Marine pollution detection through biomarkers in marine bivalves

X. N. Verlecar, N. Pereira, S. R. Desai, K. B. Jena and Snigdha*

Indian coastal waters are subjected to considerable pressure from sewage and industrial wastes, which are responsible for the contamination of the waters with consequent loss in biodiversity. Monitoring methods adopted so far have helped in documenting the level of contaminants in water, sediments and biota, but not the interaction of these pollutants with living organisms. Exposure of animals to xenobiotics brings disturbances in metabolic function, activating detoxifying enzymes and the antioxidant system, as also damage to genetic material. This could be used as potential biomarker to measure pollution stress in animals. While extensive work on biomarker research is being undertaken in several parts of the world, such studies are yet to receive sufficient attention in India. Marine bivalves such as clams, Mytelid mussel and oysters, which represent the entire Indian coast and are sentinel species, are considered to be the best organisms for such studies. This article stresses the importance of biomarker research and recommends incorporating the same in Indian coastal monitoring programmes.

Keywords: Biomarkers, coastal waters, pollution, xenobiotics.

WASTE management strategies adopted in India have failed to keep pace with the industrial growth and urbanization. This has resulted in the accumulation of contaminants with a consequent loss in coastal marine biodiversity, for the past few decades. Estimates indicate that Mumbai city itself discharges around 2200 MLD of waste to the coastal waters[1]. Similar is the case with some of the major cities such as Chennai, Kolkata and Visakhapatnam and the industrial areas of Gujarat, Pondicherry and Orissa, where the coastal and estuarine waters remain in degraded condition.

The responses of estuarine and marine organisms to waste input are manifold, but can mainly be classified on four levels of biological organization, i.e. cellular, organismal, population and community basis (Table 1). The earliest detectable changes inside the cell, in response to toxic environmental chemicals (xenobiotics), involve subcellular organelles such as lysosomes, endoplasmic reticulum and mitochondria. Significant impact to pollution exposure at cellular level are the specific biochemical responses, such as production of cytochrome P450-mediated system of mixed function oxygenation of organic compounds, antioxidants and metal-binding proteins, as also genetic changes.

Pollution along the Indian coast

Many pollution impacts on marine communities can be traced directly to the industrialized centres, which release an array of chemical contaminants to effluent systems. Others are more difficult to delineate because they are largely derived from contaminants supplied by diffused source such as run-off from land and atmospheric fallout. Of even greater concern have been the adverse environmental effects associated with waste-disposal activities, particularly sewage sludge and dredged spoil dumping, oil spills and leakages as well as municipal and industrial waste-water discharges. These wastes often contain a wide range of contaminants such as petroleum hydrocarbons, chlorinated hydrocarbons and heavy metals. Some of the previous studies in the Arabian Sea have shown that the petroleum hydrocarbons ranged from 1.8 to 11.1 μg/l in water, 1.84 to 5.81 μg/g dry wt in sediments and 0.33 to 3.67 μg/g wet wt in fish[2], while the total DDT in zooplankton samples in the Arabian Sea[3] varied from 0.083 to 0.563 μg/l. In the coastal waters off Mumbai, which receive large amounts of domestic and industrial waste, the Hg content fluctuated between 0.12 to 1.4 μg/l, in sediments from 0.08 to 0.36 μg/g dry wt and in fish muscle at Thana Creek from 0.217 to 0.512 μg/g wet wt[4]. Also, off Karwar (Karnataka) Hg was recorded as high as 2.68 μg/l in water and 1.32 μg/g dry wt in sediment[5].

Eutrophication of estuarine waters often culminates into anaerobiosis, toxic algal blooms, mass kills of benthic and epibenthic organisms and change in the abundance and diversity patterns of fish. But the extent to which contaminants enter the biotic components and interact with communities in terms of its physiological and biochemical concerns is a problem under investigation.

