ARE THE BENTHIC FORAMINIFERA SUITABLE BIOMONITORS OF MARINE SEDIMENTS CONTAMINATION? A CASE STUDY FROM THE HARBOUR OF NAPLES (SOUTHERN ITALY)

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Abstract

We propose a rigorous investigation on the distribution patterns of the benthic foraminiferal assemblage and their predominant species in the highly contaminated marine sediments of the harbour of Naples. Combined use of multivariate canonical, cluster and principal component analysis in a proper geostatistic framework provided an appropriate approach to explore the role played by a number of contaminants (Heavy elements, Polycyclic Aromatic Hydrocarbons, Volatile Organic Compounds, Heavy Hydrocarbons, PCBs) and different physical parameters (grain size, pH, Eh, Total Organic Carbon) on the spatial distribution of the benthic foraminiferal assemblage and its single species. The obtained results evidence a high and systematic non-linear response of the biota to the effects of contamination of the different classes of pollutants. Although the reduced number of specimens per sample, their small dimensions and low diversity, testify the negative effects of a highly contaminated environment, it appears very difficult to clearly identify a differential sensitivity of different benthic species to single classes of contaminants. In particular, contrary to a number of case studies which invoked a key role of the heavy metals in the control of the distribution patterns of the benthic foraminifers in the marine environment, the results here presented underline the primary effects of some classes of organic compounds (Volatile Organic Compounds, heavy hydrocarbons and Polychlorinated biphenyls) on the distribution patterns of the benthic assemblage. However, the obtained results demonstrate that any kind of oversimplification would unquestionably cancel the strong complexity of the biotic response to combined effects of different coexisting contaminants.

Keywords: Benthic foraminifera; organic compounds; heavy metals; physical and sedimentological parameters; Harbour of Naples

Introduction

Studies on pollution effects on benthic foraminifera (class Foraminifera, phylum Granuloreticulata) and of the possibile use of these organisms as proxies were initiated in 1960s, with the pioneer papers of Resig (1960), Watkins (1961) and Boltovskoy (1965). Since then, benthic foraminifera are increasingly used as environmental bioindicators, especially in polluted marine-coastal environments. In the last decades, a substantial number of investigations (e.g., Alve, 1991, 1995; Boltovskoy et al., 1991; Bresler and Yanko, 1995; Yanko et al., 1994, 1998, 1999; Stouff et al., 1999; Coccioni, 2000; Geslin et al., 2000, 2002; Debenay et al., 2001, 2005; Bergamin et al., 2003; Coccioni et al., 2003, 2005; Samir and El-Din, 2001; du Châtelet et al., 2004; Scott et al., 2001; Triantaphyllou et al*.,* 2005; Ferraro et al., 2006; Quilty and Hosie, 2006; Cearreta et al., 2002; Frontalini and Coccioni, 2008; Leorri et al., 2008; Romano et al., 2008) confirmed the reliability of this group of organisms (in terms of species diversity and density populations) as useful tracers of marine sediments contamination and suggested an intensive use of these bioindicators in integrated programs of pollution monitoring (e.g., Armynot du Châtelet et al., 2004) as early warning indicator of anthropic pollution (Kramer and Botterweg, 1991).

However, current literature lacks detailed information on benthic foraminiferal assemblage distribution modes and wide spectra of organic and inorganic contaminants, being generally restricted to analysis of a limited number of pollutants in different marine areas. That renders the potential attribution of the effects of the single classes or groups of analytes, on the distribution of benthic foraminiferal species extremely unlikely.

The dataset presented in this study shows result of a large range of contaminants and chemico-physical parameters from a highly anthropised area (the Harbour of Naples) and therefore offers a first chance for a more definitive and appropriate evaluation, solidly based on a multivariate statistical analysis, of the toxic effects of a variety of pollutants on different species of benthic foraminifera.

