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Bioaccumulation of trace metals and total petroleum and genotoxicity responses in an edible fish population as indicators of marine pollution

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ABSTRACT

The present study reports the genetic damage and the concentrations of trace metals and total petroleum hydrocarbons prevailing in natural populations of an edible fish, *Arius arius* in different seasons along the coast of Goa, India as an indicator of the pollution status of coastal water. Fish were collected from a suspected polluted site and a reference site in the pre-monsoon, monsoon and post-monsoon seasons. Physico-chemical parameters as well as the concentrations of total petroleum hydrocarbons (TPH) and trace metals in the water and sediment as well as the tissues of fish collected from these sites were recorded. The genotoxicity status of the fish was assessed employing the micronucleus test and comet assay. A positive correlation ($p < 0.001$) was observed between the tail DNA and micronuclei in all the fish collected. Multiple regression analysis revealed that tissue and environmental pollutant concentrations and genotoxicity were positively associated and higher in the tissues of the fish collected from the polluted site. Pollution indicators and genotoxicity tests, combined with other physiological or biochemical parameters represent an essential integrated approach for efficient monitoring of aquatic ecosystems in Goa.

1. Introduction

Seafood is a major source of income for the coastal state of Goa and is the staple diet of the locals. The rivers of Goa contain large qualities of pollutants, such as trace metals and petroleum hydrocarbons that end up in the coastal waters of the state. Most of these pollutants are toxic to the marine fauna and other associated organisms, including man. They can adversely affect growth, development, reproduction and behaviour (Bistodeau et al., 2006; Ginebreda et al., 2014) and can induce clastogenesis, oncogenesis and teratogenesis (Gangar et al., 2010; Yang et al., 2010). Bioaccumulation and bioconcentration can also occur in fish and may have serious health implications for humans (Singh et al., 2014). Additionally, this could affect the normal food chain, resulting in a deleterious shift in the biotic community of this coastal ecosystem, besides a drastic reduction in the quantity, quality as well as the palatability of sea food.

The concentrations of trace metals in the Goan estuaries are primarily due to mining and its associated activities (Mesquita and Kaisary, 2007; Dessai and Nayak, 2008). Mining of iron and manganese is carried out extensively in various parts of Goa and ores are transported down the Mandovi and Zuari rivers. Metal effluents through ferro-manganese mining activities find their way into Goan estuaries and pose a threat to the organisms inhabiting these waters (Attri and Kerkar, 2011). Further, in the absence of proper disposal systems, byproducts of mining processes enter and contaminate the surrounding water bodies (Koski, 2012). The surface sediments of the Zuari estuary are polluted mainly by manganese, followed by cobalt and least with copper, zinc, chromium and iron (Dessai and Nayak, 2008). The Zuari is reported to be regularly polluted by the discharge of petroleum hydrocarbons as waste materials due to various shipping activities from the Mormugao harbour and the Zuari Agrochemical Industry (Sarkar et al., 2014). Tar balls, which are often seen along the Goan coast during the monsoons are also carcinogenic and can affect water quality and the marine fauna (Suneel et al., 2013).

Metal-induced genotoxicity is predominantly due to the inhibition of the DNA repair process (Hartwig and Schwerdtle, 2002). The micronucleus test (Cavaş and Ergene-Gozukara, 2005; Bolognesi and Hayashi, 2011) and comet assay (single cell gel electrophoresis) (Fernández-Tajes et al., 2011; Frenzilli and Lyons, 2013; Praveen Kumar et al., 2014) are easy and widely used tests for *in vivo* genotoxicity assessment in aquatic organisms. They are often employed for *in vivo* as well as *in vitro* genotoxicity studies in fish and bivalves. Many studies have found a positive correlation between the comet assay and the micronucleus test (Bresler et al., 1999; de Andrade et al., 2004; Russo et al., 2004). However, there are studies which report a negative correlation between these two tests (Klobucar et al., 2003;

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Wirzinger et al., 2007). The negative correlation may depend on the fish species chosen and / or on the nature of the assays.

