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Metals in molluscs and algae: A north–south Tyrrhenian Sea baseline

Marcelo Enrique Conti [a,*], Maria Grazia Finoia [b]

[a] Dipartimento Studi per lo Sviluppo (SPES), Sapienza, Università di Roma, Via del Castro Laurenziano 9, 00161 Rome, Italy
[b] Istituto Superiore per la Protezione e la Ricerca Ambientale, Via di Casalotti 300, 00166 Rome, Italy

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ABSTRACT

We develop a 800 km long relative baseline of metal pollution for the Tyrrhenian Sea, from the north of Naples to south of Sicily (Italy), based on spatio-temporal (1997–2004) concentrations of trace metals in marine organisms and on the bioaccumulative properties of those organisms. The study concerns sites in the gulf of Gaeta-Formia, near Naples, and three islands north, west, and south of Sicily: Ustica, Favignana and Linosa. The five metals are: cadmium, chromium, copper, lead, and zinc; the species include: *Monodonta turbinata* ($n = 161$), *Patella caerulea* ($n = 244$) and the algae *Padina pavonica* ($n = 84$). We use Johnson's (1949) [15] probabilistic method to determine the type of distribution that accounts for our data. It is a system of frequency curves that represents the transformation of the standard normal curves. We find an N–S pollution gradient in molluscs considered: the lowest metal pollution occurs around the Sicilian islands. Our method can accurately characterize marine pollution by contributing to: policy-making, coastal resources management, the assessments of environmental damages from marine accidents and other events. The method here presented is a useful tool for pollution comparisons purposes among ecosystems (i.e., risk monitoring) and it is an ideal starting point for its application on a global scale.

1. Introduction

Using organisms as biomonitors for metal pollution in nature is well established [1–10]. Recently, gastropod molluscs have contributed to the more complete understanding of bioaccumulation and ingestion by humans in marine Mediterranean areas [11–14].

Additionally, bioaccumulation may not be toxic to one (or more) species [1–4,7,8] but may be an index of human exposure, particularly when the species is known to be indigenous food (e.g., *Patella*); besides, the selected species have high concentration factors (CFs) and are sedentary, easily identifiable, ubiquitous and can be used as a good biomonitors of baseline seawater trace metal pollution [11–14].

The theoretical distributions of concentrations of metals in marine species are often unknown, other than these there is an absolute lower bound (zero) on their mass. Nevertheless, empirical distributions, their shape, and the explanation for one distribution over another must be understood as those distributions are essential to descriptive and predictive work. The type of probability distribution gives insights for inference by providing a top-down understanding of the mechanisms that may control uptake and bioaccumulation processes. For example, a Gaussian distribution suggests several independent and small additive factors affecting the measured quantity; a log-normal distribution suggests multiplicative effects. A bimodal probability distribution suggests seasonal effects, time-dependent factors, or the presence of two distinct biological processes. Finally, a lower bounded distribution suggests that the most of the probability mass is assigned to low concentrations, while some of the higher concentrations may belong to a population with possibly different biological characteristics. This information, combined with a bottom-up mechanistic approach that links the distribution of concentrations in the species to a model of that species' physiological processes, can result in accurate risk assessments.

We apply a probabilistic method [15,16] with the aim to establish a baseline metal concentration ranges in an 800 km transect in the Tyrrhenian Sea based on biomonitoring studies conducted from 1997 to 2004 [11–14]. We provide normal (i.e., baseline) intervals (as well as medians and distribution) of the concentrations of metals in those species. These are necessary for: (i) comparing different findings, (ii) measuring sustainability, (iii) developing environmental standards; and (iv) in calculating ecological and economic damages from events such as spills or other marine disasters.

The probabilistic method here applied easily consent, by means of the normalization of any continuous probability distribution, to

Abbreviations: S_L, log-normal distribution; S_U, unbounded distribution; S_B, bounded distribution; S_N, normal distribution; s.s., sampling stations; Mt, *Monodonta turbinata*; Pc, *Patella caerulea*; Pp, *Padina pavonica*.

