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Heavy metal accumulation capacity of *Axinella damicornis* (Esper, 1794) (Porifera, Demospongiae): a tool for bioremediation of polluted seawaters

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Abstract

A wide range of contaminants are continuously introduced into the aquatic environment and among these, heavy metals constitute one of the most dangerous groups because of their persistent nature, toxicity, tendency to accumulate in organisms and more still, they are non-degradable. Marine organisms such as sponges represent target species for the monitoring of heavy metal contamination due their filtering activity. This study aims to evaluate the retention capacity of lead and cadmium by the sponge *Axinella damicornis* under laboratory conditions. The sponges were exposed for 144 h to seawaters artificially polluted with lead (Pb) and cadmium (Cd) separately and with a mixture of the two metals. The final goal of the experiments was to evaluate the metal uptake in the sponge body and efficiency of the sponge in removing the metals from seawater. In particular, the highest values of metal concentration in the sponges were recorded for Pb: this metal was found to be 6 times and 9 times more concentrated than Cd, respectively in the case of exposure to the single metal and to the combination of both metals. The metal concentrations found, especially for Pb, were much higher in *A. damicornis* than in other organisms investigated in the sea. Remarkable signs of stress and necrosis were recorded in the specimens when exposed to the combination of Pb and Cd, evidencing a synergistic effect of the metals mixture. This study paves adds knowledge on the contamination effects by heavy metals on the marine organisms and on the contribution from *A. damicornis* as efficient tool for bioremediation of polluted seawaters.

Keywords: trace metals; lead; cadmium; marine pollution; sponge; Mediterranean Sea.

Introduction

Aquatic ecosystems, and in particular marine ones, are important indicators of the presence and distribution of mainland pollutants. Because of anthropogenic inputs, many pollutants generally accumulate in marine coastal areas both in the abiotic and in the biotic components causing environmental concerns and toxic effects for living organisms (Johnston *et al.*, 2015).

Among the common environmental pollutants heavy metals register a continuous and significant growing concentration particularly in densely populated regions, such as the North-Western Mediterranean Sea (Danovaro, 2003; Elberling *et al.*, 2003). These pollutants bio-accumulate in aquatic organisms in concentrations higher than in their abiotic environment (van der Oost *et al.*, 2003; Gentric *et al.*, 2016; Conte *et al.*, 2015; Ferrante *et al.*, 2018; Pappalardo *et al.*, 2017). Special attention has been given to persistent and highly toxic heavy metals; in particular cadmium (Cd) and lead (Pb) are listed among the priority pollutants by the European Commission (Directive 2008/105/EC).

Among marine organisms, long-living benthic filter-feeder invertebrates, such as sponges, represent target species for research on heavy metal contamination due to their effective sea water filtering activity (Gifford *et al.*, 2007; Genta-Jouve *et al.*, 2012; Batista *et al.*, 2014). Sponges are able to filter huge amounts of water (0.002 - 0.84 mL s^{-1} cm^3 of sponge tissue) through their aquiferous system (Reiswig, 1990; Ribes *et al.*, 1999; Weisz *et al.*, 2008, Kahn *et al.*, 2015), to retain a wide range of 0.1 - 50 μm organic particles (phytoplankton, heterotrophic eukaryotes, bacteria and viruses) with a retention efficiency of up to 99% for nano-and picoplankton (Reiswig 1975; Hadas *et al*., 2006) and to accumulate and/or bioremediate organic and inorganic pollutants such as heavy metals (Hansen *et al*., 1995; Patel *et al*., 1985; Venkateswara *et al*., 2006, 2009; de Mestre *et al*., 2012; Mahaut *et al*., 2013; Davis *et al*., 2014; Gentric *et al*., 2016; Orani *et al*., 2018a, b; Espejo *et al*., 2019; Roveta *et al*., 2020). Due to their characteristics mentioned above, various species of sponges have been considered useful both as "sentinel organisms" in biomonitoring programs of heavy metal contamination (Perez *et al.*, 2004; Cebrian *et al*., 2007; de Mestre *et al*., 2012; Davis *et al.*, 2014) and as bioremediators of organic and inorganic pollutants (Milanese *et al*., 2003; Stabili *et al.*, 2006; 2008; Longo *et al*., 2010; Orani *et al*., 2018b).

Despite the important role of sponges in reducing marine pollution, many data in the literature only report studies conducted under controlled laboratory conditions on sponges exposed to a single contaminant at a time. At present, no published data concerning synergistic or antagonist effects of a mixture of contaminants on their accumulation in the sponge tissue are available to our knowledge. Starting from this consideration and keeping in mind that the living organisms are exposed to different pollutants simultaneously present in natural environments, we studied the ability of the demosponge *Axinella damicornis* to retain lead and cadmium from contaminated waters. This study can provide a deeper understanding of the effects of metal pollution in aquatic environment as well as the possible bioremediation of contaminated areas by using marine sponge species.

Materials and Methods

Sponges studied

Axinella damicornis (Esper, 1794) (Demospongiae, Heteroscleromorpha) is a widespread Axinellidae in the Mediterranean coastal water, on hard substrata, in semi-sciaphilous conditions, up to 70 m in depth on coralligenous and shelf-edge detritic bottoms (Pansini & Longo, 2003). *A. damicornis* is a massive sponge with a short stalk and flattened fan-shaped branches. These fused branches give an irregular shape in the taller specimens and a convoluted shape in the smaller ones. Its oscules are positioned at the top of the branches. The consistency is strong, and the surface appears velvety with projecting spicules. Its skeleton is composed of siliceous monoaxon spicules embedded in spongin fibers. Yellow in colour, it can reach a height of about 10 cm.

As regards this species, Roveta *et al.* (2020) reports low accumulation value of Hg (under 0.2 mg kg^{-1}) in specimens collected in the Tyrrhenian Sea. In addition, this species shows high accumulation capability for As and Cu, ranging between 55 and 85 mg kg^{-1} of total As and between 56 and 61 mg kg^{-1} of Cu compared with other sponge species (Orani *et al*., 2018a, b).

