

## Levels of metals in reared mussels from Taranto Gulf (Ionian Sea, Southern Italy)

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### Abstract

Samples of *Mytilus galloprovincialis* were collected monthly during the period July 1999–June 2000 from two mussel culture areas influenced by urban and industrial wastes. These stations, subjected to different environmental impact conditions, are located in the coastal area of Taranto Gulf (Ionian Sea, Southern Italy). Metals (Cd, Cu, Pb, Zn, Fe and As) were determined by atomic absorption spectrophotometry (AAS) in the whole soft tissue of mussels. Seasonal changes in metal concentrations were observed. Metals exhibited maximum values in later winter–early spring, followed by a progressive decrease during the summer.

Metal concentrations were similar to those detected in other Italian coastal zones, and indicate that the seafood under investigation poses no hazard to human health because metal content is within the permissible range established for safe consumption by humans. © 2007 Elsevier Ltd. All rights reserved.

**Keywords:** *Mytilus galloprovincialis*; Shellfood; Mussel farming; Metals; Taranto; Health hazard

### 1. Introduction

In the marine environment, levels of contaminants have been increasing over the last decades, as a consequence of anthropogenic activities. Pollutants are potentially accumulated in organisms and sediments, and subsequently transferred to man through the food chain. Coastal zones, particularly near large population centres, are of concern as they receive the largest exposure to chemical contamination, due to source proximity. The mussels *Mytilus* spp., as sedentary filter-feeders, are known to be good bioaccumulators of some trace elements (Rainbow & Dallinger, 1993; Viarengo, 1985). In *Mytilus* spp., metals are probably absorbed both from water and from ingested phytoplankton and other suspended particles (George, 1980). Mussels have proven to be useful for biomonitoring chemical contamination and, therefore, have been used for indicating

levels of trace metal concentrations, in the marine environment (Bryan & Hummerstone, 1977; Conti & Cecchetti, 2003; Goldberg, 1975; Phillips, 1980). The suitability of mussels as test organisms for the study of trace metal pollution results from their widespread distribution and favourable accessibility for collecting. Also, owing to their sedentary filter-feeding habit, metal concentrations in their tissues readily respond to impinging levels in the environment (Phillips, 1976; Schulz-Baldes, 1974). Moreover, molluscs are study organisms for their inclusion in the human diet.

The relatively shallow waters in the Gulf of Taranto (Ionian Sea, Southern Italy), yield large numbers of mussels. Taranto seas, Mar Piccolo and Mar Grande, represent a coastal marine ecosystem example, whose biological balances have been modified step by step, in relation to the anthropic development and, in particular, to the big industry settlement. The basin of Mar Piccolo lies to the North of Taranto town and is connected to the basin of Mar Grande through two narrow passages. It has experienced

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the negative effects of pollution because of its semienclosed shape and it has had remarkable problems of water exchange, which are mainly due to moderate sea tides. The basin is the final address of nine urban wastes. Mar Grande is a wide roadstead, which lies to the North-Eastern area of the Taranto Gulf. Twenty-eight urban and five industrial discharges drain directly into the basin. Taranto seas are a noteworthy economic resource, being the site of intensive mussel farming. Mussel farming in Taranto has a long history that dates back to the sixteenth century, so that the typical chestnut stakes, which stick out of the sea have always been part of the city view.

This industry has grown from the idea of an enterprising local to become a big export earner. Actually, the annual output amounts to 30,000 tonnes of mussels. Only a part of the locally harvested seafood is used for home consumption, while most is exported to European Economic Community countries, in particular to Spain.

The aim of this work was to determine the concentration of metals in two populations of mussels, over a one-year period, in order to investigate metals levels, accumulation of these pollutants with the population growth and public health risks associated with consuming mussels harvested from these areas.

## 2. Materials and methods

Mussels (*Mytilus galloprovincialis* Lam.) were collected monthly from July 1999 to June 2000, from two marine culture areas (Fig. 1). The Food and Agricultural Organisation for sampling recommendations, handling and specimen storage, as described in the FAO Fisheries Technical Paper (1976), were followed. Each month, 100 mussels of similar length were collected from the two sites, at the same

intertidal depth. Then, for a 24 h period, the samples were placed in filtered seawater, collected at the corresponding station to allow the depuration of the particulate matter residues present in the mantle cavity and digestive tract. Subsequently, the animals were carefully opened by cutting the adductor muscle with a plastic spatula and the soft tissues were extracted. The length, the volume of the shells and the wet weight of the soft tissue were measured for each sample. Biometric data are reported in Table 1. Fifty soft tissues samples dry weight (at 105 °C until constant weight) and ashes (at 750 °C for 2 h) were determined, in order to calculate both the content of organic substance and the “bivalve condition index” according to Matta (1968).

