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EVALUATION AND COMPARISON OF TRACE METAL ACCUMULATION IN DIFFERENT TISSUES OF POTENTIAL BIOINDICATOR ORGANISMS: MACROBENTHIC FILTER FEEDERS *STYELA PLICATA*, *SABELLA SPALLANZANII*, AND *MYTILUS GALLOPROVINCIALIS*

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Abstract: Trace metal concentrations were measured in different tissues of *Sabella spallanzanii*, *Styela plicata*, and *Mytilus galloprovincialis* collected in the Termini Imerese Harbor (Sicily, Italy) to evaluate the potential use of these species as bioindicators. Higher bioaccumulation factors (BAFs) were calculated in the tube of *S. spallanzanii*, except for As, which had a higher BAF in the branchial crown of the same species. Regarding the other species analyzed, higher BAFs were found in the digestive gland of *M. galloprovincialis*. An exception was Pb, which was significantly more concentrated in the branchial basket and tunic of *S. plicata*. The BAFs calculated in the present study show that all the species analyzed accumulate a certain amount of metals as a consequence of filter feeding mechanisms, and thus it was possible to assess the suitability of *S. plicata*, *S. spallanzanii*, and *M. galloprovincialis* as indicators of water quality. In particular, the tube of *S. spallanzanii* is an important compartment in terms of metal retention and is more suitable for the evaluation of contamination from trace elements. *Environ Toxicol Chem* 2016;9999:1–9. © 2016 SETAC

Keywords: Trace elements Bioindicator *Styela plicata* *Mytilus galloprovincialis* *Sabella spallanzanii*

INTRODUCTION

Anthropogenic activities such as fossil fuel burning, domestic effluents, and urban runoff are important sources of major and trace elements to the marine environment [1]. Estuaries and coastal zones, particularly those near large urban and industrial centers such as harbors, are exposed to higher concentrations of different contaminants because of the proximity of pollution sources. Trace metal contamination in marine ecosystems is a global problem, and exposure of marine organisms may result in bioaccumulation. Although trace metals are normal constituents of the marine environment, and some of them are essential to marine organisms, all metals are toxic above some threshold level. Thus, monitoring programs are needed to identify potential sources of contamination and to assess the current state of coastal environments, which are under anthropogenic pressures. This information is very difficult to obtain by analysis of marine water samples [2], because the metal concentrations in water often are near or below the detection limit of instruments and fluctuate drastically, depending on water flow and intermittence of discharge [3]. Thus, the pollutant concentrations in the water column are not representative parameters [4]. Bioindicator species accumulate trace metals in their tissues and may therefore be used to monitor contaminants in ecosystems [5]. Filter feeding organisms have been used successfully as indicator organisms in environmental monitoring programs because of their abundance, wide spatial distribution, sessile behavior, tolerance to changes in salinity, resistance to stress, and ability to accumulate a wide range of contaminants [5]. Sedentary organisms maximize their exposure to any harmful materials within the water column as they process relatively large amounts of water during feeding. As sedentary filter feeders, mussels are well known to accumulate a wide range of metals in their soft tissues. Among them, the mussel *Mytilus galloprovincialis* has been extensively employed as a bioindicator of metal contamination in a number of biomonitoring programs [6,7] because of its slow decontamination kinetics and ease of identification and collection; it also provides enough tissue for chemical analysis [8]. Other filter feeder invertebrates such as polychaetes and ascidians are used to a lesser extent, although it has been shown that they can provide useful information on the pollution status of a particular area [9]. The polychaete *Sabella spallanzanii* (Sabellidae) is widely distributed throughout the Mediterranean Sea, and it is commonly found in sheltered shallow areas with eutrophic conditions, such as harbors. The body of this species is differentiated into a thorax and tentacles or branchial crown, which can be extended out for filter feeding activities and respiration. The thorax is protected by a bioconstructed muddy tube, usually attached to hard bottoms or artificial substrates. Sessile habits, filter feeding behavior, and the ability to colonize disturbed environments such as harbors suggest their potential use as bioindicators for monitoring marine environmental pollution. The ascidian *Styela plicata* is a benthic filter feeder that is particularly abundant in harbors [10]. Generally, ascidians are considered as indicators of water quality and can accumulate toxicants such as heavy metals or hydrocarbons in their tissues [11]. The ability to accumulate metals can be ascribed to their sedentary nature and their filter feeding habits, as well as the presence of vanadocytes. Because information on trace metal accumulation in these species is almost completely lacking (except for the mussel *M. galloprovincialis*), the main goal of the present study is to provide systematic data on trace

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metal concentrations in different tissues of *S. spallanzanii*, *S. plicata*, and *M. galloprovincialis* collected in Termini Imerese Harbor (Sicily, Italy) and to evaluate and compare their potential use as biomonitor organisms. Trace element concentrations in water samples were also analyzed, to evaluate the bioaccumulation factor (BAF) in the different tissues.

