Determination of trace metal baseline values in *Posidonia oceanica*, *Cystoseira* sp., and other marine environmental biomonitors: a quality control method for a study in South Tyrrhenian coastal areas

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Abstract In this study, we investigated Cd, Cr, Cu, Pb, and Zn in the seagrass *Posidonia oceanica* (L.) Delile leaves and in the brown algae *Cystoseira* sp. sampled along a 280-km transect in the Tyrrhenian Sea, from the Ustica to Linosa Islands (Sicily, Italy) with the aim to determine their control charts (baseline levels). By applying the Johnson's (Biometrika 36:149–175, 1949) probabilistic method, we determined the metal concentration overlap ranges in a group of five biomonitors. Here, we propose the use of the indexes of bioaccumulation with respect to the lowest (L'_i) and the highest (L_i) extreme values of the overlap metal concentration ranges. These indexes allow the identification of the most opportune organism (or a suite of them) to better managing particular environmental conditions. *Posidonia* leaves have generally high L_i indexes for Cd, Cu, Pb, and Zn, and this suggests its use as biomonitor for baseline marine areas. Our results confirm the high aptitude of *Patella* as a good biomonitor for Cd levels in seawater. From this study, Ustica resulted with higher levels of Cd, Cu, Pb, and Zn than the other Sicilian Islands.

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Abbreviations

S_L	Log-normal distribution
S_U	Unbounded distribution
S_B	Bounded distribution
S_N	Normal distribution
s.s.	Sampling stations

Introduction

The use of living organisms as biological monitors for the investigation of metal pollution in the environment is well-established (Markert et al. 2003; Gosselin et al. 2006), and several vegetal and animal organisms are involved for this purpose. For instance, the marine phanerogam *Posidonia oceanica* is currently used in monitoring programs because it holds a relevant position in the ecology of the Mediterranean Sea (Pergent-Martini and Pergent 2000; Pergent-Martini et al. 2005); it is an endemic species in the Mediterranean and can be found from the surface to a depth of 40 m and can colonize wide surfaces of seabed in which about 350 animal and vegetable species cohabit (Campanella et al. 2001; Conti et al. 2007a). In particular, *Posidonia oceanica* leaves can give an indication of trace metal concentrations in seawater over several months to years with good accuracy (Gosselin et al. 2006; Ferrat et al. 2003). In fact, *Posidonia oceanica* leaves have the requisites for a good biomonitor of baseline seawater trace metal pollution: They have high concentration factors (CFs), and the species is sedentary, ubiquitous, and easily identifiable (Lafabrie et al. 2007; Campanella et al. 2001; Conti et al. 2010).

The good practice of biomonitoring studies generally recommends the simultaneous use of several biomonitors in order

to obtain complete information on different possible bioaccumulation patterns (Conti 2008) because it is well recognized that each species responds to a particular fraction of the contaminants present in marine waters. For instance, *Posidonia oceanica* and algae respond to metals in solution and mussels to the fractions present in particulate matter (Conti et al. 2002; Volterra and Conti 2000; Deng et al. 2007). In fact, metal uptake for *Posidonia oceanica* is strictly connected with its life cycle (Conti et al. 2010; Richir et al. 2013).

The selection of these biomonitors depends on their proved ability in metal assumption. Metals present in seawater may penetrate the cell membrane of molluscs after complexation

reactions depending on esopolysaccharide and protein contents present in their mucus secretions (Simkiss and Mason 1984; Wright 1995). After assumption, metals can be metabolized by mechanisms of metallothionein formation which is the main detoxification mechanism present in aquatic invertebrate organisms (Amiard et al. 2006)

As far as brown algae is concerned, metals exhibit great chemical affinity with their alginate (Pan et al. 2000; Davis et al. 2003) and polyphenol contents (Czechowski et al. 2004; Lewis and Yamamoto 1990), making them widely applied for biosorption studies (Davies and Cliffe 2000; Romera et al. 2007; Saravanan et al. 2011). Moreover, metals can support
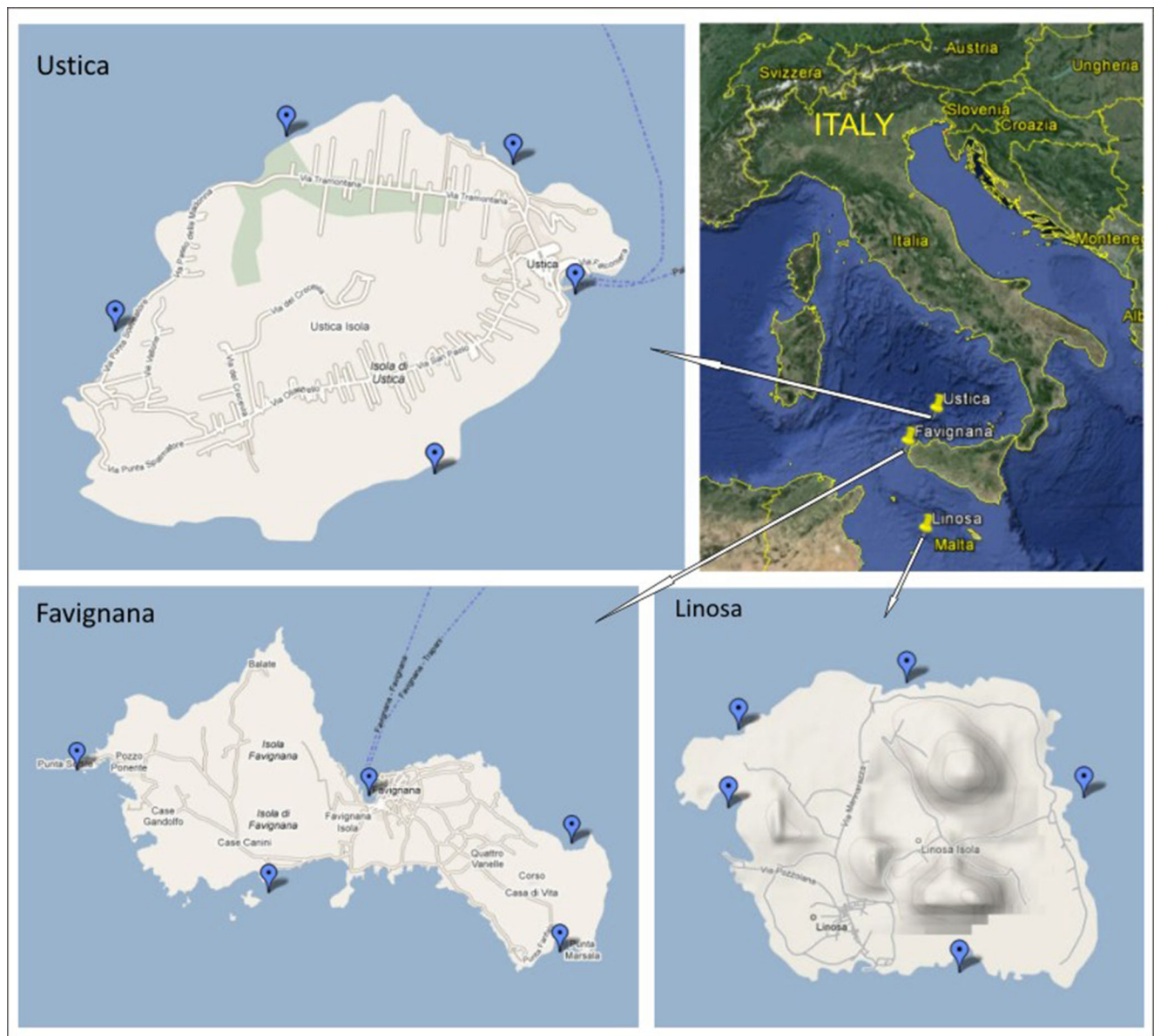