For metals determinations (Cd, Cu, Pb, Zn, Fe and As), 50 soft tissues samples were homogenised by an Ultra-Turrax T25 homogeniser. To avoid contamination, all the parts of the homogeniser, which came into contact with the sample, were covered with Teflon. The blades, made of stainless steel, were disposable.

One gram of homogenised sample was mineralised with 10 ml of concentrated HNO<sub>3</sub>, in a closed teflon vessel using a MARSX microwave oven (CEM Corporation, Matthews, NC). After mineralisation, digests were cooled and the resulting solutions were diluted to a known volume (50 mL) with Milli Q water and stored in polyethylene bottles, until analysis.

Copper, cadmium and lead were determined by graphite furnace atomic absorption spectrometry (ETAAS), using a Perkin Elmer model Zeeman 3030 spectrophotometer. Zinc and iron analysis was performed by flame atomic absorption spectrometry (FAAS), using a Perkin Elmer model 1100B spectrophotometer while arsenic was determined by hydride technique using a Perkin Elmer FIAS 100.

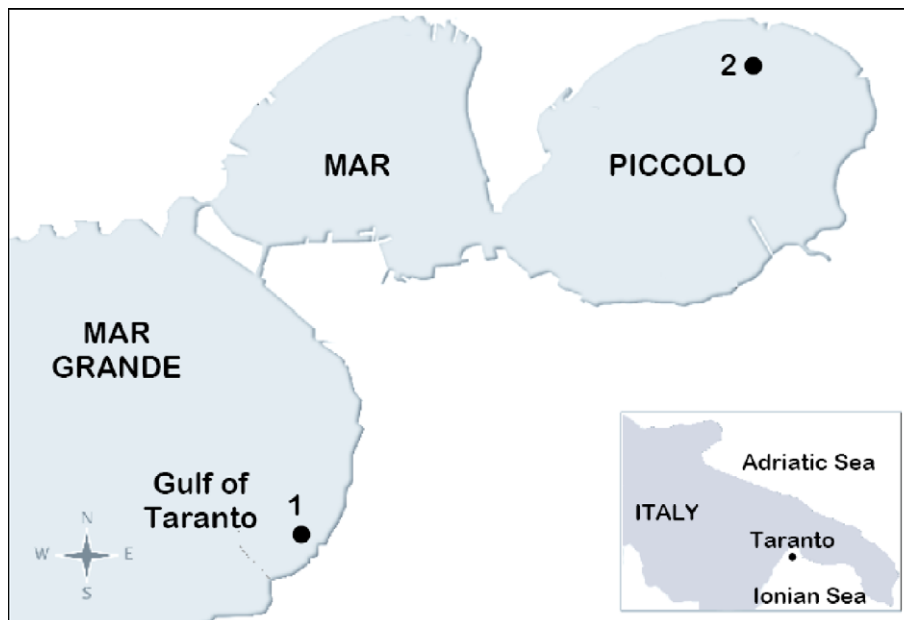


Fig. 1. Sampling stations in the study area.

Table 1  
Biometric data ( $n = 100$  specimens; mean and SD corresponding to one specimen) for *Mytilus galloprovincialis* from the two sampling stations

