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Assessing changes in physical and biological properties in a soil contaminated by oil sludges under semiarid Mediterranean conditions

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Abstract

We have studied the changes in biological and physical properties of a soil, in a semiarid area, contaminated by hydrocarbons in order to predict the potential for bioremediation of this soil. The microbial biomass C, the basal respiration and the metabolic quotient (qCO_2) of contaminated soil were significantly higher than that of the control soil, which points to a markedly reduced efficiency of substrate use and to a possible toxic effect of hydrocarbons in the soil. The low global rate of mineralisation in the contaminated soil also indicates the presence of hydrocarbons resistant to biodegradation. The dehydrogenase activity and the activities of hydrolases involved in the N and P cycles (urease, protease and phosphatase) were stimulated by the contamination with hydrocarbons. Soil total porosity was increased by the contamination, about 15-fold in comparison with the control soil. The contamination by hydrocarbons led to an increase in cracks in the 100–200 μm size classes, which can be regarded as a reservoir to hold water for plants and microorganisms. The biodegradation of hydrocarbons in this soil would require long periods of time, and it would be advisable to apply methods of bioremediation to this contaminated soil.

Keywords: Enzyme activities; Soil microbiological biomass; Metabolic quotient; Porosity; Shrinkage; Hydrocarbon

1. Introduction

Hydrocarbons are common contaminants found in soil and groundwater as a result of past and current industrial activity. The greatest concern regarding the contamination by hydrocarbons lies in the mutagenic, carcinogenic and toxic characteristics of such contaminants. The extent of environmental contamination depends on the chemical composition and concentration of the contaminant and the properties of the soil (Fine et al., 1997). In general, the presence of high molecular weight compounds characterised by very low solubility in water hinders the natural biodegradation for soils contaminated by hydrocarbons. For soils from semiarid Mediterranean regions of Spain, their low organic matter contents can directly affect sorption of hydrocarbons by the soil and indirectly affect biodegradation of hydrocarbons (Mohn and Stewart, 2000).

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The contamination of soils by hydrocarbons may induce changes in the physical condition and biological activity of the soil, and prior knowledge of these processes is considered necessary in order to choose and develop the most suitable methodology for remediation of contaminated soils. Thus, for example, measurements of soil porosity and texture are a prerequisite for applying methods of bioremediation (Morgan and Watkinson, 1989). However, few studies have examined the effect of contamination by hydrocarbons on soil structure in semiarid environments.

The quantification of soil porosity and pore size distribution is essential for characterisation of soil structure. These parameters are closely related to storage and movement of water and gases, and to ease root penetration. The micromorphometric method, based on image analysis of soil thin-sections, supplies useful information about the complexity of pore patterns in soil, the pore shape and the relative positions of the aggregates and pores (Sartori et al., 1985).

Biological and biochemical properties, including soil respiration measured by the rate of CO_2 release or O_2 consumption, microbial biomass and the activities of soil enzymes, are considered to be sensitive indicators of contamination because of their importance in cycling of organic matter and regulating active nutrient pools in soils. Contaminated soils are a system of great complexity and the behaviour of many enzymatic activities in such soils may be very variable, which has thrown doubts on the possibility of their use as reliable indicators of soil contamination. Hence, the quantification of soil contamination may require the combined determination of several biochemical soil properties (Trasar-Cepeda et al., 2000).

The objective of this study was to assess changes in biological activities, porosity and superficial shrinkage of a soil contaminated by hydrocarbons from oil sludges, under semiarid Mediterranean conditions. This information would help to predict the rate and extent of intrinsic bioremediation of the contaminated soil.

2. Materials and methods

2.1. Soil

The contaminated soil was collected from an area near an oil refinery in the province of Murcia (SE Spain), which has received uncontrolled quantities of hydrocarbons over nearly 10 years. Previous to hydrocarbon contamination the soil (clay loam) had an organic C content of 0.7% and a pH of 7.8 in H_2O. Soil samples were also collected from an uncontaminated adjacent area and were used as control. The climate of the region is semiarid, Mediterranean type, with an average 300 mm $year^{-1}$ rainfall, 1000 mm $year^{-1}$ evapotranspiration and on average annual temperature of 19.2 °C. In June 2000, four soil samples were taken from each of the sampling areas. Each soil sample consisted of a mixture of five subsamples (200 cm^3 soil cores) randomly collected from the top 20 cm depth of soil. The soil subsamples were crushed, mixed and sieved to <2 mm. A subsample of each sieved soil was air-dried to determine soil physical–chemical properties and the remainder was stored at 4 °C for analyses of biological properties.

