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ORIGINAL PAPER

Phytoremediation of soil contaminated by heavy oil with plants colonized by mycorrhizal fungi

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Abstract The purpose of this study was to investigate the effect of phytoremediation on soils contaminated with heavy crude oil using plants infected by mycorrhizal fungi. Five plant species, *Vetiveria zizanioides*, *Bidens pilosa*, *Chloris barbata*, *Eleusine indica*, and *Imperata cylindrica*, infected with the species of mycorrhizal fungi *Glomus mosseae*, were selected for this study. The degradation of total petroleum hydrocarbons in soils and several physiological parameters of plants such as shoot length and biomass were analyzed. Out of the 5 plant species tested, only *V. zizanioides*, *B. pilosa,* and *E. indica* could take up the *G. mosseae*. Out of these three, *V. zizanioides* showed the greatest growth (biomass) in soils with 100,000 mg kg^{-1} total petroleum hydrocarbons. In addition, *B. pilosa* infected with *G. mosseae* was found to be able to increase degradation by 9 % under an initial total petroleum hydrocarbons concentration of 30,000 mg kg^{-1} in soils after 64 days. We conclude that plants infected with mycorrhizal fungi can enhance the phytoremediation efficiency of soils contaminated with high concentrations of heavy oil.

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Keywords Crude oil · Mycorrhiza · Rhizoremediation · Total petroleum hydrocarbons

Introduction

Petroleum and its refined products would pollute soils when it spill out during production, transportation, and storage operations. The characteristics and compositions of spilled oils may change by time and climate. Oils, especially heavy ones, are more viscous and difficult to remove from the contaminated soils. Compared with other refined oil products, heavy oils have higher carbon numbers which means it have high heat resistance, high chemical stability, low water solubility, and low biodegradation (Fingas 2001). Thus, once soils are contaminated by heavy oils, it is usually time- and cost-consuming to clean them up.

Since traditional technologies, such as chemical, physical, and heat treatment, may cause great harm to the natural environment by disrupting soil structure and causing soil acidification, attention has focused on bioremediation techniques. In bioremediation (biostimulation and bioaugmentation), microorganisms play an important role in degrading pollutants in soils. Although this biotechnology has strong potential, the injected bacteria (bioaugmentation) might compete with native species and change the native ecological system. By contrast, phytoremediation technology uses the plant mechanisms and associated rhizosphere effects in the absorption, degradation, and stabilization of soil pollutants (Liste and Prutz 2006; Melo et al. 2011).

Phytoremediation is an environmentally friendly ecotechnology, which can be applied to remediate contaminated soils and groundwater containing heavy metals, organic pollutants, and radioactive nuclear materials

(Pradhan et al. 1998). The advantages of phytoremediation include low costs, low energy consumption, low ecological disruption, and acceptance by the public. Therefore, this ecotechnology has become a promising remediation technology for contaminated soils. Some studies concerning phytoremediation have concentrated on heavy metals, obtaining valuable results (Marchiol et al. 2004). However, while a relatively large number of studies explore phytoremediation of heavy metals, there are other studies of phytoremediation on its application to organic pollutants. Since the potential of using phytoremediation to treat soils contaminated by organic pollutants (Pichtel and Liskanen 2001; Gao and Zhu 2004) is high for both ecological and economical reasons, an increasing number of researchers have focused on this area (Glick 2003; Sung et al. 2003). Vegetative uptake and degradation in the rhizosphere can play a major role in remediation at hazardous waste sites (Burken and Schnoor 1996). Among organic pollutants in soils, chlorinated organic solvents (Alkorta and Garbisu 2001), petroleum hydrocarbons (Ferro et al. 1994; Hodson et al. 2002; Agamuthu et al. 2010; Hamdi et al. 2012; Wang et al. 2012), and polychlorinated hydrocarbons (PCBs) (Ke et al. 2003; Huang et al. 2004; Parrish et al. 2005) have drawn much attention. Phytoremediation is becoming a promising option in the application of the technique in contaminated mangrove and wetland areas (Moreira et al. 2011; Zhang et al. 2012).

