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Impact of PAH on biological health parameters of soils of an Indian refinery and adjoining agricultural area—a case study

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Abstract The present study is aimed at analysing and comparing different soil enzymes in soil samples of native contaminated sites of a Mathura refinery and adjoining agricultural land. Enzyme activities are considered as indicators of soil quality and changes in biogeochemical function due to management or perturbations. Soil samples were collected from the premises and nearby area of Mathura refinery, India. Biological health parameters (dehydrogenase, aryl esterase, aryl sulphatase, β-glucosidase, alkaline phosphatase, acid phosphatase, lipase, laccase and catalase activity) were estimated in the soil samples. Among all the samples, sewage sludge soil showed maximum activity of enzymes, microbial biomass carbon and most probable number of polycyclic aromatic hydrocarbon (PAH) degraders in soils spiked with three- to four-ring PAHs at 50 ppm. Available phosphorus, potassium and nitrogen was also exceptionally high in this sample, indicating maximum microbial bioconversion due to presence of nutrients stimulating potent PAH-degrading microorganisms.

Keywords Bio impact · MPN · PAH · Soil enzymes

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are chemicals belonging to a class of hydrophobic compounds consisting of two or more fused benzene rings in linear, angular or in cluster form. On the basis of their configuration, PAHs are divided into three classes: two- to three-ring PAHs defined as low molecular weight PAHs, four-ring PAHs as medium molecular weight PAHs, whereas five- to six-ring PAHs are defined as high molecular weight PAHs. Urbanization and industrialization has resulted in accumulation of these compounds in the ecosystem mainly through incomplete combustion of organic material, fossil fuel, petroleum product spillage and partly from natural sources such as forest fires and volcanic eruptions (Brandt and Watson 2003). Members of this class have been found to exhibit

toxic/hazardous properties (Dzombak and Luthy 1984). Based on their ecotoxicity, the US Environmental Protection Agency (US EPA) has listed 16 PAHs as priority pollutants for remediation (Liu et al. 2001). The International Agency for Research on Cancer has identified 15 PAHs as potential carcinogens. After being emitted into the atmosphere, they are redistributed between the gas and particle phase and subsequently deposited in the terrestrial environment through dry or wet deposition (Wong et al. 2004). Deposited PAHs are transported over a long distance, thus the soil is contaminated with PAHs mainly through atmospheric deposition, directly or via vegetation or through anthropogenic sources. Soil contamination by PAH is considered to be a good indicator of the level of environmental pollution by human activities (Chung et al. 2007). Concentration of PAHs present in Indian soils has been reported to be 6.7 μg g^{-1} in agricultural land, 9.3 μg g^{-1} in residential, 12.9 μg g^{-1} along roadside and 13.7 μg g^{-1} in industrial region (Fadzil et al. 2008). Few reports reveal the ambient PAH concentration in Delhi airport soil, bank of Yamuna river (Tyagi 2004; Ray et al. 2008; Agarwal et al. 2009), Mumbai and Ahmadabad (Sahu et al. 2001; Raiyani and Shah 1993). Bishnoi et al. (2009) reported 51.59–148.82 mg kg^{-1} dry weight of PAH in the Hisar region. Soil may be looked on as a biological entity, i.e. a living tissue with complex biochemical reactions depicting soil health (Quastel 1946). Each of the organic and mineral fractions in soil has a special influence on enzyme activity. In practice, the biochemical reactions are brought about largely through the catalytic contribution of enzymes and variable substrates that serve as energy sources for microorganisms (McLaren 1975). These enzymes may include aryl esterase, aryl sulphatase, glucosidase, dehydrogenase and phosphatase released from plants microbiota or microorganisms of soils. A better understanding of the role of these soil enzymes activities in the contaminated ecosystem will potentially provide a unique opportunity for an integrated biological assessment of soils. Therefore, the present study was undertaken to assess the bioimpact of PAHs in soil collected from Mathura refinery with a special reference to anthracene, fluorene, phenanthrene and pyrene, and compare the same with adjoining agricultural field soil.

Materials and method

Site location and description

Mathura refinery was commissioned in the year 1982 as India's sixth oil refinery, nestled between the historic cities of Delhi and Agra; the refinery at Mathura is situated at 27°30′N 77°41′E 27.5°N 77.68°E. For the purpose of soil sample collection, Mathura refinery premises was divided into seven parts based on the locations of different petroleum processing waste outlets and the flanking garden. Samples were collected with the help of an auger from 0 to 15 cm of topsoil from five different points within same area. A total of 35 soil samples were collected (five each from seven locations), i.e. Mathura agricultural field (village—Dhana Teja, 1 km from refinery) Mathura refinery agricultural field (100 m range), sewage sample Mathura refinery which was accumulated outside the outlet pipe discharging refinery waste, bitumen unit Mathura refinery, kerosene unit Mathura refinery, aviation turbine fuel refining unit and sulphur unit (sulphur depositions in soil through chimney of refinery). Samples from each location were pooled together, air-dried, sieved through 200 mesh sieve, stored in polybags and kept in refrigerator till further analysis.