Fig. 1 The studied ecosystems (N-S): [sampling stations (s.s.—marked in the map)] (1) Ustica Island, north Sicily, Italy [(s.s.: Punta Galera, Harbour, Marine Reserve (Protected Sea Park), Tri Petri, Giaconia)]. (2) Favignana Island, Sicily [s.s.: Punta Sottile, Preveto, Cala Azzurra, Cala Rossa, Favignana Harbour]. (3) Linosa Island, south Sicily [s.s.: Calarena, Mannarazza, Pozzolana di Levante, Pozzolana di Ponente, Faraglioni]

Table 1 Johnson's classification of probability distributions for *Posidonia oceanica* leaves

	Element (µg g^{-1} dry weight)				
	Cd	Cr	Cu	Pb	Zn
Type of distribution	S$_B$	S$_B$	S$_U$	S$_U$	S$_B$
Baseline range	1.13–8.9	0.068–0.805	0.139–65.75	0.447–7.628	33.12–283.49
Overall median	3.06±2.08	0.19±0.15	15.11±7.58	1.28±0.68	111.78±71.49
Ustica	6.25±1.71	0.33±0.12	24.76±13.20	1.63±0.74	222.00±52.67
Favignana	2.67±0.36	0.60±0.21	10.95±7.64	0.94±0.33	115.90±10.89
Linosa	2.37±1.45	0.11±0.04	11.72±4.72	1.28±0.91	66.18±40.95

Baseline interval, overall median, and medians±median absolute deviations (m.a.d.) of samples for each of the three ecosystems: Ustica, Favignana, and Linosa Islands (near Sicily)

S$_U$ unbounded, S$_B$ bounded

the aggregation of organic matter from natural particles to anomalous size aggregates called mucilage (Mecozzi et al. 2008, 2009).

Furthermore, pH and ionic strength conditions also can affect metal absorption both for molluscs and seaweeds. In many algal species, the increased ionic strength (i.e., salinity in the marine environment) and increased pH, such as the alkaline conditions of seawater, enhance metal biosorption (Pan et al. 2000; Gonzales-Davila and Millero 1989). Additionally, these aspects are also governed by seasonality (O'Leary and Breen 1997). All these factors have to be considered in the identification of metal baseline level surveys (Conti 2008).

Thus, the first goal of this study is to establish a baseline metal concentration ranges in a 280-km transect in the Tyrrhenian Sea in the north, west, and south islands of Sicily: Ustica, Favignana, and Linosa by using *Posidonia* leaves as biomonitors. Due to its suitability as a possible candidate trace metal biomonitor, the brown algae *Cystoseira* sp. was also collected in Ustica and Linosa Islands (Conti et al. 2007a, 2010). Taking into account the natural variability of metal uptakes (Richir et al. 2013), we apply the probabilistic Johnson's method (Conti and Finoia 2010; Giovanardi et al. 2006) with the aim to provide baseline

intervals (as well as medians and distribution) of the concentrations of metals in *Posidonia* leaves. These are necessary (i) for comparing different findings, (ii) for developing environmental standards, and (iii) in calculating environmental and economic damages from events such as oil spills or other marine disasters (Conti and Finoia 2010).

The study of the probabilistic distributions of trace metals concentrations in marine species can give reliable information on their bioaccumulation mechanisms. In fact, it is well known that a Gaussian (i.e., normal) distribution of metals in organisms suggests several independent and small additive factors affecting the measured quantity, while a log-normal distribution suggests multiplicative effects; these issues were discussed elsewhere (Conti and Finoia 2010). By means of the normalization of any continuous probability distribution, the probabilistic approach here applied supports the identification of the metal concentration confidence intervals at 95 % ranges of variability. As reported above, this also consents to build a quality control charts (Johnson 1949; Miller and Miller 2005) that can be interpreted as a natural baseline metal levels in the studied ecosystems by using *Posidonia* leaves and *Cystoseira* sp. as biomonitors.

In many scientific studies, the concept of environmental baseline values is not always well defined because it tends to

Table 2 Johnson's classification of probability distributions for *Cystoseira* sp.

	Element (µg g^{-1} dry weight)				
	Cd	Cr	Cu	Pb	Zn
Type of distribution	S$_U$	S$_B$	S$_U$	S$_U$	S$_B$
Baseline range	0.06–3.67	0.18–0.83	4.40–21.70	1.13–15.00	21.80–175.40
Overall median	0.36±0.29	0.40±0.19	7.96±3.16	3.72±2.71	89.58±94.80
Ustica	0.25±0.13	0.53±0.25	10.65±4.78	4.39±1.91	143.70±57.60
Linosa	0.75±0.47	0.30±0.15	7.18±1.30	2.46±1.60	25.62±5.32

Baseline interval, overall median, and medians±median absolute deviations (m.a.d.) of samples for each of the two ecosystems: Ustica and Linosa Islands (near Sicily)

S$_U$ unbounded, S$_B$ bounded

Table 3 Cd in the studied species (µg g^{-1}), Johnson's distribution class (Dbz), median ($Q_{i,50}$) calculated on species i, lower and upper bounds of baseline range calculated by Johnson's method for the species i ($Q_{i,2.5}$ and $Q_{i,97.5}$), lower and upper bounds defining the range of overlap metal concentrations, and bioaccumulation indexes calculated on species i (L_i, L'_i)

Species (Cd)	Dbz	$Q_{i,2.5}$	$Q_{i,50}$	$Q_{i,97.5}$	Overlap range	L_i	L'_i
Monodonta turbinata	SU	0.33	1.31	5.41	1.74–1.85	2.92	5.28
Padina pavonica	SU	0.497	0.773	1.74		0.94	3.50
Patella caerulea	SL	1.85	4.33	10.47		5.65	0.94
Cystoseira sp.	SU	0.066	0.35	3.67		1.98	26.36
Posidonia oceanica	SB	1.13	3.15	8.92		4.82	1.54

be misunderstood with the term background. The term background is referred to pristine sites, while the term baseline is connected to specific basal values of metals in sites not necessarily unpolluted and often used as reference sites. With the aim to establish the baseline levels for the three selected ecosystems (Ustica, Favignana, and Linosa Islands, Sicily, South Italy), we prior evaluated and then confirmed that they are comparable for the metal contents by using an unsupervised multivariate statistical method such as cluster analysis

[see Chap. 7 in Conti (2008), Conti et al. (2007b), and Conti and Finoia (2010)].

