Effect of C/N Ratio on the Phytoremediation of Crude Oil Contaminated Soils by *Puccinellia Distance*

Ezzatian, Reza
Department of Energy and Environment, Science and Research Campus, Islamic Azad University, Tehran, I.R. IRAN

Voussoughi, Manouchehr*+; Yaghmaei, Soheila
Department of Chemical and Petroleum Engineering, Sharif University of Technology, Tehran, I.R. IRAN

Abedi Koupai, Jahangir
College of Agriculture, Department of Water Engineering, Isfahan University of Technology, Isfahan, I.R. IRAN

Borghaei, Mahdi; Pazuki, Gholam Reza
Department of Chemical and Petroleum Engineering, Sharif University of Technology, Tehran, I.R. IRAN

ABSTRACTS: *Petroleum contamination of soil is a serious problem throughout the oil producer countries. Vegetation may play an important role in the biodegradation of toxic organic chemicals in soil. For petroleum compounds, the presence of rhizosphere micro flora may accelerate biodegradation of the contaminants. In a greenhouse study, petroleum contaminated soil were treated using phytoremediation . The C/N ratios and microbial populations were assessed in the beginning and the end of trials. The results showed that Puccinellia distance could tolerate the harsh condition of the soils. As MPN increases, C/N ratios decrease among trials. N, as a nutrient, had effects on both microbial populations and decreasing of organic carbon. Among six C/N ratios, organic carbon content of soil was lower at the end of the study in the vegetated pots compared with the non-vegetated ones. For the most part, the presence of plants enhanced the dissipation of the contamination. Our findings show that in level of 12.9 g crude oil per kg of dry soil we have critical point and for successful pyhtoremediation operation pollution must be below this amount. Microbial activity in loam texture had greater numbers and seedling pots had better efficiency in comparison with planting seed pots.*

KEY WORDS: *Phytoremediation, Contamination limit, Microbial populations, C/N ratio, Puccinellia distance.*

** To whom correspondence should be addressed.*
+ E-mail: vosoughi@sharif.edu
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INTRODUCTION

Phytoremediation is an in situ use of plants and their associated microorganisms to degrade, containment or render harmless contaminants in soil or ground water [1a]. It is well situated at sites with shallow contamination of organic or metal pollutants, also in very large field sites, where other methods of remediation is not cost-effective or practicable [2].

Plants stimulate the degradation of organic chemicals in the rhizosphere by releasing root exudates, enzymes, and build-up of organic carbon in the soil. Petroleum-derived hydrocarbon contamination of soil is a serious problem due to its toxic ingredients which have harmful effect on microorganism and even on some plant species. Bioremediation of petroleum in soil using indigenous microorganisms has proven effective; however, the biodegradation rate of recalcitrant and potentially toxic petroleum contaminants, such as polycyclic aromatic hydrocarbons (PAHs), is rapid at first but declines quickly [3].

Vegetation may play an important role in the biodegradation of toxic organic chemicals in soil. For petroleum compounds, the presence of rhizosphere micro flora may accelerate biodegradation of the contaminants. The establishment of vegetation on hazardous waste sites is an economic, potentially effective and low-maintenance approach to waste remediation and stabilization. The biological stability of soil organic carbon (SOC) is influenced by the chemical structure of SOC and the existence of various mechanisms of protection offered by soil minerals and their spatial arrangement within the soil matrix [4].

In general to define the potential availability of SOC to decompose organisms in soils, the chemical structure of SOC which identifies the living strength in soil is determined. The degree of physical protection is mainly a factor of soil texture, specific mineral surface area and soil mineralogy [5]. However, other soil parameters (e.g. water holding capacity, pH and porosity) can act as rate modifiers in attaining the protective capacity, set by the mineral matrix of the soil [6].