Most probable number of PAH degrading microorganism in soil samples

Samples were diluted in a saline buffer containing 0.1% sodium pyrophosphate (pH 7.5) and 2% NaCl. PAH substrate (anthracene, fluorene, phenanthrene and pyrene) was added in triplicate to a 96-well microtitre plate both individually as well as in an equimolar mixture of the four PAHs in separate wells as a solution in acetone from their respective stock solutions to provide a final concentration of 50 μg PAH in respective wells. Acetone evaporated rapidly, depositing PAH onto the surface inside each well. The Bushnell and Haas medium (Bushnell and Haas 1941; 180 μl) supplemented with 2% NaCl was added

as growth medium. Tenfold serial dilutions of soil were performed, and the wells were inoculated by adding 20 µl of soil dilution to each row of eight wells in a 96-well titre plate. The 10^{-10} dilution was inoculated into row 11, the 10^{-9} dilution was inoculated into row 10, and so on. The first row of each plate was inoculated with 20 µl of undiluted sample, and row 12 remained uninoculated to serve as sterile control. After 2 weeks of incubation at 30 ± 1°C, 50 µl of filter, sterilized iodonitrotetrazolium (INT) violet (3 g/l), was added to each well in these plates. INT is reduced to an insoluble formazan by microbial cells that deposit intracellularly as a red precipitate. Wells showing positive reaction were scored after an overnight incubation at room temperature with INT. The most probable number (MPN) values were calculated after comparing with the published MPN tabulated values of Cochran (1950).

Soil physicochemical analysis

Electrical conductivity and pH measurements were performed for suspension of soil in distilled water (1:5 *w*/*v*) by electrometric determination using handheld digital EC meter and pH meter, respectively. Organic matter was estimated by ignition method for 4 h at 550°C in a muffle furnace and total organic carbon was determined by dichromate oxidation method of Walkley and Black (1934). Available nitrogen, phosphorus and potassium of soil were estimated by standard protocols according to Jackson (1973). Humus content of soil was estimated by method of Kononova (1961).

Microbial biomass carbon

Pre-weighed freshly removed soil (17.5 g) was placed in a Schott bottle and fumigated with chloroform. One set of soil was maintained without fumigation. The soil samples were incubated in dark for 24 h. After incubation, the bottle was placed in a fume hood for 30 min, until chloroform evaporated completely and 0.5 M K_2SO_4 (70 ml) was added to both fumigated and unfumigated samples. The bottles were shaken in an end-to-end shaker for 30 min. The extract was filtered through a Whatman no. 42 filter paper. Optical density of the fumigated and unfumigated extract at 280 nm was taken immediately and the microbial biomass carbon was measured by method of Nunan et al. (1998) using aliquots of K_2SO_4 extract through dichromate digestion method. Microbial biomass carbon was calculated after back titration with ferrous ammonium sulphate using the equation:

$$\text{Biomass} = 2.64 \times C_{\text{E}}$$

where C_E = (organic C from fumigated soil) - (organic C from non-fumigated soil).

Microbial biomass carbon was expressed as micrograms carbon per gram soil.

Enzyme assay in soil

Dehydrogenase activity

Dehydrogenase activity was assayed using soil (6 g), incubated with triphenyl tetrazolium chloride (3%) for 24 h in the dark. Methanol was added to terminate the enzymatic reaction. The supernatant was filtered and absorbance taken at 485 nm (Casida et al. 1964). The values were expressed as micrograms of triphenyl formazon (TPF) per gram of soil per day.

Aryl esterase

Aryl esterase activity was assayed in soil (1 g) suspended in 4 ml of modified universal buffer and 1 ml *p*-nitrophenyl acetate (0.025 M) solution (Zornoza et al. 2009). After incubation for 1 h at 37 ± 1°C, the enzyme reaction was stopped by addition of 4 ml of 0.5 M NaOH. The suspension was filtered through a Whatman no. 42 filter paper. The absorbance was measured at 430 nm and the enzymatic activity was expressed as micrograms of *p*-nitrophenol per gram of soil per hour.

Aryl sulphatase

Aryl sulphatase activity was assayed in reaction mixture containing soil (1 g), 0.2 ml of toluene, 4 ml of acetate buffer, 1 ml of *p*-nitrophenyl sulphate solution (0.025 M), prepared in buffer (Tabatabai and Bremner 1970). After incubation at 37 ± 1°C for 1 h, the enzyme reaction was

stopped by addition of 4 ml of 0.1 M NaOH. The soil was filtered through a Whatman no. 42 filter paper. The absorbance was measured at 430 nm and the enzymatic activity was expressed as micrograms of *p*-nitrophenol per gram of soil per hour.