MATERIALS AND METHODS

Sample collection

Ten specimens of *S. spallanzanii*, 50 of *S. plicata*, and 10 of *M. galloprovincialis* were collected in the Termini Imerese Harbor, during a simple sampling session in May 2015 to minimize differences in animal physiology and seasonal variations. Organisms of approximately the same length were selected to minimize variability. Termini Imerese is a single local administrative unit of level 2 (LAU2) located in the province of Palermo, Sicily. Termini Imerese covers an area of 77.58 km^2 at latitude 37°59′20″N and longitude 13°41′56″E. The population of 27 702 is at a density of 357.08 people/km^2. The harbor has a long outer breakwater and an inner breakwater that provide good shelter for boats. The site is well sheltered from the winds of the northern quadrants, and the south wind creates a significant undertow inside the harbor. The harbor is used mainly for cargo and freight movement in support of the adjacent industrial area. Three samples of water were also collected at the same depth and location in which the organisms were collected (the inner part of the harbor). Samples of water were immediately acidified with 1 mL of concentrated nitric acid. To minimize contamination risks, acid-cleaned laboratory materials were used during sample collection and analytical determination. The tissue samples were immediately removed from each individual and stored at –20 °C. The following samples of tissues were selected to monitor trace metal accumulation: external tube and branchial crown for *S. spallanzanii*; hepatopancreas, branchial basket, and tunic for *S. plicata*; and digestive gland for *M. galloprovincialis*. For each analysis, a pool of 5 hepatopancreas and branchial basket tissues of *S. plicata* were combined, with the aim of providing a sufficient amount of samples for the analysis. The tissues were dried for 48 h at 40 °C in an oven, and their dry weights were determined. Low drying temperature was used to avoid the volatility of certain trace metals, such as Hg and As. The dried samples were ground with a mortar and pestle for subsequent analysis.

Analysis of heavy metals in tissue and water samples

Approximately 200 mg of each oven-dried and homogenized tissue samples were digested under pressure in 1 mL of ultragrade HNO_3 and 0.5 mL of H_2O_2 in Teflon vessel liners using a microwave digestion system (CEM MARS-5). Samples were prepared and analyzed to minimize contamination from glassware and reagents, all of which were of Suprapur quality. The concentrations in solution of As(III), Cd(II), Cr(III), Cu(II), Fe(II), Hg(II), Pb(II), Se(II), V(II), and Zn(II) were measured by using an inductively coupled plasma–optical emission spectrometer (ICP–OES Optima 2100), equipped with an autosampler model AS90. Analyses were carried out by external calibration using standard solutions in the same acid matrix of samples, prepared by diluting the ICP high-purity standard solutions. Reagent blanks and controls also were taken into account to monitor the appropriateness of the analytical procedures. All data are calculated as an average of 3 duplicates. The analytical precision, measured as relative standard deviation, was routinely between 5% and 6%, and never higher than 10%. All results were calculated with respect to dry weight. The ICP-OES Optima 2100 has a system (automatic dual viewing) that ensures very low detection limits for trace metal analysis. The instrument detection limit for selected elements ranges from 0.2 μg/kg to 0.9 μg/kg dry weight.

Calculations and statistical analysis

Data were tested for goodness of fit to a normal distribution using Kolmogorov–Smirnov tests. Because variables were not normally distributed, nonparametric tests were used to compare different groups in the present study. Spearman's rank correlation coefficient was used among metal concentrations. The Kruskal–Wallis test was used to compare trace metal concentrations among different tissues and species. A p value lower than 0.05 was considered to indicate statistical significance. The statistical evaluations were performed with the STATISTICA 7.0 software package. The BAF for each metal was calculated as the ratio of concentration of metal in the tissue to that in the water.

RESULTS

Trace element concentrations in the different tissues analyzed

Mean trace element concentrations in the different tissues analyzed are shown in Table 1.

Generally, low Cd, As, and Hg amounts determined in all analyzed tissues show concentration levels near to the instrument detection limit; the only exception was for the As and Hg concentrations found in the branchial crown and tube of *S. spallanzanii*, respectively, which were definitely detectable. Wide ranges of concentrations were found for Cr (0.01–1.4 mg/kg dry wt), Cu (0.1–2.94 mg/kg dry wt), Fe (0.79–286 mg/kg dry wt), Pb (0.05–2.21 mg/kg dry wt), Zn (0.2–3.38 mg/kg dry wt), and V (0.06–2.34 mg/kg dry wt). A narrower range of concentrations was found for Se (0.01–0.2 mg/kg dry wt). As shown in Table 1, tissues of *S. plicata* accumulated trace elements in the following hierarchical order: Cu > Zn > V > Pb > Cr > Se > Hg > As > Cd in the branchial basket and hepathopancreas tissues; and V > Cu > Pb > Zn > Cr > Se > Cd > Hg > As in the tunic. In terms of toxic elements, statistically significant differences in Pb concentrations were found between different tissues of *S. plicata*, with the higher concentrations found in the tunic (Kruskal–Wallis test, $p = 0.00005$). The other toxic element concentrations (Hg, As, and Cd) can be considered negligible. In terms of essential elements, statistically significant differences in concentrations were found among the different tissues, with the exception of Cu. In particular, Zn, Fe, Cr, and Se concentrations were highly abundant in branchial basket tissues (Kruskal–Wallis test, $p = 0.0004$, 0.002, 0.003, and 0.001, respectively), whereas V concentrations were highly abundant in the tunic (Kruskal–Wallis test, $p = 0.00003$). The lowest accumulation rate appeared to be in the hepatopancreas (Figures 1 and 2). As shown in Table 1, trace element concentrations in tissues of *S. spallanzanii* had the following hierarchical order: As > Zn > V > Cu > Pb > Se > Cr > Cd > Hg in the branchial crown; and Cu > Zn > V > Pb > Cr > Hg > Se > Cd > As in the tubes. As shown in Figures 1 and 2, significant differences in trace element concentrations were found between the branchial crown and tube of *S. spallanzanii* (Kruskal–Wallis test significant at $p < 0.01$ for all trace elements analyzed). In particular, higher trace element concentrations were found in

Table 1. Bioaccumulation factors (BAFs) in the different tissues analyzed and mean trace element concentration ± deviation standard (mg/kg dry wt) in water samples and tissues collected at the Termini Imerese Harbor (Sicily, Italy)