Sponge collection and maintenance

Sponge samples were collected by scuba diving in October 2019 at a depth of 14 meters in Civitavecchia along the Latium coast (Italy). Twelve specimens were carefully removed from the rocky substratum, placed in aerated and refrigerate bag and transported to the laboratory in the shortest time possible. All the specimens were subjected to starvation in an aquarium containing 100 L of sterile filtrate sea water (SFSW) at the Aquaculture and Ecology Laboratory (Department of Biology, University of Roma "Tor Vergata"), where they were maintained at temperature of 19 °C and exposed to artificial light-dark cycle, so mimicking natural conditions, until their employment in bioremediation experiment. The well-being of the sponge specimens was monitored throughout the experiment by observing the sponge surface and the osculum openings.

Reagents and materials

All the chemicals used were of analytical grade. All containers and the materials used were made of Teflon or polyethylene, soaked with HNO_3 3% v/v before utilization. PVDF filters (Minisart®, Sartorius Stedim, Biotech) of 0.45 µm pore size were used for the filtration process.

Experimental design

Twelve polyethylene beakers (2 L) previously cleaned with nitric acid 3% were set up, each filled with 1 L of artificial seawater (ASW) prepared dissolving 37 g of marine salt to reach the 36-37 PSU salinity. Each container was covered with an additional beaker and sealed with polyethylene film, to avoid the seawater evaporation. Moreover, each container was connected to a pump for air insufflating. Of these, four containers were contaminated with 500 µg/L (= 500 ppb) of Pb^{2+} (Pb single exposure), four other containers were contaminated with 500 µg/L (= 500 ppb) of Cd^{2+} (Cd single exposure) and the remaining four containers were contaminated with 500 µg/L of both the metals (Pb + Cd, double exposure). Before the time-zero of the experiment, a total of nine specimens of *Axinella damicornis* of similar size were transferred from the storage aquarium and individually placed in as many containers: three for each experimental condition. A beaker without sponge was employed for each condition as control. The dry weights (mean ± SD) of sponges exposed to lead contamination were 365 ± 139 mg and 472 ± 252 mg for single and double exposure, respectively; as for cadmium the weights were 296 ± 36 mg and 562 ± 138 mg for single and double exposure, respectively. A picture of experimental setup is showed in Figure 1.

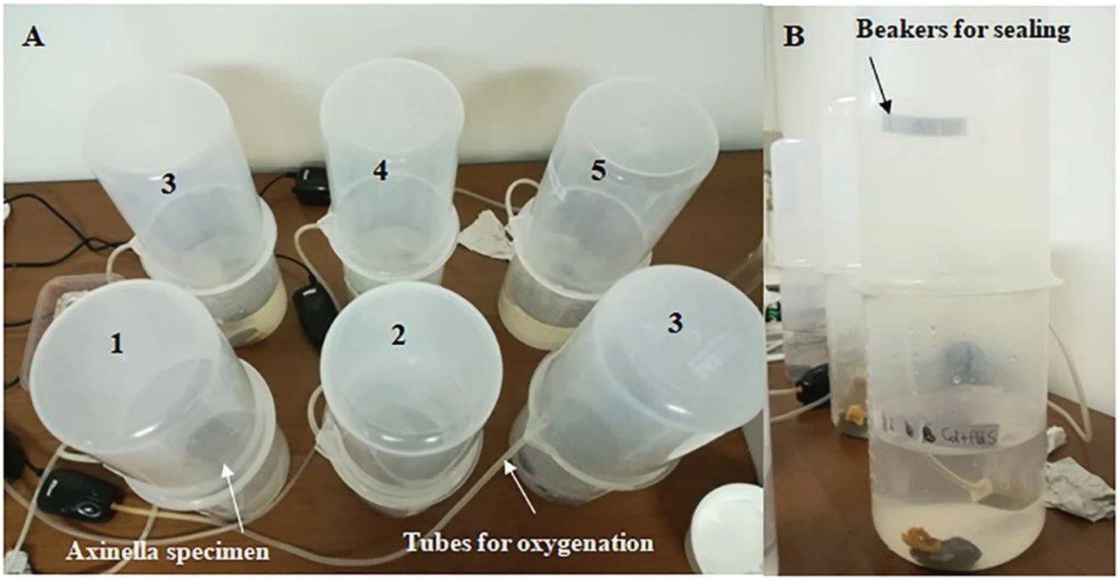

Fig. 1: A) Picture of a part of the bioremediation experiment setup. Beakers labelled from 1 to 3 contain one specimen of *Axinella damicornis* each. Beakers labelled from 4 to 6 are controls. B) Side view of the bioremediation experiment setup, showing the sequence of beakers containing *A. damicornis*.

Heavy metal analyses

Seawater concentrations of Pb and Cd were measured at the beginning of the experiment (T0) and 144 hours later (Tf) in each container (single exposure of Pb, single exposure of Cd and double exposure of Pb + Cd). Heavy metals concentrations in the sponge tissue employed in the experiment were measured at the end of the experiment. In addition, heavy metals concentrations in three wild specimens of *Axinella damicornis* were also evaluated.

The spectroscopic measurements were carried out by means of a Perkin Elmer Optima 8300 ICP-OES instrument in the Arpa Lazio laboratory, using standard parameters, according to the APAT CNR IRSA 3020 method: RF power 1400 W; plasma gas (Ar) flow rate 15 L/min; auxiliary gas (air) flow rate 0.2 L/min; nebulizer gas (Ar) flow rate 0.55 L/min; emission wavelengths 214.440 and 226.502 nm for Cd, and 220.353 nm for Pb. The sponges' tissues were left to dry for 24 h, and then weighed and mineralized. Microwave-assisted acid digestion of organisms' tissue was carried out by using an Anton Paar Multiwave 3000, by applying the standard method EPA 3051: 0.2-0.5 g of sample were mineralized with 10 mL of ultrapure HNO_3 and then filtered with a 45 μm filter up to 50 mL with distilled water. The seawater samples were acidified to 1% with HNO_3 and diluted a 1:2 v/v with distilled water.

