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Hydrocarbon degradation potential of salt marsh plant–microorganisms associations

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Abstract Estuaries are often considered sinks for contaminants and the cleanup of salt marshes, sensitive ecosystems with a major ecological role, should be carried out by means of least intrusive approaches, such as bioremediation. This study was designed to evaluate the influence of plant–microorganisms associations on petroleum hydrocarbons fate in salt marshes of a temperate estuary (Lima River, NW Portugal). Sediments un-colonized and colonized (rhizosediments) by different plants *(Juncus maritimus, Phragmites australis, Triglochin striata* and *Spartina patens)* were sampled in four sites of the lower and middle estuary for hydrocarbon degrading microorganisms (HD), total cell counts (TCC) and total petroleum hydrocarbons (TPHs) assessment. In general, TPHs, HD and TCC were significantly higher ($P < 0.05$) in rhizosediments than in un-colonized sediments. Also recorded were differences on the abundance of hydrocarbon degraders among the rhizosediment of the different plants collected at the same site (*J. maritimus* < *P. australis* < *T. striata*), with statistically significant differences ($P < 0.05$) between *J. maritimus* and *T. striata*. Moreover, strong positive correlations—0.81 and 0.84 ($P < 0.05$), between biotic (HD) and abiotic (organic matter content) parameters and TPHs concentrations were also found. Our data clearly suggest that salt marsh plants can influence the microbial community, by fostering the development of hydrocarbon-degrading microbial populations in its rhizosphere, an effect observed for all plants. This effect, combined with the plant capability to retain hydrocarbons around the roots, points out that salt marsh plant–microorganisms associations may actively contribute to hydrocarbon removal and degradation in estuarine environments.

Keywords Bioremediation · Hydrocarbons · Estuary · Salt marsh · Rhizosphere · Lima River estuary

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Introduction

Estuaries are often considered sinks for contaminants, receiving important anthropogenic inputs from the upstream catchments and from metropolitan areas and industries located on or near those areas (Almeida et al. 2004). Estuaries are dynamic,

complex and unique systems that present both seasonal and spatial variability (Chapman and Wong 2001). Petroleum hydrocarbons are among the most common contaminants bound to estuarine sediments (Chapman and Wong 2001), giving rise to significant environmental concern (Daane et al. 2001). The heterogeneity and variability of grain size estuarine sediments along with their organic matter content can influence the sequestration of hydrocarbons (Kukkonen and Landrum 1996; Wang et al. 2001). In addition, the periodic inundation of the estuarine environment due to tides, with the subsequent percolation of salt water, can enhance the sorption of hydrophobic chemicals to the sediment particles caused by "salt effects" (Brunk et al. 1997).

Temperate salt marshes, including those at estuarine sites, have an important ecological role since they are among the most productive ecosystems on Earth (Boorman 1999; Costanza et al. 1997). Simultaneously, these ecosystems are extremely sensitive to pollutants, including oil pollution (Andrade et al. 2004). In fact, some studies (e.g. Martins et al. 2008) highlighted the salt marshes capability to retain hydrocarbons in their sediments. As a result, it is important to clean and recover theses areas, which can be a difficult task (Zhu et al. 2004). Organic contaminants can undergo biodegradation as a result of the activity of sediment microorganisms giving less toxic, less mobile and/or less bioavailable products (Vidali 2001). Accelerating the biodegradation of petroleum hydrocarbons in general is thus a major challenge in order to improve the performance and acceptance of cost-saving bioremediation techniques (Liste and Felgentreu 2006).

In fact, the presence of vegetation can accelerate the bioremediation of sediments contaminated with petroleum hydrocarbons (Davis et al. 2002; Xu et al. 2006). In the specific case of soils, plants can alter the microbial community when introduced in a polluted area (Hartmann et al. 2009; Kirk et al. 2005), increasing the degradation of petroleum hydrocarbons relatively to that in bulk soil (Wang et al. 2008). It is well known that the rhizosphere is an ideal microhabitat for increasing the number of microorganisms (Hutchinson et al. 2003; Wang et al. 2008). The plant exerts a major influence on microbial communities through the release of a range of organic compounds, as root exudates, and eventually through nutrients released when the roots die and are degraded (Bais et al. 2006; Kuiper et al. 2004; Olson et al. 2003; Salt et al. 1998). Plants, on the other hand, benefit from the microbial turnover of root exudates and other soil organic and inorganic matter, which releases nutrients and enhances the soil structure (Olson et al. 2003; Prosser et al. 2006). The interactions between plant and microorganisms in the rhizosphere are complex and varied (Prosser et al. 2006; Lambers et al. 2009), being influenced by the plant species involved. Although hydrocarbon biodegradation in soils has been widely addressed, studies on salt marshes sediments are scarce, and it is still not clear how and to which extent the rhizosphere effect influences microbial communities and pollutants, namely petroleum hydrocarbon degradation (Daane et al. 2001; Muratova et al. 2003; Merkl et al. 2006) in theses estuarine ecosystems.