β−*Glucosidase*

β−Glucosidase activity was assayed by taking 1 g of soil suspended in 4 ml of modified universal buffer and 1 ml of *p*-nitrophenyl-β-glucosidase (0.025 M) solution. After 1 h incubation at 37 ± 1°C, 4 ml of 0.1 M tris-hydroxyl amino methane was added to stop enzymatic reaction and supernatant was filtered. The absorbance was taken at 430 nm against a standard curve of *p*-nitrophenol (Tabatabai 1994). The activity was represented as micrograms of *p*-nitrophenol released per gram of soil per hour.

Alkaline phosphatase and acid phosphatase

Alkaline phosphatase and acid phosphatase activity was assayed in soil (1 g), suspended in 4 ml modified universal buffer (MUB) at pH 11 for alkaline phosphatase and MUB pH 6 for acid phosphatase (Skujins et al. 1962; Tabatabai and Bremner 1969). Soil was incubated with 1 ml *p*-nitrophenyl phosphate (0.025 M). After incubation at 37 ± 1°C for 1 h, the enzyme reaction was stopped by addition of 4 ml 0.5 M NaOH. The suspension was filtered through a Whatman no. 42 filter paper. The absorbance was measured at 430 nm and the enzymatic activity was expressed as micrograms of *p*-nitrophenol per gram of soil per hour.

Lipase activity

Lipase activity was estimated by method of Margesin et al. (1999). Moist soil (0.1 g) was weighed into centrifuge tubes, mixed with 5 ml 100 mM NaH_2PO_4/NaOH buffer (pH 7.25). Then 40 μl of substrate solution (0.05 M *p*-nitrophenyl palmitate) was added. The contents were mixed and the tubes were incubated in the water bath at 37 ± 1°C for exactly 30 min. In order to measure the *p*-nitrophenol (*p*-NP) released from the substrate, a control was prepared without soil. To stop the reaction, the tubes were cooled for 10 min on ice. Afterwards, the contents were centrifuged at 2,000 rpm at 2°C to 4°C for 5 min. The supernatant was pipetted in test tubes that were held on ice. The absorbance of the released *p*-NP was measured spectrophotometrically at 430 nm against the reagent blank. Lipase activity was represented as IU g^{-1} of soil. One IU represents one micromole of *p*-nitrophenol released per minute by the enzyme.

Laccase activity

Laccase activity was determined by measuring change in absorbance at 436 nm with 5 mM ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) as substrate (Munoz et al. 1997). In a cuvette, 1 ml soil filtrate was taken along with equal volume of 0.05 M citrate buffer (pH 4.8); assay was initiated by adding 0.2 ml ABTS (5 mM) and absorbance was recorded at 436 nm for 180 s at 30-s intervals.

Catalase activity

Catalase activity was estimated by a potassium permanganate titration method (Guan et al. 1991). Forty milliliters of distilled water and 5 ml of 0.3% hydrogen peroxide solution was added to 2 g of soil in a wide-mouthed flask (100 ml) and the reaction mixture was shaken at 125 rpm for 20 min at 25°C. The action was stopped with 5 ml of sulphuric acid (1.5 M) and the suspension was filtered through a Whatman no. 42 filter paper. Twenty-five milliliters of the filtrate was extracted and subjected to potassium permanganate titration (0.1 M $KMnO_4$). Catalase activity was expressed as milliliters of $KMnO_4$ per gram of soil.

Statistical analysis

All the estimations were performed in triplicate and the mean was calculated as per standard procedure. Correlation analyses were undertaken using Microsoft Excel package. Standard deviation values are depicted in the graphs as error bars.

Results and discussion

Soil physicochemical analysis

The soil samples were analysed for pH, EC, organic carbon, humus, and availability of nutrients (Table 1). All the soil samples had nearly neutral pH, varying between 6.3 and 7.3. Higher values of organic carbon were recorded in sewage sample of Mathura refinery followed by Mathura refinery field soil. In the rest of the samples, it was 0.08% to 0.63%. Available N, P and K were found highest in the sewage sample, especially the P which was 241.92 kg ha^{-1} while in other sample it ranged between 13.44 and 87.36 kg ha^{-1}. Electrical conductivity was highest in sulphur unit sample (3.5 mS cm^{-1}) followed by kerosene unit which recorded 2.1 mS cm^{-1} of EC.