Data analyses

Statistical analyses were performed for comparison of metal concentration in the seawater samples. The null hypothesis tested was that the two set of data at time-zero and at final-time are not different. The non-parametric permutational multivariate two-way analysis of variance (PERMANOVA) was carried out to test statistical differences between metal concentration of seawater samples as a function of the factors time and exposure (presence/absence of sponge). For statistical analyses the program PAST (version 4.03) was used. The retention efficiency (R) was calculated as a percentage for the difference in heavy metal concentrations in the seawater, by the following equation:

$$R\ (\%) = 100 * \left[\frac{(C_0 - C_f)}{C_0}\right]$$

where C_0 is the initial heavy metal (HM) concentration and C_f is the HM concentration after sponge filtration.

Results

Chemical analyses

The analytical measurements were carried out by using ICP-OES. Firstly, the effect of the complex matrix of seawater on the analytical measurement of Pb^{2+} and Cd^{2+} was studied. In detail, the results obtained for ASW were compared with seawater diluted with distilled water in ratios 1:10 (v/v), 1:5 (v/v) ed 1:2 (v/v), by measuring Pb^{2+} concentration from 500 to 2500 μg/L. The results are reported in Figure 2 and show that Pb^{2+} concentration decreases when the proportion of seawater increases. This is due to the salty composition of the seawater as well as to the presence of organic matter traces, which decrease the efficiency of the atomization process at the basis of ICP-OES detection principle. The 1:2 (v/v) dilution was chosen in order to obtain a satisfactory sensitivity of the analytical measurement and to avoid higher dilution factors. Thus, the response for Cd^{2+} and Pb^{2+} was studied separately by carrying out the calibration curves in seawater diluted 1:2 (v/v), using concentrations up to 1000

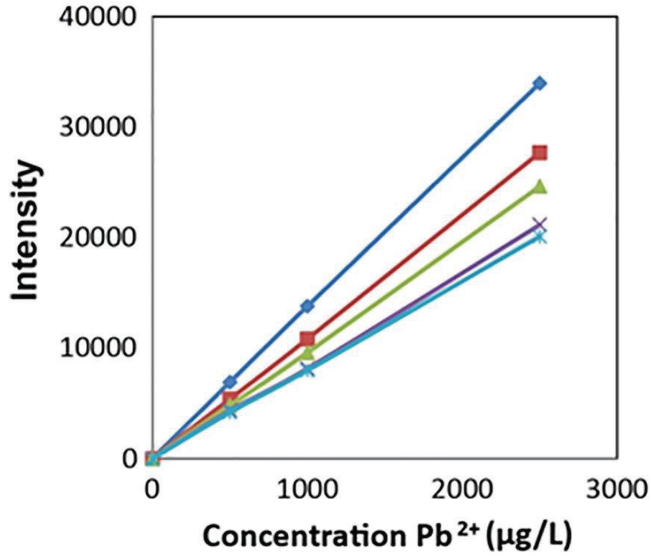

Fig. 2: Study of the matrix effect for the detection of Pb^{2+} in seawater (light blue curve), seawater diluted 1:2 (v/v) (violet curve), 1:5 (v/v) (green curve), 1:10 (red curve), and distilled water (blue curve), analyzed by ICP-OES.

Table 1. Calibration curves obtained in ICP-OES for the detection of 50, 250, 500, and 1000 µg/L of Cd^{2+} and Pb^{2+} in seawater diluted 1:2 (v/v) with distilled water.

Matrix sample	Metal	Equation	R^2
Seawater	Cd^{2+}	$y = (47.06 \pm 0.01)x + (4.59 \pm 0.01)$	0.9995
	Pb^{2+}	$y = (8.17 \pm 0.01)x + (56.39 \pm 0.01)$	0.9987
Sponge's tissue	Cd^{2+}	$y = (163.66 \pm 0.01)x + (2266.6 \pm 0.2)$	0.9992
	Pb^{2+}	$y = (13.77 \pm 0.01)x + (35.27 \pm 0.01)$	0.9997

µg/L. The equations describing the calibration curves are summarized in Table 1.

ICP-OES analysis of the sponges' tissues was carried out after the microwave-assisted acid digestion of the tissue samples. Thanks to the mineralization process, the digested samples did not present any adverse matrix effect on the spectroscopic technique. Thus, calibration curves were obtained for Cd^{2+} and Pb^{2+} concentrations up to 1000 µg/L without diluting the samples. The equations describing the calibration curves are summarized in Table 1.

Bioremediation experiments

Before the beginning of the experiment, three samples of *Axinella damicornis* were analyzed to detect the presence of lead and cadmium in their tissues originating from sampling site. Since no traces of metals were found in the samples at the detection limit of the analytical methods employed, we assumed that the metal content found in sponge tissues would come from the artificial contamination of the seawater in the containers.

Nine sponges were removed from the aquarium, placed in the experiment containers and left to acclimatize for one week so as to recover from possible stress; during this period their health state was monitored by periodic observations, these mostly concerning their position, adherence to the substratum, oscular openings, colour and sponge surface. The bioremediation experiment lasted six days (144 h): the time-zero, T(0), was taken as the sponges appeared well acclimatized. At this time the seawater of each container was contaminated with metals. At the end of the experiment, time T(f) taken after 144 h, seawater samples were gathered, and the lead and cadmium concentrations were measured. In addition, at T(f) all the sponge specimens were carefully washed with double-distilled water and frozen for proceeding with analytical analyses, to evaluate the metal content accumulated in their tissues.

The sponge health status was assessed visually during the experiment. The sponge specimens at the beginning of the experiment were perfectly healthy (Fig. 3A), while at the end of the exposure all showed strong signs of stress (Fig. 3B). In particular, some were covered by a whitish biofilm, others showed clear signs of necrosis when subjected to the double exposure of Pb + Cd. No signs of necrosis were found in the case of single exposure.

Heavy metal content in seawater

The content of Pb and Cd measured in the seawater of the two systems with and without sponges at the time-zero (T0) of the experiment and after the duration of 144 hours (Tf) are reported in Figure 4.

