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**Highlights**

- > First data of metal distribution in tissues of Antarctic and Mediterranean sponges
- > Cd Pb and Cu higher concentrations in organic than siliceous tissues
- > Similar bioaccumulation ability in polar and temperate organisms
- > Use of marine sponges as monitors of marine ecosystem in line with WFD

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

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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; Scarponi et al., 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

1 dissolved and suspended phases (Reiswig, 1971; Ribes et al., 1999; Perez et al., 2004;  
2 Genta-Jouve et al., 2012; Turon et al., 2014). Demospongiae were largely used  
3 worldwide to monitor coastal ecosystems (Patel et al., 1985; Verdenal et al., 1990;  
4 Hansen et al., 1995; Philp et al., 2003; Perez et al., 2004; Perez et al., 2005; Rao et  
5 al., 2006; Rao et al., 2007; Rao et al., 2009; Pan et al., 2011; de Mestre et al., 2012).

6  
7 In Antarctica few studies have been carried out on trace metal concentration in  
8 marine sponges (Capon et al., 1993; Negri et al., 2006) and limited to the content in  
9 organic tissues. In this area of interest we have recently published the first results  
10 about heavy metals content in spicules of different specimens of Antarctic sponges  
11 (Annibaldi et al., 2011; Truzzi et al., 2008).

12  
13 No papers compare the distribution of metals between sponge tissue and siliceous  
14 spicules.

15  
16 This feature could have an important scientific resonance because a recent paper  
17 (Batista et al., 2014), hypothesizes that differences in metal accumulation between  
18 sponges could be related to their skeletal composition and for this reason it suggests  
19 demosponges more suitable as heavy metal bioindicators, than calcareous sponges: in  
20 fact demosponges present higher collagen content in the mesohyl (Klatau et al, 2004)  
21 allowing them to accumulate more elements than calcified sponges can do. However  
22 other species could be analyzed to support and validate this hypothesis.

23  
24 Although organic tissues have been extensively studied, here we tested the hypothesis  
25 that spicules may also represent a sort of “tank” to accumulate heavy metals. We also  
26 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 *Petrosia ficiformis* and *Spongia 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).

1 All sponges were thawed and cut in the clean room laboratory (Italy). The sample  
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3 *S. antarcticus* was separated into oscula and the respective bodies, i.e. bodies that are  
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5 physically attached under oscula. Oscula are orifices of the digestive system of  
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7 sponges through which water inhaled from pores can escape. To evaluate the  
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9 homogeneity of metal concentrations in samples, six sub-samples were collected for  
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11 each sponge (*S. antarcticus*, *K. coulmani*, *Haliclona sp.*, *P. ficiformis* and *S.*  
12  
13 *officinalis*). About 1-cm depth samples (both bodies and oscula), including the  
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15 surface, were cut (using an acid-decontaminated scalpel) and weighed (about 1 g, wet  
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17 weight). Samples were then dried to constant weight ( $\pm 0.2$  mg) inside a desiccator  
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19 located in an ISO Class 5 laminar flow area (water content 75–80% for *P. ficiformis*  
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21 and *S. officinalis*, around 60% for *S. antarcticus*).  
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32 The organic compound of the sponges were digested with 5.00 ml superpure  $\text{HNO}_3$   
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34 7.3 M for 48 hours. Spicules in the digested solution were then separated by  
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36 centrifugation and treated for final analysis as explained elsewhere (Truzzi et al.,  
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38 2008; Annibaldi et al., 2011). The supernatant solution of  $\text{HNO}_3$  was diluted 200  
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40 times with ultrapure water before voltammetric analysis (final pH  $\sim 1.2$ ).  
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46 Dry organic tissues weight was determined by subtracting the spicules dry weight  
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48 (d.w.) to the overall sponge mass (d.w.). The percentage of the total (d.w.) of the  
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50 sponge represented by spicules is dominant in all sponges studied (except *K.*  
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52 *coulmani*): *S. antarcticus*  $75 \pm 6$  %, *K. coulmani* ( $49 \pm 4$  %), *Haliclona sp* ( $62 \pm 7$  %).and  
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54 *P. ficiformis*  $73 \pm 7$  %. *S. officinalis* is constituted only of organic tissue.  
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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<sub>3</sub> 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 ( $t_{\text{cleaning}}$  in min,  $i_p$  in nA  $\pm$ SD in nA): 0, 79 $\pm$ 4; 1, 60 $\pm$ 3; 2, 47 $\pm$ 5; 3, 24 $\pm$ 2; 4, 20 $\pm$ 1; 5, 15 $\pm$ 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.

