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POTENTIAL OF VETIVER (*VETIVERIA ZIZANIOIDES* L.) GRASS IN REMOVING SELECTED PAHS FROM DIESEL CONTAMINATED SOIL

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Abstract

Phytoremediation has been renowned as an encouraging technology for the remediation of polycyclic aromatic hydrocarbon (PAH)-contaminated soils, little is known about how plant species behave during the process of PAH phytoremediation. Therefore, the aim of this study was to investigate the effectiveness of vetiver (*Vetiveria zizanioides* L.) plant in PAH phytoremediation and extraction potential of *Vetiveria zizanioides* for selected PAHs from the diesel contaminated soil. The field soil samples were spiked with varying concentrations (0.5% and 1%) of diesel and used for pot experiment which was conducted in greenhouse. Vetiver grass was used as experimental plant. Physico-chemical analysis of soil was performed before and after the experiment. Concentration of selected PAHs i.e. phenanthrene, pyrene and benzo[a]pyrene in soil was determined using HPLC. Plant parameters such as root/shoot length and dry mass were compared after harvest. Concentrations of PAHs were also determined in plant material and in soils after harvesting. Result showed that initial concentration of phenanthrene was significantly different from final concentration in treatments in which soil was spiked with diesel. Initial and final concentration of pyrene in soil was also significantly different from each other in two treatments in which soil was spiked with 1% diesel. Pyrene concentration was significantly different in roots and shoots of plants while benzo[a]pyrene concentration in treatments in which soil was spiked with diesel was also significantly different from roots and shoots. Phenanthrene was less extracted by the plant in all the treatments and it was present in higher concentration in soil as compared to plant. Our results indicate that vetiver grass has effectively removed PAHs from soil consequently a significantly higher root and shoot uptake of PAHs was observed than control treatments. Study concludes *Vetiveria zizanioides* as potentially promising plant specie for the removal of PAHs from diesel contaminated soil.

Key words: Phytoremediation, Polycyclic aromatic hydrocarbons, HPLC, *Vetiveria zizanioides.*

Introduction

Hydrocarbons, primarily considered as total petroleum hydrocarbons (TPH), Polycyclic aromatic hydrocarbons (PAHs) are one target component of TPH and more concern because of carcinogenicity and toxic to the environment (Zemanek *et al*., 1997; Anon., 2005). PAHs have anthropogenic sources (Venkataraman *et al*., 2002) these are persistent organic pollutants (POPs) that resistant to degradation, can remain in the environment for long periods and have the potential to cause adverse environmental effects (Kordybach, 1999). PAHs have unique stable structures to persist in the environment (Sugiura *et al*., 1997) and highly hydrophobic with low water solubility so these have strong attraction to soil particles (Sung *et al*., 2001).

Soil PAHs pollution is primarily due to industrial accidents (spills, leaks and leaking underground storage tanks) (Lee *et al*., 2002; Kiem *et al*., 2003). In soil most of PAHs are strongly sorbed to the organic matter, making them relatively unavailable for degradation processes. The effect of sorption generally increases as the number of benzene rings in the PAH-molecule increases (Bogan & Trbovic, 2003). Most important organic matter fraction in the soil is humic like substances i.e. Humic Acid, Fulvic Acid and humins (Berthe *et al.,* 2008) and important for physico-chemical properties of soil (Vergnoux *et al.,* 2011; Dai *et al.,* 2011). SOM controls the sequestration of PAHs in soil. Sequestration occurs when PAHs are removed from bioavailable pools and stored for long periods of time. The most recalcitrant PAH fraction consists of residual PAHs in soils and seems to increase with aging or soil-PAH contact time (Vessigaud *et al*., 2007).

