The biomonitoring approach as a tool of trace metal assessment in an uncontaminated marine ecosystem: the island of Ustica (Sicily, Italy)

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Abstract

Marine organisms were tested as possible biomarkers of heavy metal contamination in a reference marine ecosystem, in Ustica (an island of the Southern Tyrrhenian Sea). The goal of this preliminary work is to evaluate the concentrations of Cu, Zn and Pb, using two gastropod molluscs, Monodonta turbinata Born and Patella caerulea L.

Samples were collected in the tidal zone, at five coastal stations, according to their availability. Namely, the stations included the Marine Reserve (a “Marine Park” area), which is an uncontaminated site, and the Harbour station, where the contamination level was expected to be higher than the other sites. In order to gain additional information on both the environmental conditions of the area and possible bioaccumulation patterns, seawater samples were also collected in each site to assess soluble metal concentrations.

Statistical analyses (one way ANOVA and multiple comparison tests) were applied to test the differences between metal concentrations in different sites and species. Results show high concentration factors (CFs) with respect to the concentrations in marine waters (soluble fraction). This confirms the suitability of these species for biomonitoring purposes.

The metal concentrations recorded at the stations generally fall in the range of the lowest values available in the literature and may be considered as useful background levels to which to refer for intraspecific comparison within the Mediterranean area.

Keywords: trace metals, biomonitoring, marine ecosystems, molluscs, Monodonta turbinata, Patella caerulea.
1 Introduction

A consequence of the growth of anthropic activities has been an increase in heavy metal concentrations in marine ecosystems, which are considered to be contaminants or a source of stress when they cause damages to living organisms. Hence the necessity to acquire information on the bioavailability of such elements in the marine environment, in order to predict the incidence of several anthropic activities on species and ecosystems. To that end, over the last few decades, there has been a constant increase in the use of cosmopolitan organisms as means for monitoring pollution levels; these organisms are known as bioindicators, [1, 2].

When choosing the species to use in biomonitoring we must take into account the necessary requirements of a bioaccumulator: it must be cosmopolitan, that is it must have a wide geographical distribution so as to allow comparison among different areas; it must accumulate the pollutant without being killed by the levels it comes in touch with; it must present a high concentration value of the contaminant under study and it must have a contaminant concentration that could be easily correlated with the mean concentration in the surrounding environment, [3]. Moreover, it is necessary to stress that each bioindicator reacts to a specific fraction of the total load of metals that are bioavailable in the marine environment, [4, 5]. Because it offers remarkable advantages in comparison to traditional analysis of abiotic matrixes (water, sediments), biomonitoring has established itself as a precious means for the assessment of environmental quality, [6].

The aim of the present study was to evaluate the concentrations of Cu, Pb and Zn present in the marine ecosystem of Ustica by making use of two species of gastropod molluscs, Monodonta turbinata Born and Patella caerulea L.

Monodonta turbinata lives on the rocky seabeds of the intertidal belt, tideland, as deep as 5-10 m. It tolerates different kinds of water and different degrees of salinity as long as it stays fairly consistent. It can resist out of the water for several hours and it tolerates high temperatures. There is plenty of it in tidal pools, stuck under rocks or hidden in shady sea ravines. It is evenly distributed in the Mediterranean and in the Western Atlantic, from Portugal to Morocco and the Canaries. It is not difficult to recognize or to capture. Patella caerulea lives on rocky substrata of tidelands at relatively shallow depths. It is able to tolerate fairly long periods of time outside the water. It is only found in the Mediterranean, where it is a popular food. It is found pretty much everywhere along the coasts and in the lagoons, provided salinity is no lower than 30°/°. Both species are herbivorous and constitute the second link of the trophic chain. They often ingest other organisms that live on the plants they feed on (epiphyte organisms).

2 Materials and methods

The island of Ustica is on the Southern Tyrrenian Sea, 36 miles North-North West off the coast of Palermo, at a longitude of 14° 21’ East and a latitude of 38°
42’ 20” North. The sea environment of Ustica enjoys a pretty much unique condition in our seas: it has no sources of eutrophication or contamination of the waters and it is exposed to a very limited anthropic impact both in terms of time and quantity. In order to preserve the wealth of marine flora and fauna, the M. D. 12/11/86 [7], decreed the creation of the Marine Reserve of Ustica. Up to recently, the body in charge of this reserve was Ustica Town Council (one and only case in Italy). Now the Harbour Master’s Office of Palermo is in charge of the administration.

