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Role of Arbuscular Mycorrhizal Fungi in Phytoremediation of Soil Rhizosphere Spiked with Poly Aromatic Hydrocarbons

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Results from an innovative approach to improve remediation in the rhizosphere by encouraging healthy plant growth and thus enhancing microbial activity are reported. The effect of arbuscular mycorrhizal fungi (Am) on remediation efficacy of wheat, mungbean and eggplant grown in soil spiked with polyaromatic hydrocarbons (PAH) was assessed in a pot experiment. The results of this study showed that Am inoculation enhanced dissipation amount of PAHs in planted soil, plant uptake PAHs, dissipation amount of PAHs in planted versus unplanted spiked soil and loss of PAHs by the plant-promoted biodegradation. A number of parameters were monitored including plant shoot and root dry weight, plant tissue water content, plant chlorophyll, root lipid content, oxido-reductase enzyme activities in plant and soil rhizosphere and total microbial count in the rhizospheric soil. The observed physiological data indicate that plant growth and tolerance increased with Am, but reduced by PAH. This was reflected by levels of mycorrhizal root colonization which were higher for mungbean, moderate for wheat and low for eggplant. Levels of Am colonization increased on mungbean > wheat > eggplant. This is consistent with the efficacy of plant in dissipation of PAHs in spiked soil. Highly significant positive correlations were shown between of arbuscular formation in root segments (A)) and plant water content, root lipids, peroxidase, catalase polyphenol oxidase and total microbial count in soil rhizosphere as well as PAH dissipation in spiked soil. As consequence of the treatment with Am, the plants provide a greater sink for the contaminants since they are better able to survive and grow.

KEYWORDS: Dissipation, Eggplant, Glomus, Microbial, Mungbean, Pollution, Wheat

Polycyclic aromatic hydrocarbons (PAHs) are byproducts from the incomplete combustion or pyrolysis of organic materials. PAHs in nature are of great environmental and cause human health concerns due to their widespread occurrence, persistence in terrestrial ecosystems and carcinogenic properties. It is expensive and time consuming to remediate persistent contaminants such as PAHs in soils. At the present time, the techniques used to remediate contaminated soils (physical removal of contaminated soil and washing of these soil with solvent; bioreactors; microbial remediation as well as phytoremediation) tend to be inefficient (Rock, 1997; Huang *et al.*, 2004b).

The advantages of phytoremediation compared with other approaches are as follows: (1) it preserves the natural structure and texture of the soil; (2) energy is derived primarily from sunlight; (3) high levels of microbial biomass in the soil can be achieved; (4) it is low in cost; and nevertheless many limitations exist for large-scale application of this technology (US EPA, 2000; Suthersan, 2002; Joner *et al.*, 2004). One serious limitation is that many plant species are sensitive to contaminants including PAHs (Huang *et al.*, 2001). Therefore, they grow slowly, and it is difficult to establish sufficient biomass for significant soil remediation. In addition, if the soil has been heavily contaminated for a long time, the levels and diversity of microorganisms in the soil will be diminished so that bacterial populations will not facilitate contaminant degradation nor enhance plant growth (Burd *et al.*, 1998; Huang, *et al.*, 2004b).

Despite the limitations, it may be possible to facilitate phytoremediation through selection and acclimation of more contaminant-tolerant plant species. Some physiological characteristics can be used as indicators for tolerance to contaminants. These traits include (but are not limited to): growth, vigorous root systems, the ability to maintain water content, chlorophyll levels, ratio of the chlorophyll a/b and root lipid content as well increasing of oxido-reductase enzyme activities. Over the last few years, enhanced remediation of soil PAHs in the presence of plants has been reported (Binet *et al.*, 2001; Yoshitomi and Shann, 2001; Mattina *et al.*, 2003). Nevertheless, the basic mechanisms involved however poorly understood, and there still exists a lack of information on the contribution of plant uptake of soil PAHs, and their translocation behaviors have not been well defined yet (Kipopoulou *et al.*, 1999; Gae and Zhu, 2004). However, phytoremediation is potentially associated with plant contamination (Sung *et al.*, 2001), therefore, information about PAH distribution and concentration in plants is essential in predicting the effectiveness of a phytoremediation operation.

Another way to improve phytoremediation is through using the plant growth promoting microorganisms in soil

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including, rhizobacteria (PGPR) (Ajithkumar *et al.*, 1998; Glick, 2003; Newman and Reynolds, 2005), ectomycorrhizal fungi (Chekol *et al.*, 2004) and Am fungi (Joner and Lyval, 2001; Rabie, 2004). Am fungi are ecologically significant because they establish relationships with the roots of a host plant in symbiotic association. Mycorrhizal associations in terrestrial ecosystems influence organic and inorganic nutrient relationships, water content and carbon cycling in the plants (Entry *et al.*, 2002). These associations have the ability to relieve environmental stress in plants and, subsequently to, increase plant growth potential under less-than-ideal conditions. Of importance to phytoremediation, Am stimulate plant root development and enhance root growth. Mechanisms of beneficial effects of Am on plants include: Am plants may subsist better under water stress. PAH-contaminated soils are more or less hydrophobic, and thus plant growth may be limited by water uptake and access to mineral nutrients dissolved in inaccessible soil water (Wilson and Jones, 1993). In addition, indirect effects of Am such as ,through modifying root architecture (Hooker and Atkinson, 1996), improving membrane integrity (Graham *et al.*, 1981) or enhanced production of oxidative enzymes (Salzer *et al.*, 1999) may improve the performance of Am plants in the presence of organic compounds.

