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Remediation of Petroleum-Contaminated Soils using Stipagrostis plumosa, *Calotropis procera* L., and Medicago sativa under Different Organic Amendment Treatments

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Authors
Jahantab E.[1] *PhD*,
Jafari M.[1] *PhD*,
Motesharezadeh B.* *PhD*,
Tavili A.[1] *PhD*,
Zargham N.[2] *PhD*

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ABSTRACT

Aims The contamination of soils and groundwater by toxic, hazardous organic pollutants is a widespread environmental problem. The use of vegetation for the treatment of contaminated soils is an attractive and cost-effective alternative, especially for petroleum-contaminated soils.
Materials & Methods Three species including *Calotropis procera* L., *Stipagrostis plumosa*, and *Medicago sativa* were selected. To evaluate the abilities of S. *plumosa, M. sativa*, and *C. procera* in the degradation of petroleum hydrocarbons, a greenhouse study was conducted with two trial factors: (1) Urban waste compost and (2) biochar (each 0, 1, and 2%). At the end of the experiment, aerial and underground parts of the plants were collected, and some important soil properties and plant morphological characteristics were measured. The total amount of hydrocarbons was measured by gas chromatography, Flame Ionization type, Agilent 7890A model.
Findings The results showed that the strongest hydrocarbon reduction by *C. procera, S. plumosa*, and *M. sativa* was 62.5%, 57.3%, and 53.5%, respectively. The results also demonstrated that control/biochar 2% had the highest/lowest (21922/14511 mg/kg) hydrocarbon level left in the soil. Therefore, biochar 1% or 2% is the best treatment for the remediation of petroleum-contaminated soils. *C. procera* L. is a good potential candidate to be cultivated for the phytoremediation of petroleum-contaminated soils.
Conclusion Overall, using the amendment seedbed including biochar and urban waste compost treatments is suitable to promote phytoremediation of petroleum hydrocarbons. Biochar and urban waste compost provide optimal conditions for plant growth and at least help to promote the process phytoremediation. Regarding plant species diversity in Iran and petroleum contamination, application of phytoremediation may apply with effective and applied solution in soils contaminated.

Keywords Biochar; Phytoremediation; Total Petroleum Hydrocarbons; Urban Waste Compost

*Department of Soil Science, Faculty of Agricultural Engineering & Technology, University of Tehran, Karaj, Iran
[1]Department of Reclamation of Arid and Mountainous Regions, Faculty of Natural Resources, University of Tehran, Karaj, Iran
[2]Department of Forestry and Forest Economics, Faculty of Natural Resources, University of Tehran, Karaj, Iran

Correspondence
Address: Faculty of Agricultural Engineering & Technology, University of Tehran, Karaj, Iran.
Phone: +98 912 202 2388
Fax: -
moteshare@ut.ac.ir

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Introduction

One of the widespread ecological problems is the pollution of soil and groundwater with toxic and hazardous organic containments. As the increasing of the petroleum hydrocarbon becomes a global problem, petroleum hydrocarbon contamination is increasingly becoming a universal problem, with spills reported across every ecosystem, containing the sparsely populated high latitude polar regions. The hazardous properties of petroleum such as high persistent in the ecosystem and its toxicity result in significant health risks to organisms subsequent to its entrance in the food chain. [1, 2] One of the main affecting parameters on the petroleum contamination problem is the continuous growth of oil extraction and associated industries.[3] Hence, the exploration to find novel methods for the remediation of such additives has got substantial interest, recently.[4]

The composition of the petroleum mixture contains thousands of hydrocarbon and non-hydrocarbon compounds, including heavy metals with potentially carcinogenic and mutagenic properties. Phytoremediation has been shown to have an impact on the degradation or removal of petroleum contaminants. However, the selection of plant species for phytoremediation is still a challenging task.[5]

Soils polluted with total petroleum hydrocarbons (TPHs) are great risk to human health and the ecologies. Phytoremediation is a powerful and cost effective alternative method to reduce pollution of soils. [6]

The production method of biochar is the charring or pyrolyzing (thermal degradation) of feedstock biomass under oxygen-limited conditions. Biochar can improve soil fertility and may also be an option for enhancing soil C stocks and reducing the emissions of greenhouse gas when combined with the agriculture soil.[7] Recently, the use of biochar has been studied for *in situ* remediation of contaminated grounds in association with plants.[8] Organic amendments including composts have long been investigated for their efficiency in improving soil characteristics such as soil structure, aggregate stability, hydraulic conductivity, and other biological and chemical properties.[9] Several researchers introduced some plant species as resistant to petroleum hydrocarbons that can survive in petroleum-polluted soils.[10-17]

The main objective of this study is to compare and evaluate the effects of organic amendments (biochar urban waste compost and urban waste compost) and three Species including *Medicago sativa*, *Stipagrostis plumosa,* and *Calotropis procera* on the remediation possibility of contaminated soils with TPHs.