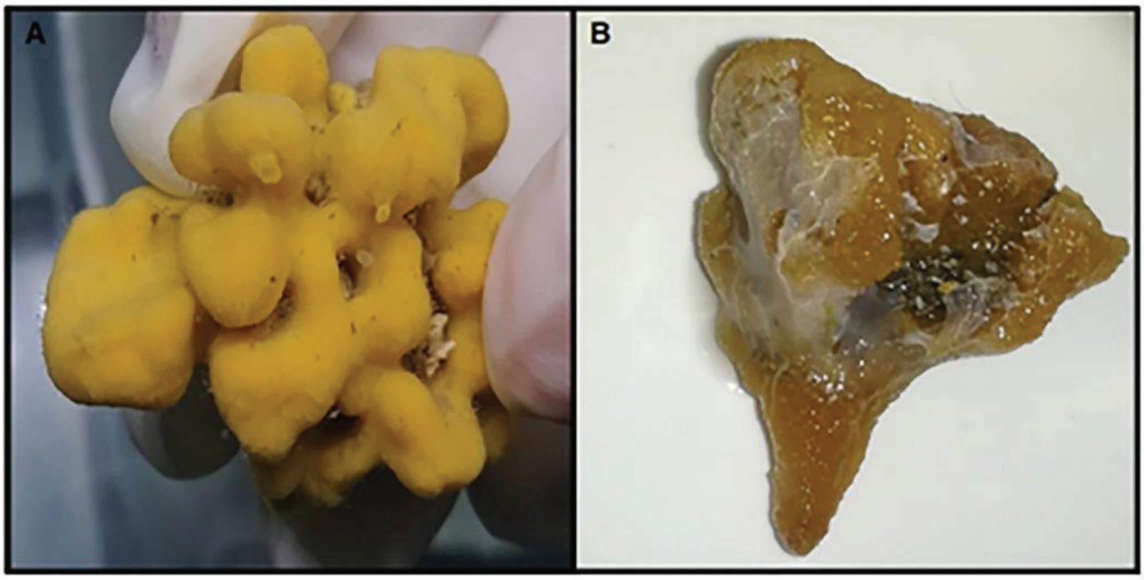

Fig. 3: A: *Axinella damicornis*, a healthy specimen before the exposure to polluted seawater. B: a specimen at the end of the experiment showing clear signs of stress.

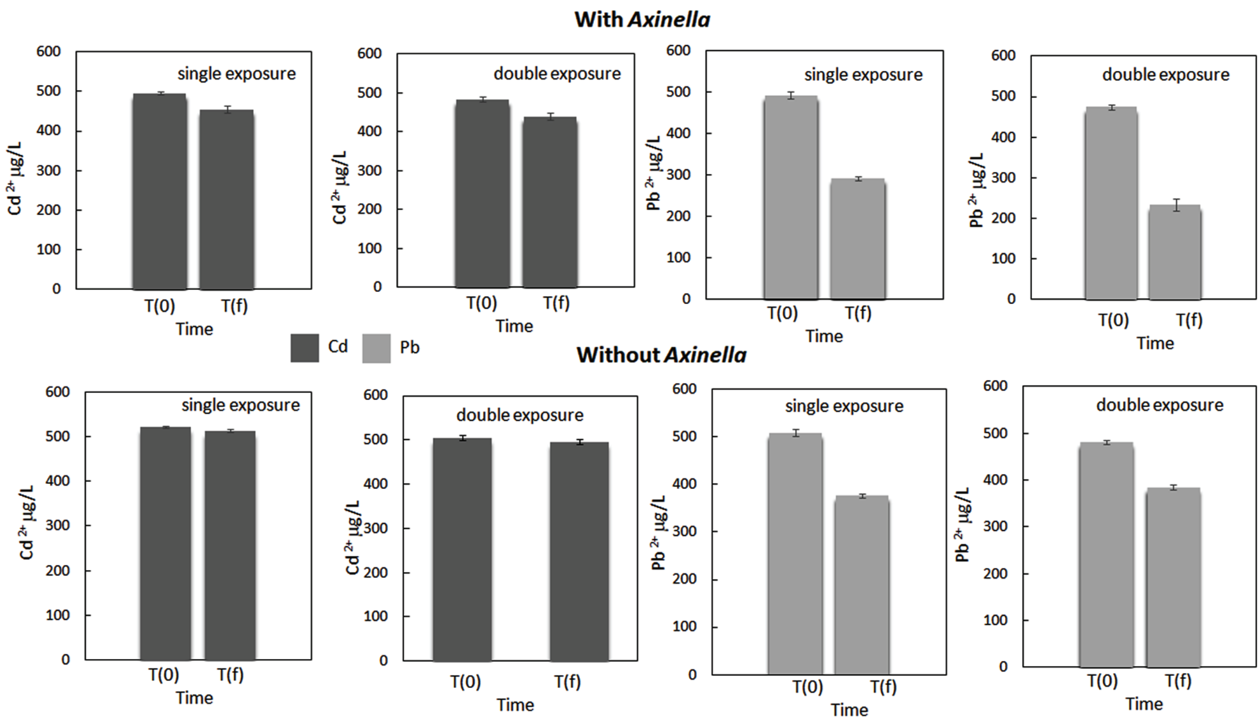

Fig. 4: Mean (±SD) values of Cd (dark grey) and Pb (light grey) concentration in the seawater measured at the time-zero (T0) and at the final time (Tf) for each condition of the experiment: single exposure of each metal and double (Pb + Cd) exposure in both the systems with and without *Axinella damicornis*.

Significant differences between the initial and final concentration of lead and cadmium in the seawater of the beakers where *Axinella damicornis* was exposed were highlighted. The results of the two-way PERMANOVA (Table 2) concerning Pb were significant for each experiment times, for each system without and with sponge and for interaction; the same results were found for Cd, save for interaction.

The highest value of heavy metals retention by *A. damicornis* was registered for lead in single exposed beakers reaching the 63.1%, while the highest mean value of retention efficiency was registered again for lead in double exposed beakers (50.8 ± 5.8%). As regards cadmium the mean retention values remained similar in single- and double-contaminated beakers with values rather low with respect to those of lead: 8.1 ± 3.4 and 8.8 ± 6.5% for single and double exposure, respectively.

