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Variations of soil enzyme activities in petroleum-hydrocarbon contaminated soil

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ABSTRACT

Petroleum hydrocarbons can affect soil ecosystems, resulting in significant losses in soil quality. The objective of this study was to evaluate the effects of different types of hydrocarbon pollution (crude oil and waste engine oil) on soil enzyme activities and determine the fate of total petroleum hydrocarbons (TPH) during an incubation period of 3 months. The possible use of wastewater sludge as a bio-stimulating agent in petroleum-contaminated soils was also evaluated. Enhanced enzyme activity levels in contaminated soils indicated that crude oil and waste engine oil (0.5% w/w) appeared to stimulate microbial growth and enzyme activity in the soil environment. The results also indicated that significant TPH degradation in both crude and waste engine oil-contaminated soils (87% and 65%, respectively) occurred after an incubation period of 3 months. The degradation of crude oil in contaminated soil was significantly enhanced by the addition of wastewater sludge, whereas no apparent biostimulating effect on TPH removal was observed in the case of engine oil contamination.

1. Introduction

Environmental pollution by petroleum and petrochemical products has attracted much attention in recent decades. Due to the increasing production of crude oil and the increasing probability of accidents, petroleum compounds are one of the most frequently encountered pollutants in soil (Banks et al., 2003). Because it contains many toxic compounds in relatively high concentration, crude petroleum is physically, chemically and biologically harmful to soil microorganisms.

Total petroleum hydrocarbons (TPH) are one of the most common groups of persistent organic contaminants (Huang et al., 2005). The relatively high hydrophobicity of petroleum hydrocarbons causes an increased ability to accumulate in soil and sediment compared to aquatic environments (Karthikeyan and Bhandari, 2001). Additionally, the high hydrophobicity of these compounds results in their binding to soil and sediment particles, thus leading to a decrease in the bioavailability of these contaminants for biological sorption (Parrish et al., 2005; Luepromchai et al., 2007).

Field studies of contaminated soils have demonstrated that elevated loading of these contaminants can induce diminished microbial biomass, reduced viable bacterial population densities, inhibition of organic matter mineralisation and decreased leaf litter decomposition (Chander and Brooks, 1991; Roane and Kellogg, 1996). Therefore, suitable solutions for the removal or control of these soil contaminants must be found.

Soil microorganisms are very sensitive to any perturbation of the ecosystem, and their diversity and activity are rapidly altered by such perturbations (Schloter et al., 2003). The measurement of microbiological parameters, such as soil respiration, microbial biomass carbon or enzyme activities, provides information on the presence and activity of viable microorganisms as well as on the extent, type and duration of the effects of hydrocarbon pollution on soil metabolic activity. Such measurements may serve as a good indication of the impact of pollution on soil health (Brohon et al., 2001; Eibes et al., 2006).

Enzyme activities have been associated as indicators of biogeochemical cycles, the degradation of organic matter and soil remediation processes thus they can determine, together with other physical or chemical properties, the quality of a soil (Gelsomino et al., 2006; Topac et al., 2009). Authors such as Nielsen and Winding (2001) and Eldor (2007) report enzymes as good indicators because a) they are closely related to organic matter, physical characteristics, microbial activity and biomass in soil and b) provide early information about changes in soil quality and c) are more rapidly assessed.

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Measurement of enzyme activities is widely used to examine nutrient cycling processes in soil (Tabatabai and Dick, 2002). Numerous enzymes are known to participate in the processes of organic matter decomposition and mineralization. The enzymes most commonly analysed include various hydrolases involved in the C, N, P and S cycles, as well as some oxidoreductases. Analysis of soil hydrolases involved in C, N and P cycles may provide some insight into the metabolic capacity of the soil, so that the potential for transformation of specific sources of energy or nutrients can be assessed (Shaw and Burns, 2006).

Oxidoreductases enzymes (dehydrogenase, polyphenol oxidase) are also important for degradation of recalcitrant compounds. Dehydrogenase enzymes are almost exclusively intracellular and they are involved in organic matter oxidation. They reflect physiologically active microorganisms and thus provide correlative information on biological activities and microbial populations in soils. It described that the concentration of petroleum hydrocarbon had significant effect on activity of soil dehydrogenase (Ueno et al., 2007).

Polyphenol oxidase plays an important role in the process that convert the aromatic organic compounds into humus in soil and it is an important measure of the soil microflora's capacity to degrade potentially recalcitrant organic ones (Gianfreda et al., 2005).

Wastewater sludge contains significant amounts of nutrients required by plants, including nitrogen, phosphorus, potassium, and micronutrients, making them an excellent fertiliser for use in agriculture and forestry. Lack of essential nutrients such as nitrogen and phosphorus is one of the major factors affecting biodegradation of hydrocarbon by microorganisms in soil and water environment (Margesin et al., 2007).

