

Review article

# Benthic foraminifera as bioindicators of pollution: A review of Italian research over the last three decades

*Les foraminifères benthiques bio-indicateurs de pollution :  
bilan de 30 années de recherches italiennes*

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## Abstract

Since the 1950s, numerous studies have demonstrated the value of benthic foraminifera in detecting ecosystem contamination. The interest in benthic foraminifera has partly been driven by government policies and programs aimed at developing suitable, non-invasive bioindicators of marine environmental quality. This paper accomplishes two things: it reveals that Italian experience has significantly contributed to the advancement of our understanding of this topic and summarizes the most important results that have served to greatly improve our knowledge in this field. Although many issues are still a matter of debate, since it is difficult to separate natural vs human-induced pollution and a foraminiferal protocol has not yet been produced, foraminifera have been proven to be successful candidates as part of an integrated monitoring program.

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*Keywords:* Benthic foraminifera; Bioindicators; Pollution; Italy

## Résumé

Depuis les années 1950, de nombreuses études ont démontré la qualité des foraminifères benthiques pour mettre en évidence les écosystèmes contaminés. Cet intérêt pour les foraminifères benthiques a en partie été guidé par les exigences gouvernementales qui demandaient des indicateurs biologiques de la qualité des environnements marins non invasifs. Les deux objectifs de cet article sont de mettre en évidence que l'expérience italienne a significativement contribué à l'avancement de notre compréhension de ce sujet et de résumer les résultats les plus importants qui nous ont conduit à l'amélioration de cette connaissance. Bien que de nombreux travaux soient toujours des sujets de débats sur le fait qu'il est difficile de séparer les contraintes naturelles des contraintes anthropiques et qu'un protocole standard d'analyse des foraminifères n'a toujours pas été proposé, les foraminifères ont largement prouvé leur valeur pour être intégrés dans les programmes de suivi environnementaux.

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*Mots clés :* Foraminifères benthiques ; Bio-indicateurs ; Pollution ; Italie

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## 1. Introduction

Coastal areas have traditionally been places of human settlement, with the accompanying development of cities, industries and other human-related activities possibly having an impact on the aquatic ecosystems. These impacts may take the form of pollution from industrial, domestic, agricultural, or mining

activities; dredging or spoil dumping; salinization; sedimentation from land clearance, forestry, or road building; and the introduction of alien plant or animal species. Agricultural runoff may be enriched in nutrients and pesticide residues, the former of which may lead to eutrophication and deterioration of water quality (Moss, 1996; Wilson et al., 1993). Nutrient enrichment is not the only factor affecting the ecosystems; trace elements may also act as contaminants of the aquatic environment, with aluminium, arsenic, cadmium, copper, lead, nickel, mercury, selenium, silver and zinc being of particular concern. Trace elements can have both natural and anthropogenic sources. Natural

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background concentrations are largely determined by catchment geology and can be highly variable (Jacinski, 1995). Anthropogenic sources of metals include point-sources (i.e., industrial discharge, sewage effluents, mining and mineral processing) and diffuse inputs (i.e. stormwater, shipping and road runoff) (Makepeace et al., 1995). Sediments act as a major repository for many natural and anthropogenic contaminants entering the coastal marine systems and can preserve a record of the pollution sources and pathways. Due to the capacity of sediments to store, immobilize, recycle and transform toxic chemicals through biological and chemical processes, the effects of pollution may not be directly evident (Campbell and Tessier, 1989; Degetto et al., 1997). The presence of these pollutants in sufficient quantities and under certain conditions may result in the sediments becoming toxic to the benthic and epibenthic organisms, which spend a large part of their life cycle in or on the sediment surface. All of these pollutants can cause major ecological changes, and assessing their concentrations on a regular basis is, therefore, of paramount importance when it comes to providing an insight into the ecosystem conditions. Data on the presence and concentration of contaminants in the environment are acquired by direct measurement of the substance (chemical approach). Due to the high temporal and spatial variability of coastal areas, single chemical measurements are useless. Therefore, repeated assessments with adequate sample density, both temporal and spatial, are required in order to monitor the parameter of concern.

Biological indicators (also called bioindicators) can be used to provide an indication of environmental conditions including the presence or absence of contaminants. Biological monitoring enables the detection of unforeseen impacts and is more directly related to the “ecological health” of an ecosystem than are chemical data. The increasing importance of bioindicators is also encouraged within the European Union’s Water Framework Directive (WFD). The directive aims to achieve a good ecological status in all European water bodies (i.e., rivers, lakes and coastal waters) and requires that the assessment of the ecological status of a system be accomplished primarily utilizing biological indicators. Among the wide range of bioindicators, five biological elements are listed within the WFD: phytoplankton, macroalgae, angiosperms, benthic invertebrates and fish. The invertebrates, which include benthic foraminifera, are one of the main biotic components of marine ecosystems and are widely used for biological monitoring. As far as benthic foraminifera are concerned, the first and most obvious rationale for their utilization is that their diversity makes them one of the most varied groups of shelled microorganisms in modern oceans (Sen Gupta, 1999). With their high number of species, probably around three to four thousand (Murray, 2007), benthic foraminifera species and genera are more likely to contain a variety of specialists sensitive to environmental change. Since foraminifera have short life and reproductive cycles, they react quite quickly to both short and long-term changes in marine and transitional-marine environments on both a global and a local scale (Alve, 1991, 1995; Armynot du Châtelet et al., 2004; Cearreta et al., 2000, 2002; Coccioni, 2000; Debenay et al., 2001, 2005; Frontalini and Coccioni, 2008; Frontalini et al., 2009; Yanko et al., 1994, 1998,