The authors are in the National Institute of Oceanography, Dona Paula, Goa 403 004, India. *For correspondence. (e-mail: verlecar@nio.org)

Table 1. Responses of marine organisms to contaminants at different levels of biological organization

Cellular response	Organismal response	Population level	Community level
Detoxification enzymes	Selected histopathologies	Changes in diversity index	Community level parameters
DNA damage	Immune system indicators	Pollution-level parameters	Food-web alterations
Antioxidant enzymes	Condition indices		Trophic-level relationships
Selected blood chemistries	Growth		
Stress protein	Reproductive parameters		

Reactive intermediates and detoxification

A relatively new concept in aquatic environmental studies that emerged recently is the analysis of changes in various physiological and biochemical parameters in resident biota. Use of the so-called 'biomarker' has been adopted from idemiology' or 'molecular toxicology' by free-radical biologists to describe changes in biological molecules out of attack by free radicals like oxygen, nitrogen or halide species, in dealing with aquatic toxicology[6]. In this context, the cytochrome P450-linked mixed function oxygenase (MFO) enzyme system has been extensively studied[7].

The metabolism of xenobiotics in animals involves oxidation by various monooxygenase reactions, including epoxidation, hydroxylation and dealkylation. These are catalysed by a number of enzyme systems such as antioxidants, cytochrome-P450, monooxygenase or MFO, and metallothionine proteins (Figure 1). The resulting products may then be converted to dihydrodioles and/or conjugated with glutathione or glucoronic acid by epoxidase hydratases, glutathione-S-transferase and UDP glucoronyl transferases respectively. Further monooxygenation of some products, conjugation with other chemicals such as sulphate and metabolism of conjugates (e.g. conversion of glutathione conjugates) to mercaptonic acid is also possible[8]. The MFO system and associated enzymes must therefore be viewed as part of the detoxication/toxication system, the value of which rests on the balance of enzymes present and chemistry of metabolites produced. The important feature of the system is that the activities of the enzymes and the concentration of cytochrome P450 may be increased by the exposure of animals to xenobiotics.

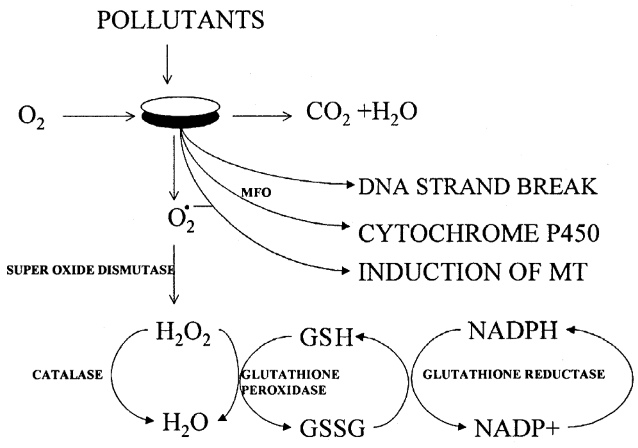

Figure 1. Flow chart of the enzyme assay.

Induction of sulphydryl-rich, MT-like proteins in organisms is often presumed to occur as a result of exposure to heavy metals. Consequently, such proteins are regarded as potential, specific indicators of metal pollution. Fish MT was found to be induced by a variety of heavy metals, including Cd, Zn, Cu, Pb and Hg[9] and was therefore suggested to be used as an essential early warning signal for their presence in the aquatic environment. MT induction is determined by chemical and immunochemical methods, whereas MT-mRNA induction is evaluated by molecular probes[9].

Some of the antioxidants mentioned above are multifunctional enzymes involved in cellular detoxication and excretion of many physiological and xenobiotic substances. Moreover, cells may respond to xenobiotic exposure by induction of particular isoenzymes. They have been mainly studied in mammals. But studies have also reported their presence in invertebrates, yeast and plants. Since cytochrome P450, MFO and antioxidants can be induced or inhibited by certain xenobiotics, they have been proposed as potential biomarkers for several aquatic species such as fishes, crustaceans and molluscs.

