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Analytical investigations on the lindane bioremediation capability of the demosponge *Hymeniacidon perlevis*

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ABSTRACT

Lindane is an organochlorine pesticide that has been widely used to treat agricultural pests. It is of particular concern because of its toxicity, persistence and tendency to bioaccumulate in terrestrial and aquatic ecosystems. In this context, we investigated the ability of the demosponge *Hymeniacidon perlevis* to bioremediate lindane polluted seawater during *in vitro* experimentation. Lindane was extracted by solid-phase micro-extraction (SPME) and determined by gas chromatography–mass spectrometry (GC–MS). Furthermore, we assessed the role exerted in lindane degradation by bacteria isolated from the sponge. Sponges showed low mortality in experimental conditions (lindane concentration 1 μg/L) and were able to remove about 50% of the lindane content from seawater in 48 h. Bacteria isolated from sponges showed a remarkable remediating capacity (up to 97% of lindane removed after 8-days). A lindane metabolite was identified, 1,3,4,5,6-pentachloro-cyclohexene. The results obtained are a prelude to the development of future strategies for the *in situ* bioremediation of this pollutant.

1. Introduction

Over the last decades, the effects of industrialization, intensive agriculture and urban development have led to the occurrence of serious pollution problems in the marine ecosystems (Bellas et al., 2005). Several pollutants, in the form of different chemicals, have been released either directly or indirectly without adequate treatment to remove their harmful effects. In this context, a problem of major concern is represented by the anthropogenic input into the environment of persistent organic pollutants (POPs). Such chemicals are usually considered resistant to photolytic, biological and chemical degradation and accumulate to hazardous levels in living organisms through the food web, causing adverse effects to human health and the environment (Szabo and Loccisano, 2012; Sheng et al., 2013). Regulatory efforts on POPs reduction or elimination worldwide are subject to multilateral environmental agreements such as the Stockholm convention (last update, UNEP, 2009). The latter was created since POPs can be subject to long-range atmospheric transport, and hence, no government alone can protect its citizens and environment from exposure to these pollutants.

Among POPs, there are many pesticides widely used all over the world to control the harmful effects of pests on agriculture production. They are mainly introduced into rivers via run-off and enter marine areas, becoming more common in coastal zones. Lindane (γ-hexachlorocyclohexane or γ-HCH), for example, with an insecticidal activity and toxic effects, is a common pollutant worldwide which was mainly produced from after the Second World War until the 1990s (Breivik et al., 1999). The low aqueous solubility and chlorinated nature of lindane contribute to its environmental persistence and resistance to degradation. Thus, the application of this pesticide has resulted in marine contamination of global dimensions (Li and MacDonald, 2005; Vijgen et al., 2011). γ-HCH was banned from production and use by the United Nations in 2009 (UNEP, 2009), yet this chemical is still used or stocked without control in developing countries (Rambo, 2013). As a consequence, it continues to be introduced into aquatic ecosystems via rain and groundwater.

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In this scenario, by 2000 the Water Framework Directive (European Commission, 2000) had already shifted its emphasis away from simply monitoring chemicals towards an approach that incorporates both chemical and ecological objectives and was designed to protect the structure and functions of aquatic ecosystems (Hagger et al., 2006). In recent years, an increasing number of studies are combining approaches of monitoring chemical contaminant levels with measurements of biological responses related to pollutants effects, allowing the assessment of environmental status across European marine regions (Hagger et al., 2008; Thain et al., 2008; Lyons et al., 2010). The Marine Strategy Framework Directive (European Commission, 2008) recommends the achievement of Good Environmental Status based upon monitoring programmes covering the concentrations of chemical contaminants and also biological measurements related to the effects of pollutants on marine organisms in each of the assessment regions.

The importance of marine invertebrates in the functioning of marine ecosystems has led to their use as test species in biological assays. Moreover, several invertebrate species in aquatic ecosystems are resistant to toxicity and have the ability to hyperaccumulate, stabilize or degrade pollutants, deserving the definition of zooremediators (Gifford et al., 2006). Among them, marine sponges have shown a remarkable ability to remediate aquatic microbial pollution (Stabili et al., 2006; Longo et al., 2010) and accumulate metals (Cebrian et al., 2003; Perez et al., 2005). Moreover, the demosponge *Spongia officinalis* is known to concentrate many organic contaminants, including polychlorinated biphenyls, to higher concentrations than bivalve mollusks (Perez et al., 2003), and to degrade the surfactant 1-(p-sulfophenyl) nonane to its main degradation products ten times more rapidly than marine bacteria (Perez et al., 2002). Thus, some sponges are presumably able to break down organochlorine pesticides as well, given their ability to produce and safely store many halogenated biomolecules within the cell (Gifford et al., 2006).

