Marine Pollution Bulletin 111 (2016) 476–482

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Baseline

Heavy metal distribution in organic and siliceous marine sponge tissues measured by square wave anodic stripping voltammetry

S. Illuminati, A. Annibaldi *, C. Truzzi, G. Scarponi

Department of Life and Environmental Sciences, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy

A R T I C L E I N F O

Article history:
Received 20 May 2015
Received in revised form 20 June 2016
Accepted 28 June 2016
Available online 4 July 2016

Keywords:
Heavy metals
Sponges
Antarctica
Mediterranean Sea
Bioaccumulation

A B S T R A C T

May sponge spicules represent a “tank” to accumulate heavy metals? In this study we test this hypothesis determining the distribution of Cd, Pb and Cu concentrations between organic and siliceous tissues in Antarctic Demospongia (*Sphaerotylus antarcticus*, *Kirkpatrikia coulmani* and *Haliclona* sp.) and in the Mediterranean species *Petrosia ficiformis*. Results show that although, in these sponges, spicules represent about 80% of the mass content, the accumulation of pollutant is lower in the spicules than in the corresponding organic fraction. The contribution of tissues to the total sponge content of Cd, Pb and Cu is respectively 99%, 82% and 97% for Antarctic sponges and 96%, 95% and 96% for *P. ficiformis*, similar in polar and temperate organisms. These results pave the way to a better understanding of the role of marine sponges in uptaking heavy metals and to their possible use as monitor of marine ecosystems, recommend by the Water Framework Directive.

Heavy metal pollution is a challenging problem for marine ecosystems. These substances are discharged into the sea by anthropic activities and their monitoring is strongly advocated by the regulation in force (European Parliament and Council of European Union, 2000) with the aim to maintain a healthy state and a good ecological and chemical status. The Water Framework Directive (WFD) (European Parliament and Council of European Union, 2000) requires the Member States of European Union to reach this status by 2015; assessing whether contamination levels comply with the Environmental Quality Standards (EQSs), and to monitor contamination trends for priority substances, using integrating matrices for bioaccumulative substances (Perez et al., 2005; Besse et al., 2012).

Filter-feeding invertebrates (e.g. tunicates, polychaetes, barnacles) are often selected to monitor trace metal contamination as they are useful tools to assess the biological impact of pollution (Davis et al., 2014). Among these, sponges represent a good biomarker thanks to their characteristics: sessility, readily available, abundance, long-living organisms, availability for sampling, high tolerance when exposed to environmental problems and a strong accumulation of metal (de Mestre et al., 2012; Batista et al., 2014).

In Antarctica, where sponges represent an essential component of benthic communities (Cattaneo-Vietti et al., 2000; Downey et al., 2012), metal trace contamination occurs in different matrices and can be influenced both by anthropogenic input of normal scientific activity and also by input from industrialized regions through atmospheric circulation and marine currents (Scarponi et al., 1995, 1997a; Barbante et al., 1998; Annibaldi et al., 2007; Bargagli, 2008). The Demospongiae are the largest class in the phylum Porifera, it includes approximately 90% of all the species of sponges (Hooper and Van Soest, 2002). Their skeletons are generally made of siliceous spicules secreted around a proteinaceous filament called silicatein (Armirotti et al., 2009) and/or collagen (Pozzolini et al., 2011).

Many species of Demospongiae are reliable bioindicators of metal contamination because they filter large amounts of water, collecting contaminants from both dissolved and suspended phases (Reiswig, 1971; Ribes et al., 1999; Perez et al., 2004; Genta-Jouve et al., 2012; Turon et al., 2014). Demospongiae were largely used worldwide to monitor coastal ecosystems (Patel et al., 1985; Verdenal et al., 1990; Hansen et al., 1995; Philp et al., 2003; Perez et al., 2004, 2005; Rao et al., 2006, 2007, 2009; Pan et al., 2011; de Mestre et al., 2012).

In Antarctica few studies have been carried out on trace metal concentration in marine sponges (Capon et al., 1993; Negri et al., 2006) and limited to the content in organic tissues. In this area of interest we have recently published the first results about heavy metals content in spicules of different specimens of Antarctic sponges (Annibaldi et al., 2011; Truzzi et al., 2008).