Materials and Methods

Characteristics of soil

The soil used in this experiment was collected from Pazanan oil refinery in Gachsaran in the south Iran. The soil was transferred to the greenhouse of the University of Tehran, College of Agriculture and Natural Resources (UTCAN). Selected chemical and physical properties of soil are presented in Table 1.

Characteristics of urban waste compost and biochar urban waste compost

To apply treatments, urban waste compost and biochar urban waste compost were added to the soil samples at three levels including 0, 1, and 2% by weight. Before the seeds were cultivated, the compost and biochar treatments were added to the soil. The properties of urban waste compost and biochar urban waste compost are shown in Table 2.

Table 1: Properties of soil in pots

Properties	Value
TPH (mg/kg)	40120
pH	7.05
EC (dS/m)	2.1
OC (%)	2.86
Total *N* (%)	0.16
K (mg/kg)	125.35
P (exchangeable) (mg/kg)	36.4
Clay	8.15
Silt	31.6
Sand	60.25

Planting of species

Species *M. sativa*, *S. plumosa,* and *C. procera* were selected for greenhouse cultivation. Seeds were planted into pots (10 seeds per pot for *C. procera* and 30 seeds per pot for *M. sativa* and *S. plumosa*). Greenhouse cultivation was carried out to assess the capability of *S. plumosa*, *C. procera,* and *M. sativa* under different treatments: Urban waste compost and biochar urban waste compost for phytoremediation of petroleum hydrocarbons. Pot cultivation was conducted in the greenhouse of UTCAN. The greenhouse was standard and its temperature was at day 5°C ± 25°C and at night ±17°C. 6 months later, the plants were collected in the pots, and the soil was shaken off the roots and mixed. Soil samples (200 g) were taken from each pot for further analyses.

Soil sampling

Soil samples (per pot) were analyzed to determine the soil properties and to define soil characteristics such as TPH, electrical conductivity (EC), pH, organic matter (OC), soil texture, phosphorus (P), total nitrogen (N), potassium (K), and cation exchange capacity (CEC) (Jafari Haghighi, 2003).[18]

TPHs

The TPH in soil was determined according to USA EPA 3550C. First, the soil was threshed in mortar; 1 g samples were next taken and immersed in 10 ml of dichloromethane + acetone solution in a centrifuge tube; tubes were then shaken for 4 min and were finally spun at a velocity of 3000 rpm for 5 min to deposit the sediments. After centrifuging, 1 ml of upper liquid was taken and used to measure the amount of hydrocarbon compounds.[19,20] The total amount of hydrocarbons was measured using the set of gaseous chromatography, the type of flame ionization detector (Agilent 7890A model).

Statistical analysis

One-way ANOVA followed by a Duncan's multiple range test was used to compare different levels of treatments ($P < 0.05$). Greenhouse cultivation was in a factorial experiment based on completely randomized design with five replications. The experimental factors included cultivation substrate, urban waste compost, and biochar urban waste compost in three levels (0, 1, and 2%). Data were analyzed using software's SPSS, MSTATC, and Excel.

Findings

Effect of application of treatments on the TPHs

The results showed that the interaction between plant species and treatments (urban waste compost and biochar urban waste compost) was significant at the 5% level on the TPHs in soil.

The results of ANOVA showed that the highest amount of TPHs remained in the soils of *M. sativa, C. procera,* and *S. plumosa* and control treatment, and the soils of *C. procera*

Table 2: Properties of urban waste compost

Urban waste compost	Rate	Biochar	Rate
Total carbon (%)	16.77	C (%)	10.81
P (%)	0.35	N (%)	0.75
K (%)	0.63	H (%)	0.19
Fe (mg/kg)	10667	Bulk density (g cm^{-3})	0.92
Mn (mg/kg)	336	Percentage yield	72
Zn (mg/kg)	174	pH	9
Ca (%)	5.53	EC (1:5) (dS/m)	8.25
Ma (%)	1.40		
EC (dS/cm)	3.66		
pH	6.89		
Moisture (%)	9.5		

and biochar 1 and 2% had the lowest TPH between the species and treatments, respectively (Table 3 and Figure 1).

