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Fate of petroleum hydrocarbons in bioturbated pristine sediments from Caleta Valdés (Patagonia Argentina): An *ex situ* bioassay

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ABSTRACT

Petroleum can pollute pristine shorelines as a consequence of accidental spills or chronic leaks. In this study, the fate of petroleum hydrocarbons in soft pristine sediment of Caleta Valdés (Argentina) subject to *ex situ* simulated oil pollution was assessed. Sedimentary columns were exposed to medium and high concentrations of Escalante Crude Oil (ECO) and incubated in the laboratory during 30 days. Levels of aliphatic hydrocarbons at different depths of the sedimentary column were determined by gas chromatography. Oil penetration was limited to the first three centimetres in both treatments, and under this depth, hydrocarbons were clearly biogenic (terrestrial plants) as in the whole sedimentary column of the control assay. Bioturbation by macrobenthic infauna was strongly impacted by oil pollution which resulted in reduced sediment oxygenation and low burial of petroleum hydrocarbons. This may partly explain the limited hydrocarbon biodegradation observed, as indicated by the relatively high values of the ratios *n*C17/pristane, *n*C18/phytane, and total resolved aliphatic hydrocarbons/unresolved complex mixture. Correspondingly, at the end of the experiment the most probable number of hydrocarbon-degrading bacteria reached $\sim 10^3$ MPN g^{-1} dry weight. These values were lower than those found in chronically polluted coastal sediments, reflecting a low activity level of the oil-degrading community. The results highlight the low attenuation capacities of Caleta Valdés pristine sediments to recover its original characteristics in a short time period if an oil spill occurs. In this work, we present a novel and integrative tool to evaluate the fate of petroleum hydrocarbons and their potential damage on pristine sediments.

1. Introduction

Marine pollution remains strongly related to petroleum exploitation whereby hydrocarbons are ubiquitous pollutants in the marine environment (i.e. NOAA, 2001), reducing worldwide pristine areas. Coastal systems are affected by oil spills depending on their geophysical, hydrodynamic, and biological characteristics. Low energy environments, where macrobenthos activity plays an important role in the fate of sedimentary organic matter, are usually more damaged by oil spills than high energy environments and their recovery is slower. Actions to mitigate ecological impacts, such as shorelines cleaning methods, are difficult to implement in highly productive ecosystems characterized by fine sediments (Duran et al., 2015), and the natural attenuation of

petroleum often constitutes the best option. The activity of macro-invertebrates significantly influences microbial activities and biogeochemical processes in sediments by modifying water and sediment fluxes at the water-sediment interface (Mermillod-Blondin and Rosenberg, 2006) and mixing physically sediments introducing oxygen by burrow ventilation, which affect nutrient cycles, fate of contaminants, and microbial metabolisms (Cuny et al., 2011; Duran et al., 2015). These processes stimulate the oxygenation of sediments (Timmermann et al., 2011) and favour the aerobic biodegradation of hydrocarbons (Duran et al., 2015). Bioturbation plays an important role in the burial and degradation of aliphatic hydrocarbons and PAHs (Christensen et al., 2002; Duran et al., 2015; Timmermann et al., 2011), whereas bacteria are considered to be the predominant hydrocarbon-

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degraders in marine environments (Mortazavi et al., 2013). Whether organic pollutants remain at the sediment surface, are buried or released to the water column, or are degraded, depends strongly on benthic macroinfauna activity (Cuny et al., 2011; Gilbert et al., 1994; Schaffner et al., 1997). The detrimental effects of hydrocarbons on benthic organisms and across trophic levels are well established (Lindgren et al., 2014). Chronic exposures lead to adapted benthic communities with higher proportion of tolerant species and/or an increased tolerance among individual species (Gilbert et al., 2014), while communities from pristine ecosystems are particularly sensitive to oil spills (Ferrando et al., 2015). The crude oil extracted in the southern Provinces of Argentina is transported by tankers to northern refineries, impacting the Patagonian coastline (Commendatore et al., 2000). Nevertheless, several areas are still pristine, such as Península Valdés which is a protected area designated as a UNESCO World Heritage Site (<http://www.unesco.org/new/>). We applied an *ex situ* experiment using microcosms to artificially simulate an oil impact on unmodified sediments from Caleta Valdés, presenting a novel tool to evaluate aspects related to the potential damage of hydrocarbons to pristine sediments. The goals of this study were: (1) to assess the fate of petroleum hydrocarbons in pristine soft sediments; (2) to assess the ability of the autochthonous microorganism community to biodegrade hydrocarbons through natural attenuation; (3) to assess the capacity of sediments to recover its original characteristics; (4) to contribute to the development of effective guidelines for the management of such pristine coastal systems in the event of hydrocarbon contamination.

2. Materials and methods

2.1. Study area

Caleta Valdés (CV) is located on the eastern sector of the Península Valdés protected area. This north-south oriented creek is 30 km long and has its mouth at the southern end. The study site (Fig. 1) was located in the muddy northern coastal area of the creek (42°15'53" S, 63°40'50" W), a low energy environment where preliminary research has demonstrated the area has not been impacted by oil pollution and is suitable for studying the fate of petroleum hydrocarbons in fully pristine sediments. In 2009, surficial sediments were sampled in five sites along the CV. The hydrocarbon concentration of the sediments ranged from less than 5–200 ng g⁻¹ dry weight (dw). Hydrocarbon fractions were devoid of polyaromatic hydrocarbons and of unresolved complex mixture (UCM). The aliphatic hydrocarbons present in these sediments have a biogenic origin, probably from vascular terrestrial plants (as determined by *n*-alkane diagnostic indices) and phytoplankton (highly

branched isoprenoids of diatom origin) (unpublished data). In addition, hydrocarbon analysis in seawater from the study area showed values below the method's limit of detection (< 5 ng mL⁻¹). Nevertheless, this pristine site faces a risk of oil contamination due to the relative proximity to the oil maritime route.

2.2. Sampling

Sediment samples were collected in April 2012 using PVC corers (10 cm diameter × 25 cm length), as previously performed in bioturbation studies (Ferrando et al., 2015; Timmermann et al., 2011). Twelve 20 cm long sediment cores were sampled and transported immediately to the laboratory, without disturbing the vertical structure of the sedimentary column. These cores are referred to as “experimental sediment”. Four Supplementary cores (“field sediment”) were sampled for the general characterization of CV sediments. In addition, 4 kg of surficial sediment (first cm) were sampled for the preparation of uncontaminated and contaminated sediment cakes, and 60 L of seawater were collected to fill the incubation tanks (Fig. 2).