No papers compare the distribution of metals between sponge tissue and siliceous spicules.

This feature could have an important scientific resonance because a recent paper (Batista et al., 2014), hypothesizes that differences in metal accumulation between sponges could be related to their skeletal composition and for this reason it suggests demosponges more suitable as heavy metal bioindicators, than calcareous sponges: in fact demosponges present higher collagen content in the mesohyl

* Corresponding author at: Department of Life and Environmental Sciences, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy.
E-mail address: a.annibaldi@univpm.it (A. Annibaldi).

http://dx.doi.org/10.1016/j.marpolbul.2016.06.098

(Klautau et al., 2004) allowing them to accumulate more elements than calcified sponges can do. However other species could be analyzed to support and validate this hypothesis.

Although organic tissues have been extensively studied, here we tested the hypothesis that spicules may also represent a sort of "tank" to accumulate heavy metals. We also addressed the following questions:

1) May exhalant areas (oscula) of sponges accumulate more contaminants than other areas of the sponge body can do?
2) May this pattern of heavy metal bioaccumulation be different between polar and temperate sponges? Could possible differences be related to different levels of metals in seawater or to a species-specific accumulation?

To answer these questions we present in this work, for the first time, a preliminary study on the distribution of three metals (Cd, Pb and Cu) between organic and siliceous tissues in the Antarctic Demospongiae specimens *Sphaerotylus antarcticus*, *Kirkpatrickia coulmani*, *Haliclona* sp. and, in addition, a comparison with two Mediterranean species: the siliceous *Petrosia ficiformis* and the protein-containing sponge, *Spongia officinalis*.

Heavy metals in Antarctic and Mediterranean seawater were determined contextually to provide useful data to calculate the bioconcentration factors; as a matter of fact, experimental studies (Richelle-Maurer et al., 1994; Hansen et al., 1995; Cebrian et al., 2003; Perez et al., 2003) have shown that accumulation is a function of the metal quantity in the environment and that bioaccumulation factors may be very high.

Cd, Pb and Cu have been selected for this study because two of them (Cd and Pb) are considered priority pollutants (PP) by the regulation in force (European Parliament and Council of European Union, 2000; Ministero dell'ambiente e della tutela del territorio e del mare, 2006) and the third one (Cu) is an element of interest, being a micronutrient for these organisms and therefore with potential differences on bioaccumulation in tissues. Square Wave Anodic Stripping Voltammetry (SWASV), used in this work, is a suitable technique for the determination of very low traces of these metals. This technique, optimized in a previous work (Truzzi et al., 2008) for the simultaneous determination of Cd, Pb and Cu in siliceous tissues was set up, in this paper, for the analyses of organic fractions.

During the Antarctic Campaign in December 2005–January 2006, sample of *S. antarcticus*, *K. coulmani and Haliclona* sp. were collected in Tethys Bay (74°41′25″ S, 164°06′07″ E), very close to the "Mario Zucchelli" Station at Terra Nova Bay, Ross Sea, Northern Victoria Land. The sponges were collected by hand at a depth of about 5 m; plastic gloves and no metallic instruments were used in order to avoid metal contamination. After collection, the sponge was immediately frozen to −20 °C and stored until analysis. The sponges *P. ficiformis* and *S. officinalis*, used for comparison, were selected because they are ubiquitous in the Mediterranean Sea and well characterized (Bavestrello et al., 1994). They were collected by hand near the rocky cliffs of the Portofino promontory (Ligurian Sea, Italy, depth ~ 15 m).

Water samples required to evaluate the total concentration of Cd, Pb and Cu in seawater were collected nearby the sites where the sponge samples were also collected using a 10-L acid-cleaned Go-Flo sampling bottle. Each seawater sample was frozen at −20 °C and stored until analysis; before analysis samples were filtered (0.45 μm pore size) and acidified with ultrapure HCl (2 mL acid in 1000 mL seawater, pH ~1.5) to determine dissolved metal content (Annibaldi et al., 2015).