Furthermore, there are three primary mechanisms by which plants and microorganisms remediate petroleum-contaminated soil and as a result affect on SOC by degradation of contaminant. These mechanisms include degradation, containment and transfer the hydrocarbons from soil to the atmosphere [1b] .Plants and micro-organisms are involved, both directly and indirectly, in the degradation of petroleum hydrocarbons into products, that are generally less toxic and less persistent in the environment than the parent compounds [7].

Though plants and microorganisms can degrade petroleum hydrocarbons independently, the literature suggests that it is the interaction between plants and microorganisms (i.e. the rhizosphere effect) which is the primary mechanism responsible for petrochemical degradation in phyto-remediation efforts. Therefore, maximum protective capacity for saving organic carbon in soil can only be achieved under ideal conditions because other soil properties, in accompany with the parameters established by the soil mineral matrix, affect carbon accumulation in soil up to its maximum capacity. Thus a realistic assessment of the degree of protection of SOC by soil minerals is possible only through a collective analysis of the individual microbial, physical, chemical and textural properties of a soil [8].

C/N ratio is most familiar indices which can explain microbial activity vs. OC and total N which may represents pollution situation.

The aim of this study included:

1- To select the suitable ratio for C/N in phyto-remediation of crude oil contaminated soil.

2- To select the best texture of soil for phyto-remediation of crude oil contaminant.

3- To evaluate performance of *Puccinellia distance* in crude oil contaminated soil.

In this study in order to assess *puccinelia distance* performance we utilize this plant as treatment species. *Puccinelia distance* is from the family of Garmina. So far 9 species of this plant have been recognized in different regions of Iran specially in flats [9]. A green house study was conducted to determine C:N ratio effect on phyto-remediation of crude oil contaminated soils by *puccinellia distance* (puccinellia distance [L.] Parl.) Statistical design was based on completely randomized blocks.

MATERIALS AND METHODS

Variables included soil texture (clay loam, loam and sandy loam), the C/N ratio (by adding crude oil and in some cases urea to soil) and vegetated and non-vegetated pots (urea was provided from Iranian petrochemical company). The soil textures were taken from south of

Table 1: The chemical and physical properties of soils used in greenhouse study.

Parameter	Soil texture		
	Loam	Clay loam	Sandy loam
Sand (%)	48	38	76
Silt (%)	40	36	15
Clay (%)	12	26	9
CEC[1] (cmol g^{-1})	9.80	13.97	10.90
Cu (mg/kg)	1.98	2.28	1.90
Zn (mg/kg)	12.8	1.72	13.0
Mn (mg/kg)	10.68	10.94	10.36
Fe (mg/kg)	4.86	6.86	4.44
K (mg/kg)	143	324	151
P (mg/kg)	7.0	18.6	7.8
N (%)	0.07	0.09	0.07
OC[2] (%)	0.73	0.90	0.71
TNV[3] (%)	8.12	8.00	7.50
pH	7.64	7.79	7.65
EC[4] (ds/m)	2.15	1.20	2.16
S.P[5] (%)	22.6	37.3	22.5

1) Cationic Exchange Capacity; 2) Organic Carbon; 3) Total Neutralizing Value; 4) Electric Conductivity; 5) Specific Gravity.

Tehran, around Tehran Refinery Plant. Each treatment was replicated four times for seedling pots and four times for planting seed ones. In each pot 2 kg of soil was placed and to enhance degradation of crude oil 0.1 g, phosphorous (super phosphate triple ,Merk company) was added. Water was added on the soil surface by sprinkler. The experiment used *Puccinellia distance* (Puccinellia distance [L.] parl.) as vegetated plants. *Puccinellia distance* is form the family of Gramina. So far 9 species of that have been recognized in different regions of Iran [10].

The soil was sampled from each pot at the end of 90 days for counting microbial populations and C/N ratios determination. Total organic carbon was determined by Valkly Black method [11]. Determination of nitrogen made by kjeltec method, Nitrogen distillation apparatus were made by Tekator model 2030 [12] .Different fractions of soils particles and physic-chemical properties of soil used in this study are given in table 1.

