isolated from oil-contaminated soil from Rayong province, Thailand and its species designation was determined by partial 16S rRNA sequence analysis at the Faculty of Science, Mahidol University (unpublished). Strain MUB1 was first tested for its ability to use two model PAH compounds (phenanthrene and pyrene) as sole carbon sources for growth. One colony of *P. putida* MUB1 growing on mineral salt agar medium was transferred to 50 ml of Basal Salt Medium (BSM) (Juhasz *et al*., 1996) containing 30 mg l^{-1} of either phenanthrene or pyrene. The cultures were incubated at 30°C in 125 ml glass Erlenmeyer flasks and shaken at 150 rpm. Degradation of PAHs was observed visually by clearing of the culture media and also by a change in the color of the media from white to brown or yellow (Binet *et al*., 2000) as compared to un-inoculated control in which the color change did not occur. After 7 days, a color change was found only in the culture of *P. putida* MUB1 in BSM plus 30 mg l^{-1} phenanthrene where the culture turned slightly yellow. None of the cultures caused any color change in the pyrene-containing cultures. This result indicated that MUB1 could degrade phenanthrene, but not pyrene. Five ml of the phenanthrene-positive culture were transferred to fresh medium and the concentration of phenanthrene was increased to 40 mg l^{-1}. The culture was grown as described above, and on observing color change after 7 days, it was subcultured again at a phenanthrene concentration which was 10 mg l^{-1} higher than before. This subculturing in gradually increasing concentrations of phenanthrene was repeated until phenanthrene concentration reached 100 mg l^{-1}. The strain was maintained by subculturing in BSM plus 100 mg l^{-1} phenanthrene until use.

Plant and bacterial preparation: Corn (*Zea may* L., commercial seeds of P.A. Seeds, Ltd.,Bangkok, Thailand) was used as the test plant in this study. It was previously selected for its ability to germinate, grow and survive well in acidic soil contaminated with phenanthrene, pyrene, and engine oil (Chouychai *et al.,* 2007). Corn seeds were immersed in water for 3 hr and then inoculated into 50 g dry weight

of acidic soil spiked with 100 mg of phenanthrene or pyrene per kg dry soil at 5 seeds per Petri dish. The dishes were kept at 29°C in a room which received natural sunlight from approximately 6.00 am to 6.00 pm. To make up for water loss, each plate received 10 to 20 ml of water at daily intervals. After 10 days, plants were randomly removed and transferred to the pot and replanted in another pot at one plant per pot for the experiment described below. At each 10-day sampling period, the corn plants were removed and the pots terminated**.** There were 15 pots for planted treatment and 3 pots for non-planted treatment.

One milliliter of a 7-day culture of *Pseudomonas putida* MUB1 grown in BSM plus 100 mg l^{-1} phenanthrene was transferred to 50 ml nutrient broth and the culture incubated for 24 hr at 30°C in a glass Erlenmeyer flask shaken at 150 rpm. One ml of the bacterial culture was suspended in 9 ml of nutrient broth and mixed with soil to a final concentration of 3.3×10^4 CFU of strain MUB1 per g dried soil. Bacterial enumeration was done by viable plate counting after serial dilution.

Experimental design and analytical method: The following 8 treatments were set up for the soil in this experiment: (a) un-inoculated (with neither plant nor bacteria) control in non-autoclaved soil; (b) inoculated with nutrient broth only in non-autoclaved soil; (c) inoculated with bacteria in non-autoclaved soil; (d) inoculated with plant (corn) only in non-autoclaved soil; (e) inoculated with plant + bacteria in non-autoclaved soil; (f) un-inoculated control in autoclaved soil; (g) inoculated with nutrient broth only in autoclaved soil and (h) inoculated with bacteria in autoclaved soil. Three replicates were done per treatment. The soil was autoclaved at 121°C for 15 min before use in the autoclaved soil treatment.