Therefore, the aim of this study was to give new insights on the influence of different salt marsh plant–microorganisms associations on petroleum hydrocarbons fate in a temperate estuarine environment, having in mind the need to increase the scientific knowledge for the development of alternative approaches to tackle coastal oil pollution, as the recent oil spill in the Gulf of Mexico highlighted.

Materials and methods

The study area and sediment sampling

Sediment samples were collected in June of 2009 as well as in July–August of 2010 in the Lima River estuary [41.41°N; 08.51°W (WGS84)], the end member of an international watershed located in NW Portugal. The urban-industrialized estuary has a large salt marsh area (Fig. 1), and a mesotidal regime.

During the 2009 survey, sub-surface sediments un-colonized and colonized (rhizosediments) by several plants [*Juncus maritimus* (P1), *Phragmites australis* (P2), *Triglochin striata* (P3) and *Spartina patens* (P4)] were collected into sterile plastic bags, at different sampling sites along the estuary (L1, L2, L3, L4, Fig. 1). All sediments were collected between 5 and 15 cm, the depth with the higher plant below-ground biomass in the case of colonized sediments. Samples were transported to the laboratory in the dark in refrigerated ice chests. At the laboratory ca. 40 g of each sediment sample was wrapped in

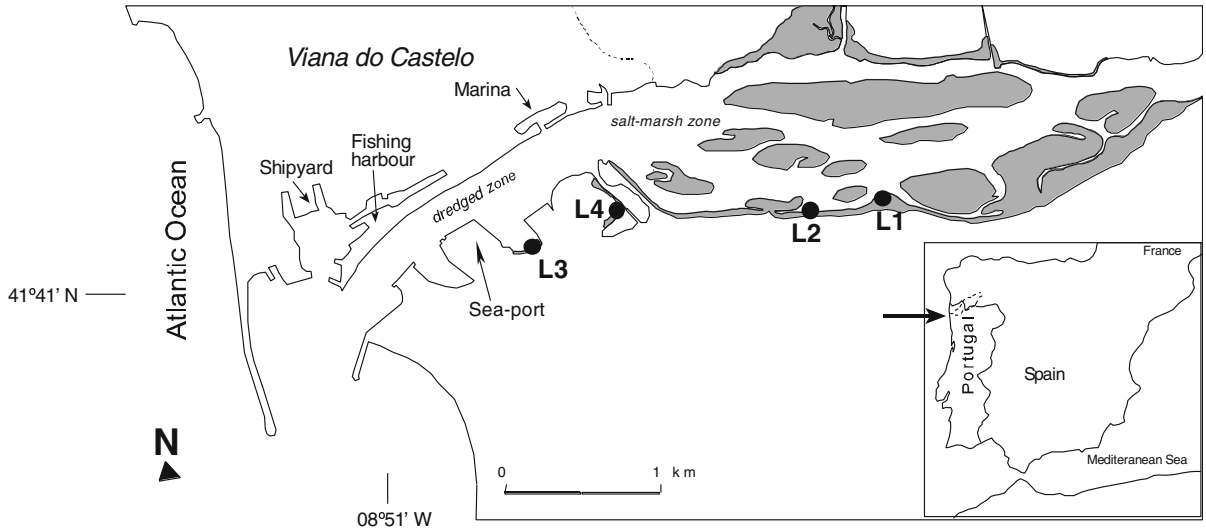

Fig. 1 Study area. Lima River estuary (North of Portugal), and the four sampling sites, L1, L2, L3 and L4

aluminum foil and frozen at −20°C until total petroleum hydrocarbons (TPHs) analysis. Remaining portions of the sediment were stored at 4°C for further treatment.

Sediment characterization

The determination of water and organic matter (OM) content (mean and respective standard deviation of three independent replicates) in the sediments was carried out according to the European Committee for Standardization (1999) methodology. OM content was determined in dry sediments (at 100°C) by loss on ignition (4 h at 500°C).

To quantify particle size distribution, sediments samples were previously treated with a 30% hydrogen peroxide solution (Mikutta et al. 2005), and divided into five fractions in a mechanical shaker for sediment sieving. Although there are several different particle size limits that can be used (Nemes and Rawls 2006), the adopted standard system was the followed: silt and clay (<0.063 mm), fine sand (0.063–0.25 mm), medium sand (0.25–1 mm), coarse sand (1–2 mm), and gravel (>2 mm). Each fraction was weighed and expressed as percentage of the total dry weight.