2.2. Physical–chemical, chemical, biological and biochemical analyses

Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous extract. Total nitrogen was determined by the Kjeldhal method, and the total organic C according to Yeomans and Bremner (1988). Available P, extracted with 0.5 M $NaHCO_3$, was determined by colorimetry, according to Murphy and Riley (1962). NO_3^- was determined on soil aqueous extracts by ionic chromatography using a DIONEX chromatograph. NH_4^+ was measured using a selective electrode for ammonium Orion Research, Cambridge, MA, Model 95-12 (Keeney and Nelson, 1982). Particle size distribution was determined using the pipette method after oxidation of the organic matter with H_2O_2 and stirring in a sodium hexametaphosphate solution (Gee and Bauder, 1986).

In soil aqueous extracts, water soluble carbon (WSC) was determined by wet oxidation with $K_2Cr_2O_7$ and measurement of the absorbance at 590 nm (Sims and Haby, 1971). Total carbohydrates was determined by the method of Brink et al. (1960).

Microbial biomass C was determined using a fumigation–extraction method (Vance et al., 1987, modified by Widmer et al., 1989).

Carbon dioxide emissions were determined using 50 g dry soil, moistened to 60% of its water-holding capacity, placed in hermetically sealed polyethylene tubes containing a vial of 10 ml 2 N NaOH and incubated for 49 days at 28 °C. Every 2 days for 49 days, the trapped CO_2 was precipitated as carbonate with excess $BaCl_2$ and the excess NaOH was titrated with 1 M HCl (Zibilske, 1994).

Dehydrogenase activity was determined according to García et al. (1997). For this, 1 g of soil at 60% of its field capacity was exposed to 0.2 ml of 0.4% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) in distilled water for 20 h at 22 °C in darkness. The INTF (iodo-nitrotetrazolium formazan) formed was extracted with 10 ml of methanol by shaking vigorously for 1 min and filtration through a Whatman No. 5 filter paper. INTF was measured spectrophotometrically at 490 nm.

Urease and *N*-α-benzoyl-L-argininamide (BAA) hydrolyzing protease activities were determined in 0.1 M phosphate buffer at pH 7; 1 M urea and 0.03 M BAA were used as substrates, respectively. Two millilitres of buffer and 0.5 ml of substrate were added to 0.5 g of sample, which was incubated at 30 °C (for urease) or 39 °C (for protease) for 90 min. Both activities were determined as the NH_4^+ released in the hydrolysis reaction (Nannipieri et al., 1980).

Phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) as substrate. Two millilitres of 0.1 M maleate buffer at pH 6.5 and 0.5 ml of substrate were added to 0.5 g of soil and incubated at 37 °C for 90 min. The reaction was stopped by cooling at 2 °C for 15 min. Then, 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were added, and the mixture was centrifuged at 4000 rpm for 5 min. The *p*-nitrophenol (PNP) formed was determined in a spectrophotometer at 398 nm (Tabatabai and Bremner, 1969). Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH.

β-Glucosidase was determined using *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M; Masciandaro et al., 1994) as substrate. This assay is based on the release and detection of PNP. Two millilitres of 0.1 M maleate buffer pH 6.5 and 0.5 ml of substrate was added to 0.5 g of sample and incubated at 37 °C for 90 min. The reaction was stopped with tris-hydroxymethyl aminomethane (THAM) according to Tabatabai (1982). The amount of PNP was determined in a spectrophotometer at 398 nm (Tabatabai and Bremner, 1969).

2.3. Physical analysis

2.3.1. Porosity

Undisturbed soil samples were dried by acetone replacement of water (Murphy, 1986), impregnated with a polyester resin and thin sections were prepared. The photographic method devised by Ismail (1975) was used on thin sections to separate pores from mineral grains. Each photograph was analysed by image-analysis (Quantimet 570) to measure total porosity and to characterise pores according to their shape and size (Pagliai et al., 1984). Pores were measured by their shape as expressed by the shape factor [perimeter^2/(4π area)] and divided into regular (more or less rounded) pores (shape factor 1–2), irregular pore (shape factor 2–5) and elongated pore (shape factor >5) categories. Pores were also subdivided into size classes according to the equivalent pore diameter for regular and irregular pores and the width for elongated pores (Giusquiani et al., 1995).