Microbial population counting was completed by pure plate count (25-35 °C, 48-72 hours), in nutrient agar media (HIMEDIA United Kingdom) [13] .

RESULTS AND DISCUSSION

The alues of C, N, C/N and MPN for different soil textures and different concentrations of crude oil are presented in tables 2 to 4. It is clearly evident that there are significant differences between OC and microbial population ($p<0.01$) in the beginning and the end of the trials. OC content of soil decreased and microbial population increased during 90 days phytoremediation operation .This is in agreement with previous findings [3]. Several published studies have evaluated the effect of plants and the associated rhizosphere on the fate of petroleum contaminants ([14-17]). As it is shown in table 2, results for vegetated pots indicated that *puccinellia distance* not only could tolerate harsh condition of crude oil contaminated soil- specially below 17 g oil/kg dry soil- but also had significant effect on cleaning up crude oil contaminated soil, which is due to microbial activity. This effect specially may be observed from C magnitude for seedling pots and planting seeds vs. blank ones. This fact is reported in tables 2, 3 and 4, which means in every type of three textures, decreasing of organic carbon is

Table 2: The Values of C,N, C/N and MPN for different concentrations of crude oil in soil with clay texture for Puccinellia distances in the beginning and end of trials(period of 90 days)

Concentration	vegetation	C (%)		N (%)		C/N		MPN	
		Beg.	End	Beg	End	Beg.	End	Beg.	End
2 g oil 4.3 g urea/kg dry soil	Planting seed	0.95	0.80	0.13	0.10	7.03	7.34	12.0×10^4	23.5×10^4
	Seedling	0.90	0.07	0.12	0.11	7.37	6.90	13.0×10^4	28.0×10^4
	blank	0.93	0.92	0.11	0.10	8.01	8.52	10.0×10^4	19.0×10^4
2g oil 0.21 g urea/kg dry soil	Planting seed	0.95	0.78	0.08	0.06	11.17	12.96	11.0×10^4	27.6×10^4
	Seedling	0.97	0.72	0.02	0.07	11.80	9.60	12.0×10^4	29.4×10^4
	blank	0.93	0.91	0.08	0.07	11.20	11.52	10.0×10^4	19.5×10^4
4g oil 0.21 g urea/kg dry soil	Planting seed	1.40	0.89	0.08	0.06	16.09	14.13	10.0×10^4	22.4×10^4
	Seedling	1.46	0.79	0.08	0.07	16.78	10.13	11.0×10^4	24.2×10^4
	blank	1.33	1.29	0.08	0.08	15.46	15.93	9.5×10^4	19.5×10^4
12.9 g oil/kg dry soil	Planting seed	2.12	1.63	0.08	0.06	25.54	27.12	9.0×10^4	17.5×10^4
	Seedling	2.16	1.23	0.08	0.07	26.34	17.32	10.0×10^4	$18/0\times10^4$
	blank	2.33	2.25	0.07	0.07	29.87	28.48	8.0×10^4	8.0×10^4
17.2 g oil/kg dry soil	Planting seed	3.18	2.46	0.09	0.06	34.37	37.27	8.5×10^4	12.0×10^4
	Seedling	3.24	2.13	0.07	0.08	41.54	25.06	9.0×10^4	13.5×10^4
	blank	3.49	3.38	0.07	0.07	44.74	48.29	7.0×10^4	6.0×10^4
27.25 g oil/kg/kg dry soil	Planting seed	4.24	3.26	0.07	0.06	54.36	50.15	5.0×10^4	4.1×10^4
	Seedling	4.32	3.23	0.08	0.07	51.42	41.41	6.5×10^4	5.0×10^4
	blank	4.66	4.50	0.08	0.07	54.82	56.97	4.0×10^4	2.1×10^4

Blank samples have the same conditions and crude oil concentrations without any vegetation.

