



Degradation of fuel oil in salt marsh soils affected by the Prestige oil spill

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ABSTRACT

We assessed natural degradation of fuel oil in three marshes from Galicia (Spain) affected by the Prestige oil spill (Baldaio, Barizo, and Muxía). Soil samples collected from polluted and unpolluted areas on four different dates were used to determine total petroleum hydrocarbon content and fuel-oil components. Natural degradation was monitored by analysing changes in the proportion of saturated hydrocarbons, aromatics, asphaltenes and resins in the soils, and also by evaluating the degree of depletion of saturated hydrocarbons on each sampling date. We additionally assessed the phytoremediation potential of *Lolium perenne* L., *Convolvulus arvensis* L. and *Raphanus raphanistrum* L. All marsh soils exhibited natural degradation of saturated and aromatic hydrocarbons to between 85 and 95% in most cases. In contrast, asphaltenes and resins were degraded to a lesser extent (viz. 64–76% in Barizo 1, Muxía and Traba; 39–44% in Baldaio; and only 12% in Barizo 2, where flooding by the river continues to introduce balls of fuel oil into the soil). Monitoring analyses revealed natural degradation to be dependent on the thickness of the pollutant layer. Field plots sown with *L. perenne* L. exhibited no significant differences in fuel-oil degradation from untreated plots.

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1. Introduction

On November 13, 2002, the oil tanker *Prestige* developed a leak off Cape Finisterre (Galicia, NW Spain); 6 days later, the vessel broke in two and sank 130 nautical miles off the coast. Overall, the Prestige spill an estimated 64,000 tonnes of heavy fuel oil, a material with high nitrogen, sulfur and heavy metal contents that forms highly viscous, poorly soluble emulsions in sea water [1–3]. The spill affected a sizeable portion of the Galician coast and coastal waters, and much of the rest of the southern Bay of Biscay. Oil removal efforts focused primarily on beaches and, secondarily, on rocks and cliffs. This paper deals with the effects of the spill on salt marshes, an ecosystem that was also severely affected and in which using conventional mechanical oil removal methods would have wiped out most existing vegetation.

Marshes, which constitute a major component of river, estuarine and coastal ecosystems, are extremely sensitive to oil pollution [4]; therefore, they can be severely damaged by spills, which block carbon fixation by stifling plant transpiration and can kill marsh vegetation through this and other mechanisms [5].

Fuel oil from spills is known to persist for at least 5 years in marsh sediments, from which it can be released into marsh water. Its persistence reflects in high hydrocarbon levels in shellfish inhabiting the polluted marshes or exposed to hydrocarbons released therefrom [6–9].

Relatively few studies have investigated the effects of crude oil spills on natural degradation in soil ecosystems [10] other than the impact on the community structure of soil bacteria [11,12]. Hydrocarbons are primarily degraded by bacteria and fungi, adaptation by prior exposure of microbial communities to hydrocarbons increasing their degradation rate [13]. Biodegradation of hydrocarbons usually requires the cooperation of more than one species [14]. This is particularly true of pollutants consisting of many different compounds such as crude oil or petroleum. The degree of hydrocarbon biodegradation in soil is influenced by various environmental factors including the nature and concentration of contaminants, soil properties, pH, temperature, water content, and nutrient availability [15,16].

The salt marshes of Baldaio and Muxía, and those associated with the beaches of Barizo and Traba Lagoon, are located in estuaries on the stretch of the Galician coast that was most severely affected by the *Prestige* spill: the Costa da Morte (Fig. 2). In a previous study [17], we reported on the effects of the spill on the physical and physicochemical properties, and total hydrocarbon and heavy metal contents, of soils in affected areas of these marshes 3 months after the spill, when the initial cleaning of the beaches had already

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Table 1
Sampling areas (salt marshes) and specific sampling locations in each area.

Site	Sample number and location	Coordinates
Baldaio marsh	1. 50 m from the low-water line (zone 1) 2. Center of marsh (zone 2)	N: 43° 17.833' W: 8° 39.733' N: 43° 17.734' W: 8° 39.672'
Barizo marsh	4. 50 m from the low-water line (zone 1) 5. Center of marsh (zone 2)	N: 43° 18.776' W: 8° 51.444' N: 43° 18.782' W: 8° 51.453'
Traba Lagoon beach	6. 50 m from the low-water line	N: 43° 11.585' W: 9° 2.422'
Muxía marsh	7. 50 m from the low-water line	N: 43° 5.948' W: 9° 12.754'

been completed. Oil pollution altered both chemical and physical soil properties: the polluted soils exhibited a dark, compact crust of significantly lower porosity and greater resistance to penetration than that of unpolluted soils at the same sites. In addition, the pollutants significantly lowered E_h and intrinsic permeability, raised hydrophobicity, and increased the concentrations of Cr, Cu, Fe, Pb, V and Ni, and total petroleum hydrocarbon contents, by a factor of 2–2500.

This previous study thus showed that the *Prestige* oil spill constituted a major source for the heavy metals currently present in the marshes. The present study was aimed to characterize natural degradation of organic components of the fuel oil over a 4-year period in the four salt marshes. To this end, we determined TPH (total petroleum hydrocarbon) contents and hydrocarbon composition in order to monitor TPH degradation in soil.

2. Material and methods

2.1. Study areas and sampling procedure

A general description of the sampling areas (Baldaio, Barizo, Traba and Muxía, four salt marshes affected by the *Prestige* spill; Fig. 2 and Table 1) and studied soils as regards physicochemical properties, and TPH and heavy metal contents, as of February 24, 2003, can be found elsewhere [17].

Table 2 summarizes the properties of the fuel-oil samples collected at sea and in the studied marshes, and Tables 3 and 4 those of the soils. The soil samples studied were obtained at polluted and unpolluted sites in the four sampling areas on four different dates, namely: February 23, 2003 (while the initial cleaning of the beaches was being performed); May 2, 2005; April 4, 2006; and February 28, 2007. Topsoil (0–30 cm) samples were collected by means of an Eijkelkamp sampler and stored in polyethylene bags at 4 °C in the dark. A total of five samples were taken at each sampling site on each date that were air-dried, passed through a 2-mm sieve, mixed and homogenized in the laboratory; subsequently, 5 subsamples from each aggregate sample were used to determine the total petroleum hydrocarbon (TPH) content and hydrocarbon composition.