2.3. Experimental design and incubation conditions

The experimental design was described in Ferrando et al. (2015). Briefly, three different treatments were considered: control cores without oil addition (E0), lowly contaminated cores (E1), and highly contaminated cores (E2). Each treatment consisted in 4 replicate cores that were placed in a 56 L tank, filled with seawater above the core levels (Caradec et al., 2004; François et al., 2002; Gilbert et al., 2007). Each corer, considered the experimental unit, had the bottom closed with a lid. Thus, no water circulation or percolation through the sediment column occurred and exchanges of oxygen and nutrients with the surrounding water were restricted to the sediment surface. The treatments were performed by depositing a cake (1 cm deep) of uncontaminated or artificially contaminated CV sediments (“sediment cakes”) on top of the cores. In order to obtain contaminated cakes, homogenized sediment was mixed with Escalante Crude Oil (ECO), to simulate a chronic (E1) or massive (E2) oil spill, respectively (UNEP/IOC/IAEA, 1992). ECO is medium oil with 0.89 g mL⁻¹ density, coming from the San Jorge Gulf basin (Chubut province, Argentina). Total Aliphatic hydrocarbon concentrations were 554.3 µg g⁻¹ dw (E1) and 6139.9 µg g⁻¹ dw (E2) for artificial contaminated sediment cakes used at zero time of the experiment, determined by gas chromatography as described in 2.4.2. Luminophores (63–355 µm particulate inert traces) were added (2 g) on top of each deposited sediment cake to assess biological reworking activity (Cuny et al., 2014). The four cores of each

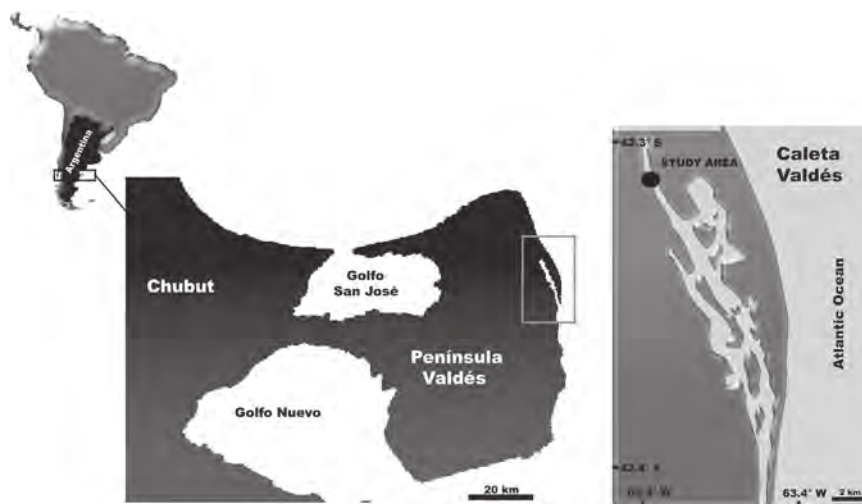


Fig. 1. Study site in Caleta Valdés, Península Valdés (Patagonia, Argentina).

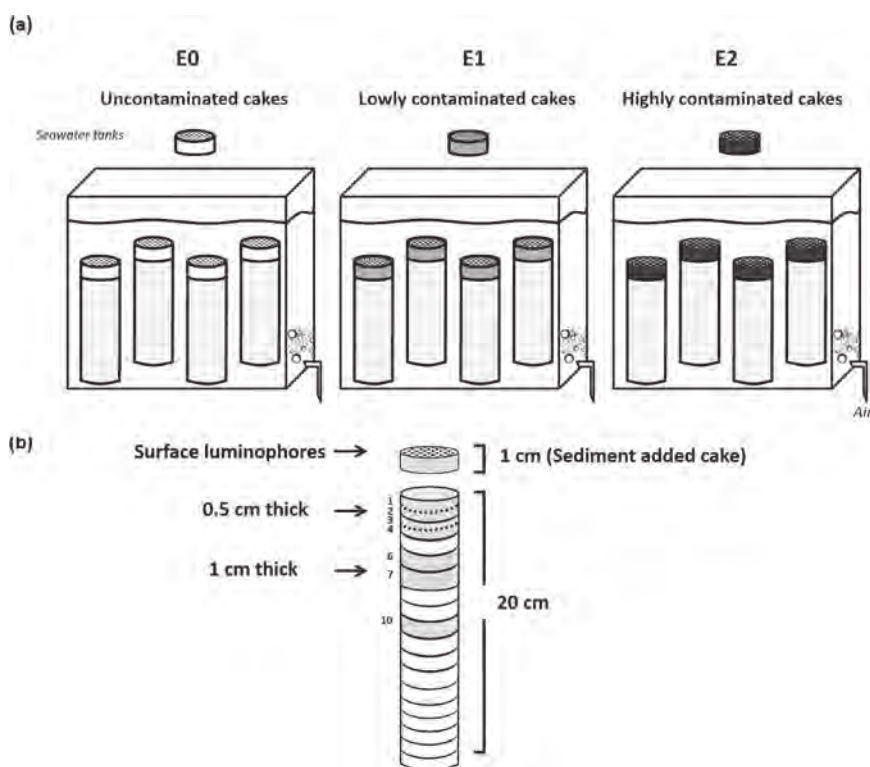


Fig. 2. Experimental system. (a) Each seawater tank contained 4 corers with uncontaminated (E0), lowly contaminated (E1), and highly contaminated (E2) cakes added. (b) Diagram of a sliced sediment core after 30 days of incubation; layers used for hydrocarbon analyses are grey colored.

experimental condition (E0, E1, and E2) were then placed in an individual 56 L tank filled with seawater from CV (Fig. 2) and incubated for 30 days at room temperature ($\sim 18^\circ\text{C}$) with continuous water oxygenation (Caradec et al., 2004; François et al., 2002; Gilbert et al., 2007). A 30 day incubation period was selected as experimentation time in order to balance the need to have a sufficiently long period to assess hydrocarbon biodegradation and a reasonable short period to study macrofauna activity (bioturbation) without them being affected by the lack of food in the closed experimental system used. Thus, a conservative “short term” 30 days period was used, based on similar studies (Brakstad and Bonaunet, 2006; Haines et al., 2005; Louati et al., 2013). Temperature, dissolved oxygen, and pH in tank water were measured during incubation. Following incubation, cores were sliced into 0.5 cm-thick layers from the surface to 2 cm deep, and into 1 cm-thick layers from 2 to 18 cm depth (Ferrando et al., 2015). Sediment humidity and organic matter (OM%) were performed for all core layers of each treatment (see Methods in Section 2.4.1). Hydrocarbon-degrading bacteria concentrations were assessed in surficial sediments of each core for all treatment (0–3 cm), by measuring a composite sample of the first three layers in each corer. In order to optimize the analytical effort, and considering that hydrocarbon penetration would be a major descriptor related with bioturbation, hydrocarbon analysis was performed in composite samples of the four cores per treatment, measuring individual layers 1–4 (0–2 cm), 6 and 7 (4–5 cm), and 10 (8 cm) as representative of the expected non-perturbed sediments (Fig. 2).

2.4. Analysis

2.4.1. Characterization of CV field sediment

In four Supplementary cores, sliced as the experimental cores, sediment humidity was determined by oven drying (105°C to constant weight) and organic matter by calcination in muffle furnace (450°C for 4 h). In addition, the granulometry was analyzed by laser beam diffraction (Partica LA-950; HORIBA Instruments, Inc.) yielding the following fractions: silt-clay $< 60\ \mu\text{m}$, fine sand $60\text{--}200\ \mu\text{m}$, medium sand

$200\text{--}600\ \mu\text{m}$, coarse sand $600\text{--}2000\ \mu\text{m}$, and gravel $> 2000\ \mu\text{m}$.

