Int. J. Hum. Capital Urban Manage., 2(2): 155-162, Spring 2017
DOI: 10.22034/ijhcum.2017.02.02.007

ORIGINAL RESEARCH PAPER

Removal of total petroleum hydrocarbons from polluted urban soils of the outskirts of Ahvaz, southwestern Iran

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Receive Date 23 December 2016; Revise Date 8 February 2017; Accept Date 24 March 2017; Publish Date 1 April 2017

ABSTRACT: Earlier phases of economic expansion and urban development have resulted in significant sources of urban soil contamination. Petroleum hydrocarbons are one of the most common groups of persistent organic contaminants in the environment. In this study, two types of treatment in 3 concentrations were prepared that were included plant treated by 1% oil pollution, treatment by 1% contamination without plant (as control), plant treated by 5% oil pollution, the 5% pollution treatment without plant (control), 10% oil pollution treatment with plant and 10% treatment without plant (control) that 3 replicates were prepared for each treatment. The obtained extracts were concentrated to 1 mL under a gentle stream of nitrogen gas, and then 2 ìg of the sample was injected into a UNICAM 610 series gas chromatograph equipped with a flame ionization detector. Primary Total petroleum hydrocarbons amount in 1%, 5% and 10% concentration was respectively: 9027.40 mg/kg, 49599.03 mg/kg and 99548.28 mg/kg. After 4 months its amount in different concentration with plant was 126.43 mg/kg, 4463.92 mg/kg and 19611.50 mg/kg. The best total petroleum hydrocarbons removal efficiency was observed in all concentration at 120th day. The results of this study showed that *vetiver* can remove petroleum hydrocarbons from contaminated soils effective.

KEYWORDS: *Ahvaz city; Flame ionization detector (FID); Petroleum hydrocarbons; Removal; Soil; Total petroleum hydrocarbons (TPH)*

INTRODUCTION

The urban environmental quality is of basic importance because the majority of people now live in cities. Urban soils are therefore an important indicator of human exposure to pollution such as total petroleum hydrocarbons and heavy metal in the urban terrestrial environment (Nriagu and Pacyna, 1988). Urban soils can vary widely over short distances, compared to naturally developed and anthropogenically influenced soils. Most urban soils are relatively young, resulting from soil exchange and mixture due to construction activities (Norra *et al.*, 2001; Janadeleh and Kardani, 2016). Petroleum hydrocarbons are one of the most common groups of persistent organic contaminants in the environment (Collins, 2007; Cook and Hesterberg, 2013; El-Sheekh and Hamouda, 2014; Janadeleh and Jahangiri, 2016). Petroleum hydrocarbon contamination of soil occurs through extraction, accidents, transportation, leakage from tanks, pipeline ruptures, consumption and refining (Soleimani *et al.*, 2010; Kokyo *et al.*, 2013). Large-scale contamination of soil and sediments with petroleum hydrocarbons is a serious problem of global scale, primarily in countries that