* Corresponding author at: SPES – Development Studies Research Centre, Sapienza, University of Rome, Via Del Castro Laurenziano 9, 00161 Rome, Italy. Tel.: +39 06 49766516; fax: +39 06 4451566.
E-mail address: marcelo.conti@uniroma1.it (M.E. Conti).

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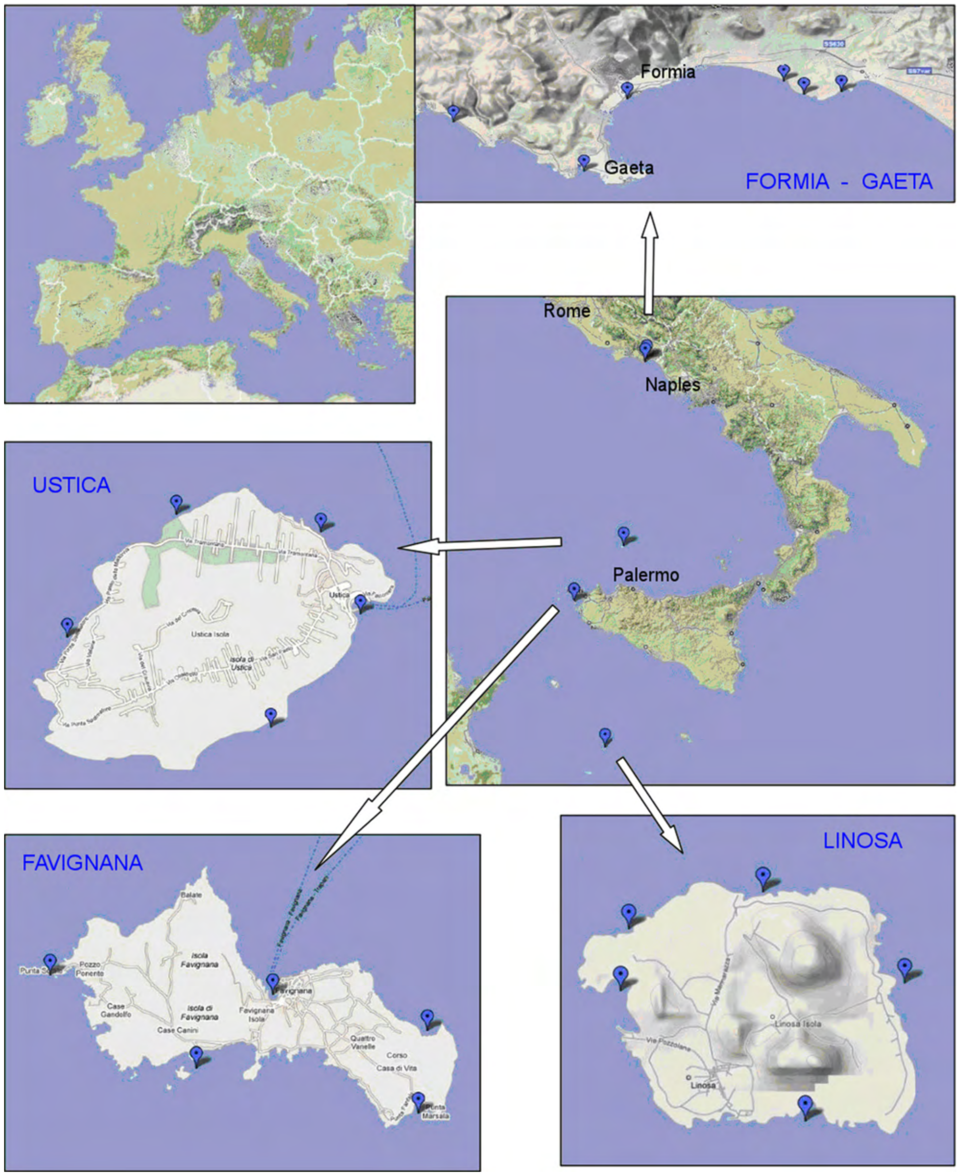

