Hydrocarbon pollution from domestic oil recycling industries in peri-urban soils. Lipid molecular assemblage

Ana-M. Álvarez, Pilar Carral, Zulimar Hernández, Gonzalo Almendros

Abstract

The impact of continuous inputs of hydrocarbons from domestic waste oil recycling industries and remediation practices like inertization (liming and addition of ashes) have been studied in peri-urban soils in Spain. Physical and chemical properties of undisturbed soils or contaminated with waste-oil were determined and the lipid fractions extracted using a dichloromethane:methanol mixture and analyzed by gas chromatography-mass spectrometry (GC/MS) in search for environmental proxies.

A pollution gradient (distance from the oil spill) was accompanied by the following chromatographic features: i) the extent of the unresolved 'hump' caused by a progressive concentration of branched/cyclic alkanes with non-biogenic signature, ii) a depletion of fatty acids and alkanol series, and iii) accumulation of phenols and polycyclic hydrocarbons. An increase in the C-preference index (odd-C/even-C numbered homologues) of fatty acids was also detected with an opposed trend in alkane chains. Concerning alkanes and fatty acids chain length, there was a generalized decrease with pollution intensity with a progressive increase of alien long-chain ($>C_{20}$) homologues. Signature compounds found in waste oil (mainly squalene, steroids and waxes) were almost absent from neighbour soils, either undisturbed or inertized, suggesting the occurrence of rapid biodegradation or condensation processes in the soil matrix probably aided by the inertization practices or by enhanced humification processes.

Keywords: waste oil; branched-alkanes; fatty acids; sterols; C-preference index

1. Introduction

The fate of lipid compounds in terrestrial and aquatic environments is a complex process intimately related with the ecosystem biogeochemical performance, which in turn also depends on the original material and the general climatic conditions [1, 2]. In some circumstances, massive inputs of lipid compounds following environmental disasters, spills or uncontrolled dumping of industrial by-products may lead to dramatic local or diffuse pollution. The spatial variability of the composition of the molecular assemblages present in the soil lipid fraction [3] may vary both in terms of the distance from the pollution focus, as well as in terms of the local application of 'inertizing' treatments or other remediation practices aiming at enhancing soil function as 'environmental filter' able to immobilize hazardous compounds that otherwise could enter the biogeochemical cycle [4].

This study was carried out in a well-publicized peri-urban contaminated site located in an industrial area near Madrid at Arganda del Rey municipality, i.e., the Boca Alta Lagoon system, including a waste oil spill (Laguna Negra or 'Black Lagoon') (Fig. 1, a, b). Although oil spilling is currently discontinued a series of industries in the vicinity continued spilling for the last 30 years that has resulted in severe landscape perturbation and the death of thousands birds [5].

The current situation began at the 1980s at the so called Boca Alta Lagoon, when oil recycling companies were dumping wastes in the lake with little control or concern about possible environmental consequences. Since the discontinuation of activities of the main recycling plant in 1997 and for more than 20 years Boca Alta Lagoon has been covered by a layer of non-volatile floating black fluid. Nonetheless, dumping of residues at the lagoon systems has continued with additions from industrial extraction companies pouring pitch-like fluid wastes generated during a number of not publicized activities. In fact, the information available suggests that the industrial activities consisted of a large variety of waste-generating operations including the mentioned domestic oil recycling, petroleum, grease, lubricants and other industrial manufactures like oil and petroleum sulfates and insulators [6]. Inertization process consisted of treating the waste oil (originally an emulsion with low pH) with a mixture of ash and lime, with the consequent formation of secondary salts [7].

The objective of this work is to assess the environmental impact of the Boca Alta Lagoon oil spill on the surrounding soil physical and chemical properties. A comparative study including both the molecular composition study of the extractable lipids in the oil floating in the lagoon with those extracted from the soils was conducted. This would inform about lipid transformations resulting either from inertization activities in sealed lagoon sites or from biodegradation and humification processes in sealed, abandoned contaminated lagoons at comparatively higher distances from the main Black Lagoon. It is also expected that the chemical characterization of the soil lipid fraction extracted from peri-urban contaminated sites would lead to define a series of molecular proxies with use in assessing the impact, resilience and degree of diagenetic transformations of the organic matter in the oil spill and its significance on the whole physico-chemical characteristics of the soils.

2. Material and methods

2. 1. Location and context

The main pollution source, the pitch lake —Black Lagoon—is located in Arganda del Rey, in the Southeast Regional Park in Madrid (40° 18' 10.1"N, 3° 29' 46.8" W). Originally was a wide natural lagoon system of high ecological value (Boca Alta Lagoon). The site is located at the confluence of Manzanares and Jarama rivers forming a wetland with high wildlife density and diversity (Fig. 1a).