A decrease in the quantity of petroleum hydrocarbons is particularly evident shortly after an accident (Chaineau et al., 2003). Although such sites are immediately restored, the frequency of accidents and limitations in restorations indicate the possibility of high petroleum hydrocarbon concentrations in the oil field soil.

The effects of petroleum hydrocarbons, particularly crude oil and engine oil on marine and freshwater algae have been well studied, but information about the effects of petroleum hydrocarbons on soil enzyme activities and indigenous populations in soil ecosystems has been scarced. The objective of this study was to examine the levels of total petroleum hydrocarbons (TPH) and the effects of different types (crude oil and used engine oil) of hydrocarbon pollution on soil enzyme activities during 3 months.

2. Materials and methods

2.1. Materials

Soil samples were collected from the top 20 cm of an agricultural field located in Bursa-Balabancık village (latitude, 40° 15′ 55.1″ N; longitude, 28° 47′ 07.55″E). The soil chemical properties are shown in Table 1.

The dairy wastewater sludge samples were obtained from a dairy company treatment plant in Bursa, Turkey. General characteristics of the canned food sludge are presented in Table 1.

Light crude oil was collected from İzmir, Aliağa refinery and has a specific gravity of 0.86 (60 F/60 F), gravity of 33.4 API, viscosity of 10.20 cs (70 F), total sulphur of 1.79% (wt), vanadium of 20.50 ppm, nickel of 4.40 ppm, and total nitrogen of 0.0980% (wt). Used engine oil (10 W/40 diesel) was collected from a car service centre.

Table 1
The chemical properties of soil and wastewater sludge from dairy industry.

Properties	Value	
	Sludge	Soil
pH (1:5 deionised water)	7.20	7.76
$EC_{25\ °C}$ (1:5 deionised water, dS m^{-1})	6.60	0.16
Organic C, %	38.4	1.23
Total-P, %	3.50	0.17
Total-N, %	6.42	0.15
Alkaline phosphatase activity (mg PNP kg^{-1} h^{-1})	144	246
β-glucosidase activity (mg PNP kg^{-1} h^{-1})	98	146
Urease activity (mg NH_4–N kg^{-1} h^{-1})	77	26
Zn (mg kg^{-1} dry weight)	418	1.35
Cu (mg kg^{-1} dry weight)	27.03	2.05
Ni (mg kg^{-1} dry weight)	21.52	<1.00
Cr (mg kg^{-1} dry weight)	32.17	<1.00

2.2. Determination of soil and wastewater sludge physical and chemical properties

Soil and wastewater sludge samples were prepared and analysed following the same procedures. The EC and pH of the samples were measured with a conductivity meter and pHmeter, respectively analysing the extracts obtained by shaking soil or wastewater sludge samples with distilled water (1:5, w/v) (Rhoades, 1982; McLean, 1982).

The Kjeldahl digestion method was used to measure the total nitrogen concentration (Bremner and Mulvaney, 1982). In addition, dichromate oxidation was used to measure the total organic carbon (Nelson and Sommer, 1982). Total phosphorus (P) was measured colourimetrically using a UV/VIS spectrophotometer (Anonymous, 1985).

The total concentrations of metals (Cr, Ni, Cu and Zn) were determined after microwave digestion of the samples with HNO_3 by using an ICP OES (Perkin Elmer 2100 DV Optima) (Isaac and Johnson, 1998).

2.3. Determination of soil enzyme activities

The alkaline phosphatase (APA), β-glucosidase (BGA) and urease (UA) enzymatic activities were determined using the methods described by Tabatabai (1994). The alkaline phosphatase activities were determined by adding a modified universal buffer (pH = 11), 0.025 M toluene and *p*-nitrophenyl phosphate solutions to the soil whereas the β-glucosidase activities were measured by adding a modified universal buffer (pH = 6), 0.025 M toluene and *p*-nitrophenyl-β-D-glucoside (PNG) solutions to the soil. The samples of both the analyses were then incubated at 37 °C for 1 h. The released *p*-nitrophenol (PNP) was quantified with a spectrophotometer at 410 nm. The β-glucosidase activity test was based on the colorimetric determination of PNP. Similarly, the assay for urease activity was based on the determination of NH_4^+ released by urease when the soil was incubated with the THAM (Tris (hydroxymethyl) aminomethane) buffer (pH = 9), a 0.02 M urea solution and toluene at 37 °C for 2 h. The formation of ammonium was determined by steam distillation. The steam distillation with MgO and Devarda alloy was performed (automatic distillation system-Velp) in order to analyse the nitrate and ammonium nitrogen concentrations in samples extracted with 2 M KCl (Keeney and Nelson, 1982).

2.4. Determination of total petroleum hydrocarbons in soil

The TPH concentration was determined by ISO 16703:2004. Petroleum-contaminated soil, 20 g was weighed and put into a glass extraction vessel with 40 ml of acetone. After briefly shaking