2.3.2. Shrinkage

Twenty grams of air-dry soil sample (sieved to <2 mm) were mixed with 20 ml distilled water until they became fluid. The suspension was put into a square box (9 × 9 cm) and was dried at constant temperature (25 °C). Cracks formed were measured optically with a Quantimet 570 apparatus using an electro-optical procedure for image-processing and analysis. Briefly, the image of the dried soil sample was scanned by a television camera and displayed on a screen. The video signal was passed to a detector where 500,000 picture points on the image were individually analysed for their grey level. Cracks were measured by setting the instrument to detect the corresponding grey level, which was different from the clods. The analysis system gave the percent of crack area, and cracks were further sub-divided into different dimensional classes (<500, 500–1000 and >1000 μm) which corresponded to average values of the crack width. All determinations were made in triplicate, with a difference, among the three measurements on the same soil sample, of about 0.1% (Pagliai et al., 1980).

3. Results and discussion

3.1. Physical–chemical and chemical parameters

The contamination by hydrocarbons decreased the clay content and increased the sand content (Table 1). Thus, the variations in the contents of soil particles caused a change in soil texture, from clay loam in the control soil to sandy clay loam in the contaminated soil. The change in soil texture may be due to adsorptions of hydrocarbons onto soil mineral colloids such as clay and humic substances (Yong et al., 1994; Pignatello and Xing, 1996), modifying their rate of sedimentation according to Stokes' law. This would have significant effects on the percentage of particles obtained through the pipette method. Changes in soil texture from clay to silt loam were recorded by Martínez and López (2001) in a soil contaminated with kerosene.

The contamination by hydrocarbons decreased soil pH and significantly increased soil electrical conductivity (Table 1). The increase observed in soil electrical conductivity could be due to salts used for the purification of waters from the oil refinery, which were incorporated in the soil together with the oil sludges.

Table 1
Changes in physical–chemical and chemical properties of a soil in response to contamination by hydrocarbons ($n=4$)

	Control	Contaminated
Clay (%)	33.3 (0.2)	21.3 (0.1)
Silt (%)	21.7 (0.1)	20.5 (0.5)
Sand (%)	45.0 (0.0)	58.1 (0.3)
Texture	Clay loam	Sandy clay loam
pH (H_2O)	7.91 (0.01)	7.31 (0.14)
EC (1:5, μS cm^{-1})	449 (19)	3460 (57)
$N–NO_3^-$ (μg g^{-1})	13 (0)	478 (75)
$N–NH_4^+$ (μg g^{-1})	0.42 (0.01)	5.86 (0.48)
Total N (g kg^{-1})	0.5 (0.0)	4.6 (0.1)
Available P (μg g^{-1})	37 (2)	28 (0)
TOC (g kg^{-1})	7.2 (0.0)	73.8 (0.0)
Total CH (μg g^{-1})	303 (10)	1965 (34)
Water-soluble C (μg g^{-1})	134 (10)	484 (41)
Water-soluble CH (μg g^{-1})	5 (1)	62 (0)
Water-soluble PP (μg g^{-1})	1.4 (0)	49.5 (2)

In parenthesis, standard error for each measure.
EC: electrical conductivity; TOC: total organic carbon; Total CH: total carbohydrates; Water-soluble C: water-soluble carbon; Water-soluble CH: water-soluble carbohydrates; Water-soluble PP: water-soluble polyphenols.

With respect to soil nutrient concentrations, only the total N was increased in the contaminated soil, by about 820% with respect to control soil (Table 1). In both soils, the majority of the total N was in organic forms. A high soil N content may be associated with high populations of hydrocarbon-degrading microorganisms (Mohn and Stewart, 2000). The high NO_3^- content of the contaminated soil could be due to chemical treatments used for the purification of waters from the oil refinery as indicated above for electrical conductivity. Available phosphorus concentration was high in both control and contaminated soils. Inorganic N/P ratio in the contaminated soil (about 17) was more than adequate for native microbial activity, which could favour the biodegradability of hydrocarbons.

As expected, hydrocarbon contamination increased significantly the soil total organic carbon by about 925% with respect to control soil (Table 1). Taking into account the total organic C and total N concentrations it appears that about 7.1% of the soil was composed of hydrocarbons.

Polysaccharides, which are mostly by-products of microbial activity, are considered the main chemical aggregate-stabilising agents (Lax and García-Orenes, 1993; Roldán et al., 1994). In the contaminated soil, the high increase in total carbohydrates with respect to control soil could be due to the biodegradation of hydrocarbons, which could contribute to improving the structure of this soil (Table 1). However, the fraction of total carbohydrates in the contaminated soil only represented about 3% of total organic carbon.

