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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.

Detection of lindane was also accomplished in selected ion monitoring (SIM) mode, using the m/z ions 181 and 183.

2.5. Lindane stability in seawater

Lindane short-term stability assessment was performed in triplicate for 7 days. Briefly, lindane solutions (1 μg/L) in AS and NS were placed in 15 mL amber vials, the vials totally filled, sealed and stored at room temperature (23°C) until SPME–GC–MS analysis that was performed at different times (0, 1, 2, 3, 5 and 7 days). In both cases, the solutions proved to be stable. Furthermore, in order to verify lindane stability in the experimental conditions, 1 L of lindane solution in NS (1 μg/L) was placed in a glass beaker (C), which was capped and equipped with an aerator. Then, 15 mL of water were sampled at different times (0, 1, 2, 3, 5 and 7 days) and immediately subjected to SPME–GC–MS analysis. In this case, a slight decrease in the lindane concentration was observed.

The compound stability at each sampling time was expressed as follows:

$$\%\text{lindane decrease} = [1 - ([L]_{t_n}/[L]_{t0})] \times 100 \quad (1)$$

where $[L]$ is the lindane concentration, t_n is the time of sampling and t_0 is time zero.

2.6. H. perlevis experiments

2.6.1. Lindane exposure

Three sponge fragments (15.0 ± 0.6 mL volume) taken from specimen 1 were moved from the laboratory tank and placed in three different glass beakers (Hp 1–3); each beaker was then filled with 1 L of a lindane solution (1 μg/L) in NS, capped and equipped with an aerator. A fourth beaker was filled only with the lindane solution and was used as a control (C 1). The same whole experiment was repeated two more times using sponge fragments taken from specimens 2 (Hp 4–6, C 2) and 3 (Hp 7–9, C 3), respectively. In order to estimate sponge survival, the fragments were observed daily with a magnifying lens to evaluate their survival and the occurrence of filtering activity. In particular, the status of the superficial channels of the aquiferous system of each sponge was carefully observed in order to detect the occurrence of water flux.

2.6.2. Lindane removal

The ability of *H. perlevis* to remove lindane from NS was estimated by withdrawing 15 mL of water from the beakers (Hp 1–9 and C 1–3) at various time intervals (0, 30 min, 1, 2, 3, 5 and 7 days) and immediately performing SPME–GC–MS analysis (each analysis was performed in triplicate). At each sampling time, the lindane removal (%) in the Hp beakers was calculated as follows:

$$\%\text{lindane removal} = [1 - ([L]_{\text{Hpn}}/[L]_{\text{C}})] \times 100 \quad (2)$$

where $[L]$ is the lindane concentration, Hpn is the sponge containing beaker and C is the control beaker.

2.7. Microbiological analyses

Microbiological analyses were performed on the homogenates of treated sponges. After 7 days, lindane-exposed specimens of *H. perlevis* were washed with AS (filtered on 0.22 μm pore size), gently squeezed with a glass stick and then the sponge pieces were homogenized in a sterile Waring blender. Afterwards, the homogenates were subjected to a series of dilutions (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}). For the isolation of heterotrophic bacteria, 100 mL of undiluted homogenate and serial dilutions of each sponge extract were plated in triplicates on Bacto Marine Agar 2216 (Difco). The plates were incubated at 22°C for 7 days. At the end of the incubation period all the colony types were isolated and sub-cultured on Bacto Marine Agar. Subsequently, before starting the screening experiment, each bacterial strain was incubated with Marine Broth for 24 h at 22°C.