2.4.2. Hydrocarbon analysis

For hydrocarbon analysis a composite sample was conformed taking sediment from each corer. The analysis was conducted according to USEPA and UNEP methodologies: extraction (EPA 3540), clean-up and fractionation (UNEP No. 20), and chromatographic determination (EPA 8015). Briefly, samples were chemically dried (Na_2SO_4) and Soxhlet extracted (8 h) with methylene chloride following the addition of surrogate standards (perdeuterated hydrocarbons, $n\text{C}_{12}\text{-}d26$ and $n\text{C}_{20}\text{-}d42$). Sulfur was removed from the total organic extract using activated elemental copper. Organic extracts were reduced in volume by rotary evaporation and dichloromethane was exchanged with hexane. Fractionation was made by using a biphasic chromatographic column (silica gel-alumina partially deactivated to 5% and 2% w/w, respectively). Aliphatic hydrocarbons were eluted with 20 mL of hexane and the fraction was reduced in volume to 1 mL under a gentle stream of nitrogen. Following the addition of $n\text{C}_{16}\text{-}d34$ as an internal standard, aliphatic hydrocarbons were analyzed by gas chromatography in a Trace GC Ultra (Thermo Fisher) chromatograph equipped with auto-sampler (TRIPLUS AS3000), flame ionization detector (GC/FID), split/splitless injector, and HP 5MS fused silica capillary column ($30\ \text{m} \times 0.25\ \text{mm i.d.} \times 25\ \mu\text{m}$ film thickness). The injector and detector temperatures were maintained at 250°C and 320°C , respectively. Nitrogen ($1\ \text{mL min}^{-1}$) was used as carrier gas. Identification of resolved *n*-alkanes and pristane (Pr) and phytane (Phy) isoprenoids was made by retention time comparison of target compounds with authentic external standards (Sigma Aldrich). Quantification was done using the internal standard method. The aliphatic unresolved complex mixture (AliUCM) and total resolved aliphatic (TRAli) were quantified based on total area calculation and the response overlying resolved peaks. Concentrations of individual *n*-alkanes (*n*-alk), Pri and Phisoprenoids, total *n*-alkanes ($\Sigma n\text{-alk}$), total resolved aliphatics (TRAli), aliphatic unresolved complex mix (AliUCM), and total aliphatic hydrocarbons (TRAli + AliUCM), were reported relative to sediment dry weight (dw). Every five samples,

a blank was analyzed to check for the absence of contamination potentially induced by the analytical procedure. The limit of detection (LOD) was 5–10 ng g⁻¹ for individual *n*-alkanes. LOD was determined as the lowest concentration that can be determined to be statistically different from a method blank (n = 7; 3.14 SD) with a 99% level of confidence. Recovery of samples ranged between 60% and 110% and the relative standard deviation between 0.4% and 9%. For *n*-alkanes, recovery assays on standard spiked pristine sediment resulted in 95 ± 12% (n = 6). Chromatographic performance was checked incorporating the injection of a control standard (5 µg mL⁻¹) in each sample sequence.

2.4.3. Aliphatic diagnostic indices

To identify hydrocarbon sources (biogenic or petrogenic), aliphatic diagnostic indices (ADIs) were used: Even to odd ratio ($\Sigma\text{Even}/\Sigma\text{Odd}$); low to high molecular weight ($\Sigma\text{LMW}/\Sigma\text{HMW}$); Carbon Preference Index (CPI); Major Hydrocarbon (MH) (Commendatore et al., 2000); and the following ratios (see Section 2.4.2 for abbreviation definitions): Pri/Phy, *n*C17/Pri, *n*C18/Phy, TRAlI/AlIUCM and $\Sigma n\text{-alk}/\text{UCM}$. Values of ADIs allowing the determination of the origin of hydrocarbons have been previously defined (e.g. Broman et al., 1987; Colombo et al., 1989; Tolosa et al., 2004). In addition, the “Natural *n*-Alkane Ratio” NAR = $[\Sigma n\text{-alk}(C_{19-32}) - 2\Sigma\text{even } n\text{-alk}(C_{20-32})]/\Sigma n\text{-alk}(C_{19-32})$ (Mille et al., 2007) was calculated. Other specific signatures such as the presence/absence of *n*-alkane homologous series and AlIUCM helped to elucidate the origin of the hydrocarbons.

2.4.4. Hydrocarbon-degrading bacteria

The enumeration of hydrocarbon-degrading bacteria was performed using the most probable number (MPN) procedure (Haines et al., 1996; Wrenn and Venosa, 1996). Briefly, a composite sample integrating the first 3-cm layer of wet surficial sediment (1 g) from each core was added to 9 mL of sterile sea water (filtered on 0.2 µm) and mixed for 3 h at 200 rpm in an orbital shaker. The suspension was allowed to settle for 30 min and ten-fold dilution was prepared with the supernatant with 1% w/v sodium pyrophosphate. Dilutions were seeded in 96 well micro plates containing 180 µL of a sterile Bushnell-Hass marine mineral salts broth (with 2% NaCl), 20 µL of sample/dilution and 5 µL of sterile light oil as growth substrate according to the procedure described in Nievas et al. (2008). Five replicates of each dilution and the corresponding sterile control were performed. Plates were incubated for 3 weeks at 16–20 °C. Positive cells were detected by reducing breathing violet indicator Iodonitrotetrazolium (INT), and the results were expressed as MPN g⁻¹ dw sediment by measuring moisture content in a sediment subsample.

2.4.5. Statistical analysis

For OM and humidity profile in field and experimental sediments, treatment and depth effect were tested by a two-way ANOVA with treatment (field sediment at initial incubation time, and E0, E1 and E2 at final incubation time) and depth as the main effects. When significant differences were detected, Tukey HSD *post hoc* test for multiple comparisons was performed to determine which treatment differed. Field sediment's granulometry by depth was tested using a one-way ANOVA and Tukey HSD *post hoc* test for multiple comparisons. Pairwise comparisons of sediment characteristics within grouped depths of interest were done with Student-*t*-tests. Petroleum-degrading bacteria concentration determined at surface sediment (0–3 cm), were compared between treatments using one-way ANOVA and Tukey *post hoc* test for multiple comparisons was applied when significant differences between treatments were found. When necessary, data were transformed to fit the assumption of homoscedasticity: OM content was logarithmic transformed, humidity was reciprocal transformed (1/*x*), exponential transformation with 0.3 as exponent was used for luminophores percentage and hydrocarbon-degrading bacteria concentration was logarithmic transformed as usually done with microbial growth data. *N*-

alkanes profiles were evaluated with the Kolmogorov-Smirnoff test (K-S test) to checked significant differences between treatments and depths, by means of pairwise comparisons.

3. Results and discussion

The temperature, dissolved oxygen concentration and pH of seawater in the incubation tanks showed little changes throughout the experiment. No statistically significant differences of means between conditions (E0, E1, and E2) were observed for the three variables, as reported by Ferrando et al. (2015). Therefore, the fate of hydrocarbons in the sedimentary matrix was not influenced by these physico-chemical parameters.

