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Influence of Petroleum Hydrocarbon Contamination on Microalgae and Microbial Activities in a Long-Term Contaminated Soil

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Abstract. Petroleum hydrocarbons are widespread environmental pollutants. Although biodegradation of petroleum hydrocarbons has been the subject of numerous investigations, information on their toxicity to microorganisms in soil is limited, with virtually no work conducted on soil algae. We carried out a screening experiment for total petroleum hydrocarbons (TPH) and their toxicity to soil algal populations, microbial biomass, and soil enzymes (dehydrogenase and urease) in a long-term TPH-polluted site with reference to an adjacent unpolluted site. Microbial biomass, soil enzyme activity, and microalgae declined in medium to high-level (5,200–21,430 mg kg^{-1} soil) TPH-polluted soils, whereas low-level ($<$2,120 mg kg^{-1} soil) pollution stimulated the algal populations and showed no effect on microbial biomass and enzymes. However, inhibition of all the tested parameters was more severe in soil considered to have medium-level pollution than in soils that were highly polluted. This result could not be explained by chemical analysis alone. Of particular interest was an observed shift in the species composition of algae in polluted soils with elimination of sensitive species in the medium to high polluted soils. Also, an algal growth inhibition test carried out using aqueous eluates prepared from polluted soils supported these results. Given the sensitivity of algae to synthetic pollutants, alteration in the algal species composition can serve as a useful bioindicator of pollution. The results of this experiment suggest that chemical analysis alone is not adequate for toxicological estimations and should be used in conjunction with bioassays. Furthermore, changes in species composition of algae proved to be more sensitive than microbial biomass and soil enzyme activity measurements.

Enormous quantities of petroleum and its products are produced annually, refined, and transported on land and sea across the world. Consequently, risks and concern over petroleum hydrocarbon contamination of soil and water with its attendant ecotoxicological effects is significant. Petroleum hydrocarbons are complex mixtures of organic compounds, including gasoline, fuel oils, diesel fuels, lubricants, and asphalts. Biodegradation of hydrocarbons in soils has been widely studied and reviewed (Atlas 1981; Leahy and Colwell 1990; Cerniglia 1992; Bossert and Compeau 1995; Mueller *et al.* 1996). Given the widespread occurrence of petroleum hydrocarbons in the terrestrial environment, it is important to examine their toxicological effects on soil microorganisms and their activities.

The effects of petroleum hydrocarbons, particularly crude oil on marine and freshwater algae have been well studied, but no information has been published on the effects of petroleum hydrocarbons on indigenous algal and cyanobacterial populations in soil ecosystems. Microalgae are ubiquitous and form an important component of the soil ecosystem comprising a significant portion (up to 27%) of the total microbial biomass in the soil (McCann and Cullimore 1979). Furthermore, cyanobacteria are more widespread than other free-living microorganisms capable of dinitrogen fixation (Burns and Hardy 1975) and are important for the nitrogen economy of soils. Thus, microalgae (including cyanobacteria) are involved in maintaining soil fertility as well as oxygen production (Bold and Wynne 1978). In addition, soil enzymes released by a wide variety of biota play an important role in organic matter degradation and nutrient cycling (Tu 1980). The measurement of intracellular dehydrogenase activity is a common method of estimating the total microbial activity in soils (Casida *et al.* 1964; Trevors 1984) and is known to be sensitive to various pollutants (Doelman and Haanstra 1979; Rossel and Tarradellas 1991; Megharaj *et al.* 1998, 1999). Urease is an important enzyme in nitrogen metabolism and is also known to be sensitive to pollution (Megharaj *et al.* 1998). Therefore, any interference of petroleum hydrocarbons with normal activities of microalgae and soil enzymes would be expected to have potentially serious consequences on the overall functioning of soil ecosystems. As microalgae and cyanobacteria are sensitive to pollution, any alteration in their composition could be useful as a bioindicator of pollution (Megharaj *et al.* 1998). Much of the published work concerning the effects of petroleum hydrocarbon (espe-