One gram of soil was collected from each pot at days 0, 10, 20, 30 and 60, and mixed with an equal weight of anhydrous Na_2SO_4 for moisture removal. The mixture was subjected to Soxhlet extraction with 150 ml of dichlorometane at 15 min per cycle for 16 hr. The extract was evaporated to 1 ml and kept at - 40°C until analysis by gas chromatography. The sample from non-spiked soil was also extracted and analyzed with GC to confirm that there was no phenanthrene in soil before spiking.

The PAH contents in soil extracts were analyzed using an Agilent model 6890 gas chromatograph version N.04.10 equipped with a FID detector. Compounds were separated on a 0.25 mm x 30 m DB-1 column using helium gas as the eluent. The column oven temperature was set at 150°C for 1 min, and then increased at a rate of 12°C per min until 280°C. The injector and detector temperatures were 280 and 320°C, respectively. Fluorene was used as the internal standard at a concentration 20 µg l^{-1}. The injection volume was 2 µl.

Statistical analysis of data: Two-way ANOVA was used to test for significant differences between treatment and subsequent mean comparison was performed by Tukey's test.

Results and Discussion

Plant health when grown in phenanthrene-contaminated acidic soil: The growth of corn seedlings in PAH-contaminated acidic soil appeared to be normal but their shoots were short and thick. The appearance of yellow leaves (chlorosis) was not seen until the fruiting period (after 45 days). There was no observable difference between the corn plants irrespective of whether the soil was inoculated with bacteria. The experiment was terminated after all the corn plants had died after 60-65 days.

Effect of plant and/or bacterial inoculation on phenanthrene degradation in acidic soil: The time course of phenanthrene degradation is shown in Fig. 1. In the first 10 days, about 35 to 60% of the spiked phenanthrene was degraded rapidly. From day 10-60, phenanthrene was degraded at a slower rate. In the first rapid phase, phenanthrene was degraded to the greatest extent (58.8%) in the medium (nutrient broth) alone and MUB1 alone treatments. The amount of phenanthrene degraded in these treatments was different from the non-autoclaved control treatment which did not receive any MUB1 or plant (36.6% degradation). During the second slower phase, phenanthrene was degraded to the greatest extent in the plant treatment and plant + MUB1 treatment, such that at the end of the experiment, 89-90% of phenanthrene was degraded. This was followed by the MUB1 treatment (81.2% degradation), medium alone treatment (72.7% degradation) and non-autoclaved control (68.7% degradation). Most of the phenanthrene (about 88%) remained undegraded in the autoclaved control soil after 60 days. In the autoclaved soil, which received a MUB1 inoculum, phenanthrene degradation was only slightly faster in that about 30% of the phenanthrene was degraded after 60 days. This amount of degradation was much lower than the treatment in which non-autoclaved soil received the MUB1 inoculum. The results suggest that indigenous microorganisms play an important role in phenanthrene degradation in this acidic soil.

Soil pH is an important factor that may control bacterial degradation capacity in soil. Phenanthrene degradation has been reported in planted or inoculated soil at pH values ranging from 6.25-10.0. In soil inoculated with *Sphingobium yanoikuyae* B1, the amount of phenanthrene degraded (initial concentration = 1,000 mg kg^{-1}) was 45.4% (pH 7.67) compared with 36.4% in un-inoculated control after 30 days (Cunliffe and Kertesz, 2006). In another study, the total degradation of 3-ring compounds (initial concentration = 5 mg kg^{-1}) in soil (pH 6.25) was 30% and 50% after 30 days in control soil and soil inoculated with PAH-degrading bacterial cultures, respectively (Li *et al*., 2008). In this study, phenathrene degradation (initial concentration = 100 mg kg^{-1}) in soil (pH 3.3) inoculated with MUB1 was 67.3% after 30 days. This showed that phenanthrene biodegradation could proceed in fairly acidic soil. However, Stapleton *et al*. (1998) showed that phenanthrene was not mineralized in extremely acidic soil (pH 2.0) and mineralization was only slightly better (less than 20% phenanthrene) in soil of pH 3.4. Phenanthrene degradation appeared to be less affected in soil of high pH. For example,