3.1. Characterization of CV field sediments

Sand, mainly fine and medium, was the predominant fraction (over 80%) in the entire sedimentary column. Silt/clay content had a statistically significant decreasing profile with depth ($p < 0.05$), with a maximum value at the surface sediment of 20.4% and a mean of 15.5 ± 6.1% in the first three cm (n = 4 layers), showing stable values around 6.4% underneath ($p < 0.05$; mean 6.4 ± 2.2, n = 17 layers, Table 1). The sediment humidity content also showed a decreasing profile with depth ($p < 0.05$), with highest content in the surficial layer (0–0.5 cm, 57%), decreasing monotonically to near 25% at 5 cm depth, and then keeping almost constant in the range 18–25% towards the bottom (Table 1). OM content decreased from 1.7% to 1.1% (mean 1.4 ± 0.23%; n = 5) from surficial CV field sediment to the third cm, and then was highly homogeneous throughout the rest of the core (0.9 ± 0.05%; n = 17) (Table 1). Silt/clay correlated positively with the OM content when considering the whole sedimentary column ($R^2 = 0.94$; $p < 0.05$; n = 22), suggesting the ability of CV surficial sediment to retain lipophilic material, since fine particles ($\phi < 60 \mu\text{m}$) tend to adsorb larger amounts of OM content (Arnarson and Keil, 2007). CV surficial sediments presented relatively high water content in accordance with the smaller size of sediment particles. Moreover, fresh ECO whose density is 0.89 g mL⁻¹ could remain in surficial layers adsorbed to sedimentary particles similarly to what happened in other comparable sedimentary systems (Olsen et al., 1982). In addition, fine-grained substrates characterized by low permeability and water-saturated sediments substantially prevents depth penetration of oil (Olsen et al., 1982).

3.2. Experimental sediments characterization at final incubation time

Grain sizes in experimental sediments were assumed identical to those determined in CV field sediments. Humidity profiles for all experimental sediments had a similar decreasing trend than those of the field sediment (Table 1), showing significant differences for treatments and depth as main factors, but without interaction between them (two-way ANOVA, $p < 0.01$ for main factors and $p > 0.48$ for interactions, see Supplemental material S1). From Tukey test, humidity showed no significant differences between treatments at all depths, except for the surficial sediments in treatment E2 and field uncontaminated sediments (38.4 ± 1.0% vs. 57.2 ± 10.6% vs. respectively). This difference was likely due to the hydrophobic character of the first layer of E2 sediment due to oil addition. No differences in humidity were found for the experimental sediments at final incubation time between treatments (E0, E1 and E2), having the same decreasing profile with depth, from 40.9 ± 2.4% at surficial sediment to 22.5 ± 0.9% at 6 cm, and keeping almost constant up to the bottom.

OM content showed overall significant changes with depth, treatment and their interactions, as expected due to the experimental oil addition (two-way ANOVA, for treatments, depth and interaction, see Supplemental material S1). For E0 and E1, OM content showed an almost flat profile with depth and no differences between treatments

Table 1

Characteristics of field sediments at initial time (0 days) and sediments of the experiments at final time (30 days). Values are mean \pm standard deviation of four replicates.

Depth (cm)	Field Sediment							Experimental Sediment at final incubation time (30 d)					
	Granulometry (%)							E0		E1		E2	
	Humidity (%)	OM (%)	Silt-Clay	Coarse silt	Fine sand	Medium sand	Coarse sand	OM (%)	Lum (%)	OM (%)	Lum (%)	OM (%)	Lum (%)
0-0.5	57.2 \pm 10.6	1.7 \pm 0.1	4.1 \pm 1.4	16.3 \pm 6.4	52.1 \pm 4.1	26.8 \pm 10.3	0.7 \pm 0.9	1.4 \pm 0.3	43.1 \pm 17.6	1.4 \pm 0.3	54.3 \pm 7.0	2.6 \pm 0.1	98.1 \pm 2.0
0.5-1	45.6 \pm 5.7	1.5 \pm 0.1	3.1 \pm 1.4	16.6 \pm 5.1	54.4 \pm 1.6	25.4 \pm 6.7	0.5 \pm 0.4	1.1 \pm 0.1	35.6 \pm 7.5	1.1 \pm 0.2	31.5 \pm 5.4	2.4 \pm 0.2	0.2 \pm 0.2
1-1.5	43.0 \pm 5.9	1.6 \pm 0.3	1.6 \pm 0.8	13.5 \pm 4.1	54.9 \pm 2.7	29.5 \pm 2.5	0.4 \pm 0.1	1.6 \pm 0.0	9.8 \pm 8.5	1.6 \pm 0.0	8.9 \pm 10.0	2.0 \pm 0.3	0.1 \pm 0.1
1.5-2	37.4 \pm 4.1	1.3 \pm 0.1	0.7 \pm 0.4	10.2 \pm 2.1	53.9 \pm 2.6	34.6 \pm 2.0	0.6 \pm 0.3	1.4 \pm 0.2	6.4 \pm 5.5	1.5 \pm 0.0	1.5 \pm 1.4	1.9 \pm 0.2	1.3 \pm 2.2
2-3	31.7 \pm 3.9	1.1 \pm 0.1	0.2 \pm 0.4	11.0 \pm 2.1	54.4 \pm 3.5	33.7 \pm 3.9	0.8 \pm 0.4	1.3 \pm 0.1	1.1 \pm 1.6	1.3 \pm 0.2	0.3 \pm 0.4	1.6 \pm 0.3	0.1 \pm 0.1
3-4	28.2 \pm 1.9	1.0 \pm 0.1	0.4 \pm 0.5	7.2 \pm 1.3	51.0 \pm 5.6	39.9 \pm 5.0	1.6 \pm 1.1	1.3 \pm 0.1	1.0 \pm 1.1	1.3 \pm 0.1	0.4 \pm 0.3	1.5 \pm 0.2	0.0 \pm 0.0
4-5	25.2 \pm 2.3	1.0 \pm 0.1	0.3 \pm 0.6	6.1 \pm 0.6	49.5 \pm 4.3	42.3 \pm 2.6	1.7 \pm 0.7	1.3 \pm 0.0	0.6 \pm 0.6	1.3 \pm 0.1	0.3 \pm 0.3	1.5 \pm 0.2	0.1 \pm 0.1
5-6	24.1 \pm 1.5	0.9 \pm 0.1	0.0 \pm 0.1	6.0 \pm 1.3	48.3 \pm 4.3	43.5 \pm 4.2	2.2 \pm 0.7	1.2 \pm 0.1	0.3 \pm 0.2	1.2 \pm 0.1	0.4 \pm 0.3	1.4 \pm 0.2	0.0 \pm 0.0
6-7	22.9 \pm 0.9	0.9 \pm 0.0	0.0 \pm 0.0	5.6 \pm 1.2	48.5 \pm 4.9	43.6 \pm 4.8	2.3 \pm 1.0	1.2 \pm 0.1	1.0 \pm 0.8	1.3 \pm 0.1	0.5 \pm 0.6	1.6 \pm 0.1	0.0 \pm 0.0
7-8	22.9 \pm 1.2	0.9 \pm 0.1	0.1 \pm 0.1	6.2 \pm 1.9	48.3 \pm 3.4	43.2 \pm 3.3	2.2 \pm 0.8	1.1 \pm 0.1	0.0 \pm 0.0	1.2 \pm 0.1	0.3 \pm 0.8	1.4 \pm 0.1	0.0 \pm 0.1
8-9	22.7 \pm 1.2	0.9 \pm 0.0	0.1 \pm 0.1	6.2 \pm 0.4	47.6 \pm 4.9	43.7 \pm 3.8	2.3 \pm 1.1	1.2 \pm 0.1	0.8 \pm 1.1	1.2 \pm 0.2	0.6 \pm 0.4	1.4 \pm 0.2	0.0 \pm 0.1
9-10	23.1 \pm 0.9	1.0 \pm 0.1	0.1 \pm 0.1	8.0 \pm 1.5	48.2 \pm 4.4	41.8 \pm 4.3	1.9 \pm 0.9	1.2 \pm 0.1	0.1 \pm 0.1	1.2 \pm 0.1	0.6 \pm 0.8	1.4 \pm 0.1	0.1 \pm 0.1
10-11	21.9 \pm 0.8	1.0 \pm 0.0	0.1 \pm 0.1	6.4 \pm 1.6	46.2 \pm 3.4	44.3 \pm 2.6	2.8 \pm 1.1	1.2 \pm 0.1	0.1 \pm 0.1	1.2 \pm 0.0	0.3 \pm 0.4	1.5 \pm 0.2	0.0 \pm 0.1
11-12	21.2 \pm 0.8	1.0 \pm 0.1	0.2 \pm 0.3	6.7 \pm 1.6	48.6 \pm 4.7	42.0 \pm 2.4	2.4 \pm 1.3	1.2 \pm 0.1	0.0 \pm 0.0	1.1 \pm 0.0	0.1 \pm 0.1	1.4 \pm 0.2	0.0 \pm 0.0
12-13	21.3 \pm 0.5	1.0 \pm 0.1	0.1 \pm 0.2	6.4 \pm 1.4	47.5 \pm 4.1	43.5 \pm 2.6	2.4 \pm 1.1	1.3 \pm 0.2	0.2 \pm 0.3	1.3 \pm 0.1	0.0 \pm 0.1	1.4 \pm 0.2	0.0 \pm 0.0
13-14	19.3 \pm 3.5	0.9 \pm 0.1	0.1 \pm 0.3	6.3 \pm 2.9	48.3 \pm 2.8	43.2 \pm 3.1	2.1 \pm 0.4	1.1 \pm 0.1	0.0 \pm 0.0	1.2 \pm 0.1	0.0 \pm 0.0	1.5 \pm 0.2	0.0 \pm 0.0
14-15	18.8 \pm 1.8	0.9 \pm 0.1	0.1 \pm 0.1	8.3 \pm 2.2	45.2 \pm 6.5	43.4 \pm 5.8	3.0 \pm 1.6	1.1 \pm 0.0	0.0 \pm 0.0	1.2 \pm 0.1	0.0 \pm 0.1	1.5 \pm 0.1	0.0 \pm 0.0
15-16	19.5 \pm 1.1	0.9 \pm 0.1	0.1 \pm 0.1	6.8 \pm 2.0	46.6 \pm 2.1	43.9 \pm 3.0	2.7 \pm 0.3	1.1 \pm 0.1 ^a	0.0 \pm 0.0 ^a	1.2 \pm 0.3 ^a	0.0 \pm 0.0 ^a	1.3 \pm 0.1	0.0 \pm 0.0
16-17	19.4 \pm 1.1	0.9 \pm 0.0	0.0 \pm 0.0	6.2 \pm 2.8	47.5 \pm 2.4	43.7 \pm 3.3	2.5 \pm 0.7	-	-	-	-	1.2 \pm 0.1 ^a	0.0 \pm 0.0 ^a
17-18	17.8 \pm 2.1	0.8 \pm 0.1	0.1 \pm 0.1	7.1 \pm 2.1	46.0 \pm 3.3	44.3 \pm 3.5	2.4 \pm 1.0	-	-	-	-	-	-
18-19	18.4 \pm 0.0	0.9 \pm 0.0	0.0 \pm 0.1	6.9 \pm 1.3	46.9 \pm 6.1	43.5 \pm 4.7	2.6 \pm 1.7	-	-	-	-	-	-
19-20	18.2 \pm 0.2	0.9 \pm 0.1	0.0 \pm 0.0	4.3 \pm 1.4	46.0 \pm 3.2	47.1 \pm 4.3	2.6 \pm 0.0	-	-	-	-	-	-