The seminatural ecosystems include typical kermes oak (*Quercus coccifera*) and oak forests (*Quercus ilex* ssp. *ballota*) accompanied by brushwood with *Retama sphaerocarpa*, *Cistus albidus* and other aromatic plants, including endemisms of the gypsiferous limestone substrate.

The studied area includes two large groups of geological formations: i) lacustrine, predominantly with Neogene sediments and, ii) coarse riverine sedimentary materials mainly Quaternary. The former Neogene lacustrine formations include gypsum and limestone, whereas the latter consists of Quaternary colluvial materials in the river channels. The soil sampled in the area corresponded to: Leptic Kastanozems (Arenic) (referred to as series R); Hortic Anthrosols (Eutric) (series M); Spolic Technosols (Toxic Densic) (series C) and Spolic Technosols (Toxic) (series L) [6]. The climate in the area is Mediterranean with continental features and tendency to aridity; average annual rainfall of 450 mm and an average annual temperature of 16 °C with marked contrast between winter and summer.

2.2. Sampling

In order to determine the chemical composition of the contaminant spill, a total of three fluid samples (series T: waste oil/pitch at the Black Lagoon) were collected from the upper most floating oily layer (Fig. 1b). Another sample series was collected from soils in the vicinity of the Black Lagoon (series L) from the surface crusts at sealed, inertized lagoons (greyish compact, but friable sediments), labelled as series C (crusts) (Fig. 1c). Soil samples were collected as composite samples taken from three spatial replications in zig-zag paths separated about 50 m each to other. The remaining samples were collected at different distance from the Black Lagoon and the inertized ones, where industrial activities requiring oil dumping were discontinued at about 20 yr ago and the soils were actually in production as olive orchards or cereal fields. These samples were labelled as series M (middle zone). Finally, one control series considered as the reference site was collected in areas of seminatural vegetation consisting mainly of a relict forest of Kermes oak (Series R) (Fig. 1d).

2.3. Soil physical and chemical analyses

Conventional physical and chemical characteristics were determined in soil samples, after air drying and further sieving through a 2 mm mesh (fine earth) [8]. Soil pH was measured in water (1:2.5, w:w) and 1M KCl with a glass-calomel combined electrode.

Soil texture was determined with the densimeter [9]. The organic matter content was measured by wet oxidation [10] and carbonates by acid dissolution [11]. Other physical properties determined were: water retention, water repellence [12] and aggregate stability [13]. All determinations were carried out in duplicate. Soil water holding capacity (WHC) was determined at 0.33 MPa [11].

The samples from the Black Lagoon (series L) showed practical difficulties for mechanical analysis due to its high content of waterproof aggregates. In order to remove the cementing substances, the samples were pre-treated with hydrogen peroxide (10 vol) in a water bath at 60 °C, and the textural tests were routinely performed. Samples from the other series (C, M, R) from undisturbed or inertized lagoons, were analysed as mineral soils.

The soil water repellence, caused by the accumulation of hydrophobic organic compounds, was measured using the water drop penetration time (WDPT) method [12] where seven classes of repellency were distinguished (from 0 to 6) [15].

2.4. Soil lipid extraction and chromatographic analysis

A 2:1 (v:v) dichloromethane-methanol mixture was used for the extraction of lipids. Samples of 3 g were suspended in 75 cm³ of extracting mixture, mechanically stirred for 10 min and subjected to ultrasonic shaking for 5 minutes. The organic extract was siphoned and filtered and the extraction was repeated with the settled residue into the same flask. The extract was dehydrated with anhydrous sodium sulphate, concentrated on a rotary evaporator and the lipid was dried in an oven at 40 °C before weighed.

For gas chromatographic analyses, the lipid samples were suspended in a mixture of methanol with a small proportion of chloroform and methylated with trimethylsilyldiazomethane [16]. The methylated lipid extracts were then analysed by gas chromatography-mass spectrometry (GC-MS) using a HP 5890 gas chromatograph equipped with a HP 5971A mass detector (EI, 70 eV). The chromatographic column was a 25 m × 0.22 mm i.d., cross-linked SE-30 column. Helium flow was 1 cm³ min⁻¹. The chromatograph oven was at 40 °C for the splitless period, then rising to 100 °C at a rate of 32 °C min⁻¹. During the chromatographic run the oven temperature was programmed from 100 °C to 270 °C (at 6 °C min⁻¹). Tentative identity of the compounds was assessed by their 70 eV electron-impact mass spectra and after searching in spectral databases. In the case of isomer compounds or series of homologues, additional confirmation was obtained after independent injection of mixtures of commercially available standards.