2.2. Hydrocarbon analysis

TPH contents were determined following ISO/TR11046(E) [18], using the methods proposed by Referentie Informatiemodel voor Ziekenhuisapotheken [19,20], Pennings [21] and Weisman [22], on soil samples stored at 4 °C in the dark. Samples were dried chemically over a hygroscopic salt, ground, and extracted with 1,1,2-trichloro-1,2,2-trifluoroethane. The extract was stirred with magnesium silicate to remove polar organic compounds and filtered; then, it was supplied with hexane for analysis by gas chromatography, using a flame ionization detector and a mixture of

Table 2
Characteristics of the fuel oil from sources S1 and S2.^a

	Method	Units	Sample and sampling site			
			S1		S2	
			Open sea (vacuum-dried)	Open sea (wet)	Salt marsh (vacuum-dried)	Salt marsh (wet)
Density (15 °C)	[52]	kg m ⁻³	977a	988a	979a	988a
Viscosity (50 °C)	[52] ^b	mm ² s ⁻¹	1630.18a	18219.80a	1273.04b	9441.55b
S			28.3a	12.68a	23.2b	9.22b
C	[53]	g kg ⁻¹	830.22a	371.94a	790.99b	314.66b
N			4.2a	2.1a	3.7b	1.6b
H ₂ O	Drying <i>in vacuo</i> [54]	%(v/v)	–	55.20a	–	54.26a
Ash	[52]	g kg ⁻¹	–	4.23a	–	1.55b
Cr			8.67a	0.20a	7.06b	0.19a
Cu			14.09b	7.73b	18.16a	15.00a
Fe			176.10a	39.80b	163.83b	141.18a
Ni	Digestion with HNO ₃ . Analysis by ICP-OES	mg kg ⁻¹	42.01b	27.20a	50.65a	30.80a
Pb			12.58a	6.73b	9.41b	8.33a
Zn			4.91b	0.67b	9.41a	3.89a
V			95.79b	58.93b	110.59a	70.19a
Saturated hydrocarbons			18.6a		19.7a	
Aromatic hydrocarbons	[23]	%	47.3a		44.8b	
Resins and asphaltenes			34.1b		35.5a	

For each sample type (wet or vacuum-dried), values of a parameter followed by a different letter are significantly different at $p < 0.05$.

^a Mean values for five samples per salt marsh.

^b American Society for Testing and Materials.

Table 3
Selected properties of the studied soils.

Marsh	Zone	pH (H ₂ O)	pH (KCl)	Bulk density (g cm ⁻³)	Soil organic matter (g kg ⁻¹)	Available P (mg kg ⁻¹)	Available K (mg kg ⁻¹)	Available Mg (mg kg ⁻¹)
Baldaio	1	7.7b	7.3b	0.42d	128a	40b	308b	1618b
	2	7.9a	7.8ab	1.20b	49b	936a	936a	1848a
Barizo	1	8.2a	8a	1.14	25c	19c	158d	612c
	2	8.1a	7.9	0.99c	28c	13c	184c	654c
Traba		7.8b	8.3a	1.88a	2e	4d	52e	172e
Muxía		8.1a	8.1a	1.26b	11d	14c	56e	304d

In each column, values followed by a different letter are significantly different at $p < 0.05$.

n-alkanes containing 6–36 carbon atoms as the external standard [22].

Fuel-oil components were extracted according to Fernandez-Alvarez et al. [23]. Residual fuel oil from soil samples, previously combined with anhydrous sodium sulfate, was recovered by ultrasonic extraction with a 1:1 (v/v) dichloromethane–acetone mixture. The resulting extracts were dried over anhydrous sodium sulfate and concentrated to dryness under vacuum. After gravimetric analysis, the residues were dissolved in *n*-hexane and fractionated by alumina column chromatography [24]. Saturated hydrocarbons were eluted with *n*-hexane, aromatic compounds with dichloromethane, and resins with methanol, while asphaltenes were retained on the column [23]. Each fraction was concentrated to dryness under a nitrogen stream and weighed in order to determine the proportions of saturated hydrocarbons, aromatics, resins and asphaltenes with respect to total weight. Resins and asphaltenes are deemed recalcitrant, while saturated hydrocarbons and aromatics are deemed degradable [25]. The overall accuracy and precision of the method of separating the components of fuel oil were verified by using undegraded fuel from the Prestige as reference [17,23].

Saturated hydrocarbons were determined on a GC-FID Varian CP-3800 system. Chromatography was done on a WCOT fused silica column (15 m × 0.25 mm i.d. × 0.25 μm CP-Sil 5 CB). The heating schedule comprised two ramps, namely: from 35 to 150 °C and from 150 to 300 °C, at 20 and 15 °C min⁻¹, respectively. Helium at a 1 ml min⁻¹ flow rate was used as carrier gas, and a 1 μl aliquot of each sample at 400 °C injected into the chromatograph by means of a Varian CP-8400 autosampler.

A Varian CP-3800 GC–MS, coupled to a Saturn 2200 GS-MS system was used for full identification of saturated hydrocarbons. Chromatography was done on a WCOT fused silica column (30 m × 0.25 mm i.d. × 0.25 μm VF-5ms). The initial temperature, 50 °C, was followed by a 50–300 °C ramp at 10 °C min⁻¹. Again, the carrier gas was helium at 1 ml min⁻¹, and a 1 μl aliquot of each sample at 250 °C was injected with the aid of a Varian CP-8400 autosampler.

Compounds were identified using NIST Standard Reference Material 1494 [26], a solution of 20 components including aliphatic hydrocarbons with odd and even numbers of carbon atoms from 10

to 20, aliphatic hydrocarbons with even numbers of carbons from 20 to 34, pristane and phytane. In addition, identification of our compounds required the use of internal standards such as isoprenoids (pristane and phytane) and hopanes. Pristane and phytane are useful monitors of alkane depletion at an early stage in degradation processes.

2.3. Assessment of fuel-oil degradation

Natural degradation was monitored by analysing changes in the proportions of saturated hydrocarbons, aromatics, asphaltenes and resins in the soils, and assessing the degree of depletion of saturated hydrocarbons with variable numbers of carbon atoms on each sampling date.

2.4. Potential for phytoremediation bioassays

These assays involved assessing germination and growth of three species (*Lolium perenne* L., *Convolvulus arvensis* L. and *Raphanus raphanistrum* L.) planted in a growth chamber. Species were selected for their ability to grow in contaminated and high salinity soils. Both three species could improve the soil structure, aeration to secondarily improve the microorganisms effects, and they could also enhance the phytostabilization. In addition, the phytoremediation potential of these species was assessed by using greenhouse trials to compare their growth in soil samples from contaminated areas with that in uncontaminated soils.

Because the Baldaio and Barizo marshes continue to be under the influence of pollution, we also assessed the phytoremediation potential of *L. perenne*, L., *C. arvensis* L. and *R. raphanistrum* L. by using contaminated marsh soil samples and uncontaminated soils in a germination and growth chamber, and in a greenhouse. Comparisons were based on biomass production and used to identify the most suitable species for *in situ* bioremediation in the affected marshes.

The soil material used in the growth chamber study was top-soil collected from Barizo (sites 1 and 2) and Baldaio (sites 1 and 2). Material was sieved to eliminate debris, air-dried and added to 0.35 l pots. Grow chambers were illuminated by fluorescent

Table 4
Characteristics of the cation exchange complex in the studied soils.

Marsh		Ca (cmol kg ⁻¹)	Mg (cmol kg ⁻¹)	Na (cmol kg ⁻¹)	K (cmol kg ⁻¹)	Al (cmol kg ⁻¹)	ClCe (cmol kg ⁻¹)	Ca/Mg	K/Mg	Ca:Mg:K
Baldaio	1	38.8b	12.94b	27.04b	0.77b	0.1a	79.65b	3e	0.1a	74/25/01
	2	91.74a	14.78a	37.12a	2.34a	0.1a	91.74a	6d	0.1a	69/27/04
Barizo	1	37.2b	4.9c	4.48c	0.40c	0.1a	47.07c	8c	0.1a	88/12/01
	2	38.2b	5.23c	4.54c	0.46c	0.1a	48.54c	7cd	0.1a	87/12/01
Traba		25d	1.38e	3.33d	0.13d	0.1a	29.93e	18a	0.1a	94/05/00
Muxía		33c	2.43d	0.78e	0.14c	0.1a	36.46d	14b	0.1a	93/07/00

In each column, values followed by a different letter are significantly different at $p < 0.05$.