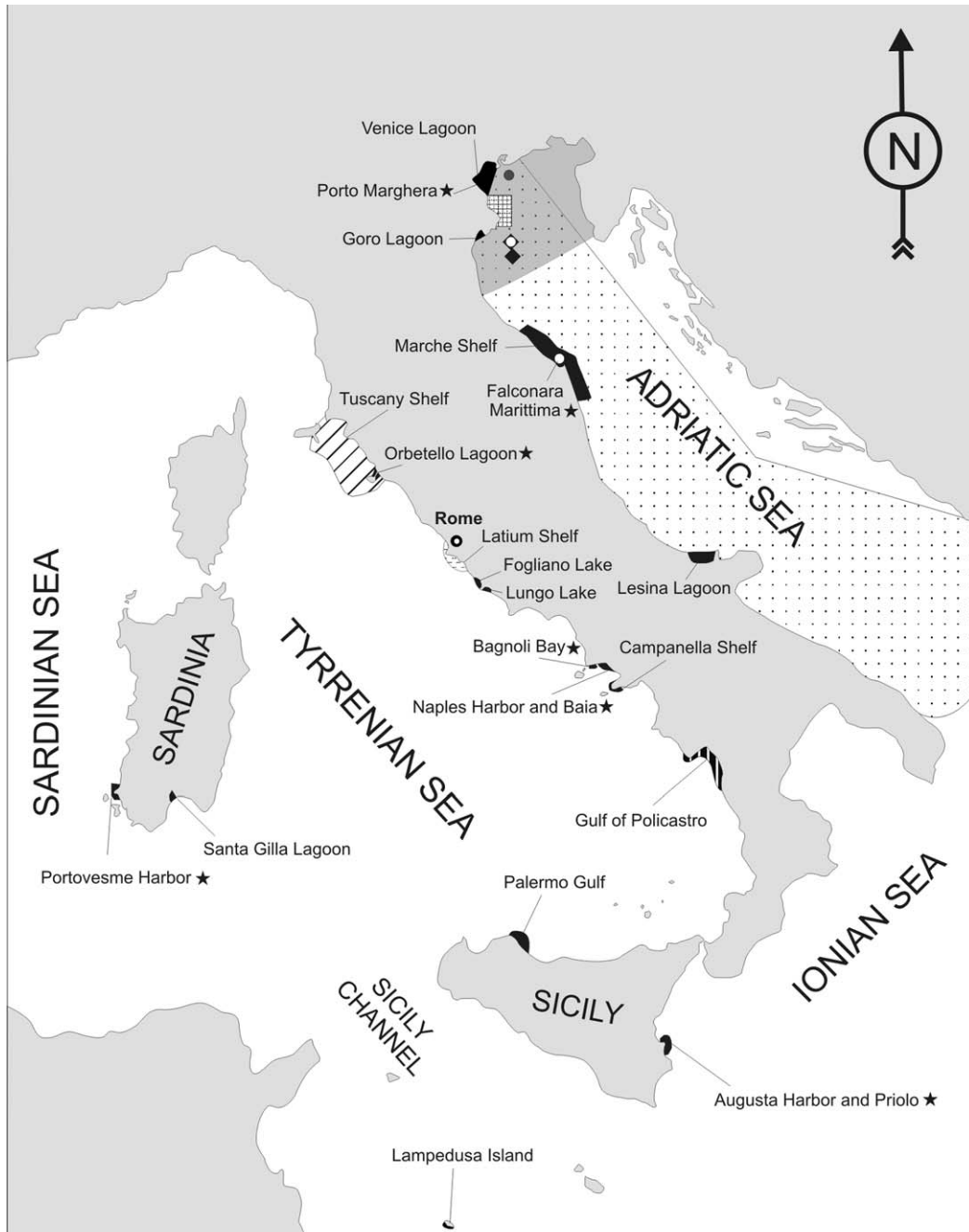
1999). Furthermore, many foraminiferal taxa secrete a carbonate shell, which can be preserved in the fossil record and used for monitoring environmental changes over longer time periods. They are generally small, highly diversified and abundant compared to other hard-shelled taxa (such as molluscs) and they are also easy to collect, providing a highly reliable database for statistical analysis, even when only small sample volumes are available.

Although the first important insight into the potential of foraminifera as a bioindicator of pollution must be credited to Resig (1958) and Watkins (1961), the first study focusing on the effect of pollution on foraminiferal distribution patterns was carried out by Zalesny (1959) in the Santa Monica Bay. Since then, both the knowledge and the number of papers published on this topic increased significantly. Many studies from different environmental settings focused on the response of benthic foraminifera to various forms of pollution such as sewage outfalls (Schafer, 1973; Seiglie, 1968), oil spills (Armynot du Châtelet et al., 2004; Mojtahid et al., 2006; Morvan et al., 2004; Seiglie, 1968), trace elements (Alve, 1991, 1995; Armynot du Châtelet et al., 2004; Coccioni, 2000; Elberling et al., 2003; Frontalini and Coccioni, 2008; Frontalini et al., 2009; Geslin et al., 2000, 2002; Sharifi et al., 1991; Samir, 2000; Samir and El-Din, 2001; Yanko et al., 1994, 1998, 1999); and pollution related to paper and pulp mills (Nagy and Alve, 1987; Schafer et al., 1991) and thermal activity (Buckley et al., 1974; Schafer, 1970, 1973). Comprehensive reviews have also been carried out by several authors (Alve, 1991, 1995; Culver and Buzas, 1995; Martin, 2000; Murray, 2006; Murray and Alve, 2002; Scott et al., 2001; Yanko et al., 1994, 1999). Although the majority of these studies highlighted the value of using benthic foraminifera in biomonitoring programs, they also conceded, in agreement with Armynot du Châtelet and Debenay (2010), that distinction between natural and human-induced stresses can be a difficult issue and is still controversial.

In the present study, the application of foraminifera for pollution monitoring in Italy will be presented and summarized. The necessity of standardizing the techniques used for tasks ranging from sample collection to the treatment of data in order to make future studies and research more comparable is also discussed. Moreover, consideration is given to the fact that once standardization and consistency are achieved, it would open up a new scientific arena within which integrated worldwide programs using benthic foraminifera as valuable bioindicators of contemporary environmental changes and disturbances caused by both natural and anthropogenic pollution could be carried out.










## 2. Review of the benthic foraminiferal monitoring in Italy


In the last decades, several studies have been carried out in Italy focusing on the use of benthic foraminifera as sensitive bioindicators for pollution monitoring with respect to different sources of pollution (Fig. 1 and Supplementary data). Some of these studies took place in heavily polluted sites that have been



Study areas for organic matter pollution

Study areas for chemical pollution

- |   |   |
|---|---|
|  1-2 |  8     |
|  3-4 |  9-10  |
|  5-6 |  11-12 |
|  7   |  13    |
|   |  14    |

-  15-44

(★) Contaminated Sites of National Relevance

Fig. 1. Location of studies carried out in Italy, focusing on benthic foraminifera as bioindicators of pollution. Study areas are subdivided by topics: organic matter (1 to 14) and chemical pollution (15 to 44). Adriatic Sea: 1-2, Jorissen (1987, 1988); 3, Van der Zwaan and Jorissen (1991); 4, Pranovi and Serandrei Barbero (1994); 5, Jorissen et al. (1992); 6, Donnici and Serandrei Barbero (2002); 11-12, Barmawidjaja et al. (1992, 1995); 13, Serandrei Barbero et al. (2003); 14, Duijnsteet et al. (2004); Tyrrhenian Sea: 7, Sgarrella et al. (1983); 8, Zampi and Gandin (1984); 9, Carboni et al. (2004); 10, Frezza and Carboni (2009); Goro Lagoon: 15, Coccioni et al. (1997); 16, Coccioni (2000); 17, Luciani (2007); Venice Lagoon: 18, Albani et al. (1998); 19, Coccioni and Marsili (2005); 20, Coccioni et al. (2009); Orbetello

listed as “contaminated sites of national relevance” by the Italian Ministry of Environment (Fig. 1).