Plants species were used to remediate PAHs from the soil from last few decades and it has been depicted as a promising approach to remediate soils contaminated with PAHs (Sung *et al*., 2004), scientists have also used plant material to clean heavy metals etc using plant material (Ali *et al*., 2013; Junaid *et al*., 2014). In this study grass species i.e. *Vetiveria zizanioides*, L. (Vetiver grass) will be used to investigate their potential for PAHs remediation from the artificially contaminated soil. Vetiver grass has successfully shown its potential towards phytoremediation of PAHs from contaminated soils since the last 2 decades (Srivastava *et al*., 2008).

Objectives of this study were:

- To determine the concentration of selected PAHs from the diesel contaminated soil.
- Assessment of plant growth aspects and subsequent uptake of PAH from contaminated soil.
- Extraction potential of *Vetiveria zizanioides* for the extraction of selected PAHs from the diesel contaminated soil.

Materials and Methods

Sample collection: As far as possible, uncontaminated soil samples were collected from field. The samples were grounded, sieved with 2 mm pore size sieve and air dried.

Treatments: Each type of soil was further divided into six treatments and replicated three times.

T1: Control
T2: Soil was mixed with 1% fruits and vegetable compost
T3: Soil was spiked with 0.5% diesel (PAHs)
T4: Soil was spiked with 1% diesel
T5: 0.5% diesel spiked soil mixed with 1% compost
T6: 1% diesel spiked soil mixed with 1% compost

Air dried soil samples were spiked with 0.5 and 1% diesel (PAHs). Then these samples were stored in polythene bags for 15 days (Park *et al*., 2011).

Physico-chemical analysis of soil: Soil samples were analyzed for physico-chemical parameters before and after the application of treatments. These analyses are as follows:

Soil moisture (SM): Pre-weighed (10 g) soil was put in a petri plate, weighed again. Soil was dried at 105^{o}C for 24 hours in digital oven to remove moisture contents. SM was calculated by using the following formula:

$$\text{Soil moisture (\%)} = \frac{\text{Loss of weight in soil samples}}{\text{Weight of oven dried soil}} \times 100$$

pH: The pH of the soil was measured by pH meter (Anon., 2005). For this purpose, 10 gram of soil sample was diluted in 90 ml of water. The pH meter was calibrated by using buffer solutions of pH 4, 7 and 10 before running the samples. The model of the pH used was (BMS pH-200L).

Electrical conductivity: Ten gram of soil diluted in 90 ml of water in order to measure electrical conductivity. Electrical conductivity was calculated in micro semen (µS). EC meter (DIST HI 98303 model) was used for the measurement of electrical conductivity (Muhammad *et al.,* 2008).

Cation exchange capacity: Four gram of air dried soil was weighed in centrifuge tube and 33ml N sodium acetate trihydate was added and shaked for 5 minutes and then centrifuged at 300 rpm until supernatant was cleared. Supernatant was decanted and 33 ml 95% ethanol was added and centrifuged at 300 rpm until supernatant was cleared, supernatant was discarded and replaced by the adsorbed sodium from the sample by extraction with 33 ml 1 N ammonium acetate solution, extract was shaked for 5 minutes. Standards of suitable concentration Na were run on flame photometer to draw a calibration curve. Then soil samples were run, Na concentration was measured by calibration curve.

Organic matter: One gram of soil was used and then it was transferred it to a conical flask and 10 ml of normal solution of potassium dichromate and about 20 ml of concentrated Sulphuric acid was added. The solution was shaked for about 10 minutes and it was allowed to stand for 10 minutes. 100 ml water and 25 ml freshly prepared solution of N/2 ferrous sulphate was added. N/10 solution of $KMnO_4$ was taken in the burette and flask contents were titrated till the colour of the solution was changed to light pink.

Total organic carbon (TOC): Total organic carbon (TOC) was analyzed by using extraction and titration methods (Nelson & Sommers, 1982). Soil samples were extracted with 25 ml of 0.5 M K_2SO_4 at 250 rpm for 60 minutes using horizontal shaker. After shaking, soil samples were centrifuged at 2000 rpm for 10 minutes followed by filtration with filter paper to obtain soil free filtrate. 4 ml of soil extract was transferred in 100 ml conical flasks for titration. 1 ml $K_2Cr_2O_7$ and 5 ml concentrated H_2SO_4 was added by continuously shaking. After cooling and adding 0.3 ml indicator, soil extract was titrated with acidified ferrous ammonium sulphate solution. The color of solution was changed from green to red. Blank titrant was prepared as sample following the similar procedure except addition of soil extract.