Furthermore, with the aim to have the general view of the metal pattern concentrations by a group of biomonitors in these marine reference areas, we built the control charts for five metals (i.e., Cd, Cr, Cu, Pb, and Zn) determined in five biomonitors collected in the three islands (two islands for *Cystoseira* sp.) by using the Johnson's probabilistic method (Conti and Finoia 2010; Giovanardi et al. 2006). The selected

Fig. 2 Control chart for Cd built for the five selected biomonitors with their obtained overlap metal concentrations (µg g^{-1}). Observed values are on *x*-axes, and values calculated by Johnson's method are on *y*-axes. Inside the plot are reported: the lower and upper bounds of baseline range ($Q_{2.5}$ and $Q_{97.5}$) and the range of overlap (see the *arrow*). The histograms of values are shown outside of the plot. The medians±median absolute deviations (m.a.d.) are reported along the curves for each species
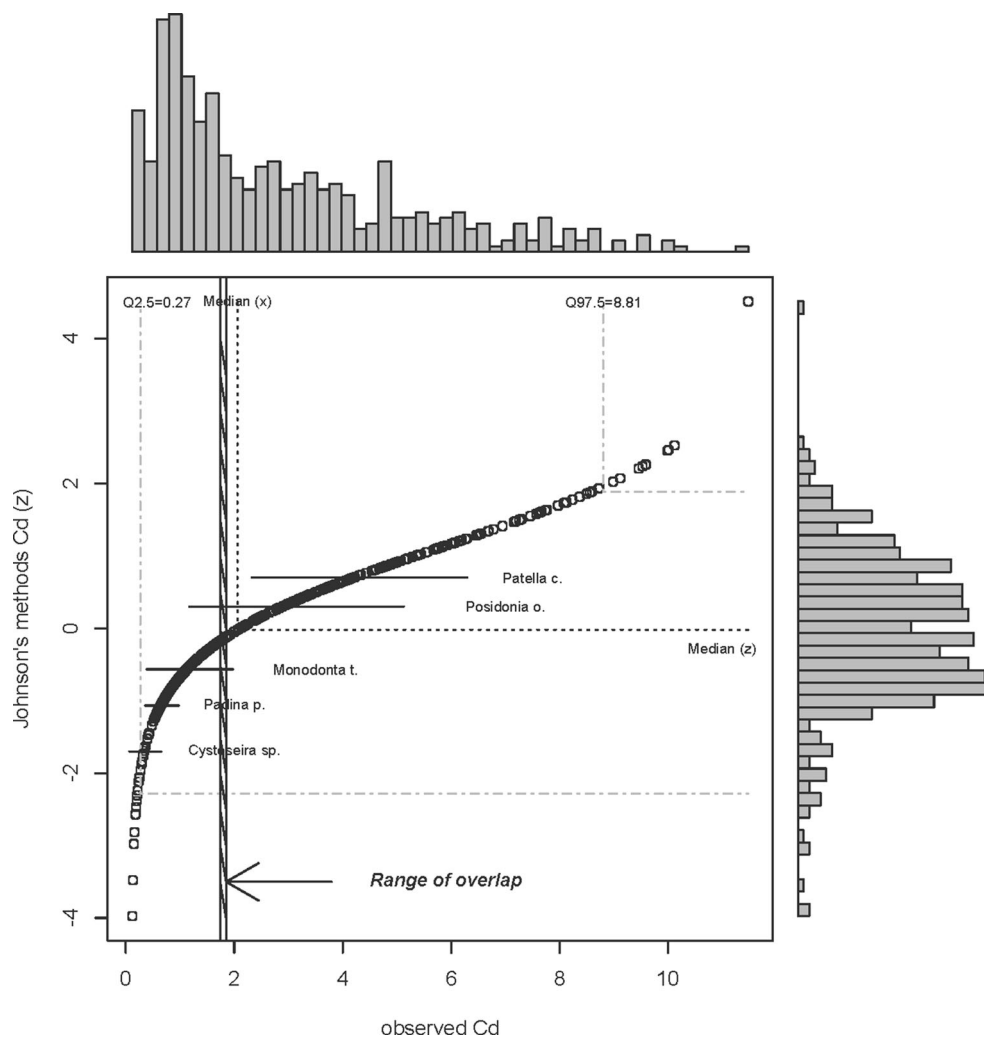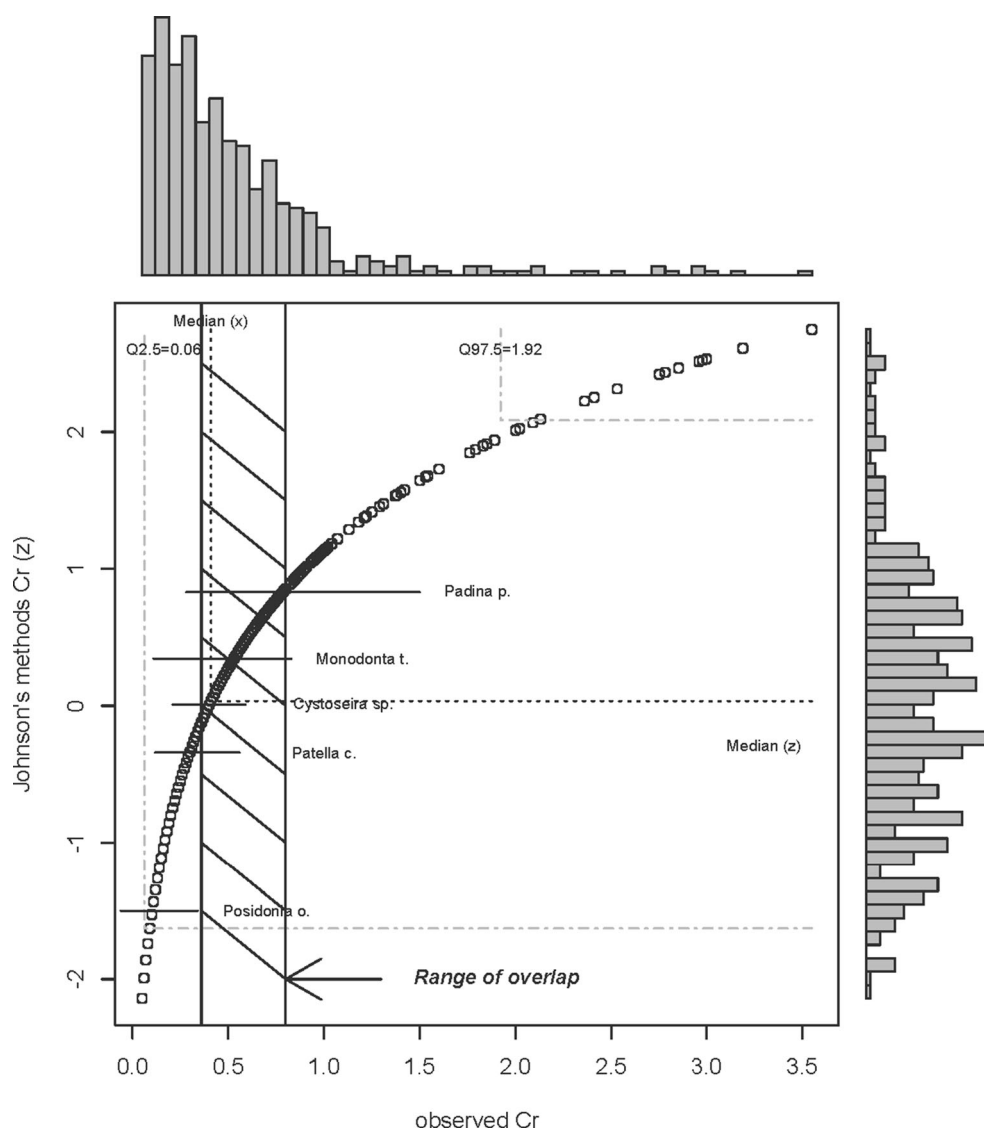
biomonitors are the seagrass *Posidonia oceanica* (leaves), the gastropod molluscs *Patella caerulea* and *Monodonta turbinata*, and the brown algae *Padina pavonica* and *Cystoseira sp.*