The water-soluble organic matter fraction consists of a heterogeneous mixture of components of varying molecular weight, such as mono- and polysaccharides, polyphenols, proteins and low molecular weight organic acids (Kuiters and Dennenman, 1987). This fraction can be used as carbon and energy source by the soil microflora (Roldán et al., 1994) and may also have a structural function (Metzger and Yaron, 1987). The soluble C-fraction (water-soluble C, water-soluble carbohydrates and water-soluble polyphenols) increased significantly due to the contamination of soil by hydrocarbons (Table 1). Among the water-soluble C-fractions, the greatest relative increase in response to the contamination was observed in the soil phenolic compounds, which may be phytotoxic (Kuwatsuka and Shindo, 1973).

Table 2
Changes in biological properties of a soil in response to contamination by hydrocarbons ($n=4$)

	Control	Contaminated
Biomass C (μg g^{-1})	370 (45)	629 (27)
ΣCO_2–C (mg g^{-1}) (day 49)	1.38 (0.02)	5.67 (0.03)
qCO_2 (mg CO_2–C g^{-1} biomass C day^{-1})	76 (3)	184 (3)
Dehydrogenase (μg INTF g^{-1})	63 (11)	125 (42)
Urease (μg NH_4 g^{-1} h^{-1})	9 (1)	27 (0)
Protease-BAA (μg NH_4 g^{-1} h^{-1})	5 (0)	7 (0)
Phosphatase (μg PNF g^{-1} h^{-1})	768 (42)	2295 (620)
β-glucosidase (μg PNF g^{-1} h^{-1})	185 (42)	183 (8)

In parenthesis, standard error for each measure.

3.2. Biological and biochemical parameters

Microbial biomass C has been used as an index to compare natural and disturbed ecosystems (García et al., 2000). In contaminated soil the biomass C was higher than in control soil (Table 2). The basal respiration reflects the activity of the soil microflora, which may be related to biodegradation of organic compounds in the soil (Brohon et al., 2001). The contamination by hydrocarbons increased the amount of CO_2 evolved during the 49-day incubation by about 310% with respect to the control soil (Table 2). The amount of mineralised substrate was calculated by subtracting the amount of CO_2–C produced in the control soil from that of the contaminated soil. Based on the calculation explained above, only 6% of C from hydrocarbons was mineralised. The hydrocarbons generally contain different constituents that exhibit a wide range of biodegradation. The low rate of global mineralisation points to the presence of high molecular weight aromatic or polyaromatic organic constituents, which are more difficult to biodegrade. Likewise, the hydrophobic characteristics of the hydrocarbons and the limited diffusion of oxygen through them make it difficult to biodegrade such compounds (Hurst et al., 1996).

The metabolic quotient (qCO_2) has been used as an ecophysiological index, and reflects the bioenergetic status of microbial biomass (Santruckova and Straskraba, 1991). The qCO_2 can also be used as a bioindicator of disturbance and stress (Anderson and Domsch, 1993). At the end of the incubation period the metabolic quotient of the contaminated soil was significantly higher than that of the control soil (Table 2). This points to a reduced substrate use efficiency in the contaminated soil, i.e. less substrate was incorporated into the biomass. The very low incorporation of hydrocarbons into the microbial biomass points to a markedly reduced efficiency of substrate use or rather reduced microbial efficiency and, thus, to toxic effects of hydrocarbons in the soil.

With the exception of the β-glucosidase activity, all enzyme activities were significantly higher in the hydrocarbon-contaminated soil than in the control soil (Table 2). The increases observed for dehydrogenase, which is considered a good indicator of microbial activity in relation to its mineralising function (Nannipieri et al., 1990), and for the enzymes involved in the N (urease and protease) and P (phosphatase) cycles would indicate the pronounced ability of the soil microbial communities to degrade peptidic and phosphoryl substrates in the contaminated soil. The contamination by hydrocarbons did not increase the β-glucosidase activity, related to the C cycle, suggesting that the organic matter of hydrocarbons contained a low content of β-glucosides compounds.

