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Multi-biomarkers approach to the assessment of the southeastern Mediterranean Sea health status: Preliminary study on *Stramonita haemastoma* used as a bioindicator for metal contamination

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HIGHLIGHTS

- A significant spatiotemporal variation in *S. haemastoma* biomarker responses from the Annaba Gulf and the El Kala coastline.
- A significant levels of trace metal elements (TME) in the digestive gland of *S. haemastoma*.
- The response of the different biomarkers studied is due to anthropogenic disturbances and to the location of the site.
- *S. haemastoma* could be used in surveillance programs as a bioindicator of contamination by TME from the Mediterranean sea.

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ABSTRACT

The present study aimed to evaluate the responses of different biochemicals parameters associated with environmental pollution in the digestive gland of the gastropod mollusc *Stramonita haemastoma*. Physiochemical parameters and trace metal elements (Copper (Cu), Zinc (Zn), Chromium (Cr), Cadmium (Cd) and Lead (Pb)) were measured in seawater. Spatiotemporal variations in reduced glutathione (GSH), malondialdehyde (MDA) and metallothionein (Mt) as well as the specific activities of glutathione S-transferase (GST) and catalase (CAT) were evaluated in digestive gland of this species during a one-year period in 2013–2014. Samples collection was conducted at three sites. The results obtained showed seasonal fluctuations in GST and CAT activities and in the rate of Mt content. In addition, intersite variations in GSH, MDA, Mt and CAT were recorded in individuals. Also, trace metal elements concentrations determined by season in the digestive gland revealed spatial and temporal variations for Cu and Zn but they are below the limit of detection for Cd and Pb. The highest values were generally recorded in spring for Cu and in winter for Zn. In this first regional study using in *S. haemastoma* as a model, the biomarkers measured were seen to be inducible parameters to evaluate the health state of the organism and the overall quality of the study sites.

1. Introduction

Marine ecosystems are highly vulnerable to pollution due to their recipient position from continents (Belabed et al., 2013b). Marine pollution has different origins. It can be of industrial (such as hydrocarbons, trace metal elements (TME) or chemicals) or agricultural origin (such as nutrients or pesticides) or simply be produced by domestic discharges following the presence of numerous wastewater outfall urban areas, wherein a wide variety of pollutants are concentrated (Valavanidis et al., 2006). The presence of these compounds in environmental media, biota and food poses nowadays a serious threat to human health and environmental integrity.

Metal pollution is one of the most abundant and dangerous forms of anthropogenic pollution threatening the littoral zone.

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Metal pollutants derive in the marine environment mainly by superficial runoff of rain, by direct atmospheric deposition and by discharges from sewage and industrial establishments. Maritime traffic is also a considerable source of metals given their presence in the antifouling paints composition of boats (Boulajfene et al., 2017). TME are micropollutants that can affect the marine environment health, as they do not undergo biological or chemical degradation. As a result, TME can accumulate in different trophic chain links at toxic concentrations in marine organisms. Due to the TME solubility, seawater analysis sample cannot be considered a reliable means of determining the pollution degree of marine environment. However, study of TME bioaccumulation in organisms exposed to them is a very important means of assessing metal pollution (Belhaouari et al., 2011). Metal contamination can have adverse effects on aquatic organisms after assimilation and accumulation. Nevertheless, not all metals have the same health impact: some (copper "Cu" and zinc "Zn") are essential at low doses and harmful at high doses, while others (cadmium "Cd" and lead "Pb") are harmful at even low doses. It is important to say that the toxic effects of Cd and Pb in marine species are multiple. Cd indirectly induces the production of reactive oxygen species (ROS) and lipid peroxidation by interference with antioxidant systems. It is also described as an inhibitor of DNA damage repair (Kamel, 2014). At the sublethal level, it can cause physiological effects (abnormalities in embryonic and larval development in bivalve molluscs) and growth inhibitions (Chiffoleau et al., 2001). Sobrino-Figueroa et al. (2007) have shown that Cd is the most toxic metal on juvenile populations of *Argopecten ventricosus*, which inhibits their growth, followed by Pb. In the mussel *Mytilus edulis*, the toxic effect of Pb can result in a competition with divalent essential metals with disruption of their metabolism: notably calcium, magnesium and Cu (Belabed, 2010). Cu is an important and essential micro-element that acts as a respiratory pigment in marine invertebrates. Its accumulation in the cell is the cause of cytotoxicity, which is manifested by enzymatic inhibition of the pyruvate oxidase system, glucose-6-phosphodehydrogenase and glutathione reductase (Kamel, 2014). High concentrations of this metal can lead to oxidative damage to lipids and proteins. It can also cause DNA deformation. Furthermore, it has been shown that, in excess, Zn becomes a prooxidant by inducing the indirect production of free radicals, and by inhibiting the enzymatic activity of certain antioxidant enzymes such as glutathione reductase and peroxidase (Sensi and Jeng, 2004).

