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Review Article

BENTHIC FORAMINIFERAL RESPONSES TO COASTAL POLLUTION: A REVIEW

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ABSTRACT
Deterioration of the coastal ecosystem due to human activities is a worldwide concern. The success of any pollution control and natural resource management strategy in the coastal zone is linked to the use of a comprehensive and inexpensive monitoring method. Benthic foraminifera are the most common micro-organism found in the surface sediments in the shallow and marginal marine environments. They are very sensitive to slight environmental changes and can reflect the health of the ecosystem they inhabit. They are increasingly used as bioindicators for pollution at various levels of investigations. The impact of pollution on foraminifera is expressed not only as the modification of their assemblage, but also as the changes in their morphology, shell chemistry and metabolic activity. These foraminiferal features have been exploited by many workers to monitor various types of pollution in the coastal areas. However, foraminiferal responses are often complex and it is difficult to distinguish between the impact of natural variability and anthropogenic pollution on foraminifera. We review the potential and limitations of using foraminiferal parameters in coastal pollution study.

***Key Words**: Coastal Pollution, Foraminifera, Community Structure, Test Morphology, Test Chemistry*

INTRODUCTION

The coastal environment is an interface between land and marine water. It represents a dynamic ecosystem with both spatial and temporal environmental gradients. It also plays a vital role in nation's economy by virtue of its resources, productive habitats and rich biodiversity. Over the years, human settlements as well as industries have concentrated near the coastal belts. Approximately 38% of the world's population lives within 100km of the coast. The concentration of development activities on such a scale threatens to destabilize the coastal ecosystem and its resources. Coastal pollution may arise from land based as well as water based sources. The major land based sources of are ports and harbors, oil terminals, metallurgical plants, power plants, paper and pulp industries, urban, commercial and residential development, tourism and beach creation, fish processing, agriculture and defense activities. The water based sources are offshore oil and gas, offshore placer mining, navigation, naval defense, water sports fishing, dredging and land reclamation. The major pollutants which are generated from the above sources are oil, sewage, garbage, pesticides, toxic chemicals, heavy metals, radioactive wastes, coolants, nutrients etc. The massive pollution causes deleterious effects on the marine ecosystem such as harm to living resources, hazards to human health, hindrance of marine activities, including fishing, impairing quality for use of sea-water and reduction of amenities and thus negatively compensates some of the benefits of industrialization. Therefore, there is a worldwide awareness to control coastal pollution. One of the basic requirements for controlling pollution is generation of data on levels of pollutants and their impacts over a period of time so that a clear picture on the increase and decrease of pollution in correspondence with measures taken can be obtained. Constant monitoring of coastal pollution is important to get continuous information on the source of pollutants as well as their routes of distribution and effects on biota. Uses of bioindicators are the cheaper way for early detection/monitoring of coastal pollution over other chemical and biological techniques. A bioindicator is an organism or biological response that reveals the presence of the pollutants by the occurrence of typical symptoms or measurable responses, and is therefore more qualitative. The suitability of a particular organism to be used as a bioindicator depends on many factors. Due to their high sensitivity to even subtle change in their ambient environment, foraminifera (a marine protist) are increasingly used as bio-indicators of marine pollution especially of coastal pollution. The study of pollution effects on foraminifera and their possible use as pollution proxies is relatively new. Since the early works by Resig (1960) and Watkins (1961), over the last five decades, publications on the foraminifera as a tool for monitoring coastal pollution have increased exponentially. Numerous papers have focused on the impact of pollution from various sources such as municipal sewage, fertilizers, aquacultures, pulp/paper mills, hydrocarbons, heavy metals, fuel ash, chemical pollutants including pesticides, thermal, radioactive, dredging and stream discharge etc. Some authors have also dealt with impact of human activities that are not directly related pollution such as construction of dams, bridges, modification in water circulation etc. Several workers have pointed out that foraminifera provide one of the most sensitive and inexpensive markers available for indicating deterioration of coastal environment. On the other hand, it is also being realised that deconvoluting the impact of pollution from natural stress and finding out one to one relationship between pollutant and its impact is a difficult task. A considerable effort has been made to develop new methodologies for monitoring of marine pollution especially in the near shore shallow environment through foraminifera.The objective of this paper is to summarize the findings on the responses of foraminifera to pollution on the coastal environment and to examine whether they can be adequately used as bio-indicators. There is also a deliberation on the limitations of the foraminiferal tools used in coastal pollution studies and the possibilities of overcoming them.