Arbuscular mycorrhiza (AM) is an ubiquitous association between soil fungi and the roots of most herbaceous plant species that permits the host plant to exploit nutrients in the soil beyond the rhizosphere through fungal transport (Joner and Leyval 2006). The rhizosphere is the narrow region of soil which directly influenced by root secretions and associated soil microorganisms. Mycorrhizal colonization can result in quantitative and qualitative changes in root exudation and in the soil microbial community, particularly in the rhizosphere. Despite the potential role of AM in the phytoremediation of soils with organic contaminants, few studies have been carried out on the interactions between AM and organic pollutants such as heavy oils in soil, with only limited and variable results obtained (Gerhardt et al. 2009).

The degradation efficiency of pollutants in soils may be affected by the different species of bacteria existing on the rhizosphere during rhizoremediation (Rahman et al. 2003). Positive effects of mycorrhizal fungi on the dissipation of heavy oils in soils have been observed (Nicolotti and Egli 1998). The solubility of several organic pollutants in water could be improved in soils treated with biosurfactants (Willumsen and Arvin 1999), which could increase the bioavailability of organic pollutants to bacteria in soils, and thus increase the efficiency of organic pollutant degradation (Bragg et al. 1994). Plant roots have been found to excrete biosurfactant to achieve that kind of function (Yoshitomi and Shann 2001). Lin and Mendelssohn (1998) also found that application of fertilizers to soils boosts phytoremediation of soils contaminated by oil pollutants. There are many other researches of mycorrhiza use in phytoremediation (Gerhardt et al. 2009). The purpose of this study was to investigate the phytoremediation efficiencies of heavy oil contaminated soils by plants colonized with mycorrhizal fungi. This study was conducted at the laboratory of National Sun Yat-sen University in Taiwan from March 2008 to April 2009.

Materials and methods

Soil preparation and plant cultivation

In this study, soil samples contaminated with different levels of 0, 5,000, 30,000, and 100,000 mg kg^{-1} of heavy oil were prepared for tests. The soil that used in the pot tests was taken from campus of university. The characteristics of soil are shown in Table 1. The heavy oil that used for imitate soil contaminant in the tests was taken from the CPC Corporation, which is the largest refinery in Taiwan. The characteristics of the heavy oil are shown in Table 2. The artificial heavy oil contaminated soil samples were prepared by dissolving 5, 30, and 100 g heavy oil, respectively, in 0.25 L dichloromethane solvent and mixing uniformly with 1 kg (dry basis) of soil. The blank test sample with 0 mg kg^{-1} level was prepared also following the same steps. The contaminated soil sample was then put in a fume hood for around 3 h to evaporate off the dichloromethane. This step was repeated until the dichloromethane concentration was lower than 50 ppm in the air. After such operation, the four different levels of heavy oil contaminated soil samples were then transferred separately to plastic pots, and then, all of them were all 12 cm in diameter and 12 cm in height.

The basis for selecting plant species used in the pot tests of this study included having (1) the ability to tolerate the toxicity of heavy oil, (2) sufficient root surface area, and (3) suitability for inoculation with some species of

Table 1 Properties of the soil

Sand (%)	47.2
Silt (%)	40.1
Clay (%)	12.7
Moisture (%)	4.2
Organic carbon (%)	0.37
Organic nitrogen (mg kg^{-1})	2.2
pH	6.32