3. Results

3.1. Physico-chemical properties of soils and sediments

The sediments at the shore of the Black Lagoon (series L) display a low pH due to the influence of the discharge (pH 2.6). Samples from series C, M and R (Table 1) showed comparatively high pH values (7.0–8.5) consistent with the high concentration of carbonates. Soils from inertized lagoons (series C) show high electric conductivity and

low pH values in some soil samples and similar to those of the floating waste oil. This indicates an incomplete neutralization of the spill after the rapid emulsion between organic effluents with $\text{Ca}(\text{OH})_2$ and calcium silicates [7].

The texture of the samples ranged from sandy to loam (Table 1). The organic matter content in contaminated sites was highly variable ranging from 20 to 300 g kg⁻¹ as compared with the seminatural soils (series R1; 130 g kg⁻¹). High soil organic matter values were also observed in samples from series L, with the pore space almost saturated with the waste organic fluid, which also showed the highest amount of plant litter. The soils showed variable physical properties depending of the distance from the Black Lagoon (Table 2), with WDPT values between 1 and 194 s. The WHC ranged between 5 and 44 g 100 g⁻¹ and were associated to an extremely high aggregate stability (55–91 %).

3.2. Molecular composition of the oil spill

The total abundance of major lipid compounds found in the studied sample series are listed in Table 3. In Figure 2 an example of the molecular composition of the waste oil pitch floating on the Black Lagoon (series T) is shown. These samples consisted mainly in long-chain alkanes (> C₂₄) series, fatty acids and alkanols. The triterpene squalene was found as a major constituent of the waste oil with c. 31 % relative abundance. The main constituents of the oil spill were fatty acids with chain length between C₁₂ to C₂₄ and a clear dominance of even-C-numbered homologues which clearly indicates a vegetable origin. Dialkyl phthalates (mainly *iso*-octyl) were systematically present in all samples. Such compounds are in many cases disregarded in lipid analysis reports due to its dubious origin from laboratory plastic ware or solvents. This is also the case with dioctyl adipate and triphenyl phosphates, which were present in all samples but not included in Table 3. The major steroid was cholesterol but minor contributions of other steroids were detected. At the end of the total waste oil chromatograms a short series of wax esters consisting of up to 7 major compounds with c. 5 % relative abundance was found.

When making a comparative study of the molecular composition of this waste oil (series T) with that of the lipid extracts from the surrounding sampled areas (series L, C, M and R) we could give light to possible transformations of the initial pollution source when entering soils and sediments. In fact the molecular composition of the waste oil dramatically changed in the soil and sediment mineral phase, mainly in sites subjected to neutralization—inertization (Table 3 and Fig. 3). A clear trend across the oil pollution gradient was observed in the order L>C>M>R.

In the lagoon sediments (series L) and inertized soils, apart from the normal chain alkanes, ca. 65 % of alkanes were in a cyclic or branched form appearing in the chromatograms as an unresolved 'hump'. These alkanes are mixtures of isomer compounds in the C₁₅ to C₃₃ range, and interpreted as derived from thermal cracking processes of alkyl compounds, or from fossil sources, e.g., fuel pollution [17]. Other branched alkanes were not included in the unresolved 'hump' of relatively long-chain homologues ranges; these correspond likely to the typical *iso*- and *anteiso*-fatty acids (frequently C₁₅ and C₁₇) which are considered of typical microbial origin [18]. The

relative contribution of this ‘hump’ clearly diminished with the distance from the pollution source, being small in the reference forest samples (series R). There were other trends detected with the distance from the pollution source, mainly a reduction of phenolic and polycyclic aromatic hydrocarbons (naphthalene) compounds and a progressive increase of fatty acids and alkanols (Fig. 3). With respect to the chain lengths of both alkanes and fatty acids, there was a generalized decrease of long-chain ($> C_{20}$) homologues with the distance from the contaminated lagoon.

The reference series R shows the typical lipid pattern of a “healthy” soil with biogenic (plant origin) long chain *n*-alkane series with maximum at C_{29} and the conspicuous odd over even chain C number preference and fatty acid series with even over odd chain C number and maximum at the palmitic acid (C_{16}) (Fig. 4).