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produce, transport and refine oil (Muratova *et al.*, 2008; Basumatary *et al.*, 2012).Over the past centuries, several factors, such as rapid growth of population, modern agricultural activities, waste disposal, mining, and industrialization, have significantly contributed to extensive soil contamination (Eapen and D'souza, 2005; Singh and Jain, 2003). Soil is the foundation of terrestrial ecosystems and a nonrenewable fundamental agricultural resource, inextricably linked to productivity, land development and environmental quality Interest in phytoremediation as a method to solve chemical contamination has been growing rapidly in recent years (Clemens *et al.*, 2002; Dhankher *et al.*, 2002; Uera *et al.*, 2007). Phytoremediation is the process of applying plants for removing contaminants from the soil, surface and ground waters, which has been developed during the past decade (Baneshi *et al.*, 2014; Moreira *et al.*, 2013; Patil and Jadhav, 2013). It is believed that the first step towards phytoremediation is to find an appropriate set of plants which enable them to grow on contaminated sites, although optimizing agronomic practice should also be considered. In contrast, phytoremediation is considered to be a less environmentally destructive, more aesthetically pleasing and solar powered treatment system. Besides, it is more cost-effective than most of the alternatives (Cunningham *et al.*, 1996; Brandt *et al.*, 2006; Janadeleh *et al.*, 2017). This method is referred to as plant-assisted remediation or phytoremediation, and it also has the benefit of contributing to site restoration when remedial action is ongoing. C. *zizanioides* (L.) Roberty, commonly known as *vetiver*, is a fast-growing perennial tussock grass member of the family Poaceae; it is considered sterile outside its natural habitat. *Vetiver* is grown worldwide for perfumery, agriculture, and bioengineering where it is used for soil and water conservation (Barbera *et al.*, 2014).*Vetiveria zizanoides*is native from native from Indian subcontinent and has the potential to meet all the criteria required for phytoremediation (Abaga, *et al.*., 2014; Danh *et al.*, 2009). *Vetiver* showed promise in removing various environmental contaminants from both aqueous media and soil (Danh *et al.*, 2009; Datta *et al.*, 2011; 2013; Janadeleh *et al.*, 2016). Many plant species are sensitive to petroleum contaminants (Chaineau, *et al.*, 1997; Huang *et al.*, 2004). "According to Merkl *et al.* (2004) a growth rate reduction of beans and wheat by more than 80%. Significant reduction of plant biomass by the presence of petroleum hydrocarbons has also been reported. Ahwaz is located in the southwest of Iran, and the capital of Khuzestan province. Ahvaz has a desert climate with long, very hot summers and mild, short winters. Ahvaz is consistently one of the hottest cities on the planet during the summer, with summer temperatures regularly at least 45 degrees Celsius, sometimes exceeding 50 degrees Celsius, with many sandstorms and duststorms common during the summer period. However, in winters, the minimum temperature can fall to around +5 degrees Celsius. Winters in Ahvaz have no snow. The average annual rainfall is around 230 mm. This study has been carried out around Ahvaz City of Iran in 2015.

MATERIALS AND METHODS

Method of artificially soil contamination

In present study the Soil samples were collected from oil field around Ahvaz city. Then in order to infect it by 1%, 5% and 10% concentration of soil contamination, soil was flat in a thin layer. Then the crude oil was dissolved in acetone in ratio of 1 to 3 and was added to soil in the form of spray. 30 cm Pot was filled by contaminated soil with various concentrations of contaminant. Soil of each pot was weighed 7Kg and was ready for planting. In this study, two types of treatment in 3 concentrations were prepared that were included plant treated by 1% oil pollution, treatment by 1% contamination without plant (as control), plant treated by 5% oil pollution, the 5% pollution treatment without plant (control), 10% oil pollution treatment with plant and 10% treatment without plant (control) that 3 replicates were prepared for each treatment.

Sampling and sample digestion

This study was evaluated in 5 period times (15, 30, 60, 90, 120 days). Some soil samples from each pot were taken for the analysis, and keep in the zippers bags and then were transferred to a laboratory for analysis.Some physical and chemical properties of the clean soil as well as contaminated soil used in this study are presented in Table 1 . Phosphorus was measured by Olsen P extracting solution (0.5 M $NaHCO_3$, pH 8.5); total nitrogen was measured by Kjeldahl digestion; EC was measured by a conductivity meter in a soil-water extract (1:5 soil: water ratio), and pH was analyzed by a glass electrode using a 1:5 soil: water ratio (Dewis and Freitas, 1970; Testing and Materials, 2000). Total Volatile Solids was measured by Using of Gravimetric according to method 1684 EPA (Telliard, 2001).

Table **1:** Physical and chemical characteristics of the clean soil used in the current study

Parameter	Value	Measurement method
pH	7.27	1:5 soil/water slurry
Electrical Conductivity(ds/m)	8.50	1:5 soil/water slurry
Total N	0.087	Kjeldahl
Phosphorus (mg/kg)	3.62	Olsen
Clay	9.00	Hydrometer measurement
Sand	80.20	Hydrometer measurement
Silt	10.80	Hydrometer measurement
Inorganic matter	92.44	Gravimetric
Organic matter	7.60	Gravimetric