Table 2 Properties of the heavy oil

Items		0.50 %	1.00 %	Quality (Test methods)	
				CNS	ASTM
Low sulfur low pour point fuel oil (LSLP 0.5 and 1.0 %)					
Density at 15 °C (kg L^{-1})	Max	0.9855	0.9855	12,017	D1298
Flash point PM (°C)	Min	60	60	3,574	D93
Kinematic viscosity, cSt at 50 °C	Max	424	424	3,390	D445
Sulfur content (wt%)	Max	0.5	1.0	13,877	D2622
				14,472	D4294
Pour point (°C)	Max	15	15	3,484	D97
				14,667	D5950
				–	D6749
Carbon residue, micro (wt%)	Max	15	15	14,477	D4530
Water content (vol%)	Max	0.5	0.5	3,517	D95
Water and sediment (vol%)	Max	1.0	1.0	6,358	D1796

mycorrhizal fungi in order to run meaningful mycorrhizal phytoremediation tests. Based on these principles, five local species of plants, *Vetiveria zizanioides* (VE), *Bidens pilosa* (BI), *Chloris barbata* (CH), *Eleusine indica* (EL), and *Imperata cylindrica* (IM), were selected in preliminary tests and planted in the prepared heavy oil contaminated soil samples. They were inoculated by the species of mycorrhizal fungi, *G. mosseae*, which has been tested the suitable species for inoculating plants selected in the previous tests (Yang et al. 2007). In the test run of selecting suitable plant species, ten treatments were used in the pot tests, including VE, VE colonized by mycorrhizal fungi (VEM), BI, BI colonized by mycorrhizal fungi (BIM), CH, CH colonized by mycorrhizal fungi (CHM), EL, EL colonized by mycorrhizal fungi (ELM), IM, and IM colonized by mycorrhizal fungi (IMM). In the experiments, each pot was irrigated with 100 mL day^{-1} of fertilizer liquid (HYPONeX No.2, HypoNeX Co., Taiwan), containing 20 % of N, P, and K; dissolved in water at 1:1,000 (w/v), in the first day of test run. The pots without inoculation of mycorrhizal fungi were used for control tests. Each treatment in this test run was repeated for seven times. For same treatment, the pot test was performed in triplicates. All test pots were placed in a greenhouse with an average temperature of 25 ± 2 °C and a relative humidity of 75 ± 5 % for cultivation. Pots were irrigated everyday with 0.1 L of tap water. The variations in the amounts of heavy oil in the soil of pots and physiological parameters (shoot length and biomass) of plants were observed and measured periodically during the test run.

Analytical methods

The survival ratios for the plants selected in this study were calculated by the number of germinated plants that survived after 39 days of growth studies, while the colonization ratios for plants by micorrhizal fungi were determined by measuring the colonized area (percentages) on plant roots through microscopic observation. The measurement of amounts of heavy oil in soil was through quantitative analyses of total petroleum hydrocarbon (TPH) concentration in the soil, in which soil samples (approximately 20 g) from each pot were collected at 0, 17, 31, 45, and 64 days, respectively, after the start of the experiment and were stored at −4 °C for drying. 1 g of sample was taken from the dried soils and mixed with 1 g anhydrous sodium sulfate (Na_2SO_4) and 20 mL dichloromethane, which was added as a solvent. The mixture was then extracted for 6 min by using the sonicator ultrasonic processor. The extraction step was repeated twice. The two extracts were then combined and analyzed for TPH concentrations by gas chromatography (GC 7890A, Agilent Technologies, USA) with a flame ionization detector (FID) (Wang et al. 2008). The operation conditions for GC-FID were as follows: HP-5 column (30 m × 0.32 mm ID × 0.25 μm); nitrogen carrier gas; injector temperature 290 °C; detector temperature 300 °C; column flow rate 2.0 mL min^{-1}; and oven temperature program of 50 °C for 2 min after injection, followed by an increase of 12 °C min^{-1}–300 °C and held for 10 min. The method detection limit for TPH was estimated to be 0.1 mg kg^{-1}.

Statistical analysis methods

Data were tested for normality and homogeneity of variance using SPSS v.17 for Windows. One-way analysis of variance (ANOVA) by Duncan's test at $\alpha = 0.05$ was conducted to determine treatment significance at each biomass and TPH concentration removal, for each treatment.