In the lipid extract of series L, apart from the hydrocarbon hump, the *n*-alkanes ranged from C_{23} to C_{31} and represented only a small amount of the total lipid (less than 14 %). The alkanes in this oil spill showed typical distributions suggesting two overlapped patterns with intermediate features between that from the waste oil and that from the soils (Fig. 4).

4. Discussion

4.1. Physico-chemical properties of soils and sediments

In general comparatively high values of hydrophobicity (WDPT) coincided with soils with large content of organic C (Tables 1 & 2). The water-repellent soils detected where those from highly contaminated, non-inertized sites (series L). This contrast with the low water repellence observed in inertized sites (series C). In fact, the water holding capacity (WHC) was inversely related to water repellence: high values were observed in seminatural soils with medium to high organic matter content (series R), but the WHC was also high in neighbour agricultural soils (series M). High values of WHC were also observed in inertized soils (series C) as compared to untreated soils. In the L series it could be hypothesized that excessive concentration of water-repellent organic matter may be filling the soil pore space affecting negatively soil WHC. Regarding aggregate stability (Table 2), the non-inertized soils close to the Black Lagoon (series L) with high oil-derived organic matter content showed an improved stability but, conversely, a weak WHC attributable to their severe water repellence.

4.2. Chemical composition of the soil and sediment lipids

The molecular composition of the lipid fraction offered, as expected, relevant qualitative and quantitative information about the environmental impact of the industrial activities. The striking concentration of squalene—a compound present in plant extracts and olive oil—could be interpreted as a selective preservation of residual hydrocarbons during the industrial processes for recycling domestic waste oils.

Concerning fatty acids, the dominance of even-C numbered homologues in all samples points out to a relatively well preserved biogenic signature [14]. In fact the

fatty acid distribution was typical of that of plant and microbial lipids, with a clear dominance of palmitic acid (Fig. 4). Minor amounts of unsaturated acids (mainly oleic and linoleic) were also present in the waste oil.

Considering the previous industrial activities in the site, largely based on processing used edible fats, it was assumed that the wax esters found in the crude waste oils could probably derive from residual epicuticular waxes from higher plants [19]. The high-range ($>C_{27}$) alkanes showed typical saw-teeth distribution with C preference index (CPI) considerably higher than the unity, as typical of biogenic epicuticular alkanes in waxes from higher plants [17]. Conversely, the medium-range alkanes (C_{23} – C_{27}) showed a bell-shaped distribution in the C_{23} – C_{21} range with maximum at *ca.* C_{25} . The presence of low-range molecular weight alkanes ($<C_{24}$) can't be assigned directly with a biogenic origin, and the presence of such molecules i.e. in series L and C, are tentatively interpreted as the product of thermal industrial treatment leading to the release of short-chain homologues. This cracking will also result in losses in parity in long chain series with CPI shifts towards the unity. Both these effects are typical and previously described for phenomena implying a cracking of soil lipids i.e. effect of forest fires or controlled heatings [20].

The changes in the quantitative distribution of the major families of lipid compounds could be tentatively interpreted as the combined effect of microbial and abiotic processes such as the insolubilization of reactive compounds with Ca^{2+} and other polyvalent cations, a clay-favoured 'resinification' or condensation (sorptive or catalytic) of reactive lipids on the mineral matrix [21], and typical selective biodegradation of the molecules suitable to be used as source of C and energy for the microbial metabolism.

In fact, precise understanding of the agents or factors determining lipid biogeochemical transformation is always largely speculative [22]. Soil lipid is a complex mixture of reactive molecules whose composition is dynamically subjected to biotic and abiotic processes [1, 14]. For instance, the concentration of a compound or group of compounds may be due to a plethora of processes such as: i) its microbial synthesis; ii) its release from the soil matrix or from particulate soil organic matter in the course of its biodegradation; iii) its origin from a series of diagenetic structural processes of individual molecules including changes in functionality and chain breakdowns, iv) its selective biodegradation or v) quantitative effects resulting from selective condensations or 'fixation' of lipid molecules onto the soil matrix or humic substances, which are therefore removed from the pool of solvent-extractable forms.