Use of Am fungi has shown a possibility in PAHs remediation. The objectives of this study were to investigate the effect of Am on plant-uptake, accumulation and efficacy dissipation of PAHs in the soil contaminated with PAHs. The levels of root colonization by *Glomus. moseae* were monitored during the phytoremediation process. Based on the studies, Am fungal contributions to the plant-enhanced remediation for soil PAH contaminants were evaluated.

Materials and Methods

Contaminated soil. An agricultural topsoil contained 74% sand, 12% silt and 14% clay, 7.9 pH, 10.2 g kg^{-1} Total organic carbon, 3.6 g kg^{-1} total nitrogen, 29 mg kg^{-1} phosphorus. 32 mg kg^{-1} potassium and 1.4 mmoh m^{-1} E.C. It was air-dried, sieved to < 2 mm, sterilized by (25 kGy gamma rays) and left un-spiked or spiked with 500 mg kg^{-1} dry soil of each anthracene, pyrene and chrysene or 50 mg kg^{-1} dry soil of benz(a, h) anthracene. Soil microorganisms were reintroduced by adding 10ml per pot of soil suspension (10 g of the corresponding soil in 200 *ml* water filtered through a 20 μm filter). The spiked soil was then stored for 5 weeks for stabilization and ageing of the PAH amendment.

Experimental treatments. The experiment examined factorial combinations of 1) two soil types (non-spiked or spiked), 2) two soil states (unplanted or planted), 3) two mycorrhizal treatments (non-inoculated or inoculated with *Glomus mosseae.* Five replications per each treatment were used to give a total 40 pots for the three plant species used in this investigation.

Seeds of wheat (*Triticum aestivum* cv. *Sakha 8*), mungbean (*Vigna radiata V 2010*) and eggplant. (*Solanium melongena L.*) plants were sanitized by soaking in 30% H_2O_2 for 20 min, washed three times in distilled water and grown in black plastic pots containing 200 g of the spiked soil. Mycorrhizal treated pots had 10 g of a mycorrhizal inoculum (15 spore's g^{-1} *Glomus mosseae*). Phosphorus fertilizer was supplied at amount of 31 kg P_2O_5 in form of calcium super phosphate (15.5%) during the soil preparation. Nitrogen and potassium fertilizers were added at rates of 90 kg N/fed. and 96 kg k_2O/fed. in forms of ammonium sulphate (20.5% N) and potassium sulphate (48% k_2O), respectively in three equal doses at 15, 30 and 60 days after emergence. Each pot was sown with 5 pregerminated seeds of one different species. Pots were sown with 5 pre-germinated seeds of each species and thinned to 3 plants in each pot after 10 days. The soil was covered with a layer of coarse sand in an attempt to minimize PAH volatilization. The pots were placed in growth chamber at 25~20°C, 11 h photoperiod day, 60~70% relative humidity, and watered 2~4 times $week^{-1}$. The plants were harvested 65 days after sowing date.

Analytical methods.

PAH analysis: The root density in the planted pots was very high and all the soil was considered as rhizospheric soil. The soil from planted (rhizospheric soil) and non-planted pots (non-rhizospheric soil) was carefully collected, homogenized and crushed. Soil samples were dried in the dark at room temperature under a fume hood. PAHs were extracted from the soil (50 g dry soil with 200 *ml* chloroform for 4 h) by Soxhlet. Soil extracts were filtered through cellulose before analysis .The root samples (100~200 mg dry weight) were ground and extracted for 4 h with 200 *ml* chloroform. The extracts were concentrated to 0.5 *ml* and analyzed on HPLC (Hewlett Packard 1050 fitted with a 250 mm C-18 vydac column, using 3D fluorescence detection (HP 1100) as described by Szolar *et al.* (2002).

Determination of soil properties and enzymatic activity of plant and soil samples: Physicochemical soil properties were determined using methods generally applied in soil chemistry laboratories. The following parameters were also determined in the soil samples: pH by a potentiometric method in 1 M KCl, and total organic carbon (TOC) content by the Tiurin' s method as modified by Simakow (Ashcroft and Browen, 1983). The activities of the following enzymes in plant roots and rhizospheric soil were measured: peroxidase (Chance and Maehly, 1955), catalase (Beers and Sizer, 1952) and polyphenol oxidase

(Alef and Nannipieri, 1995) and dehydrogenase (Thalmann, 1968).