The molluscs were sampled in July 2001 at 5 stations along the island’s coasts (figure 1). This was in order to monitor different sites of the coast, provided the stations actually offered a reasonable number of organisms of each species. Namely, the stations included the Marine Park (A Zone), a presumably uncontaminated site, and the harbour, a site with a contamination level expected to be higher than the other sites.

![Map of Ustica Island with sampling stations](image)

Figure 1: Sampling stations and areas of the Marine Park of the Ustica island (Sicily, Italy): 1) Punta Galera, 2) Harbour, 3) Marine Reserve ("Marine Park"), 4) Tri Petri, 5) Giaconia; A) Zone A, B) Zone B, C) Zone C.
Individuals of *Monodonta turbinata* and *Patella caerulea* were collected in different quantities in the tideland. In the Reserve laboratory, they were immersed (\( t = 24 \) hours) in filtered seawater to be purified. The water had been drawn at the same stations where the samples were collected. Only individuals with a similar weight and ranging within a tight weight variability interval were selected. Subsequently, the soft parts were taken out of the shell using tools (hammer and spatula) made exclusively of plastics, so as to prevent metal contamination, and then they were rinsed with deionized (MQ) water, in order to remove every residue of shell and immediately they were deep-frozen inside polyethylene bags.

In order to assess concentration factors (CFs) with the aim to observe the bioaccumulation ability of every single species, metal analyses of seawater were performed on metals in solution. Water samples (\( n = 5 \)) were collected at a depth of 2 m with precleaned sampling bottles and rinsed twice with seawater.

The calculation of dry weight (d.w.) on the studied species (10 replicates for each location) was carried out through oven drying at 105\(^\circ\) C until constant weight was achieved.

Mineralization was carried out using a microwave oven MDS-2100-CEM with a mixture of concentrated HNO\(_3\)/H\(_2\)O\(_2\) (5 + 2 mL). Metal concentrations in molluscs were measured using Perkin Elmer A Analyst 300 atomic absorption spectrophotometer with flame atomization (FAAS) and graphite furnace system (GFAAS) with HGA 800 autosampler. For Cu and Zn, flame atomization was employed, while for Pb the GFAAS with HGA 800 system was used. Pb was analysed using the standard additions method and using matrix modifier NH\(_4\)H\(_2\)PO\(_4\) at 10\% with 0.2 mg of PO\(_4\). Whenever low levels occurred, Cu in molluscs was determined by GFAAS. The procedure for water samples is described elsewhere [8].

Table 1: Analysis of certified reference materials. Certified and found values (mean ± SD, \( n = 15 \) replicates).

<table>
<thead>
<tr>
<th>Element</th>
<th>CRM 278 (mussel tissue) ( \mu g ) g(^{-1}) d.w. ± SD</th>
<th>CRM 403 (seawater) ng L(^{-1}) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Certified</td>
<td>Found</td>
</tr>
<tr>
<td>Pb</td>
<td>1.91 ± 0.04</td>
<td>1.88 ± 0.32</td>
</tr>
<tr>
<td>Cu</td>
<td>9.60 ± 0.16</td>
<td>9.27 ± 2.46</td>
</tr>
<tr>
<td>Zn</td>
<td>76 ± 2</td>
<td>72 ± 30</td>
</tr>
</tbody>
</table>

The limits of detection (LODs) were calculated on the basis of 15 determinations of the blanks as 3 times the standard deviation of the blank. Results of molluscs LODs were: Pb = 0.09 \( \mu g/L \) and Cu = 0.43 \( \mu g/L \) (GFAAS), Zn = 0.09 mg/L and Cu = 0.01 mg/L (FAAS). LODs for seawater were: Pb = 23 ng/L, Zn = 57 ng/L and Cu = 41 ng/L.
The accuracy of the mineralization procedures was controlled using certified reference materials: BCR-CRM 278 (mussel tissue) and CRM 403 (seawater). One sample of CRM and blanks were included in each analytical batch. Results are in good agreement with certified values and the standard deviations were low and confirm the good quality of analytical procedures (table 1).