The control charts for *Patella*, *Monodonta*, and *Padina* were previously reported (Conti and Finoia 2010). The control charts for *Posidonia oceanica* leaves and *Cystoseira sp.* were suitably built for this study.

Thus, the second goal of this study was to identify the following:

(i) The range of overlaps of metal concentrations among the five selected species, for comparing the common bioaccumulation capabilities of each biomonitor

(ii) The metal bioaccumulation levels with respect to the upper and lower bound of the overlap concentration range by using the bioaccumulation indexes (L_i and L'_i, see "Materials and method" section).

In environmental studies, this approach can be useful in order to identify the specific biomonitor (or biomonitors) needed for a particular condition of contamination that can arise from natural or anthropogenic activities (i.e., pollutant spills and/or marine accidents). The control charts presented in this study consent to obtain the common metal concentration ranges for the suite of the selected biomonitors in the three south Tyrrhenian Islands. Moreover, the quality control method here applied allows to determine the indexes of bioaccumulation of each species with respect to the upper and lower bound of the common overlap ranges of metal concentrations.

Materials and method

Our study concerns sites in the north, west, and south islands of Sicily: Ustica, Favignana, and Linosa based on spatiotemporal (1997–2004) concentrations of trace metals in the leaves

Fig. 3 Control chart for Cr built for the five selected biomonitors with their obtained overlap metal concentrations ($\mu g\ g^{-1}$). Observed values are on *x*-axes, values calculated by Johnson's method are on *y*-axes. Inside the plot are reported: the lower and upper bounds of baseline range ($Q_{2.5}$ and $Q_{97.5}$) and the range of overlap (see the *arrow*). The histograms of values are shown outside of the plot. The medians± median absolute deviations (m.a.d.) are reported along the curves for each species

of the seagrass *Posidonia oceanica* (L.) Delile ($n=90$) and in the brown algae *Cystoseira* sp. ($n=45$), a 280-km long relative baseline of metal pollution for the South Tyrrhenian Sea. The five metals are the above-reported cadmium, chromium, copper, lead, and zinc.

The selected experimental sites lack of any industrial site and are quite far away from the urbanized Sicilian coastline. They can be considered not affected by anthropogenic activities. Samples were collected in July, which is the month with higher levels of ionic strength and salinity with higher metal absorption (see "Introduction" section). In these conditions, there is the highest availability of dissolved metals in seawater. Only mature leaves and thalli of similar length were carefully selected.

Samples were handpicked in the subtidal zone at depths of about 2 m (Favignana Island) and 7–8 m (Ustica and Linosa Islands) (Fig. 1). In each sampling site (five sites for each island, $n=15$; Fig. 1), five plants were sampled; individuals

richly covered by epiphyta were rejected during sampling. Then, in the laboratory, epiphyta and the sediment residuals were carefully removed by using nylon brushes (Campanella et al. 2001; Conti et al. 2010). Thus, the obtained whole leaves were rinsed with filtered seawater, put into polyethylene bags, and frozen (−20 °C) until analysis. Subsequently, samples were accurately pooled, and subsamples of 0.7 g were mineralized in a microwave oven with ultrapure concentrated HNO_3 and H_2O_2 (6+2 mL). Similar approach was employed for *Cystoseira* sp. See Conti et al. (2010) for details.

All the chemicals used in sample treatments were ultrapure grade. Water used for solution preparation and cleaning was obtained from a Millipore Milli-Q system. All glassware was cleaned prior to use by soaking in 10 % HNO_3 for 24 h and rinsing with Milli-Q water. Separate dry-weight determination was conducted on *Posidonia* leaves and *Cystoseira* sp. by oven drying at 105 °C until constant weight (15 replicates).

