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Metals and As content in sediments and Manila clam *Ruditapes philippinarum* in the Tagus estuary (Portugal): Impacts and risk for human consumption

Stefania Chiesa[a], Paula Chainho[b], Ângela Almeida[a], Etelvina Figueira[a], Amadeu M.V.M. Soares[a], Rosa Freitas[a,*]

[a] Department of Biology, CESAM, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal
[b] MARE, Marine and Environmental Sciences Centre, Faculty of Sciences, Lisbon University, Campo Grande, 1749-016 Lisbon, Portugal

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ABSTRACT

The Manila clam is emerging as a relevant species for the Portuguese market. The present work was conducted in the Tagus estuary to evaluate 1) the metals and As content in the sediments of the Tagus estuary, especially on those areas subjected to Manila clam harvesting 2) the metals and As content in clams, and the risk associated with their consumption 3) the physiological and biochemical responses of the clam to metals and As contamination, and its possible role as a pollution bioindicator in the estuarine environment. The most contaminated sediments were identified nearby industrial areas, nevertheless clams collected in low contaminated areas showed high metals and As concentrations. The condition index, glycogen content, membrane oxidative damage, biotransformation enzymes and metallothioneins showed consistent responses to metals and As content in clams. Results emphasize the need for the development of a management plan for the species exploitation in the Tagus estuary.

1. Introduction

Among coastal ecosystems, estuaries are considered highly productive areas, although deeply impacted by anthropogenic pressure (Covelli et al., 2012). They represent complex and dynamic aquatic environments, frequently exploited for fisheries, aquaculture, industries, transport and recreational activities (Lei et al., 2016). Estuaries worldwide are also impacted by discharges from urban sites, since pollutants are transported by the rivers and then into the sea through estuaries (Mitchell et al., 1999). As a consequence, these environments are constantly exposed to a wide variety of pollutants, as metals and metalloids (Hoffman et al., 2002), organic (Cardoso et al., 2016) and new emerging contaminants, such as pharmaceuticals (Heberer, 2002) and micro plastics (Zhao et al., 2015).

Metals and metalloids have demonstrated to be hazardous pollutants, due to their persistency, toxicity and bioaccumulative characteristics (Du Laing, 2011; Velez et al., 2015a). Considering that sediments of coastal ecosystems can act as a sink for metals and metalloids, the benthic fauna, closely in contact with the sediments, is particularly vulnerable to their contamination (Elliott and Quintino, 2007). Metals and metalloids are readily bioaccumulated by benthic fauna, including bivalves (Freitas et al., 2012; Velez et al., 2015a; de Oliveira et al., 2016; Gao et al., 2016; Vazquez-Luis et al., 2016) and polychaetes (Dean, 2008; Freitas et al., 2012), inducing toxic effects.

Metals and metalloids are considered typical environmental pollutants with prooxidant effects, due to their potential to induce the intracellular formation of reactive oxygen species (ROS) (Regoli and Giuliani, 2014). In fact, biochemical markers, including oxidative stress markers, became popular tools for detecting early toxic effects in aquatic organisms caused by these inorganic elements (e.g. Monserrat et al., 2007; Matozzo et al., 2012; Moschino et al., 2012; Velez et al., 2015b). The comparison between biochemical performance obtained from the same species in different environments will allow to assess the toxic impacts induced by different pollution types and/or levels and the effectiveness of the biological responses to specific environmental conditions (Chandurvelan et al., 2015; Velez et al., 2016a).

Bivalves have been recognized as appropriate bioindicator and/or sentinel species of environmental quality in aquatic systems (Cajaraville et al., 2000), not only due to their filter feeding behavior and benthic ecology (for a complete list of references, see Velez et al., 2016a) but also due to their commercial importance as seafood. Among bivalve species, the Manila (Asari) clam *Ruditapes philippinarum* (Adams & Reeve, 1850) is gaining increasing attention due to its wide geographic distribution and its ecological and economic relevance (Moschino et al., 2012). As a bivalve, the Manila clam presents high ability to accumulate elements in its tissues, with known capacities as bioindicator of

* Corresponding author.
E-mail address: rosafreitas@ua.pt (R. Freitas).

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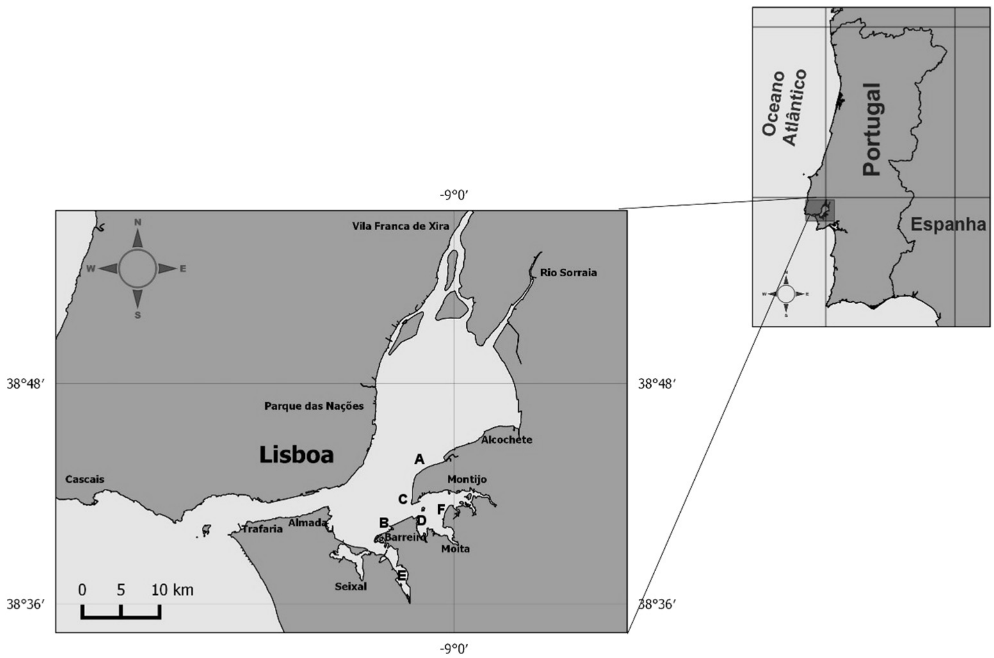