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cially crude oil) on microflora results from *in vitro* studies conducted in the laboratory with pure cultures, whereas very little is known about toxic effects under field conditions, and virtually no work exists on soil algae. The present study was therefore aimed at evaluating the impact of total petroleum hydrocarbon (TPH) pollution on soil microalgae, microbial biomass, and activity of two enzymes, namely, dehydrogenase and urease in polluted soil, and the suitability of changes in microalgal species composition as a biological indicator of pollution. Finally, this work was also aimed at evaluating the use of soil eluates from polluted soils for toxicity testing with pure cultures of algae isolated from soil.

Materials and Methods

Soils

The study was conducted on a soil from a petroleum hydrocarbon–contaminated site located north of Adelaide city, South Australia. This site, which received effluent created after cleaning of diesel engines, has now been fully remediated. Soil samples from this site (0–8 cm depth) were collected using a soil auger. Soil samples were taken from specific areas known to have varying levels of pollution from previous chemical analysis. At least five subsamples were taken at random from each sample point. Subsamples were combined, passed through a 2-mm sieve, and assayed. Uncontaminated control soils were collected from the same site approximately 10 m away from the contaminated site. Soil texture was determined by the hydrometer method (Day 1965). Total carbon and nitrogen were determined by the oxidative combustion method using a LECO CN 2000 Analyser. Soil pH was determined using 1:4 ratio of soil:deionized water. The particle size distribution of soil was: sand, 87.5%; silt, 3.75%; clay, 8.75%. Uncontaminated samples had similar textural characteristics to the contaminated soils. The other important physicochemical characteristics of the soils are presented in Table 1. All soils were stored at 4°C and analyzed within 1–2 weeks of sampling.

TPH analyses

The analyses of TPH in composite soil samples were carried out by AMDEL Laboratories Ltd., Melbourne, according to EPA method 8015B. TPH in soil samples were extracted using a mixture of dichloromethane/acetone and the resulting extracts analyzed by capillary column gas chromatography GC-FID and the analytes were fractionated according to their molecular weights.

Microalgae

Microalgal populations in the freshly collected soils were estimated by most probable number (MPN) method and algae were identified to the genus level (Muralikrishna and Venkateswarlu 1984; Megharaj *et al.* 1986). The dominant algae were streaked onto Bold's basal medium agar plates and incubated at room temperature (21 ± 2°C) under continuous illumination (approximately 2,500 lux) provided by cool white fluorescent lamps. Thus, axenic cultures of unicellular algae, *Chlorococcum* sp. and *Scenedesmus* sp., were obtained by repeated subculturing on Bold's basal medium-agar and the cultures were maintained as described earlier (Megharaj *et al.* 1986). These two algae only were used for further testing the toxicity of TPH in soil eluates because axenic cultures of other algae proved difficult to obtain. We did not use any antibiotics to obtain axenic cultures in order to avoid the possible mutations that may arise in algae due to antibiotic exposure.

Microbial Biomass and Soil Enzyme Activity Measurements

Freshly collected soil samples were used for measuring the microbial biomass and enzymatic activity. Microbial biomass carbon was determined by microwave irradiation technique according to Islam and Weil (1998). Soils were extracted with 0.5 M K_2SO_4 (pH 7.0), and organic carbon in these extracts was analyzed using a Dohrmann (DC-180) Carbon Analyser. Dehydrogenase activity in the soils was measured by incubating the soil samples at 37°C for 24 h with 2,3,5-triphenyltetrazolium chloride for the production of 2,3,5-triphenyltetrazolium formazan (Casida *et al.* 1964). The activity of soil urease was measured according to Zantua and Bremner (1975). The use of toluene and buffer solution were excluded in order to measure the urease activity in conditions closer to the actual soil conditions. For all the assays controls were included without the substrate. All the assays were performed in triplicate, and the data represent the average of triplicate values.