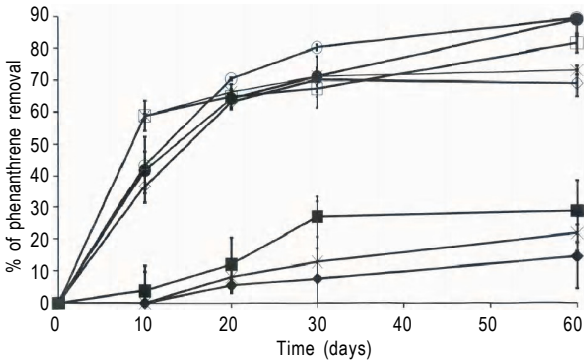

Fig. 1: The percentage of phenanthrene degradation in acidic soil spiked with 100 mg kg^{-1} phenanthrene. Values are the means ± SD for 3 independent replicates. Symbols: ♦ autoclaved soil, ■ MUB1 in autoclaved soil, x nutrient medium addition in autoclaved soil, ◇ non-autoclaved soil, * nutrient medium addition in non-autoclaved soil, □MUB1 in non-autoclaved soil, ● plant, ○ plant + MUB1

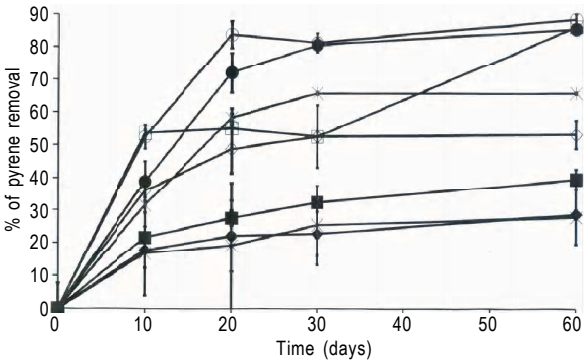

Fig. 2: The percentage of pyene degradation in acidic soil spiked with 100 mg kg^{-1} phenanthrene. Values are the means ± SD for 3 independent replicates. Symbols: ♦ autoclaved soil, ■ MUB1 in autoclaved soil, x nutrient medium addition in autoclaved soil, ◇ non-autoclaved soil, * nutrient medium addition in non-autoclaved soil, □ MUB1 in non-autoclaved soil, ● plant, ○ plant + MUB1

Betancur-Galvis *et al.* (2006), reported that at pH 6.3, phenanthrene biodegradation (initial concentration = 1200 mg/kg) in fertilized soil was 66.7% compared with 50% biodegradation in soil pH 10.0.

In the case of phytoremediation, there have been reports that phenanthrene was efficiently removed from planted soil at pH values ranging from 6.2-7.6. For example, in soil (pH 6.2) planted with barley, 76% phenanthrene was degraded in control and planted soil, while phenanthrene biodegradation improved to 82-92% (initial concentration = 5,000 mg kg^{-1}) in planted soil inoculated with *Pseudomonas* sp (Anokhina *et al*., 2004). Binet *et al*. (2000) reported that phenanthene degradation increased to 69.5% (initial concentration = 187 mg kg^{-1}) in the rhizophere of ryegrass grown soil (pH 6.6), as compared with 46.5% in non-rhizospheric soil after 40 days. Planting with corn was reported to increase phenanthrene biodegradation to 90.5% as compared with 79.2% biodegradation in control soil (pH 6.2, initial concentration = 50 mg kg^{-1}) after 60 days (Xu *et al*., 2006). In this study using acidic soil (pH 3.3), the presence of corn increased phenanthrene biodegradation to 88.6% after 60 days as compared to 68.7% in control unplanted soil (Fig. 1). Inoculation with MUB1 did not further enhance phenanthrene biodegradation (89%) after 60 days beyond what was seen in the corn-planted soil. This was likely because the high level of phenanthrene degradation already achieved was difficult to surpass. The results showed that low soil pH did not adversely affect phenanthrene phytoremediation efficiency with an acid-tolerant plant.

