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Immunotoxicological effects of environmental contaminants on marine bivalves

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ABSTRACT

Coastal areas are complex environments frequently contaminated by numerous pollutants that represent a potential threat to marine organisms, especially bivalves. These pollutants may have major ecological consequences. Although effects of different environmental contaminants on the immune system in marine bivalves have been already reported, a few of reviews summarizes these effects. The main purpose of this chapter relies on summarizing recent body of data on immunotoxicity in bivalves subjected to contaminants. Immune effects of heavy metals, pesticides, HAP, PCB and pharmaceuticals are presented and discussed and a particular section is devoted to nanoparticle effects. A large body of literature is now available on this topic. Finally, the urgent need of a better understanding of complex interactions between contaminants, marine bivalves and infectious diseases is noticed.

1. Introduction

Coastal areas are complex and highly changing environments at the interface between freshwater and marine aquatic ecosystems. Contaminants are frequently detected in these areas and represent a potential threat to marine organisms, especially bivalves. Pollutants may have major ecological consequences [4]. They can endanger organism growth, reproduction or survival. Their effects may result from direct toxic actions or from alterations of the homeostatic mechanisms including the immune system [7,20]. Among physiological processes possibly disturbed by pollutants, the immune system is likely to be one of the more sensitive [23]. Bivalves filter large volumes of seawater and their immune capacities can be adversely affected by exposure to contaminants. Therefore, bivalves have to face highly variable environmental conditions.

Investigating immunity toxicity can provide relevant information on the quality of the marine environment and facilitate understanding of the occurrence of infectious diseases affecting bivalves in coastal areas. Bivalves in culture may be weakened after contaminant exposure, potentially increasing their susceptibility to infectious diseases. It has been reported that a mixture of pesticides could induce a decrease of immune defenses in the Pacific oyster, *Crassostrea gigas*, rendering animals more vulnerable to an experimental bacterial infection [27]. In bivalves, immunity is mainly supported by cells called hemocytes found in the open hemolymphatic circulatory system. Hemocytes have been widely used to explore pollutant effects on bivalve immunity.

The monitoring of health conditions by the assessment of marine bivalve immunocompetence may serve as a criterion for the achievement of the Good Environmental Status as defined in the EU Marine Strategy Framework Directive. Bivalve molluscs such as mussels and oysters have been postulated as ideal indicator organisms because of their wide geographical distribution, and sensitivity to environmental pollutants [17]. Moreover, the development of techniques allowing the analysis of pollutant effects on bivalve immunity may lead to the development of diagnosis tools adapted to analyze pollutant transfer towards estuarine areas.

In this context, the effects of different environmental contaminants on the immune system of marine bivalves have been already reported. The condition of the immune system determines partly susceptibility to disease and survival. Measuring endpoints linked to immunity can help to identify sub-lethal effects of exposure to contaminants and provide early warning signals. However, there are at present a few of reviews summarizing these effects. The main purpose of this chapter relies on summarizing recent body of data

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on immunotoxicity in bivalves subjected to contaminants. Immune effects of heavy metals, pesticides, HAP, PCB and pharmaceuticals are presented and discussed and a particular section is devoted to the effects of nanoparticles. A large body of literature is now available on this topic. Finally, the urgent need of a better understanding of complex interactions between contaminants, marine bivalves and their infectious agents is identified.

2. Pesticides

Pesticides are used especially in agriculture. They are defined as chemical substances used to kill pests including insects, weeds, parasites or rodents. All pesticides act by interfering with the target species normal metabolism. However, some inadvertently effects may affect other organisms in the environment, either directly by their toxic effects or via elimination of the target organism.

Hemocyte activities have been extensively studied in order to investigate the effects of pesticides on bivalve immunity. As an example, 23 pollutants including several pesticides have been tested on Pacific oyster hemocytes maintained in vitro through monitoring of cell parameters by flow cytometry [26]. Several pesticides have been shown to induce a modulation of some hemocyte activities including cell mortality and non specific esterase activities. However [40], reported no significant effect of a mixture of 14 pesticides and methaldehyde alone tested at different concentrations on Pacific oyster hemocytes maintained in vitro for short-term periods. Several cell parameters including dead/alive cells, non specific esterase activities, intracytoplasmic calcium, lysosome number and activity, and phagocytosis were monitored by flow cytometry. Pacific oyster hemocytes appeared thus resistant to a pesticide exposure in the tested conditions [40]. Developing in vivo assays can be useful to better understand pollutant effects on immune system in bivalves.

After 7 days of in vivo exposure to a mixture of eight pesticides (atrazine, glyphosate, alachlor, metolachlor, fosetyl-alumimium, terbuthylazine, diuron and carbaryl) at environmentally relevant concentrations, phagocytosis was significantly reduced and 19 genes involved in *C. gigas* functions were down-regulated in treated animals [27]. Ref. [28]. investigated the effects of the same mixture of 8 pesticides on Pacific oyster, *C. gigas*, during a 7 day period. Enzyme activities including glutamine synthetase (GS), glutathione S-transferase (GST) and catalase (CAT), hemocyte parameters and DNA damages were assessed as effect biomarkers. GS, GST and CAT activities increased after pesticide exposure as well as DNA adducts. Moreover, the hemocyte phagocytic capacity decreased significantly after 7 days of the exposure.