Algal Growth Inhibition Assay

Soil eluates were prepared separately for all the contaminated samples and uncontaminated control. To 100 g of soil sample placed in a beaker, 200 ml of half-strength sterile Bold's basal medium (Bischoff and Bold 1963) was added and the resulting slurry sonicated for 10 min and shaken in an end-over-end shaker for 6 h at room temperature (21°C). The soil suspension was centrifuged at 10,000 *g* for 15 min and filter sterilized by passing through 0.45-µm millipore filters. The final pH of the eluate was adjusted to pH 7. Portions (20 ml) of soil eluates placed in sterile flasks (100-ml baffled glass flasks with Teflon-lined screw caps) were inoculated with exponentially growing cultures (1 ml) of *Chlorococcum* and *Scenedesmus* sp. Algal growth inhibition tests were performed as described elsewhere (Megharaj *et al.* 1991a). Controls containing only Bold's basal medium and algae were included in the test in addition to the eluates from uncontaminated soil. The test flasks were placed in a temperature-controlled (21 ± 2°C) orbital shaker set at 100 rpm under continuous light (approximately 2,500 lux) provided by cool white fluorescent lamps. Samples were withdrawn from each flask at 0, 24, and 96 h and growth determined as cell number per milliliter medium using a hemocytometer (Megharaj *et al.* 1991a). Growth inhibition of the alga was used as an endpoint in this bioassay. All the assays were conducted in triplicate.

Statistical Analyses

The data were subjected to analysis of variance, and the means were compared by Duncan's new multiple range test at the 5% level (Megharaj *et al.* 1991b).

Results

The physicochemical properties of the petroleum hydrocarbon–polluted and unpolluted soils are given in Tables 1 and 2. The pH values of these soils were in the range of 7.0–7.5 with not much variation among the samples. All soils were predominantly sandy consisting of >85% sand and had similar clay and silt contents, indicating they were the same soil type and should have similar microbial characteristics. Analyses of TPH content, fractionated based on their molecular weight and priority

Table 1. Chemical and biological characteristics of the TPH-contaminated and uncontaminated soil samples

Soil/Pollution Level	Total C (%)	Total N (%)	pH	Microbial Biomass Carbon (mg kg^{-1})*	Dehydrogenase Activity (%)*	Urease Activity (%)*
Uncontaminated	4.56	0.29	7.5	267.1[a]	100[a]	100[a]
Low	5.74	0.29	7.4	251.8[a]	90.4[a]	91.8[a]
Medium low	12.78	0.92	7.2	105.6[c]	35.8[c]	51.7[c]
Medium	10.83	0.53	7.2	145.6[b]	78.2[b]	70.5[b]
Medium high	13.05	0.83	7.0	152.3[b]	39.4[c]	48.2[c]
High	17.34	1.07	7.1	140.2[b]	31.3[c]	34.8[d]

* Means (n = 3) in each column followed by the same letter are not significantly different ($p \leq 0.05$)

Table 2. Analyses of hydrocarbon content in the soil samples

	Soil/Pollution Level (mg kg^{-1})				
Analyte Fraction	Low	Medium Low	Medium	Medium High	High
C6–C9	ND	ND	ND	ND	ND
C10–C14	ND	ND	ND	18	31
C15–C28	920	3,400	6,300	11,000	17,000
C29–C36	1,200	1,800	2,900	2,300	4,400
Total C6–C36	2,120	5,200	9,200	13,318	21,431
PAHs					
Naphthalene	1.45	2.39	2.52	2.79	3.97
Acenaphthene	ND	ND	ND	ND	ND
Fluorene	ND	ND	ND	ND	ND
Phenanthrene	ND	ND	ND	ND	ND
Anthracene	ND	ND	ND	ND	ND
Fluoranthene	ND	ND	ND	ND	ND
Pyrene	ND	ND	ND	ND	ND
Benz(a)anthracene	ND	ND	ND	ND	ND
Chrysene	ND	ND	ND	ND	1.96
Benzo(b) and (k)fluoranthene	ND	ND	ND	ND	ND
Benzo(a)pyrene	ND	ND	ND	ND	ND
Indeno(1.2.3-c,d)pyrene	ND	ND	ND	ND	ND
Dibenz(a,h)anthracene	ND	ND	ND	ND	ND
Benzo(g,h,i)perylene	ND	ND	ND	ND	ND
Total PAHs	1.45	2.39	2.52	2.79	5.93