3 Results

Mean metal concentrations and standard deviations in molluscs are reported in table 2. Data were reported as concentration referred to dry weight. This way it is possible to reduce variability of measurements determined by habitat, life conditions, pretreatment and conservation of organisms after sampling [2]. Also, table 2 reports the mean dissolved metal concentrations in seawater from stations 1 to 5. The data on seawater metals must be interpreted with some caution, due to the high variability of metal concentrations in seawater, which depends on physico-chemical agents as well as many other factors.

Table 2. Concentrations of metals (µg g⁻¹ d.w) in the soft tissues of *M. turbinata* and *P. caerulea* and mean dissolved metal concentrations in seawater from stations 1 to 5 (µg L⁻¹ ± SD).

<table>
<thead>
<tr>
<th>Sampling station</th>
<th>Individuals</th>
<th>Pb</th>
<th>Cu</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patella caerulea</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>0.94 ± 0.16</td>
<td>5.27 ± 2.24</td>
<td>53.28 ± 16.40</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0.85 ± 0.57</td>
<td>9.50 ± 5.63</td>
<td>55.24 ± 20.14</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>1.10 ± 0.54</td>
<td>4.87 ± 2.19</td>
<td>56.62 ± 12.80</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>1.30 ± 0.66</td>
<td>6.54 ± 3.22</td>
<td>47.60 ± 7.85</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0.89 ± 0.26</td>
<td>5.16 ± 1.99</td>
<td>44.20 ± 11.09</td>
</tr>
</tbody>
</table>

| **Monodonta turbinata**<sup>b</sup> |             |          |          |          |
| 1                | 10          | 0.75 ± 0.27 | 18.72 ± 7.56 | 72.17 ± 6.67 |
| 2                | 10          | 1.06 ± 0.44 | 20.67 ± 10.39 | 54.91 ± 5.73 |
| 3                | 10          | 0.65 ± 0.35 | 18.55 ± 6.08 | 60.47 ± 4.31 |
| 4                | 10          | 0.59 ± 0.31 | 20.07 ± 4.67 | 64.45 ± 10.59 |
| 5                | 10          | 0.39 ± 0.11 | 32.60 ± 9.00 | 68.03 ± 9.02 |

| Seawater (n = 5)  |             |          |          |          |
| Dissolved metals  | 0.71 ± 0.22 | 1.18 ± 0.18 | 12.46 ± 2.57 |

<sup>a</sup>Mean length (cm): 2.78; <sup>b</sup>shell size (cm): 1.72 (mean height)
Figure 2: Mean lead concentrations for *M. turbinata* (µg/g d.w.) (Y axis) in the 5 sampling stations (X axis); the harbour station was significantly higher (p = 0.05) than the other stations.

In order to point out the significant differences with regards to mean metal concentrations (lead, copper and zinc) found on the two species of molluscs, the possibility was considered to employ a two way ANOVA model, where the two “experimental factors” were the Site and the Species.

The different hypotheses of the two factor ANOVA model were verified through the statistical software SPSS 10.0. Results show that the model’s assumptions are not always met and also the logarithmic transformation of data did not fit the purpose.

Therefore a variance analysis was applied, according only to the Site factor (one-way ANOVA) for both species. Where significant differences were detected among the stations, and in order to assess which stations were different from the others, the statistical study was completed with multiple comparison tests (Scheffé, Hochberg's GT2, Duncan, Tamhane and Tukey); the following results emerged:

**Lead:** from the use of *Monodonta*, stations 1, 3, 4 and 5 (Punta Galera, Riserva, Tri Petri and Giaconia) show the same concentration levels, while station 2 (Harbour) is remarkably different from the others in that it shows a much higher level (figure 2). As to *Patella*, on the other hand, no meaningful differences were detected in the stations.

**Zinc:** from the use of *Monodonta*, no significant differences arise between stations 2 and 3 (Harbour and Reserve) although they both show mean concentration levels that are remarkably lower in comparison with stations 1, 4 and 5 (Punta Galera, Tri Petri and Giaconia) (figure 3). Even in this case, as far as Patella is concerned, there are no differences in the mean concentrations of each station.
Copper: with regards to this element, from the use of Monodonta, stations 1-4 (Punta Galera, Porto, Riserva e Tri Petri) turned out to have the same contamination levels, whereas in station 5 (Giaconia) the concentration is significantly higher (figure 4). As to Patella, no meaningful differences were detected in the stations.