## 2.1. Organic matter enrichment

### 2.1.1. The Adriatic Sea

Numerous studies have been performed in the last three decades on benthic foraminifera obtained from the northern and western Adriatic Sea (Barmawidjaja et al., 1992, 1995; Donnici and Serandrei Barbero, 2002; Duijnsteet et al., 2004; Jorissen, 1987, 1988; Jorissen et al., 1992; Pranovi and Serandrei Barbero, 1994; Serandrei Barbero et al., 2003; Van der Zwaan and Jorissen, 1991). Remarkably, most of this research has focused on the front of the Po River mouth (northern Adriatic Sea) because of its peculiar characteristics (Fig. 1). In particular, the work concentrated on the ecological characterization of benthic foraminiferal assemblages in relation to environmental parameters (e.g., oxygen availability and food supply), although in only a few of the published papers living assemblages were used.

The first comprehensive and detailed analysis was conducted by Jorissen (1987, 1988), who explored the distribution of benthic foraminiferal assemblages in 285 grab samples and core tops collected in 1962 along the entire Adriatic Sea. This author documented that the enormous quantities of nutrients and organic detritus that have been transported and deposited in an area (mud-belt) in front of the Italian side of the Adriatic Sea resulted from the runoff of the Po and other small Italian rivers. This input can periodically lead to algal blooms and the rapid consumption of oxygen, which ultimately causes oxygen depletion in the bottom waters. He reported that these circumstances could lead to the presence of a limited number of species with an opportunistic life strategy, such as *Nonionella turgida*, *Bulimina marginata* and *Valvulineria bradyana*.

These findings were soon corroborated by Van der Zwaan and Jorissen (1991), who documented the effect of the riverine input on the benthic ecosystem in three areas (one of which was the Adriatic Sea). The foraminiferal assemblages have revealed the occurrence of regular anoxic-dysoxic conditions at the sediment-water interface, which is further characterized by the low diversity and opportunistic life strategy adopted by benthic foraminifera. The same authors reported microhabitats and the degree of tolerance to oxygen deficiency of selected benthic foraminiferal taxa. In particular, *Epistominella* and *Bulimina denudata* were described as very tolerant taxa, followed by *Buliminella*, *Fursenkoina*, *Bolivina* spp. (smooth), *Uvigerina peregrina* and *Bulimina aculeata*. The same authors also proposed a model explaining the seasonal microhabitat variations of species living in the mud belt. These data were later con-

firmed by Jorissen et al. (1992), who investigated the relationship between the distribution of living benthic foraminifera and both oxygen and food availability in the uppermost seven centimetres of selected stations in the northwestern Adriatic Sea. In particular, *Hopkinsina pacifica*, *Bolivina dilatata*, *Bolivina seminuda* and *N. turgida* were regarded as the most opportunistic taxa, while *Stainforthia concava*, *Bolivina spathulata*, *B. marginata* and *Epistominella exigua* as the second most opportunistic. The living benthic foraminifera from a single station in a zone of active mud sedimentation (northwestern Adriatic Sea) were investigated by Barmawidjaja et al. (1992). Their aim was to document whether the strong oxygen variations in the bottom water could have been reflected in faunal density and vertical distribution fluctuations. Three groups of benthic foraminifera have been recognized on the basis of vertical distribution characteristics: an epifaunal group (striatula types of *B. dilatata*, *B. seminuda*, *N. turgida* and *Stainforthia fusiformis*), a predominantly infaunal group (*Ammoscalaria pseudospiralis*, *Eggerella advena*, *Eggerelloides scaber* (reported as *Eggerella scabra*), *Morulaeplecta bulbosa* and *Textularia agglutinans*) and a group of possibly infaunal foraminifera (*B. dilatata*, *B. spathulata*, *B. marginata*, *Epistominella vitrea* and *H. pacifica*). Smaller fluctuations in the faunal density of infaunal taxa when compared with their epifaunal counterparts were also reported. Pranovi and Serandrei Barbero (1994) investigated the living and dead benthic foraminifera affected by anoxic conditions in 20 bottom samples taken from the area between Chioggia and the Po Delta (northwestern Adriatic Sea). On the basis of principal component analysis (PCA), these authors concluded that the Po River and the lagoon of Venice exert a strong control on the benthic community (foraminifera and macrobenthos). The most important contribution as far as eutrophication is concerned was provided by Barmawidjaja et al. (1995), who accurately described the vertical distribution of benthic foraminifera in front of the Po Delta by analysing a 57 cm long core spanning 160 years. Several changes in the benthic foraminiferal and grain size patterns were noted. These changes were generated by man-induced alterations of the main outflow canals of the Po River (1840 and 1880), a steady increase of the nutrient load (from 1900 onwards), intensification of eutrophication (1930), and first signs of anoxic events (1960). Since 1880, the original taxa have been gradually replaced by more stress tolerant ones (i.e., *N. turgida*). Another substantial change occurred in 1930 when opportunistic stress tolerant species (*H. pacifica*, *Bolivina seminuda* and *Quinqueloculina stalkerii*) became dominant. The authors regarded *H. pacifica* (peaking in 1960) as the most stress tolerant taxon. A seaward increase in abundance of *Bolivina* spp., *H. pacifica* and *N. turgida* was found in front of three small rivers in the Marche Region (central Adriatic Sea),

Lagoon: 21, Succi et al. (2008); 22, Frontalini et al. (2010); Santa Gilla Lagoon: 23, Frontalini et al. (2009); Fogliano and Lungo Lakes: 24, Carboni et al. (2009); Lesina Lagoon: 25, Frontalini et al. (2010); Marche Shelf: 26–27, Coccioni et al. (2003, 2005); 28, Frontalini and Coccioni, 2008; Falconara Marittima: 29, Orsini et al. (2006); Bagnoli Bay: 30–31, Bergamin et al. (2003, 2005); 32–33, Romano et al. (2008, 2009a); Campanella Shelf: 34, Ferraro and Lirer (2006); Portovesme Harbor: 35, Cherchi et al. (2009); Baia: 36, Bergamin et al. (2009); Naples Harbor: 37–38, Ferraro et al. (2006, 2009); 39, Rumolo (2008); 40, Rumolo et al. (2009); Palermo Gulf: 41, Basile et al. (2007) and Cosentino et al. (2008), 42, Valenti et al. (2008), 43, Di Leonardo et al. (2007); Lampedusa Island: 41, Basile et al. (2007), 42, Valenti et al. (2008); and Augusta Harbor: 43, Di Leonardo et al. (2007), 44, Romano et al. (2009b). The star marks the “contaminated sites of national relevance” as defined by the Italian Ministry of Environment.