MPN of PAH utilizing microorganism in soil samples

MPN procedure permits the selective enumeration of PAH degrading bacteria in contaminated soil. Earlier studies showed the presence of PAHs in refinery soils. A study of PAH content in several locations in Agra, which is adjacent to the site of Mathura refinery revealed that the average concentration of PAH in all samples ranged from 3.1 to 28.5 μg/ g soil (Masih and Taneja 2006). Total mean concentrations of PAHs were found to be ranged from 4.43–22.53 μg g^{-1}. Fluoranthene, Chrysene and benzo (*b*) fluoranthene were found to be the most abundant PAHs at this location (Rawat and Sharma 2008). Reduction of INT was used to detect the presence of PAH-utilizing microbes in the soil samples collected from Mathura refinery. Tetrazolium dye, INT is reduced by respiratory electron transport systems to insoluble formazans that are highly coloured (Packard 1971; Rodriguez et al. 1992; Atlas 1979; Colwell 1979; Roubal and Atlas 1978). MPN method was used to count the number of culturable PAH degraders in environmental sample as this method detects either growth on a mixture of PAHs (Johnsen and Karlson 2005; Wrenn and Venosa 1996) or growth on individual PAH like anthracene, phenanthrene, fluorene and pyrene. Thus, INT reduction is a sensitive indicator of growth and is generally useful for detection of positive dilutions in MPN assay. In the present study, different soils responded in different patterns. The higher MPN of PAH degraders were recorded in sewage sludge indicating the presence of resident microflora-comprising fluorene and pyrene-degrading microorganisms (Fig. 1). The formation of a red colour was highest in the presence of 50 ppm fluorene and pyrene and decreased at higher PAH concentrations; this implies that three- to four-ring PAH degraders could grow well on individual PAHs (fluorene and pyrene) but growth was poor in PAH mixture.

Table 1 Physicochemical properties of soil collected from different locations of Mathura refinery premises and agricultural land

Location	Site description	pH (soil: water; 1:5)	Organic carbon (%)	Electrical conductivity (mS cm^{-1})	Humus (%)	Available macronutrients (kg ha^{-1})		
						P	N	K
M 1	Mathura agricultural field soil	7.1	0.58	0.6	0.34	44.8	188.16	254.91
M 2	Mathura refinery field soil	6.9	1.3	0.6	0.60	13.44	150.83	141.12
M 3	Sewage Mathura refinery	6.5	1.5	0.8	0.40	241.92	376.32	478.91
M 4	Bitumen Mathura refinery	7.1	0.54	0.5	0.10	33.6	37.63	158.14
M 5	Kerosene Unit Mathura refinery	7.2	0.63	2.1	0.70	15.68	50.18	235.65
M 6	A.T.F	7.3	0.44	1.2	0.70	17.92	–	250.43
M 7	Sulphur Unit Mathura refinery	6.3	0.08	3.5	0.13	87.36	238.34	163.07

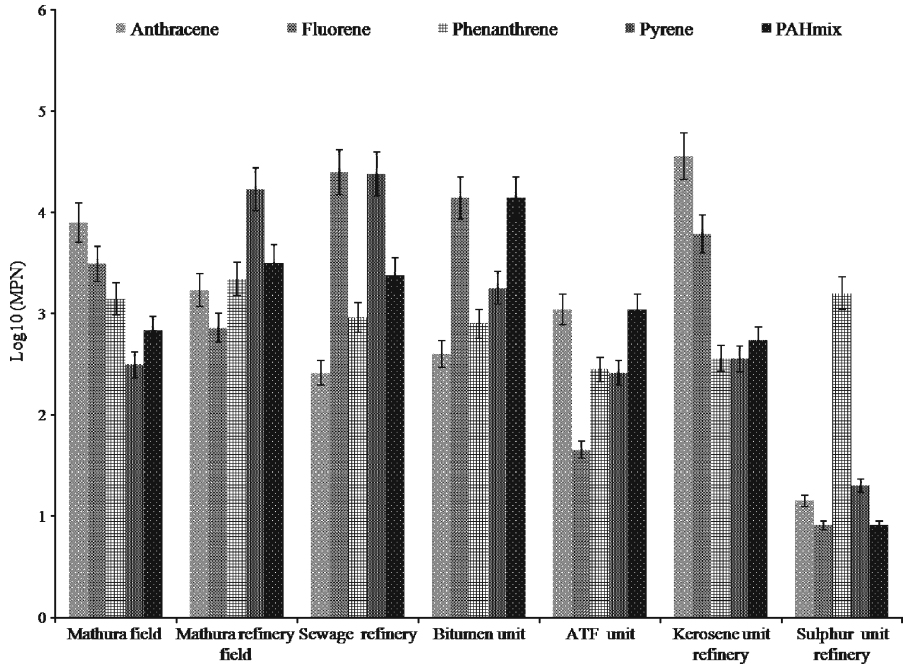

Fig. 1 Log_{10} MPN of PAH utilizing bacteria in the soil sample collected from Mathura refinery