Figure 3: Mean zinc concentrations for M. turbinata (µg/g d.w.) (Y axis) in the 5 sampling stations (X axis), stations Punta Galera, Tri Petri and Giaconia were significantly higher (p = 0.05) than the others.

Figure 4: Mean copper concentrations for M. turbinata (µg/g d.w.) (Y axis) in the 5 sampling stations (X axis). Giaconia station was significantly higher (p = 0.05) than the others.
Metals concentrated in molluscs (Monodonta and Patella) decreased in the following order (see table 2): Zn > Cu > Pb. As for Monodonta the values obtained were in the range of 47.66 – 86.17, 4.95 – 52.46 and 0.14 – 1.77 µg/g d.w. for Zn, Cu and Pb, respectively. As for Patella the concentrations intervals detected for Zn, Cu and Pb were 22.89 – 101.47, 1.41 – 22.13, and 0.24 – 2.44 µg/g d.w., respectively. These results were in good agreement with those already identified by us in other studies [2, 8].

Table 3 reports the calculation of CFs for the species examined with reference to soluble metal concentrations. Regarding net accumulation we can observe that M. turbinata was the strongest accumulator of Cu accordingly to our previous studies [2, 8]. However, results show high concentration factors (CFs) with respect to the concentrations in marine waters (soluble fraction), in both species. This confirms the good suitability of these species for biomonitoring purposes.

Table 3: CFs\(^a\) \times 10^3 calculated with reference to soluble metal concentrations in seawater.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Cu</th>
<th>Zn</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monodonta turbinata</td>
<td>19.29</td>
<td>5.21</td>
<td>1.06</td>
</tr>
<tr>
<td>Patella caerulea</td>
<td>4.70</td>
<td>3.55</td>
<td>1.13</td>
</tr>
</tbody>
</table>

\(^a\)CF = Co/Csw, where Co = mean concentration in the organism (µg g\(^{-1}\) d.w.) and Csw = mean concentration in seawater (mg L\(^{-1}\)).

4 Conclusions

The two gastropods M. turbinata and P. caerulea can be considered as potential biomonitors of trace metals in the Mediterranean area. These two species possess the necessary requisites: they are sessile and sedentary, they are available all year round, they are easy to identify and capture and they are present along all coasts of the Mediterranean.

It is important not to overlook the possibility of regulating and/or competition mechanisms typical of some molluscs. As it is, some species have the ability to regulate the concentration of essential metals (Cu and Zn) in tissues until reaching roughly constant levels, without, for that matter, reflecting with exactness the contamination of the surrounding environment. This does not seem to be the case of M. turbinata. Statistical analysis revealed that, as far as Cu is concerned, Giaconia station presents a higher concentration; as to Zn, the Harbour and the Reserve present lower concentrations with comparison to the other ones. At the Harbour, Pb concentrations in Monodonta are higher than the other sites.

On the other hand, P. caerulea presented similar concentrations in all stations for both essential metals. One way through which Patella accumulates copper is complexation with some molecules of mucus. As a matter of fact, Davies [9] found that exposure to zinc and copper reduces mucus production in Patella.
vulgata; it is therefore likely that *Patella sp.* is somehow able to defend itself against high concentrations of this element. Moreover *P. caerulea* and *P. aspera* remove Cu very quickly when they return to clean waters, [10]. As far as Zn is concerned, Boyden [11] found that *Patelle spp.* accumulate comparatively more Zn from an environment with low concentrations of this element as opposed to a contaminated environment. It is therefore not possible to rule out self-regulation abilities on the part of *Patella*. On the other hand, *Monodonta* has a remarkable tolerance to high levels of contaminants [12, 13]: as a result, we can consider it an effective biomonitor for the Mediterranean area, perhaps more effective than *Patella*.

From the reported data, it is clear that there is no one site more contaminated than another. Thus the hypothesis of the Harbour as being the most contaminated site must be reconsidered. The levels of metals detected in the examined biomonitors fall into the lower levels of the literature, which is in accord with the assumption that the Ustica site can be used as a basal reference ecosystem for the contaminants under exam. The follow-up of this study, extended to other biomonitors and contaminants, might further confirm these initial results.

References