ND, not detected

polycyclic hydrocarbons in these soil samples, are presented in Table 2. Depending on their TPH content the polluted soils were classified as low (<4,000 mg TPH kg^{-1} soil), medium low (4,000–8,000 mg TPH kg^{-1} soil), medium (8,000–12,000 mg TPH kg^{-1} soil), medium high (12,000–16,000 mg TPH kg^{-1} soil), and high (>16,000 mg TPH kg^{-1} soil). TPH fractions C6–C9 were not detected in any of the polluted soil samples, whereas a minor amount (≤31 mg kg^{-1} soil) of C10–C14 fraction was detected in only two soil samples (medium high and high). In all the soil samples the C15–C28 fraction was the predominant TPH fraction, followed by C29–C36 fraction. Of the 15 priority PAHs (polycyclic aromatic hydrocarbons) analyzed, naphthalene was present in all the polluted samples (around ≤4 mg kg^{-1} soil). In the soil sample with a high level of pollution 2 mg chrysene kg^{-1} soil was detected, but no other PAHs were detected in any of the samples.

Total carbon content increased with increasing TPH content in all the polluted soil samples except in the soil with the medium low level of pollution, which had a higher total carbon content than the medium-level soil and a similar carbon content to the medium high–level polluted soil (Table 1). Total nitrogen was high in the medium low–level polluted soil and was on a par with the medium high– and high-level polluted samples. There was no difference in the total nitrogen content among the low-level polluted and unpolluted soils.

Microbial biomass carbon decreased in all the polluted soils with the decrease being more pronounced in the soil with medium low–level pollution. Thus, there was 60, 45, 43, and 48% reduction in the biomass carbon of medium low, medium, medium high, and high polluted soils, respectively. The activity of two enzymes, dehydrogenase and urease, decreased with increasing concentrations of TPHs in soils except in the soil with medium pollution, in which the inhibition was similar to that in medium low and medium high polluted soils (Table 1).

Viable count estimates of algal populations with their 95% confidence levels in the polluted and unpolluted soils are presented in Table 3. The algae were more abundant in the low-level polluted soil with a threefold increase over control soil. A large reduction in the population size of algae was observed in the medium low polluted soil. Thus there was a

Table 3. Algal populations (MPN $\times 10^3$ g^{-1} dry soil) in the TPH-contaminated and uncontaminated soil samples

Soil	MPN	95% Confidence Limits	
		Higher	Lower
Uncontaminated	49.5	90.9	27.0
Contaminated			
Low	153.7	282.2	83.7
Medium low	7.4	13.7	4.1
Medium	34.9	64.1	19.0
Medium high	25.3	46.4	13.7
High	10.2	18.6	5.5

Table 4. Qualitative occurrence of algae in TPH-contaminated and uncontaminated soil samples

Organism	Uncontaminated	Contaminated				
		Low	Medium Low	Medium	Medium High	High
Chlorophyceae						
Chlorella sp.	+	++	+	++	−	−
Chlorococcum sp.	++	++	+	++	+	−
Scenedesmus sp.	+	+	−	−	−	−
Unidentified unicellular green	+	+	+	+	++	+
Ulothrix sp.	+	+	−	+	+	−
Cyanobacteria						
Anabaena sp.	−	+	−	+	−	−
Nostoc sp.	+	+	−	−	−	−
Nostoc sp.	+	+	−	−	−	−
Phormidium sp.	+	+	−	+	+	+

