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Uptake of Total Petroleum Hydrocarbon (TPH) and Polycyclic Aromatic Hydrocarbons (PAHs) by *Oryza sativa* L. Grown in Soil Contaminated with Crude Oil

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Abstract The purpose of this study was to determine whether total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbons (PAHs) present in crude oil contaminated sites are transferred to roots, shoots and finally the grains of rice crops (*Oryza sativa* L.) grown in those sites. Soil was artificially contaminated with crude oil at concentrations of 0, 1000, 5000, 10,000, and 15,000 mg/kg, followed by planting of rice seedlings. After harvest, TPH in plant samples were measured, and it was determined that the uptake of TPH by the plants gradually increased as the concentration of oil in soil increased. Further, from GC–MS analysis, it was observed that PAHs including naphthalene and phenanthrene bioaccumulated in rice plant parts. Vital physico-chemical properties of soil were also altered due to crude oil contamination. Our study revealed that rice plants grown in crude oil polluted sites can uptake TPH including PAHs, thus emphasising the importance of prior investigation of soil condition before cultivation of crops.

Keywords Rice · Crude oil contamination · Bioaccumulation · Physico-chemical properties · GC–MS

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Rapid industrialization and ever growing demand for petroleum fossil fuel has led to the release of hydrocarbon compounds into the environment. Total petroleum hydrocarbon (TPH) is a measure of the mixture of hydrocarbon compounds derived from substances such as crude oil. Various oil field operations such as drilling, transportation, pipeline leakage, and accidental spillage of petroleum, lead to the release of hydrocarbons into the environment, thereby possibly causing pollution. TPH includes both aliphatic (alkane, alkene, alkyne etc.) and aromatic compounds. Among the aromatic fractions, polycyclic aromatic hydrocarbons (PAHs) are given prime attention as they are considered to pose risks due to their toxic, mutagenic, and in some cases, carcinogenic properties (Patnaik 1992). PAHs in the environment can have both natural as well as anthropogenic origins (Kaushik and Haritash 2006). Around 90% of TPH and PAHs in the environment reside in soil surfaces (Wild and Jones 1995), which can lead to the alteration of several physico-chemical properties of the soil. As a result of such changes, the vegetation in those contaminated sites can be adversely affected with respect to their germination and growth pattern (Siddiqui and Adams 2002). Moreover, plants grown in polluted sites can absorb the TPH, including PAHs, thereby leading to possible entry to human and animal populations through the food web (Kang et al. 2010). Uptake of TPH and PAHs by plants from crude oil contaminated sites usually occurs through root absorption followed by their translocation to shoots via transpiration (Ashraf and Salam 2012). Once contaminants come into contact with root surfaces, their uptake or translocation to other parts of the plant depends on the hydrophobicity of the chemical. The highly hydrophobic hydrocarbon substances have a strong affinity for lipid components and therefore tend to remain sorbed on root surfaces, reducing their chances of transportation to other plant parts (Gao and

Zhu 2004). Despite such properties, reports show uptake of hydrocarbon contaminants into other parts of the plants. Parrish et al. (2006) reported uptake of PAHs by shoots of cucumber and squash plants. In the plant system, the hydrocarbon contaminants can either remain accumulated in several locations including root surfaces, interfacial nodes, or leaves, or be further metabolised within the plant by undergoing transformation, conjugation and sequestration processes (Parrish et al. 2006).

In urban areas, petroleum contaminated sites are often used for cultivation purposes. In Lakowa (27°0′44″N, 94°51′17″E; altitude 95 m), a village of Sibsagar district of Upper Assam, India, where there are numerous oil fields, it has been observed that local farmers practice rice cultivation near oil fields. In such areas, there is a high possibility of the presence of hydrocarbon pollutants which can be often released by different activities occurring in nearby oil fields. Thus there are chances for the uptake of such pollutants by rice crops grown in polluted sites. Consumption of such crops by grazing animals and humans might lead to serious health hazards. The present study investigated whether rice plants (the staple crop of most of the Asian countries), grown in petroleum hydrocarbon contaminated soil, have the ability to uptake the hydrocarbons from the soil and accumulate them in plant parts, including the grain.