Fig. 1. Time course of soil TPH contents and of fuel-oil crusts on the soil (2003–2007). (a)–(d) Barizo: 2003 (a), 2005 (b), 2006 (c) and 2007 (d). (e)–(h) Baldaio: 2003 (e), 2005 (f), 2006 (g) and 2007 (h). (i)–(l) Muxía: 2003 (i), 2005 (j), 2006 (k) and 2007 (l). (m)–(p) Traba Lagoon beach: 2003 (m), 2005 (n), 2006 (o) and 2007 (p). (a), (e), (i), and (m): general views.

lamps with an irradiance of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$. Four replicates per plant treatment/soil treatment were used in addition to a control soil obtained from an unaffected, uncontaminated marsh (Louro, Galicia, Spain) with a zero TPH concentration. Bioassays were designed in accordance with a completely random scheme, using a 5×6 factorial arrangement with three replications. Additionally, germination in Petri dishes was assessed as an external control.

The three species studied (*L. perenne*, L., *C. arvensis*, L. and *R. raphanistrum*, L.) had all previously been deemed potential phytoremediators both organic and inorganic, including heavy metals that are normally associated to the oil spills, and they are capable of growing in saline situations [27–39]. If plants are capable of growing in such situation, they can help alleviate the problem either transporting the organic pollutants to aerial parts, where they could be harvested, or just improving the soil conditions, e.g. to create more favorable conditions for breakdown by microorganisms. That means that just being capable of good growth in the field, plants can be potentially useful. Assays were done on five soils; four were of the contaminated type and obtained from the Baldaio and Barizo marshes (2 samples each).

Five replicates of each soil (Baldaio and Barizo soils, and unpolluted control soil) were incubated in a germination and growth chamber under appropriate conditions for the three species, namely: a 16/8 photoperiod, 80% RH and 24°C . Plants were watered three times a week, using water collected from a clean marsh in order to mimic environmental conditions as closely as possible.

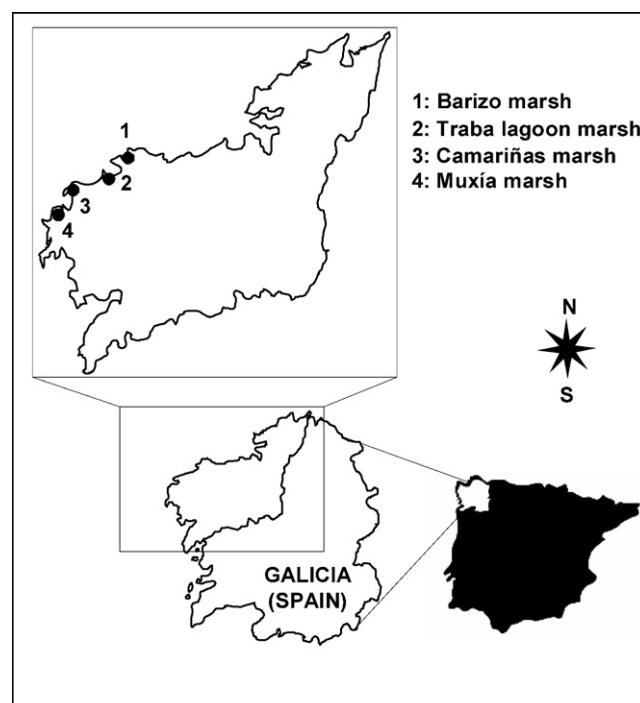


Fig. 2. Map showing the location of the study areas.

Germination parameters were recorded on a weekly basis for 30 days, and aerial biomass was cropped and stove-oven dried at 110 °C during 3 days, 45 days after sowing in order to assess growth. Determinations included the following parameters:

- Germination rate, which was calculated from the following expression:

$$\text{Germination rate} = \frac{1}{\sum(G_i \cdot D_i)/N}$$

where G_i is the number of germinated seeds with expanded cotyledons between days i and $i - 1$; D_i the number of days after sowing; and N the total number of seedlings.

- Seedling growth, which was assessed from seedling height, in cm, as measured in the bioassays.

- Net growth (g/seedling), which was taken to be the dry weight of seedlings measured in the bioassays.

- Plant stress, which was assessed with a Hansatech modulated fluorometer. To this end, the maximum quantum yield of photosystem II (F_v/F_m) was measured according to Genty et al. [40] on days 7 and 15. Chlorophyll fluorescence (more specifically, the F_v/F_m ratio, where F_v denotes variable fluorescence and F_m maximum fluorescence), which is a measure of photochemical efficiency in photosystem II, is often used as an indicator of plant stress [41], particularly in studies of pollution impacts [42,43].

Finally, *L. perenne* L. was also used in field trials in Baldaio and Barizo – the two marshes most severely affected by the spill – in order to ascertain whether planting this species would favor natural degradation of fuel oil in the soil. To this end, we planted *L. perenne*