Effect of treatments on the soil properties

The results indicated that the effect of treatments on soil pH and EC was significant (Table 4). Biochar and urban waste compost treatments increased soil pH and EC compared to control treatment. The interaction effect between species and treatments (biochar and urban waste compost) on soil pH and EC was significant. Mean comparison showed that 2% biochar and control treatment had the highest and lowest effects on pH and EC, respectively. In general, treatments (biochar and urban waste compost) increased the soil pH and EC compared to the control treatment (Figure 2). There was no significant interaction effect on soil nitrogen content (Table 4). The effect of treatments on soil nitrogen content was significant (Table 4). Mean comparisons indicated that 1 and 2% biochar-treated soils had the highest and lowest amount of nitrogen content compared to control (Figure 2). The interaction between species and treatment (biochar and urban waste compost) on the amount of soil potassium was significant (Table 4). Multiple comparisons showed that biochar 2% and control groups had the highest and the lowest amounts of potassium, respectively (Figure 2). The interaction effect between species and treatment on the amount of soil phosphorus was significant (Table 4). The effect of treatments on the amount of phosphorus was significant. Mean comparisons showed that biochar 2% and control treatment had the highest and lowest amount of phosphorus, respectively (Figure 2).

The interaction effect between species and treatment on the amount of soil organic carbon was significant (Table 4). The effect of treatments on the amount of organic carbon was significant. Mean comparisons showed that 2% compost-treated soil had the highest percentage of organic carbon and control treatment had the lowest percentage of soil organic carbon (Figure 2). The interaction effect between species and the treatment on the CEC was not significant (Table 4). The effect of treatments on the CEC was significant. Mean comparison indicated that biochar 2% treatment was the highest CEC and the control treatment had the lowest CEC (Figure 2).

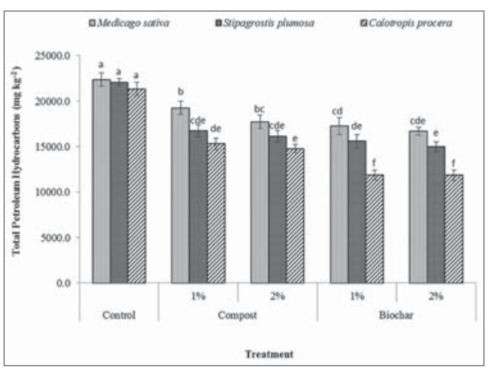

Figure 1: Mean comparison the effect of interaction species and treatment on the amount of total petroleum hydrocarbons

Discussion

The results show that *C. procera*, *S. plumosa,* and *M. sativa* were resistant to oil pollution.

Table 3: ANOVA table of F values for the effects of species and treatments and their interaction on total petroleum hydrocarbons

	Source	df	Mean square
Total petroleum hydrocarbons	Species	2	82768939.7**
	Treatment	4	132729217.1**
	Species* Treatment	8	4340552.7*
	error	60	2041283.7
	Total	74	
	CV (%)	8.43	

*,***P*<0.05, *P*<.01, NS: Not significant

Table 4: ANOVA for the effects of species and treatments and their interaction on soil pH, EC, N, K, P, OC, and CEC

Soil Factors	Source	df	Mean square
	Species	2	0.087**
	Treatment	4	0.474**
	Species*Treatment	8	0.019*
	Error	60	0.003
pH	Total	74	
	CV (%)	0.72	
	Species	2	0.241**
	Treatment	4	0.206**
	Species*Treatment	8	0.014**
	Error	60	0.006
EC	Total	74	
	CV (%)	2.92	
	Species	2	0.019**
	Treatment	4	0.012**
	Species*Treatment	8	0.0006^{ns}
	Error	60	0.001
N	Total	74	
	CV (%)	10.12	
	Species	2	24580.05**
	Treatment	4	4530.20**
	Species*Treatment	8	683.37**
	Error	60	155.94
K	Total	74	
	CV (%)	6.14	
	Species	2	32.06^{ns}
	Treatment	4	227.65**
	Species*Treatment	8	10.38^{ns}
	Error	60	19.85
P	Total	74	
	CV (%)	13.7	
	Species	2	0.253**
	Treatment	4	0.795**
	Species*Treatment	8	0.028**
	Error	60	0.004
OC	Total	74	
	CV (%)	1.83	
	Species	2	7.79**
	Treatment	4	32.11**
	Species*Treatment	8	0.619^{ns}
CEC	Error	60	0.688
	Total	74	
	CV (%)	4.96	

*,***P*<0.05, *P*<0.01, NS: Not significant, EC: Electrical conductivity, N: Nitrogen, K: Potassium, P: Phosphorus, OC: Organic carbon, CEC: Cation exchange capacity