Microorganisms enumeration

Total cell counts (TCC) were obtained by 4′,6′-diamidino-2-phenylindole (DAPI) direct count method (Porter and Feig 1980; Kepner and Pratt 1994). Triplicate sediment samples were immediately fixed with formaldehyde (0.2 μm-filtered), to reach a final concentration of 4% (v/v). Afterwards, to 0.1 g of homogenized samples were added 2.5 ml of saline solution (0.2 μm-filtered, 9 g l^{-1} sodium chloride) and 200 μl of Tween 80 solution (0.2 μm-filtered; 12.5%, v/v), being fixed with 1 ml of formaldehyde solution (0.2 μm-filtered; 4%, v/v). Samples were stirred at 150 rpm for 15 min followed by sonication for 20–30 s at low intensity (0.5 cycle, 20% amplitude). Sub-samples of fixed sediment samples were then stained with DAPI, and incubated in the dark for 12 min (Porter and Feig 1980). Samples were filtered onto black Nuclepore polycarbonate filters (0.2 μm pore size, 25 mm diameter, Whatman) under gentle vacuum and washed with autoclaved 0.2 μm-filtered distilled water. Membranes were mounted in glass slides and cells counted at 1,875× on an epifluorescence microscope (Labphot, Nikon, Japan).

Hydrocarbon degrading microorganisms (HD) were estimated using a modified most probable number (MPN) protocol (Haines et al. 1996; Wrenn and Venosa 1996), in 96-well microtiter plates. Pre-filtered (0.2 μm) of Arabian Light fuel oil (supplied by a local oil refinery) was the selective substrate for determination of total hydrocarbon degraders. Bushnell Haas medium (BH) supplemented with 2% sodium chloride was used as the growth medium for MPN procedures (180 μl BH/well). The fuel oil was

added to 5 × 12 wells (10 µl/well) after filling the wells with the growth medium. For each sample, 0.5 g of sediment was mixed in 1.5 ml BH and supernatant was diluted in a saline buffer solution containing 0.1% sodium pyrophosphate (pH 7.5) and 2% sodium chloride. Tenfold serial dilutions were performed, in the first row well of the microtiter plates, and the inoculation was made by adding 20 µl of each dilution to five wells. Five wells remained un-inoculated to serve as a sterile control. MPN plates were incubated for 2 weeks at room temperature. After incubation, 50 µl of filter sterilized Iodonitrotetrazolium violet (INT) (3 g l^{-1}) was added to each well. Positive wells were scored after overnight incubation at room temperature with INT.

Determination of total petroleum hydrocarbons concentration

Prior to TPHs analysis, sediments samples were dried at room temperature until constant weight and sieved through a nylon net of 2 mm mesh in order to remove large particles and roots. For TPHs measurements, a previous optimized method for soil samples was adapted for the sediment samples (unpublished results). Briefly, about 1 g of sediment was mixed with anhydrous sodium sulphate (1:1, w/w) and tetrachloroethylene (≥99% spectrophotometric grade) (1:10, w/v) was added, being followed by an ultrasonic (Elma, Transsonic 460/H model) extraction for 30 min. The extracts were cleaned with deactivated silica gel (70–230 mesh), to remove non-mineral oil contaminants such as animal greases and vegetable oils and other polar compounds, and refrigerated until analysis, usually within 1 h. The sample extracts were analyzed by Fourier transform infrared spectrophotometry (Jasco FT/IR-460 Plus) using a quartz cell of 40 mm path length (Infrasil I, Starna Scientific). Calibration standards (in tetrachoroethylene) were prepared using a stock standard solution of equal volumes of isooctane (≥99% ACS spectrophotometric grade) and hexadecane (99%) solutions. TPHs were quantified by direct comparison with the calibration curve. Quality control tests were conducted by analysing the certified reference material CRM350-100 (TPH in Sandy Loam Soil (C6–C35), from Resource Technology Corporation). The results were within the prediction interval of expected TPHs concentration. Sample solutions spiked with known amount of hydrocarbons, yielded recoveries between 82 and 135%. The mean and respective standard deviation of five independent replicates was calculated and the results were expressed on a dry weight basis.