TPH analysis

For TPH analysis, soil samples were air dried and stored at 4 °C prior to extraction and analysis. Ultrasonic extraction was performed using dichloromethane solvent. Dichloromethane (10 mg) was added to about 5 g of contaminated soil and then it was placed in an ultrasonic water bath for 3 min at room temperature (Walters *et al*., 1989).The obtained extracts were concentrated to 1 mL under a gentle stream of nitrogen gas, and then 2 ìg of the sample was injected into a UNICAM 610 series gas chromatograph equipped with a flame ionization detector (FID). The column used for analysis was DB-5 with 30 m length, 0.25 mm internal diameter, and 0.2ìmthickness of film. The injector and FID detector temperatures were adjusted at 280 °C and 340°C, respectively. Initial column temperature was adjusted at 50 °C for 5 min, and then increased to 250°C with 10 °C min-1 slope and remained at 250 °C for 40 min.

HPC analysis

The heterotrophic plate count (HPC), formerly known as the standard plate count, is a procedure for estimating the number of live heterotrophic bacteria in water. This test can provide useful information about water quality and supporting data on the significance of coliform test results. High concentrations of the general bacterial population may hinder the recovery of coliforms.

The HPC is useful in judging the efficiency of various treatment processes for both drinking water and swimming pools, and for checking the quality of finished water in a distribution system. 8241 Pour Plate, Heterotrophic Bacteria Plate Count Agar, Count CFU/ Ml (HACH Company, 2012)

Statistical analysis

In this study the significance of differences observed among the mean values was tested by one-way ANOVA. Significance level was considered at P = 0.05. All statistical analyses were performed using SPSS 17.

RESULTS AND DISCUSSION

Physical and Chemical analysis of both soil contaminated by petroleum hydrocarbons and untamed some physical and chemical properties of the soil were measured by standard methods (Table 1). According to the results of present study the texture of soil had 50% porosity and sandy loam, which its sand percentage was reported 80.2%. Results of TPH amount in soil without plant and with plant presence is showed in Table 2.

TPH removal rate during phytoremediation process

The results of TPH concentration in soil samples in different time period during the phytoremediation

Table 2: Different concentration of studied contaminated soil (mg/kg)

Contamination percentage made by artificially	Primary Concentration	Concentration in plant presence	TPH removal percentage
1%	9027.40	126.43	98.6
5%	49599.03	4463.92	91
10%	99548.28	19611.50	80.3

Table 3: TPH removal rate in different treatments (mg/kg)

Soil sample	Treatment with plant			Treatment without plant (control)		
Contamination percentage	1%	5%	10%	1%	5%	10%
Time interval (day)						
0	9027.40	49599.03	99548.27	9027.40	49599.03	99548.27
15	3520.07	30751.48	71674.81	6138.64	36802.49	91385.32
30	1579.89	25047.50	63213.28	4965.07	35711.31	88299.32
60	875.78	11755.59	40615.73	4694.25	30652.21	71674.76
90	343.12	9542.91	30481.72	3430.42	21575.58	67692.83
120	126.43	4463.92	19611.50	2979.05	19343.63	63013.06

process with *Vetiveria zizanioides* are presented in Fig. 1. The rate of TPH removal was evaluated in control treatment with plant and 1%, 5% and 10% concentration. The Results from this study suggest that the highest TPH removal during 4 months in all treatments with plant had significantly (*P<0.05*) difference in compared with control treatments. Primary TPH amount in 1%, 5% and 10% concentration was respectively: 9027.40 mg/kg, 49599.03 mg/kg and 99548.28 mg/kg. After 4 months its amount in different concentration with plant was 126.43 mg/kg, 4463.92 mg/kg and 19611.50 mg/kg. In addition, Peng *et al.* (2009) noted 41.61–63.2% removal of TPH by Mirabilis Jalapa, during 127 days (Peng *et al.*, 2009). The best TPH removal efficiency was observed in all concentration at 120th day and in treatments with plant. Although plants with highly branched fine fibrous root systems which have higher total rhizosphere volume have been reported to enhance biodegradation of organic contaminants more than plants with taproot systems(Soleimani *et al.*, 2010; Aprill and Sims, 1990). Euliss *et al.* (2008) found out that *Carexstricta*, Pannicumvirgatum and Tripsacumdactyloides significantly reduced TPH by 70% after 1 year of growth (Euliss *et al.*, 2008). Also, the lowest TPH removal rate was observed in all concentration at 15th day and control treatments. In 1%, 5% and 10% of oil pollution TPH removal rate was 32%, 25.8% and 8.2%, respectively. 1% oil pollution treatment had no correlation with time. 5% oil pollution treatment had positive correlation with time at 1% level, 10% oil pollution treatment had negative correlation with time at 1% level. The results showed that TPH removal amount was higher than controls in all treatment with plant in all contamination concentration treatments.