| Month of sampling | Mar Grande        |                               | Mar Piccolo       |                               |
|-------------------|-------------------|-------------------------------|-------------------|-------------------------------|
|                   | Shell length (cm) | Wet weight of soft tissue (g) | Shell length (cm) | Wet weight of soft tissue (g) |
| July '99          | 3.07 ± 0.34       | 0.72 ± 0.29                   | 3.10 ± 0.59       | 1.09 ± 0.63                   |
| August '99        | 3.13 ± 0.45       | 0.75 ± 0.33                   | 3.35 ± 0.63       | 1.15 ± 0.61                   |
| September '99     | 3.28 ± 0.36       | 0.76 ± 0.29                   | 3.74 ± 0.48       | 1.80 ± 0.44                   |
| October '99       | 3.43 ± 0.23       | 1.15 ± 0.29                   | 4.13 ± 0.43       | 2.21 ± 0.69                   |
| November '99      | 3.82 ± 0.43       | 1.25 ± 0.44                   | 4.22 ± 0.45       | 2.28 ± 0.95                   |
| December '99      | 4.29 ± 0.49       | 1.11 ± 0.46                   | 4.50 ± 0.41       | 2.52 ± 0.80                   |
| January '00       | 5.04 ± 0.43       | 3.28 ± 0.84                   | 4.98 ± 0.42       | 3.21 ± 0.60                   |
| February '00      | 5.09 ± 0.40       | 3.04 ± 0.77                   | 5.13 ± 1.33       | 4.60 ± 2.85                   |
| March '00         | 5.29 ± 0.46       | 3.11 ± 0.95                   | 5.54 ± 0.51       | 3.87 ± 1.10                   |
| April '00         | 5.34 ± 0.47       | 3.21 ± 0.77                   | 5.67 ± 0.47       | 3.95 ± 1.02                   |
| May '00           | 5.36 ± 0.31       | 2.78 ± 0.53                   | 5.96 ± 0.43       | 4.07 ± 0.89                   |
| June '00          | 5.44 ± 0.35       | 2.34 ± 0.75                   | 6.41 ± 0.46       | 6.17 ± 1.74                   |

Dry weight calculation on mussel tissues was carried out by oven drying at 105 °C until constant weight.

All the chemicals used in sample treatments were ultra-pure grade (Merck Suprapur, Daemastadt, Germany). All the glassware was cleaned prior to use by soaking in 10% v/v HNO<sub>3</sub> for 24 h and rinsed with Milli Q water. All solutions were prepared daily with ultra-pure deionised water (<0.1 µs at 25 °C), obtained by treating double distilled water in a Milli Q-system (Millipore, Milford, Ma., USA). Working standard solutions of metals were prepared by serial dilution of stock standard solutions of each metal containing 1000 mg/L (BDH, Poole, UK).

Limits of detection of measurements (LOD) are 0.06 µg g<sup>-1</sup> d.w. for Cd, 0.12 µg g<sup>-1</sup> d.w. for Cu, 0.09 µg g<sup>-1</sup> d.w. for Pb, 0.18 µg g<sup>-1</sup> d.w. for Zn, 0.11 µg g<sup>-1</sup> d.w. for As and 0.09 µg g<sup>-1</sup> d.w. for Fe. The accuracy of measurements was tested with Community Bureau of Reference (BCR) certified reference material CRM 278R (mussel tissue). Results were in agreement with certified values and the standard deviations were low, proving good repeatability of the method (Table 2).

Experimental data were elaborated by multivariate statistical analysis (Defernez & Kemsley, 1997; Frank & Todeschini, 1994; Massart, Vandegiste, Deming, Michotte, & Kaufman, 1988). Multivariate statistical analysis was performed by using the computer software package STAT-

ISTICA® (StatSoft Inc., Tulsa, OK, USA). The used statistical techniques were principal component analysis (PCA), hierarchical clustering analysis (HCA) and two-way analysis of variance (ANOVA).

### 3. Results and discussion

From the determination of the organic substance and the shell volume, the condition index (C.I. = dry weight, without ashes/shell volume × 1000) of the two mussel populations was calculated (Fig. 2). The C.I. is a common standardised indicator for the physiological state of a bivalve, because it reduces strong variance in data, which could be due to size differences. Studying the trend of the condition index, it was possible to individualise the emission of gametes and the reproduction period: the first emission of gametes, both in mussels from Mar Piccolo and from Mar Grande occurs from November to January, while the second emission from February to April (Fig. 2). This data are comparable to those found by other authors (Ceccherelli & Barboni, 1983; Hrs-Brenko, 1973; Renzoni, 1973). At the end of the reproductive cycles (January and April), both the condition index and the organic substance content in mussels of two different sites reach the lowest values (Fig. 3).

Table 2  
Precision and accuracy of analytical methods obtained using a certified mussel tissue (CRM 278R)<sup>a</sup>

| Metals  | CRM 278R      |                    |
|---------|---------------|--------------------|
|         | Certified     | Found <sup>b</sup> |
| Cadmium | 0.348 ± 0.007 | 0.351 ± 0.011      |
| Copper  | 9.45 ± 0.13   | 9.42 ± 0.18        |
| Iron    | —             | —                  |
| Lead    | 2.00 ± 0.04   | 2.12 ± 0.06        |
| Zinc    | 83.1 ± 1.7    | 83.9 ± 2.1         |
| Arsenic | 6.07 ± 0.13   | 6.09 ± 0.17        |

<sup>a</sup> Concentrations are given in µg g<sup>-1</sup> dry weight.