Laboratory experiments

For the laboratory evaluation of the TPHs biodegradation potential, experimental work involving degradation experiments was carried out with sediment samples, collected in 2010, in the L3 and L4 sampling sites (Fig. 1). These experiments were restricted to those sites colonized simultaneously by the four plants mentioned above. The same procedures for sampling and handling described before were applied. However, the Arabian Light fuel oil was submitted to an aging process to simulate an oil spill by means of shaking the fuel oil overnight in BH medium. The experimental design adopted was, briefly, 10 ml (volume) of sediment samples were placed in 50 ml flasks, supplement with 20 ml BH medium and 0.5 ml of aged Arabian Light fuel oil. Initial triplicate sediment samples were collected for analysis of TPHs, and considered as T0 samples. The remaining flasks, with triplicate sediment samples, were incubated at room temperature in the dark in a mechanical stirring at 100 rpm. The flasks were also manually shaken once every day to improve blending between fuel oil and sediment. It has been previously suggested (Aichberger et al. 2005) that shaking flasks were the faster (2–4 weeks), cheaper and less sample requiring test method to predict biodegradation performance of hydrocarbons, with a good indication of hydrocarbon degradability. Simultaneously, sediment samples, not spiked with Arabian Light fuel oil, were incubated to verify the promoting effect of BH medium in the HD microorganisms. After 15 days of incubation, the sediment samples were removed and considered as T15 samples. All samples (including T0) were frozen at −20°C (to stop microbial growth). After at least 3 days at −20°C, samples were left to dry at room temperature until constant weight. The TPHs analysis in the dried T0 and T15 sediments was performed as previously described. In the additional triplicate sediment samples, un-spiked with Arabian Light fuel, only HD MPN procedures were performed.

Statistical analyses

Microbial enumeration data were normalized by logarithm ($\log_{10}$) transformation prior to statistical analysis. Significant differences ($P < 0.05$) between two means were evaluated using t tests. Correlation factors ($P < 0.05$) were analyzed by correlation matrices. All statistical tests were performed using the commercial software Statistica (Version 9).

Results

Sediment characterization

Un-colonized sediments and rhizosediments collected around the different plants were characterized in terms of content in water and OM, and grain size distribution (Table 1).

Sediments (both un-colonized sediment and rhizosediments) collect at L1, the uppermost sampling site, had coarser particles whereas sediments from L4 sampling site, in the lower estuary, had the smallest grain size (more than 80% of total particle size was inferior to 0.25 mm). Concomitantly, L4 was usually the site with the highest water and OM content.

When comparing rhizosediments with un-colonized sediments, a general tendency to register higher contents of water, OM and/or silt and clay fraction in rhizosediments was found. Nevertheless, significant ($P < 0.05$) differences could only be considered at sites L3 and L4. At L3, P4 rhizosediment were more similar to the surrounding un-colonized sediment than to the P1 rhizosediment. Nevertheless all sediments were significantly different ($P < 0.05$) in terms of OM content and fine-grained particles. At L4, P2 and P3 rhizosediments were significantly higher ($P < 0.05$) in OM content than the surrounding un-colonized sediment and the P1 rhizosediment.

Microorganisms enumeration

The TCC and HD microorganisms abundance were estimated (Fig. 2). The level of microbial abundance ranged from 10^7 to 10^9 TCC $g^{-1}_{\text{wet sediment}}$, whereas HD ranged from 10^4 to 10^8 MPN $g^{-1}_{\text{wet sediment}}$.

The results of total microbial abundance showed significant differences ($P < 0.05$) between un-colonized

Table 1 Water (H_2O) and organic matter (OM) contents (mean and standard deviation, $n = 3$) and particle size fractions of dry un-colonized sediments (Sed.) and rhizosediments (Rhizo) of several plants [*J. maritimus* (P1), *P. australis* (P2), *T. striata* (P3) and *S. patens* (P4)]. Samples were collected at four different sampling sites (L1, L2, L3 and L4)

Sample	% H_2O	% OM	Particle size fraction percentage relatively to total weight				
			Silt + clay	Fine sand	Medium sand	Coarse sand	Gravel
L1							
Sed.	23 ± 2	2.3 ± 0.3	4.4	15	51	16	13
Rhizo P1	23 ± 3	2.6 ± 0.2	6.0	18	42	18	17
L2							
Sed.	37 ± 1	4.0 ± 0.2	1.4	51	38	5.2	4.4
Rhizo P1	39 ± 4	3.74 ± 0.01	14	38	36	5.2	7.1
L3							
Sed.	27 ± 1	0.9 ± 0.1	1.6	50	46	1.5	1.1
Rhizo P1	51 ± 2	5.85 ± 0.01	52	30	16	1.8	0.08
Rhizo P4	22 ± 2	2.0 ± 0.3	7.2	48	41	2.2	1.5
L4							
Sed.	50 ± 1	5.4 ± 0.2	50	35	16	0.20	n.d
Rhizo P1	53 ± 1	5.3 ± 0.1	26	57	17	n.d	n.d
Rhizo P2	62 ± 1	6.00 ± 0.05	50	36	14	n.d	n.d
Rhizo P3	53.2 ± 0.4	6.4 ± 0.2	41	53	6.0	n.d	n.d