The ability of their plant species to grow in these conditions suggested that these plants are, probably, useful for the phytoremediation of soils polluted with hydrocarbons. It has been proposed that crude oil's indirect effect on the plants is confined to a more or less marked reduction in growth and biomass. In other related studies, the introduced plant species that resist to oil pollution can be referred to *Trifolium repens* and *Melilotus officinalis*[21] and *Polygonum aviculare*.[17] Bramley *et al.* suggested that *Poa foliosa* is a valuable option for refining hydrocarbons in Macquarie Island.[22]

Mean comparison showed that the highest amount of TPH remained in the soil was related to the *M. sativa* and the highest amount was related to *C. procera*. The results suggest that *C. procera* plant has a better impact in reducing TPHs in soil than other plant species.

The results indicated that the effect of treatments and plant species on petroleum hydrocarbons in soil was significant; urban waste compost, biochar urban waste compost, and plant species reduced the TPH in soil; the results of this research is consistent with Doni *et al.*[23] and Muratova *et al.*[24]

The microorganism activity in the soil is probably the main mechanism of urban waste compost and biochar urban waste compost in removing pollutants of soil. Therefore, organic amendment such as urban waste compost and biochar urban waste compost provides the desired condition for plant growth.

Using the compost is an efficient way to increase bioremediation in oil-contaminated

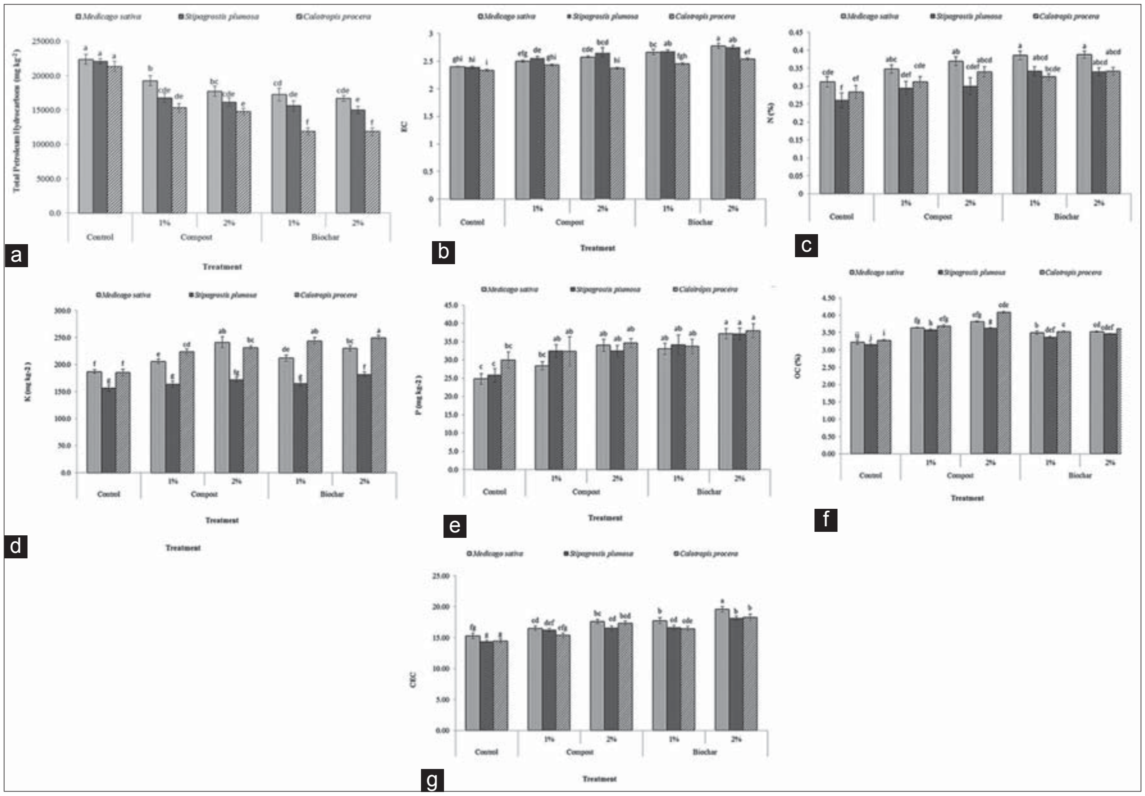

Figure 2: Effect of treatments on (a) soil pH, (b) electrical conductivity, (c) soil nitrogen, (d) soil potassium, (e) soil phosphorus, (f) soil organic carbon, and (g) cation exchange capacity

soils, and this method has advantages such as rich and fertile soil after the refining process. The results showed that fertilizer treatments (biochar and compost) reduced the amount of TPHs in the soil. The results were consistent with the studies accomplished by Qin *et al.,*[25] Wang *et al.,*[26] Feng *et al.,*[27] Ayotamuno *et al.,*[28] Hickman and Reid,[29] and Stewart *et al.*[30]