TOC (%) calculated as:

$$\text{TOC (\%)} = \{(A \times M \times 0.003)/g\} \times (E/S) \times 100$$

Soil extraction: Soil samples were air dried in the dark and passed through a 2-mm steel sieve. Five g soil was mixed with 20 ml dichloromethane and extracted for 30 min by ultrasonic agitation. Samples were centrifuged at 2000 rpm for 5 min. The supernatant was the extract for PAHs and then dried by sparging with N_2, and then re-dissolved in 1 ml methanol for high-performance liquid chromatography (Li *et al*., 2006).

HPLC analysis: HPLC (SPD-10VP Shimadzu with UV detection 254 nm) was used for to determination of selected PAHs which include phenanthrene (PHE), pyrene (PYR) and benzo-a-pyrene (BAP) in soil. Standard of 16 priority PAH pollutant was run on the HPLC. Methanol/water was used as mobile phase. Retention times was optimized with individual PAHs and mixed PAHs. Prepared samples after extraction were run on HPLC.

Pot experiments: The experiments were carried out at the green house of Department of Environmental Sciences, Arid Agriculture University, Rawalpindi. Vetiver grass (*Vetiveria zizanioides* L.) was used as test plant to see the effect of soil PAH contamination on plant growth and uptake. In pot experiment 5 kg soil was filled in each pot and small plants of 10 cm height were planted and replicated three times. Pot experiment was conducted for a period of four months from June to October. After four months, plants were harvested and growth parameters were observed which include root length (cm), shoot length (cm) and plant biomass.

PAH analysis in plant material: Pant material was subjected to water bath extractions for 20 min in ethyl acetate. Insoluble materials were removed by filtration through a membrane filter. The extract was passed through silica gel packed column and the compounds on the silica gel column were eluted with 250 ml of benzene. Elution was concentrated under reduced pressure, dried under nitrogen stream and then elute was re-dissolved in 1ml of methanol for HPLC analysis (Li-Hong *et al*., 2006).

Statistical analysis: Data was statistically analyzed by using SPSS software. T-test was used to analyze the data. For all tests, $p<0.05$ was used as level of significance.

Result

Physicochemical characteristics of soil: Table 1 summarizes the results of all characterizations done on the soils used in pot experiment. Texture of soil comes out sandy loam with pH 6.8, electrical conductivity 203 µS, cation exchange capacity 13 meq $100g^{-1,}$ microbial biomass nitrogen 17 mg kg^{-1} and microbial biomass carbon 430 mg kg^{-1}. Percentage of soil moisture, total organic carbon and organic matter was estimated as 23 %, 67 % and 2.8 % respectively.

Table 1. Physicochemical characteristics of soil used in pot experiment.

Parameters	Characteristics
pH	6.8
Soil texture	Sandy loam
Soil moisture (%)	23
EC (µS cm^{-1})	203
Organic matter (%)	2.8
Total organic carbon (%)	5.6
*CEC (meq100 g^{-1})	13 ± 5
*MBN (mg kg^{-1})	17 ± 6
*MBC (mg kg^{-1})	430±7

* CEC: Cation exchange capacity, MBN: Micobial Biomass Nitrogen, MBC: Microbial biomass carbon