	Species	Tissue	Cd	Pb	Cu	Zn	As	Se	Cr	V	Fe	Hg
Water			<dl	0.029 ± 0.002	0.001 ± 0.001	0.011 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.002 ± 0.001	0.014 ± 0.003	0.332 ± 0.02	0.001 ± 0.001
Mean	*Mytilus galloprovincialis*	Digestive gland ($n = 10$)	0.003 ± 0.001	0.123 ± 0.15	0.252 ± 0.21	0.656 ± 0.5	0.009 ± 0.004	0.031 ± 0.007	0.021 ± 0.008	0.189 ± 0.09	6.75 ± 4	0.008 ± 0.005
BAF	*M. galloprovincialis*	Digestive gland	3.75	4.27	252	59.7	9.3	30.6	10.15	13.52	20.33	8.4
Mean	*Styela plicata*	Branchial basket ($n = 10$)	<dl	0.337 ± 0.163	1.22 ± 0.92	0.946 ± 0.29	<dl	0.051 ± 0.02	0.078 ± 0.06	0.741 ± 0.46	30.7 ± 26.7	0.003 ± 0.001
BAF	*S. plicata*	Branchial basket	nd	11.62	1224	86.1	nd	50.6	39.3	52.9	92.6	3
Mean	*S. plicata*	Hepatopancreas ($n = 10$)	<dl	0.123 ± 0.06	1.072 ± 0.66	0.359 ± 0.11	0.003 ± 0.001	0.057 ± 0.01	0.028 ± 0.03	0.275 ± 0.13	9.43 ± 10.9	<dl
BAF	*S. plicata*	Hepatopancreas	nd	4.26	1072	32.6	3.6	57	14	19.7	28.4	nd
Mean	*S. plicata*	Tunic ($n = 10$)	0.001	0.491 ± 0.16	0.972 ± 0.43	0.434 ± 0.1	<dl	0.022 ± 0.01	0.092 ± 0.04	1.136 ± 0.36	38.6 ± 19.2	<dl
BAF	*S. plicata*	Tunic	1.444	16.9	972	39.4	nd	22	46.3	81.1	116	nd
Mean	*Sabella spallanzani*	Branchial crown ($n = 10$)	0.003	0.193 ± 0.31	0.216 ± 0.08	0.847 ± 0.14	1.865 ± 0.86	0.046 ± 0.009	0.013 ± 0.007	0.49 ± 0.08	4.303 ± 1.4	<dl
BAF	*S. spallanzani*	Branchial crown	3	6.65	216	77.1	1868	46.6	6.94	35	12.9	nd
Mean	*S. spallanzani*	Tube ($n = 10$)	0.031 ± 0.005	1.25 ± 0.5	2.36 ± 0.97	1.92 ± 0.63	<dl	0.145 ± 0.05	0.955 ± 0.14	1.86 ± 0.62	247 ± 30	0.811 ± 0.12
BAF	*S. spallanzani*	Tube	30.9	43.2	2364	174	nd	145	477	133	746	811

dl = detection limit; nd = not determined.

the tube, except for As concentrations. As shown in Table 1, the digestive gland of *M. galloprovincialis* accumulated trace elements in the following hierarchical order: Zn > Cu > V > Pb > Se > Cr > As > Hg > Cd. Differences between toxic metals in tissues of the different species analyzed are shown in Figure 1. Higher Cd, Hg, and Pb concentrations were found in the tube of *S. spallanzanii*, while higher As concentrations were found in the branchial crown of the same species. Significantly higher Cd concentrations were found in the digestive gland of *M. galloprovincialis* compared with tissues of *S. plicata* (Kruskal–Wallis test, $p = 0.00001$), whereas significantly higher Pb concentrations were found in the branchial basket and tunic of *S. plicata* compared with the digestive gland (Kruskal–Wallis test, $p = 0.00001$). No statistical differences were found for As and Hg concentrations. Differences between essential elements in tissues of the different species analyzed are shown in Figure 2. Higher concentrations were found in the tube of *S. spallanzanii*. Regarding the other tissues, higher essential element concentrations were found in tissues of *S. plicata* compared with the digestive gland of *M. galloprovincialis*, except for Zn, which accumulated preferentially in the digestive gland compared with hepatopancreas and tunic tissues.

Bioaccumulation factors

The BAFs are summarized in Table 1. Higher BAFs were calculated in the tube of *S. spallanzanii*, except for As, which had a very high BAF in the branchial crown (BAF = 1868). A very high BAF was calculated in the tube for Hg (BAF = 811). In contrast, very low BAFs for As, Cd, and Hg were calculated in the other tissues analyzed. Regarding the essential elements, high BAFs were calculated in all tissues of the different species, ranging from 216 to 2364 for Cu, from 32.6 to 174 for Zn, from 22 to 143 for Se, from 10 to 477 for Cr, and from 13 to 746 for Fe.

Trace element correlations

Correlations among trace elements are shown in the Supplemental Data. Many significant correlations among trace elements were recorded, with the highest correlation coefficients being observed in all tissues for Pb–Cu, Pb–Zn, Zn–Cu, and Pb–V. Positive correlation coefficients were found between essential elements in all tissues analyzed, especially between Cu and Zn (correlated at significant levels in all tissues, except for the tunic of *S. plicata* and branchial crown of *S. spallanzaniii*). Positive significant correlations were found between Cd, As, V, Zn, and Cu in the digestive glands of *M. galloprovincialis*. Negative correlation coefficients were found between As and the other trace metals in the branchial crown of *S. spallanzanii* and in all the tissues of *S. plicata*.