Table 3
Effect of contamination by hydrocarbons on soil porosity larger than 50 μm and size-classes pores distributions

	[a]Porosity (%)	[b]Size classes of pores (%)						
		50–100 μm	100–200 μm	200–300 μm	300–400 μm	400–500 μm	500–1000 μm	1000–2000 μm
Control	0.8 (0.1)	5.1 (1.4)	11.5 (3.0)	20.5 (5.8)	9.0 (2.1)	7.7 (2.9)	21.8 (3.2)	24.4 (10.8)
Contaminated	11.9 (0.1)	2.2 (0.1)	11.1 (0.7)	21.4 (3.6)	9.7 (1.7)	17.1 (7.3)	28.8 (3.7)	9.7 (1.5)

In parenthesis, standard error for each measure.
[a] Porosity is expressed as a percentage of area occupied by pores >50 μm per thin section.
[b] Size-classes pores distributions are expressed as a percentage of total porosity.

3.3. Physical parameters

It is well known that soil organic matter and microbiological activity play a very important role in soil structure formation, stability and genesis of porosity (Marinari et al., 2000; Caravaca et al., 2002). Soil total macroporosity (pores >50 μm) was increased by the contamination with hydrocarbons about 15-fold compared to the control soil (Table 3). According to the classification proposed by Pagliai (1988) for this micromorphometric method, the soil contaminated by hydrocarbons can be classified as

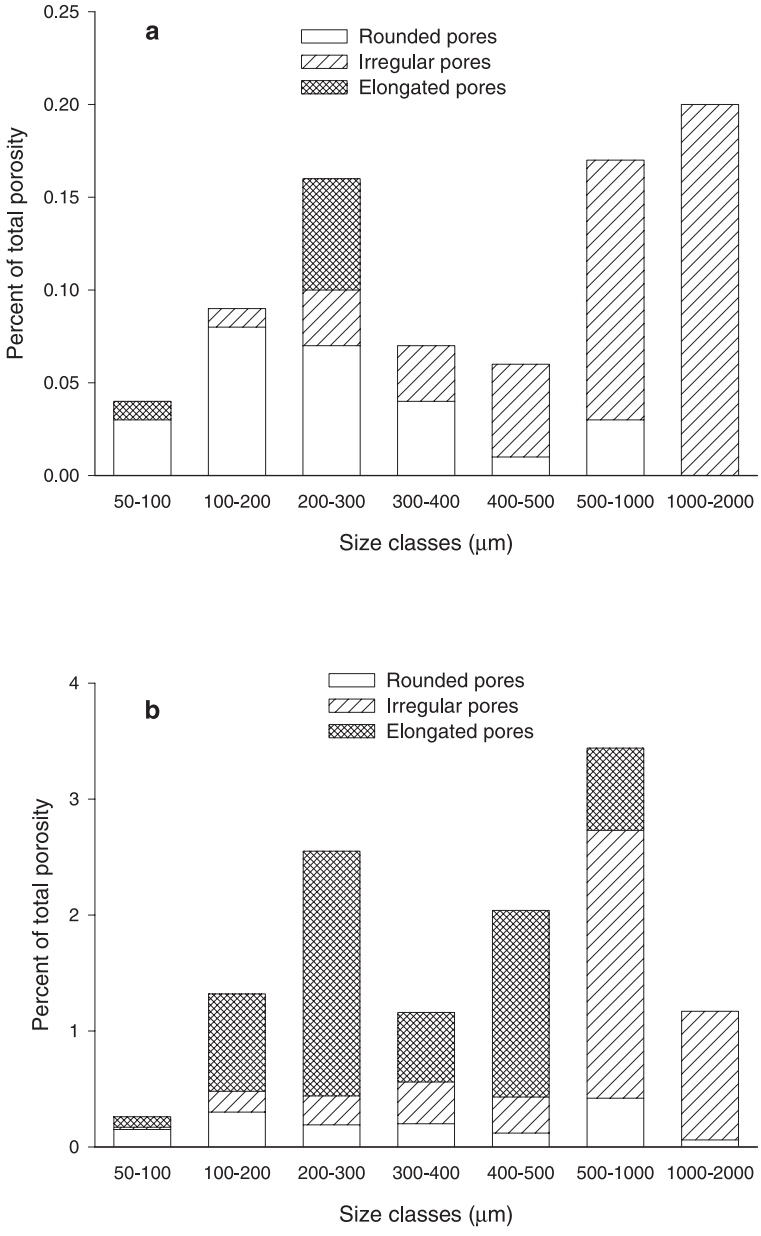

Fig. 1. Pore-size distribution according to the equivalent pore-diameter for regular and irregular pores, and to the width for elongated pores, in a thin section of control soil (a) and of soil contaminated by hydrocarbons (b).