Organic solids changes rate during phytoremediation process

The results of Volatile Solids concentrations at different time period during the phytoremediation process with *Vetiveria zizanioides* were presented in Fig. 2. The results showed that 1% treatment at 30, 60, 90 and 120 days had significant differences (P<0.05) in compared to control. 5% treatment at 60, 90 and 120 days had significant difference (P<0.05). 10% treatment at 90 and 120 days showed significant differences (P<0.05) with control. The amount of organic solid reduction in 1%, 5% and 10% oil pollution concentration was 62.96%, 65.49% and 66.41%, respectively, and 10% treatment had maximum of organic solid reduction.

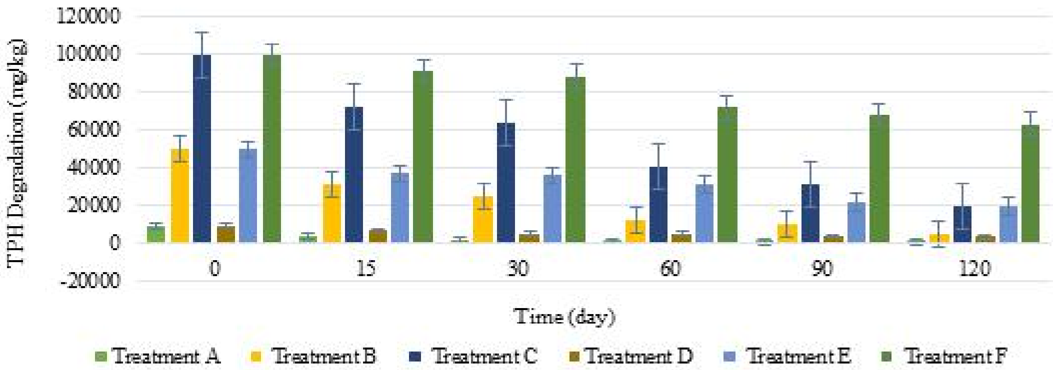

Fig. 1: Degradation of TPH pollutants at different time points during the phytoremediation process with *Vetiveria zizanioides* on 4 months. Values are means + standard deviation (SD). Treatment A: TPH -contaminated soil with concentration1% (with plant). Treatment B: TPH-contaminated soil with concentration5% (with plant). Treatment C: TPH-contaminated soil with concentration10% (with plant).Treatment D: TPH-contaminated soil with concentration1% (no plant). Treatment E: TPH-contaminated soil with concentration5% (no plant). Treatment F: TPH-contaminated soil with concentration10% (no plant).