Concentration of PAHs in soil: Table 2 shows the initial and final concentrations of selected PAHs in soil. Initial concentration of PHE was 3.71 mg $kg^{-1}$and final concentration after 120 days of pot experiment was 2.53 mg kg^{-1} while concentration of pyrene and benzo-a-pyrene was not detected in T1. Initial concentration of PHE after 15 days of amendment with compost was 3.21 mg kg^{-1} and final concentration was 2.13 mg kg^{-1} in T2. In T3 after 15 days of spiking with 0.5% diesel initial concentration of PHE, PYR and BAP were 27.23, 45.32 and 32.23 while final concentrations were 12.29, 38.11 and 24.11 mg kg^{-1} respectively. In T4 in which soil was spiked with 1% diesel and after 15 days of spiking, concentration of PHE, PYR and BAP were 42.68, 53.21 and 37.12 and final concentrations after 120 days of experiment were 26.96, 41.54 and 28.87 mg kg^{-1}. In T5 soil was spiked with 0.5% diesel and amended with 1% compost and after 15 days of spiking the initial concentrations of PHE, PYR and BAP were 31.76, 40.43 and 29.21 while final values after 120 days of pot experiment were 20.12, 20.19 and 20.32 mg kg^{-1}. After spiking with 1% diesel and mixed with 1% compost initial concentrations were 59.43, 49.12 and 41.08 while 35.24, 19.43 and 22.17 mg kg^{-1} were final concentrations of selected PAH in T6.

In Table 2 concentrations of PAHs are compared by t-test in which initial concentration of phenanthrene was significantly different from final concentration in T3, T4, T5 and T6 in which soil was spiked with diesel. Initial and final concentration of pyrene in soil was significantly different from each other in T4 and T6 in which soil was spiked with 1% diesel.

Table 2. Concentrations of PAHs (mg kg^{-1}) in soil used before and after 120 days of pot experiment.

Treatments	PAHs					
	Phenanthrene		Pyrene		Benzo[a]pyrene	
	Initial†	Final‡	Initial†	Final‡	Initial†	Final‡
T1	3.71 a	2.53 a	ND	ND	ND	ND
T2	3.21 a	2.13 a	ND	ND	ND	ND
T3	27.23 a	12.29 b	45.32 a	38.11 a	32.23 a	24.11 a
T4	42.68 a	26.96 a	53.21 a	41.54 b	37.12 a	28.87 a
T5	31.76 a	20.12 a	40.43 a	20.19 a	29.21 a	20.32 a
T6	59.43 a	35.24 a	49.12 a	19.43 b	41.08 a	22.17 b

Values sharing similar letters in a row for individual PAH are not significantly different
† Initial: Soil PAH values after 15 days of spiking
‡ Final: Soil PAH values after plant harvest (120 days)
Each value is mean of three replications

Table 3. Comparison between root and shoot mean concentrations of PAHs (mg kg^{-1}) for *Vetiveria zizanioides* grown in PAH contaminated soil for 120 days.

Treatments	PAHs					
	Phenanthrene		Pyrene		Benzo[a]pyrene	
	Root	Shoot	Root	Shoot	Root	Shoot
T1	0.81 a	0.21 b	ND	ND	ND	ND
T2	0.53 a	0.12 b	ND	ND	ND	ND
T3	2.17 a	1.52 a	9.17 a	4.52 b	5.17 a	2.52 b
T4	6.57 a	2.76 b	18.57 a	6.76 b	19.57 a	5.76 b
T5	1.75 a	0.98 a	7.75 a	3.98 b	3.87 a	1.98 a
T6	2.43 a	0.97 b	12.78 a	4.76 b	12.34 a	5.19 b

Values sharing similar letters in a row for individual PAH are not significantly different
Each value is mean of three replications

Table 4. Extraction potential of *Vetiveria zizanioides* to remediate selected PAHs from the diesel contaminated soil.