Fig. 4 Control chart for Cu built for the five selected biomonitors with their obtained overlap metal concentrations ($\mu g\ g^{-1}$). Observed values are on *x*-axes, and values calculated by Johnson's method are on *y*-axes. Inside the plot are reported: the lower and upper bounds of baseline range ($Q_{2.5}$ and $Q_{97.5}$) and the range of overlap (see the *arrow*). The histograms of values are shown outside of the plot. The medians±median absolute deviations (m.a.d.) are reported along the curves for each species
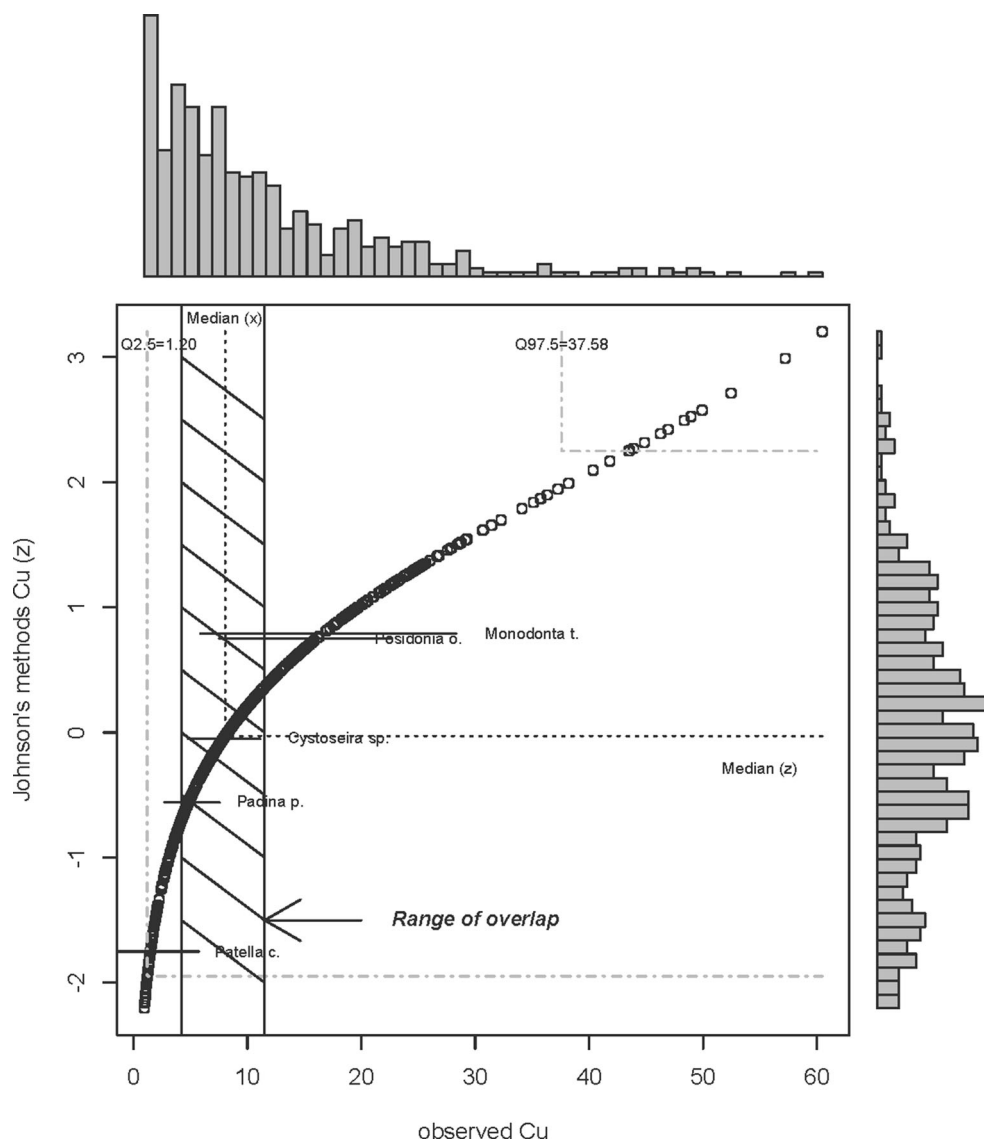
Sampling, chemical protocols, and results have been published elsewhere (Campanella et al. 2001; Conti et al. 2010).

The Johnson's method (Johnson 1949) was applied to our data by means of RSuppDists package (Slifker and Shapiro 1980; Chou and Polansky 1998; Trujillo-Ortiz and Castro-Perez 2007; Wheeler 2009). The univariate non parametric Mann-Whitney test (Siegel and Castellan 1988) was employed for testing metal bioaccumulation differences among the selected ecosystems by using *Posidonia* leaves and *Cystoseira* sp. as biomonitors. It was applied by means of the R software (Wheeler 2009).

For the determination of the overlap concentration ranges among biomonitors, we applied the same Johnson's probabilistic method. Then, an index of bioaccumulation for Cd, Cr, Cu, Pb, and Zn with respect to each overlap range is here proposed.

Definition of the overlap range for the studied metals

Given the $Q_{i,2.5}$ and $Q_{i,97.5}$ values, corresponding to the minimum and the maximum metal concentration levels, respectively, for the range determined according to Johnson's method, we build the control chart for the i_{th} species. Analogously, $Q_{j,2.5}$ and $Q_{j,97.5}$ are determined for the j_{th} species. Then, the overlap range for the i_{th} and j_{th} species is defined according to the following extreme values:

$$I_{\min} = \max\left(Q_{i,2.5}, Q_{j,2.5}\right) \text{ with } i = 1, 2, .., k \text{ and } i \neq j \quad (1)$$

$$I_{\max} = \min\left(Q_{i,97.5}, Q_{j,97.5}\right) \text{ with } i = 1, 2, .., k \text{ and } i \neq j \quad (2)$$

Fig. 5 Control chart for Pb build for the five selected biomonitors with their obtained overlap metal concentrations (μg g^{-1}). Observed values are on *x*-axes, and values calculated by Johnson's method are on *y*-axes. Inside the plot are reported: the lower and upper bounds of baseline range ($Q_{2.5}$ and $Q_{97.5}$) and the range of overlap (see the *arrow*). The histograms of values are shown outside of the plot. The medians±median absolute deviations (m.a.d.) are reported along the curves for each species