Fig. 1. The studied ecosystems (N–S): (1) Gaeta-Formia Gulf, Tyrrhenian Sea [Sampling stations (s.s. – marked in the map): Formia Vindicio beach; Porto Romano (Roman harbour); Sant'Agostino beach; Sassolini beach (Protected Sea Park); Santa Croce river; Serapo beach]. (2) Ustica Island, north Sicily, Italy [(s.s.: Punta Galera; Harbour; Marine Reserve (Protected Sea Park); Tri Petri; Giaconia)]. (3) Favignana Island, Sicily [s.s.: Punta Sottile; Preveto; Cala Azzurra; Cala Rossa; Favignana Harbour]. (4) Linosa Island, south Sicily [s.s.: Calarena; Mannarazza; Pozzolana di Levante; Pozzolana di Ponente, Faraglioni].

define metal concentration confidence intervals at 95% ranges of variability, and they can be interpreted as a natural background metal levels in the studied ecosystems (800 km long relative baseline of metal pollution for the Tyrrhenian Sea).

2. Materials and method

The study concerns sites in the gulf of Gaeta-Formia, near Naples, and three islands north, west, and south of Sicily: Ustica, Favignana and Linosa. The five metals are: cadmium, chromium, copper, lead, and zinc; the species include: *Monodonta turbinata* Born 1778 ($n = 161$), *Patella caerulea* Linné 1758 ($n = 244$) and the algae *Padina pavonica* (L.) Thivy 1960 ($n = 84$).

M. turbinata and *P. caerulea* are herbivorous and constitute the second link in the trophic chain. *M. turbinata* lives on the rocky seabeds of the intertidal belt, tideland, as deep as 5–10 m and is very tolerant to salinity and high temperatures and can live out of the water for some hours. *P. caerulea* lives on rocky substrata of tidelands and can also survive for long times outside of the water. It is found only in the Mediterranean, where it is a popular food, while *M. turbinata* is also equally distributed in the western Atlantic Ocean [14]. These molluscs often ingest epiphyte organisms, which are organisms that live on the plants they feed on, and they generally take metals mainly from the diet [14]. Due to its extensive distribution along the Mediterranean coasts and its potential suitability as trace metal biomonitor, the brown algae *P. pavonica* was also selected for this study [11–14].

Samples were collected in July, a month in which average metal concentrations in adult molluscs are possibly the highest [17]. This is consistent with establishing a precautionary baseline. The sites have several sampling stations (between five and six, depending on length of the site's shoreline) (see Fig. 1).

Molluscs were carefully collected and only strictly mature individuals in a very narrow range of weight (and size) were selected; identical procedure was done for *Padina* samples were only mature samples were collected. This was in order to have a high level of homogeneity and rigorous sampling protocols [11–14]. For instance, molluscs were immersed for 24 h in filtered seawater (of the corresponding station) to be purified so as to allow the depuration of the particulate matter residues present in the mantle cavity and their digestive tract [8,14]. Subsequently, the soft parts were taken out of the shell using plastic hammer and spatula in order to avoid metal contamination, and then they were rinsed with deionized MilliQ water, so as to remove every residue of shell. The samples were deep-frozen inside polyethylene bags and then analyzed after digestion in microwave oven. Separate dry-weight determination was performed on the different biota by oven drying at 105 °C until constant weight (10–15 replicates for each species). All chemicals used in sample treatments were ultrapure grade. The water used for solution preparation was obtained from a Millipore Milli-Q system. All the glassware were cleaned prior to use by soaking in 10% HNO_3 for 24 h and rinsed with Milli-Q water. Sampling and chemical protocols for our analyses have been fully detailed elsewhere [11–14].

