International Biodeterioration & Biodegradation 84 (2013) 86–96

Contents lists available at SciVerse ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Influence of natural rhizosediments characteristics on hydrocarbons degradation potential of microorganisms associated to *Juncus maritimus* roots

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ARTICLE INFO

Article history:
Received 1 February 2012
Received in revised form
9 May 2012
Accepted 10 May 2012
Available online 10 November 2012

Keywords:
Rhizoremediation
Hydrocarbons
Juncus maritimus
Rhizosediments characteristics
Lima river estuary

ABSTRACT

The influence of natural sediments colonized by *Juncus maritimus* (rhizosediments) on the microbial rhizosphere communities and their potential for hydrocarbons degradation was investigated. Rhizosediments from four salt-marsh sites of a temperate estuary (Lima River, NW Portugal) were sampled throughout the plant phenological cycle, for sediment characterization, total petroleum hydrocarbons, abundance and structure of microbial community, and hydrocarbon degrading microorganisms. Additionally, evaluation of hydrocarbon degradation potential was assessed from fortnight laboratory experiments, with and without crude oil amendment. Silt + Clay and organic matter (OM) content influenced the retention of hydrocarbons around plant roots by increasing their levels. Those parameters tended to correlate positively with total microbial abundance but negatively with hydrocarbon degrading microorganisms, a fact that could be related to lower hydrocarbon bioavailability. Rhizosediment laboratory experiments without crude oil amendment showed a significant ($p < 0.05$) negative correlation between hydrocarbon degradation rates and OM and Silt + Clay content, confirming the strong influence of rhizosediment characteristics on hydrocarbons bioavailability. This is the first insight into the influence of natural sediment characteristic on the hydrocarbon degradation potential of salt marsh microorganisms, a feature that should be considered when designing rhizoremediation strategies in estuaries.

1. Introduction

In the last decades, levels of petrochemical products in the environment, particularly in estuaries and coastal areas have increased (Lima et al., 2007). Marine oil spills, particularly large-scale accidents, as the recent one in the Gulf of Mexico, have drawn great attention due to their catastrophic impact on the coastal environment. However, minor oil spills on coastal zones, as a result of municipal and industrial wastewater discharges, urban runoff, and oil leakage from boats and ships, are no less threatening to the environment and to public health, although they receive much less attention. Oil contamination affects all seashores, estuaries and rivers, causing the loss of biodiversity, destruction of breeding habitats of aquatic organisms and hazard to organisms, including man (Zhu et al., 2004).

Coastal intertidal zones, like salt marshes, are sensitive ecosystems that serve many ecological functions (Boorman, 1999; Lefeuvre et al., 2003), being often affected by oil spills and extremely vulnerable to hydrocarbons (Andrade et al., 2004). It is important to clean and recover these areas, which can be a difficult task (Zhu et al., 2004). Bioremediation can be considered a less damaging and cost effective cleanup approach, when compared to alternatives such as soil washing, incineration or disposal to landfill, often more damaging than the oil itself (Lin and Mendelssohn, 1998). Bioremediation of hydrocarbons often includes the biostimulation, where the growth of indigenous oil degraders is stimulated by the addition of nutrients, giving less toxic, less mobile and/or less bioavailable products (Vidali, 2001). The use of plants

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http://dx.doi.org/10.1016/j.ibiod.2012.05.039

and their associated microorganisms is another approach for the treatment of contaminated areas, referred to as rhizodegradation or rhizoremediation (Olson et al., 2003; Gaskin and Benthan, 2010). In fact, the presence of vegetation can accelerate the bioremediation of soils/sediments contaminated with petroleum hydrocarbons (Lin and Mendelssohn, 1998; Davis et al., 2002; Xu et al., 2006).

Petroleum hydrocarbons are among the most common contaminants bound to estuarine sediments (Chapman and Wang, 2001). However, sediment heterogeneity, the presence or absence of vegetation, variability of grain size, organic matter content along with the salt water intrusion can influence hydrocarbons sequestration (Kukkonen and Landrum, 1996; Brunk et al., 1997; Wang et al., 2001), and bioavailability (Amellal et al., 2001; Talley et al., 2002), as well as microbial communities responsible for their degradation (Sessitsch et al., 2001; Marschner et al., 2004). Therefore, hydrocarbon rhizoremediation processes should have into account sediment characteristics. Nevertheless, studies on this subject are scarce, particularly in estuarine areas, where a great variability of these characteristics can be found.

In this vein, the present study aimed to investigate the influence of natural characteristics of vegetated sediments on the associated microbial communities and on their hydrocarbon degradation potential. For that, microbial communities, hydrocarbon degrading (HD) microorganisms included, and petroleum hydrocarbon distribution and degradation rates were accessed on sediments colonized by *Juncus maritimus* (sea rush), a salt marsh plant. Sediments were collected at four different locations through the plant phenological cycle. To our knowledge, this is the first report on the influence of sediment characteristics on hydrocarbon degradation potential in salt marshes.

