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Impact of petroleum hydrocarbon on soil enzymatic activities in agricultural soil around a refinery

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ABSTRACT

The accumulative levels of Total petroleum Hydrocarbons (TPH) on agricultural soil around an oil refinery were monitored. Four major sites around the refinery were chosen: east, west, north and south. Experiments were done in two seasons: pre monsoon and post monsoon. The enzymes arylsulphatase and urease activities were measured to evaluate the effects of TPH on soil biochemical characteristics. Results showed that the study area were soil were contaminated by TPH. Soil TPH and arylsulphatase activity varied in the sequence west>north>south>east in both the seasons. However, the changes in urease activity showed directional variation in both the seasons. Correlation studies illustrated that at the current pollution level, soil TPH concentration was positively correlated with arylsulphatase activities. On the contrary, the urease activities showed a significant negative correlation with the TPH concentration. This indicates that soil enzymatic activities could be used as a sensitive biological indicator of petroleum contamination. This study will be supportive to use proper bioremediation technique to detoxify the petroleum contamination for their ultimate removal from the soil.

KEYWORDS

Petroleum hydrocarbon;
Enzyme activity;
Arylsulphatase,
Urease.

INTRODUCTION

The petroleum industry is one of the major contributors of different contaminants to the environment. The emergent threat to the natural environment caused by petroleum wastes due to release of effluent in the form of wastewater and gas flaring has assumed great importance during the past few years because of increased awareness of its environmental implications. The wastewater released by the refineries are characterized by the presence of large quantities of polycyclic and aromatic hydrocarbons, phenols, metal derivatives, surface-active substances, sulphides, naphthylenic acids and other toxic chemicals. Gas flaring is the controlled burning of natural gases associated with oil production. The consistent flaring left a devastating effect on the surrounding environment. Gas flares have potentially harmful effects on the health and livelihood of the communities in their vicinity, as they release a variety of poisonous chemicals including nitrogen dioxides, sulphur dioxide, volatile organic compounds like benzene, toluene, xylene and hydrogen sulfide, as well as carcinogens like benzapyrene and dioxin. Every year coastal areas discharge 600 tons of hydrocarbons of industrial and urban origin, in addition to the 200 tons discharged by refineries alone. While such contaminant penetrates into the soil, it disturbs the structure of the soil and modifies its physico-chemical properties. They also affect the biological properties of the soil by modifying the populations of particular microorganisms. This in turn, affects the soil enzymatic activity and the content of assimilable macro and microelements forms in it. Under such conditions, the take up of macro elements by plant changes which affects the growth and productivity of the arable crops. Besides, soil enzymatic activities are sensitive biological indicator of soil pollution and could be changed under pollution stress. It is an important soil attribute and may serve as a robust measure of soil health and cultivation potential[9].

The effects of heavy metals and petroleum hydrocarbons on soil enzymatic activities have been reported earlier[1, 3, 5, 7, 9, 14]. Soil enzymes participate in many biological processes in soil and offer a useful assessment of soil 'function'. Petroleum hydrocarbons are highly recalcitrant and their biological transformation mediated by bacteria and fungi represents a potential route for their ultimate removal from the soil environment. Moreover, among several clean-up techniques available to remove petroleum hydrocarbons from the soil and groundwater, bioremediation techniques are gaining importance due to their ease, higher efficiency and cost efficacy compared to other technologies.

The aim of the present study was to determine the enzymatic activities of the agricultural soil surrounding the Bongaigaon Refinery of Assam, India. The study also includes the impact of paddy soil total petroleum hydrocarbon on soil enzymatic activity.

MATERIALS & METHODS

Site description

The area chosen for the study is in the vicinity of Indian Oil Corporation Ltd. (Bongaigaon Refinery); formerly known as Bongaigaon Refinery and Petrochemical Limited (BRPL), Dhaligaon,(latitude 26.47^{0}N and longitude 90.57^{0}E) Assam, India. The effluents of Bogaigaon refinery drains into a small rivulet the Tunia nala originating from the refinery complex and ultimately drains into river Tunia in the southern side of the refinery. This river passes through the agricultural lands of several villages located along its way. These agricultural lands are situated at a distance of about 3 km apart from the refinery. The water of this river is used by the farmers for raising crops. In the northern and western side, there are two gas flaring points surrounded by bare agricultural lands where the effect of the effluent does not come in contact. In the eastern side of the refinery there is scattered human habitation. The effects of long-term petroleum containing wastewater irrigation and also the effects of the gas flaring on the soil of agricultural land surrounding the Bongaigaon Refinery have not yet been studied. To understand if soil functions are affected by the refinery activities, soil total petroleum hydrocarbon and enzymatic activities were taken as a measure of the soil health to evaluate the pollution stress.