DISCUSSION

Trace element accumulation in tissues of S. plicata

Although relatively low trace element concentrations were found in tissues of *S. plicata*, high BAFs were recorded for the essential elements in the tissues analyzed, especially for Cu (Table 1). Copper is an essential component of the metal enzymes of Ascidians, and plays a crucial role in the catalysis of metabolic reactions because it is required in many biological enzyme systems that catalyze oxidation or reduction reactions [12]. Although Cu is required for normal growth and development of marine animals, it interferes with animal metabolism and physiology at high concentration [13]. It has been previously observed that high Cu accumulation occurs in

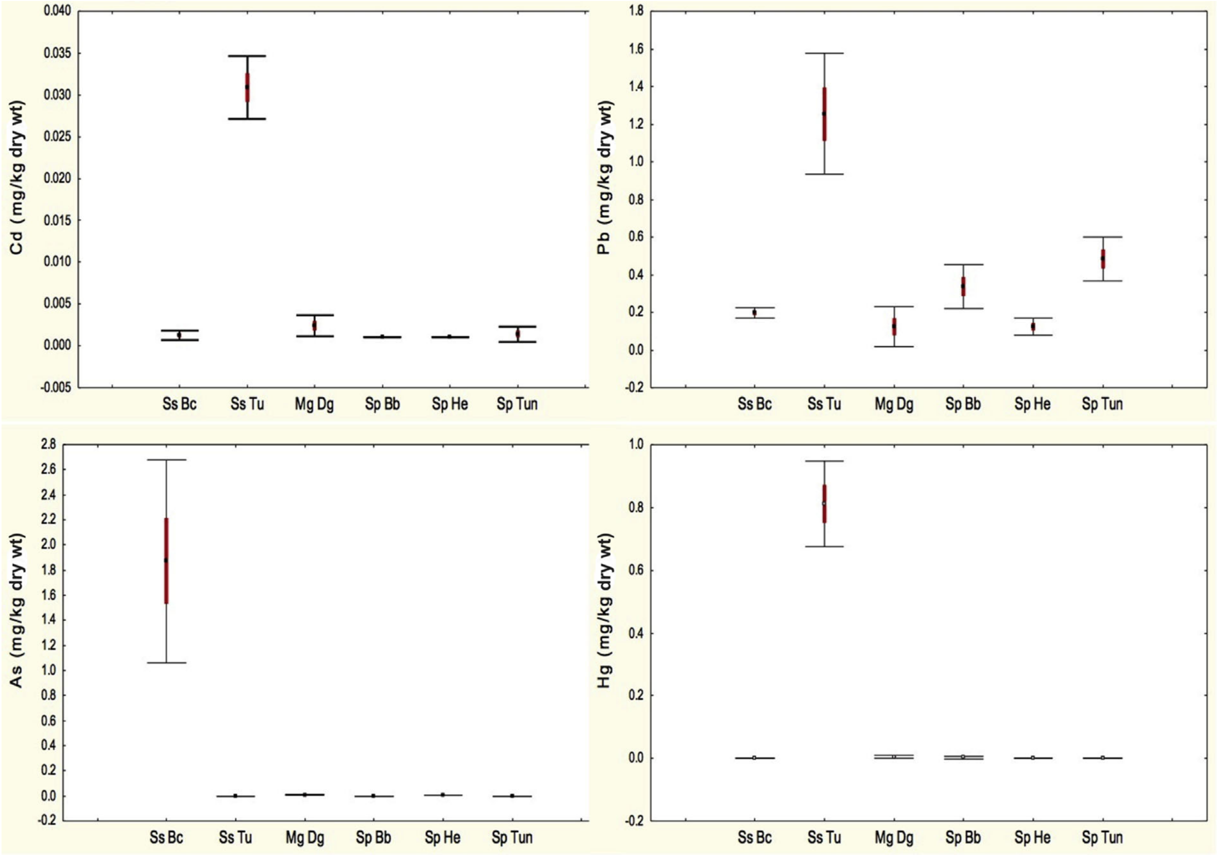

Figure 1. Difference between toxic element concentrations in different tissues of *Sabella spallanzanii, Mytilus galloprovincialis, and Styela plicata*. Concentrations are expressed in mg/kg dry weight. Data are presented as mean ± standard deviation. Ss Bc = *S. spallanzanii* branchial crown; Ss Tu = *S. spallanzanii* tube; Mg Dg = *M. galloprovincialis* digestive gland; Sp Bb = *S. plicata* branchial basket; Sp He = *S. plicata* hepatopancreas; Sp Tun = *S. plicata* tunic.

the ascidians *Herdmania pallida, Phallusia arabica*, and *Styela canopus* [12]. As discussed by Cima et al. [14], the toxic effect of Cu could be responsible for immune depression of tunicate larvae and hence lack of survival. Although the Cu concentrations found in the present study cannot be considered harmful, the very high BAFs (up to 1224 in *S. plicata* branchial basket) pose a health risk for specimens living in more polluted areas. It has long been recognized that ascidians actively accumulate essential elements [15] including Zn, Mn, Fe, Ni, Se, and Cr [16]. Our results are in agreement with those of other studies, confirming that ascidians are able to accumulate these essential elements for their metabolic profits (Table 1). As shown in Figure 2, the significant statistical differences in distribution of the essential elements suggest their preferential accumulation in the branchial basket compared with the tunic and hepatopancreas tissues. Because all the vital functions—such as absorption, circulation, storage, breeding—take place especially in the branchial basket, it is not surprising that the accumulation of essential elements would be higher in this tissue. The positive significant correlations between the essential elements in the branchial basket confirm analogous processes and dynamics of the physiological and metabolic pathways that control essential element incorporation. Moreover, many studies have documented hyperaccumulation of V for this animal taxon [17,18]. Thus, V cannot be considered a pollutant that is passively incorporated into the tissues of ascidians; instead, it is actively incorporated and involved in metabolic roles [19]. These roles may explain the preferential accumulation of this element in blood cells known as vanadocytes [19]. The biological function of V in ascidians is still disputed, although it may act against predation or as an antimicrobial agent [19]. However, our results suggest that V bioaccumulation in tissues of *S. plicata* takes place to a lesser extent than in other species. Webb [20] first proposed the hypothesis that ascidians represent a transitional stage between V users and Fe users and that the relative concentrations of V and Fe reflect phylogeny. This hypothesis was based on earlier reports that species in the suborders Phlebobranchia and Aplousobranchia contained high levels of V, whereas the evolutionarily newer taxon of Stolidobranchia contained smaller quantities of V but retained large quantities of Fe. The higher Fe concentrations found in the present study in tissues of *S. plicata* (~30-fold higher than V concentrations) confirm that this species accumulates Fe rather than V (Figure 2). As shown in Figure 2, V concentrations are higher in the tunic compared with the branchial basket and hepatopancreas. This result is in agreement with V distribution in different tissues of *S. plicata* found by Michibata [21], who found higher V concentrations in the tunic compared with internal organs. Although there are very few studies on V distribution between different tissues of *S. plicata*, we speculate that the V enrichment in the tunic could be a consequence of an antipredator strategy used by this species to make the tunic unpatalable for predators. This hypothesis has been previously suggested by Radhalaksmi et al. [12] to explain high V concentrations in tissues of *Ciona intestinalis*, justifying the invasive nature of this species. Regarding toxic elements, negligible concentrations of Hg, As, and Cd were found in all