−, absent; +, common; ++, abundant

Table 5. Effect of soil eluates on growth of microalgae

Soil Eluates	Growth (cell no. $\times 10^4$ cells ml^{-1})			
	Chlorococcum sp.		*Scenedesmus* sp.	
	24 h	96 h	24 h	96 h
Control (medium)	22[a]	144[b]	138[a]	1,861[a]
Uncontaminated	19[a]	134[b]	121[a]	1,840[a]
Contaminated				
Low	21[a]	170[a]	54[b]	325[b]
Medium low	11[b]	0[d]	18[c]	9[c]
Medium	7[b]	4[c]	8[d]	0[d]
Medium high	9[b]	8[c]	11[d]	0[d]
High	4[c]	0[d]	6[d]	0[d]

Means (n = 3) in each column followed by the same letter are not significantly different from each other ($p \leq 0.05$)

seven- and fivefold decrease in algal numbers in the medium low and high polluted soils. Also, there was a reduction in numbers of algae in medium and medium high polluted soils. The species composition of algae and cyanobacteria in the polluted and unpolluted soils are presented in Table 4. There were a total of nine genera of microalgae consisting of five green algae and four cyanobacteria in the unpolluted and low-level polluted soils. Almost all the cyanobacteria were eliminated in the medium low-level polluted soil whereas only *Phormidium* sp., and an unidentified green unicellular form were present in the high-level polluted soil. Only four out of nine algae were found in the medium high polluted soil, and only two out of these four were found in the high-level polluted soil.

Table 5 shows the results of algal growth inhibition tests conducted with aqueous eluates obtained from polluted and unpolluted soils. The growth measured in terms of cell number of algae in eluates obtained from unpolluted soil was more or less similar to that found in medium only controls. The growth of both the algae was severely affected by the eluates from medium low, medium, and medium high polluted soils. *Scenedesmus* was found to be more sensitive to the eluates obtained from all polluted soil samples. The growth of *Chlorococcum* sp. increased in eluate obtained from low-level polluted soil, while the same eluate was inhibitory to *Scenedesmus* sp. Eluate from high pollution level soil was lethal to both algae. Microscopic observation of both the cultures exposed to the eluates showed alteration in their morphology, with appearance of enlarged giant cells with irregular shapes.

Discussion

The effect of petroleum hydrocarbon contamination in a long-term contaminated soil was analyzed by using different criteria, including TPH analysis, soil microbial biomass, soil enzymes, soil algal species composition, and algal growth inhibition tests. The chemical analysis of the contaminated soil revealed the presence of high molecular weight TPH fractions (C15–C36) with the C15–C28 fraction being the predominant one, accounting for between 65 and 81% of total TPH (5,200–21,430 mg kg^{-1} soil) in medium to high polluted soils. The priority PAHs in the contaminated soils were in the range of 1.4–6.0 mg kg^{-1} soil. Among the various biological parameters tested for measurement of soil toxicity, changes in the species composition of the algae were found to be the most sensitive to the petroleum hydrocarbon pollution in the soil, followed by soil enzymes and microbial biomass carbon. Although total carbon and nitrogen increased with the total TPH content, the trend was not clear. The medium low–level contaminated soil (total TPH 5,200 mg kg^{-1}) showed high total nitrogen content (0.92%) compared with medium high–polluted soil (total nitrogen 0.83%), which had double the TPH content (13,318 mg kg^{-1}) over the medium low–level polluted soil. Likewise the total carbon contents of the medium low (12.8%) polluted soil and medium high (13.0%) polluted soil were similar even though there was twofold difference in their TPH concentrations. This suggests that carbon mineralization may have been inhibited in the medium low polluted soil. As a general observation, no inhibition of plant growth was noticed on the site, so continued deposition of organic matter from plants but depleted ability to mineralize carbon may have resulted in the increased soil carbon levels observed. However, this would require further work to confirm.