n.d not detected

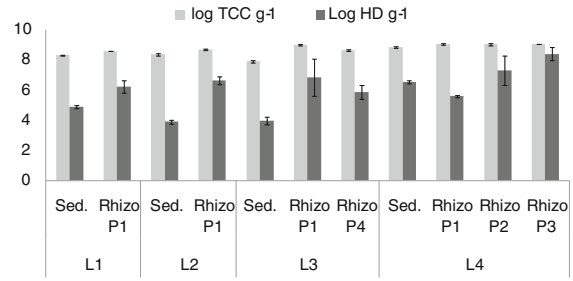

Fig. 2 Microbial abundance estimated by total cell counts ($\log_{10}$ TCC g^{-1}, mean and standard deviation, $n = 3$) and hydrocarbon degraders microorganisms estimated by most probable number ($\log_{10}$ HD g^{-1}, mean and standard deviation, $n = 2$) in un-colonized sediments (Sed.) and rhizosediments (Rhizo) of several plants (*J. maritimus* (P1), *P. australis* (P2), *T. striata* (P3) and *S. patens* (P4). Samples were collected at four different sampling sites (L1, L2, L3 and L4)

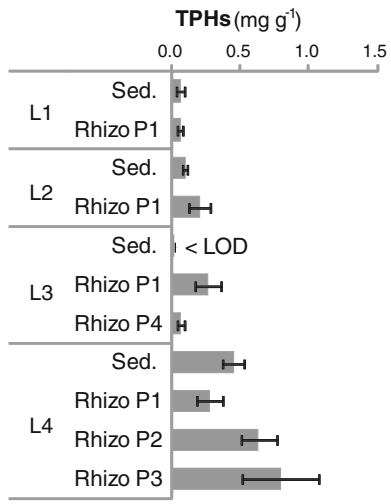

Fig. 3 Total petroleum hydrocarbons (TPHs) concentrations (mg g^{-1}, mean and standard deviation, $n = 5$) in un-colonized sediments (Sed.) and rhizosediments (Rhizo) of several plants (*J. maritimus* (P1), *P. australis* (P2), *T. striata* (P3) and *S. patens* (P4). Samples were collected at four different sampling sites (L1, L2, L3 and L4). Limit of detection (LOD) 0.032 mg g^{-1}

sediments and rhizosediments, with higher TCC in rhizosediments at all sites. Based on TCC, rhizosediments could be divided in two significantly ($P < 0.05$) different groups: A (L1 rhizo P1 $\approx$ L2 rhizo P1 $\approx$ L3 rhizo P4) and B (L3 rhizo P1 $\approx$ L4 rhizo P1 $\approx$ L4 rhizo P2 $\approx$ L4 rhizo P3).

Considering HD abundance, significant differences ($P < 0.05$) between un-colonized sediments and rhizosediments were also found in all sites. In general, higher values were found in the rhizosediments, but the opposite occurred for P1 rhizosediment in L4 sampling site. Also in L4 sampling site, HD abundance in P2 rhizosediment was not significantly higher ($P > 0.05$) than at surrounding un-colonized sediment. Generally, no important differences ($P > 0.05$) were found between rhizosediments of the different plants, although there were significant differences ($P < 0.05$) in the abundance of HD between the P1 and P3 rhizosediments collected at the same site (L4).

Total petroleum hydrocarbons concentration

The concentration profile of TPHs in un-colonized sediments and rhizosediments are presented in Fig. 3, and ranged from below detection level (0.032 mg g^{-1}) to 0.8 mg $g^{-1}_{\text{dry sediment}}$. The most notable aspect that emerged was a significantly higher ($P < 0.05$) TPHs concentration in rhizosediments, except in L1 sampling site, the uppermost location. Also, the P1 rhizosediment in L4 sampling site that was significantly ($P < 0.05$) lower than the surrounding un-colonized sediment.

Another perceptible feature was the difference between the L4 and the others sampling sites, that, with the exception of *J. maritimus* (P1), had the highest statistically significant ($P < 0.05$) TPHs concentrations.

The TPHs concentrations in *J. maritimus* (plant P1) rhizosediment did not differed significantly ($P > 0.05$) among L2, L3 and L4 sampling sites, but were significantly ($P < 0.05$) lower at L1 sampling site. The TPHs concentrations in *T. striata* (plant P3) and *P. australis* (plant P2) rhizosediments did not differed significantly ($P > 0.05$) between each other, being significantly ($P < 0.05$) higher than TPHs concentrations in the other two plants (*J. maritimus* and *S. patens*) rhizosediments.