All sponges were thawed and cut in the clean room laboratory (Italy). The sample *S. antarcticus* was separated into oscula and the respective bodies, i.e. bodies that are physically attached under oscula. Oscula are orifices of the digestive system of sponges through which water inhaled from pores can escape. To evaluate the homogeneity of metal concentrations in samples, six sub-samples were collected for each sponge (*S. antarcticus*, *K. coulmani*, *Haliclona* sp., *P. ficiformis* and *S. officinalis*). About 1-cm depth samples (both bodies and oscula), including the surface, were cut (using an acid-decontaminated scalpel) and weighed (about 1 g, wet weight). Samples were then dried to constant weight (± 0.2 mg) inside a desiccator located in an ISO Class 5 laminar flow area (water content 75–80% for *P. ficiformis* and *S. officinalis*, around 60% for *S. antarcticus*).

The organic compound of the sponges were digested with 5.00 mL superpure HNO_3 7.3 M for 48 h. Spicules in the digested solution were then separated by centrifugation and treated for final analysis as explained elsewhere (Truzzi et al., 2008; Annibaldi et al., 2011). The supernatant solution of HNO_3 was diluted 200 times with ultrapure water before voltammetric analysis (final pH ~ 1.2).

Dry organic tissues weight was determined by subtracting the spicules dry weight (d.w.) to the overall sponge mass (d.w.). The percentage of the total (d.w.) of the sponge represented by spicules is dominant in all sponges studied (except *K. coulmani*): *S. antarcticus* 75 ± 6%, *K. coulmani* (49 ± 4%), *Haliclona* sp. (62 ± 7%) and *P. ficiformis* 73 ± 7%. *S. officinalis* is constituted only of organic tissue.

Laboratory, apparatus, reagents and procedures used in this work were described in detail elsewhere (Annibaldi et al., 2011; Truzzi et al., 2008). A set-up of principal voltammetric parameters were done to optimize the procedure for the analysis of organic tissue, using 10-mL digested solution of *S. antarcticus*.

To select the optimal deposition potential for the determination of Cd, Pb and Cu in HNO_3 solution, pseudopolarographic experiments were carried out, by varying the deposition potentials and recording the respective peak currents. The results obtained (Fig. 1) showed that the pseudopolarographic half-wave potential for the three metals were about −750 mV for Cd, −500 mV for Pb and −300 mV for Cu.

From the wave shapes a deposition potential of −1000 mV was selected for the simultaneous determination of Cd, Pb and Cu.

The thin mercury film electrode (TMFE) was prepared by electrochemical deposition each day and tested according to a procedure reported elsewhere (Truzzi et al., 2008).

The optimal, minimum time required for the electrochemical cleaning of the TMFE at the end of each voltammetric scan was determined by measuring the peak current (i_p) of Cd (the most concentrated metal) after the cleaning step carried out at −50 mV for 0 to 5 min, in a new voltammetric scan performed without metal deposition. The following results were obtained ($t_{cleaning}$ in min, i_p in nA ± SD in nA): 0, 79 ± 4; 1, 60 ± 3; 2, 47 ± 5; 3, 24 ± 2; 4, 20 ± 1; 5, 15 ± 1). It can be noted that after 4 min the Cd peak current reduced by about 4-folds, and this value is negligible compared with metal content in sponge tissue (<1%). In any case, to be safe a cleaning time of 5 min was chosen for all the following experiments.

Metal determination in seawater was carried out using the optimized SWASV procedure (Truzzi et al., 2002).

The accuracy of the electrochemical procedure for organic tissues was tested using the Certified Reference Materials dogfish muscle DORM-2 and Antarctic Krill MURST-ISS-A2. The experimental values obtained are reported in Table 1; measured concentrations of Cd, Pb

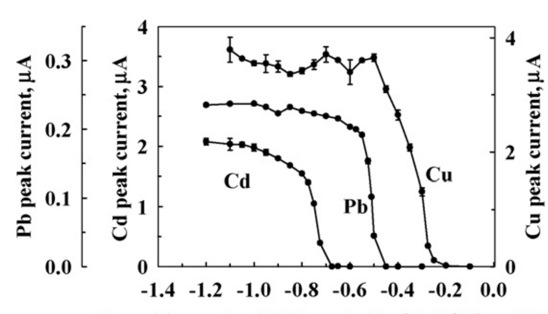

Fig. 1. Pseudopolarograms for Cd, Pb and Cu in the HNO_3 digested *S.antarcticus* diluted 200 times with ultrapure water.