DNA strand breaks, adducts and micronuclei

The major techniques used to determine damage in genetic material include the identification of DNA strand breaks, adducts and micronuclei. Genotoxicants have the ability to alter DNA and their effects may be particularly harmful as these agents can induce changes that may be passed onto future generations and have an impact on populations long after the original exposure. Environmental contaminants have been reported to induce DNA strand breaks in various mussel cells which can damage their functions[10]. Ching *et al.*[11] reported marked increase in strand breaks after one day of exposure of *Perna viridis* to benzo[*a*]pyrene concentrations between 0.3 and 3 $\mu g\ l^{-1}$. A significant increase in DNA adduct formation has also been reported by the same author, on PAH exposure of these mussels for a six-day period, but the presence of adducts decreased after 24 days of exposure, indicating that this biomarker may have limited use as a quantitative marker of chronic exposures.

In some other investigations DNA strand breaks have been observed using an alkaline comet assay and the proportion of micronucleus formation in haemocytes of *P. viridis* exposed to benzo[*a*]pyrene, over a 12-day period[12]. Overall, the results on genetic changes induced by xenobiotics in *P. viridis*, suggest that, although DNA strand breaks, DNA adducts and micronucleus formation could serve as potential biomarkers of pollution, validation of these parameters would be needed on a site-specific basis, before they could be induced as effective monitoring tools in the field.

Progress of biomarker research in developed countries

The earliest field studies[13] on the use of MFO enzymes as biological markers were carried out in the early 1970s. Its usefulness as biomarker has now been confirmed in many field studies worldwide[14,15]. Some of the studies cited in the literature were carried out in association with a variety of contamination sources which included urban run-off and industrial outfalls, pulp mills, petroleum development sites and agricultural spray programmes.

Changes in cellular processes in some sentinel species now find increasing use as biomarkers of pollution index at sub-lethal level in the environment[7]. Sea star, *Asterias rubens* was used by den Besten *et al.*[16] as a test species to measure cytochrome P-450, metallothionins and other biomolecules as indicator of pollution stress in North Sea waters. Livingstone[17] used microsomal contents of digestive glands of Mussel, *Mytilus edulis* and periwinkle, *Littorina littoria* to determine cytochrome P-450 and NADPH-cytochrome *c* reductase activity as a marker of oil and metal pollution in Langesundfjord, Norway. Similarly, Porte *et al.*[7] used *Mytilus galloprovincialis* in the Venice Lake, Italy and Galician coast in NW Spain, to study the relation of antioxidant defence system enzymes as a marker of chemical pollution. Also, many other researchers have stressed that the cytochrome P-450 and its conjugates, metallothionine proteins and antioxidant defence molecules could be possible indicators of stress in several other marine and freshwater species[18]. However, considerable amount of variation in the levels of the bio-indicator molecules could be noticed, which largely depends on the amount of stress, test species chosen and sampling season.

Need for biomarker studies in India

The presence of heavy metals (Cd, Pb and Hg) and petroleum residues in coastal waters of India is well documented. As regards the pesticides distribution, reports cover only isolated locations in sediments and biota[2,19]. Nevertheless, the existing data suggest that the Indian coast is vulnerable to pollution due to anthropogenic activities and therefore marine biodiversity may be at risk. As regards the biomarker enzyme studies, few reports are available in some confined environments such as freshwater and estuarine regions on the presence of the activity of some of the antioxidant enzymes in freshwater fish, bivalves and prawns[20].

However, there is apparently lack of information on bioaccumulation of contaminants and its correlation to antioxidant response in animal tissues along the Indian coast. Hence utmost need is felt to examine the concentration of various organic and inorganic contaminants and its response to different biomolecules such as MFO-mediated cytochrome P450, antioxidants, metallothionines as also genetic changes in sedentary organisms such as bivalves and oysters, and fishes. These studies should be conducted on organisms collected from certain identified hotspots along the east and west coast of India on seasonal basis. This will help to obtain information on the extent of bioaccumulation of pollutants and their response to biological indices as markers of contaminant-induced stress. Non-enzymatic antioxidants should also be measured to understand their role against oxidative stress and in inhibiting the effects of contaminants. The relation between the contaminants and antioxidant tissue response should further be confirmed using toxicity bioassay tests under running sea water laboratory conditions.