2.1. Probabilistic method

We use Johnson's method [15,16] to determine the type of distribution that accounts for our data. It is a system of empirical distributions, based on alternative transformations of the standard normal deviate that approximate several non-normal distributions, including those that are either bounded or unbounded. For a continuous random variable X, whose distribution is unknown, the normalizing transformations have the general form:

$$Z = \gamma + \delta \cdot g\left(\frac{X - \xi}{\lambda}\right) \tag{1}$$

where $Z \sim N(0, 1)$, γ and δ are shape parameters, λ is a scale parameter, ξ is a location parameter, and $g(\cdot)$ is one of the following functions:

$$g(y) = \begin{cases} \ln(y) & S_L \text{ -- log -- normal distribution} \\ \ln(y + \sqrt{y^2 + 1}) & S_U \text{ -- -- unbounded distribution} \\ \ln\left(\dfrac{y}{1 - y}\right) & S_B \text{ -- -- bounded distribution} \\ y & S_N \text{ -- -- normal distribution} \end{cases} \tag{2}$$

S_U is defined over an unlimited interval. S_B provides a more flexible alternative to having to assume distributions such as the uniform, triangular, and beta [18]: this is necessary in Monte Carlo simulations in risk assessment by sharpening knowledge about the distributions of concentrations.

The script provided by the R SuppDists package for fitting a Johnson distribution on sample data utilises the procedure of quantiles, and requires, firstly, a choice of translation function and, secondly, an estimate of the values to be assigned to the four parameters γ, δ, λ and ξ. They may also be estimated from the moments of data distribution. Then, we can apply the appropriate translation function $g()$, defined in (2), that is univocally identified by the third and fourth order of moments of X sample distribution, i.e., skewness and kurtosis.

Johnson's method classifies sample distributions according to four family of frequency curves that can be plotted on the $(\sqrt{\beta_1})^2$ (i.e., the square of skewness) and $\beta_2 + 3$ (i.e., kurtosis + 3) axes (Fig. 4). The normal distribution (S_N) is identified by the point $\sqrt{\beta_1} = 0$ and $\beta_2 = 3$. The log-normal distribution should lies exactly on the S_L line, which divides the domain of the unbounded curves (S_U) from that of the bounded curves (S_B), beyond which S_B curves become bimodal. Finally, the *impossible area* extends beyond the straight line labelled as $\beta_1 - \beta_2 - 1 = 0$ where the combination of β_1 and β_2 cannot occur (see Supplementary data for more details about the Johnson's method).

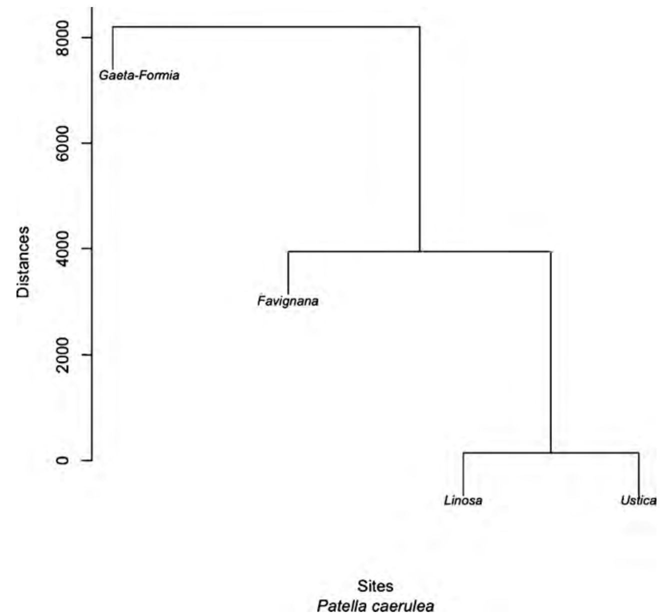

Fig. 2. Classification of the ecosystems (Cluster analysis, Ward method-euclidean distances) for *P. caerulea*.