In the context of mass mortality outbreaks affecting the Pacific oyster, *C. gigas*, in France since 2008, Ref. [37] investigated effects on immune related gene expression, enzyme activities, and hemocyte parameters in animals exposed to diuron alone and to diuron, isoproturon, and ibuprofen as a mixture. Mortality outbreaks were mainly reported in spring and summer suggesting a putative role played by the seasonal use of pesticides and freshwater inputs in estuarine areas where oysters are frequently reared. Following exposure to diuron at 1 μg L(−1), a reduction of gene expression and enzyme activities including laccase-type phenoloxidase (PO) activity and superoxide dismutase (SOD) activity was reported [37]. Catecholase-type PO activity and hemocyte phagocytosis were reduced after an exposure to the mixture containing the herbicides diuron and isoproturon, and the pharmaceutical ibuprofen [37]. Effects of metaldehyde on Pacific oyster hemocyte parameters were monitored through in vivo experiments based on a short-term exposure by Ref. [41]. Metaldehyde is used to kill terrestrial gastropods and could be potentially more toxic to oysters than other pesticides (herbicides, fungicides, insecticides, …). Metaldehyde at 0.1 μg L(−1), which corresponds to an average concentration detected in the environment, modulated hemocyte activities of Pacific oysters.

Ref. [16] explored the sublethal impact of azamethiphos, an organophosphate pesticide, on the blue mussel, *Mytilus edulis*, after a short term exposure. Azamethiphos is used to combat sea lice infestations in farmed salmonids. A significant reduction in acetylcholinesterase activity in haemolymph and gills, an alteration in cell viability and a decrease in phagocytic index were reported in mussels exposed to azamethiphos for periods of up to 24 h. These results suggested that hemocyte functions of blue mussels could be actively modulated by azamethiphos at environmentally relevant concentrations after only a few hours.

[27] reported higher mortality in pesticide-treated Pacific oysters compared to untreated oysters after a bacterial challenge by intramuscular injection of two *Vibrio splendidus*-related pathogenic strains. Gene expression was also up-regulated in pesticide-treated oysters compared to untreated oysters after the bacterial challenge. As gene expression was up-regulated in pesticide-treated oysters compared to untreated ones after the bacterial challenge, Ref. [27] suggested that gene over-expression due to an interaction between pesticides and bacteria could be related to an injury of host tissues, resulting in higher mortality rates. Although this study reported an effect of pesticides at environmentally relevant concentrations on *C. gigas* susceptibility to an experimental bacterial infection, no other studies have been then published on such interactions.

3. Heavy metals

Constant increase of industrial wastes, a source of heavy metals, results in pollutant transfer towards estuarine areas. Marine bivalve molluscs, as filter-feeding organisms, are known to accumulate metals that can produce deleterious effects on organisms.

Ref. [25] investigated the effects of cadmium and mercury (Hg) on defence mechanisms in the Pacific oysters, *C. gigas*. Pollutant effects were tested in vitro on oyster hemocytes. Although no effect of cadmium exposure was reported, mercury caused a significant hemocyte mortality after a 24 h in vitro incubation. The aminopeptidase positive cell percentage was enhanced in presence of this pollutant, and the phenoloxidase-like activity was inhibited. Ref. [44] reported high levels of apoptosis in Eastern oyster hemocytes after cadmium (Cd(2+)) exposure (10−100 μmol L(-1)). Necrosis was observed at higher concentrations (200−1000 μmol L(−1)). Enhanced apoptosis of hemocytes after Cd(2+) exposure could induce immunosuppression and result in reduced disease resistance. Ref. [45] explored immunomodulation produced by metals in the green mussel, *Perna viridis*. Mussels were exposed to copper and mercury at 20 μg L(−1) and 10 μg L(-1), respectively. Both metals affected adversely immune parameters (phenoloxidase, reactive oxygen species generation, and phagocytosis). Some level of recovery (depuration) from the toxic effects of metals was also observed. Ref. [33] reported significant effects on phagocytic activity and bacterial clearance in the blue mussel, *M. edulis*, after short-term exposure (1, 7 and 13 days) to low copper concentrations (5, 9 and 16 μg L(−1)). These authors compared also immune responses of mussels kept at two salinities (12‰ and 20‰) and showed a significant interaction between salinity and copper exposure in terms of metal accumulation. Mussels kept at the lower salinity accumulated markedly more copper than mussels maintained at the highest one.

Mercury (Hg) effects on the immunity of the bivalve *Scrobicularia plana* inhabiting a contaminated area (Laranjo basin, Ria de Aveiro, Portugal) were monitored by Ref. [2]. Animals collected from both moderately and highly contaminated sites demonstrated higher haemolymph heavy metal load and reduced plasma

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