Materials and Methods

Approximately 200 kg soil was collected from a rice field near Guwahati, India. Soil samples were dried, followed by homogenization and then sieved through a 2 mm mesh. About 5 L of crude oil was collected from the Noonmati Refinery, Guwahati, India. 18 plastic pots (18 × 30 cm) were purchased for the experiment. Soil (10 kg) was weighed for each pot, which was contaminated with crude oil at concentrations of 0 (control), 1000, 5000, 10,000, 15,000 mg/kg. For preparing the 1000 mg/kg concentration, 10 kg of soil was added in a plastic bucket followed by the addition of a mixer containing 10 g crude oil +3.5 L tapwater +50 mL of extran detergent as an emulsifier. Mixing was carried out with the aid of a magnetic stirrer. In a similar way, the other concentrations of crude oil i.e., 5000, 10,000, and 15,000 mg/kg were prepared by addition of 50,100, and 150 g crude oil, respectively. A second control set containing only detergent (extran) without oil, was used in order to investigate the possible added effects of the detergent on the development of the rice plants and its influence on the physico-chemical properties of the soil. Individual treatments were composed in triplicates and all the prepared soil samples were allowed to dry, followed by homogenization.

Healthy rice seedlings of *Aijung* (*Mahsuri* variety) were collected from a local farmer and in each pot, five seedlings were planted, followed by regular watering. After proper ripening of the grains, rice plants were harvested with intact roots and then washed thoroughly with tap water so as to remove soil, followed by the separation and drying of the rice plant parts i.e., the roots, shoot, grains, and husks. The yield of rice grains of each harvested plant was estimated.

Physico-chemical properties of soil, both prior to and after the cultivation of rice were recorded. The pH of the soil was determined in a 1:5 soil/water suspensions using a pH Meter (Mettler Toledo). Similarly, the conductivity of the soil was determined using a conductivity meter (Nics 96, µC conductivity meter 117). Water holding capacity (WHC) of soil samples was determined by the method followed by Baruah et al. (2013). The total nitrogen content was estimated by following the Micro Kjeldahl method (Jackson 1975) and the total organic carbon (TOC) content was determined by the Rapid Titration method (Walkley and Black 1934).

The TPH in the soil samples and rice plant parts was extracted with dichloromethane (DCM) using a soxhlet apparatus, followed by evaporation of the excess solvent through a rotary evaporator. The condensed solution was then poured into a previously weighed glass beaker and allowed to dry until a constant weight was achieved. The TPH present in the samples was then quantified through gravimetric analysis.

The PAHs present in extracts were identified with gas chromatography-mass chromatography (GC–MS), Shimadzu GC 2010 plus with triple quadrupole MS (TP-8030) equipped with an EB-5 column. The column and oven temperature was kept at 60–280°C with an incremental column temperature at 8°C/min and finally held at 280°C for 37 min. Helium was used as the carrier gas at the flow rate of 1 mL/min. Mass spectrometric data were acquired in electron ionization mode (70 eV). The interface temperature was 310°C and mass range was 45–600 m/z. Individual PAH components were determined by matching their retention time with those available in mass spectral (MS) library NIST 11 database.

Data were presented as the mean ± standard deviation of three replicates. Statistical analysis of the uptake of TPH by rice plants was performed using least significant difference (LSD). Results were calculated by employing Origin Pro8 software. A p value of <0.05 compared to control value was set.

Table 1 Mean ± SD physico-chemical properties of the contaminated soil before and after plantation of rice

Concentration of crude oil in soil (mg/kg)	pH		Conductivity (mS/cm)		Total organic carbon (TOC) in g/kg		Water holding capacity (WHC) in (%)		Total nitrogen (%)	
	Time of cultivation	Time of harvest	Time of cultivation	Time of harvest	Time of cultivation	Time of harvest	Time of cultivation	Time of harvest	Time of cultivation	Time of harvest
15,000	4.9 ± 0.2	5.5 ± 0.3	0.091 ± 0.015	0.095 ± 0.041	20.1 ± 4.3	18.7 ± 4.3	38.05 ± 3.5	44.50 ± 2.6	0.094 ± 0.011	0.112 ± 0.010
10,000	5.3 ± 0.5	6.2 ± 0.7	0.094 ± 0.031	0.097 ± 0.024	19.4 ± 6.4	16.9 ± 1.9	42.20 ± 4.0	48.18 ± 4.2	0.098 ± 0.006	0.126 ± 0.005
5000	5.7 ± 0.4	6.5 ± 0.5	0.096 ± 0.039	0.120 ± 0.038	14.2 ± 3.2	12.7 ± 3.5	49.94 ± 3.2	53.63 ± 5.3	0.120 ± 0.004	0.154 ± 0.008
1000	6.4 ± 0.3	6.8 ± 0.2	0.110 ± 0.026	0.142 ± 0.025	09.8 ± 7.0	07.4 ± 5.2	54.38 ± 2.8	59.41 ± 4.6	0.137 ± 0.035	0.168 ± 0.014
0	7.1 ± 0.4	7.2 ± 0.5	0.124 ± 0.054	0.149 ± 0.052	04.1 ± 1.9	04.5 ± 6.1	62.25 ± 5.1	63.50 ± 5.8	0.190 ± 0.021	0.199 ± 0.009
0 (with soap)	7.3 ± 0.1	7.3 ± 1.1	0.118 ± 0.042	0.143 ± 0.066	04.9 ± 2.7	05.8 ± 3.3	60.55 ± 4.6	61.43 ± 3.9	0.188 ± 0.009	0.196 ± 0.007