The maximum amount of INT was reduced in sewage sludge soil and the minimum amount in sulphur unit soil plate spiked with three- and four-ring PAHs. Although the MPN method probably underestimates the number of PAH degraders by detecting only those cells which are able to grow in minimal media with PAHs as the sole source of carbon and energy, INT reduction is superior to some of traditional methods such as turbidity and measurement of biomass protein (Wrenn and Venosa 1996; Chiou et al. 1998) as these methods can lead to false negative interpretations if growth is closely associated with the water–hydrocarbon interface (Johnsen and Karlson 2005). But INT reduction will occur only when cells are metabolically active as observed in these soils. Anthracene was maximally utilized by sulphur unit soil in contrast with other PAH which were poorly metabolised in this soil (Fig. 1). Fluorene and pyrene had shown less inhibition in the sewage sludge of the refinery. In the seven samples collected from the refinery area, 66 bacterial isolates were purified. Out of these, 15 potent isolates capable of utilizing high concentration of PAH (fluorene, anthracene, phenanthrene and pyrene) were identified and sequenced. Predominant communities were also identified which were as follows: In agricultural field of Mathura refinery *Bacillus* (HQ536219), *Lysinibacillus* (HQ536218) and *Serratia* sp. (HQ 536226) were dominant genera. A total of nine potent bacteria were isolated from sewage sample of Mathura refinery. *Acinetobacter* (HQ536214), *Brevibacterium* (HQ536221) and *Bacillus* (HQ536223) were the dominant genera therein. *Streptomyces rochei* (GQ904711), a potent PAH-degrading strain, was isolated from bitumen unit of refinery (Chaudhary et al. 2011). In all other refinery soil samples, *Bacillus* was the most predominant genus.

Microbial biomass carbon

PAHs tend to interact with non-aqueous soil organic matter because of high hydrophobicity and solid water–waste distribution ratios. Soil organic matter contribute to soil fertility both directly by releasing major inorganic nutrients as well as trace elements during its decomposition and indirectly by increasing cation exchange capacity and by improving soil structure and water-holding capacity. It is being acknowledged that soil organic matter is a principal factor in influencing the distribution of PAH and presence of PAH influence the bioavailability of nutrients as well as soil enzymes which are primarily expression of bacteria, fungi and plant roots responsible for the flux of carbon,

nitrogen and other essential elements in the biogeochemical cycle. In soil, organic compounds like PAHs are processed by heterotrophic microorganisms that use organic carbon as nutrients and energy sources, resulting into a healthy state of soil which is stable and resilient to stress. The percentage of microbial C in total organic carbon C in soil is closely related to the capacity of soil to support microbial life (Anderson and Domsch 1989). The microbial biomass carbon was found to vary from 27.26 to 773.11 μg C g^{-1} soils in Mathura refinery soil. The highest microbial biomass carbon was recorded in sewage sludge soil (Fig. 2) and least values were recorded in kerosene unit. The percentage of organic C and available N, P, K observed in sewage sludge is given in Table 1. The higher values of N, P and K may be a factor for higher microbial biomass carbon in this soil sample.

Quantitative evaluation of enzyme

The quantitative evaluation of nine hydrolytic enzymes (dehydrogenase, aryl esterase, aryl sulphatase, β-glucosidase, alkaline phosphatase, acid phosphatase, lipase, laccase, catalase activity) revealed the differences in soil of seven sites from Mathura refinery.

The dehydrogenase activity represents instant metabolic activities of soil microorganism. The active dehydrogenase in soils is a part of biological cells and only exists in living microbes. Dehydrogenase activity is also known to increase markedly with increasing active viable cells (Tabatabai and Bremner 1969). Dehydrogenase expressed as micrograms of TPF per gram soil per day varied in the range of 9.15 to 18.64 with maximum in bitumen unit soil as shown in Fig. 3. Interestingly, one of the potent actinomycete strains which was able to utilize and degrade all the four PAH compounds was isolated from this soil.

Aryl esterase enzyme catalysed the hydrolysis of toxic metabolites and is also involved in the degradation of xenobiotics and organophosphates (Chrost 1991; Speir and Ross 1978). Therefore, determination of aryl esterase activity in soil might be important for evaluating the response of soil microbial communities to organic contamination as well as to suggest remediation measures to assess the fate of aromatic compounds. Maximum activity of aryl esterase was recorded in bitumen unit followed by agricultural field soil (Fig. 4).

Aryl sulphatase participate in sulphur cycling and its activity was highest in the sewage unit of refinery soil (Fig. 5) which is rich in minerals as observed by its high ash content. This enzyme