Table 2. Results of PERMANOVA testing differences between Pb and Cd concentration according to time and exposure with and without sponges.

Metal	Source	Sum of sqrs	Df	Mean square	Pseudo-F	p
	Time	0.27484	1	0.27484	66.756	0.0001
Pb^{2+}	Sponge	0.06037	1	0.06037	14.663	0.0008
	Interaction	0.051666	1	0.051666	12.549	0.0011
	Residual	0.082344	20	0.0041172		
	Total	**0.46922**	**23**			
	Time	0.004637	1	0.0046373	14.354	0.0014
Cd^{2+}	Sponge	0.010556	1	0.010556	32.674	0.0002
	Interaction	2.62E-05	1	2.62E-05	0.080996	0.0814
	Residual	0.004523	14	0.0003231		
	Total	**0.019742**	**17**			

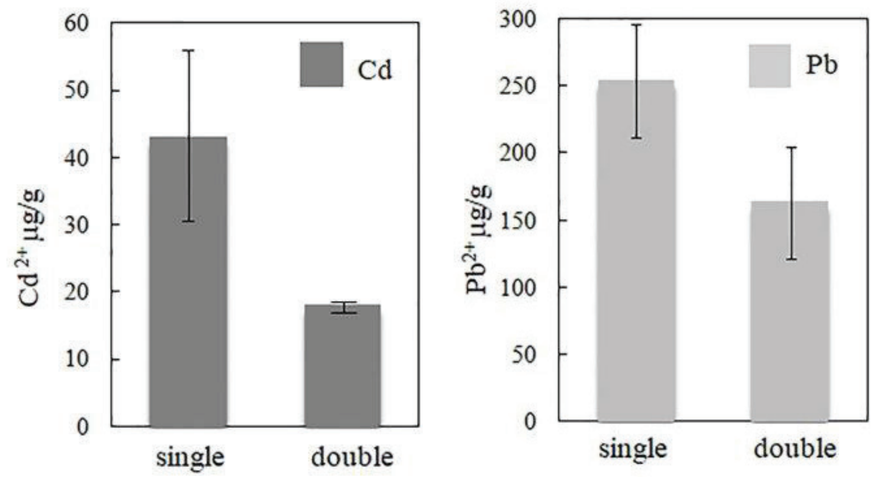

Fig. 5: Comparison of the heavy metal contents in the *Axinella damicornis* tissue after six days of exposure in the case of single and double exposure of Cd (dark grey) and Pb (light grey).

Heavy metal content in sponge tissue

After six days (144 hours) of exposure the *Axinella damicornis* specimens involved in the bioremediation experiment were analyzed to measure the Pb and Cd content accumulated in their tissues. The comparison between the heavy metal contents in the case of single and double exposure is shown in Figure 5. The accumulated amounts were calculated from the concentration values measured in the mineralized solutions (expressed in µg/L), multiplied by the total volume in which the tissues have been mineralized, and divided by the mass that have been mineralized, thus obtaining the final values expressed µg/g. The level of Pb accumulation varied between 310 and 158 µg/g (mean ± SD 254 ± 83 µg/g) in the case of single exposure and between 261and 94µg/g (mean ± SD 163± 87 µg/g) in the case of double exposure. In contrast, the mean value of Cd accumulation varied very little, ranging from 72 to 24 µg/g (mean ± SD 43 ± 26 µg/g) in the case of single exposure and from 19 to 17 µg/g for double exposure (mean ± SD 18.00 ± 2 µg/g).

Discussion and Conclusions

The sponge *Axinella damicornis* showed significant ability in removing lead and cadmium from polluted seawaters, as the results of the PERMANOVA analysis confirmed. Consequently, considerable quantities of both metals have been bioconcentrated in the sponge tissues. It is worth noting that *A. damicornis* showed a different capacity to remove Pb and Cd, since the sponge tissue concentrations found for lead were greater than those for cadmium in all the specimens; particularly the average value of Pb (254 µg/g) resulted 6 times higher than the average value of Cd (43 µg/g) in the case of single contamination and 9 times higher (163 µg/g of Pb compared with 18 µg/g of Cd) in the case of double contamination.

This study showed the high capacity of *A. damicornis* in accumulating Pb, whose tissue content at the end of the experiment varied from 254 to 163 µg/g according to single and double exposure. These very high values are expected considering the extreme concentrations tested in the experiments. Ferrante *et al.* (2018) found in the sponge *Chondrilla nucula* exposed to similar extreme concentrations of Pb (ranging from 200 to 800 µg/L) the content of lead ranging from 7.2-20.8 µg/g, which resulted to be an order of magnitude lower than in *A. damicornis* exposed to similar level (500 µg/g) of heavy metal. In addition, in the same extreme concentration of Cd (varying from 200 to 800 µg/L) Ferrante *et al.* (2018) found in *C. nucula* a content of this metal ranging from 1.2-2.7 µg/g. Meanwhile, in the specimens of *A. damicornis* tested in our experiment concentration of cadmium varied from 42.7 and 18 µg/g, respectively for single and double exposure, and resulted an order of magnitude higher, even if our sponges have been exposed to a coherent level (500 µg/L) of pollutant.

