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The Effects of Microbial Population on Phytoremediation of Petroleum Contaminated Soils Using Tall Fescue

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ABSTRACT

Petroleum contamination of soil is a serious problem throughout the south of Tehran and Khuzestan province of Iran. Vegetation may play an important role in the biodegradation of toxic organic chemicals in soil. For petroleum compounds, the presence of rhizosphere microflora may accelerate biodegradation of the contaminants. In a greenhouse study, petroleum contaminated soils from sites around Tehran Refinery Planet were phytoremediated using four native plants including *Agropyron*, *Lolium*, Tall fescue and *Puccinellia distance*. The soils were silty clay and loam. Treatments included six C: N ratios and no-vegetated pot as control. After 90 days the C: N ratios and microbial populations were assessed compared with the beginning of trials. The results revealed that all these plants behaved differently for root exudates, which directly affects the microbial activities. The rate of degradation appeared to be dependent on the selection of plant species compared to the specific root surface area. Among the plant species tested, only Tall fescue could survive the harsh condition of the soils. With increasing crude oil concentrations, the microbial population was increased. The presence of Tall fescue roots clearly improved the physical structure of the soil and could provide oxygen and energy to microbial population, which was responsible for reducing contamination.

Key Words: Rhizosphere; C: N ratio; Microbial population; Phytoremediation; Tall fescue

INTRODUCTION

The industrial revolution of the past century has resulted in significant damage to environmental resources such as air, water and soil. Healthy survival of human beings depends on the quality of physical environment (Riaz *et al*., 2002). Using plants to store, remove, degrade and metabolize environmental contaminant including metals, hydrocarbons and other toxic organic compounds is a bioprocess called phytoremediation. Phytoremediaton is an alternative to another technology, including incinerators and chemical treatment. It is well-suited at sites with shallow contamination of organic or metal pollutants, for use at very large field sites, where other methods of remediation may not cost-effective or practicable (Abedi-Koupai & Afyuni, 2003; Abedi-Koupai, 2003) and it is practicable at sites with low concentrations of petroleum contaminants. Interaction between microorganisms associated with plants and plants is known the main features of this phenomenon. There are three primary mechanisms by which plants and microorganisms remediate petroleum–contaminated soil and ground water. These include degradation, containment and transfer of the hydrocarbons from soil to atmosphere (Sims & Overcash, 1983; Cunningham *et al*., 1996; Siciliano & Germida, 1998a, b). Several studies serve as examples of rhizosphere effect in the phytoremediation of petroleum hydrocarbons. Gunther *et al*. (1996) found higher microbial numbers and activity coupled with increased degradation in hydrocarbon-contaminated soil planted to ryegrass compared to un-planted soil. They suggested that plant roots stimulated the microbial growth, which enhanced the degradation of hydrocarbon mixture. Plants provide root exudates of carbon, energy, nutrients, enzymes and sometimes oxygen to microbial populations in the rhizosphere (Cunningham *et al*., 1996). Root exudates of sugars, alcohols and acids can amount to 10 to 20% of plant photosynthesis annually (Schnoor *et al*., 1995) and provide sufficient carbon and energy to support large numbers of microbes (e.g., approximately 10^8 - 10^9 vegetative microbes per gram of soil in the rhizosphere (Erickson *et al*., 1995). Due to these exudates, microbial populations and activities are 5 to 100 times greater in the rhizosphere than in the bulk soil. This plant-induced enhancement of the microbial population is referred to as the rhizosphere effect (Atlas & Bartha, 1998), which is believed to result in enhanced degradation of organic contaminants in the rhizosphere. Plants with a fibrous root structure and therefore greater root surface area may enhance organic dissipation more than plants with simpler, less fibrous systems (Aprill & Sims, 1990).