FORAMINIFERAL ECOLOGY AND THEIR SUITABILITY FOR POLLUTION MONITORING

The foraminifera constitute the most diverse group of shelled microorganisms in modern seas (Murray, 1991). The estimated living species are about 10,000 (Vickerman, 1992). Of these, about 40 species are planktonic and the rest are benthic. Foraminifers have also an excellent fossil record since Cambrian Period (570 million years before present) and have been traditionally used for biostratigraphic correlation and paleoenvironmental reconstruction that have great significance in hydrocarbon exploration. Therefore, foraminifera have been a subject in geology and paleontology rather than in biology. Presently, with proven application of foraminifera in other fields such as pollution monitoring and climate change, a multidisciplinary approach to its study is emerging.

Foraminifera are unicellular and taxonomically designated as a Class in the Kingdom of Protista. Their soft tissues (protoplasm) are enclosed within and protected by outer hard covering known as test which may either be constructed using organically cemented detritus (agglutinating or arenaceous forms), or secreted using calcium carbonate (calcareous forms). These tests incorporate important physico-chemical properties of the ambient environment during the life. After death of the foraminifera, their tests are readily preserved as fossils in the sediment. The organism can consist of a single chamber or several chambers. Each successive chambers are connected by an opening (foramen) or many openings (foramina). The test surface is sculptures by a variety of ornamentation, some of which are species specific whereas some may be environment controlled. The identification and separation of foraminiferal species, genera and higher taxa is based on the aspect of test morphology, especially chamber arrangement and not on the living foraminifera. The size of foraminifera is generally less than 1 mm and commonly in the range of 0.1mm to 10mm. They occur in great abundance and hundreds of specimens can be found in 1cm^3 sediment.

Foraminifera are ubiquitous in marine environments (Todo et al, 2005). Their ecology embraces both planktonic and benthonic modes. Planktonic forms generally inhabit the open ocean and seldom live in coastal waters, while benthonic foraminifera exist on substrates from abyssal plains to high intertidal areas. Diversity of Foraminifera is highest in tropical waters and gradually declines towards poles (Brasier 1980). They are niche specific. The distribution of foraminifera is not random, but is controlled by environmental gradient. Planktonic forms' distribution is controlled by temperature, salinity and nutrient availability on the surface water. The factors which influence the distribution and abundance of benthic forms include bathymetry, sediment texture and physicochemical characteristics of sediments as well as water. Therefore, these organisms provide one of the best sources of proxy oceanic and climatic information.

Foraminifers exploit a great variety of environments, substrates and nutritional modes. Benthic foraminifera also differ in their mode of living. Most of them are epifauna living on the sediment (within first 1cm in the substrata) whereas some can live within the sediment in anoxic conditions (Bernhard and Sengupta, 1999). They are more abundant in silty and clayey beds than in sandy substrata due to larger pore space. Foraminifera have short reproductive cycle (six months to one year) (Boltovskoy, 1964) and rapid growth (Walton, 1964). They can reproduce both sexually and asexually which is manifested as dimorphs. As a group, foraminifera utilize a broad range of feeding mechanisms and nutritional resources including grazing, suspension feeding, deposit feeding, carnivory, parasitism, direct intake of dissolved organic carbon (DOC) and symbiotic relation with algae.

Over the last five decades many studies on benthic foraminiferal assemblages have been carried out from different parts of the world in areas exposed to different kinds of marine pollution. Through these studies, a considerable effort has been made to develop new methodologies for biological monitoring of marine pollution especially in the near shore shallow environment. Several workers have pointed out that foraminifera provide one of the most sensitive and inexpensive markers available for indicating deterioration of coastal environment. The advantage of application of foraminifera over other chemical and biological techniques, for pollution monitoring can be summarized on the following points:

1. Their tremendous taxonomic diversity gives them the potential for diverse biological responses to various pollutants. Different index species can be identified for pollution from diverse sources.
2. Due to relatively small size and great population density, statistically significant sample sizes can be collected quickly and relatively inexpensively for either assemblage assessment and for experimental studies, with minimal environmental impact.
3. Their short reproductive cycle and rapid growth makes their community structure responsive to quick environmental change.
4. Living population and surface sediment assemblages can be used to assess the current state of benthic ecosystem.
5. As the mineralised tests are readily preserved, fossil foraminifers can be studied from sediment cores to assess decadal, century and millennial scale changes in community structure at sites of interest, providing an historical record. This provides historical base line data even in the absence of background studies.
6. Some species can be readily maintained in culture, so laboratory protocols can be established to determine responses of selected taxa to pollutants of concern. Field transplant studies can also be designed for them.
7. They have biological defence mechanisms which protect them against unfavourable environmental factors, thus providing detectable biological evidence of the effects of pollution.