On the other hand, literature data has revealed that *in situ* conditions native sponges accumulate heavy metals but, as expected, at much lower levels if compared with the results of laboratory experiments. As evidence of this, Pb mean value accumulation ranged between 3 and 7 µg/g in specimens of *A. damicornis* collected in the north-western Mediterranean sea (Orani *et al*., 2018a); Pb values ranged from 0.4 to 6.9 µg/g in *Chondrosia reniformis*, *Spongia officinalis*, *Scalarispongia scalaris* (reported as *Cacospongia scalaris*), *Agelas oroides*, *Spongia lamella* (reported as *S. agaricina*) (Perez *et al*., 2004; 2005); from 0.42-0.47 µg/g in *Petrosia ficiformis* and *Spongia officinalis* respectively (Illuminati *et al*., 2016) and from 0.36 to 4.16 µg/g in *Crambe crambe* (Cebrian *et al*., 2007). Orani *et al*. (2018a) also found Pb values ranging from 1.1 and 8 µg/g for *Haliclona fulva*, from 1.1 and 4 µg/g for *Chondrilla nucula*, from 3 and 6 µg/g for *Acanthella acuta*, from 1.6 and 10 µg/g for *Halichondria panicea* (Atlantic) and from 2.7 and 7 µg/g for *Hymeniacidon perlevis* (Atlantic). Similarly, for cadmium, the bioconcentration values recorded in native Mediterranean sponges were of the same order of magnitude as those of Pb from *in situ* specimens. Indeed, values of cadmium varied from 0.2 to 0.5 µg/g in *Spongia lamella S. officinalis*, *Scalarispongia scalaris*, *Chondrosia reniformis* and to 1.4 µg/g in *Agelas oroides* (Perez et *al*., 2004); from 1.6 and 2.2 µg/g in *Haliclona fulva*, from 0.56 and 0.73 µg/g in *Chondrilla nucula*; from 1.6 and 1.9 µg/g in *Acanthella acuta*; from 0.4 and 1.3 µg/g in *Halichondria panicea* (Atlantic) and from 0.8 and 1.9 µg/g in *Hymeniacidon perlevis* (Atlantic) (Orani *et al*., 2018a); Illuminati *et al*. (2016) reported mean values of 1.7 µg/g in *Petrosia ficiformis* and 0.3 µg/g in *Spongia officinalis.*

The concentration of lead and cadmium recorded in *A. damicornis* resulted particularly high with regard to solitary, not colonial invertebrates studied *in situ*. In fact, five sabellid polychaete species exhibited average levels of Pb concentration varying from 0.2 to 0.96 µg/g in the body and from 1.1 to 5.1 µg/g in the branchial crown; regarding cadmium the concentration measures ranged from 0.08 to 1.3 µg/g in the body and from 0.2 to 3.1 µg/g in the branchial crown (Giangrande *et al*., 2017). Minor differences were recorded based on the laboratory experiment where the ascidian *Styela plicata* was employed and exposed to similar metal concentrations (Colozza *et al*., 2017): from this comparison Pb turned out to be about 5 times and Cd 1.5 times more concentrated in *A. damicornis*. These differences could be attributable to the modularity organization of the organisms; this would provide a more efficient filtration in organisms characterized by large leuconoid filtering system, such as demosponges, in comparison with other marine invertebrates with restricted filtering organs (e.g., polychaetes and ascidians).

Moreover, our results highlight the high removal capacity of *A. damicornis* particularly for lead, which reached the 41% and 51% in the case of single and double exposure, respectively. By contrast, much lower values of removal capacity were recorded for cadmium: 8.1 and 8.8 % respectively for single and double exposure. According to Orani *et al*. (2018a) the observed ability in metal removal of *A. damicornis* may be related to the peculiar behavior of this species. In particular, *A. damicornis* hosts an original bacterial community attributed to low microbial abundance species (LMA) (Vacelet & Donadey, 1977). The body architecture of LMA sponges allows large amounts of water to move through their tissues in order to quickly acquire small particles for nutritional needs (Weisz *et al*., 2008). Probably, this efficient filtration mechanism favors a similar success in the retention of dissolved pollutants, with greater selectivity for Pb than Cd due to the particular associated microbiota. Furthermore, the presence of secondary metabolites able to preferentially complex Pb with respect to Cd could be a further explanation. Confirmation of this, comes from the morphology of the aquiferous system with flagellate chambers of different sizes responsible for different filtering activity of the sponges. The symbiotic associations with bacteria and zooxanthellae have also been found to influence their metal accumulation capacity (Perez *et al*., 2004). Moreover, the spongin fibers enhance the metal up-take in sponges with developed skeleton (Perez *et al*., 2004). The significant role of the spicules in bioaccumulating metals was also highlighted in sponges with expanded organic tissues (Illuminati *et al*., 2016). Our experimental results are in agreement with the literature, since *A. damicornis* is characterized by a dense siliceous skeleton made by spicules organized in an axial and extra-axial region and embedded in low spongin fibers.

In our opinion, the high ability of metal accumulation is also attributable to the integrity of the specimens employed in the experiment. In fact, we think that the entire body architecture, preserving the structural and functioning integrity of the aquiferous system, could be the best assurance of the filtering efficiency.

In the biological systems heavy metals are dangerous because they tend to be bioaccumulated in living organisms. These pollutants are accumulated in living organisms whenever they are assimilated and stored faster than they are metabolized or excreted. As above mentioned, sponges are characterized by the presence of a communities of microorganisms composing its associated microbiota that play a fundamental role in its secondary metabolism and can actively participate in the bioaccumulation process (Santos-Gandelman *et al*., 2014; Thomas *et al*., 2016; Roveta *et al*., 2021). Moreover, in encrusting shallow-water tropical sponges and in massive and encrusting deep sea sponge species the sponge loop hypothesis has been established, which is a pathway involving DOM and POM assimilation and subsequently production of significant amounts of particulate detrital waste (de Goeij *et al*., 2013; Bart *et al*., 2021). While *A. damicornis* is attributed to the LMA species, the imbalance between heavy metals concentrations could be explained by its microbiota activity and the detrital waste produced by metabolic process. In fact, in the experimental system with high retention efficiency the imbalance is more evident than that found in the system with low retention This indicates that the sponge specimens that removed heavy metals more efficiently, filtered more efficiently activating a greater metabolic process with the production of detritus containing heavy metals which, however, was not considered in the present research.

Furthermore, our study provides relevant novelties on knowledge related to the synergistic effects of a mixture of metals to which organisms are subjected. In fact, on average Pb resulted 6 times more concentrated than Cd (253.7 against 42.7 µg/g respectively) in the *A. damicornis* specimens exposed to separate metals, whilst the content of Pb was 9 times higher than Cd in the specimens subjected to the double metal contamination (162.7 against 18 µg/g respectively). Such synergistic activity is noteworthy because it may reflect the variability in the bioaccumulation levels in the organisms living in natural environments, where different metals are present together. Thus, these results on the role of *A. damicornis* in the uptake of Pb and Cd pave the way to the increase of knowledge on both the effective effects of heavy metal contamination on the organisms and on the possible use of this species as efficient tool in bioremediation of polluted seawaters.