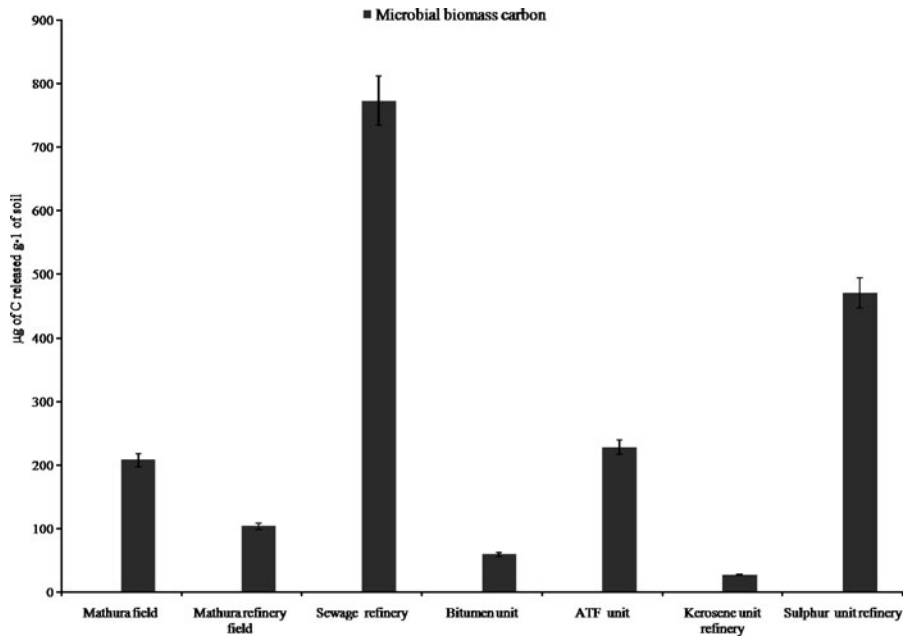

Fig. 2 Microbial biomass carbon collected in different soil samples collected from Mathura refinery

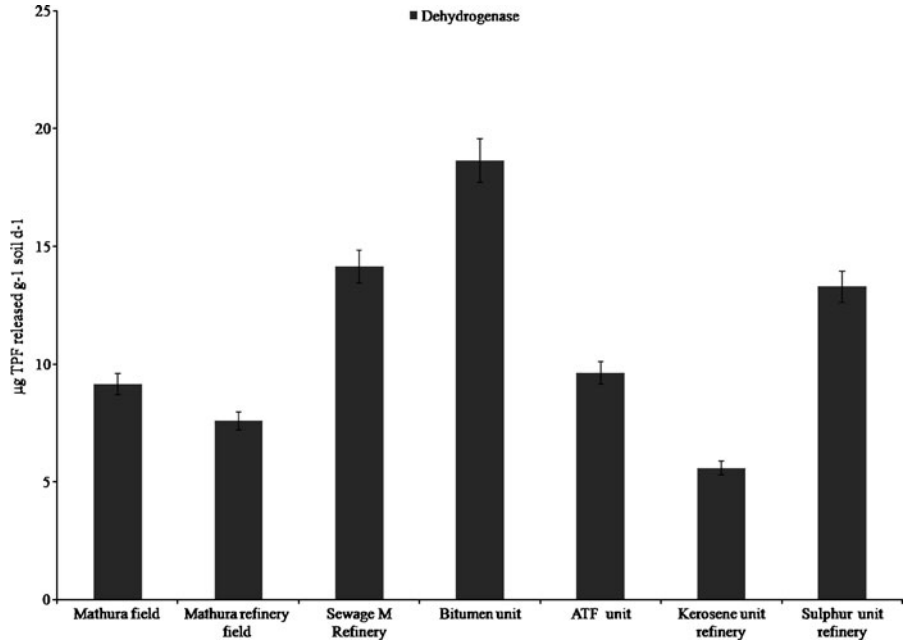

Fig. 3 Dehydrogenase activity of different soil samples collected from Mathura refinery

catalyzes the hydrolysis of organic sulphate esters and can sometimes constitute a rate-limiting step in S-cycling process. Aryl sulphatase mainly originates from fungi and bacteria, although plants and animals produce this enzyme too (Fitzgerald 1978; Dick and Tabatabai 1984).

Alkaline phosphatase activity is known to be related to soil organic matter content and highly correlated with microbial C as reported by several researchers (Tabatabai 1994; Kosaric 2001). This activity was exceptionally high in refinery sewage sludge (314 µg *p*-NP released g^{-1} soil h^{-1}) as