1 Metal determination in seawater was carried out using the optimized SWASV  
2 procedure (Truzzi et al., 2002).  
3

4  
5 The accuracy of the electrochemical procedure for organic tissues was tested using  
6 the Certified Reference Materials dogfish muscle DORM-2 and Antarctic Krill  
7 MURST-ISS-A2. The experimental values obtained are reported in Table 1;  
8 measured concentrations of Cd, Pb and Cu are in agreement with certified values  
9 within experimental errors (no statistically significant differences between certified  
10 and measured values, p-values generally >0.05), showing a good accuracy of  
11 measurements (STATGRAPHICS, 2000).  
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14 Moreover, in a further effort to ascertain accuracy, the analytical results obtained  
15 using the present SWASV procedure were compared with those obtained with the  
16 more established Differential Pulse Anodic Stripping Voltammetry (DPASV)  
17 method. Good consistency (p-values >0.05) was obtained in the intercomparison  
18 (n=4) of DPASV with SWASV for analysis of organic tissue from *S. antarcticus*  
19 (DPASV vs. SWASV): Cd  $85 \pm 9 \mu\text{g g}^{-1}$  vs  $84 \pm 3 \mu\text{g g}^{-1}$ ; Pb  $6.2 \pm 0.3 \mu\text{g g}^{-1}$  vs  $5.5 \pm 0.8$   
20  $\mu\text{g g}^{-1}$ ; Cu  $18 \pm 5 \mu\text{g g}^{-1}$  vs  $17 \pm 1 \mu\text{g g}^{-1}$ .  
21  
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23 The accuracy of the procedure for the analysis of sponge siliceous tissues and  
24 seawater samples was tested in a previous study (Truzzi et al., 2008).  
25

26 In the following section we discuss the concentrations of metals in the studied  
27 sponges. Since the water content in sponge samples may differ, and to ensure data  
28 comparability, all the results are reported as d.w. (mean  $\pm$  standard deviation (SD)).  
29

30 Results are reported in Tables 2 and 3. Concentrations are calculated as follows:  
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1.  $\mu\text{g g}^{-1}$  of spicules or tissues dry weight, to compare the accumulation capability between the two different components.
2.  $\mu\text{g g}^{-1}$  of total sponge dry weight, to study the contribution of tissues and spicules to the total concentration of heavy metals.

Regarding metal content in bodies of Antarctic sponges (*S. antarcticus*, *K. coulmani* and *Haliclona sp.*) we can note that cadmium concentration in tissues is homogeneous in all species (RSD 4-11%) with concentration higher in *K. coulmani* ( $174 \pm 7 \mu\text{g g}^{-1}$  tissue d.w.) and *S. antarcticus* ( $84 \pm 3 \mu\text{g g}^{-1}$  tissue d.w.) compared to *Haliclona sp.* ( $8.9 \pm 1.0 \mu\text{g g}^{-1}$  tissue d.w.) with similar differences in Cd content in spicules. The concentration in tissues respect to spicules is higher for all species, 90x *S. antarcticus* and ~300x for *K. coulmani* and *Haliclona sp.*

Considering the organism as whole Cd content in tissues represents the 97% for *S. antarcticus* and ~99% for *K. coulmani* and *Haliclona sp.*; so Cd accumulates much more in tissues even though the mass content of spicules in these sponges is much higher (50-75%) (details in Annibaldi et al, 2011). Lead concentration in tissues of Antarctic sponges varies from  $0.94 \pm 0.06 \mu\text{g g}^{-1}$  of *Haliclona sp.* to  $4.2 \pm 0.2 \mu\text{g g}^{-1}$  of *K. coulmani* and  $5.5 \pm 0.8 \mu\text{g g}^{-1}$  of *S. antarticus* (d.w., Tab. 2) with a good homogeneity between samples (RSD% 5-14%).

Tissues contain about 20x, 8x and 5x Pb more than spicules respectively for *S. antarticus*, *K. coulmani* and *Haliclona sp* (Table 2) giving a contribution to the total lead content of 86% (*S. antarticus*), 90% (*K. coulmani*) and 70% (*Haliclona sp.*).

Copper concentration in tissues of Antarctic sponges is very homogeneous for all species (RSD 4-6%, see Table 2) with mean values that vary from  $17 \pm 1 \mu\text{g g}^{-1}$  tissue d.w. of *S. antarcticus* to  $24 \pm 1 \mu\text{g g}^{-1}$  tissue d.w. of *Haliclona sp.* up to  $85 \pm 5 \mu\text{g g}^{-1}$  tissue d.w. of *K. coulmani*.