The Naples harbour is located in the eastern Tyrrhenian Sea margin (Gulf of Naples; Fig.1) and is one of the larger of Southern Europe. It receives municipal and multiple industrial (petroleum refineries, metallurgy, cement and food production) discharges and is characterised by multiple port activities (shipbuilding, goods stocking, commercial and tourist transaction) and commercial wastewaters. For this reason it represents a complex environment with a strong dynamicity and can be considered a suitable natural laboratory to assess the potential of benthic foraminifera as tracers of anthropic pollution.

Material and Methods

Surface sediment samples (0-20 cm) were collected from 84 stations, between the *Diga Foranea* and the *Molo San Vincenzo* external wharf (Fig. 1) in the Naples harbour in November 2004. A differential global positioning system (DGPS) was used to precisely identify each location. Sediments were collected using a hydraulic 6 meter long vibro-corer with an inner diameter of 10 cm. . Three sub-samples were collected from each site, homogenized with a plastic spoon, placed into pre-cleaned high-density polyethylene (HDPE) bottles for chemical analyses and stored at -18°C on board within an hour of collection.

Sampling for analysis of Volatile Organic Compounds (VOCs) followed the methodology reported in EPA SW 846 Method 5035. In particular, approximately 5 g sample were placed in a pre-weighed vials with a septum sealed screw-cap that already contained a sodium bisulfate preservative solution. The vials were immediately sealed and shipped to a laboratory where they were frozen at -20°C. Analyses were performed within 15 days from sampling.

Benthic foraminiferal analysis

Each analysed sample was sieved at the 90 ųm mesh size. The entire dried residue was microscopically analyzed and all the specimens were counted. The foraminifera were hand-picked and separated from the sediment. Non living foraminifera were counted and percentages utilized for statistic analysis. The Loeblich and Tappan classification (1988) was used.

For each sample, the specific abundance of the benthic foraminiferal association was computed as number of individuals per species per sample. This value was normalized for the dry weight of sample, in order to return unbiased counts (numbers of individuals per gram of sample – BF/g).

Geochemical analyses

Analytical procedures for analysis of grain size, Total Organic Carbon (TOC), Heavy metals, Polycyclic Aromatic Hydrocarbons (PAHs), Polychlorinated biphenyls (PCBs) are reported in detail in Sprovieri et al. (2007). For analysis of the total recoverable petroleum hydrocarbons (TRPHs) we used the infrared spectrophotometric method ISO/TR 11046 (ISO, 2005), which is a measure of mineral oils (petroleum hydrocarbons) only. It corresponds to the high molecular weight ($12<C<40$) of petroleum derived hydrocarbons, also known as the Diesel Range Organics (DRO) and does not include the biodegradable animal greases and vegetable oils captured in oil and grease measurements. Two grams of sediment were extracted by accelerate solvent extraction method (ASE 200) using a hexane/isooctane (1:1 v/v) mixture. The extracts, in a hexane:isooctane (1:1 v/v) solution, were purified by elution through a Florisil (5g) column using 30 ml of hexane:isooctane (1:1 v/v). The final extracts, dissolved in carbon tetrachloride, were finally analysed by a Thermo Nicolet FT-IR with narrow band MCT detector in the range 2925-2958 cm^{-1}. Laboratory quality control procedures included analyses of blanks, spiked samples and reference material. Repeatability, based on six analyses of the same certified standard, was <10% and the detection limit was estimated close to 1 $\mu g \cdot g^{-1}$. Volatile Organic Compounds (VOCs) were measured according to EPA 8260b method by Head Space-Gas Chromatography Mass spectrometry ThermoElectron DSQ. A total of 57 congeners (reported in the EPA's method) were measured with a <10% repeatability and a detection limit close to 0.001 $\mu g \cdot g^{-1}$.

Multivariate Analysis

Generally, environmental analyses are faced with multidimensional systems, where the number of variables and parameters are hardly handled with basic statistical methods (Duda, 2000). In this work, the main dataset consists of a matrix of tens of variables among benthic foraminifers, contaminants and physical parameters, for over than 80 samples. Principal Component Analysis and Canonical Correlation Analysis are widely used to synthesize (reduce the dimensions of system) and interprete (evidence variability patterns of subsets of information), in order to extract as much more information as possible from such a non linear complex system. Furthermore, cluster analysis (with K-means method) is employed in order to integrate multivariate approach with the aim to improve consistency of analyses output. Such methodology is used both in manual mode, where number of clusters is defined by the user, and in auto mode, whereas an iterative approach (v-fold method) is run extracting the optimal number of clusters.