2.7.1. Screening of lindane-degrading bacterial isolates

In order to identify potential lindane-degrading microorganisms arising from the sponge homogenates, we tested the ability of the isolated bacterial strains to grow in the presence of lindane. Briefly, a set of three tubes containing 5 mL of a lindane solution (0.05 mg/L) in AS (filtered on 0.22 μm pore size) were prepared for each bacterial strain. A 0.1 mL aliquot of the bacterial suspension previously incubated in Marine Broth was added to each tube of the set. A further set of three tubes containing only the lindane solution in AS at the same concentration level represented the control. The tubes were sealed and placed in a test tube roller. After 48 h at 22°C bacterial growth was qualitatively determined by recording the optical density of each test tube at 560 nm. The tubes were centrifuged at 5000× rpm and the resulting supernatants were diluted 1:10 with AS before being subjected to SPME–GC–MS analysis to evaluate lindane residual concentration.

The tubes showing the best bacterial growth and, consequently, the best lindane removal performances were incubated for a further 8 days and submitted again to SPME–GC–MS analysis.

2.8. Statistical analysis

Analysis of variance (ANOVA) was used to test the differences between different experimental groups; variations at 95% level of confidence ($p<0.05$) were considered significant.

3. Results

3.1. SPME–GC–MS analysis

Preliminary experiments (data not shown) were performed on lindane solutions (1 μg/L) prepared in artificial seawater (AS) in order to select the best solid phase micro-extraction conditions and to optimize the instrumental parameters, reported in the experimental section. Then, the final conditions were used throughout the work.

At first, analysis of both natural seawater (NS) samples and seawater taken from the laboratory tank containing the sponges were performed, in order to verify the absence of matrix interferences at the retention time of the target analyte and of the analyte itself, which could already be present as an environmental contaminant. All samples, which were analyzed in both total ion current (TIC) and selected ion monitoring (SIM) modes, were found to be lindane free and no significant matrix interferences were observed.

The analytical method for the determination of lindane in NS was developed working in SIM mode. A calibration curve in NS was constructed and it proved to be linear in the concentration range 0.05–5.00 μg/L, with correlation coefficients better than 0.999 and intercept not significantly different from zero at 95% confidence level. Three replicates were performed for each concentration. LOD and LOQ, calculated at a signal-to-noise (S/N) ratio of 3 and 10 (noise calculated peak to peak on a seawater blank chromatogram at the lindane retention time), were 0.02 and 0.07 μg/L, respectively.

The within-day ($n = 6$) and between-days ($n = 3$ over 7 days) coefficients of variation, estimated by an ANOVA test, were 6% and 12%, respectively, and remained practically unchanged, passing from 0.1 to 1.0 μg/L. Identical results were obtained by analyzing lindane in AS.

3.2. Lindane stability in seawater

After the optimization of the method, the stability of lindane was determined in AS and NS over a 7 day period as described in the experimental section. Lindane was found to be stable both in AS and NS sealed vials. However, as shown in Fig. 1, a slight decrease in lindane concentration was observed in NS in the experimental conditions, i.e. when it was placed in the aerated glass beaker.

3.3. H. perlevis experiments

3.3.1. Lindane exposure

Simultaneously, exposure experiments aimed at observing *H. perlevis* survival in the presence of lindane were performed (see experimental). After one day of exposure, one of the sponge fragments (Hp 6) showed opaque color, patches of necrosis and lack of water flux through the superficial channels of the aquiferous system; after 2 days the areas of necrosis were wider, and after 3 days the whole specimen was affected by necrosis, with evident areas showing putrefactive processes.

The other eight sponge specimens never showed signs of distress during the study period (7 days).

3.3.2. Lindane removal

Experiments were performed in order to evaluate the ability of *H. perlevis* to remove the organochlorine pesticide from seawater. Since a slight lindane degradation was observed in NS in the experimental conditions (see above), the *H. perlevis* compound removal was evaluated according to Eq. (2). The relevant results are shown in Fig. 2. As can be seen from the figure, a significant removal of lindane from NS was observed in all the analyzed samples. Furthermore, the removal was similar in all samples; a comparable bioremediation of the analyte was observed even in the sample containing Hp 6, the sponge which showed clear necrosis signs.