Chekole and Vough (2002) stated that planting both legume and grass species had significant impact on the

transformation of a nitroaromatic compound (TNT) and a polychlorinated biphenyl (PCB) in soil with low organic-matter and did not affect the fate of a polycyclic aromatic hydrocarbon (Pyrene) in soil with low and high organic-matter. Dominguez-Rosasdo *et al*. (2004) reported that seed germination of several grass, legume and cereal species declined with an increase in used oil concentration, at oil rates grater than 1% (w/w). They stated in terms of both germination and overall growth, leguminous plant were generally more resistant to used oil contamination than non-leguminous species mainly due to massive root system. Merkl and Schultze-Kraft (2005) reported that legumes died within 6 to 8 weeks in heavily crud-oil contaminated soil, whilst the grasses showed reduced biomass production. Furthermore, a positive correlation between root biomass production and oil degradation was found. The microbial communities and their degradative potential in rhizosphere of alfalfa, reed and un-planted soil in response to bitumen contamination of soil reduced the total number of microorganisms more significantly (by 75%) in un-planted than in rhizosphere soil (by 42% & 7%) for reed and alfalfa, respectively (Muratova *et al*., 2003). They indicated that the rhizosphere microflora of alfalfa was less inhibited by hydrocarbon pollution and had a higher degradative potential than the rhizosphere microflora of reed. Banks *et al.* (2003) indicated that the presence and type of plants and level of contamination may greatly influence microbial communities in plotted soils. Issoufi *et al.* (2006), while studying seedling growth of 6 crop species in crude oil contaminated soils reported that *Zea mays* and *Glycine max* seedlings show the greatest potential to enhance remediation compared to the *Meticago sativa, Lolium perenne, Triticum aestivum* and *Vicia villosa*. White *et al.* (2003) investigated soil amendments with different C: N ratios for their effects on both seed germination and plant growth. Addition of boiler litter (C: N ratio ~ 8) resulted in lower soil total petroleum hydrocarbon (TPHs) compared to amendments with higher C: N ratios. White *et al*. (2006) evaluated the effect of vegetation establishment on the biodegradation of alkylated polycyclic aromatic hydrocarbons in a crude oil contaminated soil and reported that there was greater degradation of the longer three-ring alkylated phenanthrenes-anthracences and dibenzothiophenes in the vegetated fertilized plots compared to the non-vegetated non-fertilized plots.

Few experiments suggest that the degradation of petroleum hydrocarbons from soil may not be enhanced by the rhizosphere effect. Ferro *et al*. (1994) reported that crested wheatgrass [*Agropyron desertorum (Fisher ex Link) Schultes*] had no effect on either the rate or extent of mineralization of [^{14}C] phenanthrene when planted and un-planted systems were compared. For this experiment, the authors speculated that rapid mineralization of the [^{14}C] phenanthrene by microbes prior to the establishment of the plant root systems and therefore, prior to the presence of a rhizosphere effect in the soil may have resulted in the lack of significant difference between mineralization in planted and un-planted systems. Ferro *et al*. (1997) reported that alfalfa (*Medicago sativa Mesa, var. Cimarron VR*) planted in artificial loamy to the soils at 40 or 662 µg kg^{-1} had no effect on either the rate or extent of mineralization of [^{14}C] benzene compared to un-planted soils.

This study was designed to examine (1) the rhizosphere effects between 4 grass species (*Agropyron, Lolium*, Tall fescue & *Puccinellia distance*) to achieve microbial activities appeared in reducing C: N ratio and (2) measurement of root surface area for different plants at the end of trials.

MATERIAL AND METHOD

A greenhouse study was conducted to determine the rhizosphere effect of different plants for decontamination of soils. Statistical design was completely randomized and treatments included crude-oil concentrations: (a) low level (10 g kg^{-1}) and (b) high level (30 g kg^{-1}) of soil. Plants used in study were *Agropyron, Lolium*, Tall fescue and *Puccinellia distance* and soil textures included silty-clay and loam (control pot without plants).

The soils used in this experiment were sampled from the crude-oil contaminated area around Tehran Refinery Planet in the south of Tehran. The pH was measured by glass electrode in a 1:1 water suspension, available-P by the Olsen method, electric conductivity with EC meter, Cu, Zn, Mn, Fe with atomic absorption (Perkin-Elmer 3110), texture by sieve analysis and hydrometer, cation exchange capacity (CEC) by NH_4 saturation, organic carbon (OC) was determined by Walkly Black method and nitrogen determination was made by Kjeltec method (Tekator company model 2030). Different fractions of soil particles and physico-chemical properties of soil used in this study are given in Table I.