3. Results and discussion

The data for Gaeta-Formia [12] were compared, through cluster analysis, with the data for the other three Sicilian sites [11,13,14] confirming that the differences among these marine ecosystems are qualitatively different. Physically, Gaeta-Formia and Linosa are the N–S extremes of the 800 km transect. The dendrograms (Figs. 2 and 3) identify the high distance among these stations, from the individual sites of these areas. Specifically, in *Monodonta* and *Patella*, the Gulf of Gaeta-Formia has higher metal contamination levels relative to the Sicilian sites.

This agrees with the fact that the area of the Gulf of Gaeta-Formia is not completely free from industrial activities, and therefore is not completely uncontaminated. It is affected by the presence of two

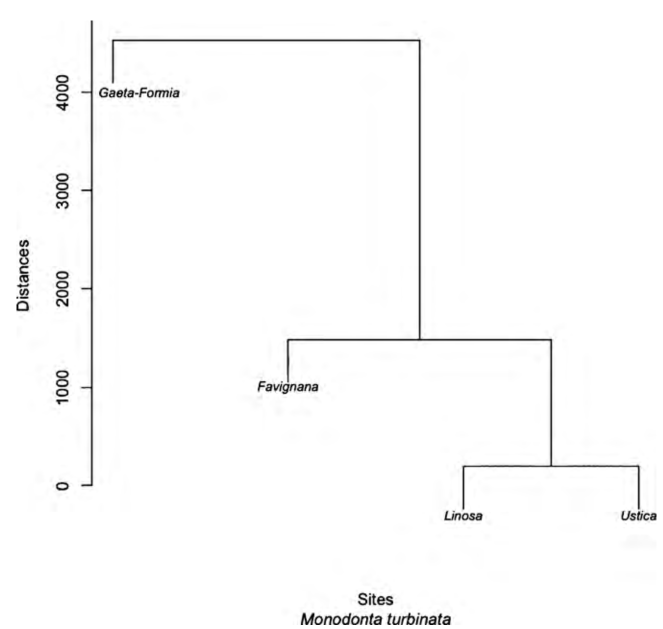

Fig. 3. Classification of the ecosystems (Cluster analysis, Ward method-euclidean distances) for *M. turbinata*.

Table 1
Johnson's classification of probability distributions for *P. caerulea*. Normal interval, overall median, medians ± median absolute deviations (m.a.d.) of samples for each of the four ecosystems: Gaeta-Formia (near Naples) and Ustica, Favignana, and Linosa islands (near Sicily). S_U – unbounded, S_B – bounded and S_L – log-normal distribution.

	Element (μg g^{-1} dry weight)				
	Cd	Cr	Cu	Pb	Zn
Type of distribution	S_U	S_B	S_B	S_L	S_B
Normality range	1.83–10.1	0.15–1.16	1.11–19.01	0.15–2.21	3.32–108.8
Overall median	3.92 ± 1.41	0.52 ± 0.41	5.57 ± 5.64	0.58 ± 0.46	46.7 ± 56.8
Gaeta-Formia	3.56 ± 0.87	0.82 ± 0.21	13.2 ± 3.71	0.82 ± 0.39	95.8 ± 10.9
Ustica	4.69 ± 1.91	0.57 ± 0.26	5.06 ± 2.47	0.93 ± 0.33	48.9 ± 12.2
Favignana	5.54 ± 2.55	0.50 ± 0.36	1.70 ± 0.55	0.23 ± 0.10	4.92 ± 1.51
Linosa	3.23 ± 1.24	0.36 ± 0.28	5.78 ± 2.74	0.60 ± 0.40	42.3 ± 7.79

moderately big towns: Formia and its harbour (37,500 inhabitants) and the town of Gaeta (24,000 inhabitants) (Fig. 1). Since 1987 this coastal area has a protected Regional Park of 285 ha and a Protected Sea area through a public concession to WWF Italy. In this area, covering 50,000 m^2, motor boats and fishing are not allowed [12]. However, from the reported results [12], the protected sea area (WWF Oasis) did not appear to be affected greatly by anthropic activities.