Geostatistics

Spatial georeferenced data were processed with the ISATIS geostatistical software package. Geodetic reference is the Universal Transverse Mercator (fuse 33) projected on DATUM WGS 1984. All the information was managed in a GIS georeferenced environment, using ArcGis 9.2 software package. Geostatistics is widely used in environmental sciences with the aim to model the spatial structure of real life variables in the most coherent way, with respect to their intrinsic behaviour. However, it is generally used as black box, with poor attention for internal complex algorithms. One of the less considered aspects of spatial modeling, mainly in complex artificial spatial domains, is the importance of physical barriers that, if ignored in the interpolation processes, can lead to incoherent results. When variogram is calculated, or the neighborhood of an unknown point is computed in the weighted average computation, the shape of a perimeter wall, or the presence of a particular structure separating any cluster of points from others, is almost crucial for quantitative implementations of geostatistical procedures. The so called *technique of fault* (or breaklines) takes into account such structures in order to return the most correct evaluation of the spatial structure of a variable, mostly reducing false homogeneities and artificial distributions (Wackernagel, 2003). In practice, the circles (or ellipses) within which samples are included, throughout the geostatistical procedure, are cut out of the screening segments of artificial barriers delimiting the spatial domain.

Results

Chemical contaminants

Results of grain size, Eh, pH, Total Organic Carbon (TOC), Heavy metals, Polycyclic Aromatic Hydrocarbons (PAHs), Polychlorinated biphenyls (PCBs) are reported in detail in Sprovieri et al. (2007). Synthetically, sands generally predominate (>50 %) in bottom sediments except from two “plumes” of fine sediments reported from the eastern part of the studied area. Eh values range between -507 and 498 mV with a median of -170mV, while pH maintains a very stable ~7.6 value with an associated standard deviation of ~0.3 (for details see Sprovieri et al., 2007).

Among the metals, Zn (17–7234 mg·kg^{-1}), Cu (12–5743 mg·kg^{-1}), Pb (19-3083 mg·kg^{-1}), V (37-2114 mg·kg^{-1}), Cr (7-1798 mg·kg^{-1}) and Ni (4-362 mg·kg^{-1}) dominate with lower concentrations measured for Sn (1-265 mg·kg^{-1}), As (1-1121 mg·kg^{-1}), Co (1-30 mg·kg^{-1}), Cd (0-3 mg·kg^{-1}) and Hg (0.01-139 mg·kg^{-1}). Total concentrations of the analysed priority 16 US-EPA PAHs range from 22 to 25,440 ng·g^{-1} dry wt with phenanthrene, fluoranthene, pyrene, benzo[a]antracene, chrysene and benzo(a)pyrene measured as the most important congeners and the 3 and 4 rings PAHs dominant in the studied sediments. The spatial PAHs distribution appear patched with highest values (4,700-7,000 ng·g^{-1}) generally localised in the shipping and commercial areas and in the internal part of most of the inner docks. ΣPCB shows the highest level (up to ~899 ng·g^{-1}) in the tourist and shipping areas localised in the central part of the harbour and substantially homogeneous values (median of 41 ng·g^{-1}) in the remaining zones. The tetra- and penta-, hexa-chlorobifenyls and hepta-chlorobifenyls, account for 75-80% of the total measured PCBs. Statistically significant correlation between grain size and heavy metals, PAHs and PCBs is lacking thus suggesting different sources in driving the distribution patterns of the different contaminants and a major impact of anthropic effect.