The one-way ANOVA test showed that there was no significant difference between the nine groups; thus the nine specimens can be treated as homogeneous. As can be seen in Fig. 2, about 50% of pesticide removal was achieved after 2 days of exposure; on the contrary, lindane concentration started to increase slightly after 3 days, eventually reaching an almost constant value.

3.4. Screening of lindane-degrading bacterial isolates

Since the pesticide removal was also observed in the case of the necrotic sponge, it was hypothesized that bacterial strains originating from the sponges might have a key role in the bioremediation. Thus, microbiological analyses were performed on the homogenates of lindane exposed sponges. After cultivation on Bacto Marine Agar, a total of 27 colony types (Li-1–27) were isolated from the sponge homogenates, on the basis of the colony morphologies and pigmentation. Among these isolates, the Gram-negative strains were predominant in comparison to the Gram-positive ones (Table 1). Then, all the strains were incubated with lindane as described in the experimental section and subsequently subjected to SPME–GC–MS analysis, in order to ascertain whether a certain amount of the recorded lindane removal was due to *H. perlevis* associated bacteria. Table 1 reports the ability of each bacterial strain to remove lindane from the medium in 48 h, using it as its only carbon source. All the strains showed a remarkable removing capacity (values ranging between 48% and 71% of lindane removal), in contrast to the control where lindane concentration remained unchanged. In addition, the strains showed appreciable growth (expressed as optical density at 560 nm) and some of them (Li-6–8, Li-11–13, Li-17, Li-18 and Li-24) reached the highest final optical density values (of 1.0 at 540 nm equivalent to 10^9 CFU (colony forming unit) mL^{-1}) associated with the highest percentages of lindane decrease (Table 1).

All samples were then subjected to further SPME–GC–MS analysis in TIC mode, searching for the presence of possible metabolites of lindane. Fig. 3 reports, for instance, the extracted-ion SPME–GC–MS chromatogram (*m*/*z* 181 and 183) obtained by analyzing the content of one tube in which a high bacterial growth was attained, clearly showing another peak (15.69 min) besides that of lindane. The spectrum was recognized using the NIST library as belonging to 1,3,4,5,6-pentachloro-cyclohexene (PCCH), a well known lindane metabolite (Quintero et al., 2005, 2008; Guillén-Jiménez et al., 2012). Identical results were obtained by analyzing all the bacterial strains incubated with lindane.

Finally, the 9 bacterial strains showing the best growth and lindane removal performances were subjected to the measurement of lindane degradation after 8 days of treatment. As shown in Table 2 a further decrease in lindane was observed, while the control still remained unchanged.

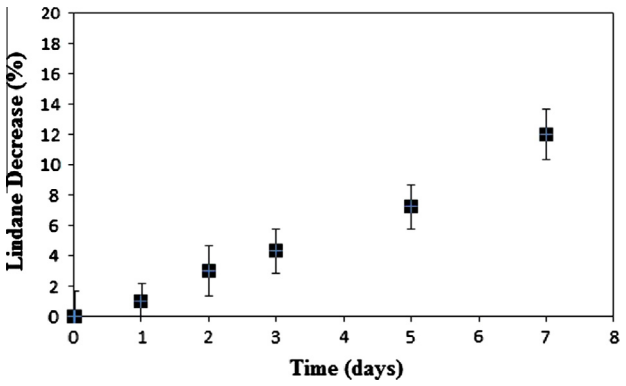

Fig. 1. Lindane decrease in aerated natural seawater, calculated using Eq. (1) (see Section 2).

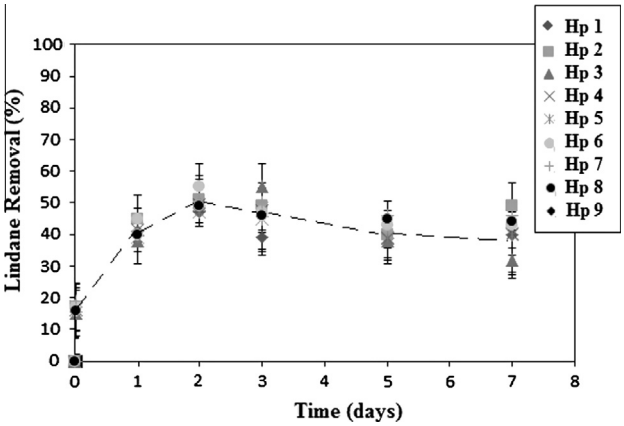

Fig. 2. Lindane removal from the Hps beakers, calculated according to Eq. (2) (see Section 2). The dotted line represents the average values.