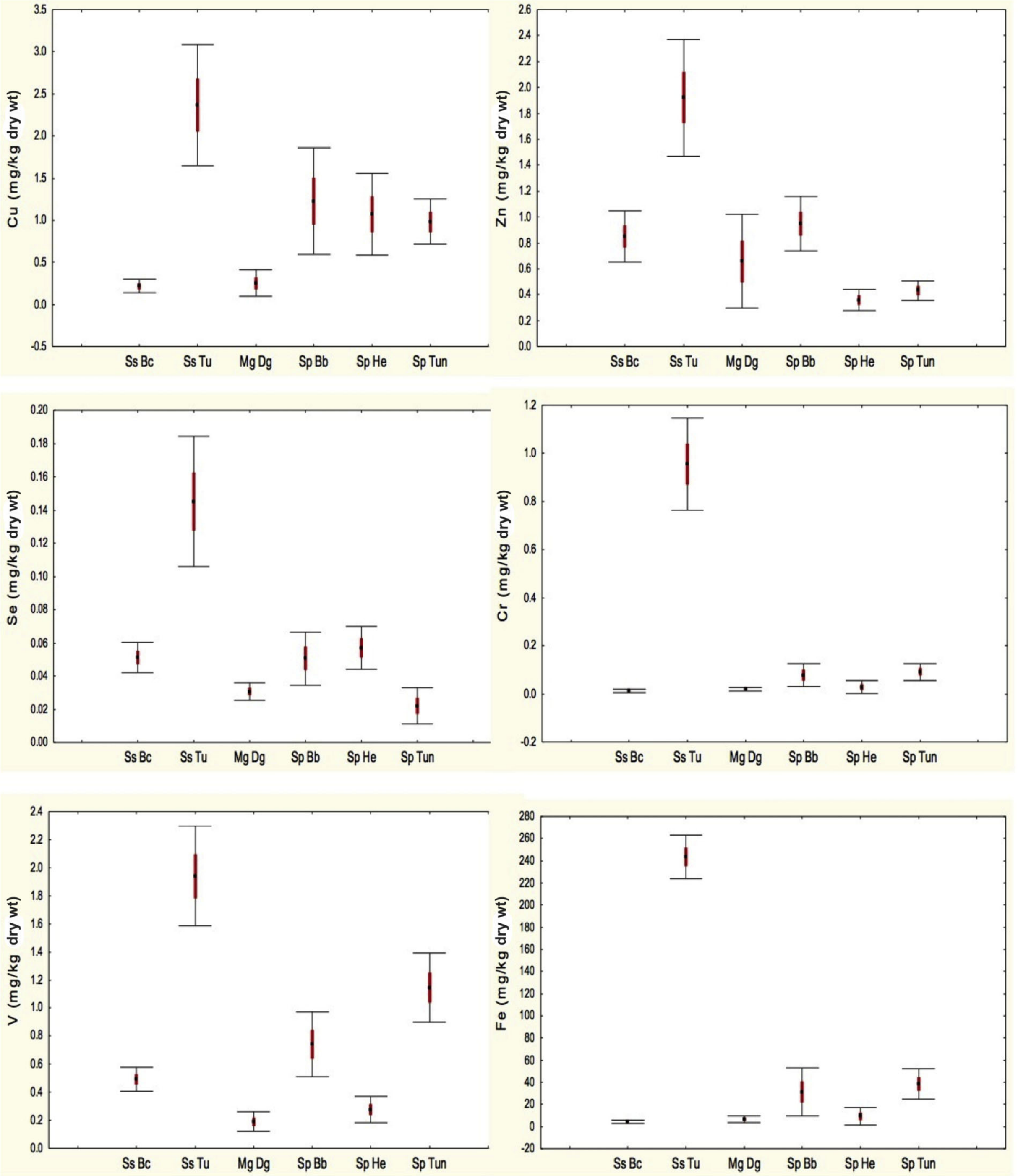

Figure 2. Differences between essential element concentrations in different tissues of *Sabella spallanzanii, Mytilus galloprovincialis, and Styela plicata*. Concentrations are expressed in mg/kg dry weight. Data are presented as mean ± standard deviation. Ss Bc = *S. spallanzanii* branchial crown; Ss Tu = *S. spallanzanii* tube; Mg Dg = *M. galloprovincialis* digestive gland; Sp Bb = *S. plicata* branchial basket; Sp He = *S. plicata* hepatopancreas; Sp Tun = *S. plicata* tunic.

the tissues analyzed, reflecting the low concentrations found in water samples, which were near the detection limit of the instrument (Table 1). Higher Hg (0.57 mg/kg dry wt), Cd (0.5 mg/kg dry wt), and As (8.2 mg/kg dry wt) concentrations were found by Philps et al. [22] in the whole body of *S. plicata* collected from a contaminated site in Florida (USA). However, higher concentrations of Pb were observed in the present study as a consequence of the higher concentrations found in water samples. Previous studies have reported higher concentrations of Pb in tissues of ascidians compared with the other toxic metals [12]. Matida and Kumada [23] found 35 mg/kg of Pb in the whole body of *S. plicata*. The relatively high levels of Pb in tissues of *S. plicata* are likely to be associated with antifouling paint residues arising from boat maintenance and vessel repair activities carried out in harbors. The antifouling paints used on small vessels are the major sources of Cu, Zn, and Pb [24] in the harbor environment. As shown in the coefficient correlations matrix, the significant positive correlations between these elements confirm the common source of pollution (Supplemental Data).