By the application of biomarker measurements the use of expensive and complex analytical chemical equipment and expertise can be reduced, as these analyses are relatively quick to perform. Furthermore, since biomarkers are a part of the detoxification mechanism, these enzymes are secreted at sublethal levels of toxic components. This provides not only early warning about degradation in environmental quality, but also specific measures of the toxic, carcinogenic and mutagenic compounds in the biological materials. While continuous improvements in the study of biomarker response in organisms are being carried out in most of the developed countries in the West, it is imperative that we should make an early beginning in this line of research.

International network programmes

The results of several worldwide studies carried out so far, have indicated that the enzymatic, non-enzymatic and genetic biomarkers are a powerful and cost-effective tool to obtain information on the state of the environment and the effect of pollution on living biological resources. Hence, many of these have been adopted in national and international monitoring programmes in Europe and USA[21,22]. Likewise, different methods for measurement of biological effects have been evaluated in a series of practical workshops organized by the International Council for the Exploration of the Sea and Intergovernmental Oceanographic Commission such as those in the North Sea[23]. The United Nations Environment Programme has funded a biomonitoring programme in the Mediterranian Sea, including a variety of biomarkers[24]. Recently, biomarkers have also been included in the joint monitoring programme of the OSPAR Convention where Portugal, Spain and other European countries are the members. BEEP (Biological Effects of Environmental Pollution in Marine Coastal Ecosystems) project, which was a three-year EU-funded project in 2002–05, mainly focused on development of new biomarkers. It involved 30 institutions from 12 countries in Europe and Scandinavia including the North Atlantic region. This brought about extensive suite of biomarkers, both traditional and novel measured in the same biological material.

Generally, biomarkers such as cytochrome P450, GST, MT, oxidative stress, etc. have been identified in environmental pollution dealing with oil and petroleum hydrocarbons, heavy metals, pesticides and organochlorine contaminants[7]. A suite of biomarkers in combination with the selected chemical analysis, can then be used to provide an integration of detoxification mechanism and bioavailability of contaminants, thus forming the basis for a more holistic interpretation of environmental health. The basis of the biomarker responses is the utilization of known biochemical and physiological events, which precede the visible organismal and population changes, as sub-lethal indicators of the first stages of toxicity and pathology. They are therefore able to act as the biological early warning system of adverse effects in line with the 'precautionary principle' adopted by the international policy makers[25].

Application of biomarker research for monitoring the Indian coast

National-level monitoring programmes initiated by the Government of India, such as COMAPS (Coastal Ocean Monitoring and Prediction System) and ICMAM (Integrated Coastal Mapping and Management) served only to document the concentration of chemical constituents in water and sediments, and the changes in biological communities in our coastal waters over the years. However, efforts to establish the relationship between pollutants and cellular damage in the resident species in the form of biomarkers are limited.

India is neither involved in any of the international collaboration programmes dealing with biomarker research nor does it have such a programme at the national level. Taking into consideration the mounting pollution pressure along our coast, the National Institute of Oceanography, Goa initiated work on biomarker research using its own research funds and has obtained encouraging results.

In order to use the biomarkers in pollution-monitoring programmes, there is a need to develop a database using various bivalve species along the Indian coast. Perhaps this shall be the first such attempt in India, to understand the molecular mechanisms by which marine organisms protect themselves from harmful effects of pollutant stress. The data generated could serve as an early warning to the serious environmental changes in biodiversity that may be occurring as a result of marine pollution along our coast. Bivalves such as green mussels, clams and oysters being sentinel in nature and having their presence all over the Indian coast are better accumulators of contaminants from the surrounding waters and these bivalves could serve as the most suitable organisms for biomarker studies.

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ACKNOWLEDGEMENTS. We thank the Director, NIO, Goa for encouragement.

Received 8 August 2005; revised accepted 5 July 2006