Author contributions

Conceptualization, MFG, CL, MS; biological methodology, CL, PP; chemical analyses, NC, MR; writing-original draft preparation, MGF, CL, PP, NC, MS; writing-review and editing, MFG, CL, NC, MS; supervision, MFG, CL, MS; project administration, MS; funding acquisition, MS. All authors have read and agreed to the published version of the manuscript.

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References

Bart, M.C., Hudspith, M., Rapp, H.T., Verdonschot, P.F.M., de Goeij, J.M., 2021. A Deep-Sea Sponge Loop? Sponges Transfer Dissolved and Particulate Organic Carbon and Nitrogen to Associated Fauna. *Frontiers in Marine Science*, 8, 604879.

Batista, D., Muricy, G., Chavez Rocha, R., Miekeley, N., 2014. Marine sponges with contrasting life histories can be complementary biomonitors of heavy metal pollution in coastal ecosystems. *Environmental Science and Pollution Research International*, 21, 5785-5794.

Cebrian, E., Uriz, M.J., Turon, X., 2007. Sponges as biomonitors of heavy metals in spatial and temporal surveys in northwestern Mediterranean: Multispecies comparison. *Environmental Toxicology and Chemistry*, 26, 2430-2439.

Colozza. N., Gravina M.F., Amendola L., Rosati, M., Akretche, D.E. *et al.,* 2017. A miniaturized bismuth-based sensor to evaluate the marine organism *Styela plicata* bioremediation capacity toward heavy metal polluted seawater. *Science of the Total Environment*, 584-585, 692-700.

Conte, F., Copat, S., Longo, C., Conti, G.O., Grasso, A. *et al.*, 2015. First data on trace elements in *Haliotis tuberculata* (Linnaeus, 1758) from southern Italy: Safety issues. *Food and Chemical Toxicology*, 81, 143-150.

Danovaro, R., 2003. Pollution threats in the Mediterranean Sea: An overview. 2003. *Chemistry and Ecology*, 19 (1), 15-32.

Davis, A.R., de Mestre, C., Maher, W., Krikowa, F., Broad, A., 2014. Sponges as sentinels: Metal accumulation using transplanted sponges across a metal gradient. *Environmental Toxicology and Chemistry*, 33, 2818-2825.

de Goeij, J.M., Van Oevelen, D., Vermeij, M.J., Osinga, R., Middelburg, J.J. *et al.*, 2013. Surviving in a marine desert: the sponge loop retains resources within coral reefs. *Science*, 342, 108-110.

de Mestre, C., Maher, W., Roberts, D., Broad, A., Krikowa, F. *et al*., 2012. Sponges as sentinels: Patterns of spatial and intra-individual variation in trace metal concentration. *Marine Pollution Bulletin*, 64, 80-89.

Elberling, B., Knudsen, K.L., Kristensen, P.H., Asmund, G., 2003. Applying foraminiferal stratigraphy as a biomarker for heavy metal contamination and mining impact in a fiord in West Greenland. *Marine Environmental Research*, 55, 235-256.

Espejo, W., Padilha, J. de A., Gonçalves, R.A., Dorneles, P.R., Barra, R. *et al.*, 2019. Accumulation and potential sources of lead in marine organisms from coastal ecosystems of the Chilean Patagonia and Antarctic Peninsula area. *Marine Pollution Bulletin*, 140, 60-64.

Ferrante, M., Vassallo, M., Mazzola, A., Brundo, M.V., Pecoraro, R. *et al.*, 2018. In vivo exposure of the marine sponge *Chondrilla nucula* Schmidt, 1862 to cadmium (Cd), copper (Cu) and lead (Pb) and its potential use for bioremediation purposes. *Chemosphere*, 193, 1049-1057.

Genta-Jouve, G., Cachet, N., Oberhänsli, F., Noyer, C., Teyssié, J.L. *et al.*, 2012. Comparative bioaccumulation kinetics of trace elements in Mediterranean marine sponges. *Chemosphere*, 89, 340-349.

Gentric, C., Rehel, K., Dufour, A., Sauleau, P., 2016. Bioaccumulation of metallic trace elements and organic pollutants in marine sponges from the South Brittany Coast, France. *Journalof Environmental Sciences*, 51 (3), 213-219.

Giangrande, A., Licciano, M., Del Pasqua, M., Fanizzi, F.P., Migoni, D. *et al.*, 2017. Heavy metals in five Sabellidae species (Annelida, Polychaeta): ecological implications. *Environmental Science and Pollution Research*, 24, 3759-3758.

Gifford, S., Dunstan, R.H., O'Connor, W., Koller, C.E., MacFarlane, G.R., 2007. Aquatic zooremediation: deploying animals to remediate contaminated aquatic environments. *Trends in Biotechnology*, 25, 60-65.

Hadas, E., Marie, D., Shpigel, M., Ilan, M., 2006. Virus predation by sponges is a new nutrient-flow pathway in coral reef food webs. *Limnology and Oceanography*, 51, 1548-1550.

Hansen, I.V., Weeks, J.M., Depledge, M.H., 1995. Accumulation of copper, zinc, cadmium and chromium by the marine sponge *Halichondria panicea* Pallas and the implications for biomonitoring. *Marine Pollution Bulletin*, 1 (3), 133-138.

Illuminati, S., Annibaldi, A., Truzzi, C., Scarponi, G., 2016. Heavy metal distribution in organic and siliceous marine sponge tissues measured by square wave anodic stripping voltammetry. *Marine Pollution Bulletin*, 111, 476-482.

Johnston, E.L., Mayer-Pinto, M., Crowe, T.P., 2015. Chemical contaminant effects on marine ecosystem functioning. *Journal of Applied Ecology*, 52, 140-149.