Results are represented as the means ± SD

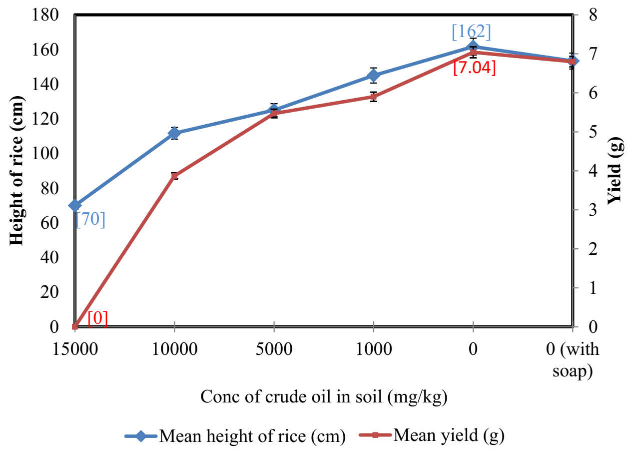

Fig. 1 Mean ± SD yield and height of rice plants grown in contaminated soil at different concentrations of crude oil

Results and Discussion

Physico-chemical properties of the contaminated soil are shown in Table 1. The pH of soil samples contaminated with crude oil was acidic as compared to the control. The acidic nature of soil gradually increased as the concentration of crude oil increased. The increase in acidity of soil could be a result of decomposition of the organic compounds present in oil that might lead to the generation of acidic intermediates (John et al. 2011). The conductivity of soil also showed a decreasing trend with an increase in crude oil concentrations, which is similar to the findings of Osuji and Nwoye (2007), who reported a decrease in conductivity of soil contaminated with oil compared to controls that did not contain crude oil. The TOC was higher in the crude oil contaminated soil as compared to the control samples. The increased level of TOC in the contaminated soil could be due to greater carbon containing oil pollutants. On the other hand, the WHC of the soil decreased with the increase in concentration of oil in the soil. This could be due to possible coating of the soil interstices with the waxy oil components and covering of the soil spaces with the same, thereby reducing the space for water accumulation. Nitrogen content decreased with increasing concentration of crude oil in the soil. This could be due to the decrease in the number of microbial nitrogen fixer and nitrifiers in the polluted atmosphere as a result of their intolerance for acidic environment that prevails in oil polluted sites (John et al. 2011). The physico-chemical properties of the same soil samples, collected after harvest, changed slightly when compared to the values obtained during prior cultivation. After the harvest of rice, the pH and conductivity of the soil were elevated. The nitrogen content and the water holding capacity also increased and the TOC decreased. The uptake of the hydrocarbon constituents by the cultivated rice plants from the contaminated soil samples, and their possible metabolism by the microflora present in the rhizosphere zone, likely resulted in such changes to the soil properties prior to and after the cultivation period. The physico-chemical properties of the soil from the second control set containing detergent (extran) were more or less similar to the properties of the soil sample of the first control set, in which crude oil and detergent were not added, and this indicated the detergent used in the experiment did not lead to significant alteration of the vital physico-properties of the soil.