Table 4
Effect of contamination by hydrocarbons on soil total shrinkage and size-classes cracks distributions

	[a]Shrinkage (%)	[b]Size classes of cracks (%)				
		100–200 μm	200–300 μm	400–500 μm	500–600 μm	700–800 μm
Control	7.9 (0.1)	35.1 (1.6)	45.2 (2.7)	16.2 (0.3)	3.1 (0.8)	0.4 (0.2)
Contaminated	3.5 (0.1)	61.7 (2.4)	29.4 (2.1)	6.6 (0.4)	1.7 (0.3)	0.6 (0.1)

In parenthesis, standard error for each measure.
[a] Total shrinkage is expressed as a percentage of area occupied by cracks in the soil surface.
[b] Size-classes cracks distributions are expressed as a percentage of total shrinkage.

moderately porous because the total porosity is in the interval 10–25%, whilst the control soil with a total porosity less than 5% is considered very dense.

Pore size distribution can be regarded as the main aspect of soil porosity. In fact, many of the most important phenomena directly related to plant growth, such as ease of root penetration and storage and movement of water and gases, depend on pore size distribution. The pores ranging from 50 to 500 μm are called transmission pores (elongated and continuous pores), which are considered the most important both in soil–water–plant relationships and in maintaining good soil structure conditions (Giusquiani et al., 1995). As a result of hydrocarbon contamination, the percentages of fissure (>500 μm) and pores belonging to the greater size class of transmission pores (400–500 μm) increased (Table 3). Pore-shape and-size distribution of the contaminated soil showed great differences with respect to control soil (Fig. 1a and b). The higher porosity found in the contaminated soil was due to the proportion of elongated pores in the range of transmission pores, which are essential for the growth of principal roots, for drainage and for soil aeration, especially in this type of soil where the total porosity is relatively low. However, there was a decrease in the elongated pores >500 μm as consequence of hydrocarbon contamination. On the other hand, the relative proportion of rounded pores, which are originated mainly from the carbon dioxide liberated by biological activity (Marinari et al., 2000) was higher in the control soil than in the contaminated soil. The presence of these pores in the control soil is an indication of an unstable and transitory soil structure characterised by the absence of soil aggregates (Giusquiani et al., 1995).

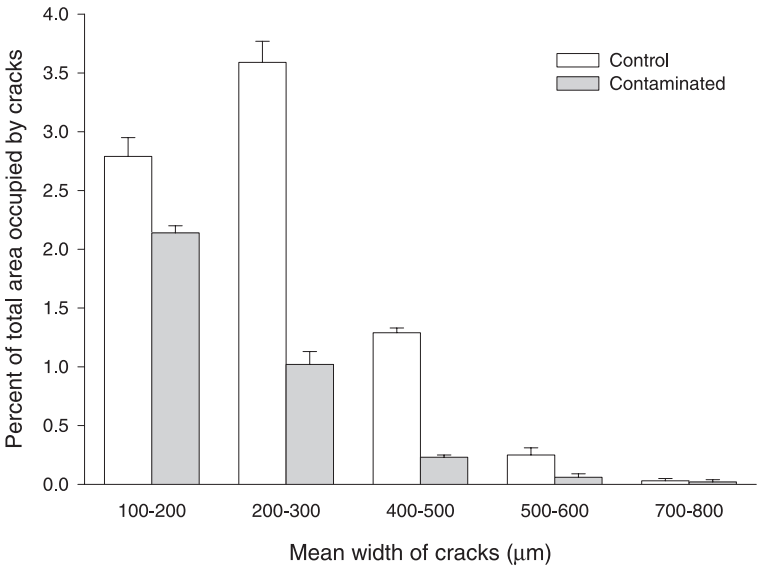

Fig. 2. Size distribution of cracks of a soil in response to contamination by hydrocarbons. Bars represent standard error for each measure ($n = 4$).

The surface shrinkage was higher in the control soil than in the contaminated soil (Table 4). The pattern of size distribution of cracks in the control soil differed from the contaminated soil (Fig. 2). The different crack patterns in the two soils could be related to the higher clay content in the control soil. Moreover, the hydrophobic nature of hydrocarbons can modify the wettability of the soil surface and therefore the interaction with the water. The contamination by hydrocarbons led to an increase in the area occupied by cracks belonging to the smaller size class (100–200 μm), which, corresponding to pores smaller than 50 μm (micropores), can be regarded as a reservoir to hold water for plants and microorganisms (Pagliai et al., 1980). The increase of this class of pores (<50 μm) together with the macropores (>50 μm) can be regarded as a clear indication of improved soil structure.