The most microbial population number (m.p.n.) was measured as microbial activity characterization at the beginning and end of trials. Colony counting was made by colony counter (Cyntex, England). Each treatment was replicated four times. In each pot 2 kg soil was put and to enhance degradation of crude oil, 0.6 g phosphorous was added. Water was added to the soil surface by sprinkler. The soils were sampled from each pot after 90 days for counting microbial population and measurement of C: N ratios. Also, plants were separated into root and shoot biomass and weighed. For measurement of specific surface area, the root samples were scanned using Delta-T scan image analysis system (Windas-Software, Cambridge U.K). The statistical analysis of data was done by the statistical analysis package SAS™ (SAS, 1998).

RESULTS AND DISCUSSION

The indices of C, N, C: N and m.p.n. for different soils, plants and concentrations of crude oil indicated significant ($p < 0.01$) effect due to soil texture, soil × time, concentration ×

Table I. The chemical and physical properties of soils used in greenhouse study

Parameter	Soil texture	
	Loam	Silty clay
Sand	48	38
Silt	40	36
Clay	12	26
CEC (C.mol g^{-1})	9.8	13.97
Cu (mg/kg)	1.98	2.28
Zn (mg/kg)	12.8	1.72
Mn (mg/kg)	10.68	10.94
Fe (mg/kg)	4.86	6.86
K (mg/kg)	143	324
P (mg/kg)	7.0	18.6
N (%)	0.07	0.09
OC (%)	0.73	0.90
pH	7.64	7.79
EC (dS/m)	2.15	1.20
S.P (%)	22.6	37.3

time, plant × time, soil × concentration × time on the C: N ratio (Table II & III). The parameters of C and C: N (by 33% & 21% for loam & silty clay, respectively) for both soils at the end of trials were significantly reduced than that at the beginning (Table IV). This may be related to more aeration in loam compared to the silty clay. Also, there was significant ($p < 0.01$) effects due to time on the C, N, C: N and m.p.n. The m.p.n in soil containing Tall fescue at the end of trials was higher than at the beginning and as a results C: N was reduced (Table V). The presence of Tall fescue roots clearly improved the physical structure of the soil provided oxygen and energy to microbial population, which spilled over in reducing contamination (Table V). Therefore, the results have implications with respect to the use of Tall fescue for remediation of low petroleum-containment sites (Epuri & Sorenson, 1997). Fiorenza *et al.* (2000) in a field trial found that using three separate planted treatments (*Rye grass*, Tall fescue & *White clover*), the greatest extent of polycyclic aromatic hydrocarbons (PAHs) was observed in the Tall fescue plots. Likewise, a field study conducted by Robinson *et al.* (2003) to assess the impact of Tall fescue on the degradation of six polycyclic aromatic hydrocarbons (PAHs). They concluded Tall fescue had a beneficial impact on the degradation of all PAHs except phenanthrene. Microbial populations on solid media plates with pyrene and chrysene as the sole carbon source were two times greater in soils from Tall fescue than from bare soils, suggesting that the increased PAHs degradation as a result of increased microbial activities in the rhizosphere.

The PNM decreased at the end of trials for *Lolium*, *Agropyron* and *Puccinellia distance*. Therefore, only Tall fescue could tolerate the harsh condition of the soils. With increasing crude oil concentrations, the microbial population was increased. Bacteria were the most abundant microbial group (including *Psedumonas*, *Klebciella*, *Bacilleuse*, *E-Coli*

Table II. The indices of C, N, C: N and m.p.n. for different soils, plants and concentrations of crude oil