In this study the main quantitative differences in the cumulative values of lipid families in the pollution gradient (i.e., ranging from the seminatural forest to closest to the Black Lagoon) are illustrated in Figs. 3–5. The most evident changes observed following this gradient were: i) the increase in concentration (*ca.* 4-fold) of the branched/cyclic alkanes (i.e., the 'chromatographically unresolved hump') which suggests heavy pollution from non-biogenic lipids, probably associated to inputs from industrial recycling processes. This effect provides a useful proxy to identify local environmental perturbation resulting from the accumulation of persistent lipids from thermal processes, ii) the marked decrease in the relative concentration of biogenic fatty acids and alkanols and, iii) accumulation of phenols and polycyclic aromatic

hydrocarbons. As regards the alkyl series there is a progressive increase in terms of the pollution gradient, in the CPI (odd-C/even-C numbered homologues) of fatty acids, with the opposed trend being observed in alkanes (Fig. 5). This is interpreted as the expected prevalence of unaltered biogenic compounds in the sites less influenced by the industrial inputs. Concerning the chain length index ($>C_{20}/<C_{20}$), there was a conspicuous decrease with the pollution gradient with long-chain ($> C_{20}$) alkanes and fatty acids homologues prevailing in less contaminated sites (series M & R). This support the above indicated less marked biogenic signature following the gradient, which is also consistent with the observed predominance of plant-derived lipids from epicuticular waxes in the control sites. In fact, in severely contaminated sites, the alkane series showed bell-shaped distribution with maximum *ca.* C_{29} suggesting accumulation of hydrocarbons from non-biological fragmentation processes (Figs. 4–5).

5. Conclusions

The strongly acid spill discharge caused significant decrease in soil pH despite of the buffering effect of carbonates in parent materials. The huge concentration of oil spill-derived organic matter in soil adversely altered soil physical properties mainly water repellence, water retention and aggregate stability.

At least the chemical composition of the uppermost floating layer of the black lagoon, consisting mainly of alkanes, fatty acids, alkanols and wax esters, with squalene as major compound, does not suggest high toxicity of the spill.

The spatial variability patterns observed in the soil lipids in the studied areas can be used to monitor the environmental impact of waste oil by taking advantage of molecular proxies, i.e., progressive appearance of a biogenic signature in sites less affected (mainly betrayed by the C-preference number, changes in chain length and in the amount of branched-chain alkanes and cyclic compounds).

Acknowledgements

The authors wish to acknowledge to Dr. José A. González-Pérez (IRNAS, CSIC) and Dr. Heike E. Knicker by their helpful suggestions and critical revision of the manuscript before its submission. This research has been financed by Spanish CICYT grant CGL2013-43845-P.

References

- [1] F.J. Stevenson, Soil Lipids, in: F.J. Stevenson (Ed.), Humus Chemistry, Wiley, New York, 2nd Edition, 1994, pp. 166–211.
- [2] H. Dinel, M. Schnitzer, G.R. Mehuys, Soil lipids: origin, nature, contents, decomposition and effect on soil physical properties, in: J.M. Bollag, G. Stotzky (Eds.), Soil Biochemistry, Dekker, New York, 1990, vol. 6. , pp. 397–429.

- [3] Y.V. Fridland, Some characteristics of soil lipids as a function of ecological conditions, *Soviet Soil Sci.* 14 (1982) 80–88.
- [4] I.A. Simpson, P.F. van Bergen, V. Perret, M.M. Elhmmali, D.J. Roberts, R.P. Evershed, Lipid biomarkers of manuring practice in relict anthropogenic soils, *Holocene*, 9, (1999) 223–229.
- [5] DSAM (Diario de Sesiones de la Asamblea de Madrid) No. 120, 9th February, 2012. (2012).
- [6] World Reference Base for Soil Resources, *World Soil Resources Reports*. No. 103. FAO, Rome, 2006.
- [7] A. Núñez Delgado, M.Y. Pousada Ferradás, F. Quiroga Lago, Procedimiento para la inertización y acondicionamiento de residuos pastosos ricos en hidrocarburos por mezclado con lodos de pizarreras. Pat. No. 2 214 969 (Spain). 2004.
- [8] H.D. Chapman, P.F. Pratt, *Methods of Analysis for Soil, Plants and Water*, 2nd. University of California, Agricultural Division, California, 1982.
- [9] Soil Survey Staff, *Illustrated Guide to Soil Taxonomy*. United States Department of Agriculture, Natural Resources Conservation Service, National Soil Survey Center, Lincoln, Nebraska, 2015.
- [10] D.W. Nelson, L.E. Sommers, Total carbon, organic carbon, and organic matter, in: D.L. Sparks (ed.), *Methods of Soil Analysis: Chemical Methods*, 3rd ed., ASA and SSSA Book Series 5, Madison, Wisconsin, 1996, pp. 961–1010.
- [11] Soil Survey Laboratory, *Methods Manual Soil Survey Investigations*, United States Department of Agriculture, Report No. 42, Version 5.0, 2014.
- [12] E. de Blas, M. Rodríguez-Alleres, G. Almendros, Speciation of lipid and humic fractions in soils under pine and eucalyptus forest in Northwest Spain and its effect on water repellency, *Geoderma* 155 (2010) 242–248.
- [13] J.R. Nimmo, K.S. Perkins, aggregate stability and size distribution, in: J.H. Dane, G.C. Topp (Eds.), *Methods of Soil Analysis: Physical Methods*, Part 4, Madison, Wisconsin, Soil Sci. Soc. America, 2002, pp. 317–328.
- [14] E. De Blas, G. Almendros, J. Sanz, Molecular characterization of lipid fractions from extremely water-repellent pine and eucalyptus forest soils, *Geoderma* 206 (2013) 75–84.
- [15] M. G. Wallis, D. J. Horne, Soil water repellency, in: B.A. Stewart (Ed.), *Advances in Soil Science*, Springer, New York, 1992, pp. 91–146.
- [16] T. Aoyama, T. Shioiri, Trimethylsilyldiazomethane: A convenient reagent for the O-methylation of alcohols, *Tetrahedron Lett.* 31 (1990) 5507–5508.
- [17] B.R.T. Simoneit, M.A. Mazurek, Organic matter of the troposphere-II, Natural background of biogenic lipid matter in aerosols over the rural western United States, *Atmos. Environ.* 6 (1982) 2139–2159.
- [18] T. Kaneda, Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance, *Microbiol. Rev.* 55 (1991) 288–302.
- [19] O.C. Braids, R.H. Miller, Fats, waxes, and resins in soil, in: J.E. Gieseking (Ed.), *Soil Components: Organic Components*, Springer, Berlin, 1975, pp.343–368.
- [20] G. Almendros, F. J. González-Vila, Wildfires, soil carbon balance and resilient organic matter in Mediterranean ecosystems. A review, *Spanish J. Soil Sci.* 2 (2012) 8–33.