Results and discussion

Plant-selection experimental results

In this study, the selection of suitable plant species for mycorrhizal phytoremediation used in heavy oil contaminated soil was achieved through plant-selection experiments. Some other studies (Barrett et al. 2011; Taheri and Bever 2011; Orłowska et al. 2012) showed that different plant species in different soil types, climate conditions, while inoculated by different species of mycorrhizal fungi, might present different effects of phytoremediation on contaminated soils. In order to simplify the environmental conditions and variables in this study, the only variable used was that of plant species with all the tests being conducted under same soil and climate conditions with inoculation with the same species of mycorrhizal fungi.

The results of calculating the survival ratio are shown in Table 3. According to this table, we found that the pots with *V. zizanioides* inoculated with mycorrhizal fungi showed the highest values of survival ratio, while the pots with *I. cylindrica* were all dead in this experiment even though the plant was inoculated by mycorrhizal fungi. Figure 1 shows the statistical analytical results in this stage of experiment by comparing the variations in biomass of different plant species with and without inoculation by mycorrhizal fungi during the test run. As seen in this figure, we found that there was no significant difference in the biomass for all five plant species with and without inoculation by mycorrhizal fungi. However, the plant selection of VE was found showing significant difference from the other plant selections ($P < 0.05$) with the highest biomass values of growing. The values of inoculation ratio(the mycorrhizal fungi area ratio in root) of mycorrhizal fungi for the plant selections of VE, CH, BI, EL, and IM were measured equal to 80, 0, 60, 60, and 0 %, respectively. It means that the positive effects of inoculation of mycorrhizal fungi on plant growth were apparent.

Table 3 Survival ratio

Treatment	%
Vetiveria zizanioides (VE)	57.1
Vetiveria zizanioides colonized (VEM)	100.0
Bidens pilosa (BI)	34.3
Bidens pilosa colonized (BIM)	10.7
Chloris barbata (CH)	0.0
Chloris barbata colonized (CHM)	0.7
Eleusine indica (EL)	21.4
Eleusine indica colonized (ELM)	16.4
Imperata cylindrica (IM)	0.0
Imperata cylindrica colonized (IMM)	0.0

The treatments: VE, *Vetiveria zizanioides*; VEM, *Vetiveria zizanioides* colonized; BI, *Bidens pilosa*; BIM, *Bidens pilosa* colonized; CH, *Chloris barbata*; CHM, *Chloris barbata* colonized; EL, *Eleusine indica*; ELM, *Eleusine indica* colonized; IM, *Imperata cylindrica*; IMM, *Imperata cylindrica* colonized

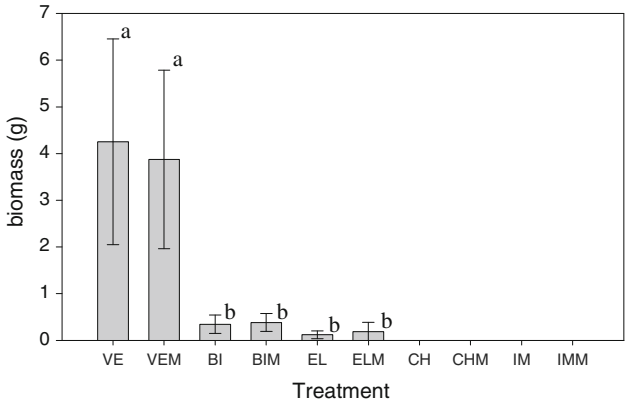

Fig. 1 Average biomass value and standard deviation of the treatments in the plant-selection experiment. VE, *Vetiveria zizanioides*; VEM, *Vetiveria zizanioides* colonized; BI, *Bidens pilosa*; BIM, *Bidens pilosa* colonized; EL, *Eleusine indica*; ELM, *Eleusine indica* colonized; CH, *Chloris barbata*; CHM, *Chloris barbata* colonized; IM, *Imperata cylindrica*; and IMM, *Imperata cylindrica* colonized. By analysis of variance, *Vetiveria zizanioides* was significantly ($P < 0.05$) different than other species