Cu also accumulates in tissues rather than in spicules (50x for *S. antarcticus*, 104x for *K. coulmani*, 71x for *Haliclona sp.*), contributing to whole sponge concentration for 94% in *S. antarcticus*, 99% in *K. coulmani* and 97% in *Haliclona sp.*

For *S. antarcticus* the same treatments made for bodies were also carried out for oscula samples (Tab. 2). The Cd concentration measured in tissue ( $82 \pm 12 \mu\text{g g}^{-1}$ ) is 170 times higher than spicule content ( $0.48 \pm 0.03 \mu\text{g g}^{-1}$  spicules d.w.); considering the whole sponge this fraction represents  $\sim 99\%$  of total Cd ( $25 \pm 3 \mu\text{g g}^{-1}$  tissue d.w. vs.  $0.30 \pm 0.10 \mu\text{g g}^{-1}$  spicules d.w.)

Even for lead, accumulation in oscula is mainly in the organic component ( $6.1 \pm 0.7 \mu\text{g g}^{-1}$  d.w)  $\sim 14$  times higher than spicule content ( $0.44 \pm 0.17 \mu\text{g g}^{-1}$ ) with an homogenous distribution in sub-samples (RSD% 11%); the contribute of organic tissue to the total Pb metal content in whole sponge is about 90%, a percentage slightly lower than the cadmium contribute (99%, Table 2).

Cu concentration is higher in tissues than in siliceous component, too, of about 80 times ( $19 \pm 2 \mu\text{g g}^{-1}$  tissue d.w vs.  $0.24 \pm 0.02 \mu\text{g g}^{-1}$  spicules d.w., Table 2) with homogenous values for both ones (RSD  $\sim 10\%$ ). When the organism as the whole is considered, the Cu contribution due to organic tissue is the most important (97%) with  $5.6 \pm 1.3 \mu\text{g g}^{-1}$  d.w., 35 times higher than the contribution from spicules

( $0.16 \pm 0.02 \mu\text{g g}^{-1}$  tissue d.w.)(Table 2). Even for oscula samples, heavy metals can accumulate more easily in the organic matrix.

In *S. antarcticus* bodies and oscula show approximately the same concentration levels of the three heavy metals in organic tissue. Indeed we found  $84 \pm 3 \mu\text{g g}^{-1}$  d.w. and  $82 \pm 12 \mu\text{g g}^{-1}$  d.w. for Cd;  $5.5 \pm 0.8 \mu\text{g g}^{-1}$  d.w. and  $6.1 \pm 0.7 \mu\text{g g}^{-1}$  d.w. for Pb;  $17 \pm 1 \mu\text{g g}^{-1}$  d.w. and  $19 \pm 2 \mu\text{g g}^{-1}$  d.w. for Cu in tissue samples (see Table 2). No significant differences were found for the three metals;  $p$ -values for two-sided  $t$ -test are  $>0.05$  for Cd, Pb and Cu ( $p_{\text{Cd}}=0.80$ ,  $p_{\text{Pb}}=0.19$ ,  $p_{\text{Cu}}=0.17$ ), respectively. Considering the contribution given by the organic fraction to the total metal content in sponge, very close values were revealed: percentages of metal in bodies and oscula are  $97 \pm 1$  and  $99 \pm 1$  for Cd;  $86 \pm 4$  and  $85 \pm 2$  for Pb;  $94 \pm 1$  and  $97 \pm 1$  for Cu. Therefore, a similar trend is found in the bioaccumulation of heavy metals in bodies and oscula, which is unrelated to the specific biological function of the examined parts.

Both in bodies and corresponding oscula, metal concentrations are higher in the organic component than in the siliceous part. In the following section we discuss metal concentrations in tissues and spicules (where present) of the Mediterranean sponges, *P. ficiformis* and *S. officinalis*: results are reported in Table 3.

In *P. ficiformis*, Cd shows a mean value of  $7.5 \pm 2.5 \mu\text{g g}^{-1}$  (RSD 33%) for organic tissue, 100 times higher than spicules ( $0.071 \pm 0.007 \mu\text{g g}^{-1}$ ). Considering the entire organism Cd content in organic tissues gives a contribution of 97% (i.e.  $1.7 \pm 0.2 \mu\text{g g}^{-1}$  d.w. against the  $0.053 \pm 0.011 \mu\text{g g}^{-1}$  d.w.).