- 411 [21] G. Almendros, J. Sanz, F. J. González-Vila, F. Martin, Evidence for a polyalkyl
412 nature of soil humin. *Naturwissenschaften* 78 (1991) 359–362.
- 413 [22] M.C. Zancada, G. Almendros, J. Sanz, R. Román, Speciation of lipids and humus-
414 like colloidal compounds in a forest soil reclaimed with municipal solid waste
415 compost, *Waste Manag. Res.* 22 (2004) 23–24.
- 416

Figure captions

Fig. 1. Lagoon system used as oil dumping (Arganda del Rey, Madrid): (a) geographical location of the sampling sites in the Iberian Peninsula, (b) Black Lagoon (sampling series T) and soils neighbor to the Black Lagoon (series L), (c) sampling sites at sealed, inertized, lagoon plots (series C), (d) and control soils in middle zone (series M) and a seminatural soil (series R).

Fig. 2. Example of gas chromatogram obtained for the volatile compounds in oil spill (series T). Major compounds are labelled on the peaks and the homologous series (A: alkanes, FA: fatty acid methyl esters, OH: alkanols) are depicted with carbon number indicated as subscript.

Fig. 3. Quantitative distribution of main compounds families of aliphatic hydrocarbons and aromatic and polar/alkyl compounds. Total abundances are referred to the total ion chromatographic area. Error bars indicate least significant differences ($P < 0.05$) calculated from spatial replications. Soils in the neighbour of the Black Lagoon (series L), inertized sealed plots (series C), control soils in middle zone (series M) and seminatural soil (series R).

Fig. 4. Homologous series for *n*-alkanes (a) and *n*-fatty acids (b) found in the free lipid fractions. Black Lagoon (series T) and soils in the neighbour of the Black Lagoon (series L), inertized sealed plots (series C), control soils in middle zone (series M) and seminatural soil (series R).

Fig. 5. Trends across the oil pollution gradient of typical ratios between alkyl compounds. Series labels refer to Fig. 4. Error bars indicate least significant differences ($P < 0.05$) calculated between spatial replications.

445 **Table 1**
 446 Analytical characteristics of soil and sediment samples

References	Sampling sites	pH (H ₂ O)	pH (1M KCl)	Electric conductivity (×10 ⁻³ dS m ⁻¹)	Soil texture (g·kg ⁻¹)			Organic matter (g·kg ⁻¹)	Carbonates (g·kg ⁻¹)
					Clay	Silt	Sand		
R (relictual oak forest)	Seminatural	8.2	6.9	254	42	153	805	152	126
M (middle zone)	Cultured	8.3	6.5	94	102	266	632	67	83
C (crusts)	Inertized	7.1	6.5	1851	62	236	702	49	296
L (lagoon)	Tar swamp ^a	6.2	6.5	284	47	73	880	178	308
LSD ^b		0.3	0.4	533	32	61	65	49	47

447
 448 ^a Samples pretreated with hydrogen peroxide before determining pH, conductivity and texture.