Fig. 6 Control chart for Zn build for the five selected biomonitors with their obtained overlap metal concentrations ($\mu g\ g^{-1}$). Observed values are on *x*-axes, and values calculated by Johnson's method are on *y*-axes. Inside the plot are reported: the lower and upper bounds of baseline range ($Q_{2.5}$ and $Q_{97.5}$) and the range of overlap (see the *arrow*). The histograms of values are shown outside of the plot. The medians±median absolute deviations (m.a.d.) are reported along the curves for each species
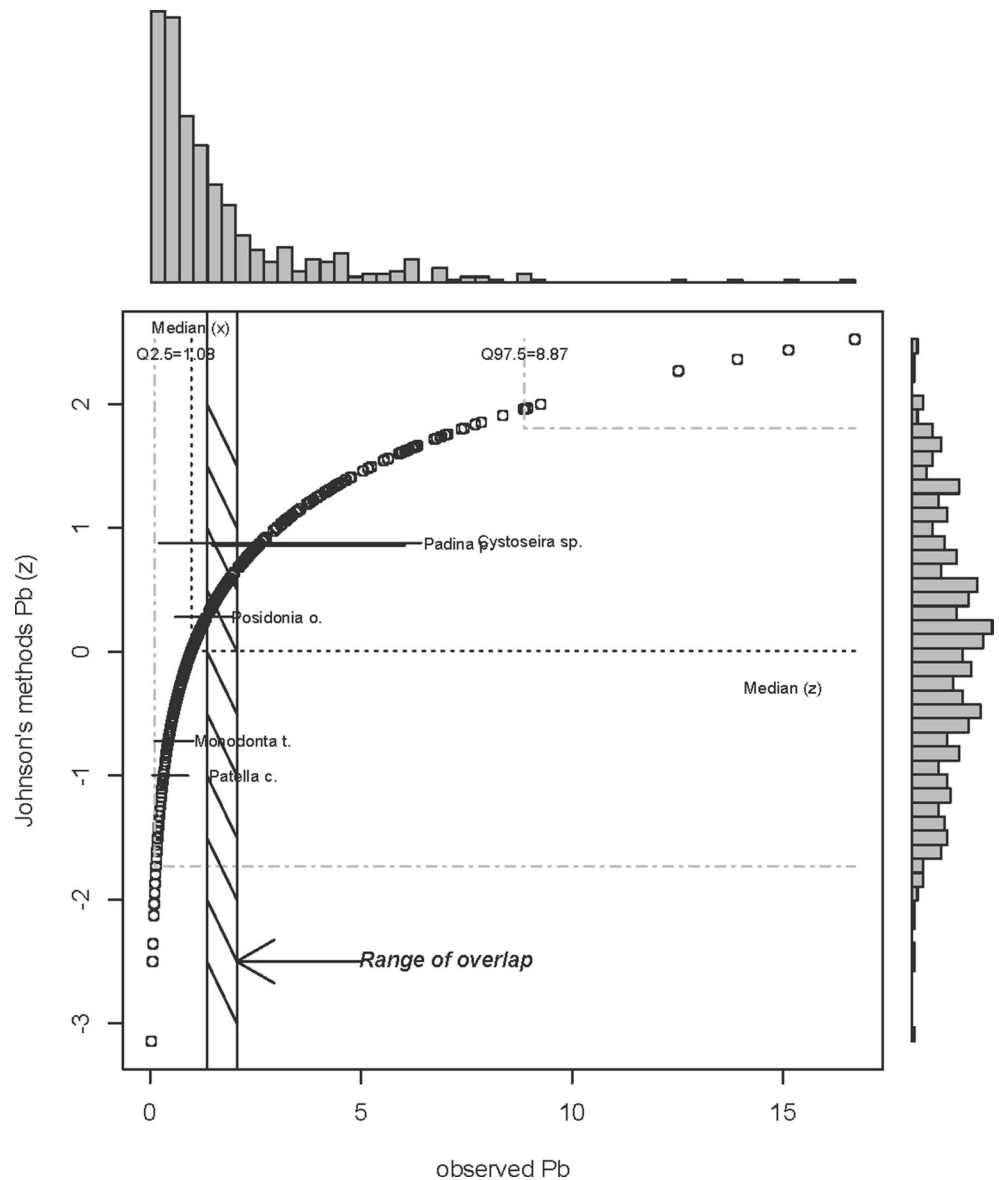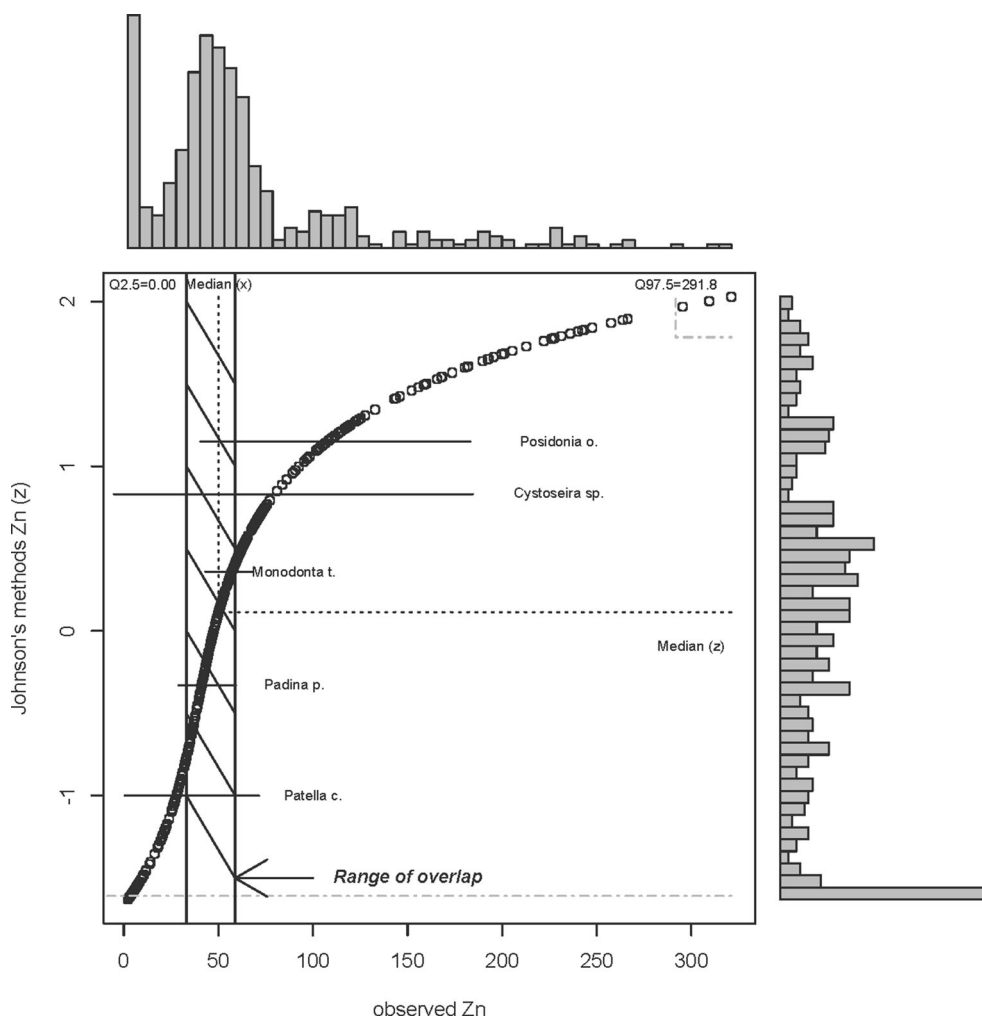

Definition of bioaccumulation index with respect to the maximum and minimum overlap range

The indexes of bioaccumulation (L_i) for the i_{th} species with respect to $Q_{i,97.5}$ is defined as

$$L_i = \frac{Q_{i,97.5}}{I_{max}} \text{ with } i = 1, 2, .., k \qquad (3)$$

L_i is generally ≥ 1 and becomes 1 when $Q_{i,97.5}=I_{max}$.

In case $I_{min}=\max(Q_{i,2.5}, Q_{j,2.5})>I_{max}=\min(Q_{i,97.5}, Q_{j,97.5})$, the L_i can be <1.

The index of bioaccumulation (L'_i) for the i_{th} species with respect to $Q_{i,2.5}$ is defined as

$$L'_i = \frac{I_{min}}{Q_{i,2.5}} \qquad (4)$$

Table 4 Cr in the studied species ($\mu g\ g^{-1}$), Johnson's distribution class (Dbz), median ($Q_{i,50}$) calculated on species i, lower and upper bounds of baseline range calculated by Johnson's method for the species i ($Q_{i,2.5}$ and $Q_{i,97.5}$), lower and upper bounds defining the range of overlap metal concentrations, and bioaccumulation indexes calculated on species i (L_i, L'_i)

Species (Cr)	Dbz	$Q_{i,2.5}$	$Q_{i,50}$	$Q_{i,97.5}$	Overlap range	L_i	L'_i
Monodonta turbinata	SB	0.071	0.45	1.56	0.36–0.80	1.95	5.07
Padina pavonica	SB	0.36	0.96	3.43		4.28	1.00
Patella caerulea	SB	0.12	0.34	1.08		1.35	3.00
Cystoseira sp.	SB	0.16	0.40	0.86		1.07	2.25
Posidonia oceanica	SB	0.07	0.19	0.80		1.00	5.14

Results and discussion

Table 1 reports the baseline range that falls between $Q_{2.5}$ and $Q_{97.5}$ percentiles. They give the variability for the five metals determined in *Posidonia* leaves ($n=90$) from Ustica Island (north Sicily), Favignana Island, and Linosa Island (south Sicily). Supplementary material (Table S1) reports the descriptive statistics for the trace metals in *Posidonia* leaves, and Supplementary material for Figs. S1–S5 show the control charts obtained for each metal by using *Posidonia* leaves as biomonitor. Data available on trace metals for *Posidonia* leaves is scarce in these marine areas (see Campanella et al. (2001) and Conti et al. (2010) for data in Mediterranean Sea for comparison).