In the following we present a more detailed description of concentration and distribution of TPHRs and VOCs, unprecedentedly reported. TPHRs show a wide range of variability with a median value of 266 μg·g^{-1}, a maximum of about 17,140 μg·g^{-1} and minima corresponding to the detection limit (~1 μg·g^{-1}; Tab.1). No statistically reliable correlation emerges with grain size and distribution of the other analysed contaminants. VOCs show a median value of 1,715 μg·g^{-1} with a maximum of 5,105 μg·g^{-1} and standard deviation of 0.962 μg·g^{-1}. As reported in Tab. 1 and Fig. 2, six of the 57 analysed congeners (specifically Methylene chloride, 2,2Dichloropropeno, Toluene, Bromomethane, Chloromethane and 1,1,2,2 Tetrachloroethane) constitute more than 40% of the total of VOCs content. Benzene, Toluene, Ethyl-benzene, and Xylens averagely represent about 15% of the total of volatile compounds (Tab.1and Fig. 2).

Benthic foraminiferal assemblage

The original faunal data set contains 34 benthic species (Tab.2). However only eight species and/or genera occurring with frequencies above 2% were here considered for statistical analysis (Tab.2). The selected species are: *Ammonia beccarii, Ammonia tepida, Bulimina* spp., *Cibicides lobatulus, Quinqueloculina* spp.*, Elphidium* spp. and *Stainforthia fusiformis*. All the specimens are relatively small in dimension and range from 90 to 125 μm. Deformed individuals are virtually absent (<2% of the total assemblage). For only four species it has been possible to determine the correspondent deformed varieties: *A. tepida*, *C. lobatulus*, *Elphidium* spp., *Quinqueloculina* spp., in only four samples (NO26, NO49, NO75, NO082; Tab.3).

A quick glance at the abundance values of the benthic foraminifera distribution table (Tab.2), reveals the presence of several empty columns which yields a marked incidence of a reduced number of dominant species and reduced biodiversity. *A. tepida* (65.2%) represents the most abundant benthic species followed by *Quinqueloculina* spp. and *Elphidium* spp.*,* with respectively 24.4% and 10.5% of the whole population. Such percentages are calculated computing the average values of the BF/g dataset, in order to avoid the effects of a few outlier samples. Moreover, these three species exceed 70% of non-zero values of BF/g. Differently, *C. lobatulus* and *S. fusiformis* lie between 40% and 50% of the same parameter, while all the other species are below 25% with a mean values of ~ 78 empty samples in a picked totaly 84 . Benthic foraminifers show low mean abundance (mean values of individuals per gram of dry sediment), with the predominant *A. tepida* presenting 1.18 BF/g and *Quinqueloculina* spp. and *Elphidium* spp. presenting 0.44 and 0.19 BF/g, respectively.

Shannon diversity index *H’* (Shannon, 1948) reveals a men value of 1.03 for all the samples, with an average maximum theoretical value of 3.22 (median value of H'_{max}; Tab. 2). Computing the relative median divergence from equiprobability (median $(H'_{max} – H')/H'_{max}$) we obtain 67.2%, that is a median divergence of two thirds from the maximum of biodiversity. Such a result is mostly due to the scarce abundance of species (a median value of 19% of present species of the 21 found) rather than to a real low evenness.

Calculated correlation matrix among the benthic species (Tab.4), evidences the high correlation values between *A. tepida* and the following species: *A. beccarii* (ρ = 0.85), *Bulimina* spp. (ρ = 0.87), *Elphidium* spp. (ρ = 0.71), *Quinqueloculina* spp. (ρ = 0.52) and *S. fusiformis* (ρ = 0.81). However, a closer look at the whole dataset reveals the presence of two main outliers (among the samples – NO51 and NO90 – mainly responsible for the high correlation values); by filtering them out, the correlations drastically collapse to respectively 0.35, 0.29, 0.26, 0.11 and 0.47. Only the pair *A. tepida* and *S. fusiformis* seems to keep a certain degree of covariance. Generally, once such samples are filtered out, the behavior of the different species seem to be statistically independent with reliable correlations measured in a few samples and on small sub-areas of the whole spatial domain.