4. Discussion

In the present study we chose lindane as representative of organochlorines, a large class of multipurpose chlorinated hydrocarbon chemicals, which are important persistent water pollutants extensively used in agriculture (Lal et al., 2010). In this scenario, at first we studied lindane stability in natural (NS) and artificial (AS) seawater and in natural seawater under experimental conditions (i.e. in an aerated beaker). Subsequently, we evaluated the effects of the

Table 1
Colony types isolated from lindane exposed sponge homogenates, with details on their cell morphology, pigmentation, Gram reaction and percentage of lindane decrease in the medium.

Colony types	Cell morphology	Pigmentation	Gram reaction	Lindane decrease (%) ($n = 3$)
Control				*3 ± 2*
Li-1	r	Brown	–	60 ± 5
Li-2	r	–	–	63 ± 4
Li-3	r	Brown	–	58 ± 6
Li-4	r	Orange	–	62 ± 4
Li-5	r	–	–	61 ± 5
Li-6	r	–	–	66 ± 4
Li-7	r	Yellow	–	66 ± 4
Li-8	r	–	–	70 ± 5
Li-9	r	–	–	61 ± 4
Li-10	c	–	+	62 ± 4
Li-11	r		–	71 ± 5
Li-12	r	Salmon	–	66 ± 6
Li-13	c	–	+	68 ± 4
Li-14	r	Orange	–	60 ± 5
Li-15	c	White	+	61 ± 4
Li-16	r	–	–	54 ± 4
Li-17	r	Orange	+	67 ± 5
Li-18	r	–	–	66 ± 6
Li-19	r	–	–	61 ± 5
Li-20	c	Orange	+	63 ± 4
Li-21	r	Yellow	+	60 ± 6
Li-22	r	Orange	–	62 ± 4
Li-23	r	Brown	–	48 ± 5
Li-24	c	–	+	66 ± 4
Li-25	c	–	+	63 ± 4
Li-26	r	–	–	60 ± 5
Li-27	r	–	–	64 ± 4

r: Rod shaped; c: cocci.
In italics, lindane decrease referred to the control.