Total microbial biomass is an important parameter in soil ecosystems because microbial biomass is vital for soil health. In addition, soil enzymes play an important role in element

transformations. There was a drastic reduction in the microbial biomass carbon and the activity of soil enzymes (dehydrogenase and urease) in the medium low–level polluted soil. At the same time there was no appreciable change in the microbial biomass of the medium, medium high, and high polluted soils, whereas dehydrogenase and urease were significantly reduced in medium high and high polluted soils. Microbial biomass carbon seemed to be a relatively less sensitive parameter than soil enzymes and therefore appears difficult to use as an indicator of toxicity, as it is a total measure of different microbial types with different responses to toxicants. It is distinctly possible that biomass carbon represents mainly pollutant-resistant organisms. Overall, there was no uniform trend among all these parameters.

The toxicity of petroleum hydrocarbons to algal populations (viable count estimates) as observed by a large reduction in the population size, followed by the change in their species composition in medium and high polluted soils was of particular interest. Many of the algal forms present in the unpolluted soil were sensitive to medium- and high-level polluted soils, whereas a unicellular green alga and a cyanobacterium, *Phormidium* sp., were dominant in high-level polluted soils. The replacement of sensitive algae with resistant species can have adverse effects on the ecosystem by reducing its biodiversity. Here some species may be totally eliminated; at the same time other species can become dominant and contribute to the high density of the populations as well. Consequently, it is doubtful whether the resistant organisms will perform the necessary ecological functions (such as N_2-fixation, polysaccharide production, which helps soil structure, etc.). For example, N_2-fixation studies on *Rhizobium* revealed that a particular *Rhizobium* genotype had been selected in metal-polluted soil and that this genotype did not fix nitrogen (McGrath 1994). Similar phenomena, *i.e.*, reduction in species diversity, have been observed in soils treated with organophosphate pesticides (Megharaj *et al.* 1986, 1988), DDT (Megharaj *et al.* 1999) and in a long-term pentachlorophenol-contaminated soil from a former timber processing facility (Megharaj *et al.* 1998). Algal species exhibit differential sensitivity to toxic pollutants and petroleum hydrocarbons have been shown to be inhibitory to the growth of some sensitive species while promoting the growth of tolerant ones in aquatic environment (Morales-Loo and Goutz 1990). Aqueous oil extract has been shown to inhibit growth and nitrogen fixation of the cyanobacterium *Anabaena doliolum* grown under laboratory culture conditions, the inhibitory effect on growth being dose-dependent (Gaur and Singh 1990). Hellebust *et al.* (1982) have observed severe inhibition in the heterotrophic growth of algae by hydrocarbons and oils. Also, microalgal response to different petroleum hydrocarbons can vary. Heavy-duty marine diesel oil (at 10% concentration) has been shown to prevent the growth of a marine microalga, *Isochrysis* sp., whereas crude oil at a similar concentration caused little effect on the growth of this alga (Ansari *et al.* 1997). This shows that the nature of the hydrocarbon is important in exerting its toxicity. Shailaja (1988) studied the effect of dissolved petroleum hydrocarbon residues on natural phytoplankton biomass in the northern Indian Ocean and found that the effect of dissolved petroleum hydrocarbons was species-specific and dependent on the nature of petroleum hydrocarbons rather than total hydrocarbons present. The observed toxicity to soil algae in the present study agrees with the above cited report (Ansari *et al.* 1997) because the polluted soils examined here originated from diesel engine cleaning.