In general, environmental or ecotoxicological monitoring in a marine ecosystem is based on two complementary approaches: biomarkers and bioindicators (Valavanidis et al., 2006).

Biomarkers can indicate links between contaminants and ecological responses and can be used to indicate the presence of harmful substances in the marine environment (Fernández et al., 2010), but data obtained are sometimes difficult to interpret due to the large amount of natural variables affecting biological processes, wich could act as confounding factors on biomarkers responses (González-Fernández et al., 2015). So, the use of a biomarkers battery is a trust approach to assess the environment health state and to increase the possibility for the detection of early biological changes (Almamoori et al., 2013).

Many species have been used as bioindicators of pollution, especially the bivalves: *M. galloprovincialis*, *Perna perna* and *Donax trunculus* (Abbes et al., 2003; Sifi et al., 2007, 2013; Amira et al., 2011; Soltani et al., 2012; Bensouda and Soltani-Mazouni, 2014).

The littoral is highly vulnerable to a wide assortment of contaminants and micropollutants directly released into the seas and oceans, to which are added those released into the air and drained by soils and rivers (Bensouda-Talbi, 2015).

The east Algerian coastline is the most important touristic and economic zone. It is continuously affected by various contaminants from urban, agricultural, harbor and industrial activities (Boucetta et al., 2016a).

The Annaba Gulf and the El Kala coastline, which represent the extreme northeastern part of the Algerian coastline, know as well as the rest of the latter, the same environmental problems. They are exposed to the risks of the different types of anthropogenic pollution that have an impact on the organisms that live there and on humans.

The Annaba Gulf is a standout amongst the most vital vacationer and financial focuses on the east coast of Algeria. It is considered as the receptacle for all residues, toxic or not, produced by the various industrial units located along the coast. This has made fishery stocks threatened by pollution linked to burgeoning economic activity. In addition, previous work has shown that the Annaba Gulf region is influenced by metal-rich effluents and is subjected to agricultural, industrial and urban activities as well as tourism development (Semadi and Deruelle, 1993; Abdenour et al., 2000; Beldi et al., 2006); with the impact of many chemicals and stressors, making the assessment of the marine ecosystem quality essential.

The El Kala coastline is minimally influenced by anthropogenic inputs, given the low urbanization of the region. Moreover, there is a notable absence of industries and consequently, little or no industrial atmospheric or continental pollution (Ounissi and Khelifi-Touhami, 1999).

Stramonita haemastoma, a gastropod mollusc commonly known as "Bakouma" in Algeria, has been the subject of several studies concerning its use as a bioindicator of tributyltin pollution (imposex phenomenon) (Chiavarini et al., 2003; Lemghich and Benajiba, 2007; El Mortaji et al., 2011). To date, however, much less attention has been paid to this species in ecotoxicology.

In this context, a multiparametric approach was implemented using more than one biomarker for the evaluation of the oxidative stress potential of *S. haemastoma* population. For this purpose, we chose to monitor seasonal variations in the rate of reduced glutathione (GSH), malondialdehyde (MDA), and metallothionein (Mt), as well as the specific activity of glutathione S-transferase (GST) and catalase (CAT) in this gastropod mollusc from three study sites: Cap de Garde (Annaba Gulf), Aouinète beach and Messida beach (El Kala coast) in the East coastal zone of Algeria.

Despite the importance and the large diversity of gastropods and their ability to bioaccumulate TME, they only interested few ecotoxicological studies (Yüzereroğlu et al., 2009; Belhaouari et al., 2011; Rabaoui et al., 2013; Boulajfene et al., 2017). This led us to conduct a seasonal study on the accumulation of Cu, Zn, Cd and Pb in the digestive gland of the same species and to analyze these same TME as well as Chromium (Cr) in the seawater to assess the environmental quality of the marine ecosystem.

2. Materials and methods

2.1. Sampling sites

The study area corresponds to the extreme northeastern part of the Algerian coastline (extreme southeast of the Mediterranean), which is bounded on the west by Cap de Garde and on the east by Cap Segleb and includes the Annaba Gulf and the El Kala coastal zone (Fig. 1). The Annaba Gulf is a bay open to the Mediterranean Sea on the north, bounded by two headlands: Rosa to the east (8°15′E, 36°58′N) and Garde to the west (7°47′E, 36°58′N), which are approximately 40 km apart with a maximum depth not exceeding 65 m (Sifi et al., 2007; Belabed et al., 2008; Belabed et al., 2013b; Amri et al., 2017a). The Annaba Gulf receives fresh water through two wadis: the Mafrag in the east and the Seybouse in the