Mycorrhizal phytoremediation effect experimental results

Since *V. zizanioides*, *B. pilosa,* and *E. indica* were found to have higher amounts of biomass inoculation ratio of mycorrhizal fungi, the three plant species were selected in the next stage of experiment of running pot tests for heavy oil contaminated soil through mycorrhizal phytoremediation. The experiment was run for 64 days. At the end of the test, the plant tissues from each pot were collected for measuring biomass. The experimental results are shown in Fig. 2. As seen in this figure, root biomass for VE, VEM, BIM, EL, and ELM in pot soils with heavy oil contaminated level of 5,000 mg kg^{-1} were found increased comparing to the control ones. In addition, we also found that the values for the plant species *V. zizanioides* in soil pots with higher contamination level of 100,000 mg kg^{-1} were higher than in the ones with lower contamination level of 30,000 mg kg^{-1}. This meant that such plant species could survive in a high level of heavy oil contaminated soil and even might grow well in the soil contaminated with higher levels of heavy oil. The reason for this is unknown and merits further studies in the future.

The quantitative analytical results of amounts of heavy oil expressed as TPH in different contamination levels of pot soil during the test run are shown in Figs. 3, 4, and 5. In Fig. 3, the pot soils with contamination level of

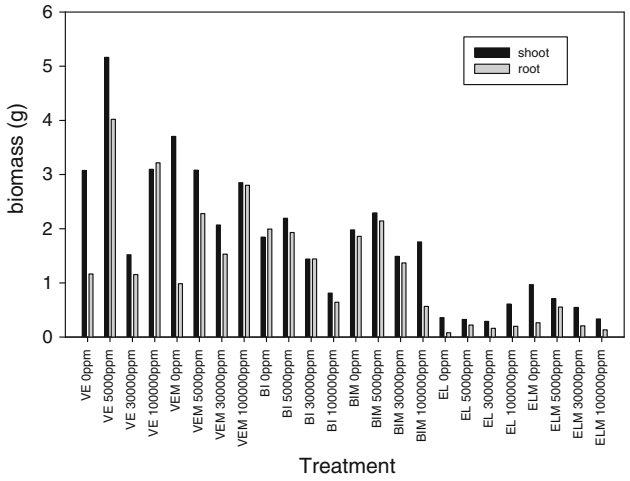

Fig. 2 Shoot and root biomass of the treatments with different TPH concentrations in the contaminated soil pot experiment. VE, *Vetiveria zizanioides*; VEM, *Vetiveria zizanioides* colonized; BI, *Bidens pilosa*; BIM, *Bidens pilosa* colonized; EL, *Eleusine indica*; ELM, *Eleusine indica* colonized

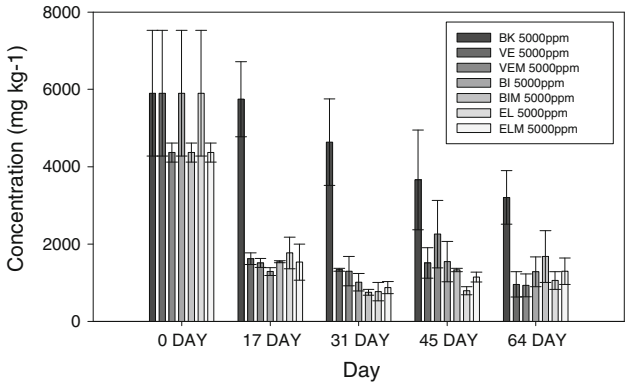

Fig. 3 Effect of plant species on the TPH degradation of the treatments of 5,000 TPH concentration in the contaminated soil pot experiment. BK, without plant; VE, *Vetiveria zizanioides*; VEM, *Vetiveria zizanioides* colonized; BI, *Bidens pilosa*; BIM, *Bidens pilosa* colonized; EL, *Eleusine indica*; ELM, *Eleusine indica* colonized