Kahn, A.S., Yahel, G., Chu, J.W.F., Tunnicliffe, V., Leys, S.P., 2015. Benthic grazing and carbon sequestration by deep-water glass sponge reefs. *Limnology and Oceanography*, 60, 78-88.

Longo, C., Corriero, G., Licciano, M., Stabili, L., 2010. Bacterial accumulation by the Demospongiae *Hymeniacidon perlevis*: A tool for the bioremediation of polluted seawater. *Marine Pollution Bulletin*, 60, 1182-1187.

Mahaut, M.L., Basuyaux, O., Baudinière, E., Chataignier, C., Pain, J. *et al.*, 2013. The Porifera *Hymeniacidon perlevis* (Montagu, 1818) as a bioindicator for water quality monitoring. *Environmental Science and Pollution Research*, 20 (5), 2984-92.

Milanese, M., Chelossi, E., Manconi, R., Sara, A., Sidri, M. *et al.*, 2003. The marine sponge *Chondrilla nucula* Schmidt, 1862 as an elective candidate for bioremediation in integrated aquaculture. *Biomolecular Engineering*, 20, 363-368.

Orani, A.M., Barats, A., Vassileva, E., Tomas, O.P., 2018a. Marine sponges as a powerful tool for trace elements biomonitoring studies in coastal environment. *Marine Pollution Bulletin*, 131, 633-645.

Orani, A.M., Barats, A., Zitte, W., Morrow, C., Thomas, O.P., 2018b. Comparative study on the bioaccumulation and biotransformation of arsenic by some northeastern Atlantic and northwestern Mediterranean sponges. *Chemosphere*, 201, 826-839.

Pansini, M., Longo, C., 2003. A review of the Mediterranean Sea sponge biogeography with, in appendix, a list of the demosponges hitherto recorded from this sea. *Biogeographia*, 24, 57-73.

Pappalardo, A.M., Copat, C., Ferrito, V., Grasso, A., Ferrante, M., 2017. Heavy metal content and molecular species identification in canned tuna: insights into human food safety. *Molecular Medicine Reports*, 15, 3430-3437.

Patel, B., Balani, M.C., Patel, S., 1985. Sponge "sentinel" of heavy metals. *Science of the Total Environment*, 41, 143-152.

Perez, T., Longet, D., Schembri, T., Rebouillon, P., Vacelet, J., 2005. Effects of 12 years' operation of a sewage treatment plant on trace metal occurrence within a Mediterranean commercial sponge (*Spongia officinalis*, Demospongiae). *Marine Pollution Bulletin*, 50, 301-309.

Perez, T., Vacelet, J., Rebouillon, P., 2004. *In situ* comparative study of several Mediterranean sponges as potential biomonitors for heavy metals. *Bollettino dei Musei e degli Istituti Biologici dell'Università di Genova*, 68, 517-525.

Reiswig, H.M., 1975. Bacteria as food for temperate-water marine sponges. *Canadian Journal of Zoo1ogy*, 53, 582-589.

Reiswig, H.M., 1990. *In Situ* Feeding in Two Shallow Water Hexactinellid Sponges. p. 504-510. In: *New perspectives in sponge biology*. Rützler, K. (Ed). Smithsonian Institution Press, Washington, DC.

Ribes, M., Coma, R., Gili, J.M., 1999. Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. *Marine Ecology Progress Series*, 176, 179-190.

Roveta, C., Annibaldi, A., Afghan, A., Calcinai, B., Di Camillo, C.G. *et al.*, 2021. Biomonitoring of Heavy Metals: The Unexplored Role of Marine Sessile Taxa. *Applied Science*, 11, 580.

Roveta, C., Pica, D., Calcinai, B., Girolametti, F., Truzzi, C. *et al.*, 2020. Hg levels in marine Porifera of Montecristo and Giglio Islands (Tuscan Archipelago, Italy). *Applied Science*, 10 (12), 4342.

Santos-Gandelman, J.F., Giambiagi-deMarval, M., Oelemann, W.M.R., Laport, M.S., 2014. Biotechnological potential of sponge associated bacteria. *Current Pharmaceutical Biotechnology,* 15, 143-155.

Stabili, L., Licciano, M., Giangrande, A., Longo, C., Mercurio, M. *et al.*, 2006. Filtering activity of *Spongia officinalis* var. *adriatica* (Schmidt) (Porifera, Demospongiae) on bacterioplankton: implications for bioremediation of polluted seawater. *Water Research*, 40, 3083-3090.

Stabili, L., Licciano, M., Longo, C., Corriero, G., Mercurio, M., 2008. Evaluation of microbiological accumulation capability of the commercial sponge *Spongia officinalis* var. *adriatica* (Schmidt) (Porifera, Demospongiae). *Water Research*, 42, 2499-2506.

Thomas, T., Moitinho-Silva, L., Lurgi, M., Björk, J.R., Easson, C. *et al*., 2016. Diversity, structure and convergent evolution of the global sponge microbiome. *Nature Communication*, 7, 1-12.

Vacelet, J., Donadey, C., 1977. Electron microscope study of the association between some sponges and bacteria. *Journal of Experimental Marine Biology and Ecology*, 30 (3), 301-314.

van der Oost, R., Beyer, J., Vermeulen, N.P., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology*, 13, 57-149.

Venkateswara Rao, J., Kavitha, P., Chakra Reddy, N., Gnaneshwar Rao, T., 2006. *Petrosia testudinaria* as a biomarker for metal contamination at Gulf of Mannar, southeast coast of India. *Chemosphere,* 65, 634-638.

Venkateswara Rao, J., Srikanth, K., Pallela, R., Gnaneshwar Rao, T., 2009. The use of marine sponge, *Haliclona tenuiramosa* as bioindicator to monitor heavy metal pollution in the coasts of Gulf of Mannar, India. *Environmental Monitoring and Assessment*, 156, 451-459.

Weisz, J.B., Lindquist, N., Martens, C.S., 2008. Do associated microbial abundances impact marine demosponge pumping rates and tissue densities? *Oecologia*, 155, 367-376.