Laboratory experiments

The characteristics of the 2010 batch of sediments were similar to those retrieved in the previous year (data not shown), with the exception of HD abundance at site L4. In the latter case, no important differences ($P > 0.05$) were found between rhizosediments of the different plants, being the HD numbers of all three rhizosediments significantly ($P < 0.05$) higher than the surrounding un-colonized sediment.

Analysis of T0 TPHs (Table 2) showed differences between the several sediments, despite identical fuel oil addition. Nevertheless, a significant positive correlation ($r = 0.91$, $P < 0.05$, $n = 7$) between these initial concentrations and those in un-spiked sediments was found, reflecting different capacities to retain hydrocarbons. Moreover, rhizosediments of all the studied plants presented higher rates of TPHs degradation than un-colonized sediments. In addition, some differentiation in TPHs degradation rates among different plants occurred. In the same vein, the stimulation effect of BH medium on HD abundance, after 15 days of incubation, varied among plants being the HD numbers from the *T. striata* rhizosediment (Rhizo P3) the lowest. Finally, the HD abundance in medium supplemented with fuel oil could not be quantified because of methodological saturation.

Correlation factors

Considering both un-colonized sediments and rhizosediments, correlation factors between TPHs concentrations and both biotic (HD microorganisms) and abiotic (OM and clay + silt grain size fraction percentage) parameters were assessed and plotted in Fig. 4a significant positive correlation ($r = 0.805$, $P < 0.05$, $n = 11$) between HD and TPHs concentrations were obtained, as well as between abiotic parameters and TPHs concentrations (OM content: $r = 0.836$, $P < 0.05$, $n = 11$ and clay + silt grain size fraction: $r = 0.810$, $P < 0.05$, $n = 11$).

Discussion

The results obtained in this study suggest that salt marsh plants can positively influence the microbial community, by increasing total microbial abundance, and promoting the development of hydrocarbon-degrading microbial populations on its rhizosphere. The results also pointed to a higher incorporation of TPHs in sediments in contact with the plant belowground tissues in comparison to un-colonized sediments.

Relationships among TPHs, OM and grain size

Discharges from municipal and industrial wastewater, urban runoff and oil leakage from boats and ships are possible sources of the TPHs contamination in estuaries, including the Lima River estuary. Different sediments can have different capacities for collecting contaminants (Chapman and Wang 2001), and it has been demonstrated that sediment properties influence the distribution and concentration of hydrocarbons (Kim et al. 1999; Wang et al. 2001). The organic carbon fraction has been identified as the most important factor for the control of the concentrations of organic contaminants like hydrocarbons (Chapman and Wang 2001). Several studies concerning PAHs

Table 2 TPHs concentrations and HD microorganisms enumeration (mean and standard deviation, $n = 3$) in laboratory experiments using un-colonized sediments (Sed.) and rhizosediments (Rhizo) of several plants [*J. maritimus* (P1), *P. australis* (P2), *T. striata* (P3) and *S. patens* (P4)]. Samples were collected at two different sampling sites (L3 and L4). HD microorganisms were enumerated only in BH medium without fuel oil

	L3			L4			
	Sed	Rhizo P1	Rhizo P4	Sed	Rhizo P1	Rhizo P2	Rhizo P3
TPHs (mg g^{-1})							
T 0	1.9 ± 0.1	6.7 ± 0.6	1.7 ± 0.1	26 ± 2	36 ± 3	45 ± 3	28 ± 3
T 15	1.80 ± 0.07	5.7 ± 0.9	1.2 ± 0.2	22 ± 4	25 ± 5	29 ± 10	22 ± 3
TPHs Degradation (%)	3	15	28	15	30	35	23
HD (log MPN)							
T0	4.0 ± 0.3	5.4 ± 0.4	5.2 ± 0.2	3.9 ± 0.2	6.2 ± 0.4	6.1 ± 0.4	6.7 ± 0.3
T15	7.5 ± 0.5	9.36 ± 0.09	8 ± 1	8.1 ± 0.5	11 ± 3	9.49 ± 0.01	8 ± 3
Promotion of HD (%)	86	72	63	111	79	56	27