<sup>b</sup> Number of replicates is 5.

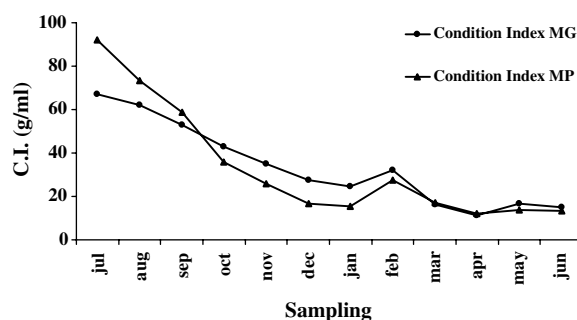


Fig. 2. Seasonal variations of condition index in mussels from Mar Piccolo (MP) and from Mar Grande (MG).

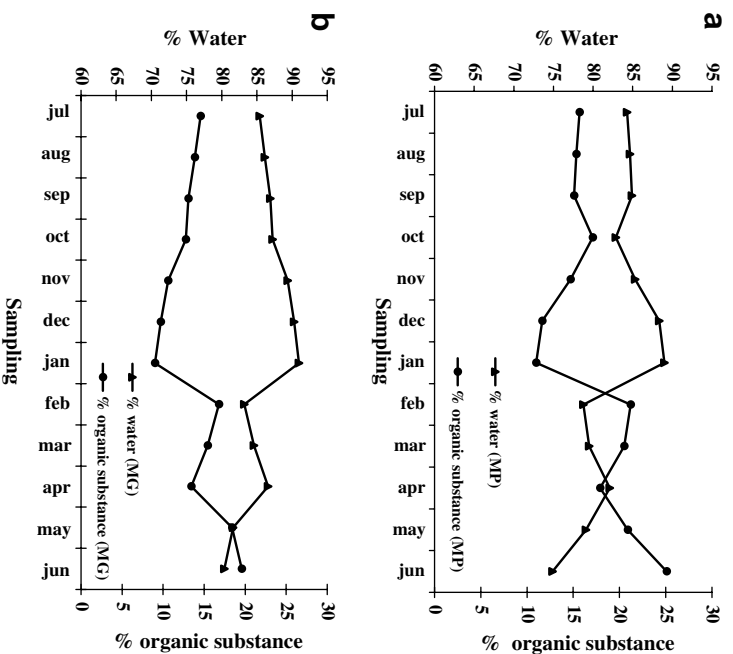


Fig. 3. Organic substance and water percentage in mussels: (a) Mar Piccolo (MP); (b) Mar Grande (MG).

Beginning from November, the percentage of the organic substance decreases, while the water content increases. This is due to the emission of gametes, as well as to the adverse environmental conditions (poor water content of nutrients in winter), which slow growth down. In February, mussels are ready for the new emission of gametes, which will occur in March; in this second reproductive cycle, the percentage of the organic substance does not decrease, due to the better environmental conditions and the algae bloom. The higher availability of phytoplankton together with the warmer water temperature allow constant growth.

Metal concentrations, expressed in  $\mu\text{g g}^{-1}$  dry weight, in the whole soft tissue of mussels, are reported in Table 3. Metal concentrations in the soft tissues of mussels from Mar Grande were slightly higher than in Mar Piccolo. The Fe, Zn, Cu, Pb, As and Cd levels in the Mar Grande mussels were in the range of 181–987, 73–109, 6.35–15.72, 1.19–4.29, 0.22–5.32 and 0.28–0.95 dry weight, respectively, while in mussels from Mar Piccolo the Fe, Zn, Cu, Pb, As and Cd values were in the range of 223–897, 68–107, 6.9–12.76, 1.31–3.35, 0.22–3.38 and 0.23–0.77 dry weight, respectively. Metals (with the exception of Zn) showed higher values between December and January, in both populations.