most likely occurring in response to a change in either sediment texture or enhanced nutrient availability (Frontalini et al., *in press*). Donnici and Serandrei Barbero (2002) examined 25 grab samples from four transects in the northwestern Adriatic Sea, where 110 species in the total foraminiferal assemblages and 90 in the biocenosis were recognized. According to the correspondence analysis, three biotopes were documented. Biotope 1 (depths between 5 and 13.5 m), in which nutrient inputs can periodically lead to oxygen deficiency, was characterized by a high abundance of *Ammonia beccarii* and *Aubignyna perlucida* (reported as *Valvulinera perlucida*) with subdominant *E. scaber* (reported as *Eggerella scabra*). Biotope 2 (nutrient rich pelitic bottom) was characterized by taxa such as *Nonionella opima*, *Bulimina* and *Brizalina*, which can adopt an opportunistic life strategy and are able to tolerate periodic reductions in dissolved oxygen. Finally, Biotope 3 (depths between 21 and 46 m) was characterized by a sandy bottom station and low organic matter content, and this biotope was characterized by *Textularia* as the dominant genus and other epiphytic species i.e. *Asterigerinata mammilla*, *Lobatula lobatula* (reported as *Cibicides lobatulus*), *Neoconorbina terquemi* and *Quinqueloculina agglutinans*.

A 16 m water depth station located on the Adriatic inner shelf was investigated from February 1991 to January 1995 by Serandrei Barbero et al. (2003) to study the temporal changes in benthic foraminiferal assemblages. They pointed out that the main controlling factor of the productivity of benthic foraminifera is the occasional availability of phytoplankton. A negative trend of *A. beccarii* with respect to *Planorbulina mediterraneensis*, most likely induced by the presence of phytoplankton blooms in areas where the more opportunistic strategy of *A. beccarii* might have prevailed over *P. mediterraneensis*, was also shown. Living benthic foraminifera and several environmental parameters have been monitored for a period of two years at two stations in the northwestern Adriatic Sea by Duijnsteet et al. (2004), who reported dysoxic conditions leading to low foraminiferal density, with most species appearing to be affected. The only exception is *S. fusiformis*, a taxon that can be regarded as highly opportunistic and able to occupy a vacant microhabitat created by the anoxia-induced mortality of other species (Alve, 1994, 1995).

### 2.1.2. The Tyrrhenian Sea

Unlike the Adriatic Sea, only a few studies of the effects of organic matter on benthic foraminifera have been carried out in the Tyrrhenian Sea (Carboni et al., 2000, 2004; Frezza and Carboni, 2009; Sgarrella et al., 1983; Zampi and Gandin, 1984) (Fig. 1). Oligotypic assemblages in freshwater discharges were documented in the gulf of Policastro (Sgarrella et al., 1983). However, no significant changes in foraminiferal parameters were found in front of the Tiber mouth area by Zampi and Gandin (1984). The distribution of recent benthic foraminifera has been investigated in the Ombrone river basin by Carboni et al. (2004), who used a statistical approach to demonstrate that the distance from the river is a very important factor for foraminifera distribution. They focused, in particular, on a zone associated with the fluvial input of the Ombrone River that stretches parallel to the coast and is characterized by *V. bradyana* assemblages, low

diversity, and high dominance values in its most eutrophicated area. According to Jorissen (1987, 1988), Bergamin et al. (1999) and Fontanier et al. (2002), *V. bradyana* is an opportunistic taxon living in sediments with high amounts of organic matter and has been found in the mud belt parallel to the Adriatic Sea coast in response to the Po River run-off. The same conclusions were reached for this area by Frezza and Carboni (2009) on the basis of a larger sample set.

## 2.2. Trace elements and other chemicals

### 2.2.1. The Goro Lagoon

The first Italian contribution to the research issue of the impact of pollution on benthic foraminifera was made by Coccioni et al. (1997), who investigated 15 surface sediment samples from the Goro Lagoon (southernmost bay in the Po River Delta) (Fig. 1). Their preliminary results documented a marked enrichment in Cr, Ni, Zn, Pb, As and total organic carbon (TOC) at some of the sites, which were also characterized by a general reduction in foraminiferal test size, a decrease in the richness of the benthic species, and a concurrent increase in the abundance of tolerant and opportunistic species such as *Criboelphidium translucens*. The same authors also reported high numbers (up to 10% of the entire assemblages) of deformed tests in the biocenosis in which higher trace element concentrations were prevalent. The degree of sensitivity to trace element pollution was reported and can be ordered as follows (from more to less affect): *Ammonia* sp. 1, *A.*, *Haynesina depressula*, and *C. translucens*. A subsequent and more detailed study in the same lagoon was conducted by Coccioni (2000), who analysed 16 surface grab sediment samples collected at three different times over a period of six months in order to document the benthic foraminiferal assemblages and the concentration of 19 trace elements. A remarkable number of specimens exhibiting a variety of morphological abnormalities within the very low-diversity living assemblages were documented. Although a clear relationship between trace element contents, morphological abnormalities, and foraminiferal distributions was not in evidence at the Goro Lagoon, this study constitutes the first comprehensive Italian attempt to document the influence of pollution on benthic foraminifera. The same lagoon has been the subject of multi-year monitoring by Luciani (2007), who documented very low diversity assemblages dominated by opportunistic species including *H. germanica*, *Ammonia tepida* and *Criboelphidium gunteri*. The same author found extremely high levels (up to 100%) of abnormality in living specimens.

### 2.2.2. The Lagoon of Venice

Albani et al. (1998) investigated the sedimentary provinces and foraminiferal biotopes of 174 sampling sites in the Gulf of Venice and found that industrial waste dumping sites had no effect on the distribution of the benthic foraminiferal assemblages, which are primarily influenced by water quality (Fig. 1). They also concluded that benthic foraminifera were not affected by the geochemistry of the substrate. A preliminary investigation of the living benthic foraminifera in the heavily polluted lagoon of Venice and their response to trace element pollution was con-

ducted by Coccioni and Marsili (2005), who documented low diversity assemblages dominated mainly by *A. tepida*, *Criboelphidium oceanensis* and *H. germanica*. Higher percentages of abnormal forms were found at stations located closer to the industrial area of Porto Marghera. These data were integrated and statistically analysed by Coccioni et al. (2009), who demonstrated a strong relationship between selected trace elements (Mn, Pb and Hg) in the Adriatic Sea and the Foraminiferal Abnormality Index (FAI), an index which was defined by Coccioni et al. (2005). A negative relationship between trace element concentrations, species richness and the relative abundance of *A. parkinsoniana* was also noted.