We can conclude that the contamination by hydrocarbons increased biochemical and microbial activities and improved soil structure. However, the rate of mineralisation of the pollutant was low due possibly to the high chemical complexity of hydrocarbons, which would require long periods of time to degrade. The metabolic quotient of the contaminated soil was higher than that of the control soil, pointing to a reduced microbial efficiency, which is the result of physiological stress imposed by the pollutant.

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References

Anderson, T., Domsch, K.H., 1993. The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of the soil. Soil Biology and Biochemistry 25, 393–395.

Brink, R.H., Dubach, P., Lynch, D.L., 1960. Measurements of carbohydrates in soil hydrolyzates with anthrone. Soil Science 89, 157–166.

Brohon, B., Delolme, C., Gourdon, R., 2001. Complementarity of bioassays and microbial activity measurements for the evaluation of hydrocarbon-contaminated soils quality. Soil Biology and Biochemistry 33, 883–891.

Caravaca, F., García, C., Hernández, M.T., Roldán, A., 2002. Aggregate stability changes after organic amendment addition and mycorrhizal inoculation in the afforestation of a semi-arid site with *Pinus halepensis*. Applied Soil Ecology 19, 199–208.

Fine, P., Graber, E.R., Yaron, B., 1997. Soil interactions with petroleum hydrocarbons: abiotic processes. Soil Technology 10, 133–153.

García, C., Roldán, A., Costa, F., 1997. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Communications in Soil Science and Plant Nutrition 12, 123–134.

García, C., Hernández, T., Roldán, A., Albaladejo, J., Castillo, V., 2000. Organic amendment and mycorrhizal inoculation as a practice in afforestation of soils with *Pinus halepensis* Miller: effect on their microbial activity. Soil Biology and Biochemistry 32, 1173–1181.

Gee, G.W., Bauder, J.W., 1986. Particle-size analysis. In: Klute, A. (Ed.), Methods of Soil Analysis: Part 1. Physical and Mineralogical Methods, 2nd ed. American Society of Agronomy, Madison, WI, pp. 383–411.

Giusquiani, P.L., Pagliai, M., Gigliotti, G., Businelli, D., Benetti, A., 1995. Urban waste compost: effects on physical, chemical, and biochemical soil properties. Communications in Soil Science and Plant Nutrition 24, 175–182.

Hurst, C.J., Sims, R.C., Sims, J.L., Sorensen, D.L., McLean, J.E., Huling, S., 1996. Polycyclic aromatic hydrocarbon biodegradation as a function of oxygen tension in contaminated soil. Journal of Hazardous Materials 51, 193–208.

Ismail, S.N.A., 1975. Micromorphometric soil porosity characterization by means of electro-optical image-analysis (Quantimet 720). Soil Survey Papers, vol. 8. Soil Survey Institute Wageningen, The Netherlands, pp. 140–142.

Keeney, D.R., Nelson, D.W., 1982. Nitrogen-inorganic forms. In: Page, A.L., Baker, D.E., Ellis, R., Keeney, D.R., Miller, R.H., Rhoades, J.D. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, 2nd ed. American Society of Agronomy, Madison, WI, pp. 643–698.

Kuiters, A.T., Dennenman, C.A.J., 1987. Water soluble phenolic substances in soils under several coniferous and deciduous tree species. Soil Biology and Biochemistry 19, 765–769.

Kuwatsuka, S., Shindo, H., 1973. Behaviour of phenolic substances in the decaying process of plant. Identification and quantitative determination of phenolic acids in rice straw and its decayed products by gas-chromatography. Soil Science and Plant Nutrition 19, 219–227.

Lax, A., García-Orenes, F., 1993. Carbohydrates from municipal solid wastes as aggregation factor of soils. Soil Technology 6, 157–162.

Marinari, S., Masciandaro, G., Ceccanti, B., Grego, S., 2000. Influence of organic and mineral fertilisers on soil biological and physical properties. Bioresource Technology 72, 9–17.

Martínez, V.E., López, F., 2001. Effects of hydrocarbon pollutants on the physical and chemical properties of clay soil. Terra 19, 9–17.

Masciandaro, G., Ceccanti, B., García, C., 1994. Anaerobic digestion of straw and piggery wastewater: II. Optimization of the process. Agrochimica 3, 195–203.