Effect of plant and/or bacterial inoculation on pyrene degradation in acidic soil: Pyrene was also degraded readily in the acidic soil. As shown in Fig. 2, pyrene was degraded to the greatest extent (85-88%) after 60 days in the two treatments (plant alone and plant + MUB1) in which corn was planted. This is similar to that seen for phenanthrene degradation in the same acidic soil. The treatment which received the MUB1 inoculum in non-autoclaved soil also exhibited a similar level of pyrene degradation after 60 days. It differed from the other two plant treatments in that the initial rate of pyrene degradation after 10 days was statistically significantly lower than the plant alone treatment. However, the pyrene level then remained relatively unchanged in this treatment through day 30 before dropping dramatically to 15% remaining by 60 days. The control treatment which received nutrient broth (medium alone) showed a progressive decrease in pyrene concentrations to about 35% of the initial level at 30 days. It then remained at this level for the remainder of the experiment. Pyrene concentration in the non-autoclaved soil followed a similar trend, except that it leveled off at a higher pyrene concentration of about 46% after 30 days. Pyrene was poorly degraded in soil which had been autoclaved. This again illustrates the importance of indigenous microorganisms in pyrene degradation.

Pyrene degradation has been reported in planted or inoculated soil at several pH values ranging from 6.25-7.67. About 10% of pyrene (initial concentration = 500 mg kg^{-1}) was degraded in soil (pH 7.67) inoculated with *Sphingobium yanoikuyae* B1 and un-inoculated control after 30 days (Cunliffe *et al*., 2006). Similarly, total degradation of 4-ring compounds (initial concentration = 5.5 mg kg^{-1}) in soil (pH 6.25) was 54.5% after 30 days in control soil and soil inoculated with PAH-degrading bacterial cultures (Li *et al*., 2008). One study showed that pyrene mineralization occurred in acidic soil (pH 3) by adding pyrene-degrading bacterial cultures (isolated from acidic soil, pH 2.0), while pyrene degradation did not occur if the culture was not added (Uyttebroek *et al*., 2007). In our study, degradation of pyrene (initial concentration = 100 mg kg^{-1}) in MUB1-inoculated acidic soil (pH 3.3) was 52.5% after 30 days and this increased to 85.9% after 60 days. This showed that pyrene biodegradation in the acidic soil was not delayed.

In the case of phytoremediation, there have been reports that pyrene was removed from planted soil at pH values ranging from 6.2-7.6. Liste and Alexander (2000) found that 62.7-77.8% of the pyrene (initial concentration =100 mg kg^{-1}) was degraded in planted soil compared with only 31.5% in non-planted soil. In another study, corn was reported to increase pyrene (initial concentration = 50 mg kg^{-1}) degradation to 86.7% as compared to 47.6% in control

unplanted soil (pH 6.2) after 60 days (Xu *et al.*, 2006). In our study, the presence of corn increased pyrene (initial concentration = 100 mg kg^{-1}) biodegradation to 85.2% after 60 days as compared to 53.2% in control unplanted acidic soil. Inoculation with MUB1 slightly increased pyrene biodegradation to 88.2% after 60 days. The results showed that low soil pH did not adversely affect to pyrene phytoremediation efficiency with acid-tolerant plant.

Plants may assist PAH biodegradation in two ways. Firstly, plants may support the growth of PAH degraders within the rhizosphere and increase their degradation rates. Secondly, plant root exudates can serve as co-substrates for microorganisms during PAH degradation (Gramss, 2000). It has been shown that corn root exudates, flushed from hydroponic system, could increase pyrene mineralization in non-autoclaved soil (Yoshitomi and Shann, 2001). This is corroborated by this study in which we found the corn plant enhanced the biodegradation of both pyrene and phenanthrene as compared to other non-plant treatments.

The greater extent of PAH degradation in planted soil is believed to be due to the co-operation between the plant and soil microorganisms. Changes in microbial communities were reported when ryegrass (*Lolium perenna* var. "Affinity") and alfalfa (*Medicago sativa* L.) were planted in petroleum-contaminated soil (Kirk *et al*., 2005). Likely, the presence of corn plants also affected the soil microorganisms and this may have been responsible for increased PAH biodegradation. As expected, the addition of PAH-degrading bacterial inoculum in planted soil further increased PAH degradation compared to having the corn planted alone.