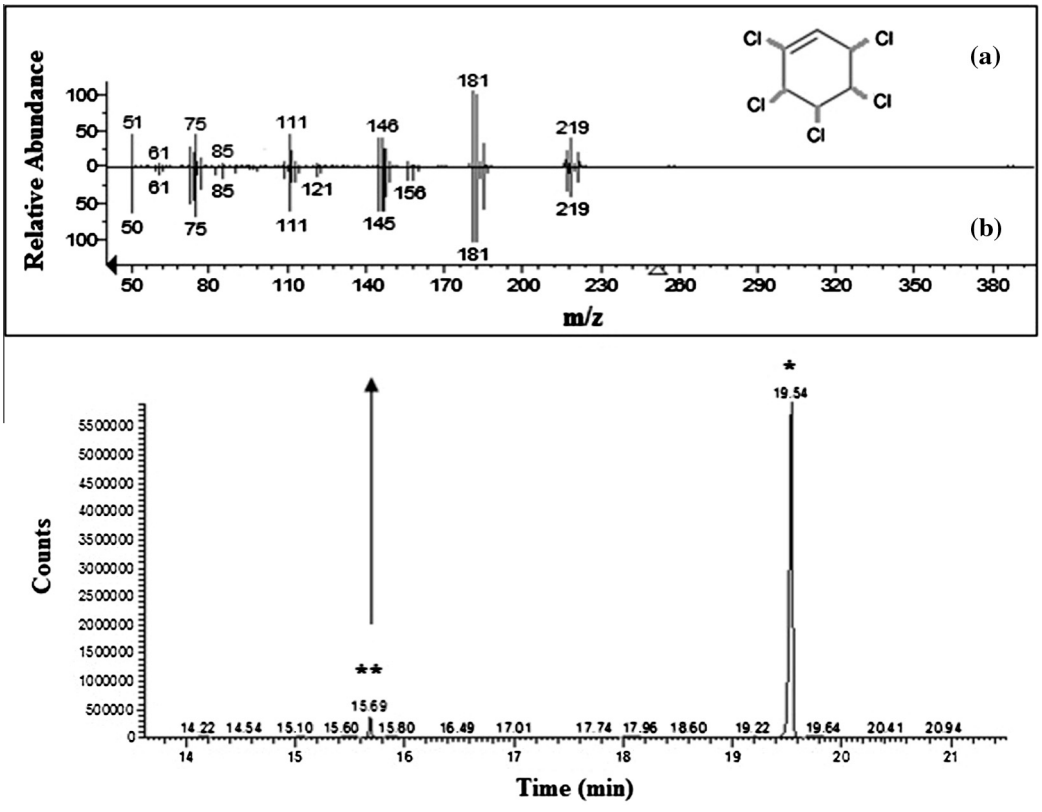

Fig. 3. Extracted-ion chromatogram (m/z 181 and 183) obtained by SPME–GC–MS of Li-6 sample after 48 h incubation. The insert shows the spectrum of the metabolite peak (a) compared with that recognized by the NIST library as 1,3,4,5,6-pentachloro-cyclohexene (b). *Lindane peak; **metabolite peak.

Table 2
Lindane decrease after 8 days in the colony types selected for their best growth and pesticide removal performances.

Colony types	Lindane decrease (%)
Control	*12 ± 4*
Li-6	97 ± 4
Li-7	89 ± 6
Li-8	95 ± 5
Li-11	93 ± 4
Li-12	90 ± 5
Li-13	97 ± 3
Li-17	87 ± 7
Li-18	88 ± 7
Li-24	91 ± 4

In italics, lindane decrease referred to the control.

pesticide on the survival of the demosponge *H. perlevis* at a concentration of 1 μg/L. We selected this concentration since it was well below the Maximum Acceptable Toxicant Concentrations (MATC) of lindane reported for different aquatic invertebrates and fish (values ranging between 2.2 and 23.5 μg/L) (U.S. EPA, 2012). Moreover, we took into consideration that *H. perlevis* is often abundant in polluted seawater such as that of ports and harbors, where the sponge thrives despite the strong anthropogenic impact (Longo et al., 2010). On account of these features and considerations, we also tested the ability of *H. perlevis* to remove lindane from seawater.

Lindane determination in seawater samples was performed using the SPME–GC–MS approach. SPME was very suitable for the purpose, since it is able to extract organic compounds from aqueous samples at trace levels with negligible depletion. Furthermore, this extraction technique is very simple, fast, solvent-free, and therefore eco-friendly (Pawliszyn, 1997).

Our results about lindane stability in NS under experimental conditions showed a slight and progressive decrease of the pesticide concentration, while it remained stable in AS and NS placed in sealed vials. Further studies will be undertaken to clarify the mechanisms involved in this phenomenon, though it is possible to hypothesize that this latter is due to the interaction between lindane and aerobic microorganisms present in the water, which probably utilize lindane for their metabolism. In fact, several studies have shown the capacity of some aquatic microorganisms to grow in the presence of lindane (González et al., 2012; Rambo, 2013), which on the contrary can determine a decrease in diversity and abundance in benthic invertebrate communities (Evans and Nipper, 2007). The lindane concentration used in this study could be considered non toxic for the target species, since the mortality (about 10%) recorded during the observation period was coherent with data on the survival of sponges collected from their natural environment and moved to laboratory tanks (Schippers et al., 2012).