Metzger, L., Yaron, B., 1987. Influence of sludge organic matter on soil physical properties. Advances in Soil Science 7, 141–163.

Mohn, W.W., Stewart, G.R., 2000. Limiting factors for hydrocarbon biodegradation at low temperature in Arctic soils. Soil Biology and Biochemistry 32, 1161–1172.

Morgan, P., Watkinson, R.J., 1989. Hydrocarbon degradation in soils and methods for soils biotreatment. CRC Critical Reviews in Biotechnology 8, 308–310.

Murphy, C.P., 1986. Thin section preparation of soils sediments. A B Academic Herts England, 149–155.

Murphy, J., Riley, J.P., 1962. A modified single solution method for determination of phosphate in natural waters. Analitica Chimica Acta 27, 31–36.

Nannipieri, P., Ceccanti, B., Cervelli, S., Matarese, E., 1980. Extraction of phosphatase, urease, protease, organic carbon and nitrogen from soil. Soil Science Society of America Journal 44, 1011–1016.

Nannipieri, P., Grego, S., Ceccanti, B., 1990. Ecological significance of the biological activity in soils. In: Bollag, J.M., Stotzky, G. (Eds.), Soil Biochemistry. Marcel Dekker, New York, pp. 293–355.

Pagliai, M., 1988. Soil porosity aspects. International Agrophysics 4, 215–232.

Pagliai, M., Guidi, G., La Marca, M., 1980. Macro- and micromorphometric investigation on soil-dextran interactions. Journal of Soil Science 31, 493–504.

Pagliai, M., La Marca, M., Lucamante, G., Genovese, L., 1984. Effects of zero and conventional tillage on the length and irregularity of elongated pores in a clay loam soil under viticulture. Soil and Tillage Research 4, 433–444.

Pignatello, J.J., Xing, B., 1996. Mechanisms of slow sorption of organic chemicals to natural particles. Environmental Science Technology 30, 1–11.

Roldán, A., García-Orenes, F., Lax, A., 1994. An incubation experiment to determine factors involving aggregation changes in an arid soil receiving urban refuse. Soil Biology and Biochemistry 26, 1699–1707.

Santruckova, H., Straskraba, M., 1991. On the relationships between specific respiration activity and microbial biomass in soils. Soil Biology and Biochemistry 23, 525–532.

Sartori, G., Ferrari, G.A., Pagliai, M., 1985. Changes in soil porosity and surface shrinkage in a remolded, saline clay soil treated with compost. Soil Science 139, 523–530.

Sims, J.R., Haby, V.A., 1971. Simplified colorimetric determination of soil organic matter. Soil Science 112, 137–141.

Tabatabai, M.A., 1982. Soil enzymes. In: Page, A.L., Miller, E.M., Keeney, D.R. (Eds.), Methods of Soil Analysis. ASA and SSSA, Madison, WI, pp. 501–538.

Tabatabai, M.A., Bremner, J.M., 1969. Use of *p*-nitrophenol phosphate in assay of soil phosphatase activity. Soil Biology and Biochemistry 1, 301–307.

Trasar-Cepeda, C., Leirós, M.C., Seoane, S., Gil-Sotres, F., 2000. Limitations of soil enzymes as indicators of soil pollution. Soil Biology and Biochemistry 32, 1867–1875.

Vance, E.D., Brookes, P.C., Jenkinson, D., 1987. An extraction method for measuring microbial biomass carbon. Soil Biology and Biochemistry 19, 703–707.

Widmer, P., Brookes, P.C., Parry, L.C., 1989. Microbial biomass nitrogen measurements in soils containing large amounts of inorganic nitrogen. Soil Biology and Biochemistry 21, 865–867.

Yeomans, J.C., Bremner, J.M., 1988. A rapid and precise method for routine determination of organic carbon in soil. Communications in Soil Science and Plant Analysis 19, 1467–1476.

Yong, R.N., Mohamed, L.F., Mohamed, A.M.O., 1994. Retention and transport of oil residue in sandy loam soil. In: O'Shay, T.A., Hoddinott, K.B. (Eds.), Analysis of Soils Contaminated with Petroleum Constituents. ASTM, Philadelphia, pp. 98–100.

Zibilske, L.M., 1994. Carbon mineralization. In: Weaver, R.W., Angle, J.S., Bottomley, P.S. (Eds.), Methods of Soil Analysis: II. Microbiological and Biochemical Properties. Soil Science Society of America, Madison, WI, pp. 835–863.