For humidity there was a significant effect of depth and treatment (two-way ANOVA, $p < 0.01$), with no interaction effect ($p = 0.48$). For percentages of organic matter (OM) and luminophores (Lum) there was a significant effect of depth and treatments, and also interaction effect (two-way ANOVA, $p < 0.01$). For percentages of granulometry there was a significant depth effect (one-way ANOVA, $p < 0.01$). See *post hoc* Tukey results of multiple comparisons for OM, Lum, humidity and granulometry in [Supplemental material \(S1\)](#). Abbreviation: E0: control cores without oil addition, E1: lowly contaminated cores and E2: highly contaminated cores.

^a Value that corresponds to the deepest layer recovered.

were found (see Supplemental material S1). E2 OM profile showed higher statistically significant values at the three top cm sediment (from 2.6% to 1.6%), being constant below that depth with no significant differences. At the end of the experiment, OM in bioassay surficial sediments (0–2 cm) was significantly higher for E2 than for E0, E1 and field sediments (Table 1, $p < 0.05$) due to the addition of a large amount of crude oil. In E2, the OM content at deeper layers than 3 cm showed no significant differences with E1 and E0, and from 6 cm depth remained practically constant for all treatments (E0: $1.2 \pm 0.1\%$, $n = 11$; E1: $1.2 \pm 0.1\%$, $n = 11$; E2: $1.4 \pm 0.1\%$, $n = 12$). In E0, no significant differences ($p < 0.05$) with uncontaminated field sediment profiles were observed, the same as for E1 (except for the 6 and 7 cm depths, but with very little differences 0.9% vs. 1.2%), suggesting the absence of petroleum hydrocarbon burial (Table 1). On the other hand, E2 treatment showed significant differences with uncontaminated field sediments for most depths ($p < 0.05$, except for layers 3 and 14), with differences varying from 1% at the top to 0.4% at the bottom.

3.3. Hydrocarbons in field CV sediment and control cores (E0 treatment)

The level of total aliphatic hydrocarbons (TAlIH) in field sediments from CV (Fig. 3a) and in E0 after 30 days of incubation (Fig. 3b) was low (maximum of $0.061 \mu\text{g g}^{-1} \text{dw}$), in accordance with unpolluted sediments. The higher hydrocarbon concentrations corresponded to the presence of high molecular weight C-odd *n*-alkanes (*n*C25, *n*C27, *n*C29, and *n*C31), indicative of an input of terrestrial hydrocarbons from marsh vascular plants present in the study area, such as *Salicornia* sp. and *Spartina alterniflora* (Isacch et al., 2006). The occurrence of C-odd low molecular weight hydrocarbons such as *n*C17 was attributed to biogenic contributions of marine origin (phytoplankton and/or macroalgae (Commendatore et al., 2000; Horel et al., 2012; Liu and Liu,

2016)). In addition, a series of compounds eluting just before *n*C21, with retention time and mass spectra characteristics of highly branched C25 alkenes and of linear C21 monoenes, was also observed. Highly Branched Isoprenoids (HBI) could be derived from diatoms (Grossi et al., 2004). The biogenic origin of hydrocarbons in CV sediments was further supported by the absence of a homologous series of C-*n*-alkanes and of AliUCM.