FORAMINIFERAL TOOLS IN POLLUTION STUDIES

Application of foraminifera in pollution monitoring is mainly based on attribution of peculiar foraminiferal features at any location to the circumstantial presence of pollutants at those sites. Pollution can affect on foraminifera in four ways: by modifying their community structure, morphology, test chemistry and cytology. Accordingly, different technique have developed to study these changes and to infer the underlying reasons for it. Our present understanding of the foraminiferal behaviour to pollution is discussed below.

Community structure

Foraminifera respond to pollution as well as to the environmental gradients (such as salinity) either by changes in the density and diversity of the assemblage or by changes in the assemblage composition. The community structure of the foraminifera can be known by studying the assemblage picked up from the surface sediment samples after wet sieving. Straining methods such as Rose Bengal technique (Walton, 1952) is used for differentiating living from the dead specimens. The quantitative analysis of benthic foraminifera is generally performed on the >63 μm fraction Various foraminiferal parameters such as species diversity (number of species per sample), foraminiferal density (number of specimens per 1g of dry sediment), Fisher index (relationship between the number of species and the number of individuals in an assemblage), and Shannon–Weaver's dominance index are used to study the assemblage (Vilela et al., 2004; Frontalini, 2009).

Pollution effects on foraminiferal assemblage have been assessed by comparing surface samples from known polluted and non-polluted locations. Multivariate statistical methods especially R-mode Factor analysis are commonly used to distinguish the effect of pollution from natural environmental variations. Quantitative comparison of faunal data in surface sediments with sufficient time gap can be used to study prolonged pollution in large areas (Scott et al., 2005). Surface sediments with a time gap of five to six years have been used to compare the degradation or recolonisation of the species (Alve 1995, Schafer et al 1991, Alve & Murray 1995, Frontalini, 2009). Comparison of the present foraminiferal assemblage with that of previously published accounts has also been attempted to determine the biotic change. A shift in dominance from long-lived, algal symbiont bearing taxa in 1960s to small, fast growing, heterotrophic taxa in 1992 has been reported from south Florida's coastal water and is attributed to the increasing nutrient flux (Cockey et al, 1996). In another study, the effect of urban and agricultural influence on a subtropical estuary in Biscayne Bay, Florida was studied through foraminiferal data analysis. The study reveals that, in the past 65 years, populations of symbiont-bearing taxa, which are indicators of normal-marine conditions, have decreased while stress-tolerant taxa, especially *Ammonia spp*., have increased in predominance (Carnahan et al., 2009). The benthic foraminiferl census surrounding Los Angels county sewage outfalls has been compared with a study 30 years before by Stott et al 1996. Foraminifera assemblage from sediment cores has also been studied and faunal shifts in the cores representing different time period in a single location has been inferred (Alve, 1991). Changes in foraminiferal assemblages were also associated with eutrophication, bottomwater hypoxia, and changes in red tide-causing algae. The impact of eutrophication on shallow marine benthic foraminifers over the last 150 years in Osaka Bay, Japan is clearly reflected in foraminiferal assemblages from the short sediment cores (Tsujimoto et al., 2006).

Many workers have studied the impact of various pollutants such as coal from thermal power station (Yanko, 1994), organic pollutants from marine aquaculture operations (Schafer et al, 1995), fish farming (Angel et al., 2000; Vidovic et al., 2009), shrimp ponds (Debenay et al., 2009), Hg, PAH and PCBs contamination (Leonardo et al., 2007; Bergamin et al., 2009), domestic sewage effluents (Teodoro et al., 2010). Jayaraju et al., (2008) have studied the response of benthic foraminifera to various pollution sources (industrial wastes, agricultural and aquacultural drainage water) from Nellore coast, India. Panchang et al., (2005) have studied the reduction in mining activities in the catchment area of the Zuari Estuary, Goa through foraminiferal study. The foraminiferal data and TSM (total suspended matter) data suggest an improvement in the environmental health of the estuary. Similarly, the impact of millennial mining activities on sediments and microfauna of the Tinto River estuary (SW Spain) was studied through sediment and microfauna (foraminifera and ostracods) data analysis (Ruiz et al., 2008). Many workers have used foraminifera as a bioindicator to study the impact oil pollution on coastal environments (Ernst et al., 2006; Jorissen et al., 2009). Sabien et al., (2009) have also monitored oil spill bioremediation using marsh foraminifera as indicators.