On the other hand, Linosa (Sicily) is not influenced by anthropogenic activities, it is positioned equidistant in the Sicilian channel 167 km away from Sicily and 165 km from the African continent. Moreover, it has high ecological relevance because the common turtle – *Caretta caretta* – uses this site for laying its eggs every summer. Since 2003, some coastal areas of this island (A, B and C zones) were appointed Protected Sea Areas from the Ministry of Environment of Italy. Linosa Island proved to have low levels of contamination and, to a certain extent, levels similarly lower than those of Favignana Island, Sicily (uncontaminated site).

Fig. 4 suggests that Pb concentrations in *P. caerulea* are log normally distributed and Zn is trimodal (multiplicative effect bioaccumulation). Additionally, the distributions Cr and Cu are bimodal suggesting two subpopulations: the first is Linosa-Ustica-Favignana, the second is Gaeta-Formia. The histogram of Zn is trimodal suggesting three subpopulations: Favignana, Ustica-Linosa, and Gaeta-Formia (Table 1). Cd is characterized by an unbounded distribution (see Supplementary data for Figs. S1–S5 and for validation tests results).

For *M. turbinata* Cd medians for Linosa and Ustica were different (Mann–Whitney test = 285.5, $p < 0.001$), although all other comparisons were statistically insignificant; in particular, the medians for Favignana and Formia were the same with the overall median for this site being 1.27 μg g^{-1}. For Cr, the overall median value was 0.45 μg g^{-1} (see Table 2 and Supplementary data for Figs. S6–S10 and for validation tests results).

Sea life depends on algae at the base of the trophic chain; the rate of net total oxygen produced by algae with photosynthesis is estimated at 30–50% [17]. Algae bind only free metal ions, whose concentration depends on the nature of suspended particulate matter which is formed by organic and inorganic compounds [8]. However algae, once they have accumulated toxic elements, may simply transfer them to the higher levels of the trophic chain

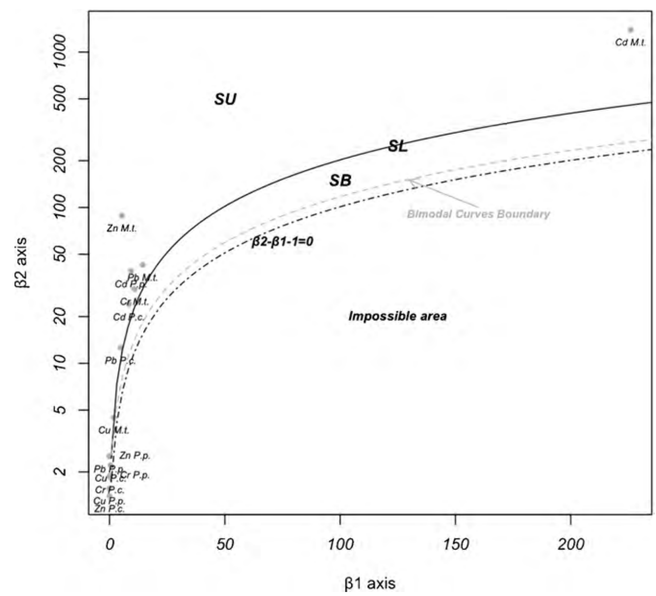

Fig. 4. The Johnson's scheme of curves classification system depending on β_1 and β_2 moments computed for each variable and species (Mt: *M. turbinata*; Pc: *P. caerulea*; Pp: *P. pavonica*). β_2 axes is plotted on logarithmic scale.

[17]. In fact each species responds to a particular fraction of the contaminant present in seawater (i.e., mussels respond to the fractions present in particulate while algae to metals in solution) [14]. For this reason we need to have a suite of biomonitors in order to have relevant information on different metal bioaccumulation patterns.

We also report results for *P. pavonica* (see Table 3; and Supplementary data for Figs. S11–S15). Cr, Cu and Zn distributions were bimodal; their frequency distributions suggest two subpopulations: the first for Linosa-Ustica, and the second for Favignana-Formia (see Supplementary data for validation tests results).