5,000 mg kg^{-1} the amounts of heavy oil removed were calculated be equal to approximately 2,000 mg kg^{-1} at the end of the experiments in all the three plant species. The concentrations of heavy oil left in the pot soils for both plant species of BI and EL were found reduced to 1,000 mg TPH/kg after 31 days of test run. After 64 days of test run, the final concentration (removal percentage) of heavy oil left in pot soils of different treatments was as follows: control treatment: 3,205.56 ± 690.50 mg kg^{-1} (46 %), VE treatment: 956.56 ± 325.72 mg kg^{-1} (84 %), VEM treatment: 930.19 ± 296.79 mg kg^{-1} (79 %), BI treatment: 1,281.05 ± 387.34 mg kg^{-1} (78 %), BIM: treatment 1,675.92 ± 667.64 mg kg^{-1} (62 %), EL: treatment 1,054.75 ± 227.39 mg kg^{-1} (82 %), and ELM treatment:

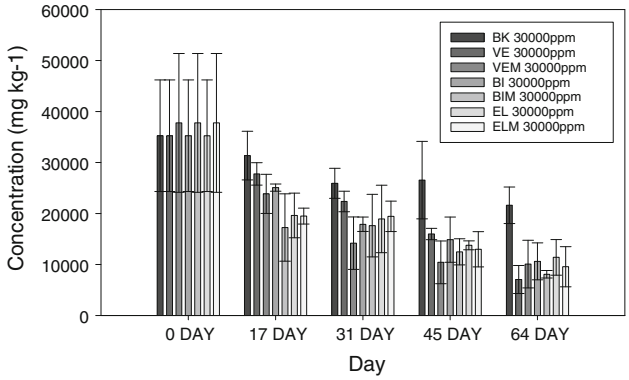

Fig. 4 Effect of plant species on the TPH degradation of the treatments with 30,000 TPH concentration in the contaminated soil pot experiment. BK, without plant; VE, *Vetiveria zizanioides*; VEM, *Vetiveria zizanioides* colonized; BI, *Bidens pilosa*; BIM, *Bidens pilosa* colonized; EL, *Eleusine indica*; ELM, *Eleusine indica* colonized

1,294.38 ± 341.07 (70 %). The amounts of heavy oil removed as TPH for the treatments of VE, VEM, BI, EL, and ELM were more significantly than BK ($P < 0.05$).

In Fig. 4, we found that all treatments in the pot tests with heavy oil contaminated level of 30,000 mg kg^{-1} exhibited greater than 50 % removal efficiencies for heavy oil after 64 days at the end of test run. The concentration (removal percentage) of the control treatment on the 64th day was 21,610.77 ± 3,583.45 mg kg^{-1} (39 %). However, the concentrations of VE, VEM, BI, BIM, EL, and ELM treatments on the 64th day were 7,058.45 ± 2,751.45 mg kg^{-1} (80 %), 10,090.20 ± 4,671.80 mg kg^{-1} (73 %), 10,615.48 ± 3,612.13 mg kg^{-1} (70 %), 8,073.87 ± 738.19 mg kg^{-1} (79 %), 11,397.45 ± 3,497.04 mg kg^{-1} (68 %), and 9,566.41 ± 3,929.28 mg kg^{-1} (75 %), respectively. It was also found that the amounts of heavy oil (TPH) removed for all treatments with plants were more significantly than BK ($P < 0.05$).

Figure 5 showed that the experimental results for pot tests with heavy oil contaminated level of 100,000 mg kg^{-1} had the same removal trends as those for pot tests under contaminated level of 30,000 mg kg^{-1}. According to Fig. 5, we found that the pot tests treated with inoculation by mycorrhizal fungi exhibited higher removal efficiencies than those with non-colonized ones. The treatments which had higher removal percentages of TPH for heavy oil were the pot tests of BI and BIM. The concentration (removal percentage) of the each treatment after 64-day test run was as follows: control treatment: 64,508.49 ± 7,417.74 mg kg^{-1} (51 %), VE treatment: 55,516.79 ± 7,751.08 mg kg^{-1} (57 %), VEM treatment: 54,566.66 ± 17,589.52 mg kg^{-1} (55 %), BI treatment: 42,323.85 ± 6,420.22 mg kg^{-1} (68 %), BIM treatment: 41,162.21 ± 5,347.98 mg kg^{-1} (66 %), EL treatment: 64,414.44 ± 19,776.76 mg kg^{-1} (51 %), and ELM treatment: 63,312.60 ± 17,589.70 mg kg^{-1} (47 %).