Sample collection

Soil samples were collected from all the four directions (east, west, north and south) during pre monsoon (in March) and post monsoon (in October) season. Five different locations had been randomly identified within 250m vicinity from the boundary wall of the refinery. Only top soil (0-25 cm) from each site was collected at an interval of 50m. The collected samples were brought to the laboratory in polythene bags and stored in the refrigerator at 4^{0}C until analysis.

The collected samples were analyzed for total petroleum hydrocarbon (TPH) and the arylsylphatase activity (ASA) and urease activity (UA).

Experimental

Soil total petroleum hydrocarbon was extracted by soxhlet extraction using dichloromethane as solvent.

Arylsulphatase activity as µg p-nitrophenol/g dry sample was measured as follows: 0.25 ml toluene, 4 ml acetate buffer (pH 5.5) and 1 ml of 0.115 M p-nitrophenyl sulphate (potassium salt) solution were added to the 1 g sample. After incubating the samples for 1 h at 37°C, the formation of p-nitrophenol was determined spectrophotometrically at 410 nm[11, 12].

Urease activity was measured by the method of Hoffmann und Teicher as follows: 7.5 ml citrate buffer (pH 6.7) and 10 ml of 10% urea substrate solution were added to 5 g soil, and subsequently the samples were incubated for 3 h at 37 °C. The volume was made up to 100 ml with distilled water at 37 °C. Following filtration through Whatman No. 42 filter papers, 1 ml of filtrate was diluted to 10 ml with distilled water, and 4 ml of sodium phenolate (12.5% (w/v) phenol + 5.4% (w/v) NaOH) and 3 ml of 0.9% sodium hypochloride were added. The released ammonium was determined spectrophotometrically at 578 nm.

All the results obtained from the above analysis were compared with a soil sample (control C) taken from an area where the affect of the refinery does not come in contact.

RESULTS AND DISCUSSION

Total petroleum hydrocarbon

The average concentrations of TPH for both the seasons are shown for in TABLE 1.

TABLE 1 : Mean concentrations of TPH

direction	season	TPH (mg/kg)	Control
South	Pre monsoon	720.12	BDL
	Post monsoon	589.99	
North	Pre monsoon	942.22	
	Post monsoon	663.20	
West	Pre monsoon	1231.31	
	Post monsoon	956.73	
East	Pre monsoon	210.10	
	Post monsoon	159.30	

The first observation to make from the analyzed data is that all the soil samples were highly contaminated by TPH with respect to the control soil and the contamination is higher in pre monsoon season than the post monsoon. The reason for this variation may be due to the fact that higher fractions of hydrocarbons evaporate more or less rapidly specifically in hot climates, and contribute to atmospheric pollution, which ultimately deposits in the soil. In premonsoon season it is very likely that they stay more time in suspension with the environment than the post-monsoon. On other hand, in the rainy season, (postmonsoon) the particles act as cloud condensation nuclei (CCN), performed moist deposition. For this reason, the pollution particles remain in the atmosphere less time than dry season (premonsoon). Total petroleum hydrocarbon accumulation in the soil is affected by the absorption of soil particles and the degradation of microorganisms.

Another major observation is that contamination was higher in the western direction and varies in the sequence west>north>south>east. This variation may be due to the presence of gas flaring points in the western and the northern direction which increases the amount of TPH in these directions.

A comparative representation of the spatial and seasonal variation pattern of TPH in all the four directions is shown in Figure 1.

Enzymatic activity

The average arylsulphatase activity and the urease activity in all the four directions of the soil samples are shown in Figure 2 and Figure 3 respectively.