Seasonal changes in metal concentrations were observed. As shown in Table 3, the metals (Fe, Cu, Pb, As and Cd) in mussels both from Mar Piccolo and from Mar Grande exhibited maximum value, in later winter–

Table 3

Metal concentrations ( $\mu\text{g g}^{-1}$  dry weight) and standard deviation in the whole soft tissues of *Mytilus galloprovincialis* from Mar Piccolo (MP) and from Mar Grande (MG)

| Month of sampling | Cu           |              | Cd          |             | Fe       |          | Zn       |          | Pb          |             | As          |             |
|-------------------|--------------|--------------|-------------|-------------|----------|----------|----------|----------|-------------|-------------|-------------|-------------|
|                   | MP           | MG           | MP          | MG          | MP       | MG       | MP       | MG       | MP          | MG          | MP          | MG          |
| July              | 8.31 ± 1.61  | 7.73 ± 1.65  | 0.38 ± 0.02 | 0.35 ± 0.04 | 387 ± 25 | 241 ± 22 | 90 ± 12  | 98 ± 14  | 3.13 ± 0.54 | 2.19 ± 0.34 | 0.85 ± 0.06 | 1.02 ± 0.05 |
| August            | 8.91 ± 1.82  | 8.1 ± 1.85   | 0.50 ± 0.02 | 0.40 ± 0.03 | 268 ± 21 | 288 ± 21 | 92 ± 13  | 109 ± 14 | 1.76 ± 0.25 | 2.00 ± 0.12 | 0.83 ± 0.05 | 1.36 ± 0.06 |
| September         | 9.12 ± 1.85  | 6.70 ± 1.62  | 0.44 ± 0.03 | 0.46 ± 0.05 | 306 ± 21 | 199 ± 23 | 96 ± 15  | 96 ± 12  | 1.70 ± 0.23 | 1.46 ± 0.16 | 0.48 ± 0.04 | 0.22 ± 0.05 |
| October           | 9.07 ± 1.64  | 9.49 ± 1.83  | 0.43 ± 0.03 | 0.53 ± 0.03 | 236 ± 22 | 415 ± 28 | 107 ± 13 | 91 ± 13  | 1.36 ± 0.22 | 2.42 ± 0.15 | 0.22 ± 0.04 | 1.50 ± 0.07 |
| November          | 9.73 ± 1.75  | 8.38 ± 1.64  | 0.50 ± 0.03 | 0.55 ± 0.05 | 443 ± 31 | 746 ± 31 | 74 ± 12  | 73 ± 11  | 1.91 ± 0.29 | 3.72 ± 0.15 | 1.07 ± 0.06 | 3.01 ± 0.08 |
| December          | 10.73 ± 1.92 | 14.62 ± 1.98 | 0.70 ± 0.03 | 0.79 ± 0.05 | 471 ± 29 | 987 ± 45 | 79 ± 11  | 93 ± 14  | 3.01 ± 0.34 | 4.26 ± 0.26 | 3.38 ± 0.08 | 5.32 ± 0.08 |
| January           | 12.76 ± 1.86 | 15.72 ± 1.84 | 0.77 ± 0.04 | 0.95 ± 0.03 | 897 ± 38 | 981 ± 32 | 99 ± 14  | 93 ± 12  | 3.35 ± 0.41 | 4.29 ± 0.15 | 2.30 ± 0.07 | 3.41 ± 0.07 |
| February          | 12.43 ± 1.94 | 12.79 ± 1.95 | 0.39 ± 0.03 | 0.40 ± 0.03 | 408 ± 28 | 548 ± 38 | 68 ± 11  | 79 ± 11  | 2.80 ± 0.41 | 2.96 ± 0.45 | 1.11 ± 0.07 | 2.68 ± 0.07 |
| March             | 11.38 ± 1.92 | 10.42 ± 1.93 | 0.37 ± 0.04 | 0.38 ± 0.04 | 338 ± 26 | 289 ± 29 | 69 ± 12  | 100 ± 16 | 2.08 ± 0.48 | 1.19 ± 0.38 | 1.23 ± 0.06 | 1.48 ± 0.04 |
| April             | 10.48 ± 1.66 | 9.83 ± 1.65  | 0.35 ± 0.02 | 0.50 ± 0.03 | 364 ± 25 | 248 ± 21 | 89 ± 10  | 83 ± 12  | 1.31 ± 0.32 | 2.02 ± 0.31 | 1.50 ± 0.06 | 1.92 ± 0.06 |
| May               | 9.54 ± 1.82  | 9.10 ± 1.61  | 0.36 ± 0.02 | 0.38 ± 0.02 | 223 ± 22 | 380 ± 21 | 90 ± 13  | 79 ± 11  | 1.72 ± 0.22 | 1.60 ± 0.31 | 0.92 ± 0.07 | 1.25 ± 0.06 |
| June              | 6.90 ± 1.62  | 6.35 ± 1.61  | 0.23 ± 0.02 | 0.28 ± 0.02 | 229 ± 21 | 181 ± 18 | 71 ± 11  | 78 ± 12  | 1.88 ± 0.22 | 1.35 ± 0.11 | 0.63 ± 0.05 | 0.83 ± 0.04 |