In this study, the plant + MUB1 treatment led to faster rates of PAH degradation than either the MUB1 or plant alone treatments. For example, more than 80% of the phenanthrene was degraded within 30 days in the plant + MUB1 treatment, but in the MUB1 or plant alone treatments, more than 80% phenanthrene degradation was achieved only within 60 days. Similarly, the plant + MUB1 treatment led to more than 80% pyrene degradation within 20 days, but this level of pyrene degradation was achieved much later, at 60 and 30 days, respectively, in the MUB1 and plant treatment alone. While indigenous microorganisms are known to play an important role on PAH biodegradation, the addition of a competent microbial inoculant, plant or both can further enhance degradation. For example, in this study, 68.7% of phenanthrene was degraded in control soil after 60 days (Fig. 1). In the plant, MUB1, or plant + MUB1 treatments, phenanthrene degradation were further enhanced by 19.9, 12.5 and 20.3%, respectively, relative to the control soil (Fig. 1). Because MUB1 was inoculated in nutrient medium, the effect of adding the nutrient medium only without MUB1 was tested. The result showed that in non-autoclaved soil during the first 20 days, there was slight difference in phenanthrene and pyrene removal between soil receiving the nutrient broth and MUB1 inoculum. This difference was more pronounced after 30 days (Fig. 1,2). In autoclaved soil, the phenanthrene and pyrene biodegradation rate between soil receiving the nutrient broth and MUB1 inoculum were not different. This showed that the nutrient medium did affect PAH biodegradation.

Soil pH is an important factor that may control bacterial degradation capacity and microbial communities in soil. At low pH, the population of fungi increases while that of bacteria decreases (Blagodatskaya and Anderson, 1998). Neale *et al.* (1997) reported that in acidic soil with pH ranging from 3.2-3.8, the fungal population accounted for 41.5-99.9% of the total microbial biomass and this percentage decreased to 15.2–99.2% after the addition of lime. Thus, in the acidic soil used in our study, fungi may have been the dominant microorganisms responsible for PAH degradation. Further investigations are needed to confirm this. Fungi are known to degrade PAHs, especially the ectomycorrhiza in rhizosphere (Joner *et al*., 2006). Merkl *et al*. (2006) reported that fungi play an important role in crude oil degradation in soil (pH 5.9) planted with the tropical grass *Brachiaria brizantha*. Inoculation of *Glomas mosseae* BEG 69 into soil (pH 6.6) planted with ryegrass and white clover decreased chrysene concentration by 66% in soil, while in planted soil which did not receive the mycorrhizal inoculum chrysene concentration decreased by 56% (Joner *et al*., 2001).

In this study, the PAHs were not completed removed from soil. At the end of the experiment, the amount of the two PAHs remaining in soil ranged from 11-19%. It may be that the remaining PAHs were not bioavailable due to adsorption to soil. How one can completely degrade this residual PAH remains a challenge in bioremediation. Replanting or re-inoculation with fresh microbial inocula may be interesting approaches with which to enhance the degradation of residual PAHs and these should be investigated in future studies.

This study demonstrated the ability of plants and a competent bacterial inoculum to enhance PAH biodegradation in an acidic soil. At the end of experiment, about 68-72% of phenanthrene was degraded in non-autoclaved soil while 81- 88% was degraded in the MUB1-inoculated, plant alone, and plant + MUB1 treatments. Notably, the treatment receiving plant + MUB1 inoculum removed more than 80% of phenanthrene within 30 days while this level of degradation was achieved within 60 days in the plant alone or MUB1 alone treatments. For pyrene, about 53 - 65% was degraded in non-autoclaved soil, while 85 - 88% was degraded in the MUB1-inoculated, plant alone, and plant + MUB1 treatments. The plant + MUB1 treatment removed more than 80% pyrene within 20 days while this level of pyrene degradation was achieved within 30 and 60 days, respectively, in the plant alone or MUB1 alone treatments. The results clearly show the additional benefit of adding both a plant and competent microbial inoculant in PAH degradation in acid soil.

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