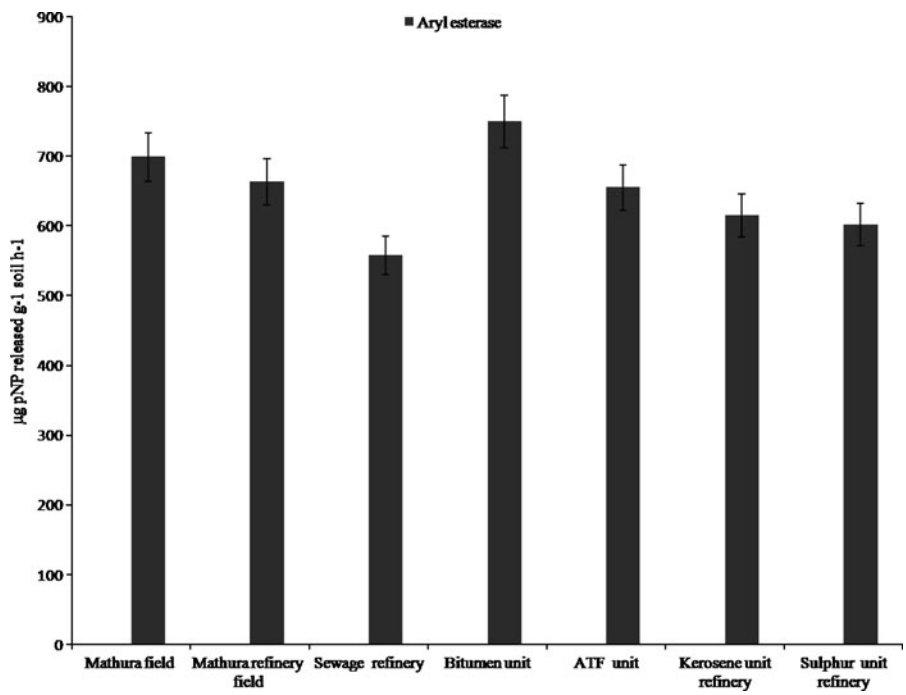

Fig. 4 Aryl esterase activity in different soil sample collected from Mathura refinery

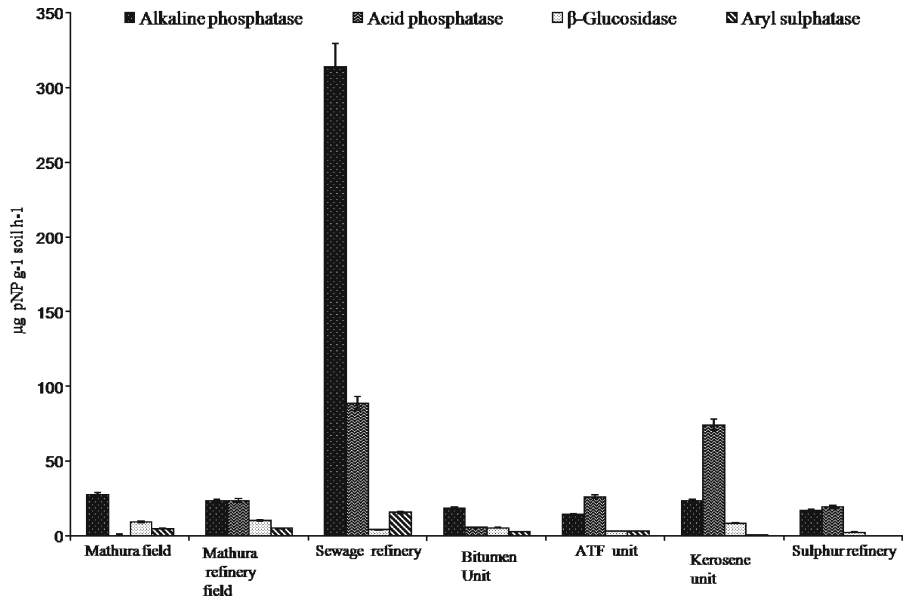

Fig. 5 Alkaline–acid phosphatase, β-glucosidase and aryl sulphatase activity in different soil sample collected from Mathura refinery

compared to rest of the soils (Fig. 5). In the rest of the soils, activity varied between 14.55 and 27.9 µg *p*-NP released g^{-1} soil h^{-1}. Acid phosphatase activity was also highest (89.04 µg *p*-NP released g^{-1} soil h^{-1}) in refinery sewage sludge followed by the soil from kerosene unit and minimum in Mathura agricultural field. Acid and alkaline phosphatase activities were therefore considered as the most satisfactory choice for determining the relative and total mass of microbial population in soils.

Soil β-glucosidase ranged between 2.727 and 10.69 µg p-NP released g^{-1} soil h^{-1} and was highest in Mathura refinery field. β-Glucosidase mainly hydrolyses dimers of glucose produced by cellulolytic microorganisms in soil. Its final product is glucose, an important C energy source for microbes in the soil. With β-glucosidase being sensitive to pH changes, the property can be used as a good biochemical indicator for measuring ecological changes resulting from soil acidification in situations involving activities of this enzyme. Consequently, deeper understanding of the β-glucosidase enzyme activities and factors influencing them in the ecosystem may contribute significantly to soil health.

The lipases are produced by varieties of soil microorganisms which increase the bioavailability of PAH to soil microbes. In general, products released from hydrocarbon degradation produces the substrates for hydrolases including esterase-lipases (Kosaric 2001). The measurement of lipase activity (Fig. 6) might represent a valid tool in monitoring the biodegradation of organic pollutants (Lin et al. 2009; Margesin et al. 2000). Lipase activity in these soils was very low; among seven soil samples, maximum activity was recorded in sulphur unit soil (0.023 IU $g^{-1}$of soil).