In this regard, Stewart *et al.*[30] said that soil modifiers such as biochar improve the decomposition of hydrocarbons, refining, and reclamation soils contaminated with hydrocarbons. Jahantab *et al.* suggested that biochar 2% treatment demonstrated the highest effect on promoting the phytoremediation of *Stipagrostis plumosa* of Ni.[31]

Interaction effects of the roots of plants, microorganisms, and compost and biochar, in rhizosphere soils, could be facilitated as a result of improved bioavailability and reduced its petroleum hydrocarbons. In this respect, the results of Wang *et al.* showed that application of compost increased decomposition of pyrene significantly.[26]

Plants are able to discharge through the releasing of nutrients and oxygen to the root zone in the soil, cause irritation, and increase the activity of the microbial population of degradation petroleum. Interaction between the soil and plant roots is a critical success factor in the tolerance of plant species and removal of the soil pollution.

Biochar and urban waste compost treatments increase both soil pH and EC compared to the control treatment. The results indicated that biochar 2% has the highest amounts of N, K, and P among all treatments. Mean comparison showed that 2% urban waste compost treatment was the highest percentage of organic carbon and control treatment was the lowest percentage of soil organic carbon.

Organic amendments including composts have long been studied for their effectiveness in improving soil properties such as soil structure, aggregate stability, hydraulic conductivity, and other chemical and biological properties.[2]

It seems that urban waste compost and biochar with nutrient elements such as N, P, and K have significant effects on the physical and chemical properties of soil. These also improve the soil structure and increase the permeability and water-holding capacity in soil.

The results are consistent with the findings of various researchers, including Singer *et al.,*[32] Uzoma *et al.,*[33] and Fellet *et al.*[34] Singer *et al.* in their researches mentioned to increase of pH, EC, and CEC in addition of compost.[32]

Results showed that increases in biochar and compost will increase soil fertility and plant biomass compared to the control treatment. Furthermore, the effect of compost on the soil surface is not only effective on soil fertility but also prevents the formation of crust on the soil surface to prevent water loss through evaporation.[35,36] Some researchers have been mentioned on the positive effects of compost on soil physical and chemical properties. In addition, different researchers[32] in their works have been mentioned to increase in pH, EC, and CEC of the soil by adding compost.

Conclusion

The amount of TPHs in soil residual is the highest in the species *M. sativa*, *C. procera,* and *S. plumosa* and control treatment but it is the lowest amount in plant *C. procera* and treatment residual biochar 1 and 2%.

The results of the treatments on the amount of TPHs in soil showed that the highest amount of residual hydrocarbons belongs to the control treatment and the minimum residual hydrocarbons belongs to the treatment of biochar 1 and 2%.

Biochar and urban waste compost treatments increase both soil pH and EC compared to the control treatment. The results indicated that biochar 2% has the highest amounts of N, K, and P among all treatments. Mean comparison showed that 2% urban waste compost treatment was the highest percentage of organic carbon and control treatment was the lowest percentage of soil organic carbon.

The treatments used in this study increased the stem length, root length, root volume, root dry weight, shoot dry weight, and total dry weight compared to the control treatment. Generally speaking, the treatment biochar 2% was the highest root length, stem length, root volume, root dry weight, and shoot dry weight and total dry weight.

As petroleum-contaminated regions are numerous and different all over the world as well as Iran, for phytoremediation purposes, cultivation of tolerable and local plant species is necessary. In general, plants are able to absorb different pollutants including petroleum ones from the environment. Besides, plants' role in avoiding pollutant transmission to different places through wind or water is noticeable.

Overall, using the amendment seedbed including biochar and urban waste compost treatments is suitable to promote phytoremediation of petroleum hydrocarbons. Biochar and urban waste compost provide optimal conditions for plant growth and at least help to promote the process phytoremediation. Regarding plant species diversity in Iran and petroleum contamination, application of phytoremediation may apply with effective and applied solution in soils contaminated.

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Ethical Permissions

None declared by authors.

Conflicts of Interest

The authors state that there are no conflicts of interest.

Authors' Contributions

Esfandiar Jahantab designed the work. Mohammad Jafari designed the work. Babak Motesharezadeh designed the work. Ali Tavili helped in experimental study. Nosratollah Zargham helped in experimental study.

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