Trace element accumulation in tissues of S. spallanzanii

Very few studies have been conducted on trace element concentrations in tissues of *S. spallanzaniii*, which makes comparison difficult. Bocchetti et al. [25] found higher trace metal concentrations in the branchial crown of *S. spallanzanii* in both nonpolluted and polluted areas. Generally, higher trace metal concentrations were found worldwide in other polychaete species from areas affected by anthropogenic inputs [26,27], confirming the low degree of contamination in the area of the present study. As shown in Table 1, higher concentrations of essential elements compared with toxic elements were found in tissues analyzed (except for As concentrations in the branchial crown). The elevated values of such bioessential elements are expected, as they play important roles in the metabolic processes of these organisms. These elements are essential for survival, are a component of many metalloenzymes and respiratory pigments, and play an important role in cellular metabolism [28]. Thus, the body Zn and Cu concentrations in *S. spallanzanii* could be considered relatively independent from surrounding water concentrations and appear to be internally regulated. At the same time, the lower Cu concentrations found in the present study compared with other reports exclude the possibility that this metal may also be involved in the deterrence of predators in tissues of *S. spallanzanii*, as suggested in previous studies. For example, Gibbs et al. [29] considered that the high Cu concentration (52 mg/kg dry wt) in the body of *Perinereis cultifera*, collected at Gangasagar (India), was associated with antipredatory strategies. However, the consistently high BAF values of these 2 elements suggest their high potential for biomagnification during transfer to the top predators, especially for Cu (Table 1). The results of the correlation coefficient matrix show that both Cu and Zn are positively and significantly correlated with a majority of the metals, suggesting a common source or chemical similarity (Supplemental Data). Data on V reveal a range of concentrations that falls within the range found by previous authors in some polychaete species from the Mediterranean Sea. Polychaetes from the Mediterranean, including Sabellidae, exhibited V concentrations ranging between 0.5 mg/kg and 7 mg/kg dry weight [30]. Vanadium hyperaccumulation has also been reported in another sabellid polychaete. Specimens of *Phyllidia ocellata* from the Sanriku coast, northeastern Japan, were shown to concentrate extremely high levels of V in branchial crowns, ranging from 3000 mg/kg to over 7000 mg/kg, values approximately 100-fold higher than those measured in the rest of the body tissues [31]. Fattorini and Regoli [32] found that V preferentially accumulated in the branchial crown of *Perkinsiana littoralis* and *P. ocellata*, within the epithelial layer covering the internal cartilaginous-like structures of the branchial filaments, which contain fibrous proteins, sulfonated mucopolysaccharides, and matrix components. However, the absence of V hyperaccumulation reported in the present study confirms that unusually elevated tissue levels of this element are not a constant feature within sabellid species, as postulated by Fattorini et al. [30]. The relative high V concentrations found in the present study compared with other trace elements suggest a biological role for this element (Table 1). For example, the high values of V concentrations in branchial crowns of the sabellid *P. ocellata* has been interpreted to be a mechanism to facilitate oxygen absorption on the surface of bipinnate radioles, or to maintain oxygen when the worm is withdrawn into the tube [33]. Recent investigations on the defensive strategies of several polychaete species revealed that various sabellid species from the Caribbean and temperate western Atlantic, including *Bispira brunnea*, *Bispira variegata*, *Anamobaea orstedii*, *Branchiomma nigromaculata*, *Megalomma* sp., *Sabellastarte magnifica* and *Sabella* sp., all had unpalatable branchial crowns through the accumulation of chemical deterrents [34]. The highest As concentrations found in the present study in the branchial crown compared with the other metals (from 5- to 200-fold higher) suggest that a chemical antipredatory strategy is associated with the elevated concentrations of this element (Figure 1). As shown in the coefficient correlations matrix, the negative correlation between As and the other trace metals confirms a unique and selective mechanism of As incorporation in the branchial filaments (Supplemental Data). Arsenic hyperaccumulation is a quite typical characteristic for several polychaetes. The Mediterranean worm *S. spallanzanii* was shown to contain exceptionally high levels of As in branchial crowns (up to 1500 mg/kg) [32,35]. Elevated levels of As involved in the deterrence of predators were found also in the sabellida *P. cultifera* [36]. The deterring function of As in the exposed tissues of *S. spallanzanii* was demonstrated by the evidence that the branchial crowns of this species were unpalatable for the white seabream *Diplodus sargus*, which consumed without hesitation the other body tissues of the sabellid [32]. The predominant chemical form of As observed in *S. spallanzanii* was dimethylarsinic acid, a moderately toxic compound that the sabellid can synthesize by methylation of inorganic As normally accumulated from phytoplankton or more probably through filter feeding mechanisms of abiotic matrices (sediments and water column) [37]. Thus, the very low As concentrations found in water samples could explain the relatively lower As concentrations found in the present study in the branchial crown of *S. spallanzanii* compared with the unusually high As concentrations found inthe other studies. In the present study, lower levels of Cr were found compared with Cr concentrations found in tissues of other polychaete species considered as Cr bioaccumulators. For example, some capitellid worms are able to accumulate concentrations of Cr of up to 164 mg/kg dry weight. [38]. Although the Cr concentrations were relatively low, the very high BAFs found in tube samples suggest a high potential for bioaccumulation of this element (Table 1). In terms of toxic elements, ranges of Pb concentrations reported for other polychaete species from the German Wadden Sea indicate similarly low minimal values (~<1.3 mg/kg). This is even more pronounced in data reported from UK estuaries with a documented pollution history and also by reported data from the Mediterranean Sea or a mangrove wetland in India [27]. These authors postulated that low Pb concentrations of 1.3 mg/kg dry weight in polychaetes might serve as a regional or even global background value for comparison in biomonitoring studies. Although the Pb concentrations found in the present study fall within the baseline range of concentrations proposed by Hans et al. [27], we found higher Pb concentrations compared with Cd and Hg in the branchial crown and with As in the tube, confirming that antifouling paint residues arising from boat maintenance and vessel repair activities represent a non-negligible source of this metal in Termini Imerese Harbor (Table 1). A particularly high Pb BAF was found in the tube (Table 1). It is interesting to note that polychaetes are able to bioaccumulate Pb from the soluble phase, as demonstrated in previous laboratory experiments [39]. The coefficient correlations matrix shows significantly positive correlations among Pb, Cu, and Zn, confirming the common source of pollution represented by antifouling paints (Supplemental Data). Negligible Cd concentrations were found in both branchial crown and tube samples of *S. spallanzanii*. In accordance with our results, other authors have reported that Cd and Hg had a lower degree of accumulation in all the polychaete species than the other metals [40]. This might be related to the unique adaptive strategies of these worms, who secrete mucus in response to these metals, which helps to reduce metal availability for uptake. However, we found that the tube of *S. spallanzanii* accumulates higher Hg concentrations than the branchial crown. To our knowledge, this is the first report of trace metal concentrations in the tube of this species. Except for As, the tube samples had the highest trace metal concentrations, according to Eça et al. [40], who found higher trace metal concentrations in the tube of *Chaetopterus variopedatus*. This is especially true for Hg, which had a very high BAF of 811 in tube samples (Table 1). Instead of the very low Hg concentrations in water samples (near the detection limit), we found higher Hg concentrations in tube samples compared with concentrations in internal organs of polychaete species found in other studies worldwide, even in contaminated sites [26,41]. Buried in the sediments, polychaetes produce their tubes, made of a mixture of mucus and sediments rich in carbonate. Polychaete mucus contains strong complex ligands for organic and inorganic pollutants, which could be involved in the depuration of toxic pollutants from the internal organs and their consequent accumulation in the tube. Various histochemical investigations carried out in other polychaete species have shown that the mucus secreted by the epidermal cells is rich in many different substances, for example, glycosaminoglycans, different mucopolysaccharides, methallothioneins, and mucoproteins rich in sulfur ligands with a high metal affinity [42]. Moreover, as suggested by Eça et al. [40], the high Fe concentrations in the polychaete tube may reflect the high concentration of oxyhydroxides of Fe that accumulate around and within the tubes; these confer a brownish color to the external wall [43]. The highest Fe concentrations found in the tube of *S. spallanzanii* (50-fold higher than in other tissues) confirm the incorporation of Fe oxyhydroxides from particulate suspended matter in the tube matrix. The high affinity of metals to either carbonate or Fe oxyhydroxides, which are usually abundant in sediments, is well known [44]. Thus, the accumulation of contaminants in the tube may have occurred because of the complexation of metals by this organic matrix composed of biological secretion, carbonate, and Fe oxyhydroxides rich in metal binding sites.