Table 3: The Values of C,N, C/N and MPN for different concentrations of crude oil in soil with Loam texture for Puccinellia distance in the beginning and end of trials

Concentration	vegetation	C (%)		N (%)		C/N		MPN	
		Beg.	End	Beg.	End	Beg.	End	Beg.	End
2 g oil 4.3 g urea kg^{-1} soil	Planting seed	0.91	0.81	0.13	0.10	6.84	7.94	12.5×10^4	26.0×10^4
	Seedling	085	0.71	0.11	0.10	7.14	6.57	13.6×10^4	28.0×10^4
	blank	0.94	0.91	0.11	0.12	8.17	7.28	10.1×10^4	20.0×10^4
2gr oil 0.21 g ureakg^{-1}soil	Planting seed	0.95	0.80	0.84	0.06	11.31	12.50	11.5×10^4	26.5×10^4
	Seedling	0.98	0.68	0.08	0.07	11.67	8.95	12.8×10^4	29.0×10^4
	blank	0.87	0.90	0.08	0.07	10.24	11.39	10.1×10^4	20.5×10^4
4 g oil 0.21 g urea kg^{-1}soil	Planting seed	1.51	1.90	0.09	0.06	15.41	19.35	10.2×10^4	24.2×10^4
	Seedling	1.49	1.35	0.09	0.07	16.56	17.53	11.4×10^4	25.5×10^4
	blank	1.61	2.42	0.07	0.08	12.99	30.25	9.8×10^4	18.0×10^4
12.9 g oil/kg soil	Planting seed	2.16	1.68	0.09	0.06	23.48	24.35	9.7×10^4	22.0×10^4
	Seedling	2.32	1.31	0.08	0.07	26.67	16.79	11×10^4	24.0×10^4
	blank	2.01	2.05	0.09	0.08	20.51	25.63	8.5×10^4	17.0×10^4
17.2 g oil/kg soil	Planting seed	3.24	2.87	0.08	0.06	39.04	44.84	8.9×10^4	14.2×10^4
	seedling	3.48	2.52	0.09	0.07	35.51	32.31	9.5×10^4	15.1×10^4
	blank	3.02	3.08	0.07	0.09	38.23	32.42	7.2×10^4	10.1×10^4
27.25 g oil/kg soil	Planting seed	4.32	3.82	0.08	0.06	48.54	55.36	5.5×10^4	8.5×10^4
	Seedling	4.64	3.36	0.09	0.07	50.77	43.08	7.1×10^4	9.2×10^4
	blank	4.02	4.10	0.08	0.08	48.28	51.25	4.2×10^4	4.8×10^4

Blank sample with the same condition and crude oil concentrations but without any vegetation.

Table 4: The indices of C,N, C/N and MPN for different concentrations of crude oil in soil with sandy texture for Puccinellia distance in the beginning and the end of trials.

Concentration	vegetation	C (%)		N (%)		C/N		MPN	
		Beg.	End	Beg.	End	Beg.	End	Beg.	End
2g oil 4.3 g urea	Planting seed	0.87	0.61	0.12	0.09	7.13	6.16	13×10^4	26.5×10^4
	Seedling	0.91	0.78	0.10	0.11	7.00	7.09	14×10^4	29×10^4
	blank	0.92	0.86	0.15	0.18	7.36	7.96	10.5×10^4	21×10^4
2 g oil 0.21 g urea	Planting seed	0.95	0.63	0.08	0.05	11.31	11.05	11.8×10^4	27.2×10^4
	Seedling	0.91	0.53	0.08	0.07	11.23	7.46	13.2×10^4	30.4×10^4
	blank	0.89	0.80	0.09	0.07	9.08	10.26	10.5×10^4	20.6×10^4
4 g oil 0.21 g urea	Planting seed	1.55	1.01	0.081	0.06	19.14	16.56	10.4×10^4	25.1×10^4
	Seedling	1.49	0.94	0.08	0.07	17.13	12.21	12×10^4	26.4×10^4
	blank	1.66	1.58	0.09	0.08	18.44	19.75	10.1×10^4	18.2×10^4
12.9 g oil/kg	Planting seed	2.18	1.76	0.08	0.05	24.22	30.34	10×10^4	23×10^4
	Seedling	2.31	1.48	0.09	0.07	23.57	21.14	11.6×10^4	25×10^4
	blank	2.25	2.09	0.09	0.07	25.00	26.79	9.1×10^4	17.5×10^4
17.2 g oil/kg	Planting seed	3.27	2.64	0.08	0.06	38.93	43.27	9.3×10^4	15.1×10^4
	Seedling	3.47	2.32	0.07	0.08	44.49	27.95	10.1×10^4	16.2×10^4
	blank	3.38	3.13	0.09	0.07	35.58	44.79	8.2×10^4	11×10^4
27.25 g oil/kg	Planting seed	4.36	3.52	0.08	0.06	50.11	52.54	5.8×10^4	9.1×10^4
	Seedling	4.62	3.42	0.07	0.07	59.23	45.00	7.4×10^4	10.1×10^4
	blank	4.50	4.18	0.08	0.07	50.56	54.29	4.5×10^4	5.2×10^4