Each value corresponds to the mean of five determinations, in a sample of 50 animals.

early spring, followed by a progressive decrease during the summer. Several studies on mussels (Cardellicchio, Brandini, Di Leo, Giandomenico, & Annicchiarico, 1998; La Touche & Mix, 1981; Lobel & Wright, 1982) have evidenced seasonal dynamics, showing similar trends of metal burdens. This could be partly related to the reproductive activity of mussels. Indeed, the reproductive cycle of the Mediterranean mussel *M. galloprovincialis* is characterised by a long gametogenesis (from March to October), a spawning season (from October to February) and a short resting period in February–March (Regoli, 1992; Regoli & Orlando, 1993; Renzoni, 1963). During gametogenesis, the progressive penetration of gonadic tissues biologically dilutes metal concentration. On the other end, similar concentrations of Zn found in the soft tissue of mussels from Mar Piccolo and Mar Grande could reflect a capacity to regulate the internal concentrations of this essential element, as it has been reported for some mytilids (Amiard, Amiard-Triquet, Berthet, & Métayer, 1986; Phillips & Rainbow, 1988).

Tables 4 and 5 summarise the correlation coefficients among the determined metals for the Mar Piccolo and the Mar Grande, respectively. Significant correlation coefficients ( $p < 0.05$ ) are in italics. A considerable number of significant correlations in the Mar Piccolo are observed between Fe and other metals such as Cu, Cd, Pb, As and between As and Cd, Pb ( $r \approx 0.60$ – $0.79$ ). For the Mar Grande, the most noticeable correlations were found between the pairs Pb–Fe ( $R = 0.95$ ), As–Fe ( $R = 0.92$ ), As–Pb ( $R = 0.89$ ), Fe–Cd ( $R = 0.87$ ), Pb–Cd ( $R = 0.84$ ), Fe–Cu ( $R = 0.82$ ), As–Cu ( $R = 0.82$ ), Cd–Cu ( $R = 0.80$ ), As–Cd ( $R = 0.76$ ) and Pb–Cu ( $R = 0.75$ ). This suggested

Table 4  
Pearson correlation coefficients between metal levels in mussels in Mar Piccolo

|    | Cu          | Cd          | Fe          | Zn    | Pb          | As |
|----|-------------|-------------|-------------|-------|-------------|----|
| Cu | 1           |             |             |       |             |    |
| Cd | 0.55        | 1           |             |       |             |    |
| Fe | <i>0.68</i> | <i>0.79</i> | 1           |       |             |    |
| Zn | –0.12       | 0.29        | 0.10        | 1     |             |    |
| Pb | 0.45        | 0.56        | <i>0.71</i> | –0.18 | 1           |    |
| As | 0.56        | <i>0.73</i> | <i>0.64</i> | –0.18 | <i>0.60</i> | 1  |

Significant correlations ( $p < 0.05$ ) are in italics.

Table 5  
Pearson correlation coefficients between metal levels in mussels in Mar Grande

|    | Cu          | Cd          | Fe          | Zn    | Pb          | As |
|----|-------------|-------------|-------------|-------|-------------|----|
| Cu | 1           |             |             |       |             |    |
| Cd | <i>0.80</i> | 1           |             |       |             |    |
| Fe | <i>0.82</i> | <i>0.87</i> | 1           |       |             |    |
| Zn | 0.04        | 0.09        | –0.15       | 1     |             |    |
| Pb | <i>0.75</i> | <i>0.84</i> | <i>0.95</i> | –0.14 | 1           |    |
| As | <i>0.82</i> | <i>0.76</i> | <i>0.92</i> | –0.14 | <i>0.89</i> | 1  |

Significant correlations ( $p < 0.05$ ) are in italics.

that the correlated metals share a common accumulation process in bivalves in Mar Piccolo and Mar Grande.

Instead no significant correlation was found between the index of condition and the metals ( $p > 0.05$ ).