Microbial frequency: Number of bacteria and fungi of fresh soil suspension were determined by counting colony forming units on selective media Bacterial colony were grown on tryptic soy agar (Martin, 1975) and Fungal colonies were estimated on Czapex agar with rose Bengal (Martin, 1950). Colonies were counted at the appropriate dilutions after 2 and 5 days of inoculations, respectively. The results are reported as count gram soil^{-1}.

Plant growth and physiological parameters: After sowing, the plants monitored every day to for any changes in appearance. Plant samples were taken on day 65 for plant analysis. There were three replicates per treatment and triplicate samples per replicate. Dry weight of roots and shoots, R/S ratios were determined. Chlorophyll content of plants was measured according to Harbon, 1984. The moisture content of plant tissues was determined as, an aliquot of plant sample was weighed, dried at 105 -C for 24 h, and weighed again; the difference gave the percent moisture. The lipid content of each fresh plant or plant part were measured as previously described by (Folch *et al.*, 1957).

Mycorrhizal analysis: Immediately after harvest, part of the root systems of non-Am and Am plants were washed carefully with cold water to remove the adhering soil particles and cut into 0.5~1.5 cm segments, followed by washing with 10% KOH and staining with trypan blue in lactophenol (Phillips and Hayman, 1970). To estimate the percentage of mycorrhizal colonization (F), intensity of infection (M) and arbuscular development (A) in the infected region of the roots were estimated in root samples stained for total infection by the method of Trouvelot and Gianinazzl, 1986.

Statistical analysis: Statistical analysis of the results was subjected to least significant difference (L.S.D.), ANOVA and Pearson correlation coefficient. Significance was set at $*P < 0.05$ and $**P < 0.01$.

Results

Behavior of the Am fungus *Glomus moseae* in rhizoshere of wheat, mungbean and eggplant plants grown in soil spiked with PAHs is showen in Table 1. The levels of root colonization by *G. moseae* are expressed in three ways: First, frequency of root segments (F%) reflecting the proportion of roots colonized with VA-mycorrhizal fungi. Second, intensity of mycorrhizal colonization in root tissues (M%). Third, the rate of arbuscular formation in root segments (A%) reflecting the potentiality of exchange with the symbiosis. Table 1 shows that the levels of root colonization by *G. moseae* were dependent on PAHs present in the soil. After 65 days there was an apparent decline in levels of root colonization in the spiked soils relative to the unspiked soil. Highly decreasing levels were shown in eggplant by 31%, 38%, and 39% for F, M and A respectively. On the other hand, mungbean highly responded to root colonization with this fungus in both spiked and unspiked soil followed by wheat plants. Based on levels of root colonization (F, M and A), the order of potential symbiosis ability of plants to VA-mycorrhiza in spiked soil is mungbean > wheat > eggplant.

The results in Table2 evinced that the physiological measurements of plants significantly correlated with the levels of Am colonization in soil spiked with PAH. Water content, root lipid, peroxidase, catalase and polyphenol oxidase in mungbean, root lipid and polyphenol oxidase in wheat plant showed highly significant correlation with the rate of arbuscular formation (A). Moreover, microbial frequency in rhizosphere of wheat and mungbean plants showed highly significant correlation with the rate of arbuscular formation (A). The correlation between the levels of Am colonization with other measurements was listed in Table 2.

Dissipation of PAHs in various planted and un-planted spiked soils was listed in Table 3. The loss of PAHs denoted the abiotic dissipation of these compounds by surface sorption, photo-oxidation or volatization. After 65 days, the loss of PAHs in unplanted soil was averaged 173 mg kg^{-1} soil pot^{-1} and the mean dissipation ratios of these compounds were 11.2%. In microb-inhibited unplanted soil, the mean dissipation amount of PAHs was 126 mg kg^{-1} soil pot^{-1} with dissipation ratio 8.3%. This implied that abiotic dissipation of these chemicals was a minor pathway of their disappearance in soils comparing with the dissipation of these compounds in planted soil (Table

Table 1. Percentage of mycorrhizal levels (as indicated by non-vital staining technique) in wheat (W) mungbean (M) or eggplant (E) grown in unspiked and spiked soil

Am levels	F %			M %			A %		
Treatments	W	M	E	W	M	E	W	M	E
Unspiked planted soil + VAM	84	97	62	32	45	22	21	29	13
Spiked planted soil + VAM	69	83	46	24.9	33.4	13.7	15.8	25.7	7.9
LSD 5%	±11.6	±7.9	±15.2	±6.4	±10.7	±5.1	±2.84	1±.58	±4.22

F%, Frequency of mycorrhizal root segments; M%, intensity of mycorrhizal infection and A%, rate of arbuscular development.
LSD, least significant difference.