Treatments	PAHs					
	Phenanthrene		Pyrene		Benzo[a]pyrene	
	Coefficient value *	Extraction potential	Coefficient value *	Extraction potential	Coefficient value *	Extraction potential
T1	0.86	< 1	ND	ND	ND	ND
T2	0.60	< 1	ND	ND	ND	ND
T3	0.25	< 1	1.90	> 1	0.95	< 1
T4	0.59	< 1	2.17	> 1	3.07	>1
T5	0.23	< 1	0.58	< 1	0.66	<1
T6	0.14	< 1	0.59	< 1	0.68	< 1

*Coefficient value is based on (root PAH + shoot PAH)/(initial soil PAH – final soil PAH)

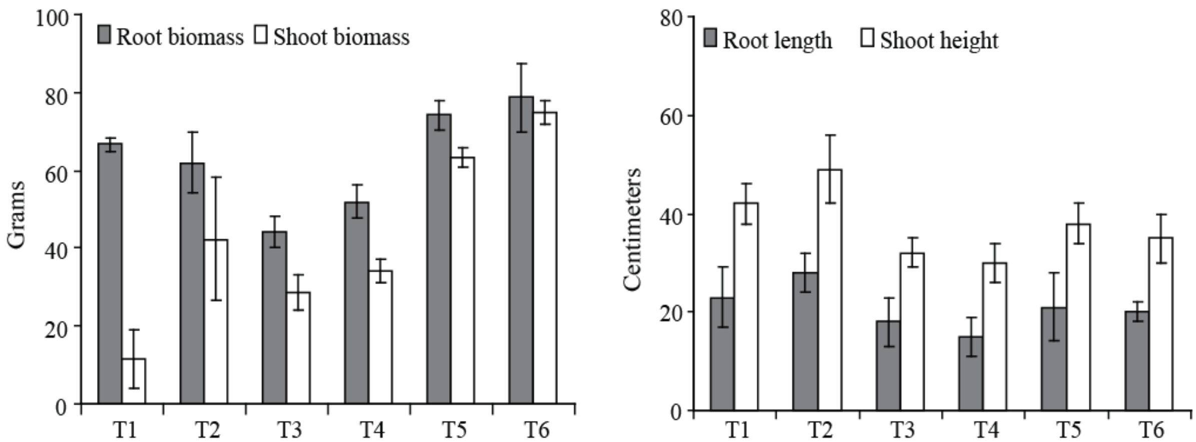

Fig. 1. Response of growth characteristics by *Vetiveria zizanioides* in different soil PAH treatments. Error bars showing means with standard deviation (n=3).

Concentration of PAH in roots and shoots of *Vetiveria zizanioides*: Table 3 shows the concentrations of selected PAHs in Roots and Shoots of *Vetiveria zizanioides* after 120 days of pot experiment. The higher concentration of PHE, PYR and BAP in roots and shoots were observed in T4.

T-test was used for the comparison of mean concentration of PAHs in root and shoot of plant *Vetiveria zizanioides.* Concentration of phenanthrene was significantly different in roots and shoots of *Vetiveria zizanioides* of treatment T1, T2, T4 and T6. Pyrene concentration was significantly different in roots and shoots of plants while BAP concentration in treatment T3, T4 and T6 is also significantly different from roots and shoots.

Extraction potential of *Vetiveria zizanioides*: Extraction potential of *Vetiveria zizanioides* for the extraction of selected PAHs from the diesel contaminated soil is presented in Table 4. Phenanthrene was less extracted by the plant in all the treatments and it is present in higher concentration in soil as compared to plant. Pyrene extracted in less amount in T5 and T6 while Benzo-a-pyrene present in low concentration in roots and shoots of *Vetiveria zizanioides* in T2, T5 and T6.

Plant growth characteristics: Response of growth characteristics by *Vetiveria zizanioides* in different soil PAH treatments are presented in Fig. 1. Statistically significant decrease was observed in root biomass of *Vetiveria zizanioides* grown on PAHs contaiminated soil as compared to control. While soil contain both, PAHs (T5 and T6) and compost showed significant increase in root biomass. while treatment 3 and 4 shows significantly decrease the shoot bimass as comapred to treatments amended with compost.