449 ^b Least significant difference between spatial replicates ($P < 0.05$)
 450

451 **Table 2**
 452 Physical characteristics of soil and sediment samples
 453

Reference	Sampling sites	WDPT, Water Drop Penetration Time (s)	Water repellence class ^a	WHC, Water Holding Capacity (g 100g ⁻¹)	Aggregate stability (%)
R (relictual oak forest)	Seminatural	23	1	44	81
M (middle zone)	Cultured	1	0	42	55
C (crusts)	Inertized	6	1	35	64
L (lagoon)	Tar swamp ^a	194	3	5	91
LSD ^b		33	0.9	14	34

454
 455 ^a Repellence classes: class 0, wettable or non-water-repellent (WDPT <5 s); class 1, slightly water-repellent (5–60 s);
 456 class 2, strongly water-repellent (60–600 s); class 3, severely water-repellent (600–3600 s) and class 4, extremely water-
 457 repellent (>3600 s).

458 ^b Least significant difference ($P < 0.05$).
 459

460 **Table 3**
461 Total abundances^a of major lipid compounds extracted from soils and sediments
462

	Series T	Series L	Series C	Series M	Series R
Compound (as methyl esters)	Crude waste oil (floating fluid)	Sediment of the 'Black Lagoon' (tar swamp)	Soil from inertized oil dump	Neighbour soil with no waste oil	Reference (oak forest)
Phenol		4.78	7.60		
Trimethylnaphthalene		0.64	7.68		
Methyl dodecanoate (laurate) (C ₁₂)		0.80			0.00
Tridecanol (C ₁₃ alkanol)	0.17				
Dimethoxybenzenecarboxylic acid, methyl ester					
Hexadecane (C ₁₆ alkane)					
Tetradecanol (C ₁₄ alkanol)	1.01				
<i>Nor</i> heptadecane (C ₁₇ alkane)					
Methyl tetradecanoate (myristate, C ₁₄)	3.11	0.14	0.18	0.85	0.90
Methyl <i>iso</i> -pentadecanoate (C _{15-b})			0.28		
Methyl <i>anteiso</i> - pentadecanoate (C _{15-b})			1.83		
Pentadecanol (C ₁₅ alkanol)	1.17				
<i>Nor</i> octadecane (C ₁₈ alkane)		0.12			
Methyl pentadecanoate (C ₁₅)	3.31	0.07		0.59	
Methyl hexadecenoate (C _{16:1}) (palmitoleate)	3.08			0.62	1.38
Hexadecanol (C ₁₆ alkanol)	0.36				
Nonadecane (C ₁₉ alkane)		0.55			
Methyl hexadecanoate (palmitate, C ₁₆)	22.08	1.11	0.88	21.01	11.20

Methyl <i>iso</i> -heptadecanoate (C _{17-b})					0.41
Heptadecanol (C ₁₇ alkanol)	0.28				
<i>Anteiso</i> -methyl heptadecanoate (C _{17-b})					0.26
Eicosane (C ₂₀ alkane)		0.25			
Methyl heptadecenoate (C _{17:1})	1.27		0.11		
Methyl heptadecanoate (margarate, C ₁₇)	2.32	0.15	0.35	0.58	0.75
Methyl octadecenoate (oleate, C _{18:1})	8.64		0.72	1.93	4.24
Octadecanol (alkanol C ₁₈)	0.10				
<i>Nor</i> heneicosane (C ₂₁ alkane)		0.31			
Methyl octadecanoate (stearate, C ₁₈)	4.34	2.01	2.08	8.28	3.17
Nonadecanol (C ₁₉ alkanol)	0.99				
<i>Nor</i> docosane (C ₂₂ alkane)		0.14		0.69	0.20
Methyl nonadecanoate (C ₁₉)	0.11	0.11		0.23	0.76
Triphenyl phosphate (TPP)		1.67	4.77		
Dioctyl adipate	0.40	3.82	4.46		
Eicosanol (C ₂₀ alkanol)	0.33				
<i>Nor</i> tricosane (C ₂₃ alkane)	0.08		0.00	0.52	1.09
Methyl eicosanoate (arachidate, C ₂₀)	0.52	0.20	0.14	1.40	2.12
Heneicosanol (C ₂₁ alkanol)					1.93
<i>Nor</i> tetracosane (C ₂₄ alkane)	0.87	0.35	0.40	0.56	1.34
Methyl heneicosanoate (C ₂₁)	0.31			0.64	0.66
Docosanol (C ₂₂ alkanol)	2.72				1.94
<i>Nor</i> pentacosane (C ₂₅ alkane)	0.90	1.01	1.22	1.00	0.34
Methyl docosanoate (behenate, C ₂₂)	0.35	0.20		3.26	1.80
Squalene (C ₃₀ H ₅₀)	31.17				
C ₂₆ branched alkane		0.60			
C ₂₆ branched alkane		0.82			