Table 2. Correlation coefficient (r) between levels of mycorrhizal colonization and physiological parameters of measured after 65 days of sowing

Am levels	F %			M %			A %		
measurements	W	M	E	W	M	E	W	M	E
Dry weight	.599*	.534*	.433	.254	.496	.533*	.373	.469	.034
% of water content	.548*	.621*	.461	.568*	.635*	.135	.683*	.882**	.372
R/S ratio	.736**	.661*	.521*	.631*	.577*	.023	.422	.513*	.129
Chlorophyll content	.436	.573*	.634*	.186	.376	.247	.457	.141	.248
Root lipid	.531*	.764**	.131	.672*	.781**	.468	.792**	.849**	.395
Peroxidase	.519*	.773**	.581*	.431	.579*	−.345	.557*	.759**	−.115
Catalase	.547*	.612*	−.136	.489	.364	−.381	.511*	.753**	−.320
Polyphenol oxidase	.639*	.531*	−.274	.694*	.723*	−.223	.751**	.711**	−.158
Dehydroge-nase	.562*	.522*	−.314	.359	.256	−.165	.436	.530*	−.361
Microbial count	.839**	.515*	−.128	.527*	.783**	−.425	.584*	.746**	−.326

W = wheat M = mungbean E = Eggplant.
F%, Frequency of mycorrhizal root segments; M%, intensity of mycorrhizal infection and A%, rate of arbuscular development.
* = significant difference ($P < 0.05$) ** = highly significant difference ($P < 0.01$).

Table 3. Dissipation ratio in pot (A) and soil concentrations of PAHs (mg kg^{-1}) in rhizosphere of plants grown in soil spiked with initial PAHs concentration of 1550 mg kg^{-1} soil after 65 days.

Treatments	PAH Conc.	(W)	$\%A_w$	(M)	$\%A_m$	(E)	$\%A_e$
Spiked unplanted soil (control)	PAHs residual Conc.	1374	11.4	1378	11.1	1380	11
	Sig.F	0.721		0.738		0.761	
Spiked unplanted soil + Hg cl_2	PAHs residual Conc.	1423	8.2	1431	8.3	1419	8.5
	Sig.F	0.721		0.739		0.806	
Spiked planted soil rhizosphere	PAHs residual Conc.	857	44.7	832	46.3	926	40.0
	Sig.F	.632		.236		.853	
Spiked planted soil rhizosphere + VAM	PAHs residual Conc.	609	60.7	412	73.4	874	43.6
	Sig.F	.035*		.006**		.26	

Dissipation amount, represent the mean of the three replica.
Dissipation amount T = (Ci − Ce) × kg soil pot^{-1}.
Dissipation ratio (% A) = T × 1 kg soil pot^{-1} × 100/(C_i × 0.5).
Where Ci was the soil initial concentration (mg kg^{-1}).
C_e was the soil residual concentration (mg kg^{-1}) after 65 days.
* = significant difference ($P < 0.05$).
** = highly significant difference ($P < 0.01$).
W : wheat, M : mungbean, E : eggplants.

3) .Three Am and non-Am plant species, wheat, mungbean and eggplant, were tested for their ability to remove PAHs from contaminated soil. After 65 days, there was no significantly difference between the three species of non-Am plants with respect to PAH removal (Table 3). Inoculation of the plants with Am fungus increased PAH removal from the soil and there was significantly difference between Am and non-Am mungbean and wheat plants (Table 3). The dissipation ratio in pot of mungbean increased from 46.3% without Am to 73.4% with Am, and wheat with the ratio of 44.7% without Am to 60.6% with Am while for eggplant there was no significant change in the ratio.

Total concentrations of PAHs in the plants either uninoculated and inoculated with Am fungi based on dry weight were listed in Table 4. Am plants showed high significant PAHs concentration than non-Am one. In the presence of Am fungi, the concentrations of PAHs in wheat, mungbean and eggplant plants increased by nearly 174%, 215% and 24% respectively, compared with those of in absence of Am fungi. Root accumulation of PAHs also significantly enhanced by Am infection in mungbean and wheat plants. The concentrations of these compounds in shoots were significantly lower than in roots. This indicated that the transfer of tested PAHs from root to shoot was considerably restricted.

Dissipation of PAHs in planted soils included leaching, a biotic dissipation, biodegradation and plant uptake and accumulation. By contrast, the dissipation of these compounds in unplanted soils was the sum of leaching, a biotic dissipation and biodegradation. Thus the loss of PAHs in vegetated and non-vegetated soils could be expressed as

$$T_p = T_i + T_a + T_b + P_a \quad (1)$$

$$T_{unp} = T_i + T_a + T_{b0} \quad (2)$$

Table 4. F-test and root and shoot concentration (mg kg^{-1}) of PAHs in roots and shoots of plants grown for 65 days in soil spiked with PAH of 1550 mg kg^{-1}