Arylsulphatase enzymes are responsible for the hydrolysis of aromatic sulphate esters to phenols and sulphate or sulphate sulphur in the soil. This enzyme plays an important role in the cycling of sulphur and can be an indicator of the mineralization of sulphur compounds in soil[6].

$$R-O-SO_3^- \xrightarrow[\text{Arylsulphatases}]{\text{Hydrolysis}} R-OH+SO_4^{-2}$$

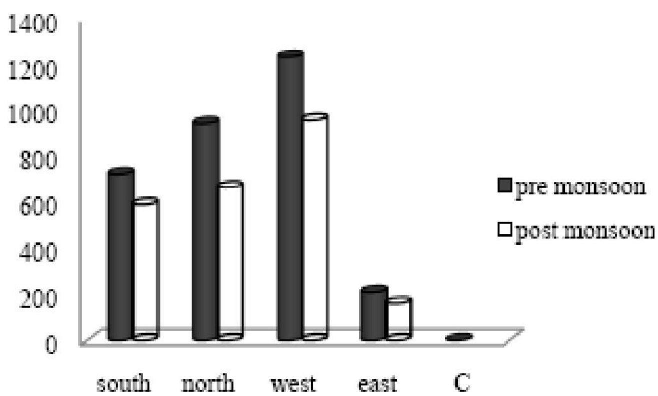

Figure 1 : Mean TPH concentration

Studies have shown that the release of sulphate from soluble and insoluble sulphate esters in the soil is affected by various environmental factors such as heavy metal and petroleum hydrocarbon pollution, p^H changes, organic matter content and its type in the soil.

The present study indicates that arylsulphatase activities were higher in post monsoon season than the

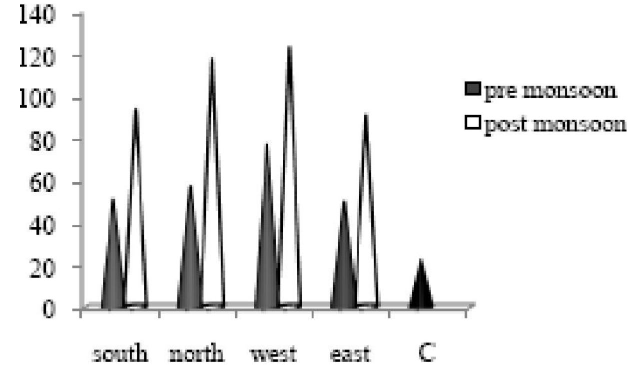

Figure 2 : Arylsulphatase activity

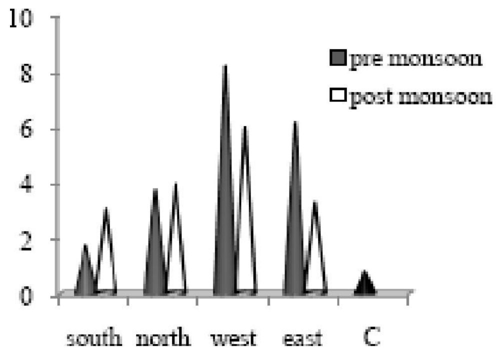

Figure 3 : Urease activity

pre monsoon. The value for the control soil was 22.14 mg p- nitrophenol produced/g soil/hr, which is much lower than the collected soil samples. These findings are similar to those of[2] and Jenkinson[8] who reported that arylsulphatase activity increased in wet conditions. They concluded that this phenomenon is largely due to more mineralization of organisms killed or damaged in dry season. Cooper[4] also concluded that during rainy season, when soils were continually moist, activity increased, but at the end of the rainy season, as the soils dried out, aryl sulphatase activity was again reduced. It was also observed that the activities for both the season followed the sequence west>north>east> south.

Urease enzyme is responsible for the hydrolysis of urea fertilizer applied to the soil into NH_3 and CO_2 with concomitant rise in soil p^H.

$$NH_2CONH_2 + H_2O \rightarrow 2NH_3 + CO_2$$

This in turn, results in a rapid N loss to the atmosphere through NH_3 volatilization. Due to this role, urease activities are considered vital in the regulation of N supply to plants after urea fertilization. However, urease activity in soils is influenced by many factors like organic matter content of the soil, soil amendments, heavy metals, petroleum hydrocarbons etc.

It was observed from the present research that the changes of urease activities were not uniform for both the seasons. The value for the control soil was 0.782 mgN hydrolyzed/g soil/3 hr. The activities for the southern and the northern direction did not show a regular trend while for the western and the eastern direction the activities were lower in post monsoon season. This seasonal variation may be due to the urea fertilizer applied on the soil for the cultivation.

These variations for both the enzymes can be well understood by the correlation study with Total Petroleum Hydrocarbons.