Most of the studies carried out in polluted environments have shown that a lowering in density and diversity can be viewed as a measure of environmental stress on benthic foraminiferal communities caused by pollution (e.g. Schafer, 1973; Yanko et al., 1998, Samir, 2000; Elberling et al., 2003; Vilela et al., 2004; Bergamin et al., 2005; Ferraro et al., 2006; Bergamin et al., 2009; Jayaraju et al., 2010; Debenay and Fernandez, 2009; Chatelet and Debenay 2010). Increased pollution has also been reported as being the cause of a high number of individuals belonging to a few opportunistic species (Murray, 1973; Pearson and Rosenberg, 1976; Ellison et al., 1986). In another study Frontalini and Coccioni (2008) have pointed out that, *A. parkinsoniana* and *A. tepida* can be reciprocally considered good bioindicators of heavy metal pollution as sensitive and opportunistic species respectively. Romano et al., (2009) have pointed out the pollution-tolerant character in some species like *Haynesina germanica* and *Quinqueloculina parvula* and their test deformation positively correlated with the concentration of PAHs, Mn and Zn. In some cases especially in organic pollutions, an initial increase of abundance of foraminifera has been detected and is attributed to increase the food availability. Pollution favours the increase in the proportion of agglutinated species (e.g., Watkins, 1961; Bandy et al., 1964; Schafer Cole, 1974; Alve, 1995). Higher proportions of agglutinated species have been reported from shrimp farming sites as well as with runoff from rice culture. Unfavourable acidic conditions may lead to a decline in living calcareous species or to post-mortem dissolution of calcareous tests during taphonomic processes (Murray & Alve, 1999). Foraminifera are more tolerant to pollution than other meio and macrofauna. Benthic foraminifera are more sensitive to industrial wastes containing heavy metals than agricultural waste (Samir 2000). *Buliminella elegantissima, Ammonia tepida, Bolivina lowmani* are commonly encountered in restricted environments under pollution stress. (Yanko et al, 1999, Alve, 1995, Debenay et al, 2000). Armynot (2011) identified four key species (*Haynesina germanica, Bolivina pseudoplicata, Elphidium excavatum* and *E. magellanicum* as potential bioindicators in the port ecosystem of Boulogne-sur-Mer (Northern France). Foster et al (2012) from a case study in the Mediterranean Bages-Sigean lagoon proposed that *Quinqueloculina bicostata* may be used as an indicator of heavy metal pollution.

Seasonal fluctuations in hydrolological profile in estuaries are reflected in foraminiferal distribution. The pollution impact also show a seasonal fluctuation being most pronounced in summer. Foraminiferal recolonization and attained seasonal stability has been reported after the cause of pollution is removed. Hallock (1996) developed a FORAM Index utilising foraminiferal assemblages from surface sediments of reef associated environment to monitor the coral health. Densities of living Amphistigina spp. on reef rubbles have the potential to be used as a simple, low cost indicator of reef vitality. Benthic foraminiferal assemblages are known to be sensitive to coastal nitrification; large symbiont bearing foraminifera lose dominance to small, fast growing herbivorous and detrivorous species when nutrient supply increases in tropical reef- associated environments (Hallock, 2000). Many workers have attempted to find the impact of specific pollutant such as effluent (LeFurgey and Jean, 1976), hydrogen sulphide (Moodley et al 1998), ri-n-butyltin (TBT) – an antifouling paint (Gustafsson et al, 2000) on experimental basis. Although the foraminiferal assemblage exhibited a high tolerance to short-term exposure (21d), prolonged (66d) exposure to sulphide conditions resulted in significant reduction of total foraminifera densities with time. Tolerance of sulphidic conditions was restricted to survival and not to reproduction.

