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Seasonal variation in biomarker responses of *Donax trunculus* from the Gulf of Annaba (Algeria): Implication of metal accumulation in sediments

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ABSTRACT

The aim of the present study was to test biomarker responses in an edible mollusk, *Donax trunculus* L. (Mollusca, Bivalvia) associated with environmental pollution in the Gulf of Annaba (northeastern Algeria). The biomarkers selected were glutathione S-transferase (GST), acetylcholinesterase (AChE) and metallothioneins (MTs). Samples were collected seasonally (September 2014, and January, April and July 2015) from two sites located over the Gulf of Annaba: El Battah and Sidi Salem. The results obtained reveal that autumn and winter were the two seasons that show an increase in GST activity, an inhibition of AChE activity and a high rate of MT. In addition, a decrease in AChE activity, an increase in both GST activity and MT levels in *D. Trunculus* collected from Sidi Salem in comparison with those of El Battah were observed. The biomarker responses at the Sidi Salem site reflect the presence of certain pro-oxidative compounds such as metals (Cd, Cu, Pb, Zn, Mn and Fe) determined in sediments in winter (January) 2015. Moreover, metal concentrations, except Fe, were higher at Sidi Salem than at El Battah. Overall, the Gulf of Annaba remains contaminated by heavy metal. However, this metallic contamination is relatively low and the risks for local population via this edible species were also low.

1. Introduction

Industrial and municipal wastewater, agricultural activities and atmospheric inputs are the main routes by which chemical contaminants enter the marine environment (Gherras Touahri et al., 2016). Organisms in aquatic environments are generally exposed to a complex mixture of chemicals, including the parent compounds and their transformation products that cause multiple damages to organisms, populations and ecosystems (Bolognesi and Cirillo, 2014). Therefore, monitoring approaches should have an integrative character combining chemical and biological evaluations with abiotic and biotic parameters (Schettino et al., 2012). Bivalves belong to the first-choice species as bioindicators for environmental and chemical stress. They are sentinel benthic organisms living as filter-feeders and exposed to different environmental compartments (Helmholz et al., 2016). Due to their wide distribution, resistance to variable environmental conditions, which

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predispose them to the direct absorption and accumulation of a wide spectrum of waterborne chemicals, they are prolific tools for the biomonitoring of chemical pollution (Boillot et al., 2015). Moreover, the possibility to measure several biochemical, cellular and physiological biomarkers makes bivalves suitable organisms for investigating the effects of chemical pollutants (Regoli et al., 2014). Biomarkers include a variety of molecular, cellular, or physiological alterations measurable in organisms in response to pollutants or other environmental stress factors (Moore et al., 2006). The glutathione S-transferase (GSTs) are a multiple-enzyme family involved in phase-II detoxification processes and are used as biomarkers of exposure to several groups of pollutants, including organochlorine pesticides, PCBs and petrochemical products in invertebrates (Hoarau et al., 2001; Lima et al., 2007). They have been recently identified as a suitable biomarker for monitoring chemical pollution in highly productive marine coastal ecosystems (Vidal-Liňán, 2010). Acetylcholinesterase (AChE) is involved in the hydrolysis of the neurotransmitter acetylcholine. The measurement of AChE inhibition in marine organisms has been widely used as an indicator of environmental contamination by organophosphate and carbamate pesticides (Blaise et al., 2016; Magni et al., 2006). Metallothioneins are low-molecular-weight, cysteine-rich cytosolic proteins able to bind to many metals, such as Ag, Cd, Co, Cu, Hg, Ni, Pb, Pd and Zn (Frank et al., 2008; Ng et al., 2007). In general, MTs are involved in different biological processes, i.e. Homeostasis of essential metals, detoxification of toxic metals and cell protection against oxidative stress (Geffard et al., 2005; Ng et al., 2007).