Blank sample with the same condition and crude oil concentrations but without any vegetation.

affected by *puccinellia distance* seedling. As tables 2 to 4 reveal organic carbon contents for seedling pot were less than planting seed and blank ones and in all cases MPN was greater for seedling treatments than others.

As table 5 indicates adding urea lead to decrease C, N and therefore C/N ratio due to microbial activities and consequently consumption of N and C. Seedling lead to decrease C, N and C/N ratio. At the first of trial, C and N had the greatest amount. Plant could affect on C , N and decreasing C/N ratio. This finding is in agreement with other recent findings ([18,19])

The prominent issue is that when C/N ratio comes up to constant value, it means that no amount of nitrogen is released or recovered (nitrogen recovery stopped). This ratio was named critical ratio of C to N. Critical ratio is dependant to some parameters like temperature, time, N % and organic materials in soil [20] .Proper range for C/N ratio is achieved between 15 to 33 [20].

As table 5 shows, Plant, crude oil concentration and time have significant effect on microbial population ($p<0.01$). This clearly shows that in the beginning of trials, microorganisms had less populations and with proceeding of trials MPN increased . Decreasing in N was resulted in microbial growth.

Radwan et al. [21] identified that the roots of several plants from the Kuwaiti desert and crop plants were densely associated with hydrocarbon-utilizing bacteria. The rhizosphere soils of all plants contained greater numbers of these hydrocarbon-utilizing bacteria in comparison with the bulk soils. Our finding accompanies with this.

Table 5: Analysis of variance of the indices of C, N, C/N and MPN in different soils and concentrations of crude oil for Puccinellia distance

Source of variations	Df[1]	C (%)	N (%)	C/N	MPN
Soil	2	70.03^{ns}	0.006^{ns}	4.34^{ns}	6840620370^{ns}
Concentration	5	55.05^{ns}	0.004^{ns}	5198.40**	31446987037^{ns}
Plant	2	55.66^{ns}	0.002^{ns}	120.20**	11630398148^{ns}
Soil×Concentration	10	65.45^{ns}	0.005^{ns}	10.76^{ns}	2306264814^{ns}
Soil×Plant	4	66.95^{ns}	$0{,}005^{ns}$	20.89^{ns}	1840217592^{ns}
Concentration ×Plant	10	65.92^{ns}	0.005^{ns}	7.50^{ns}	2618275925^{ns}
Time	1	101.53^{ns}	0.020*	17.21^{ns}	251430750000**
Soil×Time	2	59.17^{ns}	0.005^{ns}	26.43^{ns}	4272527777^{ns}
Concentration×Time	5	62.59^{ns}	0.005^{ns}	4.88^{ns}	7470394444**
Plant×Time	2	80.89^{ns}	0.009^{ns}	215.57^{**}	1255194444^{ns}
Soil×Concentration ×Time	10	66.08^{ns}	0.005^{ns}	8.80^{ns}	2202105555^{ns}
Soil×Plant×Time	4	67.17^{ns}	0.005^{ns}	1.69^{ns}	2053930555^{ns}
Concentration×Plant ×Time	10	64.24^{ns}	0.005^{ns}	16.49^{ns}	2689538888^{ns}

*Ns) Not significant, *) Significant ($p<0.05$), **) Significant ($p<0.01$).*

Table 6: Least square differences of the indices of C, N. C/N and MPN. in different soils for Puccinellia distance.