<i>Nor</i> hexacosane (C ₂₆ alkane)	0.71	1.19	1.39	1.30	1.01
Methyl tricosanoate (C ₂₃)	0.18		0.16	0.92	0.88
Tetracosanol (C ₂₄ alkanol)					4.58
C ₂₇ branched alkane		0.29			
C ₂₇ branched alkane		0.35			
<i>Nor</i> heptacosane (C ₂₇ alkane)	0.60	1.90	1.66	3.87	2.63
Methyl tetracosanoate (lignocerate, C ₂₄)	0.42		0.38	3.24	5.03
C ₂₈ branched alkane		0.61			
C ₂₈ branched alkane		0.15			
<i>Nor</i> octacosane (C ₂₈ alkane)	0.20	1.37	1.77	3.3	1.60
Cholest-5- <i>en</i> -3-ol (3β)	4.18				
Methyl pentacosanoate (C ₂₅)			0.06	0.94	0.70
Pentacosanol (C ₂₅ alkanol)				1.03	0.77
C ₂₉ branched alkane		0.51		3.88	
<i>Nor</i> -nonacosane (C ₂₉ alkane)	0.48	1.96	1.83	2.4	13.04
Methyl hexacosanoate (cerotate, C ₂₆)		0.67	0.10	1.20	1.47
Wax ester	0.48				
Wax ester	0.48				
<i>Nor</i> triacontane (C ₃₀ alkane)	0.14	1.95	1.53	1.55	1.78
Wax ester	0.82				
Wax ester	0.40				
Wax ester	0.79				
C ₃₁ branched alkane		0.74			
C ₃₁ branched alkane		1.54			
<i>Nor</i> hentriacontane (C ₃₁ alkane)	0.34	1.57	1.43	1.20	8.18
Methyl octacosanoate (C ₂₈)				0.64	1.33
C ₃₂ branched alkane		1.04			

C ₃₂ branched alkane	0.23		
<i>Nor</i> dotriacontane (C ₃₂ alkane)	0.80	0.83	0.81
<i>Nor</i> tritriacontane (C ₃₃ alkane)	0.48		

463 ^a As methyl esters. Total abundances relative to the total ion chromatographic area. Compounds representing less than
464 0.1% (blank cells) were not taken into account.

Figure

Fig. 1. Lagoon system used as oil dumping (Arganda del Rey, Madrid): (a) geographical location of the sampling sites in the Iberian Peninsula, (b) Black Lagoon (sampling series T) and soils neighbor to the Black Lagoon (series L), (c) sampling sites at sealed, inertized, lagoon plots (series C), (d) and control soils in middle zone (series M) and a seminatural soil (series R).

Fig. 2. Example of gas chromatogram obtained for the volatile compounds in oil spill (series T). Major compounds are labelled on the peaks and the homologous series (A: alkanes, FA; fatty acid methyl esters, OH: alkanols) are depicted with carbon number indicated as subscript.

Fig. 3. Quantitative distribution of main compounds families of aliphatic hydrocarbons and aromatic and polar/alkyl compounds. Total abundances are referred to the total ion chromatographic area. Error bars indicate least significant differences ($P < 0.05$) calculated from spatial replications. Soils in the neighbour of the Black Lagoon (series L), inertized sealed plots (series C), control soils in middle zone (series M) and seminatural soil (series R).

Alkanes (a)

Fig. 4. Homologous series for n-alkanes (a) and n-fatty acids (b) found in the free lipid fractions. Black Lagoon (series T) and soils in the neighbour of the Black Lagoon (series L), inertized sealed plots (series C), control soils in middle zone (series M) and seminatural soil (series R).

Fatty acids (b)

← Soil contamination gradient →

Fig. 5. Trends across the oil pollution gradient of typical ratios between alkyl compounds. Series labels refer to Fig. 4. Error bars indicate least significant differences ($P < 0.05$) calculated between spatial replications.