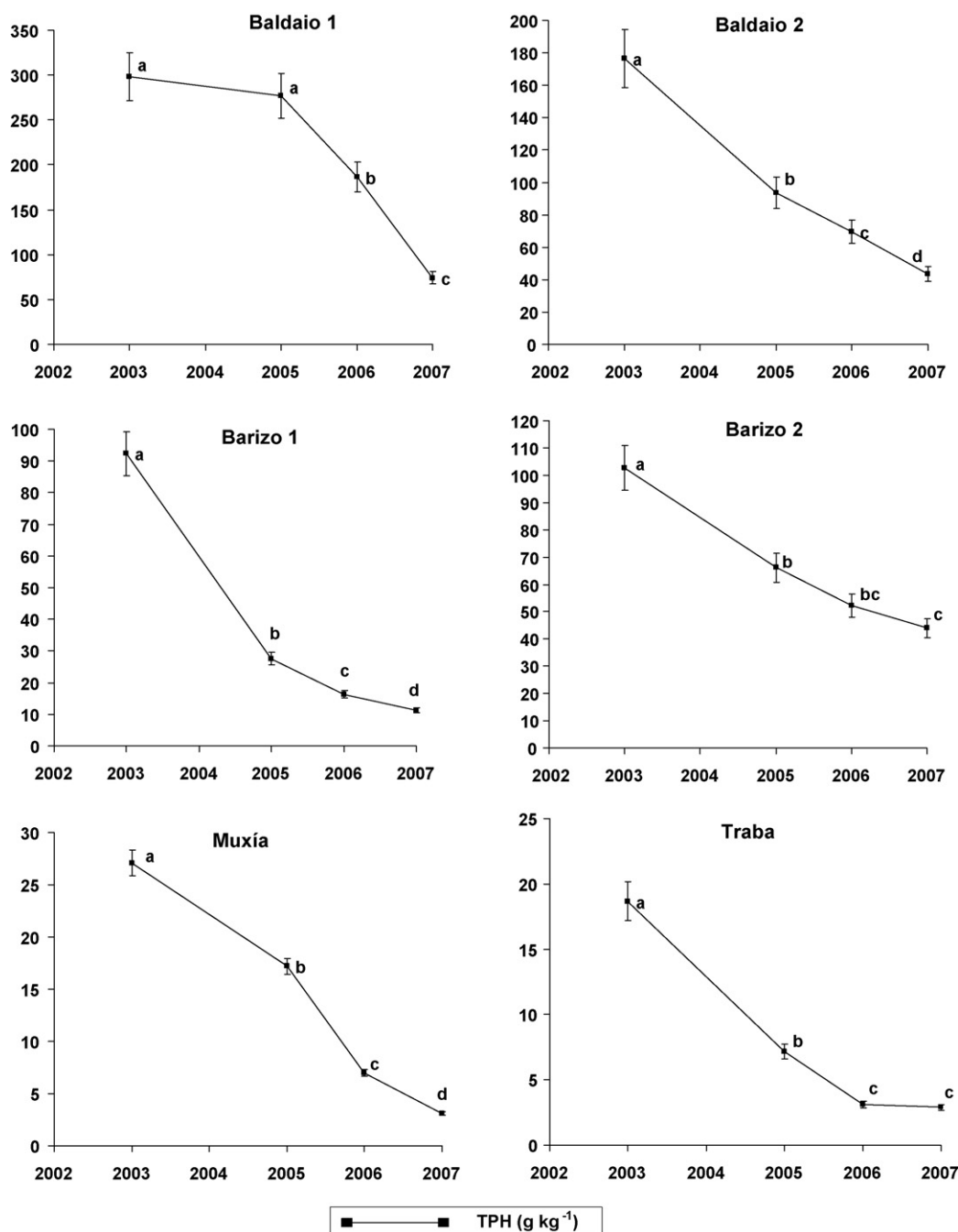


Fig. 3. Time course of the decrease in total petroleum hydrocarbon (TPH) content (g kg^{-1}) in the salt marsh soils over the studied period (2003–2007). (Means of 15 analyses: three replicate analyses of each of five replicate samples from each sampling area. For each site and date, means with different letters are significantly different at $p < 0.05$.)

in three 4 m × 4 m plots in each marsh leaving them grow for 6 months (from April to October, 2006).

2.5. Statistical analyses

Following testing for normality and homogeneity of variances, all data were subjected to one-way ANOVA and LSD as implemented in the software SPSS/PC+ for Windows in order to compare multiple means.

3. Results and discussion

3.1. Macroscopic effects of the fuel-oil spill

Sampling in February 2003 – very soon after the spill – in the Baldaio, Barizo, Traba Lagoon and Muxía salt marshes revealed that the fuel oil had impregnated the roots and lower stems of *Juncus* sp. (rushes) that formed the dominant vegetation at the studied sites, “gluing” soil particles to the roots and the stems to one another [17].

These areas exhibited a compact crust (Fig. 1) at or just below their surface; the crust had a low porosity, was 1–40 cm thick and crossed by cracks 0.3–1.0 cm wide. It ranged from black (7.5YR2/0), with high resistance to penetration, in Barizo and Baldaio, through various shades of gray in Muxía (10YR5/2), where it was thinner and rather less resistant to penetration, to yellowish brown (10YR5/8) at the Traba Lagoon. The crust consisted of aggregates of fine material bound by the oil, which, in coating the finer particles, must have facilitated their penetration and permanence in the larger pores and channels, thereby “gluing” larger particles together and reducing porosity and permeability while increasing resistance to penetration. Under normal circumstances, particles in sandy salt marsh soils reorganize during each cycle of wetting and drying rather than adhering to each other, which results in a very low resistance to penetration [43–45,17].

Subsequent sampling revealed that the thickness of the fuel-oil crusts at Muxía and Traba had decreased to such an extent that these crusts had virtually disappeared. At Baldaio and Barizo, however, traces of the crusts still remained in 2007: a distinct crust about 20 cm thick somewhat less compact and lighter in color remained in Baldaio; at Barizo, where neither the marsh nor the river bottom had been cleaned, little balls of oil continued to appear sporadically in the soil (Fig. 1c), however, giving a rusty appearance to crusts about 2 cm thick present at variable depths (Fig. 1d).

3.2. TPH contents

All samples collected February 2003 from Baldaio 1 and 2, Barizo 1 and 2, Traba and Muxía had high TPH contents (298.5, 176.5, 92.3, 102.8, 18.7, and 27.1 g kg⁻¹, respectively; Fig. 3). Overall, their fractionation revealed a high proportion of aromatics, followed by asphaltenes and resins, and also by saturated hydrocarbons but in rather low proportion (Fig. 4). Natural degradation of the fuel oil in the four marshes is clearly apparent Figs. 2–5.

Total hydrocarbon contents declined over the studied period at all sites (Fig. 3). Although TPH remained high in Baldaio and Barizo (74.3, 57.2, 11.2 and 44.0 g kg⁻¹ in Baldaio 1, Baldaio 2, Barizo 1, Barizo 2, respectively), the values were 75.4, 75.4, 87.9 and 57.2% lower, respectively, than those measured in February 2003 (Fig. 4), consistent with pronounced natural degradation. The February 2007 TPH contents of the Muxía and Traba soils were approximately 3 g kg⁻¹, which is 88.6 and 84.5% lower, respectively, than the original values.

The analysis of the different fractions revealed natural degradation of saturated and aromatic hydrocarbons of between 85% and as 95% in most soils (Figs. 5 and 6). A degradation pattern for

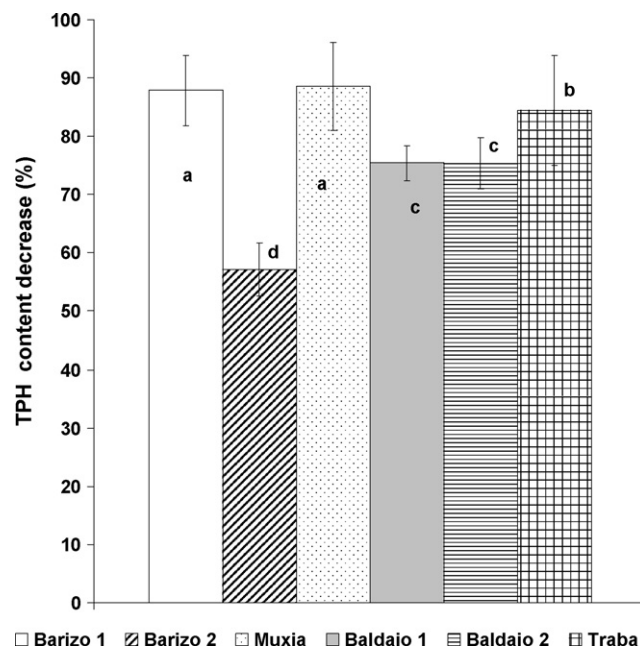


Fig. 4. Overall decrease in total petroleum hydrocarbon (TPH) contents (%) in the salt marsh soils over the studied period (2003–2007). (Means of 15 analyses: three replicate analyses of each of five replicate samples from each sampling area. Within each group, bars with different letters are significantly different at $p < 0.05$.)

saturated hydrocarbons was established by comparing the chromatograms for soil extracts obtained on different sampling dates. Gas-chromatographic analyses of the saturated fraction showed that the samples obtained in 2003 were very similar in this respect and also to the reference standards for C14, C15, C16, C17, C18, C19, C20, C22, C24, C26, C28, C30, C32 and C34; this indicates that all samples contained tetradecane, pentadecane, hexadecane, heptadecane, octadecane, nonadecane, eicosane, docosane, tetracosane, hexacosane, octacosane, triacontane, dotriacontane and tetratriacontane.