Besides, in order to evaluate the results obtained, principal component analysis (PCA) and hierarchical clustering analysis (HCA) have been applied on the data set of twenty-four sampling and six variables (Cu, Cd, Fe, Zn, Pb and As). For principal component analysis, five principal components have been extracted by covering 95.2% of the cumulative variance. The variance of the five principal components is 62.9%, 14.1%, 8.0%, 5.5% and 4.7%, respectively. The scatter plot of the scores on the first two principal components PC1 and PC2 (Fig. 4) shows a separation among the months. Indeed, the months of January, December, November and February have a positive score on the PC1, while all other months are characterised by negative scores, on the same principal component. There is no high difference on components on PC2. Loading of the variables on the first two principal components shows that Cu, Cd, Fe, Pb and As from Mar Piccolo and from Mar Grande are the dominant variables on the PC1, while Zn is the dominant variable on the PC2 both from Mar Piccolo that from Mar Grande (Fig. 5).

HCA was carried out using Ward's method procedure, applied on the Euclidean distances. The resultant dendro-

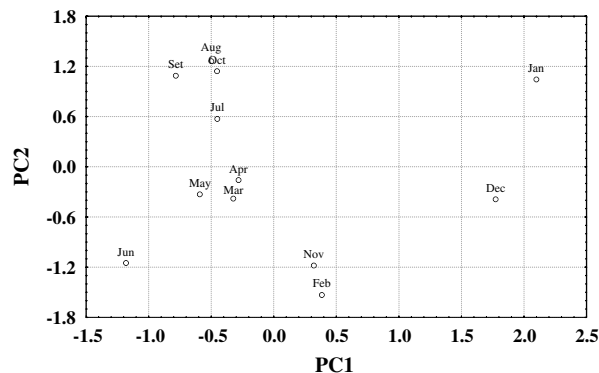


Fig. 4. Scatter plot of the scores on the first two principal components obtained using Cu, Cd, Fe, Zn, Pb and As.

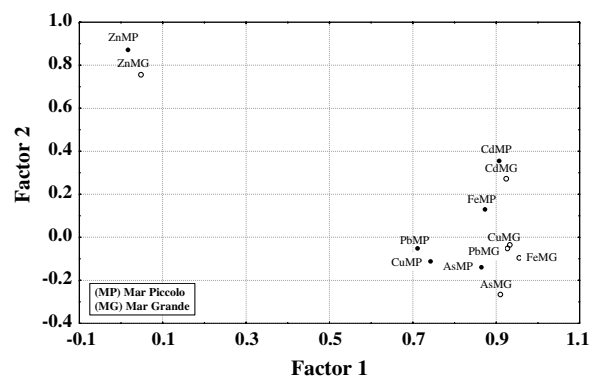


Fig. 5. Loading of the variables on the first two principal components.

gram (Fig. 6) confirms the results obtained with PCA. Indeed, there are four clusters, which can be identified as follows: the first cluster contains the months of January, December, November and February, the last three clusters include the other months. Results obtained by HCA, as well as PCA, show that mussels sampled both in Mar Piccolo and in Mar Grande has Cu, Cd, Fe, Pb and As concentrations are higher in the months of January, December, November and February. Cluster analysis results gave further evidence that the Mar Piccolo Station was not noticeably different from the Mar Grande Station.

Indeed, ANOVAs did not show a statistically significant difference in the observed levels of heavy metals and condition index ( $p > 0.05$ ) between the two sites.

Total metal contents in mussels, found in this work, have been compared to those found in literature from different Italian locations (Table 6). Comparing metal levels from different areas, one must pay attention, as data are often reported in wet weight. While the content of water is not so important for fish, it is very variable for molluscs, being affected by factors such as: habitat, vital conditions and pretreatment and sample conservation procedures, after sampling. Data concerning dry weight are susceptible to lower variability than those of wet weight. However, in order to have a clear comparison among values, in Table 4 there are also reported metal concentrations, (mean and range), found in this work, expressed in wet weight.

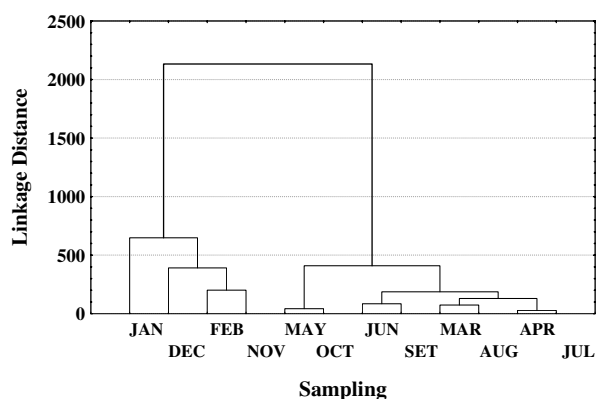


Fig. 6. Hierarchical clustering dendrogram of metal concentrations in mussels from Mar Piccolo and from Mar Grande.