Multivariate statistical analysis on the benthic foraminiferal assemblage

Principal Component Analysis (PCA) was applied to the standardized benthic foraminiferal dataset to verify the dominance of a reduced number of species. The PCA reveals the closeness of the species *A. tepida, Elphidium* spp.*, Quinqueloculina* spp., *A. beccarii*, *S. fusiformis* and *Bulimina* spp. with the variable *Total Foraminifera* (Fig. 3a). However these findings call for a careful interpretation: firstly, PCA application is based on correlation parameter (regarding mean and standard deviation) while the percentages of species in the samples are computed in relation to median values (thus correctly allowing for asymmetry in the distribution); secondly, the included species *A. beccarii* and *Bulimina* spp. show very low percentages of non-empty samples, with respectively 16.7% and 10.7%. These two considerations reduce the dominant species in the studied samples. to *A. tepida, Elphidium* spp. and *Quinqueloculina* spp.

The PCA, applied to cases, reveals the presence of 4 evident outliers (NO51, NO75, NO82 and NO90 – box in Fig 3a). After filtering , only *A. tepida* maintains its leading position in the Total Foraminifera assemblage variable (Fig. 3b).

In order to confirm results of suspicious samples identification procedure (PCA applied to cases) and focus on the subset of outliers for all three dominant benthic species, an automatic cluster analysis (with v-fold method) was applied. The method returned a total number of three clusters, of which one is composed by only samples NO75 and NO90. Moreover, once applied to only the predominant species *A. tepida*, cluster analysis evidenced that one of the 6 extracted clusters contains only two samples: NO51 and NO90, both of them showing values that are three orders of magnitude higher than the median. According to these results, samples NO51 and NO90 were filtered out by the *A. tepida* column.

Multivariate statistical analysis applied to the integrated geochemical and faunal dataset

Canonical Analysis is a PCA-like technique based on the maximization of variance between two groups of variables (Duda, 2000), rather than among variables. It is very useful and generally mostly applied in biotic-abiotic comparing systems. Frequently, Canonical Analysis is used to delineate modes of correlation between living structures and contaminants in the environment.

A combined use of PCA and Canonical Correlation Analysis has been used in this work. In particular, the first technique was employed to inspect the presence of outliers in the available dataset and to enhance consistence of analysis, while the function of correlation pattern recognition is granted by thesecond. PCA reliably suggested filtering out samples NO44, NO51, NO75 and NO90 from the whole dataset and on the reduced set, Canonical Analysis was applied. This approach has been conveniently employed for analysis of organic compounds, heavy elements and physical/sedimentological parameters covariance modes with the three dominant benthic species.

PCA applied to the group of benthic foraminifers and three grains size classes (Fig. 4) reveals a substantial absence of structured spatial variability and a reduced control of these sedimentological parameters on the distribution patterns of the different benthic species. Inspection of the potential relationships among Eh and pH and benthic species once again excludes evident covariance patterns (Fig.5). Only *A. tepida* evidences a certain degree of opposite variance with pH in the sediments, although the statistical significance of such a kind of relationship is questionable. In this case PCA rather than Canonical Correlation Analysis is used because of the low number of variables (two in one group in the case of Eh and pH) and the anticovariating nature of grain size variables. In fact, to assume the minimization of within-variance in a small group of antithetic variables would be unreliable.

An evident inverse correlation between *A. tepida* and TOC and a substantially inverse dependence with VOCs, TRPHs and ΣPCB emerges by the analysis of the benthic foraminifers compared to the different classes of organic compounds (Fig. 6), while *Quinqueloculina* spp., *Elphidium* spp. show an opposite covariance only with VOCs and an apparently limited effect of the other organic compounds. On the other hand, canonical analysis performed on the biotic dataset and the group of heavy elements (Fig. 7) indicates opposite distribution patterns between the three benthic species and Co, Ni and V. Unexpected scarce influence is recorded for Hg, Cr, Zn, Sn, Cu, Cd and Pb on the distribution patterns of *A. tepida*, *Elphidium* spp. and *Quinqueloculina* spp. yelds the larger “distance” with Co, Ni and V and a certain statistical distance with the distribution of the other elements.