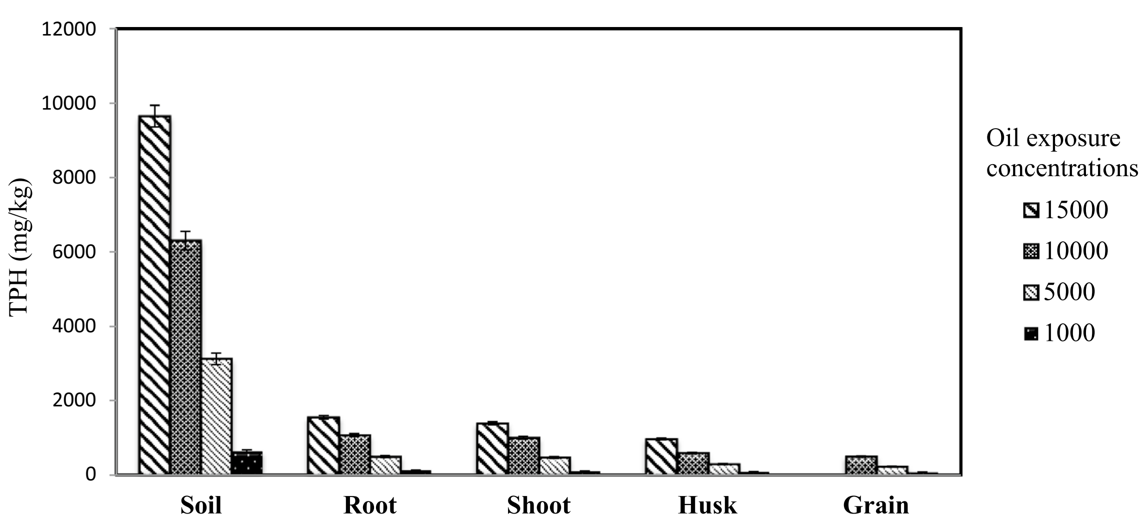

Fig. 2 Estimation of TPH in soil and rice plant samples grown in different concentration of crude oil. The TPH for soil and plant samples from the control sets, i.e., 0 mg/kg (with and without extran) are not shown as TPH were not detected in those samples. The TPH of the grains of rice plants grown in 15,000 mg/kg concentration could not be estimated as grains did not develop in that concentration

Table 2 Poly aromatic hydrocarbons (mg/kg) detected in soil and rice plant samples

PAHs	15,000 (mg/kg)						10,000 (mg/kg)						5000 (mg/kg)						1000 (mg/kg)					
	Soil		Root	Shoot	Husk	Grain	Soil		Root	Shoot	Husk	Grain	Soil		Root	Shoot	Husk	Grain	Soil		Root	Shoot	Husk	Grain
	(A)	(B)					(A)	(B)					(A)	(B)					(A)	(B)				
Naphthalene and derivatives	318	206	39.7	1.3	0.6	ND	153	94	14.2	0.4	–	0.9	75	41	4.6	–	–	–	32	19	–	–	–	–
Chamazulene	24	11	0.78	–	–	ND	–	–	–	–	–	–	–	–	–	–	–	–	1.6	–	–	–	–	–
Azulene	138	64	10.45	–	–	ND	87	38	3.6	–	–	0.03	23	9	–	3.1	–	–	6.1	–	–	–	–	–
Indene	18	10	–	0.6	–	ND	9	2.4	–	–	–	–	2	–	–	–	–	–	1.9	–	–	–	–	–
Anthracene	4.5	2.4	–	–	–	ND	3.1	1.9	–	–	–	–	1.5	–	–	–	–	–	–	–	–	–	–	–
Phenanthrene	6	4.8	–	–	–	ND	3.0	1.2	–	–	–	0.002	–	–	–	–	–	–	–	–	–	–	–	–
Flourene	15	12.5	–	–	–	ND	10	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Concentrations of PAHs are expressed in mg/kg. Here, "–" indicates absence of particular PAHs mentioned in the table, and "ND" denotes not development of rice grains in the 15,000 mg/kg. The 'A' and 'B' in the table denote the soil samples prior and after rice cultivation respectively

The record of yield and height of rice plants is presented in Fig. 1. Growths of rice plants cultivated in soil contaminated with higher concentrations of crude oil were less than controls. The height of the rice plants and the yield of grains decreased gradually with increasing concentration of crude oil. The average height of rice plants grown in the pot containing the highest concentration of crude oil was recorded to be 70 cm; whereas, for the control set, the average height was found to be 162 cm. A similar report by Odjegba and Okunnu (2012) suggested the growth of plants grown in oil polluted sites was generally stunted, and this could be due to the low availability of mineral elements and increased acidity in the contaminated soil (Njoku et al. 2012). Grain development in rice plants grown in the soil contaminated with the highest concentration of crude oil (15,000 mg/kg) was not observed; whereas, in the control, the yield of grain was around 7.04 g. This could be the result of excessive stress condition and detrimental effect caused by the high oil content. The yield of rice plants gradually decreased as the concentration of oil in the soil increased. The yield and height of the rice plants grown in the second control set (containing detergent) were comparable to the first control set (soil in which detergent was not added).This signifies that the added detergent in the experiment did not pose a noticeable effect on growth and yield of rice plants.