1 Lead in organic tissue shows a non-homogeneous distribution ( $1.7 \pm 0.6 \mu\text{g g}^{-1}$  d.w.,  
2 RSD 35%) in contrast with Pb in spicules ones (i.e.  $0.025 \pm 0.003 \mu\text{g g}^{-1}$ , RSD 12%)  
3 with a concentration  $\sim 70$  times higher than the siliceous tissue. In fact, the  
4 contribution of organic tissue related to whole sponge is about 96% ( $0.42 \pm 0.19 \mu\text{g g}^{-1}$   
5 tissue d.w. against  $0.018 \pm 0.002 \mu\text{g g}^{-1}$  spicules d.w).  
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8 Even for Cu we observe higher concentration in organic tissues ( $134 \pm 57 \mu\text{g g}^{-1}$  tissue  
9 d.w., RSD 42%) (Table 3) than in siliceous components ( $1.3 \pm 0.1 \mu\text{g g}^{-1}$  spicules d.w.)  
10 or about 100 times. The tissue contribution to Cu in total sponge is 97%, with  $30 \pm 8$   
11  $\mu\text{g g}^{-1}$  d.w.; 30 times higher than the contribution due to spicules ( $1.0 \pm 0.2 \mu\text{g g}^{-1}$   
12 tissue d.w.).  
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15 For all three metals, a non-homogeneous content is measured in organic tissues: this  
16 is probably due to the presence of symbiont such as cyanobacteria (Arillo et al.,  
17 1993); in fact the presence of microorganism in these invertebrates can play an  
18 important role in the process of heavy metal accumulation (Genta-Jouve et al., 2012).  
19 In *S. officinalis* the concentration of Cd is fairly homogeneous (RSD 7%) in the  
20 sample analysed and is around  $0.27 \pm 0.02 \mu\text{g g}^{-1}$ , the same order of magnitude of the  
21 other Mediterranean sponge (Table 3).  
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24 Even for Pb the concentration is quite homogeneous in the sub-samples ( $0.47 \pm 0.05$   
25  $\mu\text{g g}^{-1}$ ) (RSD 11%), with values very close to the *P. ficiformis* content ( $0.42 \pm 0.19 \mu\text{g g}^{-1}$ ).  
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28 Copper presents a mean value of about  $42 \pm 2 \mu\text{g g}^{-1}$  (RSD 5%) (Table 3), comparable  
29 to concentration ( $30 \pm 8 \mu\text{g g}^{-1}$ ) measured in *P. ficiformis*.  
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1 The comparison between Antarctic and Mediterranean sponges shows that in all  
2 sponges studied tissues shows higher concentrations than siliceous spicules (see  
3 Tables 2 and 3). Considering tissues, Mediterranean sponge *P. ficiformis* shows a  
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5 lower concentration of Cd and Pb than *S. antarcticus* and *K. coulmani* (10-20 times  
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7 for Cd and 5 times for Pb, respectively) but similar levels of *Haliclona sp.* Opposite  
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9 situation for Cu where higher concentrations were measured in Mediterranean sponge  
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11 of about 2-7 times than Antarctic ones.  
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19 Considering spicules, in the Mediterranean sponge *P. ficiformis*, generally (except  
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21 *Haliclona sp.*) lower concentrations of Cd and Pb (Table 3) of about one order of  
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23 magnitude with respect to *S. antarcticus* and *K. coulmani* (Table 2), but higher  
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25 concentrations of Cu, of about four times were found: this is the same concentration  
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27 trend noticed for the organic component. For the three metals, the differences  
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29 between tissue and spicule concentrations in the Mediterranean sponge are similar to  
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31 those found in Antarctic ones. This is a significant finding because it indicates that,  
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33 different species of Demospongiae, in deeply different kinds of ecosystems, show the  
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35 same behaviour related to accumulation ratio between organic and siliceous  
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37 component, with the major contribution for all three metals ascribable to tissues  
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39 (mean percentage of about 94% in all sponges, Tables 2 and 3).  
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51 Even in the case of *S. officinalis*, the Mediterranean sponge shows lower  
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53 concentrations of Cd and Pb than Antarctic ones, but generally higher concentrations  
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55 of Cu (except for *K. coulmani*).  
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For a complete overview metals in seawater were also determined. Antarctic seawater metal concentrations are reported in detail in Annibaldi et al, 2011; in brief these are as follows: Cd  $35 \pm 2 \text{ ng L}^{-1}$ ; Pb  $18 \pm 3 \text{ ng L}^{-1}$ ; Cu  $93 \pm 5 \text{ ng L}^{-1}$  (n=3-6). Regarding the seawater sampled at the time of collection of the Mediterranean sponges *P. ficiformis* and *S. officinalis*, the total metal concentrations are Cd  $13 \pm 3 \text{ ng L}^{-1}$ ; Pb  $23 \pm 2 \text{ ng L}^{-1}$ ; Cu  $600 \pm 52 \text{ ng L}^{-1}$  (n=3-6). All data are consistent with those obtained from the literature (Capodaglio et al., 1989; Capodaglio et al., 1991; Scarponi et al., 1997b; Capodaglio et al., 1998; Migon and Nicolas, 1998; Pesavento et al., 2001; Illuminati et al., 2010). Table 4 compares the bioaccumulation of metals of different Demospongiae in different ecosystems: our data, for both Antarctic and Mediterranean sponges, agree with previous studies. Generally higher content of Cd are measured in Antarctic sponge tissues, when compared with Mediterranean and other marine organisms, whereas Cu levels are generally higher in non-Antarctic sponges, especially these from the Mediterranean Sea. Except for *H. oculata*, significant concentrations of Pb in all specimens both from remote and anthropized areas were found.