Laccase have been suggested to play a key role in PAH degradation (Vares et al. 1994). Most of the aromatic compounds are degraded by lignin-degrading enzymes and H_2O_2 serving as co-substrate for lignin peroxidases (Arun et al. 2008). Lignin enzymes produced by basidiomycete fungi as lignin peroxidase, manganese peroxidase and catalase efficiently participate in PAH degradation as lignin structure is very similar to PAHs. In the present study, laccase activity was found to be highest in the sulphur unit followed by sewage sludge. In general, catalase activity has a role in catalyzing the decomposition of hydrogen peroxide, which is harmful to the organism and was found to be highest in refinery sewage sludge followed the sulphur unit (Fig. 6).

The microbial activities are affected in different ways depending on the type of pollutant and their concentration in soil. The resident flora of soil plays an important role in degradation of contaminations and their activity is linked to the soil

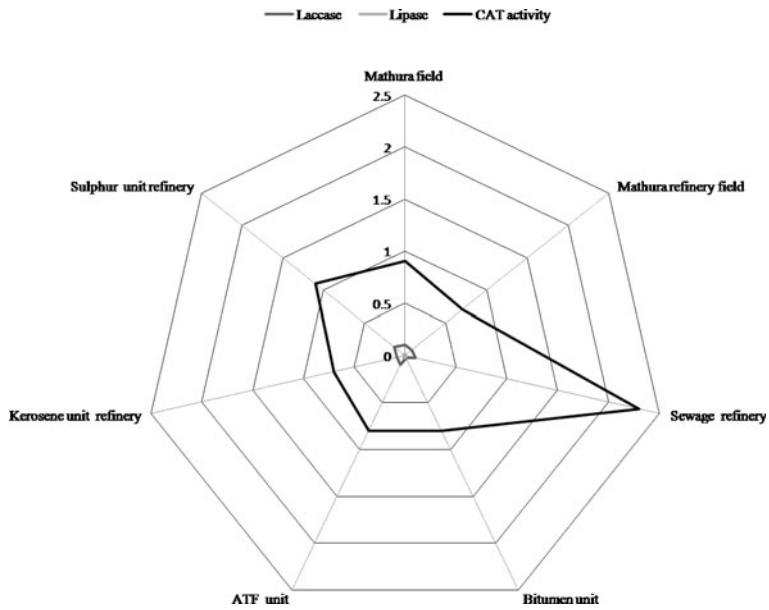

Fig. 6 Enzymatic profile of lipase, laccase and catalase in soil collected from Mathura refinery

organic matter content. Soil enzymes are useful indicators of microbial activity because they are sensitive to slight change in soil environment. Activities of aryl sulphatase, alkaline phosphatase, acid phosphatase, laccase and catalase were found to have higher correlation ($r = 0.931$) with organic C and available NPK. Correlation between enzyme activities and pH was low, the highest being for aryl esterase activity ($r = 0.576$) and lowest with β-glucosidase activity ($r = 0.4989$). Catalase activity was also correlated with microbial biomass carbon ($r = 0.931$), available P ($r = 0.995$), available N ($r = 0.859$) and available K ($r = 0.873$). These results support the fact that enzyme activity increases with organic inputs, regardless of source (Bandick and Dick 1999). Therefore, in other words, fertility status mainly affects the soil microbial populations and continuous exposure of soil to pollutants may help in enrichment of PAH degraders in soil. It has been suggested that synthesis rates of enzyme as well as its release by soil microorganisms and its stability in soil are soil pH-dependent as slightly acidic pH promotes the growth of microorganism in sewage sludge but decreasing pH decreases the growth of microorganisms as recorded in sulphur refinery soil. In general, soil enzyme activities were highest in refinery sewage soil indicating PAH-degrading population build up with instant metabolic activities in sludge. The higher microbial activity might be due to added contaminants from refinery waste which served as carbon sources for microbial growth. The applications of sewage sludge bring changes in soil chemical and biological properties. The addition of sewage sludge increases the biological activity, introduction of microorganisms adapted to degrade PAHs, altogether resulting in a faster disappearance rate of PAH (Wilcke 2000).

Conclusions

Out of seven samples collected from Mathura refinery, maximum enzyme activity, available macronutrients and most probable number was recorded in sewage sludge sample. This sample preferably contained three- to four-ringed PAH degraders. Slightly acidic pH (6.5), maximum percentage of organic carbon (1.5%) and available N, P and K may be the favourable factors for survival of potent microbial population in this sample. Soil enzymes play key biochemical function in the overall process of nutrient/geochemical cycling in soil system. In general, the enzymes estimated in the present study represent the soil metabolic process. Thus, PAH degradation may not be directly correlated by the soil enzyme activity but related to soil indigenous population of

PAH-degrading microorganism. Therefore, overall increase in microbial number will enhance the degradation directly or co-metabolically and the assay of enzymes in such soil can be used as a simple method to examine the degradation potential of microbial mass. A significant representation of Gram-positive members, as against Gram-negative microorganisms is a novel finding and should be taken into consideration while developing bioremediation strategies.

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