Through the analysis of changes in abundance of marker species, the introduction of new species, serious loss of previously existing species, changes in species diversity, dominance and abundance outside the established limits of variability, it is possible to document the environmental change that have taken or are taking place. Living benthic foraminifera have potential as robust elements of assemblage models that can be used to monitor the temporal and spatial impact of pollution flux from various sources of on local benthic environments. However, the detailed understanding of the precise controls on distribution and on niche of each species (Alve 1995; Divrikli et al. 2003; Soylak et al. 2004) is lacking.

Test morphology and test abnormality

Several authors have concluded that the evaluation of deformation in the foraminiferal tests could be used as a bioindicator of heavy metal pollution (Alve, 1991; Yanko et al, 1998; Geslin et al, 1998; Ferraro et al., 2006; Leonardo et al., 2007; Cherchi et al., 2009; Martins et al., 2010). Measures of deformity rely on the kind, degree, frequency and species specificity of deformity. The frequency expressed as the percentage of the total is the easiest method to quantify. Morphological deformity is common in small numbers within the range of natural variability of a given species in given environmental conditions. However several species display an increase in the proportion of deformed foraminifera in living assemblages that can be caused by low salinity or by increase in concentration of heavy metals within the sediments. Above-background percentages of deformed tests, and a relatively high number of species exhibiting deformities, are common features of foraminifera populations that inhabit contaminated marine environments (e.g., Boltovskoy et al. 1991). Seiglie (1975) concluded that test abnormalities appeared to be of greater significance than the species composition of indigenous assemblages in establishing differences among the closely similar contaminated environments. The power of the deformed test approach may be of particular value in those environments where species diversity is too low to permit the effective application of mapping and monitoring protocols that exploit the community structure parameter. Coccioni et al. (2005) introduced the Foraminiferal Abnormality Index (FAI) to index and compare the percentages of morphological abnormality occurring at different sites. Reddy et al**.** (1980) noticed seasonal variations in the size of foraminifera.

Different authors have identified different types of deformation and have tried to classify them. Alve (1991) distinguished seven modes of deformation while Yanko et al. (1998) described 11 distinct types of morphological deformities in foraminiferal tests. Jayaraju and Reddy (1996) considered magnitude of corrosive effect, lower than normal ornamentation, sutural thickening, pores enlargement and widening apertures in foraminifera as indices of pollution impact on the coastal zone of Tuticorin. Additional modes of test deformation include cavities, compressed tests, and the formation of a bulla like chamber covering the umbilicus. The various deformations have been classified according to the affected part of the test and to the nature of deformation. Polovodova and Schonfeld (2008) assessed 18 different types of abnormalities, which were classified into five groups: chamber, apertural, umbilical, coiling and test abnormalities. Test abnormalities may be species-specific For example a bulla-like chamber covering the umbilicus and spiroconvex occurrs only in *Ammonia beccarii*.

Few studies thus far have addressed the relationship between modes of deformities and environmental variables (Bhalla and Nigam 1986; Alve 1991). The mode of deformation depends upon the nature of the pollutant. Forms having corrosion, cavity development, broken peripheries and reduction in the overall growth are associated with high trace metal levels. Twinned and reduced chamber size forms, which represent the minimal response of benthic foraminifera to pollution, occur largely in sites subjected to agricultural and aquacultural runoff drainage water. Angular-asymmetrical morpho-groups get adversely affected by high turbulence associated with increased fresh water river discharge whereas, rounded-symmetrical forms respond in a reverse manner. Karlsen et al, (2000) studied the sediment core spanning last five centuries and found 10 to 20% deformed tests of *Ammonia spp* occur in all cores, suggesting unprecedented stressful benthic conditions in Chesapeake Bay. Heavy metal pollution has a more deleterious effect upon the foraminiferal test morphology than agricultural and aquacultural wastes.