Plants	VAM state	Root mg kg^{-1}		Shoot mg kg^{-1}		Total mg kg^{-1}	
		value	F-test	value	F-test	value	F-test
Wheat	–VAM	12.68	.254	1.53	.587	14.21	.274
	+VAM	36.53	.005**	2.13	.174	38.66	.014*
Mungbean	–VAM	17.71	.121	1.89	.217	19.6	.113
	+VAM	59.31	.002**	2.42	.148	61.73	.002**
Eggplant	–VAM	9.33	.349	ND	.285	9.33	.167
	+VAM	11.45	.283	0.19	.147	11.54	.043*

* = significant difference ($P < 0.05$) ** = highly significant difference ($P < 0.01$).

where T_{unp} and T_p were the dissipation of chemicals in spiked unplanted and planted soils (mg pot^{-1}). T_i and T_a denoted the dissipation by leaching and a biotic dissipation respectively. T_b and T_{b0} were the loss by biodegradation in vegetated and non-vegetated soils, respectively. P_a denoted the removal of chemicals by plant uptake and accumulation. Thus the dissipation enhancement (T_d) of PAHs in planted versus unplanted soils was

$$T_d = T_p - T_{unp} = (T_b - T_{b0}) + P_a \quad (3)$$

$$T_{pb} = T_b - T_{b0} \quad (4)$$

In equation (4), T_{pb} denoted the loss of PAHs by the plant-promoted biodegradation. The results by these calculations were summarized in Table 5. The presence of Am would increases plant promoted removal of PAHs from the spiked soil. PAHs accumulation was increased from 14.2 to 38.7 mg pot^{-1} by wheat, from 17.7 to 59.3 mg pot^{-1} by mungbean and from 9.3 to 11.5 mg pot^{-1} by eggplant. Based on the data in Table 5, mycorrhizal efficiency enhanced dissipation of PAHs in plants increased in eggplant < wheat < mungbean PAHs in plants greatly diminished dry weight accumulation (Table 6). The dry weight of wheat, mungbean and eggplant plants decreased about 40%, 41% and 45% in spiked soil, respectively. With the addition of Am fungi, the total biomass accumulated significantly increased for the three plants species. Am wheat plant in soil spiked with PAHs appeared healthy and accumulated biomass increased to 16% more than non-Am wheat that grew in un-spiked soil. Percent plant dry weight reduction of mungbean in the presence of PAHs in soil was lowered from 41% to 10% and for eggplant from 45% to 33% (Table 6). Of particular importance was the mycorrhizal effect in wheat dry weight was reduced from 124% to 93% in un-spiked and spiked soil respectively. On the other hand, mycorrhizal effect was increased from 36% to 52% for mungbean and for eggplant from 10% to 21% in un-spiked and spiked soil respectively.

Table 5. Contributions of plant to the remediation of PAH in planted versus unplanted grown in soil spiked with PAHs at concentration of 1550 mg kg^{-1}

Plants	VAM state	P_a mg·pot^{-1}	T_p mg·pot^{-1}	T_d mg·pot^{-1}	T_{pb} mg·pot^{-1}
Wheat	–VAM	14.2	693	517	502.8
	+VAM	38.7	945	769	730.3
Mungbean	–VAM	17.7	718	546	526.4
	+VAM	59.3	1138	966	904.3
Eggplants	–VAM	9.3	624	454	444.7
	+VAM	11.5	676	506	494.5

P_a : amount of PAHs accumulated in plant; T_p : amount of PAHs Dissipated in planted spiked soil; T_d : enhancement of PAHs dissipated in planted versus unplanted spiked soil; Tpb : the loss of PAHs in planted soil by plant promoted biodegradation.

Under stress conditions, it is important for plants to maintain water content in the tissue. It was found that the of water content of non-Am plants in spiked soil increased by 2.8% and 49% for wheat and mungbean plants respectively (Table 6). In non-Am eggplants water content was decreased by 21% in spiked soil. Inoculation of Am fungi increased the water content of wheat and mungbean plants while the water content decreased in eggplants. The results in Table 6 showed that the water content of mycorrhized wheat and mungbean significantly increased from 9% and 12% in un-spiked soil to 172% and 200% in spiked soil, respectively.

Root/shoot dry weight ratios of the plant species under investigation significantly reduced as a result of PAHs amendment the soil (Table 6). Root/shoot ratio in non-Am wheat, mungbean and eggplant were 52%, 56% and 40% respectively. With inoculation of Am fungi, the inhibitory effect of PAHs on root/shoot ratios was greatly diminished in wheat (3% reduction) and mungbean (3% increase) while in eggplant it was increased to 50% of control. The results also revealed the VAM symbionts had more pronounced effect in spiked soil than un-spiked one on root/shoot ratios of wheat and mungbean. Mycorrhizal effect on root/shoot ratio was increased from 17% to 100% for wheat and from 15% to 133% for mungbean but it decreased from 27% to 11% for eggplant in un-spiked and spiked soil respectively (Table 6).