To evaluate the capability of bioaccumulation of the studied species regarding the three metals determined in sponges, a bioconcentration factor (BF)(see Table 5) was calculated from the formula:  $\text{BF} = [\text{metal}] \text{ sponge tissue or spicule} / [\text{metal}] \text{ seawater}$ .

Table 5 reports the BF values for body (and oscula where present) of the species studied; these are compared to other Antarctic organisms; BF values for tissue do not show significant differences ( $p > 0.05$ ) between body and oscula.



Antarctic tissues present generally greater bioconcentration capability for Cd and Pb (BF of  $\sim 3200$  and  $\sim 300$   $[(\text{ng kg}^{-1})_{\text{tissue}}/(\text{ng L}^{-1})_{\text{seawater}}] \times 10^{-3}$ , respectively) (*Haliclona* *sp.* excluded) than for Cu that presents lower BF, about  $\sim 200$   $[(\text{ng kg}^{-1})_{\text{tissue}}/(\text{ng L}^{-1})_{\text{seawater}}] \times 10^{-3}$  (*K. coulmani* excluded). Comparison between spicules' and tissues' BF's clearly shows greater bioconcentration values in tissues of 1-2 orders of magnitude for all the metals examined in this study. The Mediterranean sponge *P. ficiformis* shows lower bioconcentration factors of  $\sim 5$ -9 times for Cd and  $\sim 4$ x Pb compared to *S. antarcticus* and *K. coulmani* and conversely an higher content compared to *Haliclona* *sp.*; these results highlight a species-specific bioaccumulation for these metals. The same trend is observed for spicules' BF with a factor of 3-5 times for Cd and about 20 times for Pb, higher in Antarctic sponges *S. antarcticus* and *K. coulmani* (Table 5). For Cu, comparable BF values are found for both tissues and spicules in Antarctic (*K. coulmani* excluded) and in *P. ficiformis* tissues; BF's values are around 200 and it is likely due to the particular feature of Cu as micronutrient for sponges and, therefore, it could be accumulated in similar way even in different ecosystems. Comparison between organic tissues of Antarctic sponges and other Antarctic organisms (*L. ellittica* and *T. bernacchii*) shows greater bioaccumulation in sponges especially for Cd (*Haliclona* *sp.* excluded) and Cu (4x-8x for Cd and 2x for Cu) For lead (*Haliclona* *sp.* excluded) a slight bioaccumulation than *T. bernacchii* is found in sponges; conversely *L. ellittica* presents 1.5x-2x more Pb than *S. antarcticus* and *K. coulmani*.