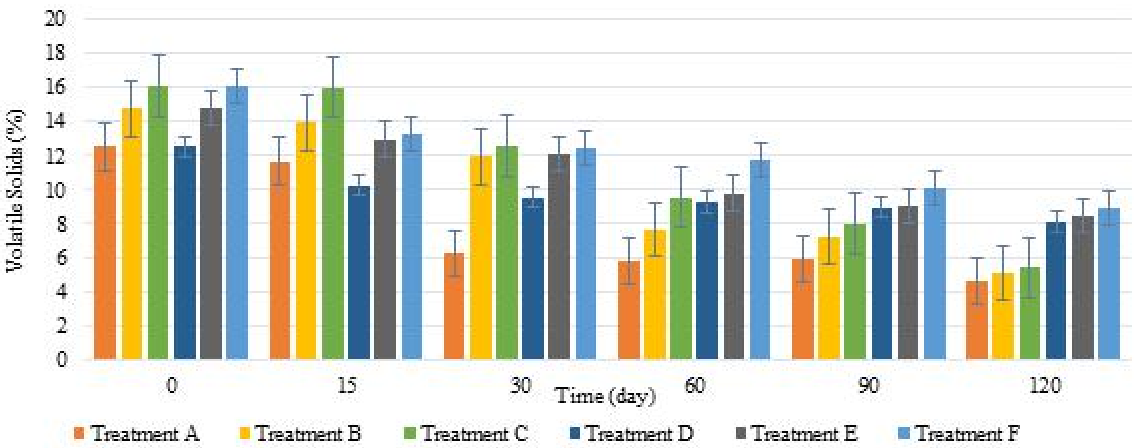

Fig. 2: Concentration of Volatile Solids at different time points during the phytoremediation process with *Vetiveria zizanioides* on 4 months. Values are means + standard deviation (SD). Treatment A: TPH-contaminated soil with concentration1% (with plant). Treatment B: TPH-contaminated soil with concentration5% (with plant). Treatment C: TPH-contaminated soil with concentration10% (with plant).Treatment D: TPH-contaminated soil with concentration1% (no plant). Treatment E: TPH-contaminated soil with concentration5% (no plant).Treatment F: TPH-contaminated soil with concentration10% (no plant).

Changes of microbial activity during phytoremediation process

Bacterial cell in CFU/g of soil (10^6) on Day0 and Day120were showed in Fig. 3. The soil heterotrophic microbial populations studied were counted at the first day and 120th days .Studies show that all treatments were significantly different to each other at 15th days and 120th days (P<0.05) and only 5% treatment had significantly different with controls (P<0.05). Soil microbial population increased during 120 days in treatments with plants. Number of microbial population in 1%, 5% and 10% contamination treatments with plant was 8.6×10^6CFU/g, 9.46×10^6CFU/g and 8.1×10^6CFU/g, respectively. Organic matter with hydrocarbon in soil as food resource for bacteria was reason of increasing in number of heterotrophic bacterial populations during 120 days. But number of heterotrophic bacteria in 10% oil contamination treatments had reduction in compared to other treatments that was due to more contamination and inhibition of bacteria growth.

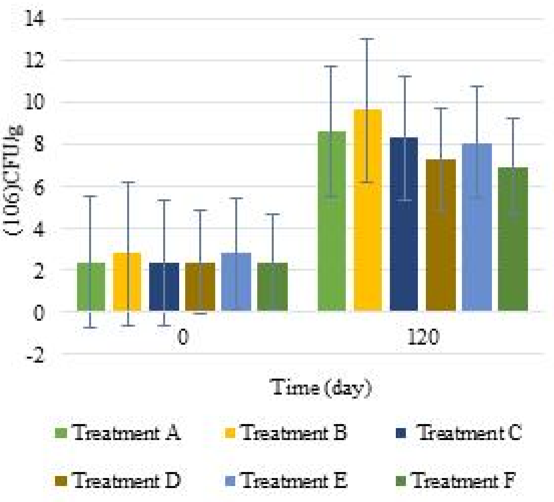

Fig. 3: Bacterial cell in CFU/g of soil (10^6) on Day 0 and Day120 Values are means + standard deviation (SD). Treatment A: TPH-contaminated soil with concentration1% (with plant). Treatment B: TPH-contaminated soil with concentration5% (with plant). Treatment C: TPH-contaminated soil with concentration10% (with plant). Treatment D: TPH-contaminated soil with concentration1% (no plant). Treatment E: TPH-contaminated soil with concentration5% (no plant). Treatment F: TPH-contaminated soil with concentration10% (no plant).

CONCLUSION

The results showed that *with Vetiveria zizanioides* can remove petroleum hydrocarbons from contaminated soils. Due to the characteristics of *Vetiveria zizanioides* plants with high tolerance to extreme climate change, long-term drought and as well as a wide geographic area in the world and also, according to the results of this study the *Vetiveria zizanioides*can be using for removal petroleum hydrocarbons in soil. The use of vegetation as a feasible remediation approach for soils contaminated with petroleum hydrocarbons may become attractive in Iran as well as in other developing countries because it is inexpensive and requires minimum maintenance and little management.

ACKNOWLEDGEMENT

The authors acknowledge the financial support provided by the National Iranian Drilling Company.

CONFLICT OF INTEREST

There is no conflicts of interest for publication of the manuscript.

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