Algal growth inhibition test on aqueous eluates obtained from unpolluted soil showed no toxicity compared to the growth medium control. Eluates were toxic to both the algal species tested. The results clearly demonstrate a varying toxicity of eluates to different algal species. It was observed that eluates of all contaminated soils except low-level polluted soil inhibited algal growth. Furthermore, these eluates induced changes in the morphology of algae with the appearance of giant-like cells with irregular shapes, suggesting that the cell division or cell permeability is affected by the toxicants. On the other hand, eluates of low-level polluted soil after 96 h exposure stimulated algal growth in the case of *Chlorococcum* sp., while growth of *Scenedesmus* sp. was inhibited. Fuel oil extract has been shown to induce changes in the morphology of *Scenedesmus* in terms of increase in cell size (Tukaj *et al.* 1984), which might be a consequence of the inhibition of cell division. Further, Tukaj (1987) also reported a decrease in cell number of *Scenedesmus* exposed to water-soluble fractions of fuel oil, which supports the observed effects of aqueous eluates from TPH-polluted soils on growth and morphology of *Scenedesmus* sp in the present study. The stimulation of growth in the case of *Chlorococcum* sp. might be due to its ability to detoxify or metabolize some of the dissolved organic compounds present in the soil. Also, microalgae can assimilate petroleum hydrocarbons. For example, *Chlorella* and *Scenedesmus* have been shown to assimilate petroleum hydrocarbons although the mechanism was not clearly known (cited in Petkov *et al.* 1992). A cyanobacterium, *Phormidium foveolarium*, has been reported to degrade tetradecane (Xing-ming *et al.* 1982). Thus, petroleum compounds in general have been shown to either inhibit or stimulate algal growth, depending on the type and level of petroleum product and the algal species concerned (Gordon and Prouse 1973; Karydis 1979; Hellebust *et al.* 1982; Chan and Chiu 1985; Tukaj *et al.* 1987; Shailaja *et al.* 1988; Gaur and Singh 1990; Petkov *et al.* 1992; Ansari *et al.* 1997). The higher toxicities observed by eluates of polluted soils in this study might be due to the presence in high concentrations of a complex mixture of pollutants, as high concentrations of oils are expected to disrupt the structure and function of the plasma membrane and thus affect cell membrane permeability (Van Overbeek and Blondeau 1954).

The total inhibition of algal growth by eluate from medium low–level polluted soil supports the observed toxicity of this soil to the microbial parameters tested. The fact that this soil contained a lower TPH compared to medium and medium high polluted soils, yet exhibited higher toxicity than the latter soils, cannot be explained solely by chemical analysis; this merits further consideration. The bioavailability and accessibility of the toxicant to the organism is likely to be different in the presence of soil. Nevertheless, testing with eluates is somewhat close to the soil situation in that the former represents the soil solution containing the theoretically bioavailable portion of soil contaminants. These data demonstrate the limitations for predicting the toxicity arising due to interactions of complex mixtures by chemical analysis alone. Although chemical analysis is an

important factor in site assessment and remediation, it has to be kept in mind that different transformation products and other chemicals may be present that may not be detected. Antagonistic or synergistic toxic effects caused by complex pollutant mixtures are also difficult to predict using analytical chemistry alone. However, chemical analysis is useful to examine the persistence of a specific chemical or its major degradation products and should be used in conjunction with toxicological assays. The importance of bioassays in toxicity determination lies in the fact that these are measures of the organism's response to simultaneous influences of various environmental parameters affecting its toxicity. Although we did not attempt to measure pollutant concentrations in the aqueous eluates, algal growth inhibition tests with eluates can be very useful to detect the bioavailable fractions of the test compounds since the bioavailable fractions are expected to be responsible for toxicity. Although several studies have been carried out on physiological responses to oil/petroleum hydrocarbon pollution of aquatic algae in cultures or in natural phytoplankton, to our knowledge, this is the first report that petroleum hydrocarbon pollution severely affects the species composition and density of indigenous microalgae in polluted soil.

The results of our study indicate that changes in soil algal composition can be used to identify potential environmental hazards at polluted sites and may be useful to establish guidelines for soil quality. In view of the limited knowledge on exposure and toxicity of pollutants to microorganisms in terrestrial environments, the soil algal tests assume special importance.

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