### 2.2.3. The Orbetello Lagoon

High percentages of abnormal specimens were documented by Succi et al. (2008) in three cores and 13 surface samples from the Orbetello Lagoon (Fig. 1). This lagoon has been contaminated by multiple pollutants originating from sewage, aquaculture and an artificial fertilizer plant. Three main groups of benthic foraminifera have been recognized on the basis of statistical analysis. The first two are dominated by *A. parkinsoniana* and different species diversities corresponding to differing degrees of confinement. The third group, oligotypic, which is dominated by *H. germanica* and *A. parkinsoniana*, can be regarded as reflecting the greatest degree of confinement. In the three cores, a barren zone confined below 40 to 50 cm, most likely induced by very high environmental stress, was also reported. A comparative analysis of the Lesina and Orbetello Lagoons has been performed by Frontalini et al. (2010), who found oligotypic assemblages characterized by species such as *A. tepida*, *H. germanica* and *C. oceanensis*, which are typical of a transitional environment. By comparing the trace element concentrations with the FAI, these authors were able to infer a possible influence of these pollutants on the benthic foraminiferal assemblages.

### 2.2.4. The Santa Gilla Lagoon

Benthic foraminifera have been successfully used by Frontalini et al. (2009) as bioindicators of trace element pollution in the heavily contaminated Santa Gilla Lagoon of Sardinia (Fig. 1), in which assemblages dominated by *A. tepida*, *C. oceanensis* and *H. germanica* were found. The assemblages were also characterized by high FAI values at the heavily polluted stations, as well as high numbers of megalospheric forms. *Rosalina globularis* showed significantly negative correlations with Cd, Pb, Zn and Cr. The same authors reported negative correlation coefficients among the different diversity indices and most trace elements. They also documented higher concentrations of trace elements in the porcelaneous foraminiferal tests than those measured at an unpolluted site in Capitana by Cherchi et al. (2009). Specifically, foraminiferal tests in the Santa Gilla Lagoon exhibited concentrations of Fe, Mn, Pb and Zn that were one order of magnitude greater than those found at Capitana. The occurrence of nanoparticles within the tests of abnormal specimens of *A. tepida* has also been documented in the Santa Gilla Lagoon, revealing the presence of Fe and S; Ba and S; and La, Ce and Nd.

### 2.2.5. The Fogliano and Lungo Lakes

Complete benthic foraminiferal assemblages in two brackish water lakes (Fogliano and Lungo) located along the coast of southern Latium have been studied by Carboni et al. (2009) (Fig. 1). Medium FAI values (~4%) were found in the inner and intermediate assemblages and were attributed to natural origins. The occurrence of *H. germanica* in the Fogliano Lake has been suggested as a possible indication of pollution. Other than in its outer portion, the Lungo Lake is uninhabited by foraminifera, suggesting highly stressful conditions and a possible anthropogenic influence. According to the authors, however, the nature of the stress cannot be assessed without conducting geochemical analyses.

### 2.2.6. The Adriatic Sea

The relationship between living benthic foraminifera and trace element contents in a shelf area of the central-western Adriatic Sea was documented by Coccioni et al. (2003, 2005) (Fig. 1). Coccioni et al. (2005) introduced the FAI and the Foraminiferal Monitoring Index (FMI) enabling the quantification and comparison of the percentages of abnormal specimens and species, respectively. These data were then integrated and summarized by Frontalini and Coccioni (2008) who, by using a statistical approach, noted a possible influence of these pollutants both on the taxonomic composition of the assemblages and the development of test abnormalities. Increasing trace element contents were reported as leading to increases in the relative abundance of *A. tepida*, *A. perlucida*, *N. turgida* and *E. scaber* (reported as *E. scabra*), as well as to a relative and concurrent decrease both in the relative abundance of *A. parkinsoniana* and the higher percentages of the FAI and FMI. The authors speculated that *A. parkinsoniana* prefers clean to modestly polluted environments and is very sensitive and intolerant to pollution, being highly affected by trace elements, even at low concentrations. On the other hand, the authors also confirm the ability of *A. tepida* to tolerate increasing trace element concentrations and point out that *A. perlucida*, *N. turgida* and *E. scaber* could be regarded as tolerant species, at least in environments with relatively low levels of pollution. Living and dead benthic foraminifera were studied in box cores recovered in shallow water off Ancona (central Italy) by Orsini et al. (2006). These authors described both a general distribution pattern controlled by the discharge of the Po River and a local pattern influenced by the freshwater input of two small rivers. They also documented the absence of living benthic foraminifera, very low diversity, and the dominance of highly opportunistic species at stations close to the Falconara Marittima oil refinery.

### 2.2.7. Bagnoli Bay

Important contributions were made by Bergamin et al. (2003), who conducted studies in the Bagnoli Bay (southern Tyrrhenian Sea, Naples), which has been heavily affected by pollution from an industrial plant (Fig. 1). These authors demonstrated that some trace elements (Hg, Mn, Ni, Pb and Zn) are well tolerated by certain benthic foraminiferal species, including *H. germanica*, *Miliolinella subrotunda* and *Quinqueloculina parvula*, while *Bulimina sublimbata*, *Elphidium macellum* and

*Miliolinella dilatata* are capable of tolerating the presence of polycyclic aromatic hydrocarbons (PAHs). They also reported the presence of several barren samples from areas close to the plant and speculated that no foraminiferal species can tolerate copper and iron-polluted environments. In a subsequent paper, based on work carried out in the same area, Bergamin et al. (2005) investigated the foraminiferal response to trace element, PAH and polychlorinated biphenyl (PCB) pollution in seven short cores. They reported that in one of the cores high concentration of PAHs could not be compared to foraminiferal assemblages due to their absence. PCB concentrations do not seem to affect foraminiferal abundance, whereas trace elements were regarded as being responsible for the variation therein. The authors suggested that the combination of Cu and Fe contamination might have caused the absence of benthic foraminifera in the shallower cores. They proposed that the ratio between deformed and normal specimens of *M. subrotunda* and *Elphidium advena* can be used as an index indicating trace element pollution. In addition, they suggested the percentages of abnormal specimens of *M. subrotunda* as a potential bioindicator of Cu pollution and those of *E. advena* for Cr, Mn and Zn pollution. These investigations were refined by Romano et al. (2008), who documented low foraminiferal abundance in the more polluted area of Bagnoli by investigating 27 grab samples. In doing so they confirmed the pollution-tolerant nature of *H. germanica*, *M. subrotunda* and *Q. parvula*, and demonstrated the relationship between the levels of abnormality and some pollutants (PAHs, Mn, Pb and Zn). The same authors revealed a significant correlation between both the percentage of deformed *M. subrotunda* and the concentration of PAHs, Mn and Fe, and between the percentage of deformed *E. advena* and Pb. Compositional analyses performed on the crystalline reticulum of deformed specimens of *M. subrotunda* revealed the presence of Fe ions and led to the conclusion that this species may be used as a bioindicator for Fe pollution. It was also hypothesized that the inclusion of the Fe ion in the crystalline reticulum, due to the inability of some species to exclude foreign elements, could be responsible for the potentially toxic effect of pollutants (PAHs, Mn and Zn). A wider section of this disused industrial site was further investigated by Romano et al. (2009a), who documented a possible correlation between the FAI and selected trace elements, and also found that increased pollution levels lead to an increased abundance of pollution-tolerant species. To that effect, they recognized *Q. lata*, *Q. stelligera*, and *Q. parvula* as pollution-tolerant species, while *L. lobatula*, *Asterigerinata mammilla* and *R. bradyi* were deemed to be pollution-sensitive. The same researchers reported that the FAI is the only parameter that can be correlated with the degree of pollution.