Mycorrhizal infection is significant on chlorophyll content at un-spiked but insignificant in spiked soil (data not

Table 6. Effect of PAHs in spiked soil on Am and non-Am plant and mycorrhizal effect in spiked and non spiked soil

Measurements	Plant species	PAH effect on plant %		Mycorrhizal effect on plant %	
		–Am	+Am	Un-spiked soil	Spiked soil
Dry weight (g plant^{-1})	Wheat	–40	16	124	93
	Mung	–41	–10	36	52
	Eggplant	–45	–33	10	21
Water content (%)	Wheat	2.8	179	9.2	172
	Mung	49	346	12	200
	Eggplant	–21	–16	4	9
R/S dry weight ratios	Wheat	–52	–3	17	100
	Mung	–56	3	15	133
	Eggplant	–40	–50	27	11
Total chloro-phyll (Ug g^{-1})	Wheat	46	83	139	25
	Mung	–27	44	102	98
	Eggplant	2	9	98	7
Root lipid (mg gm fresh weight^{-1})	Wheat	4	38	25	33
	Mung	4	52	13	46
	Eggplant	4	10	10	2

PAHs effect on non-Am plant = non-Am plant in unspiked soil – non-Am plant in spiked soil × 100 ÷ non-Am plant in unspiked .soil
PAHs effect on Am plant = non-Am plant in unspiked soil – Am plant in spiked soil × 100 ÷ non-Am plant in unspiked .soil
Mycorrhizal effect in unspiked soil = non-Am plant – Am plant × 100 ÷ non-Am plant.
Mycorrhizal effect in spiked soil = non-Am plant – Am plant × 100 ÷ non-Am plant.

shown). However, the chlorophyll content of mycorrhized plants increased in either spiked and un-spiked soil. On the other hand, the chlorophyll content of wheat and eggplant without Am increased by 46% and 2% compared with control in spiked soil respectively, while in mungbean it decreased by 27% compared with control. The chlorophyll content of Am wheat, mungbean and eggplants were increased to 83%, 44% and 9% compared with control in spiked soil respectively. It is of important that the mycorrhizal effect was lowered from 139%, 102% and 98% in un-spiked soil to 25%, 98% and 7% in spiked soil for wheat, mungbean and eggplant respectively (Table 6).

The lipid content of roots increased slightly in spiked soil than that of unspiked soil for the plants without Am. With the inoculation of Am root lipid contents increased to 38%, 52% and 10% of control wheat, mungbean and eggplant respectively (Table 6). Mycorrhizal effect on root lipid content was significantly increased from 25%, 13% and 10% in unspiked soil to 33%, 46% and 2% in spiked soil for wheat, mungbean and eggplants respectively.

Enzyme activities in soil and root increased when Am fungus was added to soil spiked with PAHs (Table 7). All enzymes tested were significantly inhibited (12~63%) in rihizospheric soil and roots of plants grown without Am. On the other hand, activities of peroxideses, catalase and polyphenol oxidase increased in both soil and root of wheat and mungbean with Am. The reversed results were obtained in rhizospheric soil and root of eggplant, where activities of these enzymes still decreased in lesser extent with Am fungi than those without Am fungi. However, dehydrogenase activities decreased in soil and roots of wheat and eggplant with Am fungi, whereas increased in mungbean root but decreased in rhizospheric soil with Am fungi.

The effect of planting with or without Am fungi on soil enzyme activities in soil spiked with PAHs was presented in Table 7. The results showed that the presence of plants significantly increased the soil enzyme activities in presence of PAHs. Higher soil enzyme activities significantly correlated with types of plants (data not shown). Based on enzyme activities the order of stimulating enzymatic activities was mungbean > wheat > eggplant. This is consistent with the order of PAHs dissipation in the soil. When Am was inoculated to plants soil enzyme activities significantly increased in rhizosphere of wheat and mungbean as compared with non-Am plants. For example, Am mungbean increased the activities of peroxidase, catalase, polyphenol oxidase and dehydrogenase by 144%, 461%, 69% and 81% respectively.

It was found that rhizosphere and root enzyme activities of Am plants increased as compared with non-Am plants. Unexpected results were present in spiked soil the case of : rhizosphere and root enzyme activities of Am plants showed much higher as compared with those in unspiked soil. For example, mungbean plants with mycorrhiza increased activities of peroxidase from 33% to 119% in soil and from 26% to 170% in root, catalase from 18% to 354% in soil and from 24% to 515% in root, polyphenol oxidase from 17% to 75% in soil and from 36% to 252% in root and dehydrogenase from 5% to 103% in root but in soil dehydrogenase activity decreased from 16% to 12% in un-spiked and spiked soil respectively. Mycorrhizal effect on increasing enzyme activities was significant with the order mungbean > wheat > eggplant.