Hydrocarbon contents changed over the studied period. Gas-chromatographic analyses of the saturated fraction of the samples collected in February 2007 showed that nearly all GC resolved peaks were degraded. The Muxía and Traba soils were found to contain virtually no saturated hydrocarbons except for very small amounts of C14 and C15 alkanes which possibly came from a more recent source than the *Prestige* spill. These results suggest a nearly complete natural degradation of saturated hydrocarbons in these two marshes. In contrast, samples obtained in 2006 and 2007 from Baldaio (sites 1 and 2) and Barizo (sites 1 and 2) still contained C24, C28, C30, C32 and C34 saturated hydrocarbons (i.e. docosane, tetracosane, hexacosane, octacosane, triacontane, dotriacontane and tetratriacontane), which suggests that both marshes remained severely affected and only the lighter *n*-alkane fraction (C13 to C23) was depleted. The difference can be ascribed to environmental conditions favoring natural degradation in Muxía and Traba, and also to the smaller thickness of the fuel-oil crusts that formed on the soils after the spill (Fig. 1).

There was strong depletion not only of the lighter *n*-alkanes, as expected in natural environments [46,14], but also, in line with the results of Medina-Bellver et al. [47] and Gallego et al. [48], of the aromatic fraction. Depletion of asphaltene and resins was much less marked: 64–76% in Barizo 1, Muxía and Traba; 39–44% in Baldaio; and only 12% in Barizo 2, where flooding of the river continues to introduce balls of fuel oil into the marsh soils.

The persistence of recalcitrant compounds (resins and asphaltenes) may have hindered complete degradation of the lighter saturated hydrocarbons in soils with high hydrocarbon

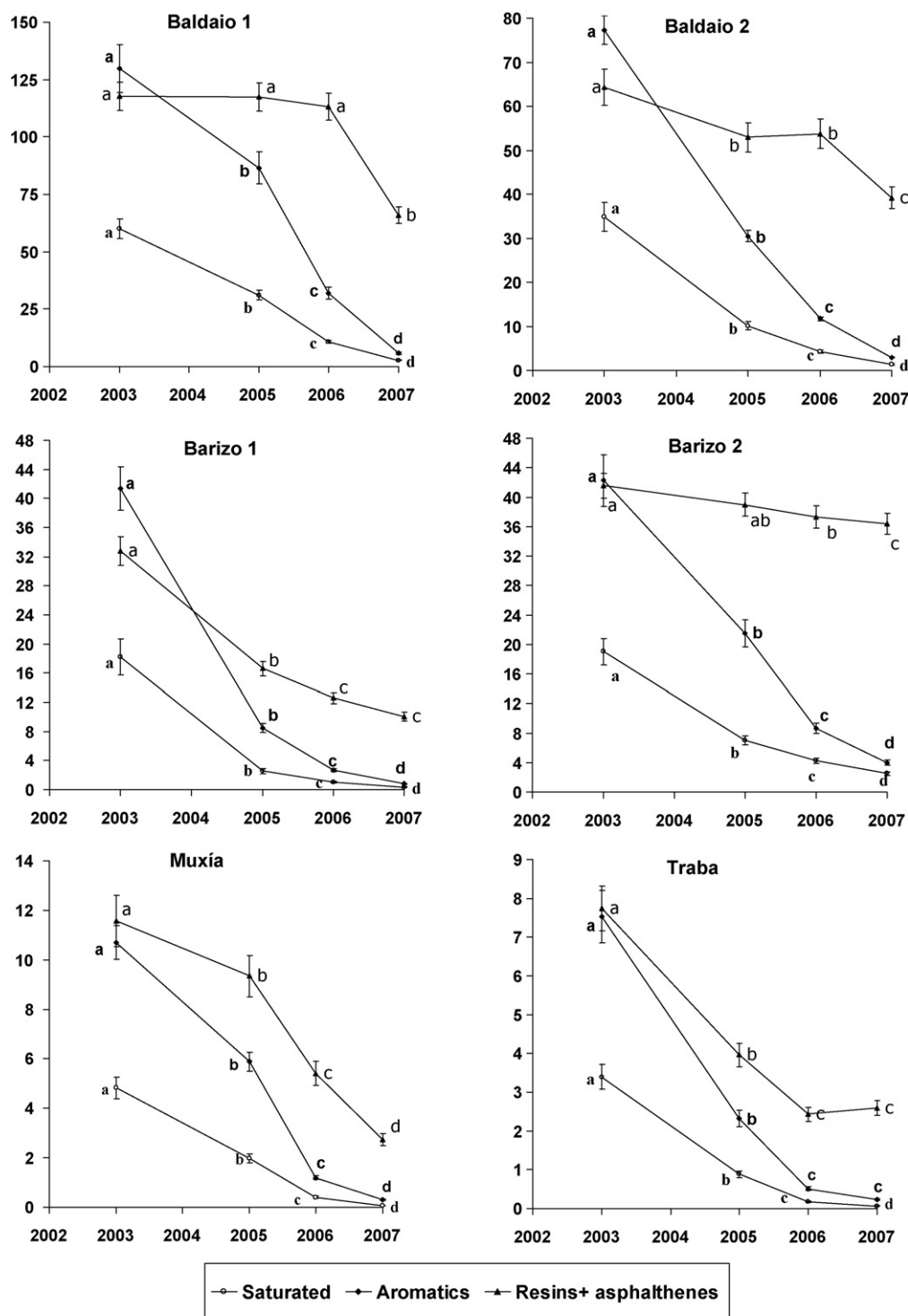


Fig. 5. Decrease in specific TPH fractions (saturated, aromatic, resins + asphaltenes) (g kg^{-1}) in the salt marsh soils over the studied period (2003–2007). (Means of 15 analyses: three replicate analyses of each of five replicate samples from each sampling area. For each date, bars with different letters are significantly different at $p < 0.05$.)

contents (i.e. the soils most severely affected by the spill). Such light fractions may have been trapped in the polar organic matrix, as previously observed by other authors at the surface of residual fuel oil deposited on rocks by the *Prestige* spill, where an asphaltic layer hindered access of biodegrading microorganisms [48].

Four years after the spill, natural degradation of the spilt fuel oil is thus apparent from the loss of lighter weight components; however, our results suggest that these salt marshes continue to be

significantly affected by hydrocarbons. Wherever the fuel-oil layer on the soil was very thick, hydrocarbon contents have only been reduced to a small extent.

3.3. Potential for phytoremediation bioassays

Fig. 7 shows the results obtained in bioassays involving germination and growth of the three studied species under the stressing conditions of the contaminated soils.