Mode of test deformation depends on the degree of pollution and type of pollutants. Alve and Olsgard (1999) performed a colonisation experiment for 32 weeks period with Cu- contaminated sediments and found out that Cu contaminated sediments alone do not seem to promote development of deformed foraminiferal test beyond normal range. Contrastingly, Bregamin et al. (2005) reported that *Miliolinella subrotunda* could be a potential bioindicator for copper pollution, since the abundance of irregular specimens of this species could be related to copper concentrations. Geslin et al. (1998) studied the abnormal wall texture and aberrant test morphology using SEM. Crystalline disorganisation may be caused by a stress imposed to the crystalline frame work by introduction of alien trace elements and cavities in the wall probably result from a thickening of the organic matrix that can be caused either by change in physical and chemical conditions or by food shortage in the environment. The size and density of pores have been considered as indicators of dissolved oxygen concentration (Sen Gupta and Machain-Castillo, 1993). X-ray microanalysis by Samir and El-Din (2001) reveals that living deformed specimens contain higher levels of heavy metals (Pb, Zn, Cu, Cr, and Cd) than non-deformed ones. This strongly suggests that heavy metals are responsible for the abnormalities in foraminiferal tests. Controlled laboratory culture experiments coupled with studies of the biochemical and crystallographic mechanisms of the development of test deformities can help to resolve the morphological reaction of the foraminiferal tests to specific degrees and types of pollution.

Test chemistry

Foraminifer shells are composed of extremely pure calcite and trace elements such as Mg, Sr, Ba and Cd comprise about 1%. Geochemical analyses of the carbonate tests calcified by foraminifera have provided much of the foundation for reconstructions of past ocean and climate conditions, and for chemostratigraphy. The possibility of using them as a reliable tracer of environmental quality has also been explored by some workers (Rumolo et al., 2009). Heavy metals could penetrate the foraminiferal cell together with food; they can also be incorporated from seawater (Yanko et al., 1998). While building up their skeletons, benthic foraminifera selectively consume metallic ions present in the surrounding water and sediments and thus the chemistry of their bioskeletal materials helps in deciphering the heavy metal pollution in the habitat. Shell composition reflects both sea water composition and the physical and biological conditions present during precipitation. There are two general mechanisms for trace element incorporation in foraminiferal calcite: direct solid solution in which the trace element substitutes directly for Ca^{2+} in the calcite structure and trapping in which the trace elemnt occurs as a discrete phase or absorbed ion (Pingitore, 1986). The presence of nanoparticles, including Fe and S, Ba and S, and La, Ce, Nd and S in abnormal specimens of *A. tepida* is also observed along with trace elements in their tests (Frontalini, 2009).

Several studies have reported enhanced Mg/Ca ratios in abnormal foraminifera, especially in severely polluted areas (Sharifi et al., 1991; Yanko and Kronfeld, 1993, Yanko et al., 1999). Increases of Mg/Ca values can also be attributed to calcification of shells in warm waters (Lea, 1999). Other cations (e.g. Ba and Cd) can also be included in the crystal structure of the test (Lea and Boyle, 1989). The introduction of alien elements into the crystalline framework during calcification may produce a crystalline disorganization leading, ultimately, to test abnormalities. Sharifi et al. (1991) conducted a set of culturing experiments which revealed higher concentrations of trace elements (in particular Cu and Zn) in deformed specimens than in their non-deformed counterparts. Enhanced concentrations of Cd, Co, and Pb were found in abnormal specimens of *Ammonia* by Banerji (1992), who also observed that Cu, Zn and Cr are better absorbed in foraminiferal tests than Ni and Pb. The absorption of Pb by foraminifera is very limited, whereas, Cu, followed by Zn and Cr are more easily absorbed, regardless of their concentrations in the sediment (Samir and El-Din, 2001). The oceanic behaviour of Cd closely mimics that of phosphate in the water column and is the most direct nutrient analogy available. Many culture studies of foraminifera have also been conducted in controlled conditions to study the impact of specific pollutant. Culturing of live individuals the potential usefulness of trace elements can be verified. Nigam et al., (2009) carried out culturing experiments to understand the response of benthic foraminifer *Rosalina leei* to gradual as well as sudden addition of heavy metal mercury into the media. During this experiment, growth was found to be inversely proportional to the mercury concentration. In addition to this, irregularities were also observed in the rate of reproduction, number of juveniles produced, the survival rate of the juveniles and deformation of their tests. The environmental conditions in multi-element culture experiments are more similar to natural growing conditions than in commonly used single element experiments. Munsel et al., (2010) conducted a multi-element culture experiment for heavy metal uptake in foraminiferal calcite by using *Ammonia tepida* as test species and observed a drop of the Ni and Cu incorporation into foraminiferal calcite which implies quenching effects that have to be considered, if results from single element experiments are transferred to the natural environment.

