

Measurement of DNA strand breaks as a biomarker of Genotoxic Pollutants

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Abstract

This paper deals with the development of a molecular biomarker technique by measurement of DNA integrity in marine organisms for biomonitoring of pollution due to genotoxic compounds. The marine environment is continuously being polluted by different genotoxic contaminants. Effect of these genotoxic contaminants is predicted with the help of biomarkers, which acts as early warning signals for marine pollution. These contaminants have shown adverse effects on the genetic material of various marine organisms. Various genotoxic compounds like polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls(PCB) etc interact with DNA molecule to form DNA adduct leading to DNA strand breaks. Hence DNA integrity can be used as a potent biomarker to measure pollution stress in marine organisms. In this paper we have studied the DNA integrity of marine gastropod, Nerita chameleon along the Goa coast. The DNA integrities in marine organism at different locations were compared with those at the reference site (Betul). The measurement of level of DNA integrity in marine organisms along the Goa coast exposed to enhanced levels of various genotoxic contaminants clearly indicated the extent and nature of pollution at the sampling site along coastal environment.

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Introduction: -

The pollution of the marine environment by various types of contaminants is a matter of global concern because of accumulation of their residues into the tissues of marine organisms leading to serious damage in the ecosystem. The concentration of these contaminants increases at an alarming rate due to urbanization and industrial development. The bio magnification of these contaminants through the food chain may cause serious threat to human health consuming seafood.

Thus the marine pollution due to highly persistent organic and inorganic contaminants results into deleterious effects to living resources, hazards to human health, hindrance to marine activities including fishing, impairment of quality for use of seawater, and reduction of amenities.(GESAMP,1983). Marine pollution poses a range of threats including from land-based sources, oil spills, untreated sewage, heavy siltation, eutrophication (nutrient enrichment), invasive species, persistent organic pollutants (POP's), heavy metals from mine tailings and other

sources, acidification, radioactive substances, marine litter, overfishing and destruction of coastal and marine habitats .

In order to assess the impact of highly persistent pollutants such as polychlorinated biphenyls (PCB), polychlorinated dibenzodioxins (PCDD), polychlorinated dibenzofurans (PCDF), polynuclear aromatic hydrocarbons (PAH), tributyltin (TBT) and other toxic metals (which are transferred up the food chain (bio magnification), and they can reach levels of concern in fish species that are eaten by humans) on the marine ecosystem a suite of biomarkers are being extensively used worldwide.(Sarkar A et al.1992,1991,1991a,1991b,1989). There are various types of biomarkers; they are acetylcholine esterase, superoxide dismutase, glutathione s transferase, cytochrome p 450, catalase, lipid peroxidase. Among the various types of biomarkers, the following have received induction, DNA integrity, acetylcholinesterase activity (AChE) and metallothionein induction. These biomarkers are being used to evaluate exposure of various species of sentinel marine organisms (e.g. mussels, clams, oysters, snails, fishes, etc.) to and the effect of various contaminants (organic xenobiotics and metals) using different molecular approaches (Sarkar A *et al.*2006)

It is of great significance to analyze the effects of present coastal xenobiotics on a relevant and primordial part of an organism: its genetic material. 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, mussels and oysters, which represent the entire Indian coast and are

sentinel species, are considered to be the best organisms for such studies.

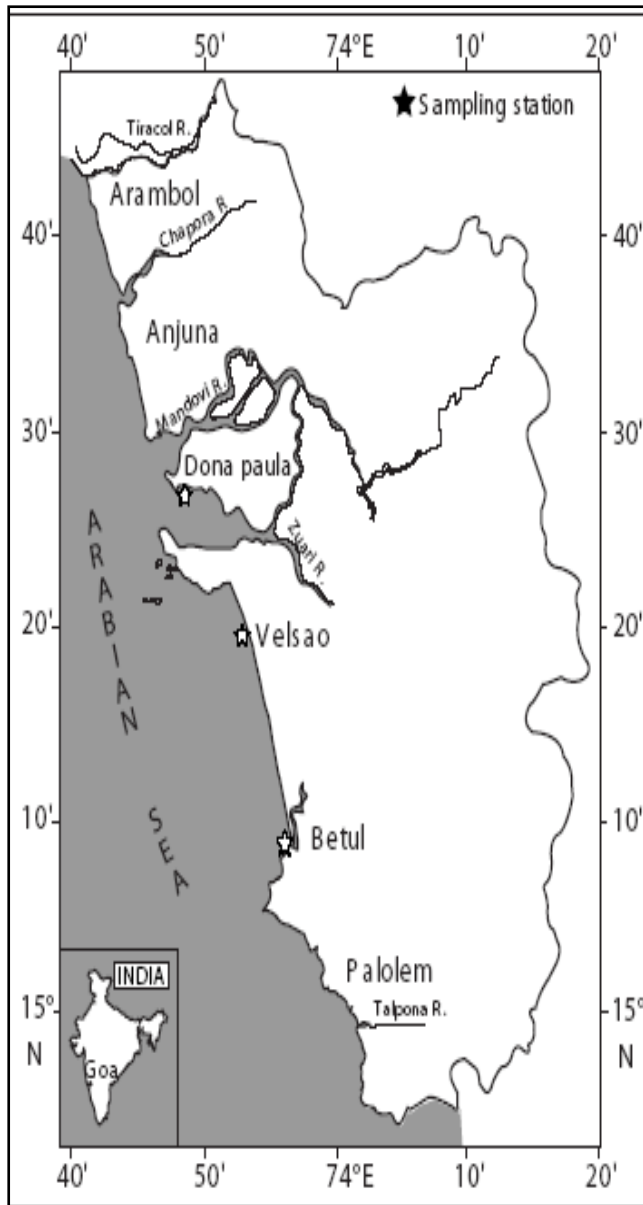
DNA integrity as potential biomarker.

