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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 ~ 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 geophysic, 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

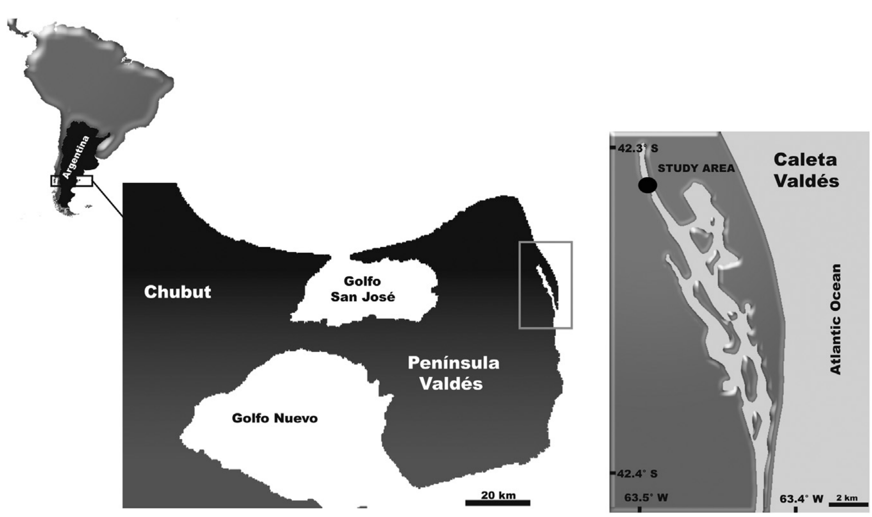

Fig. 1. Study site in Caleta Valdés, Península Valdés (Patagonia, Argentina).