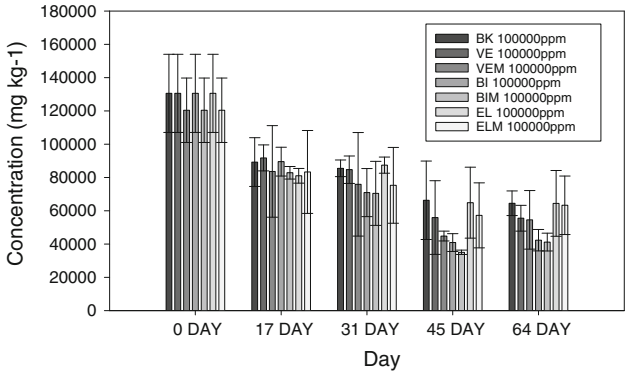

Fig. 5 Effect of plant species on the TPH degradation of the treatments with 100,000 TPH concentration in contaminated soil pot experiment. BK, without plant; VE, *Vetiveria zizanioides*; VEM, *Vetiveria zizanioides* colonized; BI, *Bidens pilosa*; BIM, *Bidens pilosa* colonized; EL, *Eleusine indica*; ELM, *Eleusine indica* colonized

Same as previous results, the removal of TPH for treatments with plants under the highest contaminated level of heavy oil in pot soils still presented insignificant difference with BK ($P > 0.05$).

In the plant-selection experiment, the germination percentage of CH, CHM, IM, and IMM were close to zero. Several factors (seasonal factors, temperature, moisture, illumination, etc.) appeared to slow the germination of *C. barbata* and *I. cylindrica* in this experiment. In this study, a wild field observation on the growth of CH and IM was proceeded during the whole experimental year of 2009. We observed large amounts of CH and IM germinated in all seasons of this year. Therefore, it could be inferred that the growth of both plants should not be affected by climate. However, CH and IM could not germinate in this study (see Table 3). Therefore, we may conclude that the growth of CH and IM should be related to soil characteristics, not climate. For the other three plant species colonized by mycorrhizal fungi in Table 3, the observed survival percentage of germination of VEM fell, but those of BIM and ELM increased. These results suggest that the mycorrhizal inoculation for seed germination may have an inhibiting effect, though this remains to be studied. There was no significant difference in biomass between plants with or without colonization by mycorrhizal fungi. This phenomenon may be related to the shoot biomass. The plants in the colonized treatments had greater shoot biomass than in the non-colonized treatments (except VE 5,000 mg kg^{-1}, VE 100,000 mg kg^{-1}, and EL 100,000 mg kg^{-1}). For example, the VEM treatment had almost 75 % biomass on shoot. Though the shoot physiology did not fare better than in the non-colonized treatments, the colonized treatments showed greater apparent effects on the shoot.

In addition to its benefits on root growth, the results presented that mycorrhizal fungi colonization had positive effects on pollutant removal. The colonization percentage of mycorrhizal fungi implies a symbiotic capacity of mycorrhizal fungi and plants. However, the symbiotic capacity and efficiency showed no significant correlation. That is because the plant does not always depend on mycorrhizal fungi for growth, especially when unaffected by environmental stress.

In contaminated soil pot experiment, most treatments showed apparent growth on the plant height by mycorrhizal fungi, except for *E. indica*. Inoculation with mycorrhizal fungi had a significant impact on the biomass, especially in the 5,000 and 100,000 mg kg^{-1} concentration treatments. Therefore, it appears that the effects of mycorrhizal fungi colonization of plants in contaminated soil are positive. Plants colonized by mycorrhizal fungi not only exhibit increased plant survival, but also resistance to toxic pollutants (Gao et al. 2011).