2. Materials and methods

2.1. The study area

Atlantic coast of the Iberian Peninsula is one of the main routes of oil cargo; therefore, there is a potential hazard due to oil spill accidents (Solana-Ortega and Solana, 2007). In the last 40 years, six major oil spills occurred in NW Iberian Peninsula, as a result of tanker accidents such as *Polycommander* (1970); *Jakob Maersk* (1975); *Urquiola* (1976); *Andros Patria* (1978); *Aegean Sea* (1992); and *Prestige* (2002). The NW Atlantic coast of Portugal is also exposed to petrochemical contamination due to the presence of oil refining industry and two major seaports (Leixões and Viana do Castelo).

The Lima River Estuary (41.41°N; 08.48°W (WGS84)) is the end member of an international watershed located in NW of Portugal (Fig. 1). In 2000, the bulk carrier 'Coral Bulker' ran aground at the river mouth, spilling 630 tons of heavy fuel oil and 70 tons of diesel oil, and severely affected the area (Moreira et al., 2004). Also, there is an important harbor, leading to continuous petrochemical contamination through the activity of commercial and fishing vessels (Lima et al., 2007). The Lima Estuary is also a receiver of diffuse pollution originated from agriculture and from domestic (46,000 inhabitants) and industrial waste discharges, including a paper mill (Guimarães et al., 2009; Costa-Dias et al., 2010).

The estuary has a semidiurnal and mesotidal regime, being vertically stratified only during the freshet period of the year (winter). During spring tides, salt intrusion can extend up to 20 km upstream (Ramos et al., 2006). A large salt marsh area spreads over the middle estuary, colonized by different plants, where *J. maritimus* (sea rush) is commonly found (Rede Natura, 2000; Costa et al., 2009a). The sampling sites (L1, L2, L3, L4; Fig. 1) within the salt marsh area had different sediment characteristics and were colonized by individual assemblages of *J. maritimus*. These perennial plants, belonging to Juncaceae family, are an example of salt marsh native and ubiquitous specie of Portuguese salt marshes (Costa et al., 2009b). *J. maritimus* presented adventitious roots borne on the horizontal rhizome. The water/substrate temperature never goes below the freezing point in this estuary. Therefore, *J. maritimus* plants do not experience a true dormancy period, and do not present a live/death cycle with belowground (roots and rhizomes) and aboveground structures being quite stable during the phenological cycle (Almeida et al., 2006). In fact, individual plants died but simultaneously, in the same site, a new batch of plants grow up. Flowering occurs mainly in June–September (Sampaio, 1988).

2.2. Sediment sampling and characterization

Sediment samples were collected, at low tide, in the beginning of March, June, September and December of 2009 as well as in summer (July–August) 2010. In each sampling site, approximately 1 kg of sediments colonized by *J. maritimus* (sediment in contact with the plant roots forward named rhizosediment) were collected into sterile plastic bags. In March, L4 rhizosediments were not sampled due to methodological/logistical problems. All rhizosediments were collected between 5 and 15 cm, the depth with the higher plant belowground biomass. Samples were

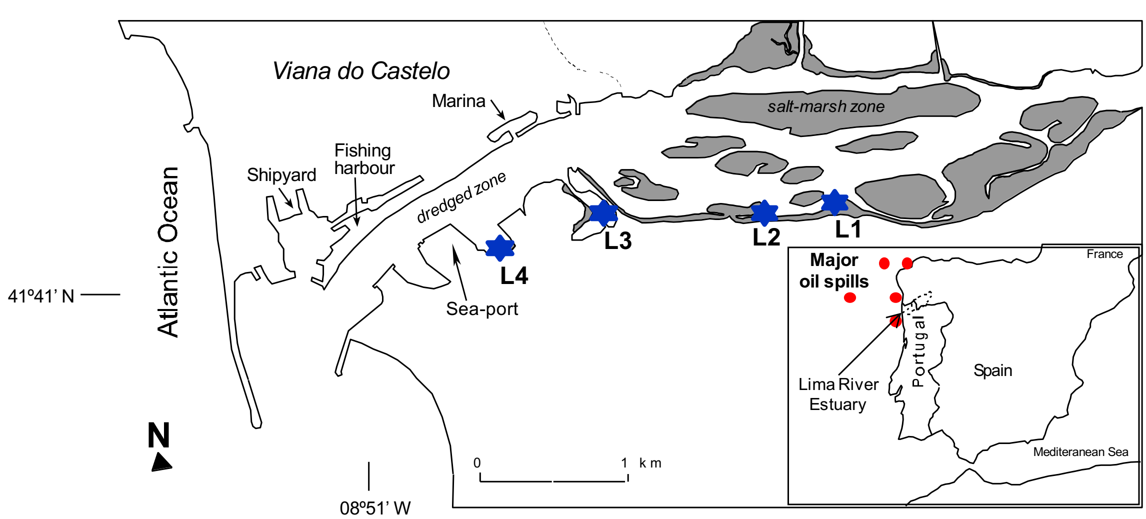

Fig. 1. Study area. Lima River Estuary (NW of Portugal) with the four sampling sites (blue star): L1, L2, L3 and L4; and the major oil spills (red dot); shaded area represents salt marshes.