#### 2.2.8. The Tyrrhenian Sea

Morphological abnormalities in benthic foraminiferal tests of the Punta Campanella Shelf (south Tyrrhenian Sea) were investigated by Ferraro and Lirer (2006) (Fig. 1). Although this location has been included within a Marine Protected Area, it is affected by the discharge of several pollutants from the surrounding coast and, in particular, by multiple pollution sources of an industrial, domestic and agricultural nature from the Sarno River. The

authors reported low percentages of deformed species. Unfortunately, no trace element data were provided in this study and it was, therefore, not possible to determine the likely factors influencing the benthic foraminiferal environment. A reduction in species diversity and specimen density, which was probably induced by increasing pollution, was found near the industrial complex of Portoscuso-Portovesme (south-western Sardinia) by Cherchi et al. (2009) (Fig. 1). These authors also documented contrasting behaviour of the hyaline and porcelaneous species, with the latter increasing in abundance in more polluted samples. Although they explained this in terms of the occurrence of pores leading to greater sensitivity of the hyaline taxa, an influence exerted on their distribution by sea grass (i.e., *Posidonia oceanica*) could not be excluded. Trace elements were measured in abnormal (Portovesme area, polluted) and normal (Capitana Gulf of Cagliari, unpolluted) miliolid tests revealing concentrations (Cd, Fe, Pb and Zn) that differed by more than one order of magnitude between these two sites. High percentages of pyritized and abnormal tests were also documented. These researchers used ESEM images to illustrate strong bio-erosion, caused by the epilithic-endolithic microbial community, in the calcareous foraminiferal tests of the most polluted environments, as contrasted with the mildly polluted/unpolluted sites which did not show any signs of microbial infestations. Bergamin et al. (2009) investigated the protected coastal-marine area of Baia (Naples), which has in the past been affected by anthropogenic action mainly related to commercial harbor activity. On the basis of 36 grab samples, they documented the response of living benthic foraminifera to trace elements, PAHs, and PCBs. They confirmed the pollution-tolerant nature of taxa, including *A. tepida*, *H. germanica*, *Cornuspira involvens* and *B. variabilis*, and reported FAI values above background levels of 1% and 1.75% according to Stouff et al. (1999) and Morvan et al. (2004), respectively.

#### 2.2.9. The Naples Harbor

A comprehensive investigation of three docks (Levante, Granili and Diaz) in the Naples Harbor was conducted by Ferraro et al. (2006) (Fig. 1). Reductions in foraminiferal density and species richness were revealed as a response to an increase in trace element pollution. In particular, they documented a completely barren area at the Diaz dock, which corresponded to the greatest concentrations of trace elements (Pb, Hg, Ni and Zn from two to nine times higher than at the other two docks). The same authors reported assemblages dominated by *A. tepida* and characterized by high percentages of deformed specimens for the second, most polluted dock (Levante). A well-diversified assemblage was found in the Granili dock, which was the least polluted. *Quinqueloculina* spp. have been proposed as taxa that are very sensitive to pollution. In order to investigate the potential contamination of the marine environment by organic matter and trace elements in the harbor of Naples, new geochemical tools, including the analysis of trace element contents in the carbonate shell of the benthic foraminiferal species of *A. tepida*, were evaluated by Rumolo (2008). High trace element concentrations were found and related to fluxes of some dissolved trace elements directly from land. Although the process of trace element incor-

poration within the foraminiferal test is largely unknown, it was hypothesized that the calcium cations were replaced by the most labile fraction of the dissolved trace elements. These data were updated by Rumolo et al. (2009), who found that carbonate tests of *A. tepida* taken from the Naples Harbor are extremely enriched in some trace elements (Cd, Cu, and Zn) when compared to the samples collected from unpolluted Mediterranean seawaters. Moreover, on the basis of partition coefficients, the authors were able to estimate the total dissolved metal concentrations in seawater.

More recently, Ferraro et al. (2009) made a very important contribution to the understanding of this issue by testing for the presence of trace elements, PAHs, PCBs, total recoverable petroleum hydrocarbons, and volatile organic compounds (VOCs) in the Naples Harbor. Despite the large dataset, they documented a non-linear response by the biota to the effects of contamination with the different classes of pollutants. The authors, however, suggested that *Quinqueloculina* is the genus that is most sensitive to high concentrations of some trace elements when compared to *A. tepida* and *Elphidium*. They also reported an opposite distributional pattern for *Elphidium* spp. and *Quinqueloculina* spp. with VOCs.