Soil	C (%)	N (%)	C/N	MPN
Sandy loam	2.022^{a}	0.08448^{a}	23.94^{b}	149472^{ab}
Loam	1.967^{b}	0.10858^{b}	18.12^{a}	155389^{a}
Clay loam	2.409^{a}	0.08519^{ab}	28.28^{ab}	129111^{b}

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

As it is observed in table 6, microbial population in clay loam texture is less than other textures. Root exudates provide sufficient carbon and energy to support large numbers of microbes. Due to these exudates, microbial populations and their activities are greater in the rhizosphere than in the bulk soil , i. e., soil not in contact with plant roots ([22-25]). Our finding accompanies with this.

Initial sensitivity studies on the rhizosphere volume and root turnover rate, suggest that the establishment of a stable root mass, is more important than inducing rapid growth rate and senescence of the root system. As table 6 shows, soil with clay loam texture had greater C/N ratios compared to the other textures because clay particles can capture organic materials. Nitrogen content of sandy texture can be easily washed by water and cause C/N to be increased [20] .Due to good ventilation and OC as nutrient, in loam and sandy loam textures, growing was developed and population increased slowly. table 6 also shows that loam texture had better performance on microbial population growth and C/N ratio decreasing.

It is evident from table 7 that different concentrations of Urea (main source of N) affect the rate of degradation and it does not indicate significant effect ($p<0.01$) due to time×soil and time × concentration. This means that plants are responsible for organic carbon decreasing in soil (table 5). This concept accompanies with Durmishidze finding [26] who summarized various studies about direct role of plants in degradation of organic matters. It was significant effect ($p<0.01$) due to time and plant on C/N ratio and microbial population. The results indicate that the nitrogen was not a limiting factor as C/N ratio remained in the range of 24:1 to 16:1 (table 7). It is resulted from table 7 that, as C/N ratio increases

Table7: Least Square differences of the values of C, N, C/N and MPN in different crude oil concentrations.

Components	C (%)	N (%)	C/N	MPN
2 (gr oil)+4.3(mg urea)	5.489^{a}	0.116^{b}	6.979^{c}	183167^{a}
2 (gr oil)+0.21(mg urea)	0.842^{b}	0.120^{b}	10.810^{b}	185333^{a}
4 (gr oil)+0.21(mg urea)	1.426^{a}	0.080^{ab}	16.881^{b}	165500^{ab}
12.9(gr oil)+0(mg urea)	1.962^{a}	0.080^{a}	24.620^{ab}	143833^{b}
17.2(gr oil +0(mg urea)	3.017^{a}	0.079^{a}	38.257^{a}	106056^{c}
27.25(gr oil)+0(mg urea)	4.059^{a}	0.800^{a}	51.008^{ab}	84056^{c}

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

Table 8: Comparison of Least Square differences of indices C,N,C/N and MPN for Puccinellia distance vegetation.

Treatment	C	N	C/N	MPN
Planting seed	2.197^{a}	0.08833^{a}	24.8726^{a}	146833^{a}
Seedling	1.972^{c}	0.08717^{a}	22.6220^{a}	161444^{a}
Blank	4.229^{b}	0.10275^{a}	41.1581^{b}	125694^{b}

from 16 to 24, there is no significant difference ($p<0.01$) between microbial populations. The microbial population in seedling pots clearly was more than seed planting table 7 clearly shows effects of phytotoxicity of crude oil, so that after 90 days of trials, pots containing more than 12.9 g oil per kg of soil lead to decrease number of microbes. Table 7, also indicates that in treatments which contain more than 12.9 g crude oil, C/N ratio increased continuously due to reduction in microorganism activities. As it is revealed in table 7 by decreasing microbial population, which is consequent of toxicity of crude oil ingredients, C/N ratios raised up.

