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The sea urchin *Paracentrotus lividus* immunological response to chemical pollution exposure: The case of lindane

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HIGHLIGHTS

- We evaluated the effect of lindane on *P. lividus* immune system defence.
- Lindane affected both cellular and humoral components of the sea urchin.
- Coelomocytes number decreased and the red cells increased in number.
- Antibacterial activity decreased after lindane treatment.
- Modifications in defence mechanism might represent biological pollution indicators.

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ABSTRACT

In the marine environment organochlorine insecticides can be broadly detected in water, sediments, and biota. These pollutants may have major ecological consequences since they may affect marine organisms and endanger organismal growth, reproduction or survival. In this study we investigated the modification of some sea urchin immunological parameters in response to subchronic lindane (γ-HCH) exposure. Adult specimens of the sea urchin *Paracentrotus lividus* were exposed to two different concentrations (0.1 and 0.5 mg L^{-1}) of lindane. After 24 and 48 h of treatment, we examined the lindane influence on coelomocytes vitality and enumeration as well on some humoral parameters. Our results showed that the presence of the pesticide affected both cellular and humoral components of the immune system. In particular, *P. lividus* coelomocytes vitality did not change but a decrease of the total cell number and an increase of the red cells was recorded. Haemolytic and lysozyme-like activities as well as antibacterial activity on *Vibrio alginolyticus* of treated animals decreased. Sea urchin immunological competence modifications might represent a tool for monitoring disease susceptibility thus providing biological criteria for the implementation of water quality standards to protect marine organisms.

1. Introduction

Coastal waters are usually under intense variety of pressures due to anthropogenic impact such as industrialization, intensive agriculture, and uncontrolled urban sprawl. Natural and man-made toxicants enter marine ecosystems by various routes comprising land run-off, atmospheric deposition, *in situ* production, abiotic and biotic movements and food-chain transfer (UNEP, 1996; Bellas et al., 2005; UN WWAP, 2009). Unlike many other chemicals, pesticides are deliberately added to the environment and are devised to be lethal to some organisms. Pesticides contamination of the marine environment is monitored worldwide through the analysis of water, sediment and marine species samples in order to elucidate the contamination status, the distribution and to assess the risks on aquatic organisms and humans (Strandberg et al., 2000; Zhulidov et al., 2000; Buisson et al., 2008). In this framework the Stockholm Convention (2009) encourage the production of monitoring data to effectively evaluate the presence of the persistent organic pollutants (POPs), including some pesticides, in all the regions in order to identify changes in levels over time. Highly persistent and bioaccumulating pesticides indeed may have major ecological consequences because of their high toxicity and could endanger organismal growth, reproduction or survival (Banerjee et al., 1996, 2001). In particular hexachlorocyclohexane creates problems of environmental toxicity

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since its isomer gamma hexachlorocyclohexane (γ-HCH or lindane) with an insecticidal activity and toxic effects is a common pollutant worldwide produced mainly after the Second World War until the 1990s (Breivik et al., 1999). The application of this pesticide has resulted in environmental contamination of global dimensions (Li, 1999; Li et al., 2003; Li and MacDonald, 2005; Vijgen, 2006a, 2006b; Vijgen et al., 2011). The use of lindane has been banned in at least 52 countries, and various bilateral and multilateral international agreements and treaties have addressed this pesticide, including, e.g. the Rotterdam Convention, the OSPAR Commission for the Protection of the Marine Environment of the Northeast Atlantic and the Stockholm Convention (Vijgen et al., 2011). However, the use of the organochlorines, including lindane, is still common in developing countries.

In many of the lindane producing countries the consequences for human and environmental health have virtually been ignored. The low aqueous solubility and chlorinated nature of lindane contribute to its environmental persistence and resistance to degradation and photodegradation. This pesticide is very stable in both fresh and salt water, it accumulates and persisting in sediments, can pose a hazard to sediment dwelling organisms. It could likely be ingested by marine populations thus constituting a threat to people who harvest and consume polluted seafood (Ospar Commission, 2002).