The quantified amounts of TPH in soil and rice plant parts are shown in Fig. 2. The TPH content in the soil that contained the highest crude oil concentration (15,000 mg/kg) was reduced to 9652 mg/kg at the time of harvest, which is after 4 months of initial contamination. Maximum TPH amount was detected in the root samples with 1561 mg/kg, followed by the shoots with 1400 mg/kg for the rice plants grown in the highest concentration of crude oil. TPH was also extracted from the grain and the husks of the rice plants. TPH was not found in the soil from either of the control sets. It was observed that the TPH concentration that was up taken by rice plants and gradually increased with increasing concentration of crude oil in the soil. Such increase in the uptake was also found to be significant with p value less than 0.05% in all the rice samples.

The PAHs present in the TPH samples are presented in Table 2. Prior to cultivation, a total of seven PAHs (naphthalene, azulenes, chamazulene, indene, anthracene, phenanthrene and flourene) were present at the 15,000 mg/kg concentration. The number and concentration of PAHs present in the soil samples at lower concentrations of crude oil decreased. In the 1000 mg/kg, only naphthalene, chamazulene, azulene and indene were detected. Again, after the cultivation of rice plants, it was found that the concentration of PAHs reduced in the soil samples, and such decrease could be due to their accumulation in rice plant parts or degradation in the soil by the microflora of the rhizosphere. Certain PAHs present in the soil during prior to cultivation were absent in the soil at the time of harvest, which could be the result of their degradation or conversion to some other metabolic intermediates during the interval period. It was found that among all other detected PAHs, naphthalene was predominantly present in the soil samples, which could be due to their strong association with the soil organic matter (Fairbanks et al. 1987). Roots of rice grown in 15,000 mg/kg of crude oil contained naphthalene, chamazulene, and azulene. Roots of the rice grown in 10,000 mg/kg of crude oil contained naphthalene along with their derivatives and azulene. Roots of rice grown in 5000 and 1000 mg/kg of crude oil contained only naphthalene. Shoot samples of rice grown in 15,000 mg/kg crude oil accumulated two-ring PAHs including naphthalene and indene, and the rice shoots grown in 10,000 and 5000 mg/kg crude oil accumulated naphthalene and azulene, respectively. PAHs were not detected in rice shoot samples grown in 1000 mg/kg crude oil. There was no proper correlation among the PAHs detected in the root and those in the shoot samples. The absence of other PAHs in the shoot samples, which were present in the roots, could be due to the inefficient transport of the contaminants through the xylem and high adsorption of the organic pollutants by the hydrophobic lipid components of the root (Li et al. 2005). Husk samples of the rice grown in 15,000 mg/kg crude oil were detected to have accumulated naphthalene whereas husk samples of plants grown at the lower concentrations did not accumulate PAHs. Absence of PAHs in husk samples could be due to lack of proper translocation from the roots and aerial parts of the plant. The PAHs present in grains of rice grown in 10,000 mg/kg were naphthalene, azulene and a trace amount of phenanthrene. The presence of trace amounts of phenanthrene in the grain samples, which was absent in the root samples of the rice plant grown in 10,000 mg/kg, could be due to the direct uptake via a gas phase absorption (Su and Zhu 2007) by the grains. PAHs were not detected in grains of rice plants grown in the lower concentrations of crude oil, signifying the chances of accumulation of PAHs in the rice grains increases as the their concentrations increase in the soil. Grains did not develop in rice plants exposed to 15,000 mg/kg crude oil. This could be a result of the changes in soil physico-chemical properties caused by crude oil contamination that can have an adverse effect on vegetation and hence hinder the proper development of the grain. Ekundayo et al. (2001) also reported reduction of grain yield in maize grown in oil polluted sites. Gabriel and Kasali (2014) reported poor growth in maize and cowpea grown in crude oil polluted sites. Furthermore, it was observed that PAHs bioavailable in the plant system were basically two-ring PAHs such as naphthalene, along with its derivatives such as methyl naphthalene, ethyl naphthalene, and isomers such as azulene, indene, chamazulene.

Our study demonstrated rice plants grown in soil contaminated with crude oil can uptake TPH including PAHs present in the contaminated soil, which leads to the possibilities of health hazards to humans and grazing animals that use the plants as their fodder. Moreover, increased levels of hydrocarbon contaminants in the agricultural soil lead to alteration of some of the vital physico-chemical properties of the soil, which have an adverse effect on the growth of crop plants leading to reduction of crop yield. This study demonstrated it is advisable to examine or evaluate the area to be utilized for crop cultivation prior to the practice of cultivation in order to ensure health safety and achieve better crop yield.

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