1 *Haliclona* sp. and *K. coulmani* show different BF<sub>s</sub> of Cd and Pb the one and of Cu  
2 the latter (Tab. 5) but similar concentrations (Tab. 4) compared to other Antarctic  
3 sponges, to underline the specie-specific bioaccumulation of the metals studied. No  
4 data for these species are present in literature and so no possible explanation could be  
5 hypothesized.  
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13 When comparing the PP's substances, the Mediterranean sponge *P. ficiformis* has  
14 lower BF values than the other Antarctic organisms taken into account in (about 1.5  
15 for Cd, respect to *L. ellittica* and, moreover of about 7 and 3 times for Pb respectively  
16 to *L. ellittica* and *T. bernacchii*) whereas for Cu BF values are comparable.  
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25 A separate discussion is necessary for *S. officinalis* that presents very low BF values  
26 for Cd, Pb and Cu than all the other organisms, showing a characteristic behaviour in  
27 metal uptake. Possible explanation is related to its particular feature; in fact for this  
28 species Perez et al. (2005) demonstrated how the accumulation of Cd, unlike other  
29 species occurs with no correlation to its environmental levels (Olesen and Weeks,  
30 1994; Hansen et al., 1995; Mueller et al., 1998). This may explain the great difference  
31 in bioaccumulation factor when a comparison with the other Mediterranean species  
32 *P. ficiformis* is made. Even for Cu Verdenal et al. (1990) found similar trend; for this  
33 metal a very low BF value was calculated when compared with *P. ficiformis*.  
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51 On the other hand, *S. Officinalis* shows a good correlation with environmental level  
52 of Pb (Perez et al., 2005); in fact our BF factor is closer to that of *P. ficiformis* (Table  
53 5).  
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Variability of trace metal levels in sponge could be explained by variations in species-specific, which are well reported in literature (Mayzel et al., 2014), depending on type, size and chemical properties of particles fed on, by differences in mineral preferences and selective incorporation of foreign particles.

In summary these are the first data ever reported for the distribution between organic and siliceous tissues of sponge. Further work will be carried out on a larger set of specimens with the aim of gathering more systematic results both for the species studied here and also for others from the Antarctic and the Mediterranean regions.

Results show that the studied heavy metals have enough homogeneous concentrations in both matrices (tissues and spicules), but they are much more accumulated in the organic tissue (one to two orders of magnitude more than in spicules). Therefore the results reject our hypothesis, indicating that spicules do not have a significant role in bioaccumulating metals. This trend is clear when the structural function of the siliceous component is considered. As a matter of fact, it works as a skeleton, allows the spicules to exchange and accumulate less metal ions from water than the organic tissues. The organic components were shown to be more suitable for biomonitoring studies; they are more exposed to the water flow and, as a consequence, to the pollutants dissolved in it.

There are no significant differences between exhalant and others areas of sponge, to prove that bioaccumulation in organic part is greater independently of functional role.

In the Mediterranean Demospongia *P. ficiformis* Cd and Pb show generally lower concentrations whereas Cu shows higher concentrations than in Antarctic sponges.

1 The bioaccumulation ability related to organic and siliceous ratio is similar in both  
2 sponges (polar and temperate), because they show the same behaviour: tissues  
3 accumulate higher quantities of pollutants, from about one to two orders of  
4 magnitude, than spicules. This suggests that the organic matrix may be the best  
5 component to be analysed in biomonitoring studies.  
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13 The bioconcentration factor in Antarctic sponge is greater than that of the  
14 Mediterranean species for Cd and Pb, underlying a species-specific bioaccumulation.  
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16 For Cu generally, the same bioaccumulation factor is reported for remote and  
17 anthropic area: this suggests that different Demospongiae, living in so different  
18 ecosystems may accumulate metals both in organic and in siliceous tissue, in a  
19 similar manner.  
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30 *S. antarcticus* and *K. coulmani* have shown to have higher bioaccumulation capability  
31 than other Antarctic organisms largely used as biomarkers, therefore they could be a  
32 potential biomarker candidate.  
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40 These results pave the way to a better comprehension of the role of marine sponges  
41 both in the uptake and in the distribution between organic and siliceous tissues of  
42 heavy metals, and to their possible use as monitors of marine ecosystems, in line with  
43 Water Framework Directive objectives.  
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## Figure Captions

Fig. 1. Pseudopolarograms for Cd, Pb and Cu in the HNO<sub>3</sub> digested *S.antarcticus* diluted 200 times with ultrapure water.



Table1

**Table 1.** Results (in  $\mu\text{g g}^{-1}$ , mean $\pm$ 95% tolerance interval) of the analysis of certified reference materials by SWASV (n=4).

		Cd	Pb	Cu
DORM-2	Analyzed	0.042 $\pm$ 0.002	0.064 $\pm$ 0.009	2.23 $\pm$ 0.21
	Reference	0.043 $\pm$ 0.008	0.065 $\pm$ 0.007	2.34 $\pm$ 0.16
Krill MURST	Analyzed	0.73 $\pm$ 0.08	1.16 $\pm$ 0.06	62.3 $\pm$ 4.2
	Reference	0.73 $\pm$ 0.08	1.11 $\pm$ 0.11	65.2 $\pm$ 3.4

Table2

**Table 2.** Cd, Pb and Cu concentrations in organic and siliceous tissue of bodies and oscula (where present) of Antarctic sponges (*S. antarcticus*, *K. coulmani* and *Haliclona sp.*). Mean $\pm$ SD (RSD%),  $\mu\text{g g}^{-1}$  d.w (n=6).