Fig. 1. Six sampling areas in the Tagus estuary (A–F).

elements pollution in estuarine environments (Sfriso et al., 2008; Matozzo et al., 2012; Moschino et al., 2012; Yang et al., 2013; Velez et al., 2015a,b). Its worldwide distribution is strictly connected to its economic relevance for fisheries and aquaculture: originally distributed in the Indo-Pacific region, specifically in Japan, Korea and China (Gosling, 2003), Manila clam was introduced into the west coast of North America, Atlantic (Portugal, France, Spain, Ireland, England) and Mediterranean European coasts (France, Italy) (Gosling, 2003). As for Portugal, the Manila clam was firstly detected in 1984 in Ria Formosa (Algarve) (Ruano and Sobral, 2000) and, since then, established populations have been reported in estuarine systems all over the country including in the Óbidos lagoon (Velez et al., 2015b), Albufeira lagoon (Gaspar, 2010), Ria de Aveiro (e.g. Figueira et al., 2012), the Sado estuary (Gaspar, 2010), and the Tagus estuary (Gaspar, 2010; Chainho et al., 2015). Today this species represents one of the most important commercial bivalve, with the highest economic value among aquaculture shellfish products (FAO, 2013). A special concern regarding the occurrence of Manila clam in the Tagus estuary is its wider distribution and increasing abundance in recent years (Ramajal et al., 2016).

The occurrence of intensive Manila clam fishing - especially with mechanical dredges - can have negative effects on environments, particularly concerning impacts on benthic communities, sediment resuspension and increase of water turbidity (Pranovi et al., 2003; Pranovi et al., 2004). These effects have been already observed in Venice Lagoon, where intense Manila clam collection has been carried out for > 20 years, with dramatic effects on the lagoon environment, followed by a collapse of the clam production (see Boscolo Brusà et al., 2013 for a complete list of reference). Manila clam fishing in the Tagus Estuary is lacking a specific regulation, and many of illegal harvesters are actually involved in its collection and commerce (Ramajal et al., 2016). Moreover, the occurrence of illegal fishing can determinate a serious risk for human consumption, due to clam collection in microbiology or chemically polluted areas. It is well known that elements contamination in edible bivalves can determinate bioaccumulation in the trophic chain, especially in human consumers (Figueira et al., 2011) with consequent poisoning effects (Santos et al., 2014).

Taking into account the intense harvesting and commercial exploitation of Manila clam currently ongoing in the Tagus estuary, and the scarce informations regarding the environmental characterization of the exploited areas in terms of metals and As pollution, there is an urgent need to investigate the contamination levels in clams at the harvesting areas, and to reveal their use as bioindicator species and role as source of pollutants along the ecosystems trophic chain.

In this way, the aims of the present work were 1) to evaluate sediments contamination in the Tagus estuary, namely in terms of metals and As, within the areas used for Manila clam harvesting; 2) to quantify the metals and As content in the Manila clam, and the human health risks associated with its consumption; 3) to assess the physiological and biochemical performance of the species to different elements and concentration levels, and its possible role as a bioindicator of pollution in the Tagus estuary.

2. Materials and methods

2.1. Study area

The Tagus estuary is one of the largest estuaries in Europe, covering an area of 320 km^2: 40% of the estuary is composed of intertidal mudflats, and its eastern and southern areas contain extensive areas of salt marshes (Caçador et al., 1996). The main source of freshwater onto the Tagus estuary is the Tagus river, draining water from a total area of 86,629 km^2, and representing the second most important hydrological basin in the Iberian Peninsula (Duarte and Caçador, 2012) in terms of dimensions. The Tagus estuary also represents a major seaport, hosting both commercial and fishing activities (Chainho et al., 2014; Duarte et al., 2014). This system used to be adversely affected by untreated urban sewage discharges, together with industry (chemicals, petrochemicals, metallurgic, shipyard and repair and cement manufacture) and agriculture-fertilizers and pesticides (Duarte et al., 2014). Although the treatment of both industrial and urban effluents has been improved in the latest decades (Chainho et al., 2014; Duarte and Caçador, 2012), this area represents a sink of historically accumulated metals and metalloids (Caçador et al., 1996). Previous studies identified high levels of zinc in the surface sediments (Vinagre et al., 2008), chromium, nickel, cobalt and also mercury (Vale et al., 2008; Mil-Homens et al., 2014). Moreover, elements like cadmium, copper, lead and zinc exhibited enhanced aluminium ratios mirroring the presence of anthropogenic local inputs associated with harbour, urban and industrial activities (Mil-Homens et al., 2014).

Six different areas located in the Southern branch of the Tagus