Recently, an increasing number of studies are combining approaches of monitoring chemical contaminant levels with measurements of biological response related to pollutants effects, allowing the assessment of environmental status across marine regions (Hagger et al., 2008; Thain et al., 2008; Lyons et al., 2010). Sessile and filter feeder organisms inhabiting coastal waters, are constantly in contact with various pollutants and therefore are proposed as sentinel species in environmental risk assessment. The effects of environmental contaminants in marine invertebrates may result from direct toxic actions on tissues or cells or from alterations of the homeostatic mechanisms (Cajaraville et al., 2000). Among physiological processes possibly disturbed by pollutants, the immune system is likely to be one of the more sensitive (Baier-Anderson and Anderson, 2000; Fournier et al., 2000). Studies on the effect of pollutants including heavy metals, nanoparticles and other chemicals on invertebrate immunological parameters have demonstrated their utility as tools for monitoring environmental hazards (Matranga et al., 2000, 2005; Stabili and Pagliara, 2009; Luna-Acosta et al., 2010; Pagliara and Stabili, 2012). Some immune parameters such as antimicrobial peptides, phenoloxidases and lysozyme may indeed be modulated by contaminants (Stabili and Pagliara, 2009).

The impact of lindane has been well documented, but data on the effects of this compound on invertebrates immune system are scarce and studies have been carried out mainly on molluscs, crustaceans and oligochaetes (Anguiano et al., 2010, 2006; Mydlarz et al., 2006). The only studies on the effect of lindane on echinoderms considered its effect on fertilization and early development of sea urchins (Pesando et al., 2004; Bellas et al., 2005). By contrast, little information is known about the impact of this pesticide on echinoderms immune system. The immune response of echinoderms has an important defence function against bacteria, fungi, and parasites. In these invertebrates, cellular and humoral components are present and operate in a coordinated way. Cell-based immunity is carried out by the celomocytes. Celomocytes present diverse morphologies and functions, which include phagocytosis, encapsulation, clotting, cytotoxicity, wound healing among others. Humoral immunity is mediated by a wide variety of secreted compounds such as lectins, agglutinins, perforins, complement, antimicrobial peptides and some cytokines (Ramírez-Gómez and García-Arrarás, 2010).

In the present study we analysed the effects of two different subchronic concentrations of lindane on some immunological parameters of the sea urchin *Paracentrotus lividus*, a sentinel species used in several environmental monitoring programs. In particular, we evaluated the effects on coelomocytes, haemolytic and lysozyme-like activities as well as antibacterial activity on *Vibrio alginolyticus*, in order to obtain information on validating the use of these parameters as valid biomarkers of environmental stress.

2. Materials and methods

2.1. 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®. An ethanol stock solution of pesticide was prepared and stored in the dark at 4 °C. Working solutions were prepared immediately before use by dilution with filtered seawater (FSW) (0.2 μm).

2.2. Sea urchins collection and treatment

Adult specimens (mean diameter size 3–4.5 cm) of *P. lividus* (Echinodermata, Echinoidea) were collected, by using SCUBA equipment, in an unpolluted coastal area of the Northern Ionian Sea (S. Caterina, Lecce–Italy), where lindane was absent (Spagnoli et al., 2010). A significant number of sea urchins (70) were collected before the spring reproduction season (March 2012) according to Tenuzzo et al. (2012) and immediately analysed in order to evaluate their reproductive state and ascertain the absence of mature gametes. After the sampling, the sea urchins specimens were divided into three sets of 48 individuals each. Individuals of each set were separated into six groups (8 individuals each one). The first set (control = C) was placed in six aquaria filled with FSW, the second set in six aquaria with FSW and lindane (final concentration of 0.1 mg L^{-1} = treatment T1) and the third set in six aquaria with FSW and lindane (final concentration of 0.5 mg L^{-1} = treatment T2). Aquaria were maintained at the same environmental parameters of the collection coastal area (20 °C and 37‰ salinity).

We exposed sea urchins to lindane concentrations higher than those recorded in contaminated areas (from 0.1 to 7.6 ng L^{-1} in seawater and from 3.8 to 80 ng g^{-1} in sediments) (Ospar Commission, 2002; Marini et al., 2010) taking into account that in marine organisms a bio-concentration factor of about 1000 (on a lipid basis) was estimated for this pesticide (El-Dib and Badawy, 1985). Moreover, it was proved that a lindane concentration of 0.003 mg L^{-1} does not affect marine organisms in short time (Ju et al., 2011).

In order to verify the wellbeing of the urchins, the spines and ambulacral feet stiffness and movement degree were observed for all the specimens (both control and treated) during the experimental period. In addition, the response of the urchins to a stimulus was evaluated by gently touching the urchins with tweezers and observing their spines and ambulacral feet movement towards the tweezers.

2.3. Samples collection and preparation

After 24 and 48 h, 8 individuals from 3 aquaria of each set (C, T1 and T2, total individuals 72) were respectively removed and analysed. Each sea urchin was bled through the peristomial membrane, the coelomic fluid was then harvested and divided into two