Table 8 shows the effects of root. The development of seedling roots is responsible for decreasing N as well as for C content of soil. Planting seed can not provide stable root system to support microbial population, because of low root density table 8 shows microbial growth and its effects on OC. As it is shown in this table there is significant difference between both seedling and seed planting and control pots (blank).

C/N ratio can explain changes which occur in mineralization and consumption cycle of both C and N in bulk soil by microorganisms. If C exceeds than its normal level, microbial activity will consume it by mineralized nitrogen and therefore amount of carbon will decrease. Inversely, if nitrogen exceeds, microbial activities will release it as mineralized nitrogen. Relative availability of nitrogen and carbon of added organic material to soil, can be demonstrated by C/N ratio. Advantage of this ratio is its ability to illustrate situation of both element in one time [20] .The concern at low C/N ratios is the loss of ammonia (NH_3) [27] ,but a higher levels of slow rates of decomposition can be anticipated [28] .

In this manner, phytoremediation is similar to composting which is mass of interdependent biological processes carried out by a myriad of microorganisms essential for the decomposition of organic matter. In composting like soil, microorganism leads to make an equilibrium between C and N amounts. Most systems are aerobic- meaning the microorganisms require oxygen (O_2).

Intensity of denitrification (release of nitrogen in form of ammonia) is related to C/N ratio. From this ratio we can predict amount of nitrogen released or consumed. If C/N ratio would be less than 10, then nitrogen released. If C/N ratio would be more than 20, then nitrogen is consumed by microorganisms and therefore degradation and berthing (CO_2 release) decreased C/N ratio [29] .

As table 8 indicates, seedling treatments have the least C and C/N ratio and greater MPN which accompanies with other findings as mentioned. As table 9 indicates, organic carbon in the end of trial reduced which can be explained by microbial activities and crude oil ingredient toxicity is responsible for decreasing in MPN in the end of trial in pots containing more than 12.9 g oil / kg dry soil.

The presence of clay particles in soil provides the most significant surface area and such rigid matrix, which

Table 9: Comparison of pollution of indices C,N,C/N and MPN in different times

Treatment	C	N	C/N	MPN
Beginning	3.769[a]	0.10643[a]	35.41[a]	196407[a]
End	1.830[b]	0.07908[b]	23.14[b]	192907[b]

It is significant differences ($p<0.05$) between MPN in the beginning and the end of trials.

OC may be adsorbed ,thus this two mechanisms is responsible for keeping number of microbes higher in loam and sandy in comparison with clay soils, briefly, micro flora bioavailability restriction is responsible for this events [30] (*Brady* and *Weil*,1996). *Carmichael et al.* [31] findings suggested that sandy soils have more ability for mineralization of PAHs. Our findings accompany with this.

Good ventilation and microbial growing in loam and sandy texture lead to apparently small C and therefore small C/N ratio as a result of microbial activity. *Puccinellia distance* had better performance in loam than sandy texture, therefore rhizosphere effect is more clear in that. This also can be observed from apparently greater MPN in loam texture. In clay texture, poor bioavailability, may lead to decrease in microbial population.

CONCLUSIONS

In a greenhouse study, phytoremediation by using *Puccinellia distance* was found to be a feasible method for improving the treatment of petroleum-contaminated soils. However some limitations due to phytotoxicity and damage of microbial population was observed. Our findings show that in level of 12.9 g crude oil per kg of dry soil (1.29 %) we have critical point and for successful phytoremediation operation pollution must be below this amount. Microbial activitiy in loam texture was shown more effective and therefore has better efficiency. Comparison between seedling and planting seed pots clearly shows that the seedling ones have greater root surface area and therefore have better performance on rhizosphere effect.

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