Donax trunculus L. is an edible mollusk species found in high densities in the sand beaches of the Gulf of Annaba in Algeria (Hafsaoui et al., 2016) and widely used as bioindicator species for the assessment of marine pollution through the measurement of several biomarkers (Amira et al., 2011; Sifi et al., 2013; Soltani et al., 2012; Tlili et al., 2013). Their habitats are exposed to several pollutants from different sources (Abdennour et al., 2000, 2004; Beldi et al., 2006; Merad and Soltani, 2017). Two rivers (the Seybouse River and the Mafrag River) drain into the Gulf of Annaba and receive agricultural water discharges from cereal farming, market gardening, and arboriculture, as well as domestic releases from important conurbations (Khelifi-Touhami et al., 2006) and untreated sewage (Abdennour et al., 2000). Metallic pollution was major, and cadmium one of the most common pollutants in our area (Belabed et al., 2013; Beldi et al., 2006; Larba and Soltani, 2014; Merad et al., 2016).

Sediments are an important sink for metals and other pollutants (Belhadj et al., 2017); they have been described as a non-point source of metals and sediment-bound metals that can be released into overlying waters and adversely affect aquatic organisms (Wang et al., 2004). Benthic invertebrates are known to take up and accumulate metals, both essential and nonessential, from water and sediment as well as from their food supply (Buzzi and Marcovecchio, 2016; Wang and Fisher, 1999).

In this context, the main objective of this work was to study the *in situ* seasonal responses of some biochemical biomarkers (GST, AChE, MTs) in *D. trunculus*, a locally prevalent edible mollusk sampled at two sites in the Gulf of Annaba: a polluted site (Sidi Salem) and a relatively clean site (El Battah). In addition, to evaluate the metallic pollution reported previously (Abdennour et al., 2000, 2004; Beldi et al., 2006; Larba and Soltani, 2014; Merad et al., 2016), the concentrations of heavy metals (Cd, Cu, Pb, Zn, Mn and Fe) were determined in sediments originating from these two sites.

2. Materials and methods

2.1. Study area

The Gulf of Annaba is located in the Northeast of Algeria. It is limited by the Rosa Cap (8°15′E and 36°38′N) to the east and by the Gard Cap (7°16′E and 36°68′N) to the west. The El Battah site (36°50′N–8°50′E), is located about 30 km to the east of Annaba far from any human activities and is considered as a relatively clean site. Sidi Salem site (36°50′N–7°47′E), located about 1 km to the east of Annaba city, receives industrial and domestic wastewater, and is considered as the polluted area (Fig. 1).

2.2. Samples collection

Mollusk bivalves (*D. trunculus*) with the same range shell length (25 ± 1 mm) were collected seasonally in the of autumn of 2014 (September), and in the winter (January), the spring (April), and the summer (July) of 2015 from two sampling sites (El Battah and Sidi Salem) and transferred to the laboratory.

Sediment samples were also collected at the two sampling sites in winter 2015 (January) for metal analysis. They were taken manually with a grab near the bivalves collecting points over a depth not exceeding 0.5 m (5–10 cm). In the laboratory, they were put to dry at room temperature until constant weight.

2.3. Biomarkers analysis

The mantle of six bivalves was dissected and samples were prepared for biomarker analyses. GST activity was measured according to Habig et al., 1974, using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The activity rate was expressed as μmol/min/mg protein. AChE activity was determined following the procedure of Ellman et al., 1961, with the use of acetylthiocholine (ASCh) as substrate. The activity was expressed as μmol/min/mg protein. The protein content was evaluated according to Bradford (1976) using serum albumin as standard (BSA, Sigma). MTs were determined according to the method of Viarengo et al., 1997, previously described (Merad et al., 2016). The mantles samples were homogenized in three volumes of 20 mM Tris-sucrose buffer with 0.1% of β-mercapto ethanol and 0.5 mM of PMSF, followed by ethanol/chloroform extraction, after incubation with DTNB. The levels of MT (MT-SH) were calculated assuming the relationship: 1 mol MT-SH = 20 mol GSH and expressed as μg of MTs per mg of fresh weight (FW).

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