TABLE 2 : Correlation coefficient between TPH (pre monsoon)

		ASA	UA	TPH
	ASA	1		
South	UA	-0.756	1	
	TPH	0.985*	-0.634	1
	ASA	1		
North	UA	-0.827	1	
	TPH	1.000*	-0.956*	1
	ASA	1		
West	UA	-0.715	1	
	TPH	0.787**	-0.998*	1
	ASA	1		
East	UA	-0.879	1	
	TPH	0.999*	-0.894*	1

TABLE 3 : Correlation coefficient between TPH (post monsoon)

		ASA	UA	TPH
	ASA	1		
South	UA	-0.999*	1	
	TPH	0.998*	-1.000*	1
	ASA	1		
North	UA	-0.827	1	
	TPH	0.984*	-0.724	1
	ASA	1		
West	UA	-0.715	1	
	TPH	0.994**	-0.636*	1
	ASA	1		
East	UA	-0.879	1	
	TPH	0.882*	-1.000**	1

Correlation between Enzymatic Activity and TPH

Enzyme activity is a sensitive indicator of petroleum contamination[9]. The community structure and diversity of microbial communities and enzymatic activities in the soil ecosystem could be changed under the pollution stress. In this research, TPH shows significant positive correlation with arylsulphatase activity while it shows negative correlation with urease activity in both the seasons (TABLE 2 & TABLE 3).

For arylsulphatase activity highest correlation was found in the western direction for both the seasons ($r=0.787$ and 0.994; $p<0.01$). This may be explained by the fact that both arylsulphatase activities and TPH concentrations were higher in this direction for both the seasons. The increase in arylsulphatase activity indicates that petroleum hydrocarbon utilizing bacteria were present in the soil which can tolerate oil contaminated environments because they possess the capability to utilize oil as energy sources. The high content of organic carbon compounds in petroleum wastes stimulates the activity of the enzyme by increasing the microbial population. This in turn helped in mineralization and degradation of organic matter present in the petroleum wastes. In the present study it was found that TPH concentration was lower in postmonsoon than the premonsoon while arylsulphatase activity was higher in postmonsoon than the premonsoon. Thus it may be concluded that the enzyme arylsulphatase facilitates the degradation of petroleum hydrocarbon present in the soil. In pre monsoon season due to hot and dry climatic condition the microorganisms present in the soil dies and as a result the fractions of petroleum hydrocarbons get hydrated and agglomerate in the soil. In the postmonsoon season the activity increases due to the regrowth of the microorganisms which degraded the petroleum hydrocarbons present in the soil. However, it was reported that only the intermediate fractions of petroleum hydrocarbons are biodegradable while heavy fractions are not easily degradable[10]. Thus heavy fractions of hydrocarbons may accumulate in the soil forming a superficial films which hinder the normal functioning of the soil.

Urease activity shows negative correlation with TPH. The correlation is significant in all the directions except in the pre monsoon season of southern side and post monsoon season of northern side. One of the possible explanations of this phenomenon can be based on the recent assumptions that lipophilic toxic compounds such as polycyclic aromatic hydrocarbon present in refinery wastes may interact with lipophilic components of cytoplasmitic membrane of bacteria, thus affecting their permiability and structure, which reduces their activity. Further, it may be due to the high concentration of available nitrogen in the petroleum wastes. Decline in urease activity in presence of TPH prevents the utilization of urea fertilizer applied to the soil for cultivation which deteriorates the soil health.

CONCLUSION

The present investigation showed that refinery operations had contaminated the soil by petroleum hydrocarbons. The western direction is highly contaminated by TPH and the contamination is higher in pre monsoon season. The correlation studies indicate that aryl sulphatase activity shows significant positive correlation with TPH while urease activity shows negative correlation. Biodegradation by natural populations of microorganisms is the basic and the most reliable mechanism by which xenobiotic pollutants, including crude oil are eliminated from the environment. Growth stimulation of indigenous microorganisms inoculated with foreign oil degrading bacteria is a promising way of accelerating decontaminating and degrading activities at a polluted site with minimum impact on the ecological systems. The study will be helpful to use proper bioremediation technique to detoxify the petroleum hydrocarbons for their ultimate removal from the soil. This will also help to assess soil fertility status for long term sustainable agricultural productivity with minimal environmental impact. However, the study suggests that chemical analysis alone is not adequate for toxicological estimations and should be used in conjunction with bioassays to decontaminate the polluted soil.

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