Trace element accumulation in digestive glands of M. galloprovincialis

In accordance with the low trace element values found in tissues of *S. plicata* and *S. spallazanii*, relatively low trace element concentrations were also found in the digestive gland of *M. galloprovincialis* (Table 1). All of the mean values (mg/kg wet wt) of the metals analyzed were lower than the permissible limits set for Cd (1.0 mg/kg wet wt), Hg (0.5 mg/kg wet wt), and Pb (1.5 mg/kg wet wt) [45], and for Cu (30.0 mg/kg wet wt), Cr (1.0 mg/kg wet wt), and Zn (30.0 mg/kg wet wt) [46]. Higher concentrations of essential elements compared with toxic elements were found in digestive glands, as would be expected because of their biological roles (Figure 1). High Zn and Cu values in bivalve molluscs have already been observed by other authors [47,48], confirming that these metals could be involved in and could regulate many biological processes in the digestive gland of mussels [49]. The digestive gland is the main tissue of metal storage in bivalves, a consequence of detoxification mechanisms such as binding to metal-chelating proteins like metallothionein (MT) or storage in an insoluble form such as metal-rich granules [50]. Although low Cd levels were recorded in the present study, a BAF higher than 1 was recorded in digestive gland samples (Table 1). Previous studies have shown that in mussel whole soft tissues, bioaccumulation of Cd occurs over time after exposure, indicating a very slow or negligible elimination rate of this element [51]. This slow rate can be explained by the fact that, as MT degrades, the Cd released induces synthesis of a new protein, to which the metal becomes resequestered [52]. Localization of Cu–MT and Zn–MT within lysosomes has been reported for the digestive glands of mussels, suggesting that this is the principal site for MT synthesis induced by Cu and Zn. Because the basic MT pool in the digestive gland of *M. galloprovincialis* is high as a result of high cytosolic Cu and Zn contents, positive correlation coefficients were found in the present study between these metals and Cd concentrations. Previous studies have also indicated that the digestive gland was a more suitable indicator of Hg concentrations in the environment than the whole organism or gills of *M. galloprovincialis* [53]. A similar distribution of total Hg was recorded by Roesijadi et al. [54] and Odzak et al. [55]. Inorganic Hg is probably transformed in the digestive gland into some less toxic (inactive) form prior to excretion or storage (e.g., by production of byssus) [56]. Moreover, Roesijadi [57] has suggested that the marine mussel possesses low molecular weight, metal-binding proteins that can be induced to bind Hg when individuals are exposed to elevated concentrations. Gailer et al. [58] examined the uptake of 9 As compounds by mussels and showed that 4 of the compounds (arsenate, arsenite, methylarsonate, and dimethyl-arsinate) were not accumulated. In contrast, arsenobetaine was readily taken up by the mussels and accumulated unchanged. In accordance with these results, non-negligible BAFs were recorded in the present study for Hg and As accumulation in digestive gland samples (Table 1), compared with the very low Hg and As concentrations in water samples (near the detection limit). In terms of the Pb bioaccumulation mechanism in *M. galloprovincialis*, previous studies have shown that after experimental exposure to Pb under conditions representative of natural environmental Pb levels in water, the shell compartment contained the major fraction of the total Pb accumulated by mussels [59]. Despite these assertions, the Pb concentrations found in digestive gland samples in the present study were higher than levels of other toxic metals because of leaching from the antifouling paints used to reduce corrosion of boat hulls (Table 1). Strong positive correlation coefficients among Pb, Zn, and Cu were found also in this tissue.