#### 2.2.10. Sicily

Trace element pollution and benthic foraminiferal assemblages were investigated by the Regional Environmental Protection Agency of Sicily (ARPAS) (Basile et al., 2007; Cosentino et al., 2008) (Fig. 1) in order to evaluate sediment quality in different coastal areas including the gulf of Palermo. *Lobatula lobatula* (reported as *Cibicides lobatulus*) was suggested as the species more sensitive to trace element pollution. In fact, in the heavily polluted gulf of Palermo, high numbers of abnormal specimens of *L. lobatula* were found. On the other hand, at Lampedusa Island, which can be regarded as an unpolluted environmental site, very low percentages of abnormalities were discovered. The researchers found a negative correlation between selected trace elements (Zn, Cr and Pb) and foraminiferal density, and they also reported that *Ammonia* spp., *Bulimina* spp. and *Elphidium* spp. were species tolerant to trace element pollution while *Quinqueloculina* spp. and *Rosalina* spp. were regarded as the most sensitive. In a subsequent paper, and on the basis of the same samples, Valenti et al. (2008) investigated the spatial distribution of trace elements and two foraminifera groups (*Quinqueloculina* spp. + *Adelosina* spp. and *Elphidium* spp.). They concluded that *Quinqueloculina* spp. + *Adelosina* spp. appeared to be more sensitive to pollution, whereas *Elphidium* spp. was more tolerant and can be regarded as opportunistic. The relationships between benthic foraminiferal assemblages and two pollutants (Hg and PAHs) were investigated by Di Leonardo et al. (2007) in vertical profiles from three box cores recovered from the industrial area of Augusta and the Palermo Gulf. These authors found a reduction in benthic foraminiferal abundance, an increase in the percentage of abnormal species, and the dominance of opportunistic species in the more recent sediments, which are more affected by pollution. Miliolids and *Brizalina* dominated the abnormal assemblages and were reported to be very sensitive

to pollution. Unfortunately, only a limited number of samples were used for the foraminiferal analysis, which meant that a statistical analysis could not be applied. The response of benthic foraminifera to pollution (trace elements, PAHs, PCBs, and TOC) in surface samples from the Augusta Harbor was later investigated by Romano et al. (2009b) (Fig. 1). On the basis of species and pollution distributions, several species have been recognized as pollution-tolerant, namely, *Q. lata*, *A. tepida*, *C. involvens*, *H. germanica*, *Bolivina aenariensis*, *B. seminuda* and *B. variabilis*. The same researchers also documented both, stunted assemblages for the most polluted sites and FAI exceeding the reference values provided by Stoff et al. (1999) and Morvan et al. (2004).

#### 2.3. Stress induced by other environmental factors (e.g., salinity, oxygen and temperature variations)

##### 2.3.1. The lagoon and the gulf of Venice

Many studies of benthic foraminifera from the lagoon of Venice have been carried out (Albani and Serandrei Barbero, 1982, 1990; Albani et al., 1984, 1991, 1998, 2007, 2010; Cita and Premoli Silva, 1967; Donnici et al., 1997; Serandrei Barbero et al., 1989, 1999; Silvestri, 1950). Albani and Serandrei Barbero (1982) used foraminiferal fauna (biotopes) to define the hydrological and ecological conditions in several sections of the lagoon. These biotopes were described in further detail by Albani et al. (1984) and Serandrei Barbero et al. (1989) for the northern, by Albani et al. (1991) for the central, and by Serandrei Barbero et al. (1999) for the southern portions of the lagoon. The distribution of the benthic foraminifera was presented in a monograph by Albani and Serandrei Barbero (1990), while the first analysis was carried out by Donnici et al. (1997) in the central portion of the lagoon documenting that the spatial variation of the biocoenosis is in agreement with biotopes obtained through the total assemblages. The same authors also reported a change in the progression to a more marine condition, as testified by the presence in the biocoenosis of inner shelf species that had not been identified earlier. More recently, an important contribution was made by Albani et al. (2007), who compared the foraminiferal assemblages of samples collected in 1983 and 2001 in the northern portion of the lagoon. They documented unchanged conditions for ~50% of the site and ascribed the main variations to the disappearance of some salt marshes and the effect of water quality improvements in the area of Porto Marghera.

##### 2.3.2. Santa Gilla

Benthic foraminiferal distributions in six surface sediments were analysed by Zampi and D'Onofrio (1984), who found that oligotypic assemblages are dominated by *A. beccarii*, *C. gunteri* (reported as *Elphidium gunteri*) and *H. germanica* (reported as *Protelphidium anglicum*), and are characterized by great numbers of abnormal specimens. They also noted major morphological variability and a high number of megalospheric forms of *A. beccarii*. The morphology of this species was correlated with the peculiar environmental conditions of the lagoon. More recently, Foresi et al. (2006) investigated the relation-



ship between the morphology of *Triloculina rotunda* and the environmental conditions in the area. Abnormal test shapes and assemblages dominated by megalospheric forms were observed. The authors also reported incompletely calcified test walls in response to stressful conditions and suggested that reproduction occurs before adult characteristics have developed.

### 2.3.3. *The thermal pool of Montecatini*

Test morphology and the organic layer of *Discorinopsis aguayoi* were investigated by Foresi et al. (2004) in peculiar environmental conditions. Four populations, of which two were modern and brackish (the thermal pool of Montecatini and the lagoon of Orbetello) and two were fossils (in Messinian and Pliocene sediments of two quarries near Leghorn and Florence, respectively), were collected. It was concluded that the environmental conditions influence the morphology and organic layer of this species. The authors also found greater thickness in the organic lining of the Montecatini individuals, with a thin calcitic wall and microcavities in the organic thickening of those living in zones of extreme conditions within the thermal pool, which is characterized by strongly hyposaline and probably oxygen-depleted waters.

## 3. Organic matter vs chemical pollution

The benthic environment as all other pelagic and terrestrial environments is a system where different domains (biotic and abiotic) interact. As a consequence, foraminiferal ecology is an impervious ground for biologist, chemist and ecologist alike as its understanding is mostly anchored to cause-effect relationships. The identification of foraminiferal response to specific environmental factor(s) is sometimes hampered by the synergic and antagonistic relationships among them, which makes the isolation of any single factor all but impossible. A clear example is the complex relationships and covariances between the nature of the substrate, and the oxygen and organic matter contents within it, which certainly affect the standing crop of benthic foraminifera. This, however, just represents a scale using which the complexity can be observed but understood only superficially. Although the use of lab-experiments, such as the meso- and microcosms, has recently began playing a major role in environmental studies, most of our knowledge is based primarily on field data. On the basis of these field studies, several parameters have been regarded as primary controlling factors in foraminiferal distribution. These include: salinity, temperature, oxygen, tidal currents, substrate, food supply, pollution, depth, competition and predation (Murray, 2006).