DNA integrity as a biomarker of pollution The integrity of DNA can be greatly affected by genotoxic agents due to DNA strand breaks, loss of methylation and formation of DNA adducts (Pisoni et al. 2004). The level of double strandedness with respect to total DNA determined the effect of various genotoxic chemicals on the integrity of DNA. In fact, DNA strand breaks may occur due to direct DNA damage by an exogenous agent or may be produced during DNA repair process or other physiologic responses in the cell. Benzo(a)pyrene (BaP), a representative PAH is oxidized to chemically reactive diolepoxide (BaPDE), which subsequently interact with DNA to form both stable and unstable adducts with DNA (Pisoni et al. 2004; ERIC.W.K.CHING et al 2001). Each type of adduct may contribute towards the eventual transformation of the cell (Pisoni et al. 2004).

Materials and Methods:

In order to assess the integrity of DNA in different species of marine organism's biological samples will be collected from different sites along the Goa (Fig-1). The samples will be subjected for isolation of DNA from different species of the marine organisms. Each of the species will be identified first with the help of the literature available.

Fig-1. Locations of the sampling site



Isolation of DNA:

DNA will be isolated from the tissues of the marine organisms using CIP (chloroform:Isoamyl alcohol and phenol), triton-X-100 in ammonium hydroxide solution followed by purification with sephadex (G-50) column chromatography. The purified DNA will be preserved in the fridge for further analysis.

Measurement of DNA integrity

The DNA integrity of each of the species of the marine organisms will be determined by measuring the double strandedness, single strandedness and partially unwinded double strandedness of the DNA from each of the species from different sites. For this purpose, hoescht dye will be used for measurement of the amount of different fractions of the DNA using Dyna Quant DNA analyzer.(Sarkar A et al 2008).

The DNA integrity will be measured in terms of the fraction of double stranded DNA remaining after a specified time of incubation (30 to 90 mins) under defined condition of pH (neutral to alkaline condition) and temperature (33° to 85°C).

Integrity of DNA, $I = (X_{AUdna} - X_{SSdna}) / (X_{DSdna} - X_{SSdna})$ where DS - double stranded DNA, AU - alkaline unwinded DNA and SS - single stranded DNA. (ZHANG H.et al 2009)

Where X stands for observed fluorescence.

The data on the integrity of DNA will be compared with those from the reference site.

Results and Discussion:

For assessment of pollution along the Goa coastal region, we have selected three different sites along the Goa coast. From these sites, Betul was considered as the reference site as it is away from human population thus less contaminated, whereas two tourist sites Dona Paula and Velsao were considered as experimental sites. Sample organism, *Nerita chameleon* was collected from each site and was checked for DNA integrity. Interesting results were obtained with an 'I' value of 0.62 and 0.44

for Dona Paula and Velsao respectively as compared to the reference site Betul (I value: 0.83). Relatively low 'I' value for Dona Paula and Velsao clearly indicates that, these sites have major impact of genotoxic compounds as compared to Betul. Similar findings were obtained in earlier studies done by Sarkar A et al 2008 on *Cornia contacta* along the Goa coastal region. Thus DNA integrity was successfully developed as biomarker for assessment of marine pollution along the Goa coast.

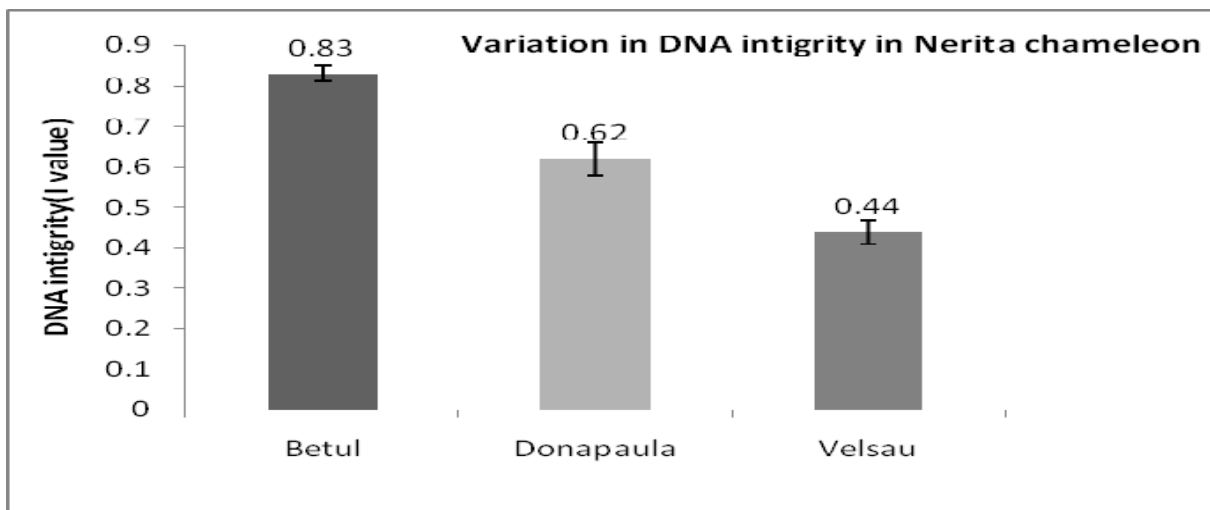


Fig: Variation of DNA integrity in *Nerita chameleon*

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