Metal/Sponge	Concentration with respect to tissue type		Concentration with respect to whole sponge	
	Tissue ( $\mu\text{g g}^{-1}$ of tissue)	Spicules ( $\mu\text{g g}^{-1}$ of spicules)	Tissue ( $\mu\text{g g}^{-1}$ of sponge)	Spicules ( $\mu\text{g g}^{-1}$ of sponge)
Cd				
<i>S. antarcticus</i>				
body	84 $\pm$ 3 (4%)	0.90 $\pm$ 0.12 (15%)	20 $\pm$ 2 (10%)	0.68 $\pm$ 0.09 (13%)
oscula	82 $\pm$ 12 (14%)	0.48 $\pm$ 0.03 (6%)	25 $\pm$ 3 (10%)	0.30 $\pm$ 0.10 (33%)
<i>K. coulmani</i>	174 $\pm$ 7 (4%)	0.54 $\pm$ 0.02 (4%)	116 $\pm$ 4 (3%)	0.26 $\pm$ 0.02 (8%)
<i>Haliclona sp.</i>	8.9 $\pm$ 1.0 (11%)	0.034 $\pm$ 0.015 (44%)	2.6 $\pm$ 0.6 (23%)	0.023 $\pm$ 0.010 (43%)
Pb				
<i>S. antarcticus</i>				
body	5.5 $\pm$ 0.8 (14%)	0.28 $\pm$ 0.08 (29%)	1.3 $\pm$ 0.2 (15%)	0.21 $\pm$ 0.06 (29%)
oscula	6.1 $\pm$ 0.7 (11%)	0.44 $\pm$ 0.17 (39%)	1.8 $\pm$ 0.39 (21%)	0.31 $\pm$ 0.14 (45%)
<i>K. coulmani</i>	4.2 $\pm$ 0.2 (5%)	0.53 $\pm$ 0.13 (24%)	2.4 $\pm$ 0.4 (17%)	0.26 $\pm$ 0.08 (31%)
<i>Haliclona sp.</i>	0.94 $\pm$ 0.06 (6%)	0.20 $\pm$ 0.01 (5%)	0.33 $\pm$ 0.07 (21%)	0.14 $\pm$ 0.02 (14%)
Cu				
<i>S. antarcticus</i>				
body	17 $\pm$ 1 (6%)	0.33 $\pm$ 0.02 (6%)	4.2 $\pm$ 0.4 (10%)	0.25 $\pm$ 0.01 (4%)
oscula	19 $\pm$ 2 (13%)	0.24 $\pm$ 0.02 (8%)	5.6 $\pm$ 1.3 (24%)	0.16 $\pm$ 0.02 (12%)
<i>K. coulmani</i>	85 $\pm$ 5 (6%)	0.82 $\pm$ 0.11 (13%)	53 $\pm$ 6 (11%)	0.41 $\pm$ 0.08 (20%)
<i>Haliclona sp.</i>	24 $\pm$ 1 (4%)	0.34 $\pm$ 0.02 (6%)	7.5 $\pm$ 0.6 (8%)	0.23 $\pm$ 0.03 (13%)

Table3

**Table 3.** Cd, Pb and Cu concentrations in organic and siliceous tissue (where present) of Mediterranean sponges (*P. ficiformis* and *S.officinalis*). Mean±SD, µg g<sup>-1</sup> (RSD%) d.w. (n=6).

Metal	Concentration with respect to tissue type		Concentration with respect to whole sponge	
	Tissue (µg g <sup>-1</sup> of tissue)	Spicules (µg g <sup>-1</sup> of spicules)	Tissue (µg g <sup>-1</sup> of sponge)	Spicules (µg g <sup>-1</sup> of sponge)
Cd				
<i>P. ficiformis</i>	7.5±2.5 (33%)	0.071±0.007 (10%)	1.7±0.2 (12%)	0.053±0.011 (21%)
<i>S.officinalis</i>			0.27±0.02 (7%)	
Pb				
<i>P. ficiformis</i>	1.7±0.6 (35%)	0.025±0.003 (12%)	0.42±0.19 (45%)	0.018±0.002 (11%)
<i>S.officinalis</i>			0.47±0.05 (11%)	
Cu				
<i>P. ficiformis</i>	134±57 (42%)	1.3±0.1 (8%)	30±8 (27%)	1.0±0.2 (20%)
<i>S.officinalis</i>			42±2 (5%)	

Note: values are mean±SD obtained from at least 3 measurements.