Plant	Soil	Concentration (g)	Time	C (%)	N (%)	C:N	m.p.n.
Lolium	Loam	10	Beginning	1.95	0.09	22.94	20000.00
Agropyron				2.73	0.08	33.70	20000.00
Puccinellia				3.10	0.07	41.89	10000.00
Tall fescue				3.12	0.08	37.59	10000.00
Lolium	Loam	30	Beginning	1.04	0.08	13.33	15000.00
Agropyron				1.66	0.08	20.78	20000.00
Puccinellia				1.42	0.07	19.19	10000.00
Tall fescue				1.98	0.08	26.05	15000.00
Lolium	Silty clay	10	Beginning	2.43	0.08	30.00	5000.00
Agropyron				1.88	0.09	22.10	20000.00
Puccinellia				2.47	0.09	29.06	15000.00
Tall fescue				2.52	0.09	28.96	10000.00
Lolium	Silty clay	30	Beginning	1.41	0.09	16.21	15000.00
Agropyron				1.64	0.08	12.03	15000.00
Puccinellia				1.08	0.10	10.80	10000.00
Tall fescue				1.29	0.10	15.18	10000.00
Lolium	Loam	10	End	1.53	0.09	17.99	5000.00
Agropyron				1.26	0.08	15.57	10000.00
Puccinellia				1.15	0.09	12.74	5000.00
Tall fescue				0.86	0.08	11.03	15000.00
Lolium	Loam	30	End	2.18	0.09	25.64	7000.00
Agropyron				1.84	0.08	22.94	10000.00
Puccinellia				1.80	0.09	21.13	10000.00
Tall fescue				1.28	0.08	16.62	10000.00
Lolium	Silty clay	10	End	1.36	0.09	15.08	5000.00
Agropyron				1.57	0.09	16.67	5000.00
Puccinellia				0.99	0.08	11.96	10000.00
Tall fescue				0.92	0.09	10.79	15000.00
Lolium	Silty clay	30	End	1.36	0.09	15.60	10000.00
Agropyron				2.14	0.09	23.52	10000.00
Puccinellia				1.59	0.09	18.23	10000.00
Tall fescue				1.49	0.09	17.54	10000.00

ns: not significant, *: significant ($p<0.05$), **: significant ($p<0.01$)

Table III. Analysis of variance of the indices of C, N, C: N and m.p.n. in different soils, plants and concentrations of crude oil

Source of variations	df	C (%)	N (%)	C:N	m.p.n.
Soil	1	0.24^{ns}	0.0004*	133.66*	9031250^{ns}
Concentration	1	0.67*	0.00001^{ns}	125.1*	1531250^{ns}
Plant	3	0.05^{ns}	0.00002^{ns}	2.56^{ns}	24031250^{ns}
Soil×Concentration	1	0.004^{ns}	0.00005^{ns}	1.87^{ns}	2801250^{ns}
Soil×Plant	3	0.042^{ns}	0.00006^{ns}	10.93^{ns}	12364583^{ns}
Concentration ×Plant	3	0.062^{ns}	0.00002^{ns}	6.99^{ns}	6531250^{ns}
Time	1	2.2**	0.00005^{ns}	356.2*	166531250*
Soil×Time	1	0.1^{ns}	0.0001^{ns}	42.4*	16531250^{ns}
Concentration×Time	1	5.05**	0^{ns}	820.7**	1531250^{ns}
Plant×Time	3	0.38*	0.000025^{ns}	56.6*	49031250^{ns}
Soil×Concentration×Time	1	0.068^{ns}	0.00001^{ns}	2.8^{ns}	281250^{ns}
Soil×Plant×Time	3	0.31*	0.0001^{ns}	57.7*	9031250^{ns}
Concentration×Plant Time	3	0.09^{ns}	0.000008^{ns}	13.6^{ns}	17364582^{ns}

ns: not significant, *: significant (p<0.05), **: significant (p<0.01)

Table IV. Analysis of variance of the indices of C, N. C: N and m.p.n. in different soils and times

Soil	Time	C (%)	N (%)	C:N	m.p.n
Loam	Beginning	2.125^{a}	0.078^{b}	26.93^{a}	15000^{a}
	End	1.48^{c}	0.85^{ab}	17.95^{c}	9000^{b}
Silty clay	Beginning	1.84^{b}	0.09^{a}	20.54^{b}	12500^{ab}
	End	1.43^{c}	0.088^{a}	16.17^{c}	9375^{b}

