Available online at www.sciencedirect.com
SCIENCE DIRECT
Science of the Total Environment 359 (2006) 135–144
Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Humankind
www.elsevier.com/locate/scitotenv

Responses of *Hexaplex* (*Murex*) *trunculus* to selected pollutants

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Received 15 March 2005; received in revised form 20 September 2005; accepted 23 September 2005
Available online 27 October 2005

Abstract

Cadmium, copper and zinc concentrations (in whole soft body and in tissues) were measured in *Hexaplex trunculus* collected from the Bizerta lagoon in Tunisia. An evaluation of the biological effects of the most toxic metals (cadmium and copper) and of two organics (carbofuran and lindane), present in the sediments of the Bizerta lagoon, was attempted by measuring biomarkers (acetylcholinesterase: AChE, catalase: CAT and glutathione *S*-transferase: GST activities) in animals experimentally exposed for 48 or 72 h. The concentration ranges as follows: Zn>Cu>Cd. Copper concentrations are highly variable (8.0 to 235 μg g^{-1} d.w.) whereas cadmium (range 1.35–4.86 μg g^{-1}) and zinc (range 360–1320 μg g^{-1}) concentrations are less variable. The digestive gland and the gill take up more metal than the muscle. AChE activity in *H. trunculus* is decreased by exposure to carbofuran or the mixture carbofuran and cadmium, in the digestive gland and muscle and by copper and by lindane in the digestive gland. AChE is generally inhibited by carbamates but some other compounds may also decrease this activity as observed in this paper. An increase in CAT activity associated with a decrease in GST activity is noted in the muscle of *H. trunculus* exposed to cadmium, to carbofuran and to the mixture of cadmium and carbofuran, and in the digestive gland of animals exposed to lindane. These pollutants may act upon glutathione and decrease the GST activity that cannot detoxify them and CAT activity has a protective effect. On the contrary, copper increases CAT and GST activities in the digestive gland of exposed gastropods; these enzymes seem to cooperate and play together their rôle of anti-oxidant enzymes. If *H. trunculus* is not a bioindicator species for metal concentrations, due to a high variability in metal concentrations, nevertheless the biochemical responses to pollutants (cadmium, copper, carbofuran and lindane) represented by AChE, CAT and GST activities may act as biomarkers of exposure in this species.
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Keywords: *Hexaplex trunculus*; Cadmium; Copper; Carbofuran; Lindane; Biomarker

1. Introduction

Marine pollution has been traditionally documented in terms of chemical concentrations of contaminants and especially heavy metals. Different invertebrates species take in markedly different concentrations of metals. The species *Hexaplex trunculus*, which was called before *Murex trunculus*, was chosen as a model in this study for a number of reasons. First, these animals are abundant in the Bizerta lagoon where they were collected. The other reasons for the choice of (*Hexaplex) Murex* are the following: the animals have been found both in relatively clean and polluted areas; they are easy to spot and sample, they are predatory gastropods and are consumed by man. Due to the importance of *Hexaplex*, studies have been developing on its potential culture (Ramon and Flos, 2001). Several papers have already dealt with their ability to concentrate cadmium (Bouquegneau et al., 1988; Dallinger et al., 1989) but also copper and mercury (Catsiki and Arnoux, 1987).

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0048-9697/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2005.09.071

In the present paper, cadmium, copper and zinc concentrations were measured in *H. trunculus* collected from the Bizerta lagoon in Tunisia. Concentrations in the whole soft body of animals as well as their distribution among different tissues were taken into consideration. An evaluation of the biological effect of these metals, and especially the most toxic i.e., cadmium and copper was attempted by measuring biomarkers in experimentally exposed animals. The effects of two organics (carbofuran and lindane) were also researched in terms of biomarkers. The pollutants which were chosen in this study are present in the sediments of the Bizerta lagoon, among them copper seems to be a major source of contamination (Mitsuo et al., 2002) as well as other metals and pesticides. Wastes of agricultural origin may reach the lagoon as the result of leaching of inland cultivated lands, which occupy an area of 172 km^2 and are mainly devoted to cereal crops (78 km^2, which utilize 20.5 tons of fertilizer per year, ANPE, 1989). The carbofuran is a well-known carbamate pesticide. The use of lindane (isomer gamma hexachlorocyclohexane) is nowadays prohibited in most countries but this organochlorine persists in soils and may reach the marine environment through erosion processes. In this area, multi-biomarker approaches were carried out in two bivalve species : the Mediterranean clam *Ruditapes decussatus* (Dellali et al., 2001a,b, 2004) and the mussel *Mytilus galloprovincialis* (Khessiba et al., 2001, 2005).