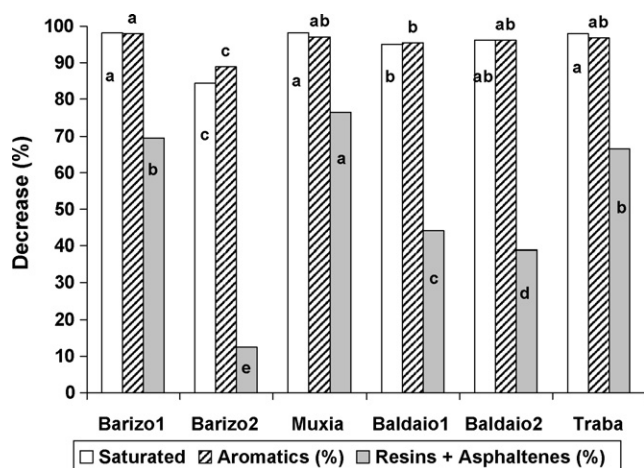


Fig. 6. Overall decrease in specific TPH fractions (saturated, aromatic, resins + asphaltenes) (%) in the salt marsh soils over the studied period (2003–2007). (Within each group, bars with different letters are significantly different at $p < 0.05$.)

The best, most promising results were obtained with *L. perenne* L., which exhibited a high germination rate and good growth; the results, however, should be confirmed in tests on adult plants. Recently [49,50] it has been proposed as one of the most promising phytoremediator species in soils contaminated around petroleum refineries, both being able to grow and also diminishing the pollutants soil concentration. Main aspects of the capacity to reduce pollution is related to its high germination rate and to the extensive root system, where microorganisms can detoxify the pollutants due to the great surface and improving of the structure, aeration and exudation [50]. *R. raphanistrum* L. was unable to germinate in soil samples from the most polluted sites by effect of its being under a highly compact, thick crust of residual oil (Baldaio 2).

None of the three species exhibited statistically significant differences in fluorescent properties when grown in the test soils (control included); rather, all values were normal and comparable to those for non-stressed plants. This suggests that these species can withstand the conditions in the contaminated soils for at least 2 weeks, so they may be useful under the prevailing field conditions.

Non-parametric comparison of means revealed highly significant differences in all studied parameters. No statistical test was done in the case of zero germination. Therefore, using these plant species to facilitate degradation showed all three to have the potential to survive in a growth chamber; this was particularly so of *L. perenne* L., which exhibited not only efficient germination, but also good aerial growth and root soil penetration, thereby improving aeration of the marsh and boosting decomposition and recovery processes. *L. perenne* chlorophyll fluorescence values were suggestive of an increased ability to cope with the stressing conditions in the marshes. According to literature, *L. perenne* has also been shown to develop aerenchyma that could be involved in a medium-term sequestration of pollutants [51].

Our greenhouse trials showed that all three plant species have the potential to survive, especially the ryegrass *L. perenne* L., which exhibited good germination and aerial growth in addition to a high capacity of root penetration in the soil. These will result in improved aeration of marsh soil and facilitate decomposition and recovery processes.

The field plots sown with *L. perenne* L. exhibited no significant differences in fuel-oil degradation with respect to the untreated plots. Seeds germinated well, the aerial parts of the plants grew acceptably, and root soil penetration was good.

According to the results obtained in the 6 months field experiment, we expect a favorable effect on fuel degradation in the longer term, particularly in the soils of the Baldaio marsh, where the spill destroyed virtually all vegetation, and also in those of the Barizo marsh, where fuel oil continues to enter the soil from the river bottom as suggested by the presence of oily layers at various depths.

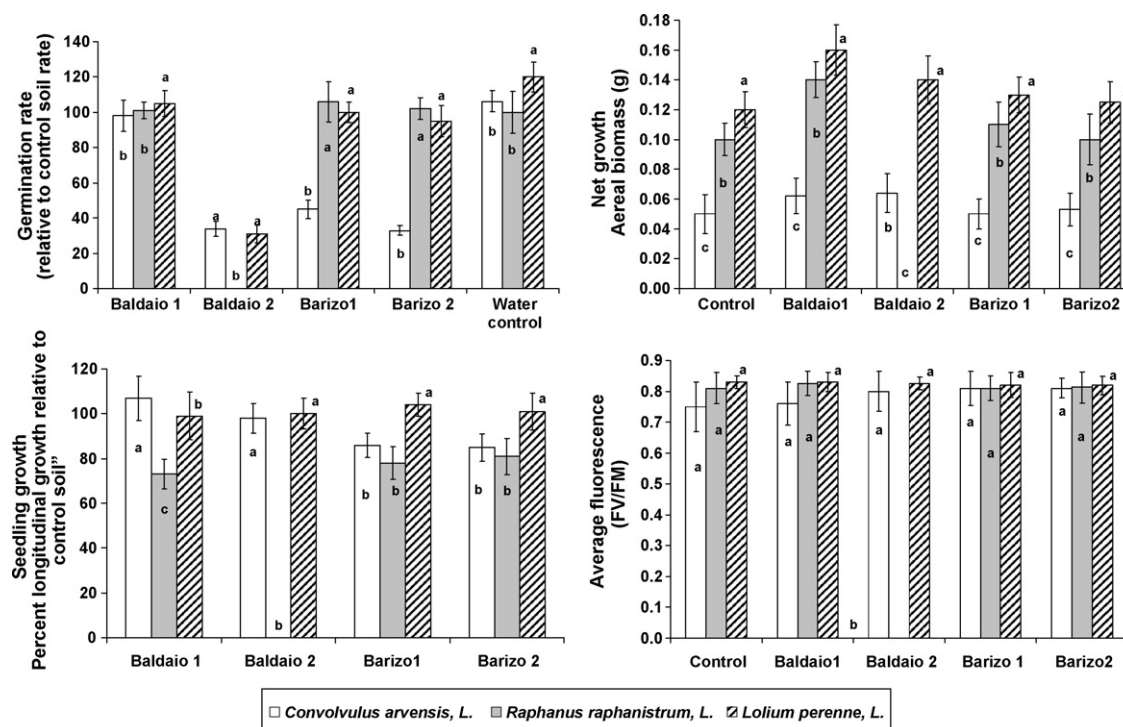


Fig. 7. Germination rate, seedling growth, net growth, and photosynthetic activity. (Bars with different letters for each species and soil are significantly different at $p < 0.05$.)

4. Conclusions

- The samples taken in February 2003 from the Baldaio 1 and 2, Barizo 1 and 2, Traba and Muxía marsh soils had high TPH contents. Their fractionation revealed a high proportion of aromatics, followed by asphaltenes and resins, and, finally, saturated hydrocarbons in rather low proportions.
- The total hydrocarbon content (TPH) decreased over the studied period in all marshes. However, TPH contents are still high in Baldaio (sites 1 and 2) and Barizo (sites 1 and 2). The decrease amounted to 75.4, 75.4, 87.9, and 57.2%, respectively, of the February 2003 values, consistent with strong natural degradation.
- The analysis of the different soil fractions revealed that saturated and aromatic hydrocarbons have been degraded by at least 85% in all soils, and by 95% in most.
- Asphaltenes and resins were less markedly degraded than the previous fractions, namely: by 64–76% in Barizo 1, Muxía and Traba; 39–44% in Baldaio (sites 1 and 2); and only 12% in Barizo 2, where flooding of the river continues to introduce balls of fuel oil into the soil.
- Four years after the spill, the fuel oil has seemingly degraded naturally through the loss of its lighter weight components; however, our results suggest that the salt marshes continue to be significantly affected by hydrocarbons. Monitoring analyses revealed natural degradation to be dependent of the thickness of the pollutant crust.
- Finally, phytoremediation tests showed *L. perenne* L. to grow efficiently in these polluted salt marshes, where it exhibited effective aerial growth and an excellent metabolic behaviour. Also, field plots planted with *L. perenne* L. exhibited no significant differences in fuel-oil degradation from untreated plots.
- It is advisable to continue the monitoring of the affected areas because there are still marshes with high pollution after 5 years of the oil spill. Also it is necessary to promote natural degradation and continue with recovery actions such as phytoremediation.