Analogously, Table 2 shows the baseline metal intervals obtained from Ustica Island (north Sicily) and Linosa Island (south Sicily) for *Cystoseira* sp. ($n=45$). Supplementary material (Table S2) reports the descriptive statistics of the trace metals determined in *Cystoseira* sp., and Supplementary material for Figs. S6–S10 show the control charts obtained for each metal by using *Cystoseira* sp. as biomonitor.

The probability distributions of the metal concentrations in *Posidonia* leaves (baseline ranges) and *Cystoseira* sp. allow their use as quality control charts in order to support decisions about environmental protection policies for these marine areas.

The median for Cd (6.25 ± 1.71 μg g^{-1}) in *Posidonia* leaves was higher in the Ustica Island with respect to Favignana (Mann-Whitney U test$=11$ $p<0.001$) and Linosa Island (Mann-Whitney (M-W) U test$=56$, $p<0.001$). Similarly, the median for Cu (24.76 ± 13.20 μg g^{-1}) was higher in the Ustica Island with respect to Favignana (M-W U test$=68$, $p<0.001$) and Linosa Island (M-W U test$=57$, $p<0.001$). The median for Pb (1.63 ± 0.74 μg g^{-1}) was higher in the Ustica Island with respect to Favignana Island (M-W U test$=47.5$, $p=0.001$) and not significant with respect to Linosa. The median for Zn (222.00 ± 52.67 μg g^{-1}) was higher in the Ustica Island with respect to Favignana (M-W U test$=21$, $p=0.001$) and to Linosa Island (M-W U test$=2$, $p<0.001$). Eventually, Favignana Island showed the higher Cr levels (0.60 ± 0.21 μg g^{-1}) with respect to Ustica (M-W U test$=256$,

$p=0.004$) and Linosa Islands (M-W U test$=3.5$, $p<0.001$). From these results, we can infer that the higher levels of Cd, Cu, Pb, and Zn in *Posidonia* leaves were determined in the Ustica Island (see Table 1 and Supplementary material for Figs. S1–S5).

For Cr, Cu, Pb, and Zn, the median levels determined in *Cystoseira* resulted to be significantly higher in the Ustica Island with respect to Linosa Island. In particular, for Cr, the median level in *Cystoseira* sp. in Ustica Island was 0.53 ± 0.25 μg g^{-1} and significantly higher than the median level of Linosa (M-W U test$=91.5$, $p<0.001$). Analogously, for Cu, the level was 10.65 ± 4.78 μg g^{-1} and higher than Linosa Island (M-W U test$=101$, $p<0.001$). For Pb, the median was 4.39 ± 1.91 μg g^{-1} and higher than Linosa Island (M-W U test$=146$, $p<0.02$). Similarly, for Zn, the median was 143.70 ± 57.60 μg g^{-1} and higher than Linosa Island (M-W U test$=1$, $p<0.001$).

On the contrary, the median level for Cd (0.75 ± 0.47 μg g^{-1}) in *Cystoseira* sp. was higher in the Linosa Island with respect to Ustica Island (M-W U test$=460.5$, $p<0.001$) (see Table 2 and Supplementary material for Figs. S6–S10). This result agrees with the fact that *Cystoseira* has high selectivity for some particular metals such as Cd [see Table 3 and Conti et al. (2010)].

Linosa is located in the Sicilian channel, and it can be considered as a key reference ecosystem because it is 167 km away from Sicily and 165 km from the African continent. These three islands are not directly influenced by anthropogenic activities and can be considered as low contaminated sites for the south Tyrrhenian seas. In fact, some coastal zones were appointed protected sea areas from the Italian Ministry of Environment.

Figures 2, 3, 4, 5, and 6 show the control charts built for each metal for the five selected biomonitors with their obtained overlap metal concentrations. Observed values are on the x-axes, and values calculated by Johnson's method are on the y-axes. Inside the plot are reports on the lower and upper bounds of baseline range ($Q_{2.5}$ and $Q_{97.5}$) and range of overlap (see the arrow). The histograms of values are shown outside of the plot. The medians±median absolute deviations (m.a.d.) are reported along the curves for each species. The control charts

Table 5 Cu in the studied species (μg g^{-1}), Johnson's distribution class (Dbz), median ($Q_{i,50}$) calculated on species i, lower and upper bounds of baseline range calculated by Johnson's method for the species i ($Q_{i,2.5}$, $Q_{i,97.5}$), lower and upper bounds defining the range of overlap metal concentrations, and bioaccumulation indexes calculated on species i (L_i, L'_i)

Species (Cu)	Dbz	$Q_{i,2.5}$	$Q_{i,50}$	$Q_{i,97.5}$	Overlap range	L_i	L'_i
Monodonta turbinata	SB	4.30	16.67	37.74	4.30–11.52	3.27	1.00
Padina pavonica	SB	3.59	5.69	12.38		1.07	1.19
Patella caerulea	SB	1.07	3.20	11.52		1.00	4.02
Cystoseira sp.	SU	4.21	8.04	23.02		1.99	1.02
Posidonia oceanica	SU	0.14	14.74	65.75		5.70	30.71

Table 6 Pb in the studied species (μg g^{-1}), Johnson's distribution class (Dbz), median ($Q_{i,50}$) calculated on species i, lower and upper bounds of baseline range calculated by Johnson's method for the species i ($Q_{i,2.5}$, $Q_{i,97.5}$), lower and upper bounds defining the range of overlap metal concentrations, and bioaccumulation indexes calculated on species i (L_i, L'_i)

Species (Pb)	Dbz	$Q_{i,2.5}$	$Q_{i,50}$	$Q_{i,97.5}$	Overlap range	L_i	L'_i
Monodonta turbinata	SB	0.08	0.57	2.06	1.35–2.06	1.00	16.88
Padina pavonica	SB	1.35	3.69	7.49		3.63	1.00
Patella caerulea	SB	0.14	0.47	2.42		1.17	9.64
Cystoseira sp.	SU	1.24	3.82	18.09		8.78	1.09
Posidonia oceanica	SU	0.45	1.26	7.63		3.70	3.00

are similarly concocted, but they are different for (i) the metal concentration ranges ($Q_{2.5}$–$Q_{97.5}$) according to Johnson's method, (ii) the size of the overlap range values, and (iii) the distribution of medians determined for each species along the curve shape.