Biomarkers consist of biochemical and/or physiological changes in organisms exposed to contaminants, and thus represent initial responses to environmental perturbations and contamination (Mc Carthy and Shugart, 1990; Roy et al., 1996). In contrast to the simple measurement of contaminants accumulating in body tissues, biomarkers can offer more complete and biologically more relevant information on the potential impact of contaminants on the health of organisms (Van der Oost et al., 1996). Various biomarkers have been measured in different groups of aquatic organisms, particularly in fish and some bivalve molluscs but few papers have dealt with biomarkers measured in gastropod molluscs. Three biomarkers (acetylcholinesterase AChE activity, catalase CAT activity and glutathione *S*-transferase GST activity) were thus measured in those animals collected from the Bizerta lagoon and exposed to the chosen contaminants as well as a mixture of them. Acetylcholinesterase activity, inhibited by the presence of organophosphorus compounds and carbamates, is considered as an exposure biomarker to these substances (Galgani and Bocquené, 1989). It has been used in coastal biomonitoring programs (Escartin and Porte, 1997; Stien et al., 1998; Mora et al., 1999, Roméo et al., 2003). CAT activity (EC 1.11.1.6), although not responding specifically to a group of contaminants but to oxidative stress, was also used in this work. This is because the primary defense against oxidative damage consists of the induction of some low molecular weight compounds (vitamins A, C, E and glutathione) and antioxidant enzymes such as CAT, which has been studied in molluscs (Pellerin-Massicotte, 1997). The glutathione *S*-transferase isoforms (GSTs) are involved in the metabolism of organochlorine pesticides (Fitzpatrick et al., 1997). GSTs (EC 2.5.1.18) have begun to be used as biomarkers of these substances and PCBs in molluscs (Fitzpatrick et al., 1997; Hoarau et al., 2001) since EROD, ethoxyresorufin *O*-deethylase, activity as an exposure biomarker of organic compounds, does not give satisfactory responses in these animals (Cajaraville et al., 2000). The biomarkers were measured in two tissues : the digestive gland, where cadmium is particularly bioaccumulated (Bouquegneau et al., 1988; Lorenzini and Orlando, 1994), and the muscle.

2. Materials and methods

2.1. Sampling and animal maintenance

Specimens of gastropods (of shell length ca 6 cm) were collected from the site Menzel Abderrahmen, located in the Bizerta lagoon in mid-September 2003. Animals were sampled in the same place from one population in order to avoid genetic diversity. Animals were transported to the laboratory and kept in 4 l aquaria filled with continuously aerated seawater. The temperature was maintained constant at $18° \pm 1$ °C, and the salinity at 38‰ for 72 h. Animals were not fed during the experiments. Animals were divided into two groups.

The first group (20 animals) was devoted to metal determinations. Each gastropod was measured (shell maximum length) and weighed, ten samples were dissected to obtain the digestive gland, the gill and the muscle. All samples were dried to constant weight (50 °C) until digestion and analysis.

The second group was exposed to the contaminants. Cadmium was introduced into the medium as $CdCl_2$ at 150 μg l^{-1}. This concentration was chosen after preliminary assays with 50 μg l^{-1} that was too low to evoke a biochemical effect and 200 μg l^{-1} which was too high. An organic contaminant, carbofuran which is a carbamate was also tested. This pollutant was introduced at the concentration of 1 mg l^{-1}, this level refers

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