Discussion

The reduced number of specimens in the benthic assemblage and the low species diversity in the studied samples clearly suggest a significant role played by the complex ensemble of organic and inorganic contaminants on the wildlife of the marine bottom sediments. However, surprisingly, the classic highly toxic contaminants (PAHs and several of the analysed toxic heavy elements) do not seem to have significant influence on the distribution of the three dominant species of the benthic assemblage. Also Eh and pH, as well as grain size distribution, seem to show an independent variability with respect to the three benthic species. On the other hand, the TOC content as well as the hydrocarbons (the volatile and semivolatile fractions) and ΣPCB seem to have a strong impact on the distribution modes of *A. tepida*, that appears uninfluenced by high concentration and potential availability (Adamo et al., 2005) of highly toxic heavy metals. It is worth noting that also *Quinqueloculina* spp. and *Elphidium* spp. show a statistically significant sensitivity to the distribution patterns of VOCs thus firstly and solidly suggesting a primary control of such a class of contaminants on the benthic foraminiferal system. This class of contaminants, generated by incomplete combustion of organic matter related to industrial processes and vehicular traffic, was demonstrated to cause acute toxic effects on human health (primarily neurological), cancer (such as leukaemia), neurobehavioral effects and adverse effects on the kidney (Edwards, 1997) and many of the congeners in this class are listed as priority pollutant ones (e.g., MINDEC, 1990). Although the concentration of highly toxic VOCs is low in vehicle exhaustes and industrial waste gas emission, it might seriously affect human health due to long-term exposure and/or bioaccumulation. As known, there are some standard methods (e.g., Microtox® and Mutatox and Ames test) that can quantitatively evaluate toxicity of pollutants. However, these methods are not cost-effective and so not ideal for initial screening of suspected toxicants in public areas.

The anti-covariance between the three dominant benthic species in the harbour of Naples and the distribution of VOCs reveals a unpredicted *dose–mortality* effect of this class of contaminants on the benthic species. Though the volatility and comparatively high solubility of the many chlorinated VOCs, will not tend to partition to aquatic sediments in the same way as the chlorinated pesticides, they will however be present in some regions at least in concentrations which could exert an effect on benthic life and organisms that in contact with the sediments over prolonged periods with potential toxic *ramifications* for the local ecosystem of unknown magnitude.

In Fig. 8 we reported a comparison between the concentration of VOCs in the studied sediments and the distribution map of the three groups of *A. tepida* obtained by application of K-means cluster analysis to this species. In particular Clusters 1 and 2, with 17 and 8 samples respectively, are representative of medium and high abundances of *A. tepida,* while Cluster 3, with its 57 samples, is widely distributed all over the harbour, mainly in the most eastern and western areas, where abundances of this species are generally low. Though the correspondence between relatively low VOCs concentration in the sediments and clusters 1 and 2 of *A. tepida* is generally verified, some evident exceptions suggest that a number of potential interactions with the ensemble of chemico-physical parameters may deeply influence the distribution patterns of this species.

Associated to the negative effects of VOCs on the distribution of *A. tepida* are the TRPHs that in the harbour of Naples are extremely abundant. In Fig. 9 we reported the distribution map of the three extracted clusters of *A. tepida* versus the concentration of TRPHs in the sediments that visually confirms the robustness of that result. Therefore, the totality of hydrocarbons seem to represent a primary limiting class of pollutants for *A. tepida* that conversely stays substantially uninfluenced by toxic metals. Once established by further detailed investigations on other suitable selected case studies and sound based on appropriate toxicity tests, the potential of benthic foraminifers as bio-monitors of marine sediments contaminated by hydrocarbons could be adopted as suitable and low-cost early warning technique in large-scale survey programmes.