Sponges are filter-feeders that retain nutrients from the water circulating within their aquiferous system. These invertebrates usually harbor a rich symbiotic community of bacteria, which in some cases represents up to 60% of sponge biomass (Hill et al., 2006) and can significantly contribute to its host metabolism (Hentschel et al., 2006). This population consists mostly of extracellular bacteria that are enclosed within the mesohyl matrix. Microbiological and molecular biological studies support the view that sponge-microbe associations are obligatory and species-specific. Moreover, sponge associated bacteria are passed on from generation to generation and over geological time (Thiel et al., 2002). Among the symbiotic functions attributed to sponge bacteria are nutrient acquisition, stabilization of the skeleton, secondary metabolite production and processing of metabolic waste (Hentschel et al., 2006).

Hymeniacidon perlevis (Montagu) is a Mediterranean demosponge rather common along Italian coasts in shallow waters. A previous study by this research group (Longo et al., 2010) reported that this species can filter several groups of bacteria, contributing substantially to their removal from the water column through accumulation and digestion. On account of these results we suggested *H. perlevis* as a potential candidate for bioremediation of microbial polluted seawater (Longo et al., 2010). In 2007 Fu et al. demonstrated that the clearance rates for *H. perlevis* were higher than those reported for other species of sponges (e.g., *Chondrilla nucula* and *S. officinalis*) (Milanese et al., 2003; Stabili et al., 2006). Moreover, the same authors (Fu et al., 2007) proved the ability of this species to remove total organic carbon in integrated aquaculture systems. In this framework, in the present work we estimated, by using solid-phase microextraction (SPME) followed by gas chromatography–mass spectrometry (GC–MS), the ability of *H. perlevis* to remove the organochlorine pesticide lindane from seawater. In addition, the capability of different bacteria isolated from this sponge to utilize the pesticide was investigated with the goal of inferring some environmental implications as well as developing a possible strategy for *in situ* bioremediation of this pollutant.

2. Materials and methods

2.1. Sponge collection

Sponges were collected from shallow waters in the Mar Piccolo of Taranto (Ionian Sea). In particular, three specimens (1, 2, and 3) of the demosponge *H. perlevis* were detached from the substrates and each specimen was cut into different fragments. Then, each group of fragments was rinsed in natural seawater (NS) and promptly transferred into a single laboratory tank filled with seawater of the same origin, equipped with an aquarium pump and an aerator for water oxygenation. Seawater temperature and salinity were 23°C and 37 psu, respectively. The specimens were left in the tank for at least seven days in order to obtain a complete cicatrisation of their cut surface and the reorganization of the sponge aquiferous system (Cardone et al., 2010).

2.2. Chemicals and standards preparation

All chemicals and solvents were purchased from Sigma–Aldrich (Italy). Lindane (1α,2α,3β,4α,5α,6β-hexachlorocyclohexane) was of PESTANAL® Grade. Sodium chloride (NaCl) had a purity of more than 99.5%. Methanol and water were of CHROMASOLV® Grade.

A methanol stock solution of pesticide was prepared and stored in the dark at 4°C. Working solutions were prepared immediately before use by serial dilution with artificial seawater (AS) (37 psu, obtained by dissolving NaCl in water) or with NS.

2.3. Equipment

The solid phase micro-extraction (SPME) device and 100 μm thick polydimethylsiloxane (PDMS) coated fibers were supplied by Supelco (Bellefonte, PA, USA).

The gas chromatography–mass spectrometry system (GC–MS) consisted of a Finnigan TRACE GC ultra gas chromatograph equipped with a split/splitless injector coupled to an ion trap mass spectrometer (MS) (Finnigan PolarisQ). A Supelco SPB-5 fused silica capillary column (30 m × 0.25 μm i.d., 0.25 μm film thickness) was used, with helium as carrier gas (flow rate 1 mL/min).

2.4. SPME–GC–MS analysis

Before use, each fiber was conditioned in the GC injector at 300°C for 3 h, as suggested by the supplier. Samples (15 mL) were placed in 15 mL amber vials and the vials sealed with hole caps and Teflon-faced silicone septa (Supelco). Extraction was carried out at 50°C for 30 min by direct immersion of the fiber in the solution under magnetic stirring. Thermal desorption (5 min desorption time) was performed into the GC injection port at 275°C. To eliminate carry over, the fiber was subjected to a second thermal desorption after each chromatographic run.

The oven temperature program was: 50 (5 min)–180°C at 12°C/min, 180–230°C at 5°C/min, 230–245°C at 2°C/min. The GC transfer line was maintained at 250°C.

The mass spectrometer was operated in the electron impact positive ion mode (EI+) with the ion source temperature at 250°C. The electron energy was 70 eV and the filament current 150 μA. Mass spectra were acquired in the *m*/*z* range 50–300.

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