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References

- [1] G.W. Hodgson, Vanadium, nickel and iron trace metals in crude oils of western Canada, AAPG Bull. 38 (1954) 2537–2554.
- [2] K.H. Wedepohl, The composition of the upper earth's crust and the natural cycles of selected metals. Metals in raw materials—natural resources, in: E. Merian (Ed.), Metals and Their Compounds in the Environment, Part I, John Wiley & Sons, New York, 2000, pp. 3–19.
- [3] A.H. Bu-Olayan, M.N.V. Subrahmanyam, M. Al-Sarawi, B.V. Thomas, Effects of the Gulf War oil spill in relation to trace metals in water, particulate matter, and PAHs from the Kuwait Coast, Environ. Intern. 24 (1998) 789–797.
- [4] E.R. Gundlach, M.O. Hayes, C.H. Ruby, L.G. Ward, A.E. Blount, I.A. Fisher, R.J. Stein, Some guidelines for oil-spill control in coastal environments, based on field studies of four oil spills, in: L.T. McCarthy Jr., G.P. Lindblom, H.F. Walter (Eds.), Chemical Dispersants for the Control of Oil Spills, ASTM STP 659, ASTM, Philadelphia, PA, 1977, pp. 98–118.
- [5] S.R. Pezeshki, M.W. Hester, Q. Lin, J.A. Nyman, The effects of oil spill and clean up on dominant US Gulf coast marsh macrophytes: a review, Environ. Pollut. 108 (2000) 129–139.
- [6] K.A. Burns, J.M. Teal, The West Falmouth oil spill: hydrocarbons in the salt marsh ecosystem, Estuar. Coast. Mar. Sci. 8 (1979) 349–360.
- [7] H.L. Sanders, S.F. Grassle, Q.R. Hampson, L.S. Morse, S. Garner-Price, C.C. Jones, Anatomy of an oil spill: Long term effects from the grounding of the barge Florida off West Falmouth, Massachusetts, J. Mar. Res. 38 (1980) 265–380.
- [8] A.W. Maki, The Exxon Valdez oil spill: initial environmental impact assessment, Environ. Sci. Technol. 25 (1991) 24–29.
- [9] T.L. Wade, Oysters as biomonitors of the Apex Barge oil spill, in: Proceedings of the 1993 Oil Spill Conference (prevention, preparedness, response), American Petroleum Institute, Washington, DC, 1993, pp. 313–317.
- [10] K. Duncan, E. Jennings, P. Buck, H. Wells, R. Kolhatkar, K. Sublette, W.T. Potter, T. Todd, Multi-species ecotoxicity assessment of petroleum-contaminated soil, Soil Sediment Cont. 12 (2) (2003) 181–206.
- [11] J.G. Bundy, G.I. Paton, C.D. Campbell, Microbial communities in different soil types do not converge after diesel contamination, J. Appl. Microbiol. 92 (2) (2002) 276–288.
- [12] J.G. Bundy, G.I. Paton, C.D. Campbell, Combined microbial community level and single species biosensor responses to monitor recovery of oil polluted soil, Soil Biol. Biochem. 36 (7) (2004) 1149–1159.
- [13] A.W. Jackson, J.H. Pardue, R. Araujo, Monitoring crude oil mineralization in salt marshes: use of stable carbon isotope ratios, Environ. Sci. Technol. 30 (4) (1996) 1139–1144.
- [14] F.M. Ghazali, R. Abdul Rahman, A. Bakar Salleh, M. Basri, Biodegradation of hydrocarbons in soil by microbial consortium, Int. Biodeterior. Biodegrad. 54 (2004) 61–67.
- [15] J.L. Walworth, C.R. Woolard, K.C. Harris, Nutrient amendments for contaminated peri-glacial soils: use of cod bone meal as a controlled release nutrient source, Cold Regions Sci. Technol. 37 (2) (2003) 81–88.
- [16] R. Riffaldi, R. Levi-Minzi, R. Cardelli, S. Palumbo, A. Saviozzi, Soil biological activities in monitoring the bioremediation of diesel oil-contaminated soil, Water Air Soil Pollut. 170 (2006) 3–15.
- [17] M.L. Andrade, E.F. Covelo, F.A. Vega, P. Marcet, Effect of Prestige oil spill on salt marsh soils on the coast of Galicia (N.W. Spain), J. Environ. Qual. 33 (2004) 2103–2110.
- [18] International Organization for Standardization, ISO/TR 11046(E), In: ISO Standard Compendium, Environmental Soil Quality, General aspects, Chemical and Physical Methods of Analysis, Biological Methods of Analysis, ISO, Geneva, Switzerland, 1994, pp. 105–111.
- [19] Referentie Informatiemodel voor Ziekenhuisapotheken, RIZA, Quantification of the mineral oil content in extracts from environmental samples: comparison of various standardized methods, RIZA note 88.040x, Lelystad, NL, 1980.
- [20] Referentie Informatiemodel voor Ziekenhuisapotheken, RIZA, Quantitative determination of mineral oil in watersoil, RIZA note 87.062x, Lelystad, NL, 1987.
- [21] M. Pennings, The Influence of Column Choice and Method of Integration when Determining Mineral Oil Gas Chromatographically, ICW note 1824, The Winand Staring Centre, Wageningen, NL, 1987.
- [22] W.H. Weisman, Total Petroleum Hydrocarbon Criteria Working Group Series, vol. 1 Analysis of Petroleum Hydrocarbons in Environmental Media, Amherst Scientific Publishers, Amherst, MA, 1998.
- [23] P. Fernandez-Alvarez, J. Vila, J.M. Garrido-Fernandez, M. Grifoll, J.M. Lema, Trials of bioremediation on a beach affected by the heavy oil spill of the Prestige, J. Hazard. Mater. 137 (3) (2006) 1523–1531.
- [24] US EPA, Method 3611B—Alumina Column Cleanup and Separation of Petroleum Wastes, <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3611b.pdf>, 1996.
- [25] X. Zhu, A.D. Venosa, M.T. Suidan, K. Lee, Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands, US-EPA, Office of Research and Development, Cincinnati, OH, USA, 2001.
- [26] NIST 1494, Certificate Analysis. National Institute of Standards and Technology, Gaithersburg, MD 20899, USA, 2004.
- [27] P. Ramos, N. Pedrol, M.J. Reigosa, Photosynthesis of natural cocksfoot populations under water and salt stresses, Biol. Plantarum 38 (1996) 413–420.
- [28] N. Pedrol, P. Ramos, M.J. Reigosa, Phenotypic plasticity and acclimation to water deficits in velvet-grass: a long-term greenhouse experiment. Changes in leaf morphology, photosynthesis and stress-induced metabolites, J. Plant Physiol. 157 (2000) 383–393.
- [29] M. del Rio, R. Font, C. Almela, D. Velez, R. Montoso, A. de Haro Bailon, Heavy metals and arsenic uptake by wild vegetation in the Guadiamar river area after the toxic spill of the Aznalcóllar mine, J. Biotechnol. 98 (1) (2002) 125–137.
- [30] S. Siddiqui, W.A. Adams, The fate of diesel hydrocarbons in soils and their effect on the germination of perennial ryegrass, Environ. Toxicol. 17 (1) (2002) 49–62.
- [31] J.L. Gardea-Torresday, J.R. Peralta-Videa, M. Montes, G. De la Rosa, B. Corral Díaz, Bioaccumulation of cadmium, chromium and copper by *Convolvulus arvensis* L.: impact on plant growth and uptake of nutritional elements, Bioresour. Technol. 92 (3) (2004) 229–235.
- [32] A. de Haro, R. Font, M. del Río, F. Ferchaud, Field trials of wild plant species growing in polluted soils of the Guadiamar river area for phytoremediation purposes, Fresenius Environ. Bull. 13 (11b) (2004) 1197–1203.
- [33] S. Jian, Z. Yang, W. Jian, Establishment and growth of *Lolium multiflorum* for phyto-remediation of Pb/Zn tailings, Chin. J. Appl. Ecol. 15 (2) (2004) 255–260.
- [34] Q. Lin, Z. Wang, S. Ma, Y. Chen, Evaluation of dissipation mechanisms by *Lolium perenne* L. and *Raphanus sativus* for pentachlorophenol (PCP) in copper co-contaminated soil, Sci. Total Environ. 368 (2–3) (2006) 814–822.
- [35] Q. Lin, Z.W. Wang, S. Ma, Evaluation of dissipation mechanisms by *Lolium perenne* L. and *Raphanus sativus* for pentachlorophenol (PCP) in copper co-contaminated soil, Sci. Total Environ. 368 (2–3) (2006) 814–822.
- [36] G. Chiapusio, S. Pujol, M.L. Toussaint, P.M. Badot, P. Binet, Phenanthrene toxicity and dissipation in rhizosphere of grassland plants (*Lolium perenne* and *Trifolium pratense* L.) in three spiked soils, Plant Soil 294 (1–2) (2007) 103–112.
- [37] E.R. Cerda, R.A.A. Nuncio, J.A.M. Trejo, Exploitation and improvement of saline soils through the establishment of forage grasses, Técnica Pecuaria en México 45 (1) (2007) 19–22.
- [38] M.A. Kashem, B.R. Singh, S.M.I. Huq, Cadmium phytoextraction efficiency of arum (*Colocasia antiquorum*), radish (*Raphanus sativus* L.) and water spinach (*Ipomoea aquatica*) grown in hydroponics, Water Air Soil Pollut. 192 (1–4) (2008) 273–279.