No significant difference (p<0.01) between the treatments with the same alphabet signs

Table V. Analysis of variance of the indices of C, N. C: N and m.p.n. in different plants and times

Plant	Time	C (%)	N (%)	C:N	m.p.n
Lolium	Beginning	1.7^{bc}	0.083^{a}	20.62^{cd}	13750^{ab}
Agropyron	Beginning	1.97^{ab}	0.081^{a}	22.15^{bc}	18750^{a}
Puccinellia	Beginning	2.02^{ab}	0.082^{a}	25.23^{ab}	11250^{ab}
Tall fescue	Beginning	2.23^{a}	0.087^{a}	26.94^{a}	11260^{ab}
Lolium	End	1.60^{c}	0.09^{a}	18.57^{de}	6750^{b}
Agropyron	End	1.70^{bc}	0.085^{a}	19.67^{cd}	8750^{b}
Puccinellia	End	1.38^{cd}	0.087^{a}	16.01^{ef}	8760^{b}
Tall fescue	End	1.14^{d}	0.081^{a}	13.99^{f}	12500^{ab}

No significant difference (p<0.01) between the treatments with the same alphabet signs

Table VI. Effects of type of plant and crude oil concentration on shoot and root biomass and specific root area

Plant	Crude oil concentration	Specific root area surface (mm^2)	Shoot biomass (g)	Root biomass (g)
Lolium	Low	1445^{b}	0. 29^{a}	0. 082^{a}
	High	2842.6^{a}	0. 049^{cd}	0. 029^{bcd}
Agropyron	Low	728^{bcd}	0.074^{c}	0.06^{ab}
	High	1334.2^{b}	0.068^{cd}	0.044^{bc}
Puccinellia	Low	-	-	-
	High	66.3^{d}	0.0065^{d}	0.003^{d}
Tall fescue	Low	1009. 8^{bc}	0.146^{b}	0.057^{ab}
	High	480.5^{cd}	0.027^{cd}	0.015^{cd}

No significant difference (p<0.01) between the treatments with the same alphabet signs.

& *Entrobacter*) in most treatments compared to the fungi (including *Yeast*).

After 90 days, both shoot and root biomass was greater for plants in lowly than highly contaminated soils except for Tall fescue. The plants grown in highly contaminated soils had consistently higher root surface area those plants grown in low contaminated soils unless for Tall fescue (Table VI). This indicated no-clear relationship between specific root area and phytoremediation of contaminated soil. Further long term studies are imperative on this issue.

Merkl *et al*. (2005), while studying root morphological characteristics of three tropical graminoids (*Grachiaria brizantha*, *Cyperus aggrgatus* & *Eleusine indica*) reported that *G. brizantha* and *C. aggrgatus* showed coarser roots in polluted soil than control as expressed in an increased average root diameter. *G. brizantha* had a significantly greater specific root surface area in contaminated soil. Additionally, oil contamination caused a significantly smaller specific root length and root surface area in the *C. aggrgatus*. Root structure of was not significantly affected by crude oil, whereas higher specific root surface area was related to higher degradation of petroleum hydrocarbons as noted in the previous studies.

Some evidence was found about serious phytotoxicity effects especially for *P. distance* after 8 weeks in the form of yellowing of leaves in 30 g kg^{-1} treatment (Table VI), which may be related to low level of soil salinity (EC = 1.2 - 2.15 dS m^{-1}). It is imperative to note that this species has been reported as more adjustable to saline and alkaline soils (Abedi-Koupai & Charkhabi, 2005).

CONCLUSIONS

Phytoremediation was found to be a feasible method for improving petroleum-contaminated soils, although all the species behaved differently in order to their exudates and root biomass and surface area, which directly affects the microbial activities. There was no clear response for decrease in C: N ratio with increased specific root area. The rate of oil degradation was dependent on the type plant species but not the. Root length, biomass and specific root surface area may be important parameters, which needs further investigation.

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