Values calculated for ADIs in control sediments (E0) supported the biogenic origin of hydrocarbons, likely from terrestrial plant sources (*Salicornia* sp. and *S. alterniflora*) (Table 2), as in CV field sediments. An exception was found in the 0–1 cm layer where the *n*C24-alkane was the major hydrocarbon (MH), together with the plant markers *n*C27, *n*C29, *n*C31, and *n*C35. Furthermore, the ratio $\Sigma\text{Even}/\Sigma\text{Odd}$ was higher than 1 due to the relatively high proportion of *n*C24. The absence of other C-even hydrocarbons suggests an artifact peak matching with *n*C24 retention time or an origin from marine phytoplankton. Bieger et al. (1997) already suggested that *n*C24 could be produced by marine phytoplankton, more specifically by diatoms. This is in agreement with the presence in CV sediments of a series of branched C25 alkenes and C21 monoenes probably derived from diatoms. In deeper sediment layers, the ratio $\Sigma\text{Even}/\Sigma\text{Odd}$ ratio was less than 1, corroborating the biogenic origin of hydrocarbons (Table 2). The presence of *n*C21 alkenes indicated an input of phytoplankton and/or macroalgae at the depths of 4–5 cm. As in field sediments, the absence of a homologous series of *n*-alkanes and of an UCM in E0 bioassays demonstrated the pristine nature of CV sediments. The hydrocarbon distribution pattern observed in chromatographic profiles (Fig. 3) reflected the values calculated for ADIs (Table 2), as well as specific compositional parameters (absence/occurrence of homologous series of *n*-alkanes and UCM).

In these pristine sediments, hydrocarbon concentrations at every depth never exceeded the value suggested by UNEP/IOC/IAEA (1992)

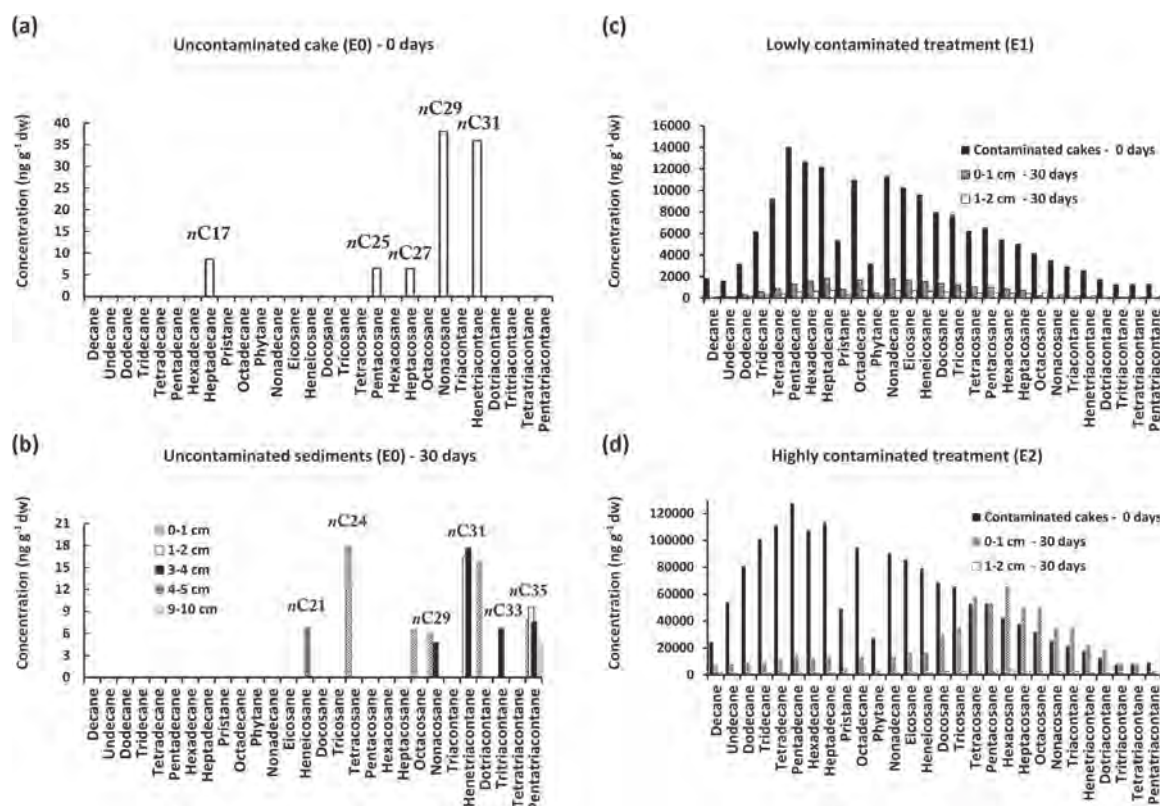


Fig. 3. Hydrocarbon distribution profile of uncontaminated sediment cake (E0) at the initial experimental time (0 days) (a) and in the different depth layers after 30 days of incubation (b). *n*-alkane hydrocarbon distribution for lowly (E1) and highly (E2) contaminated treatments (c and d, respectively) in the top layer at the beginning of the experiment (0 days, black bars) and in the first two sediment layers [0–1 cm (grey bars) and 1–2 cm (white bars)] after 30 days of incubation. Values correspond to a composite sample of the four corers. All hydrocarbons profiles are statistically different ($p < 0.05$) as determined by the Kolmogorov-Smirnov test, except for E0 (1–2 cm) and E0 (3–4 cm) at 30 days; and for E0, E1 and E2 at 9–10 cm at 30 days (data not shown).

Table 2
Aliphatic Diagnostic Index values (ADIs).^a

Indices	MH	ΣEven/Σodd	ΣLMW/ ΣHMW	CPI	Pri/Phy	nC17/ Pri	nC18/ Phy	TAlI/ UCM	Σn-alk/ UCM	NAR
Source										
Biogenic Marine	nC15, nC17 & nC19	< 1	1							1
Vascular Plants	nC27, nC29 & nC31	< 1	0.3–0.4	5–8	1.2–1.7					1
Petrogenic	nC15 & nC18	Approx. 1	1–5.7	1–1.5	0.5–1					0
References^b	Colombo et al. (1989)	Volkman et al. (1992)	Wang et al. (2006)	Tolosa et al. (2004)	Wu et al. (2001)					Mille et al. (2007)
This study										
ECO	nC15	1.0	1.9	1.3	1.6	3.0	4.2	0.7	0.3	0.1
Uncontaminated cake (0 d)	nC29	0.2	0.1	13.5	–	–	–	–	–	0.9
0–1 cm (30 d)	nC24	3.4	0.0	0.9	–	–	–	–	–	– 0.8
1–2 cm (30 d)	nC31	0.0	0.0	–	–	–	–	–	–	1.0
Lowly contaminated cakes (0 d)	nC25	1.0	1.0	1.4	1.7	2.3	3.4	1.0	0.6	0.1
0–1 cm (30 d)	nC17	0.9	1.0	1.3	1.5	2.3	3.3	0.5	0.2	0.1
1–2 cm (30 d)	nC17	1.0	0.7	1.2	1.5	2.3	3.5	0.6	0.3	0.1
Highly contaminated cakes (0 d)	nC15	1.0	1.3	1.3	1.7	2.3	3.3	0.5	0.5	0.1
0–1 cm (30 d)	nC26	1.2	0.2	1.1	1.4	2.4	3.5	0.7	0.7	– 0.1
1–2 cm (30 d)	nC26	1.2	0.4	1.2	2.0	2.4	4.2	0.8	0.8	– 0.1

(–) not calculated, values below of the analytical detection level.