- [39] J. Rezek, C.I.D. Wiesche, M. Mackova, The effect of ryegrass (*Lolium perenne*) on decrease of PAH content in long term contaminated soil, *Chemosphere* 70 (9) (2008) 1603–1608.
- [40] B. Genty, J.M. Briantais, N.R. Baker, The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence, *Biochim. Biophys. Acta* 990 (1989) 87–92.
- [41] G. Öqwist, R. Wass, A portable, microprocessor operated instrument for measuring chlorophyll fluorescence kinetics in stress physiology, *Physiol. Plantarum* 73 (1988) 211–217.
- [42] T. Saarinen, Chlorophyll fluorescence, and nitrogen and pigment content of Scots pine (*Pinus sylvestris*) needles in polluted urban habitats, *Ann. Bot. Fen.* 30 (1993) 1–7.
- [43] A.M. Odasz-Albrigtsen, H. Tømmervik, P. Murphy, Decreased photosynthetic efficiency in plant species exposed to multiple airborne pollutants along the Russian–Norwegian border, *Can. J. Bot.* 78 (2000) 1021–1033.
- [44] M.C. Marley, G.E. Hoag, Induced soil venting for recovery restoration of gasoline hydrocarbons in vadose zone, in: Water Well Assoc. Am. Pot. Inst. (Ed.), Proceedings of Petroleum Hydrocarbons and Organic Chemicals in Ground Water, National Water Well Association, Houston, TX, 1986, pp. 473–503.
- [45] L. Andrade, M.L. Reyzábal, P. Marcet, M.J. Montero, Industrial impact on marsh ecosystem at the Bahía Blanca Ría (Argentina), *J. Environ. Qual.* 31 (2002) 532–538.
- [46] S. Bordenave, R. Jézéquié, H. Budzinski, F.X. Merlin, T. Fourel, M. Goñi-Urriza, R. Guyoneaud, R. Grimaud, P.A. Caumette, Degradation of the “Erika” oil, *Aquat. Living Resour.* 17 (2004) 261–264.
- [47] J.I. Medina-Bellver, P. Marín, A. Delgado, A. Rodríguez-Sánchez, E. Reyes, J.L. Ramos, Evidence for in situ crude oil biodegradation after the Prestige oil spill, *Environ. Microbiol.* 7 (2005) 773–779.
- [48] J.R. Gallego, E. González-Rojas, A.I. Peláez, J. Sánchez, M.J. García-Martínez, J.E. Ortiz, T. Torres, J.F. Llamas, Natural attenuation and bioremediation of Prestige fuel oil along the Atlantic coast of Galicia (Spain), *Org. Geochem.* 37 (12) (2006) 1869–1884.
- [49] B.S. Dear, K.F.M. Reed, A.D. Craig, Outcomes of the search for new perennial and salt tolerant pasture plants for southern Australia, *Aust. J. Exp. Agric.* 48 (4) (2008) 578–588.
- [50] A.Yu. Muratova, T.V. Dmitrieva, L.V. Panchenko, O.V. Turkovskaya, Phytoremediation of oil-sludge-contaminated soil, *Int. J. Phytoremediat.* 10 (6) (2008) 486–502.
- [51] N.M. McFarlane, T.A. Ciavarella, K.F. Smith, The effects of waterlogging on growth, photosynthesis and biomass allocation in perennial ryegrass (*Lolium perenne* L.) genotypes with contrasting root development, *J. Agric. Sci.* 141 (2003) 241–248.
- [52] American Society for Testing and Materials (ASTM), ASTM D 1998, 95, 445, 92, 97, 1796, and 482 methods, in: Annual Book of ASTM Standards; Section 5, Petroleum Products, Lubricants, and Fossil Fuels, 05.01. Petroleum Products and Lubricants (I), ASTM, Philadelphia, PA, 1995.
- [53] American Society for Testing and Materials (ASTM), ASTM D 4239C method, in: Annual Book of ASTM Standards, Section 5, Petroleum Products, Lubricants, and Fossil Fuels, 05.02, Petroleum Products and Lubricants (II). ASTM, Philadelphia, PA, 1995.
- [54] American Society for Testing and Materials, ASTM D 95 method, Test Method for Water in Petroleum Products and Bituminous Materials by Distillation, in: Annual Book of American Society for Testing and Materials, ASTM Standards, Section 5, Petroleum Products, Lubricants, and Fossil Fuels, 05.01, Petroleum Products and Lubricants (I), ASTM, Philadelphia, PA, 1995, pp. 52–56.