Comparison of S. plicata*,* S. spallanzanii*, and* M. galloprovincialis *as biomonitor organisms*

As postulated by Phillips and Rainbow [5] species to be chosen as bioindicators should fulfill several criteria. An ideal bioindicator should be sedentary, easy to identify, abundant, available for sampling throughout the year, large enough to provide sufficient tissue for individual analysis, and a net accumulator of metals. The species analyzed in the present study adequately fulfill the criteria of being sessile, abundant, and readily sampled throughout the year. Moreover, the BAFs calculated in the present study (Table 1) show that all 3 species are able to accumulate a certain amount of metals as a consequence of filter feeding mechanisms, and are thus suitable as indicators of water quality. This is confirmed by the fact that all 3 species had higher Pb tissue concentrations compared with the other toxic metals, reflecting the major source of contamination in the study area, represented by antifouling paints. In particular, after comparison of trace metal concentrations between the different tissues analyzed, it is clear that the tube of *S. spallanzanii* represents the compartment with the highest trace metal accumulation. Thus the present study postulates that the tube of *S. spallanzanii* is an important compartment in metal retention and is more suitable for evaluation of contamination as a result of trace elements. Because of the complexation of metals by an organic matrix (composed of biological secretion, carbonate, and Fe oxyhydroxides rich in metal-binding sites), the metals chelated in the matrix cannot be eliminated in the same way as may occur in other tissues, so bioaccumulation even of toxic elements builds up over time without active or passive mechanisms of excretion. An exception is represented by the As hyperaccumulation in *S. spallanzanii* specifically in the branchial crown. Moreover, as previously discussed, the elevated As concentrations in the branchial crown of sabellids would not reflect a gradual accumulation of this element with age of the organism, avoiding variability as a result of the size effect. Thus the physiological roles (such as antipredatory strategies) performed by As in the branchial crown make this tissue more suitable for biomonitoring studies specifically focused on As. In terms of the other species analyzed, the BAFs calculated for the different tissues (Table 1) and the trace metal distribution among the different tissues (Figures 1 and 2) show that the digestive gland of *M. galloprovincialis* seems to bioaccumulate toxic metals more efficiently than tissues of *S. plicata*, in which no accumulation of Cd, As, and Hg was noted. An exception is Pb, which was significantly more concentrated in the branchial basket and tunic of *S. plicata*, suggesting a higher Pb accumulation rate. Previous studies have demonstrated that the relatively slow uptake of Pb limits the ability of mussels to accurately record Pb concentrations in the surrounding waters [59], a fact that should be taken into consideration in defining the appropriate species and/or sampling of tissues used in biomonitoring programs involving Pb. In terms of the essential elements, higher concentrations were found in tissues of *S. plicata* compared with the digestive gland of *M. galloprovincialis*. In particular, the very high BAFs calculated in the tunic and branchial basket especially for Cu, Zn, V, Se, Cr, and Fe suggest the suitability of these tissues to evaluate essential element concentrations in surrounding waters (Table 1). In this regard, we suggest that the tunic would be more useful because a sufficient amount of tissue can provide for individual analysis. It should be noted that environmental and biological conditions may affect trace metal bioaccumulation pathways in aquatic invertebrates, such as assimilation efficiency, ingestion rates of food, and growth rates [60,61]. The influence of such factors was not considered in the present study, and further investigations are needed.

CONCLUSIONS

The present study has provided data on trace metal accumulation in different tissues of 3 poorly studied benthic species, with the aim of evaluating their potential use as biomonitor organisms for trace metal pollution. We showed that the ascidian *S. plicata* and the polychaete *S. spallanzanii* can be used in biomonitoring programs, as both species were metal accumulators, even in an area of low contamination. We demonstrated that the use of *S. spallanzanii* in biomonitoring programs offers advantages over the mussel *M. galloprovincialis* because of the higher metal uptake and retention of the tube; thus, *S. spallanzanii* should be more widely used to assess metal contamination in both the water column and sediments. Although we assume that the ascidian *S. plicata* could be used as a sentinel of trace metal contamination, more studies, possibly in more contaminated sites, are needed to assess whether this species could be used as a valid alternative to mussels in biomonitoring surveys aimed at accurately reporting Cd, As, and Hg contamination.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3494.

Data availability—Data, associated metadata, and calculation tools are available on request from the corresponding author (daniela.piazzese@unipa.it).

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