Stable isotope proxies (e.g., $\delta^{18}O$, $\delta^{13}C$) can also be employed along with other trace element parameters in pollution studies. Oxygen isotopic data of foraminifera spp. can indicate the salinity change (Thomas et al, 2000). Carbon isotope data provide the supply of organic matter as nutrient input by waste water plants causes algal bloom and episodes of anoxia/hypoxia.

Cytology

As with other organisms, foraminifera have a number of defence mechanisms that can protect them against xenobiotics (foreign chemicals in their environment). The health of foraminiferal species can be objectively characterized by biological parameters (e.g. biophysical, morphophysiological, histopathological, cytogenetic, physiological and biochemical bioindicators). Changes in these parameters can be used as early response indicators of exposure to environmental pollutants. The state of the defence system against xenobiotics in benthic foraminifera can serve as a very sensitive biomarker for monitoring and prediction of ecological consequences of anthropogenic pollution. The non-destructive cytophysiological and cytochemical examination of living foraminifera using biophysical methods, fluorescent probes, markers, fluorogenic substrates and fluorescent microscopy makes it possible to visualise and study metabolic reactions, enzyme activity, transport processes. The cytophysiology, biochemistry, molecular biology and chemical ecology of foraminifera are studied little and poorly understood due to more attention has been paid to the foraminiferal shells than the living specimens. Bresler and Yanko (1995) used different vital cytophysiological and cytochemical methods and recognised that the presence of unidentified natural organic compounds (UNOC) derived from seaweeds decreased acute toxicity of heavy metal ions for foraminifera. MDRtr/MXRtR (Multidrug/ or mutixenobiotic resistance transporters and System of Active Transport of Organic Anionic xenobiotics (SATOA). Comparing the activity of these export pumps in foraminiferal population living in polluted and non-polluted sites can be interesting. SATOA eliminates many xenobiotics from the cytoplasm congruently. Therefore, the quantitative study of SATOA in foraminifera can be used for ecological monitoring.

Cellular ultra-structure of deformed specimens after their exposure to sub-lethal contaminations shows following cytological modifications in the two species, *A. beccarii* and *A. tepida*: (1) thickening of the organic lining (2) increasing number of fibrillar vesicles; (3) increasing number and volume of lipidic vesicles (4) disruption of the plasma membrane (5) increasing number of residual bodies and (6) sulfur enrichment of the cell. These cytological modifications seem to be related to defence mechanisms, notably the thickening of the organic lining and the sulfur enrichment detected by microprobe analysis. The detection of sulfur in deformed specimens suggests that foraminifers may have a detoxification\ mechanism with production of a metallothionein-like protein. Thus, foraminifers seem to develop defence mechanisms. Saraswat et al. (2004) cultured juvenile specimens of *Rosalina leei* that had been exposed to different Hg concentrations and documented the adverse effect of this element on both the normal functioning of the foraminifers' cytoplasm and on the addition of abnormal chambers. In another study, the effect of graded concentrations of copper was analyzed at morphological and cytological levels on two species of Ammonia (foraminifera) often found in polluted areas through a culturing experiment (Cadre and Debenay, 2006). Increasing concentrations lead to (1) increasing delay in production of new chambers, explaining dwarfism in polluted areas; (2) increasing delay in reproduction and decreasing number of juveniles, explaining low density; and (3) increasing proportion of deformed tests. Cytological modifications occurred only in deformed specimens. They may be responsible for anomalies in biomineralization processes.

LIMITATIONS AND FUTURE CHALLENGES

The literature review amply demonstrates that the proxy indicator value of benthic foraminifera is a powerful tool in environmental assessment. However, the heterogeneity of nearshore marine settings calls for a precautionary approach that recognizes our limited knowledge of the plethora of diagenetic effects, and of biological interactions, at both the species and community levels. Some of the important limitations in foraminiferal studies are discussed.