The normal intervals in Tables 1–3 fall between 2.5 and 97.5 percentiles, which give the variability of magnitude of background (normal concentration ranges), for the five metals in these species,

Table 2
Johnson's classification of probability distributions for *M. turbinata*. Normal interval, overall median, medians ± median absolute deviations (m.a.d.) of samples for each of the four ecosystems: Gaeta-Formia (near Naples) and Ustica, Favignana, and Linosa islands (near Sicily). S_U – unbounded, and S_B – bounded distribution.

	Element (μg g^{-1} dry weight)				
	Cd	Cr	Cu	Pb	Zn
Type of distribution	S_U	S_U	S_B	S_U	S_U
Normality range	0.32–4.68	0.10–1.54	5.35–84.17	0.11–1.89	14.2–123.3
Overall median	1.27 ± 0.58	0.45 ± 0.31	22.81 ± 16.4	0.58 ± 0.36	59.4 ± 19.1
Gaeta-Formia	1.10 ± 0.38	0.37 ± 0.16	59.8 ± 18.9	0.58 ± 0.14	95.6 ± 16.7
Ustica	1.66 ± 0.36	0.57 ± 0.26	21.02 ± 7.9	0.56 ± 0.33	62.7 ± 10.1
Favignana	1.10 ± 1.04	0.20 ± 0.15	8.88 ± 2.68	0.19 ± 0.11	35.2 ± 16.5
Linosa	1.03 ± 0.30	0.47 ± 0.43	14.9 ± 10.3	1.03 ± 0.58	55.2 ± 7.01

Table 3
Johnson's classification of probability distributions for *P. pavonica*. Normal interval, overall median, medians ± median absolute deviations (m.a.d.) of samples for each of the four ecosystems: Gaeta-Formia (near Naples) and Ustica, Favignana, and Linosa islands (near Sicily). S_U – unbounded and S_B – bounded distribution.

	Element (μg g^{-1} dry weight)				
	Cd	Cr	Cu	Pb	Zn
Type of distribution	S_U	S_B	S_B	S_B	S_B
Normality range	0.24–1.66	0.44–4.21	3.9–13.4	1.43–7.44	29.1–67.7
Overall median	0.71 ± 0.23	1.83 ± 1.68	8.0 ± 4.4	3.77 ± 1.71	48.5 ± 12.8
Gaeta-Formia	0.49 ± 0.16	3.42 ± 0.73	11.2 ± 1.33	3.9 ± 1.13	52.0 ± 10.4
Ustica	0.74 ± 0.12	0.89 ± 0.47	5.04 ± 1.19	3.11 ± 1.88	49.2 ± 12.5
Favignana	0.98 ± 0.53	2.82 ± 0.49	10.4 ± 0.58	6.05 ± 1.10	54.6 ± 12.3
Linosa	0.83 ± 0.2	0.69 ± 0.19	4.86 ± 1.01	3.02 ± 1.70	32.5 ± 5.39

from Gaeta-Formia (north of Naples) to the south of Sicily. Data available for these areas is very scarce (see Ref. [14] for data in Mediterranean sea biomonitors for comparison).

The determined probability distributions of the metal concentrations of the selected biomonitors (normality ranges) can consent the improvement of the environmental protection policies for Tyrrhenian areas.

4. Conclusions

The normal metal pollution concentration ranges determined, in an 800 km long baseline for the Tyrrhenian Sea, are relevant for environmental monitoring programmes (risk monitoring, environmental policies, environmental impact assessment, etc.).

The determined normality ranges of trace metal pollution in a large marine areas is of high relevance because the simply analysis of trace metal content in these species could indicate a possible risk of pollution; for instance, in the case of marine accidents (i.e., oil spills, industrial wastes pollution) or other related events (including environmental crimes).

This is consistent with our previous studies where these species showed that they can supply information on the bioavailability of contaminants at baseline levels, and then they can act as sentinel biomonitors of quality ecosystems. The here presented probabilistic approach is a useful tool for pollution comparisons purposes among ecosystems and it is an ideal starting point for its application on a global scale.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2010.05.022.

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