Table4

**Table 4.** Selection of literature data for metal concentrations in organic tissue of sponges.

Location	Methodology	Cd, µg g <sup>-1</sup>	Pb, µg g <sup>-1</sup>	Cu, µg g <sup>-1</sup>	Reference
<b>Antarctica</b>					
<i>S. antarcticus</i>	Mean±SD	20±2	1.3±0.2	4.2±0.4	This study
<i>K. coulmani</i>	Mean±SD	116±4	2.4±0.4	53±6	This study
<i>Haliclona</i> sp.	Mean±SD	2.6±0.6	0.33±0.07	7.5±0.6	This study
<i>S. antarcticus</i>	Mean (Min-Max)	19 (12-23)	2.7 (0.1-7)	10.2 (4.5-12.9)	Negri et al., 2006
<i>M. acerata</i>	Mean (Min-Max)	13 (8-16)	1.2 (0.1-2.4)	5.4 (3.8-7.1)	Negri et al., 2006
<i>H. balfourensis</i>	Mean (Min-Max)	34 (19-42)	0.82 (0.5-2.4)	15 (9-22)	Negri et al., 2006
<i>Rossella, Tedania and Axociella</i> <sup>a</sup>	Min-Max	10.3-79.9	-	-	Bargagli et al, 1996
<b>Mediterranean Sea</b>					
<i>P. ficiformis</i>	Mean±SD	1.7±0.2	0.42±0.19	30±8	This study
<i>S. officinalis</i>	Mean±SD	0.27±0.02	0.47±0.05	41.7±2.2	This study
<i>S. officinalis</i>	Mean±SD	0.3±0.1	0.8±0.4	36.6±7.1	Perez et al., 2005
<i>C. reniformis</i>	Mean±SD	-	2.1±0.7	11.3±0.8	Cebrian et al., 2007
<i>Crambe Crambe</i>	Mean±SD	-	0.4±0.2	9.5±0.6	Cebrian et al., 2007
<i>P. tenacior</i>	Mean±SD	-	0.6±0.04	34±10.1	Cebrian et al., 2007
<i>D. avara</i>	Mean±SD	-	0.8±0.2	47.4±10.3	Cebrian et al., 2007
<b>Other Sea</b>					
<i>M. prolifera</i> (Gulf of Mexico)	Min-Max	1.45-3.17	-	9.61-16.46	Philp et al., 1999
<i>H. bowerbanki</i> (Gulf of Mexico)	Mean	0.72	1.78	4.00	Philp et al., 2003
<i>Dysidea camera</i> (Gulf of Mexico)	Mean	0.93	1.80	2.80	Philp et al., 2003
<i>H. panicea</i> (Gulf of Mexico)	Mean	6.50	0.78	1.70	Philp et al., 2003
<i>Chynachyra</i> (Atlantic Ocean)	Mean±SD	2.045±0.730	-	18.4±8.5	Gomes et al., 2006
<i>H. Oculata</i> (Poole Harbour, UK)	Min-Max	0.3-0.8	10-18	30-170	Aly et al., 2013

<sup>a</sup>Pooled across species

**Table 5.** Comparison of Bioconcentration Factor (BF) (obtained by mean value of each sample) between species studied and other Antarctic organisms.

Species	BF-tissue			BF-spicules			Reference
	[(ng kg <sup>-1</sup> ) <sub>tissue</sub> /(ng L <sup>-1</sup> ) <sub>seawater</sub> ] $\times 10^{-3}$			[(ng kg <sup>-1</sup> ) <sub>spicules</sub> /(ng L <sup>-1</sup> ) <sub>seawater</sub> ] $\times 10^{-3}$			
	Cd	Pb	Cu	Cd	Pb	Cu	
<i>S. antarcticus</i> (body)	2400	306	183	26	16	3.5	This study
<i>S. antarcticus</i> (oscula)	2343	339	204	14	24	2.6	This study
<i>K. coulmani</i>	4971	233	913	15	29	8.8	This study
<i>Haliclona sp.</i>	254	52	258	1	11	3.7	This study
<i>P. ficiformis</i>	577	74	223	5.5	1.1	2.2	This study
<i>S. officinalis</i>	21	20	70				This study
<i>L. elliptica</i> (total body)	820	520	130				De Moreno et al., 1997
<i>T.bernacchii</i> (liver)	400	250	160				Illuminati et al, 2010

Figure1