Tables 3, 4, 5, 6, and 7 show the Johnson's classification of probability, the median value ($Q_{i,50}$) of the ith species, the extremity values of the baseline range according to Johnson test for the ith species ($Q_{2.5}$, $Q_{97.5}$), the extremity values of the overlap range, and the bioaccumulation indexes (L_i) and (L'_i) (see "Definition of the overlap range for the studied metals" and "Definition of bioaccumulation index with respect to the maximum and minimum overlap range" sections for definitions).

From Table 3 and Fig. 2, we observe that *Patella* and *Posidonia* have higher bioaccumulation Cd surplus (L_i) that is 5.65 and 4.82, respectively, with respect to the upper extreme bioaccumulation overlap range (see the arrow in Fig. 2). For instance, the L_i for Cd in *Patella* was obtained after dividing the $Q_{i,97.5}$ value (i.e., 10.47 μg g^{-1}) by the upper extreme value of the overlap range (i.e., 1.85 μg g^{-1}) according to Eq. 3 (see "Definition of bioaccumulation index with respect to the maximum and minimum overlap range" section and Table 3). Table 3 also shows that *Cystoseira*, *Monodonta*, and *Padina* have the higher Cd bioaccumulation L'_i indexes according to Eq. 4 (see "Definition of bioaccumulation index with respect to the maximum and minimum overlap range" section) that are 26.36-, 5.28-, and 3.50-fold lower with respect to the minimum overlap range, respectively (see Table 3 and Fig. 2).

However, as far as Cd bioaccumulation is concerned, *Cystoseira* sp. has an L'_i=26.36, which means that it detects 26-fold lower Cd levels with respect to the minimum overlap range. This agrees with the high aptitude of this biomonitor to detect very low Cd levels in marine waters in spite of its nonuniform distribution in coastal areas (Riedl 1991). Conversely, *Cystoseira* has the highest L_i index (8.78) value for Pb, which means that it detects 8.78-fold higher Pb levels with respect to the maximum overlap range for the five studied biomonitors. This implies that this biomonitor is more specific for marine sites with high levels of Cu and Pb and sites with very low Cd levels.

From Table 4 and Fig. 3, we observe that *Padina pavonica* has the highest Cr L_i index that is 4.28 with respect to their upper extreme bioaccumulation overlap range. Furthermore, we observe that *Posidonia oceanica* and *Monodonta turbinata* have the higher and similar Cr L'_i indexes that are 5.14- and 5.07-fold, respectively, lower than the minimum overlap range.

From Table 5 and Fig. 4, we observe that *Posidonia* and *Monodonta* have the highest Cu L_i indexes that are 5.70 and 3.27, respectively. Furthermore, we observe that *Posidonia* has the highest Cu L'_i index that is 30.71.

From Table 6 and Fig. 5, we observe that *Cystoseira* has the higher Pb L_i index that is 8.78. Furthermore, we observe that *Monodonta* and *Patella* have the higher Pb L'_i indexes that are 16.88 and 9.64, respectively.

From Table 7 and Fig. 6, we observe that *Posidonia* and *Cystoseira* have the higher Zn L_i indexes that are 4.82 and 3.27, respectively. Additionally, we observe that *Monodonta*

Table 7 Zn in the studied species (μg g^{-1}), Johnson's distribution class (Dbz), median ($Q_{i,50}$) calculated on species i, lower and upper bounds of baseline range calculated by Johnson's method for the species i ($Q_{i,2.5}$, $Q_{i,97.5}$), lower and upper bounds defining the range of overlap metal concentrations, and bioaccumulation indexes calculated on species i (L_i, L'_i)

Species (Zn)	Dbz	$Q_{i,2.5}$	$Q_{i,50}$	$Q_{i,97.5}$	Overlap range	L_i	L'_i
Monodonta turbinata	SU	2.20	55.59	81.84	33.12–58.85	1.39	15.05
Padina pavonica	SB	27.30	44.04	71.90		1.22	1.21
Patella caerulea	SB	3.16	33.40	58.85		1.00	10.48
Cystoseira sp.	SB	20.98	84.69	192.72		3.27	1.58
Posidonia oceanica	SB	33.12	112.84	283.84		4.82	1.00

and *Patella* have the higher Pb L'_i indexes that are 15.05 and 10.48, respectively.

From these results, we can infer the following:

(i) For Cd, Cu, Pb, and Zn, *Posidonia* leaves have generally high L_i indexes with respect to the other biomonitors. This is reasonably linked with the chemical affinity (i.e., complexation ability) among these metals and the carboxylic group of alginates and polyphenolic compounds present in their tissues (Gosselin et al. 2006; Czechowski et al. 2004; Lewis and Yamamoto 1990).

(ii) These results confirm again the high aptitude of *Patella* as a good biomonitor for Cd in seawater (L_i=5.65). In fact, it has usually very high CF levels in these marine areas (Campanella et al. 2001; Conti et al. 2010).

(iii) *Monodonta* showed high L'_i values for Cd, Cr, Pb, and Zn, while *Patella* showed high L'_i values for Cu, Pb, and Zn. This agrees with our previous studies (Campanella et al. 2001; Conti et al. 2010) that show the high aptitude of these organisms to detect very low concentration metal levels in seawater.

Conclusions

From this study, we can draw several conclusions. By means of the Johnson's probability method, we built the quality control charts for *Posidonia oceanica* leaves and the brown algae *Cystoseira* sp., and then we determined the baseline intervals in 280-km transect sampling area in south Tyrrhenian marine areas.

We included in this survey the quality control charts for *Patella caerulea*, *Monodonta turbinata*, and *Padina pavonica* previously published (Conti and Finoia 2010). Thus, we determined the range of overlaps of metal concentrations among the five selected species. By means of this new approach, we can find the indexes of bioaccumulation with respect to the lowest (L'_i) and the highest (L_i) extreme values of the determined overlap range.

These indexes allow the identification of the most opportune organism (or a suite of them) in order to better manage different environmental conditions. For Cd, Cu, Pb, and Zn, *Posidonia* leaves have generally high L_i indexes that suggest its use as biomonitor for quite contaminated marine areas. Similar results can be drawn for *Patella* showing high L_i index value for Cd. Thus, *Patella* can be used as biomonitor in marine contaminated areas were high Cd levels can be present.

Monodonta showed high aptitude to detect very low levels of Cd, Cr, Cu, Pb, and Zn, whereas *Patella* was for Cu, Pb, and Zn. In fact, these two species showed the highest L'_i values.

Cystoseira showed high selectivity for some trace metals such as Cd, Cu, and Pb. However, further studies are necessary to confirm *Cystoseira* as a candidate biomonitor for these marine areas. Ustica Island resulted with higher levels of Cd, Cu, Pb, and Zn than the other two Sicilian Islands by using *Posidonia* leaves as biomonitor.

These studies are necessary (i) for comparing different findings, (ii) for developing environmental standards, and (iii) in calculating environmental and economic damages from events such as oil spills or other marine disasters.

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