Table 7. Effects of PAH, plant and Am on microbial count and enzyme activities in soil and plants after 65 days of sowing

Measurements	Plant type	Plant tissue	PAHs effect %		Plant effect %		Mycorrhizal effect %	
			–Am	+ Am	–Am	+ Am	un-spiked	spiked soil
Peroxidase unit nmol $h^{-1}g^{-1}$	Wheat	Soil	–40	16	10	33	48	92
		Root	–43	54	–	–	29	168
	Mung	Soil	–42	26	15	144	33	119
		Root	–46	45	–	–	26	170
	Eggplant	Soil	–58	–48	7	14	18	24
		Root	–60	–44	–	–	4	41
Catalase *ml* 0f 0.1 N $KMno_4$ g^{-1} dry weight	Wheat	Soil	–46	67	33	299	22	210
		Root	–14	67	–	–	22	93
	Mung	Soil	–47	142	41	461	18	354
		Root	–52	159	–	–	24	515
	Eggplant	Soil	–53	–40	5	41	5	29
		Root	–63	–46	–	–	10	44
Polyphenol oxidase mg of pure pyrogallol h^{-1} g^{-1} dry weight	Wheat	Soil	–24	10	21	21	26	46
		Root	–34	116	–	–	44	229
	Mung	Soil	–16	47	33	69	17	75
		Root	–34	131	–	–	37	252
	Eggplant	Soil	–24	–19	1	3	7	7
		Root	–31	–11	–	–	17	28
Dehydrogenase (cm^3 H_2 kg^{-1} 24 h^{-1})	Wheat	Soil	–28	–20	39	55	14	11
		Root	–40	–10	–	–	7	50
	Mung	Soil	–27	–18	65	81	16	12
		Root	–22	–59	–	–	5	103
	Eggplant	Soil	–46	–36	11	7	7	15
		Root	–12	70	–	–	17	93
Total fungal and bacterial count (colony gm $soil^{-1}$)	Wheat	Rhizosphere	–95	–23	24	585	31	1563
	Mung	Rhizosphere	–93	43	81	1472	26	2100
	Eggplant	Rhizosphere	–93	–91	32	54	17	95

PAHs effect on non-Am plant = non-Am plant in unspiked soil – non-Am plant in spiked soil × 100 ÷ non-Am plant in unspiked .soil
PAHs effect on Am plant = non-Am plant in unspiked soil – Am plant in spiked soil × 100 ÷ non-Am plant in unspiked .soil
Mycorrhizal effect in unspiked soil = non-Am plant – Am plant × 100 ÷ non-Am-plant.
Mycorrhizal effect in spiked soil = non-Am plant – Am plant × 100 ÷ non-Am-plant.
Non-Am Plant effect in spiked soil = unplanted – planted × 100 ÷ unplanted.
Am plant effect in spiked soil = Am-unplanted – Am planted × 100 ÷ Am-unplanted.

This is also consistent with the order of PAHs dissipation in the soil.

Table 7 clearly showed that the PAHs had inhibitory effects on microbial numbers in the soil rhizosphere where microbial counts in spiked soil decreased by 93~ 95% of that in unspiked soil. Am colonization of the tested plants would minimize the inhibitory effects of these pollutants in soil especially in rhizosphere of mungbean and wheat plants. In polluted soil, the microbial numbers in rhizosphere of mungbean, wheat and eggplants were 1472, 585 and 54 folds in planted than those of un-planted soil respectively. In spiked soil, mycorrhizal effect followed similar pattern to planting effect. The number of microbial colony increased up to 2100%, 1563% and 95% in mungbean, wheat and eggplant respectively in spiked soil compared with those of the unspiked soil.

Discussion

The results in this study revealed that the plant did enhance the remediation in contamination of soil with PAH. Inoculation of plants with Am increased plant survival and growth in contaminated soil. A toxic effect of PAHs on wheat, mungbean or eggplant was evident at the level growth examined, and this negative effect reduced by the presence of Am. The levels of root colonization by Am were decreased by the presence of PAHs in soil. The effect of Am on plants grown in the soil spiked with PAHs have been shown to contribute to the maintenance of plants to grow in spiked soil (Binet *et al.*, 2001; Rabie, 2004).

This study showed that Am fungal inoculation enhanced dissipation amount of PAHs in planted spiked soil, plant promoted removal PAHs and loss of PAHs by the plant-promoted biodegradation. In addition, the results also revealed that the enhanced dissipation of PAHs would overwhelmingly drive direct uptake and accumulation of PAH by plant and promoted biodegradation. However, amount of PAHs directly accumulated by the tested plant contributed 2% to 6% in the enhancement in dissipation

of PAHs. In contrast, plant-promoted biodegradation of PAHs was a major contribution, and larger than 94% of the enhancement of PAHs dissipation in planted spiked soil. The beneficial role of Am fungi in the dissipation enhancement of PAHs in planted soil was previously shown by Binet *et al.*, 2001; Joner and Leyval, 2001; Rabie, 2004 who found that Am inoculated to wheat-mungbean system may significantly increase the efficiency to enhance the dissipation of these pollutants in the soil.