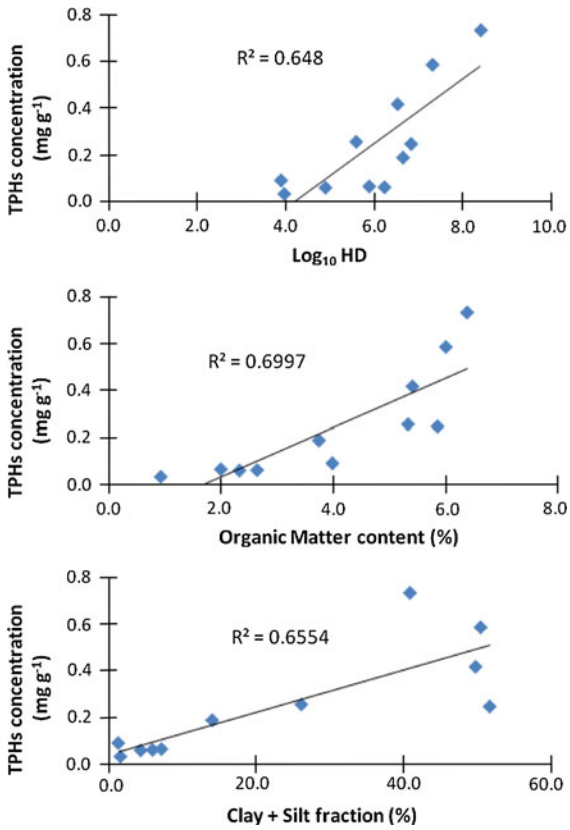

Fig. 4 Correlation plots of total petroleum hydrocarbons (TPHs) concentrations vs hydrocarbon degraders microorganisms estimated by most probable number (Log_{10} HD), TPHs versus organic matter content and TPHs versus clay + silt grain size fraction

have demonstrated significant correlation between hydrocarbon concentration and OM content (Ke et al. 2005; Kim et al. 1999; Xu et al. 2007; Yang 2000; Wang et al. 2001; Zhang et al. 2004). Our results have also shown a positive correlation ($P < 0.05$) between TPHs concentrations and the OM contents. In fact, sediments (colonized and un-colonized) with higher OM contents were characterized by higher values of TPHs (Table 1, Fig. 3). A positive correlation ($P < 0.05$) between TPHs concentrations and the silt + clay fraction was also observed, as expected as sediments with higher OM contents were in general those with an higher percentage of the silt and clay fraction.

The obtained results clearly showed that sediments containing high OM content might play an important role in adsorption, control, distribution and concentration of TPHs, and in addition, grain size could also influence TPHs distribution in sediments.

Plant influence on rhizosediment TPHs retention

TPHs concentrations were, in general, statistically higher in rhizosediments than in un-colonized sediments (Fig. 3). Plants are a major source of organic matter into sediments since root exudates can polymerize with humic materials to form large and stable aggregate structures. These structures are conducive to sequestration of organic carbon, which increases OM content and, consequently, the binding of hydrocarbons (Gregory et al. 2005). We also observed, a significant trend ($P < 0.05$) of higher OM content in rhizosediments, comparatively to the surrounding un-colonized sediments (Table 1). Moreover, the differences in the TPHs concentrations among the different plants and the different sampling sites rhizosediments followed the same trend of the OM content (Table 1 and Fig. 2). In fact, plants like *T. striata* (P3) and *P. australis* (P2) had not only higher OM but also higher TPHs concentrations in their rhizosediments than the other two plants. Also, *J. maritimus* (P1) had similar TPHs concentrations among the different sampling sites rhizosediments, except at L1 where rhizosediment OM content was lower. The evidence that plants may be responsible for the movement of compounds into the rhizosphere, proposed by several authors (Liste and Alexander 2000; Martins et al. 2008), may contribute to the importance of plants in the rhizosediments TPHs retention. Consequently, the significant ($P < 0.05$) higher concentrations of TPHs in rhizosediments, found in our study, may be explained by the movement of compounds towards the roots, due to the plant uptake of water and dissolved nutrients, from the surrounding sediments (Clothier and Green 1997), followed by the subsequent sequestration onto OM content, as discussed above. Additionally, the exudation by plant roots of compounds like organic acids, aromatic acids and phospholipidic surfactants (Liste and Felgentreu 2006 and references therein) may also facilitate the mobility of hydrophobic contaminants from the bulk sediment to the rhizosphere. Therefore, our results demonstrated that plants can effectively influence the distribution of TPHs retaining, in general, more TPHs around their belowground tissues than un-colonized sediments.

Hydrocarbons degradation potential by plant–microorganisms associations

The present study demonstrated a significantly ($P < 0.05$) higher total microbial abundance in rhizosediments comparatively to un-colonized sediments, presumably because plants roots release oxygen and nutrients (especially small molecules such as amino acids, sugars and organic acids), creating an aerobic, nutrient-rich environment in which microbial activity was stimulated (Bais et al. 2006; Kuiper et al. 2004; Olson et al. 2003; Salt et al. 1998). This assumption is corroborated by the observed rhizosphere effect (Olson et al. 2003). Generally, in our study the total amount of microorganisms increased an order of magnitude in the rhizosediment, at the vicinity of plants roots, relatively to the surrounding un-colonized sediments. These findings are consistent with results reported in recent studies (Muratova et al. 2003; Ho and Banks 2006; Nichols et al. 1997) but performed in soil rhizosphere.