The main sources of food for benthic foraminifera (not taking into account the endosymbiotic species) are organic matter and the entire bacterial community that proliferates on it. Although these sources can act as limiting factors in deep-sea areas, they also exert a strong influence in coastal and marginal settings where periodically enhanced production and the input of nutrients may lead to an increase in the standing crop of the benthic community. The quality of organic matter (labile vs. refractory and C/N ratio) is, however, another parameter influencing

the benthic foraminiferal fauna (Armynot du Châtelet et al., 2009). Where the oxygen demand to metabolise the organic matter exceeds the supply, dysoxic-anoxic conditions may be established. In these circumstances, an excess of organic matter is not a pollutant in itself, but the induced consumption of oxygen certainly is, especially when it approaches the tolerance limit for a species. A species-specific response is expected according to the degree of eutrophication. On the basis of all of the results from the published papers mentioned above, some benthic foraminiferal taxa, including *H. pacifica*, *N. turgida*, *V. bradyana*, *U. peregrina* and several species belonging to the genus *Bulimina*, *Buliminella*, *Fursenkoina*, *Bolivina* and *Epistominella*, can be considered as the most tolerant to organic matter. A degree of tolerance to trace element pollution among species was first suggested by Schafer (1973) and Sharifi et al. (1991). Since then, plenty of species and genera have been inferred to be sensitive or tolerant to pollution. Although most of these inferences require laboratory confirmation, an enhanced degree of tolerance to trace elements and other chemicals has been inferred for several taxa, including *Ammonia*, *Criboelphidium*, *Haynesina*, *Brizalina* and *Bolivina*, as reported by Yanko et al. (1999) and confirmed by Armynot du Châtelet et al. (2004), Armynot du Châtelet and Debenay (2010) and Carnahan et al. (2008, 2009). Certainly, these inferences represent an oversimplification of the species response to both organic matter vs. oxygen stress and contaminants since other factors such as depth and salinity variations must also be considered, particularly in paralic environments. In fact, the discharge of sewage is normally associated with freshwater that may play a multiple role in influencing the foraminiferal distribution and composition due to the establishment of a hypertrophic zone and the lowering of its salinity. Not only does salinity influence the foraminiferal assemblages, but its variation has been described as responsible for the development of deformation in foraminiferal tests. Under these circumstances, assessing the role played by a single factor is undoubtedly a difficult task and the influence of additional factors on foraminiferal assemblages cannot be ruled out. The comparison of polluted with unpolluted sites, “control-impact design”, as defined by Murray (2006) may help in solving this issue, though, the same conditions are hardly encountered in the same area and an effective evaluation of the stress induced by a single factor can only rarely be achieved. A further strategy would be to compare the data of the site in question with those before the pollution event (pre-pollution conditions) or to extend the record back to a time predating human activity. The adoption of this strategy, however, does not exclude some minor pitfalls, such as the comparison of actual assemblages with ones that experienced diagenetic alteration (i.e., selective dissolution) and there is no prove that the prevailing conditions (i.e. physico-chemical parameters of water) were constant through the record, at least for paralic environments.

Consequently, laboratory culture experiments may represent an accurate tool in deciphering the foraminiferal response under controlled conditions. When a single parameter can be varied and all other parameters kept fixed, the specific response of foraminiferal assemblages to that parameter can be determined, potentially adding supporting evidence to field data. Moreover,

laboratory experiments may also identify specific interactions between species and selected pollutants permitting researchers to experimentally resolve the problems presented by covariation. Although the laboratory culture studies constitute an important tool, they would need to be integrated with field data along with advanced crystallographic and geochemical analyses of the tests, molecular investigations and the study of organic matter quality (i.e.,  $C_{org}$ , C/N ratio and lipids).

#### 4. Towards uniformity and standardization

Most of the Italian foraminiferal studies referred to above have relied on different techniques and methods, making most of them incomparable (Supplementary data). Monitoring using biological indicators (biomonitoring) is based on comparative studies of the taxa at a particular site and time (with the same or different characteristics). However, to make these studies readily comparable and reliable the same set of techniques must be used from the initial sampling to the final treatment of data, at least for studies in the same environmental setting. This can only be guaranteed if there is an agreement among scientists and a protocol(s) is developed. These protocols are imperative if researchers are to ensure that samples are collected consistently and the data obtained are accurate, reliable and scientifically well founded. The suite of techniques used must be equally applicable in the same environmental conditions (i.e. bottom sediments) and at all sampling times, and they must be strictly adhered to. To this end, several issues have to be addressed before foraminiferal-based biomonitoring can be introduced in the form of governmental and international protocols. These are unique to each environmental setting and include the choice of sampler, the sieve size, the minimum quantity of foraminifera to be selected from each sample, number of replicates, the sediment sampling depth and the total (dead + living) vs living assemblages. Most of the studies cited above have been carried out in temperate coastal and transitional environments where the same techniques would be adopted. For instance, since the size of the mesh used to process a sample affects the number of species, foraminiferal density, and the composition of the foraminiferal assemblages, the same mesh size should be utilized (i.e., 63.5 to 500  $\mu\text{m}$ ). According to Murray (2006), the total foraminifera count and the number of species will decrease with an increase in mesh size, which could result in misleading conclusions (Alve, 2003). A specimen number of 250–300 for each sample is normally quoted as the minimum figure for accurately estimating the composition of an assemblage. The sediment sampling depth affects both the number of specimens and the assemblage compositions, since it has a direct effect on the numbers of infaunal and epifaunal taxa. Therefore, the sampling depth should be standardized for similar environments. One of the most controversial issues in the foraminiferal debate is whether total or living assemblages should be used. This concern has been discussed by Murray (2006) and Martinez-Colon et al. (2009), and we certainly concur that the choice depends upon the aim of the research. The use of benthic foraminifera as reliable indicators of the health of marine and transitional marine environments should be based on living assemblages, even though this makes the task time-

consuming (Murray, 2006), because of the influence of sample thickness, post-mortem transportation, the dissolution of fragile tests, and reworking, all of which could influence the original distribution of species and specimens. Although the Rose Bengal technique has provided reliable results for shelf foraminifera, Debenay et al. (2009) documented a lower accuracy in paralic and highly dynamic environments. The use of the fine fraction (63–125  $\mu\text{m}$ ) and of an appropriate number of specimens is typically sidestepped because of its time-consuming nature; however, it is the only way to satisfactorily assess the ecological quality of environments. In order to ease the comparison of results, any scientific paper should include either the absolute abundance of foraminiferal taxa or their relative abundances, including the number of specimens. In order to readily make a comparison between different localities and different ecological environments, the species name should be followed by the author's name and date. Although scientific agreement has not yet been reached, the development of a protocol will be a milestone that will lead to foraminifera being included in government and international programs regulating environmental surveys of marine and transitional marine environments.

#### 5. Conclusions

This paper provides a synthesis of Italian research onto the emerging field of benthic foraminifera as pollution bioindicators over the last three decades and reveals that the Italian contribution has, on the whole, played a considerable part in the advancement of our knowledge of environmental micropaleontology. In addition, it has been possible to qualitatively summarize the degree of sensitivity of selected taxa to different sources of pollution (organic vs. chemical pollution), as well as to confirm that benthic foraminifera are sensitive bioindicators and successful candidates for inclusion in an integrated pollution-monitoring program. Although our experience and knowledge have been greatly enhanced in recent decades, this field is still far from being completely understood or exploited, as is evident from the absence of a protocol.

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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.revmic.2011.03.001.

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