Faunal assemblages rather than individual species of foraminifera are diagnostic environmental indicators as many species range over several faunal zones. Foraminiferal province should involve determination of ecological pattern of family distribution, similarities of test structure, rations between family groups and general trends of populations. (Lidz & Rose, 1989). Natural and seasonal variability in foraminiferal assemblage , microhabitats, covary. The variability of the standing crop during a year and one year to another is an important topic. There are few studies in which foraminiferal samples have been collected from the same locality monthly for a period of one or more years. The foraminiferal assemblage is also affected by taphonomic and digenetic processes such as bioturbation and dissolution. Because the generation time of foraminifera are inherently much shorter than the net rate of sediment accumulation and burial, fossils of different ages mixed into a single assemblage called time averaging. Post mortem transportation of tests has been demonstrated to vary with respect to such factors as test size, shape and density. Taphonomic effects can limit the resolution and reliability of nearshore foraminiferal proxy data in both space and time domains. These potential effects should be continuously assessed to determine when additional chemical or physical measurements will be needed to fully understand the system under study. Only living specimens should be considered for pollution study. Species and pollutant specific responses interfere with other physical, chemical properties as well as the substrata. For example, organic matter accumulates especially in the belt of active clay sedimentation. (Van der Zawaan and Jorissen, 1991) and presence of organic pollutants interferes with the heavy metal distribution. It is often difficult to separate effects caused by heavy metals from those caused by organic material because most polluted areas are subjected to some kind of organic enrichment (Alve 1995). Bioavailability, uptake rates, speciation, clay mineralogy, pH, complexation, and other factors control the behavior of heavy metals in marine systems, especially in estuaries. The high correlation between heavy metals and the silt and clay fraction makes it difficult to determine whether sediment characteristic or pollution have the stronger influence on foraminiferal assemblages. (Debenay et al, 2000).

Environmental stress may also be exerted by natural factors such as, hypersalinity, periodical acidification and strong hydrodynamics. Abnormal test shapes in benthic foraminifera are known to occur under natural conditions in all environments (Alve, 1991). High deformation rate may result from abnormal environmental conditions such as low pH (Le Cadre et al.,2003), hypersalinity (e.g., Debenay et al., 2001; Seiglie, 1964; Zaninetti, 1984) or high energy (Geslin et al., 2002) rather than pollution. The deformations occurring in non-polluted environments may also result from the regeneration of the cell and of the test after reproduction, when a small quantity of cytoplasm with a nucleus remains in the test (Stouff et al., 1999a,b). It is suggested that coincidence of salinity changes with a reproduction period might be harmful, leading to development of abnormal tests. Short-term changes in the salinity of seawater could result in morphological anomalies in benthic foraminifer tests (Debenay et al, 2001, Geslin et al., 2002; Nigam et al., 2006; Meric¸ et al., 2009). Thus, high ratios of abnormal to normal tests can also be observed in environments protected from human impact. Abnormal tests as an indicator of environmental pollution have to be used cautiously in areas with strong environmental instability. Increasing pollution leads to increasing delay in the construction of new chambers (growth) and reproduction. Increasing pollution also leads to increasing deformation of new chambers. Conversely, growth inhibition resulting from pollution may lead to the absence of new chambers and therefore absence of deformation.

Conclusions

The distribution pattern of foraminifera species demonstrates that they can be very sensitive and inexpensive biomarkers capable of indicating of deterioration of shallow marine environment. However, foraminiferal responses are often complex and nonlinear. Biotic patterns often co-vary with natural and anthropogenic environmental gradients that are difficult to distinguish. Deeper understanding of biological and ecological processes is needed, to achieve the full potential of foraminifera as environmental indicators. It is still a speculative subject and future investigations including both field studies and culture experiments under controlled conditions may provide a new insight into the coastal pollution. There is a need to understand the forminiferal ecological dynamics in polluted environment and to characterize the specific foraminiferal features to the specific pollutants. Culture of foraminifera in specific polluted conditions supplemented with their crystallographic and molecular studies will be helpful in this regard.

Applications of foraminifers as bioindicators require strong scientific models based on both field and laboratory experiments and which specifically examine the influence of toxic elements and other pollutants at community, assemblage, population, individual, and gene-expression levels. The use of foraminifera (or any other bioindicators) for environmental monitoring needs a careful selection of reference stations. To assess the impact of different types of pollutants on the biota, a comparison of different areas would be useful if they have the same faunal assemblage and comparable hydrographic and physical characteristics, but are exposed to different types of pollution. Monographs/atlas of foraminiferal species should be prepared for different coastal regions to study the diversity. There is also a need to develop standard and convenient techniques for sampling, straining, counting and selecting foramineferal specimens for study.

Thus, many major challenges exist to fully realize the potential applications of foraminifers in coastal pollution. Nevertheless, their global importance in the past and present argues strongly for further development of these promising tools ideally as a component of comprehensive monitoring programs.

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