Interestingly was the division of TCC data for rhizosediments into two significantly ($P < 0.05$) different groups: group A—L1 rhizo P1 $\approx$ L2 rhizo P1 $\approx$ L3 rhizo P4) and group B—L3 rhizo P1 $\approx$ L4 rhizo P1 $\approx$ L4 rhizo P2 $\approx$ L4 rhizo P3. The TCCs values in the group B were significantly ($P < 0.05$) higher than those of group A, a difference probably related to the OM content. In fact, we found a significantly ($P < 0.05$) higher OM content in the B group, and we also found a strong positive correlation ($r = 0.951$, $P < 0.05$, $n = 7$) between all TCC in rhizosediments and the OM contents. These results demonstrated the likely influence of OM, as an available carbon source on the growth and metabolic activity of rhizosphere microorganisms, which could be potentiated by plant roots.

Several studies have demonstrated the importance of the rhizosphere effect on the degradation of hydrocarbons, being most of these studies focused on terrestrial plants (see below). Recently, Wang et al. (2008) concluded that petroleum pollutants and plant rhizosphere promoted the increase of microorganisms that could degrade soil petroleum hydrocarbons. Corgié et al. (2003) also observed a bacterial gradient with higher numbers of hydrocarbon degrading bacteria in the soil closest to plant roots.

In marine ecosystems, a positive correlation between the number of hydrocarbon-degrading microorganisms and oil pollution was found (Braddock et al. 1995 and references therein). Moreover, Leahy and Colwell (1990) have suggested that the levels of hydrocarbon degrader microorganisms generally reflect the degree of contamination of the ecosystem. Information regarding the influence that the rhizosphere of salt marsh plants might have on hydrocarbon degrading microorganisms is scarce. Nevertheless, salt marshes rhizosphere are interesting sites to investigate the degradation of hydrocarbons because several factors favors their retention (Martins et al. 2008), and it contains a diverse population of hydrocarbon degrading bacteria (Daane et al. 2001). The present study revealed a significantly ($P < 0.05$) higher HD abundance in the rhizosediments which, attending to the earlier mentioned studies on soil rhizosphere, suggests the potential for higher degradation of hydrocarbons in this environment, compared to un-colonized sediments. In fact, we found a significant positive correlation ($P < 0.05$) between HD and TPHs concentrations, demonstrating the salt marsh plant capabilities to retain hydrocarbons around their roots, and for fostering hydrocarbon-degrading microorganisms in its rhizosphere. Differences in degradation potentials were confirmed by the laboratory experiments, which showed higher TPHs degradation rates in rhizosediments than in un-colonized sediment (Table 2).

Not all plant species have the same potential for enhancing rhizoremediation, and climate, soil/sediment characteristics and salinity can also influence the success of the remediation process by one particular plant (Hutchinson et al. 2003). Although in our study *J. maritimus* (P1) rhizosediments had different sediment characteristics along the four sampling sites, the hydrocarbon content and HD enumeration were, in general, not significantly different ($P < 0.05$) among the four sampling sites. On the other hand, plants can alter the microbial population, and these changes can be plant-specific (Kirk et al. 2005). These is a possible explanation for the differences in the numbers of hydrocarbon degraders among the rhizosediments of the different plants collected at the same site (*J. maritimus* < *P. australis* < *T. striata*), with statistically significant differences ($P < 0.05$) between *J. maritimus* and *T. striata*. This study suggests that the interaction between microorganisms and *P. australis* and *T. striata* probably have higher hydrocarbon degradation potential than the interaction with *J. maritimus*

and *S. patens*. Differences in degradation potentials among plants were confirmed by the laboratory experiments, which showed different degradation rates among the different rhizosediments (Table 2). However, slightly lower degradation rates were observed for *T. striata* comparatively to the remaining plants. This fact can reflect an experimental limitation as, after 15 days incubation, a lower stimulation of HD microorganisms by the BH medium was observed for this plant.

More insights on plant–microorganisms interactions with regard to degradation process are needed, in particular the influence among salt marsh plant species, in order to fully ascertain the interactions of rhizosphere on hydrocarbon biodegradation. In fact, little information is available on salt marsh rhizoremediation, therefore, this report is one of the first attempts to describe the effect that salt marsh plants might have on hydrocarbon degradation in a temperate estuarine environment.

Conclusion

The results obtained in this study suggest that salt marsh plants can influence microbial communities by fostering the development of hydrocarbon-degrading microbial population in its rhizosphere, an effect observed for all plants species selected. This effect, combined with the plant capability to retain hydrocarbons around the roots, points out that salt marsh plant-microorganisms associations may actively contribute to hydrocarbon removal and degradation in temperate estuarine environment.

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