On the other hand, as already suggested by Ferraro et al. (2006) in limited areas of the same harbour, *Quinqueloculina* spp. appears the most sensitive genera to high concentration of heavy metals in the sediments in comparison to *A. tepida* and *Elphidium* spp. However it appears extremely difficult to quantify the effects of the single heavy elements on the distribution of that species and to thus separate the effects of VOCs on the spatial distribution patterns of the same species. Moreover, the higher sensitivity of *A. tepida* (and partially of the other two benthic species) to Co, Ni and V that are generally present in the studied sediments at values close to the natural background (Sprovieri et al., 2007) compared to the more toxic concentrations measured for Hg, Cd and Pb, suggests extreme caution in defining linear oversimplification in the interpretation of the effective toxic role of heavy elements on the benthic assemblage. Appropriate analysis on the speciation of the different toxic metal in the studied sediments are needed to demonstrate a potential higher bioavailability of the different elements to the associated specific benthic foraminifera.

In its ensemble, the evident high-level of complexity which characterizes the available dataset, reveal a some degree of indeterminacy in identifying clear patterns of correspondence among the different variables and this limits the reliability of definitive biotic-abiotic relationships. Furthermore, we cannot exclude that a number of other unexplored factors, related for example to a significant influence of a highly dynamical environment, may directly and in some case primarily influence and drive the distribution of the different benthic species. We consider these results an important contribution to the understanding of the potential role played by organic and inorganic contaminants on the distribution patterns of the benthic foraminiferal assemblage although we believe that caution is needed to correctly approach the use of this class of organisms as biomonitors of polluted marine systems. Only an accurate micro-cosmos investigation on the lethal effects of single contaminants on the benthic foraminiferal species will offer more definitive information on the potential of this assemblage as monitor of environmental pollution, thus verifying the indirect results obtained by multiple observations and interpretation of real case studies.

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Figure captions:

Fig. 1: Location map of the study area with sampling stations.

Fig. 2: Concentration values ($\mu g \cdot g^{-1}$) of the 57 analysed congeners.

Fig. 3: PCA (Principal Component Analyses). (a) Projection of the variables (benthic species) with Total Benthic Foraminifera and PCA applied to cases with four filtered distinct outliers (in the box). (b) Plot of the four outliers with Total Benthic Foraminifera.(Apla= **Ammodiscus planorbis**; Amtedef= *Ammonia tepida* deformed; Abec= *Ammonia beccarii*; Bucgra= *Buccella granulata*; Bultot= *Bulimina* total; Cascar= *Cassidulina carinata*; Ciblob= *Cibicides lobatulus*; Clobdef= *Cibicides loabatulus* deformed; Elphdef= *Elphidium* deformed; Melbar= *Melonis barleanum*; Milspp= *Miliolinella* spp.; Plame= *Planorbulina mediterranensis*; Quidef= *Quinqueloculina* deformed; Rosspp.= *Rosalina* spp.; Sphebul= *Spheroidina bulloides*; Stafus= *Stainforthia fusiformis*; Trilosp= *Triloculina* spp.)

Fig. 4: PCA (Principal Component Analyses) applied to Total Benthic Foraminifera and Grain size.

Fig. 5: PCA (Principal Component Analyses) applied to Total Benthic Foraminifera and Eh/pH values.

Fig. 6: CCA (Canonical Correlation Analysis) of *A. tepida*, *Elphidium spp*. and *Quinqueloculina* spp. with TOC, VOCs, ΣPAH, ΣPCB and TRPH values.

Fig. 7: CCA Canonical Correlation Analysis) of *A. tepida*, *Elphidium spp*. and *Quinqueloculina* spp. with heavy metals values.

Fig. 8: Distribution map of the three extracted clusters of *A tepida* (obtained by application of K-means cluster) versus the concentration of VOCs (mh/kg) in the studied samples.

Fig. 9: : Distribution map of the three extracted clusters of *A. tepida* (obtained by application of K-means cluster) versus the concentration of TRPHs.

Table captions:

Tab. 1: Basic statistics of VOCs, TRPHs and of 12 main analysed congeners.

Tab. 2: Abundance values of benthic species, total of individuals, Shannon index, H'max, Relative divergence and Pielou index, recorded in each station with main basic statistics (mean, median, min, max, proportion, % non empty samples, empty samples and Skewness),

Tab. 3: Abundance (indiv/g) and contribution (%) of four deformed species in 4 selected samples.

Tab. 4: Correlation matrix calculated among the benthic species.