In the contaminated soil pot experiment, all experimental treatments of 5,000 mg kg^{-1} concentration were removed to an approximate concentration of 2,000 mg kg^{-1} by the end of the experiment. The concentration of BI and EL treatments showed reductions to 1,000 mg kg^{-1} after 31 days and slight recovery after 64 days. That might be because the high molecular weight of hydrocarbons in heavy oil was initially adsorbed onto the root area of plants and then was decomposed latterly and released back into soils. This phenomenon could be explained further by GC spectrum analysis for hydrocarbons in soil samples. According to the GC spectrum analytical results, we found that the amounts of high molecular weight petroleum hydrocarbons (more than C_{16}) were decreased, while low molecular weight petroleum hydrocarbons ($<C_{16}$) were increased.

In addition, the experimental results for all treatments in contaminated level of 30,000 mg kg^{-1} pot tests exhibited more than 50 % removal by the end of the experiment. By comparing the TPH concentrations (remained in soil) between colonized and non-colonized treatments at the 0th day and the 64th day (see Fig. 4), we confirmed that the TPH removal percentage of treatments colonized by mycorrhizal fungi was significantly better than non-colonized treatments (except VE) ($P < 0.05$). This phenomenon indicates that mycorrhizal fungi not only enhance the individual tolerance of plants, but also lead to further changes in the root-zone environment, thus increasing the removal efficiency. By comparing the TPH concentrations (remained in soil) between colonized and non-colonized treatments at the 0th day and the 64th day (see Fig. 5), we confirmed that the TPH removal percentage of 100,000 mg kg^{-1} concentration treatments colonized by mycorrhizal fungi was significantly better than non-colonized treatments. The EL and ELM displayed no significant difference from the control treatment. Biodegradation may

not be the only one process in the experiment. Photodissociation and plant absorption may also occur in the system. The BI and BIM treatments exhibited the best results for removal percentage. It is inferred that low molecular weight petroleum hydrocarbons in the polymer removal process might be uptaken into the plant tissues. In almost, all treatments of the plants could maintain removal percentage of over 70 % at the level of 30,000 mg kg^{-1} TPH concentration. Significantly, positive effects of plants were found under the 100,000 mg kg^{-1} TPH concentration in soil.

For the experiment of selecting suitable plant species, we found that the species of *E. indica* could not performed well in this study for the reason of that the TPH concentrations applied into the systems might be too high to cause the plant's death. However, the species of *V. zizanioides* was found still within its tolerance, but its root growth was inhibited, which meant that the contaminant of heavy oil in soil might provide a worse environment in root zone for plants. However, in this study, we found that the inoculation of mycorrhizal fungi onto plant root surface for some species of plants might be helpful to recover such problem to increase the ability of plant root to tolerate high levels of heavy oil through symbiotic relationship, which might reinforce the effects of phytoremediation for soil contaminated by high levels of heavy oil.

Conclusion

Usually, the plants inoculated by mycorrhizal fungi can increase the growth of their root. However, in this study, it was found that inoculum by mycorrhizal fungi for the plant species of *E. indica* exhibited negative impact, while the plant species of *V. zizanioides* and *B. pilosa* were found presenting positive effects, which were helpful for these plant species to tolerate heavy oil pollution in soil. In addition, the pot tests with plant species of *V. zizanioides* presented the lowest contaminant concentrations of TPH in pot soil after 64-day test run. Besides, according to the experimental results of this study, the systems with plants colonized by mycorrhizal fungi could speed up the phytoremediation rates and increase the removal efficiencies significantly, resulting in greater removal amounts of heavy oil from soil than the systems without inoculum. In this study, it was concluded that the ecotechnique of phytoremediation was practical used for treating contaminated sites by high levels of heavy oil. However, the experimental results also suggested that plant species should be selected to match site conditions in order to improve their survival ability and pollution removal potential, especially for those plant species that were easily inoculated by mycorrhizal fungi, such as *V. zizanioides* and *B. pilosa*.

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