This survey shows that concentrations of Cd and As found in the mussels from Mar Piccolo and Mar Grande were lower than those reported by Favretto, Favretto, and Felician (1987), Majori, Nedoclan, Modonutti, and Daris (1978) Zatta, Gobbo, Rocco, Perazzolo, and Favara (1992), whereas the levels of Cu, Fe, Zn and Pb were higher or comparable than those reported by Favretto et al. (1987), Licata, Trombetta, Cristiani, Martino, and Naccari (2004), Majori et al. (1978), Renon and Malandra (1991) and Zatta et al. (1992).

Fe, Cu and Zn are essential elements in organism life, while Cd, Pb and As can be considered as xenobiotic metals, with tendency to bioaccumulation, although distributed in the marine environment at low concentrations.

For Pb, the maximum residual level recommended by the European Community (Commission Regulation (EC) No. 466/2001) is  $1 \mu\text{g g}^{-1}$  of wet weight. The highest levels of lead are observed at the end of the first reproductive period, (which occurs in January) but they always remain well under the specified limit.

The same maximum residual level ( $1 \mu\text{g g}^{-1}$  of wet weight) is recommended by the European Community for Cd (Commission Regulation (EC) No 466/2001) and no sample exceeded this limit.

Fluctuations in Pb and Cd concentration during one year of investigation, depending upon physiological and metabolic factors, are always below levels recommended by the European Community.

For monitoring programs, in which mussels are used as bioindicator organisms, some criteria should be adopted in the mussel sampling phase. Mussels should be taken in the same period and at the end of the reproductive cycle, when the percentage of metal concentration gets to the highest value (late winter). Otherwise, comparisons between mussels, at different stage of the reproductive cycle, would show different concentrations of metals, which could simply be a function of the seasonality in the reproductive cycle. Furthermore, mussels should have the same size and be sampled at the same depth. Only by following this instruction it is possible to obtain, in different geographical areas, homogeneous data, which can be easily compared to each other.

Concentrations found for Cd and Pb indicate that molluscs populations under investigation pose no health risk to

Table 6  
Selected references of metal concentrations ( $\mu\text{g g}^{-1}$  wet weight) of *Mytilus galloprovincialis* from different Italian locations (ranges)

| Locations                      | Cd        | Cu        | Pb        | Zn          | Fe          | As        | References                |
|--------------------------------|-----------|-----------|-----------|-------------|-------------|-----------|---------------------------|
| Gulf of Trieste (Adriatic Sea) | 0.21–0.29 | 1.26–1.48 | 0.84–3.29 | 16.5–27.4   | 22.6–29.9   |           | Majori et al. (1978)      |
| Gulf of Trieste (Adriatic Sea) | 0.12–0.38 | 0.77–2.23 | 0.48–1.79 | 6.90–29.60  |             |           | Favretto et al. (1987)    |
| Cagnano Varano (Adriatic Sea)  | 0.13–0.35 |           | 0.23–0.80 |             |             |           | Renon and Malandra (1991) |
| Manfredonia (Adriatic Sea)     | 0.09–0.23 |           | 0.23–0.75 |             |             |           |                           |
| Trani (Adriatic Sea)           | 0.07–0.20 |           | 0.26–0.58 |             |             |           |                           |
| Venice Lagoon (Adriatic Sea)   | 0.01–1.64 | 0.01–2.53 | 0.14–1.18 | 7.10–37.32  | 1.94–167.53 | 0.29–2.35 | Zatta et al. (1992)       |
| Faro basin (Tyrrhenian Sea)    | 0.04–0.06 | 0.19–0.40 | 0.06–0.09 | 11.0–18.5   |             |           | Licata et al. (2004)      |
| Mar Piccolo (Ionian Sea)       | 0.06–0.18 | 1.79–3.35 | 0.30–0.77 | 12.23–22.12 | 58.38–204.5 | 0.03–0.80 | Present work              |
| Mar Grande (Ionian Sea)        | 0.08–0.23 | 1.64–3.68 | 0.28–0.99 | 12.67–25.29 | 48.35–233   | 0.02–1.23 |                           |

seafood consumers, because its metal contents are within the permissible range established for safe human consumption. Nevertheless, the continued monitoring of sea areas having mussel farming activity, particularly those affected by both urban and industrial pollution, is necessary in order to prevent eventual hazard.

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