Abbreviations: n-alk = n-alkanes; nCx = n-alkane with x carbon number; Pr = pristane; Phy = phytane; TRAlI = total resolved aliphatic; UCM = unresolved complex mixture; ΣEven/ΣOdd = Even to odd ratio; ΣLMW/ΣHMW = Low to high molecular weight; CPI = Carbon Preference Index; MH = Major Hydrocarbon; NAR = Natural n-Alkane Ratio. ECO = Escalante Crude Oil.

^a See text for indices definition (Section 2.4.3).

^b References indicate which were taken for comparison with this study.

as representative of unpolluted sediments ($< 10 \mu\text{g g}^{-1} \text{ dw}$) (Fig. 3a). Concentrations found in CV sediments were similar to those reported by different authors in pristine areas worldwide (i.e. Tolosa et al., 2004; Volkman et al., 1992).

3.4. Aliphatic hydrocarbons of ECO

A chromatographic profile of an ECO aliphatic fraction shows the presence of a series of homologous n-alkanes extending from nC8 to nC35 and of an Unresolved Complex Mixture (UCM). More precisely, n-alkanes, total resolved aliphatic hydrocarbons (identified and unidentified compounds), and the UCM, represented $19.6 \pm 0.3\%$, $40.7 \pm 2.6\%$ and $59.3 \pm 2.6\%$ of the aliphatic fraction, respectively (Marino et al., 2012). Aliphatic diagnostic indices (ADIs) were applied to obtain reference values (see Section 2.4.3).

3.5. Hydrocarbons in contaminated cores (E1 and E2 treatments)

In cores contaminated with ECO (E1 and E2 treatments), relatively high concentrations of TAlIH were recovered in the first two cm of sediment after 30-d of incubation. In E1, TAlIH concentrations reached 142.86 and $56.98 \mu\text{g g}^{-1} \text{ dw}$ for 0–1 and 1–2 cm layers, respectively (Fig. 3c), while in E2 these were 2034.70 and $124.61 \mu\text{g g}^{-1} \text{ dw}$ (Fig. 3d). Although the 2–3 cm layer could not be analyzed for hydrocarbons, several biotic and abiotic parameters indicated the lack of burial of crude oil beyond the depth of three cm. For instance, OM values showed no significant differences between treatments (E0, E1 and E2) at the 2–3 cm layer (two-way ANOVA, Tukey test $p > 0.61$) with a mean of $1.4 \pm 0.1\%$ ($n = 3$) indicating the absence of oil migration beyond the 3 cm layer. In fact, below three cm depth, TAlIH values in analyzed layers were in the same order of magnitude than those found in the control cores (E0; TAlIH $< 10 \mu\text{g g}^{-1} \text{ dw}$) corresponding to unpolluted sediment (UNEP/IOC/IAEA, 1992). In addition, maximum macrobenthic community parameter values, such as density and specific richness, were found in the first two cm, and 96% of luminophores were located in the first 5 cm (Ferrando et al., 2015). Also,

the silt/clay sediment fraction which was maximum in the first three cm of the sedimentary column contributed with the retention of hydrocarbons by adsorption processes. Typical indices such as ΣEven/Σodd n-alkanes, Pri/Phy, and CPI in the 0–1 and 1–2 cm layers presented values coherent with a petrogenic origin, and they were similar to those determined in the Escalante Crude Oil (Table 2). Other signatures of clear petrogenic character were the presence of the complete homologous series of n-alkanes and of an AliUCM.

The ratios nC17/Pri and nC18/Phy are generally used as indicators of the degradation state of petroleum hydrocarbons at early stage. The isoprenoid alkanes, such as pristane and phytane, are generally more resistant to biodegradation than linear n-alkanes (nC17 and nC18) due to their branched structure (Commendatore et al., 2000). In the first two sediment layers of cores E1 and E2, these ratios remained almost the same as in the initial contaminated cakes, indicating that under current experimental conditions n-alkanes were not degraded preferentially relative to isoprenoid alkanes (Table 2). N-alkane/isoprenoid indices are very sensitive, and usually show a quick response in biodegradation systems. In laboratory experiments, for example, nC17/Pri showed a variation of up to 27% of its initial value (1,86) in only 6-day incubation of fertilized marine sediments that were contaminated with oil (Singh et al., 2014). Although in our system we used a relative short incubation period and measured only natural attenuation (without fertilization), and taking into account that experimental systems cannot be directly compared, CV sediments still showed very limited biodegradation activity. Gilbert et al. (1994) did not observe any biodegradation of Arabian light crude oil after 15-d incubation in sediments from the Mediterranean Sea, but the ratios nC17/Pri and nC18/Phy calculated for the buried hydrocarbons had decreased after 45-d, indicating a microbial degradation of n-alkanes in polychaete burrows where microbial activities were enhanced. In CV sediments, ostracods predominated over polychaetes (Ferrando et al., 2015) and the smaller size of *Axiiothella* compared to *Hediste diversicolor* used by Gilbert et al. (1994) could partly explain the absence of biodegradation, as *Axiiothella* activity does not include the construction of burrows. The absence of biodegradation in the present study was further supported

by the ratio $\Sigma n\text{-alk/UCM}$, which showed values in the same order of magnitude than polluted cores (between 0.21 and 0.83) and ECO (0.33) (Table 2). In previous studies carried out along the coast of Patagonia, sediments where hydrocarbon biodegradation occurs showed values as low as 0.02 (Commendatore et al., 2000). This clearly indicates a lack of marked biodegradation in the present CV oiled sediments. Below the three cm depth, odd-carbon numbered hydrocarbons clearly prevailed for both high molecular weight compounds (nC_{31} , nC_{33} and nC_{35}) of terrestrial origin and low molecular weight compounds (nC_{19} and nC_{21}) of marine origin similar to those found for original CV sediments and control cores (E0). This indicates the lack of burial of petroleum beyond a depth of three cm. Interestingly, the proportion of hydrocarbons that was recovered in the 1–2 cm depth with respect to the initial amount added was systematically higher ($\sim 29\%$) in the less contaminated cores (E1 treatment) than in the highly contaminated cores ($\sim 6\%$; E2 treatment), while recovery in the 0–1 cm layers was 94.2% and 70.3% for E2 and E1, respectively.

The difference in the amount of buried hydrocarbons was associated to a greater toxicity of hydrocarbons to macrobenthic communities in the E2 treatment, in accordance with the level of contamination. Indeed, Ferrando et al. (2015) reported that the presence of oil affected the structure and the activity of the macrobenthic community inhabiting CV sediments, even for the lowest level of contamination tested (corresponding to chronically contaminated sites). In the cores with the highest level of contamination (E2), corresponding to that recorded in intertidal areas following an oil spill, the amount of oil added almost completely inhibited the reworking activity due to a high mortality of macro-organisms (Ferrando et al., 2015). The lowering or absence of reworking activity in the contaminated cores compared to control cores was further supported by the percentages of luminophores recovered in the top sediment layer by the end of the incubation, which decreased when the degree of contamination decreased (98%, 54% and 43% of the amount deposited for E2, E1 and E0, respectively; Ferrando et al., 2015). Quantification of sediment reworking and of hydrocarbon burial thus clearly showed a negative impact of oil contamination on the transport and mixing of particles by organisms.

The macrofauna activity in sediments (bioturbation) plays notably a key role in oxygen and organic matter transportation through the sedimentary column and therefore, in the fate of hydrocarbons (Cuny et al., 2011). According to Ferrando et al. (2015), the main activity of the macrofauna was associated with the first two centimetres of the sedimentary column, where the highest values of organism richness and density were recorded.