The results obtained about the ability of *H. perlevis* to remove lindane from seawater showed that the pesticide decrease was significantly higher in sponge-containing beakers than in control ones. It must be emphasized that the beaker containing the only necrotic specimen showed the same drop in lindane concentration as the others, suggesting that at least a part of lindane removal might not be caused by the sponge itself. Indeed, it is well known that most demosponges host microbial symbionts able to carry out several functions usually attributed to the sponge. This evidence led us to investigate the role played by *H. perlevis*-associated bacteria in lindane biodegradation. We assessed the capability of different bacteria isolated from the sponge to remove the pesticide from the culture medium, utilizing it as the only carbon source for their growth.

The results obtained demonstrated that all the bacterial strains isolated grew in the experimental medium, removing between 48% and 71% of the lindane present in 48 h. Moreover, the 9 selected strains caused an almost complete lindane removal (from 87% to 97%) after 8 days of incubation. Literature data (Camacho-Pérez et al., 2012; Guillén-Jiménez et al., 2012) report that the aerobic degradation pathway of lindane is initiated with dechlorination to form 1,3,4,5,6-pentachloro-cyclohexene (PCCH), followed by further reactions that lead to various products. Although other non-detectable metabolites might be present in the system, the presence of a small amount of PCCH is demonstrated in the present study, which is clear evidence of bacteria-mediated degradation in progress. This result is encouraging for the applicative relapses related to the bioremediation of lindane polluted seawater. In fact, most of the lindane-degrading bacteria known to date have been isolated from contaminated soils (Girish and Kunhi, 2013). As regards heterotrophic marine bacteria, little work has been performed on the pathways of lindane degradation (Rambo, 2013). The bacterial strains isolated from *H. perlevis* and able to degrade lindane presumably establish a symbiotic relationship with the sponge and exploring this bacterial diversity as well as the metabolic capabilities of symbiotic bacteria represents an interesting challenge, showing great potential for diverse applications including biotechnologies. It is well known that the ability of microbes to degrade lindane *in situ* can be optimized by the process of induction and acclimatization, where the enzyme systems of the biodegradation pathways get induced, facilitating the effective removal of the pollutant (Girish et al., 2000; Elcey and Kunhi, 2010).

The interesting results obtained by the present study, together with the evidence that *H. perlevis* may accumulate, metabolize and remediate bacteria (Longo et al., 2010), suggest this demosponge as a potential candidate for the bioremediation of polluted seawater. Up to now, bacteria capable of degrading HCH isomers have received considerable attention as they provide the possibility to be utilized for *in situ* remediation. Some authors (Camacho-Pérez et al., 2010; Elcey and Kunhi, 2010) have reported the removal and degradation of lindane by generic consortia of aerobic bacteria. For instance, a consortium of 10 bacterial species comprising 7 species of *Pseudomonas* and one species of each of the genera *Flavobacterium*, *Vibrio* and *Burkholderia* was tested. This consortium showed the ability to degrade nearly 90% of γ-HCH within 72 h of incubation (Afsar et al., 2005). However, the process raises some issues that should be considered, such as the long-term impact of introducing microorganisms into the environment. In the case of *H. perlevis*, this problem is overcome since the sponge itself harbors symbiotic lindane degrading bacteria. In addition, the observation that at least nine bacterial strains isolated from the sponge were able to completely degrade lindane suggests the hypothesis that this consortium of symbiotic bacteria could act simultaneously and probably synergistically in the degradation of the pesticide.

On the basis of our results, *H. perlevis*, a marine demosponge able to live in polluted environments, proved to be a microcosm hosting symbiotic bacteria effective in lindane bioremediation and thus could be considered a promising tool for low-cost *in situ* bioremediation of lindane polluted seawater. Further studies will be carried out in the future to test the ability of *H. perlevis* to degrade other important organochlorine pollutants.

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