The ability of Am fungi to colonize the tested plants in spiked soil varied in the three species tested in this study (Table 1). Since growth conditions of these plants grown in spiked soil were identical, thus the disparity of Am colonization would be attributed to plant themselves and/or their responses to mycorrhizal infection. Mungbean plants showed higher levels of Am colonization than the two other plants in spiked and non spiked soils. Of particular importance was the behavior of mycorrhizal activity in spiked soil. For example, in wheat and mungbean plants the intensity of mycorrhizal colonization in root tissues and rate of arbuscular formation in root segments showed significant positive and negative correlations with the PAH accumulation in plants and in the soil, respectively. In addition, both showed positive correlations with water content, R/S ratio, root lipid content, peroxidase and polyphenol oxidase in wheat and mungbean plants grown in spiked soil (Table 2). These correlations were highly significant in wheat and mungbean and attributed to rate of arbuscular formation in root segments, which reflect symbiotic activity of Am fungi within plants. Although evaluation of efficacy of plant-mycorrhizal associations to remidiate soils contaminated with PAHs was previously discussed by many authors (Leyval and Binet, 1998; Jouner and Leyval, 2001; Entry *et al.*, 2002; Rabie, 2004 and others). Hitherto, this is the first report to discuss the role of levels of mycorrhizal colonization on the behavior of infected plants responses grown in spiked soil with PAHs.

The data suggest that certain responses might be indicative of plant resistance and acclimation to contaminants in the environment and these responses were found to be much higher in Am than that of non-Am plants. However, since the growth conditions of Am and non-Am plants grown in spiked soil were identical, the disparity of these responses would come from Am fungal inoculation to plants. In the context of these responses, the more resistant species, mungbean, can increase dry weight, water content, biomass accumulation of root, root : shoot ratio, total chlorophyll and root lipid content (Table 6) as well as concentrations of peroxidase, catalase, polyphenol oxidase and dehydrogenase in plant roots (Table 7). These characteristics are very important for plants grown in heavily contaminated soils. With this regard, Huang *et al.* (2004a) revealed that the strategy of the more tolerant species, grown in soil heavily contaminated with creosote, includes increasing water content, biomass accumulation in roots, maintaining chlorophyll content and constant chlorophyll a/b ratio.

Among the three plant species tested, the most tolerant and effective plant to PAHs and their dissipation in the soil was the Am mungbean. This finding may be related to increasing water content and root lipid in Am mungbean plants. This increase is very important for plant where the toxic chemicals in the tissue would be diluted, and thus the toxic effects of contaminants would be diminished. Therefore, the plant would be able to maintain their growth. Chiou *et al.* (2001) elucidated that for roots with high water contents, the root-water phase acted as the major reservoir for highly water-soluble contaminants. By contrast, the lipids in roots, even at small amounts, were usually the major reservoir for highly water insoluble contaminants. Results of our study indicate that root lipid content might be a good predictor for root accumulation of PAHs. This is in some way, consistent with the observations by Paterson and Mackay, 1994; Simonich and Hites, 1995; Petersen *et al.*, 2002. Another strategy for coping with the stress is the expansion and biomass increasing of root system. Large and deep root systems will increase nutrient and water uptake to maintain vigorous growth, to overcome stress induced by PAHs. This results in agreement with what was previously reported by Hooker and Atkinson, 1996; Joner and Leyval, 2001; Chekol *et al.*, 2004; Licht and Isebrands, 2005.

It is expected that plant roots enhance the pools of extractible oxido-reductases enzymes as well as other types of enzymes by root exudation as well as by the propagation enzyme producing rhizosphere microorganisms indirectly. In addition, the presence of rhizosphere microorganisms increase root exudation by plants (Gramss *et al.*, 1999; Newman and Charles, 2005). The results in this study showed increases in activities of several enzyme and microbial number in the rhizospheric soil and plants with mycorrhiza, suggesting that enzymatic oxidation with the microbial degradation is one of the important effects in PAH remediation strategy. The positive correlation observed between Am infection and the enzyme activity in the present study could be indicative of the enzyme activity stimulated by the presence of Am fungi. These correlations could be an indication for the biological role of Am fungi in biodegradation of PAHs in soil. The role of enzyme activity in dissipation of different organic pollutants in the soil was previously discussed by several researchers (Salzer *et al.*, 1999; Gramss *et al.*, 1999; Ma *et al.*, 2003; Joner *et al.*, 2004).

The results of this study showed that microbial numbers in rhizosphere of Am plants were much higher than

that of non-Am one. This is consistent with the order of enzyme activity as well as PAHs dissipations in the spiked soil. The plant-promoted biodegradation of soil organic chemicals has been studied in the past. Plants may contribute to the biodegradation of organic compounds by an increase in microbial number (Reilley *et al.*, 1996; Binet *et al.*, 2001), a promotion in microbial activity (Binet *et al.*, 2000; Newman and Charles, 2005), and a modification in microbial community in rhizosphere (Joner *et al.*, 2002; Joner and Leyval, 2003).

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