3.6. Abundance of hydrocarbon-degrading bacteria

The abundance of hydrocarbon-degrading bacteria at final incubation time in the first 3-cm layer of CV sediments were $1.3 \times 10^3 \pm 2.6 \times 10^2$, $3.6 \times 10^3 \pm 4.9 \times 10^3$ and $1.3 \times 10^4 \pm 7.4 \times 10^3$ MPN g^{-1} dw for E0, E1 and E2 treatments, respectively. Significant differences (one-way ANOVA, $p < 0.05$) were found, being higher the hydrocarbon-degrading bacteria concentration in the highly contaminated (E2) cores than in the control (E0) ($p < 0.05$), while there were no significant differences between treatment E1 and, the control (E0) or treatment E2 (Fig. 4). The abundances found in our work were similar to those previously reported in different pristine sediments, as the absence of crude oil contamination history limits the development of hydrocarbon-degrading microorganisms (Horel et al., 2012; Mortazavi et al., 2013; Rosenberg, 2006). In contrast, in sediments chronically polluted with petroleum hydrocarbons, hydrocarbon-degrading bacteria concentration can be four orders of magnitude higher, as reported for marshes in Louisiana and Eagle Harbor, USA (Geiselbrecht et al., 1996). A direct contamination generally results in a several order of magnitude increase in biomass of hydrocarbon-degrading microorganisms (Horel et al., 2012). The relatively low increase in MPN values in the present study may be due to the limited duration of the experiment (30 days) and/or to the low potential of adaptation of the

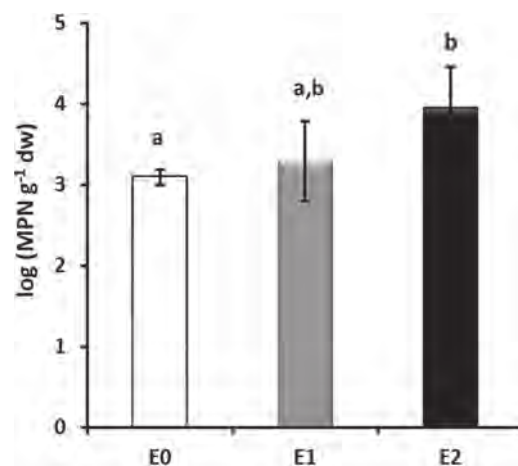


Fig. 4. Hydrocarbon-degrading bacteria MPN enumeration (mean values \pm standard deviation of four replicates) in the first 3 cm of the sediment. Different lowercase letters indicate significant differences ($p < 0.05$) between treatments as determined by one-way ANOVA and *post hoc* Tukey's test.

microbial community from CV sediments to an abrupt oil contamination. However, despite of the relatively short experimental time, the permanence of unaltered crude oil for a month in studied sediments is very likely a problem for this coastal ecosystem.

3.7. Hydrocarbons mass balance in bioassays

The mass of aliphatic hydrocarbons in the whole sediment cakes (E0, E1, and E2) at initial time and in analyzed layers of the sedimentary column at final time, were calculated. Biogenic hydrocarbons constituted the initial mass in E0 (10.18 μg), which was negligible in comparison to initial masses in E1 (55,629 μg) and E2 (629,350 μg) where petrogenic hydrocarbons were dominant. The total mass of aliphatic hydrocarbon at the end of the experiment for the 0–1, 1–2, 2–3, and 3–4 cm depth layers were 5.7, 3.0, 4.9, and 4.3 μg for E0, 12,545, 6,684, 3,296, and 1.1 μg for E1, and 183,995, 13,364, 6,695, and 1.5 μg for E2. The mass of aliphatic hydrocarbons at the end of the experiment were calculated for the combined top 4 cm of sediment. Below 3 cm depth, sediments recovered a biogenic imprint in both E1 and E2 treatments. The percentage of TAlIH lost resulted in 60% and 68% in E1 and E2, respectively. This indicates mainly an exportation of hydrocarbons from the top sediment to the surrounding seawater by dissolution and/or dispersion during the incubation. In experiments carried out with crude oil-contaminated sediments and the polychaete *Hediste (Nereis) diversicolor*, Gilbert et al. (1994) found that 76.5% of the initially added hydrocarbons were exported to the water after 45 d of incubation, whereas 10% remained in the top layer (0–2 cm), and 13.5% were buried to 10 cm depth (mostly in the first 4 cm). Strictly, the hydrocarbon loss from sediments to surrounding seawater would be associated to dissolution, evaporation, degradation (bio or photo-chemical), diffusion and/or dispersion process (Kus et al., 2017). Moreover, macrobenthic organisms are able to solubilize hydrocarbons by effect of digestive biosurfactant production, which besides enhancing hydrocarbons biodisponibility, increases their transport to the water column (Ahrens et al., 2001). Also, the microbenthic community can produce biosurfactants, altering hydrocarbon dispersion and favouring the solubilisation process (Hassanshahian, 2014).

Other processes such as evaporation and dissolution which are mainly restrained to low molecular weight compounds could not by themselves justify the high hydrocarbon loss recorded in oiled treatments. In addition, biodegradation processes were not active, as confirmed by the values of biodegradation diagnostic indices as well as by the low number of hydrocarbon-degrading bacteria, as similarly found by Mortazavi et al. (2013).

3.8. Bioremediation in CV soft sediments

Oil in marsh environments is not easily removed and its fate is mostly governed by macrofauna activity which buries petroleum and/or favours its exportation to the water column. Soft sediments show a greater richness, abundance, and diversity of macrobenthic organisms which can show more or less resilience to disturbances due to oil pollution. Macrobenthic communities in CV sediments have low resistance capacities to oil contamination (Ferrando et al., 2015) and, consequently, petroleum hydrocarbons could cause severe damage to the ecosystem. Our results highlight the low capability of CV pristine sediments to recover their original characteristics following oil pollution, at least in the short term after contamination. According to NOAA (2001), manual oil removal in the case of light to moderate oiling conditions can be considered without neglecting some potential adverse impacts.

Sediments from CV have not been exposed to oil spills or to chronic inputs of petrogenic hydrocarbons, which may explain a low adaptation of the autochthonous community of microorganism to use these compounds as carbon source. Because of this low bioremediation potential, clean-up of oil-contaminated CV soft sediments should involve an early response with manual surficial sediment removal, after which the natural environmental recovery should be monitored.

4. Conclusions

Petroleum hydrocarbons had an important impact on marsh pristine sediments from the northern coastal zone of Caleta Valdés under *ex situ* experimental conditions, highlighting the harmful consequences if an oil spill reaches this highly vulnerable environment. Microorganisms did not respond quickly to an input of oil, contrary to what is usually observed for chronically polluted sites. In addition, oil contamination strongly reduced sediment reworking by macrobenthic organisms, thus decreasing sediment oxygenation and aerobic biodegradation. In this context, it is likely that an oil spill will have significant deleterious effects on the benthic ecosystem of CV, with long term effects due to weak self-cleaning capacity of the system. The vulnerability of soft sediment environments and the necessity of having prevention measures and adequate contingency plans to control spilled hydrocarbons in coastal pristine areas worldwide undoubtedly deserve further attention.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ecoenv.2018.06.069](https://doi.org/10.1016/j.ecoenv.2018.06.069).

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