Fig. 1 also shows the effect of different soil PAH treatments on mean root and shoots length of *Vetiveria zizanioides.* At the beginning of the greenhouse studies, all plants had a height of 15 cm. As the experiment progressed, plant height increased in all treatments. However, differences between contaminated and uncontaminated soil were clearly evident throughout the study. Average root and shoot length in contaminated soil reached its maximum at 4 months. Plant root and shoot length is recorded in uncontaminated soils (T1) and (T2) and it shows highest length as compared to PAH contaminated soils.

Discussion

Phytoremediation is a viable choice for PAH remediation, if sufficient time is allowed for plant establishment and contaminant degradation (Mehmood *et al*., 2013). In the process, plants can be used to extract and detoxify toxic pollutants from soil (Chen *et al*., 2003; Pilon-Smits, 2005; Aslund *et al*., 2007). In the present study *Vetiveria zizanioides* was used to remove the selected

PAHs from diesel contaminated soil. A significant decrease in concentration of selected PAHs *i.e*., phenanthrene, pyrene and benzo[a]pyrene in soil was observed after 120 days of pot experiment. It might be due to plants uptake of PAHs from diesel contaminated soil. Similar trend was observed by Park *et al*. (2011), in which plants grown on the polluted soil significantly enhanced the dissipation of PAHs in the soil and planting of alfalfa along with tall fescue in mixture achieved the highest value for PAHs decrease in soil. Denys *et al*. (2006) also found 25% decrease in soil total PAHs in a mixture of herbaceous species grown in PAH contaminated soil in the field.

Plants may uptake or sequester PAHs in their tissues and transport in roots and shoots. We observed that considerable amount of soil phenanthrene, pyrene and benzo[a]pyrene has transferred from root to shoot tissues in *Vetiveria zizanioides* in all the treatments which demonstrate the efficiency of this plant to remove PAHs from soil. Accumulation of phenanthrene and pyrene were reported in ryegrass roots, with concentrations in shoots much lower than those in roots and PAHs were mainly accumulated by plant roots and rarely transported to above ground plant parts (Yu *et al.*, 2011). However, we found that the concentration of phenanthrene, pyrene and benzo-a-pyrene was higher in roots as compared to shoots as root accumulates more PAHs. Furthermore, in our study benzo[a]pyrene concentration in roots was observed highest as compared to shoot and other PAHs. It may be due to low solubility and extractability. Li *et al*. (2006) also reported that translocation of BAP appeared to be weak from root to shoot due to low solubility of BAP in soil.

Extraction potential exhibited by *Vetiveria zizanioides* in most of the treatments was less than 1. Soil treatments with compost addition have lowered the extraction potential of *V. zizanioides*. Organic matter has sequestered the PAHs in soil and thus less amount of PAH was taken up by plants and hence we observed < 1 values for extraction potential. Plants have taken up certain quantities of PAHs, the absolute amount of PAHs stored in plant compartments makes little contribution to the removal of total soil PAHs. Regarding phytoremediation efficiency, it is reported that plants take up less than 2% of total soil PAHs (Gao & Zhu, 2004; Meng *et al*., 2011). Findings of this study revealed that removal of phenanthrene, pyrene and benzo[a]pyrene by *V. zizanioides* through phytoextraction is 1% of total PAH mass in the soil which seems a promising evidence for usefulness of this plant species for phytoremediation of PAHs.

The negative effect of diesel contamination on growth *Vetiveria zizanioides* was obvious as significant reduction of plant biomass was observed in diesel treated soil than control. The biomass of *V. zizanioides* grown in all PAH treated soils was decreased and this decline in plant biomass was highest in maximum diesel contamination treatments. The behavior of *V. zizanioides* to organic matter amendments in soil has mixed effect with treatments having no diesel addition showed positive influence on growth and biomass production as compared to those treatments in which soil was only spiked with diesel. Biomass and plant height of vetiver were reduced in the presence of heavy crude oil in soil (Brandt *et al*., 2006). Although growth of *Vetiveria zizanioides* was slightly suppressed when exposed to soil diesel contamination but our study concludes that vetiver plants were able to remove PAHs from soil to a considerable extent.

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