Micronuclei result from acentric chromosome fragments or whole chromosomes lagging behind during metaphase/anaphase transition induced by clastogens or by spindle dysfunctions, respectively. The single-cell gel electrophoresis (SCGE) is a rapid and sensitive technique that detects DNA strand breaks, measuring the migration of nicked DNA fragments from immobilized individual cell nuclei. Both these tests are routinely used as biomarkers for monitoring aquatic pollution by genotoxic contaminants and can be combined with other physiological and biochemical biomarkers to fully assess the pollution status of various water bodies (Praveen Kumar et al., 2015; Bolognesi and Cirillo, 2014).

We hypothesized that pollutants such as trace metals and petroleum hydrocarbons induce genotoxicity in *Arius arius* and that DNA damage could be affected by seasonality. Thus, the primary goal of this study was to report the genotoxicity status of this native fish population at a probable polluted site (Vasco) and an unpolluted site (Palolem) and also to estimate the quantities of total petroleum hydrocarbons (TPHs) and trace metals such as Fe, Mn, Cu, Cd and Pb in the water and sediment as well as the tissues of the fish. The rationale for selecting these pollutants is that they are found in the waters as a result of mining from iron and manganese mine sites and effluents released from agrochemical industries and induce toxicity and biochemical changes in estuarine and marine fauna. We further assessed the relationship between DNA damage and pollutants from the tissues of fish at different sites during various sampling seasons.

2. Materials and methods

2.1. Study sites

Palolem and Vasco were selected as the unpolluted (reference) and polluted sites, respectively, along the coast of the Indian state of Goa (Fig. 1), based on the reports of Sarkar et al., (2008, 2010) and Sarker and Sarkar (2015). Palolem is a clean, pristine beach with no known industrial activity at this site. Vasco, an industrial hub, is dependent on the Mormugao harbour for most of its economic activities. This harbour serves as a port for carrier barges, ore barges, oil liners and cruise liners. Thus, the water and sediment around this area suffers considerable anthropogenic stress.

2.2. Sample collection

2.2.1. Water and sediment collection

Surface and bottom water (2 L each) were collected with the help of a Niskin Sampler. The temperature, pH and salinity of the water samples were immediately recorded with the help of an automated water and sediment analysis kit (Labtronics, India) consisting of probes for measuring the required parameter. Water samples for dissolved oxygen were taken in amber-coloured bottles (300 ml) and fixed with Winkler's solution. The samples for the analysis of phosphates, nitrates, trace metals and TPHs were stored in clean, labelled polypropylene bottles. Sediment samples (~ 500 g) were collected with the help of a Van-Veen Grab and stored in clean, labelled polythene bags. These samples were stored at 4 °C and carried to the laboratory for further analysis. Samples were collected from three sampling stations in triplicates at each site during the pre-monsoon, monsoon and post-monsoon periods from March 2012 to February 2014.

2.2.2. Fish collection

Arius arius (Thread-fin sea catfish) was chosen for the present study due to its bottom feeding niche, availability in Goan waters all year round and its consumption by a majority of people living along the coast. Both sexes of fish were collected with the help of a trawl net (mesh size 40 mm), which was mechanically operated by a trawler at the sites as described for water and sediment collection. The sample size of the fish collected from these sites and across the seasons is given in Table 1. Blood was immediately drawn from the caudal vein, smeared on clean glass slides and stored in slide boxes. Excess blood was stored in microfuge tubes containing phosphate buffered saline (pH 7.4) at 4 °C. The fish were then stored in clean labelled polythene bags and transported in ice bags to the laboratory for further analyses of the metal and TPH content in their tissues.

2.3. Analysis of samples

2.3.1. Physico-chemical parameters

The physico-chemical parameters temperature, pH, salinity and dissolved oxygen were analysed and recorded as mentioned earlier. Phosphates were analysed Nitrates were analysed spectrophotometrically using the Brucine method (EPA, 1971). Phosphates were estimated by adding ammonium molybdate and stannous chloride to the water sample and reading the intensity of a blue coloured complex at 690 nm (Bureau of Indian Standards, 1988).

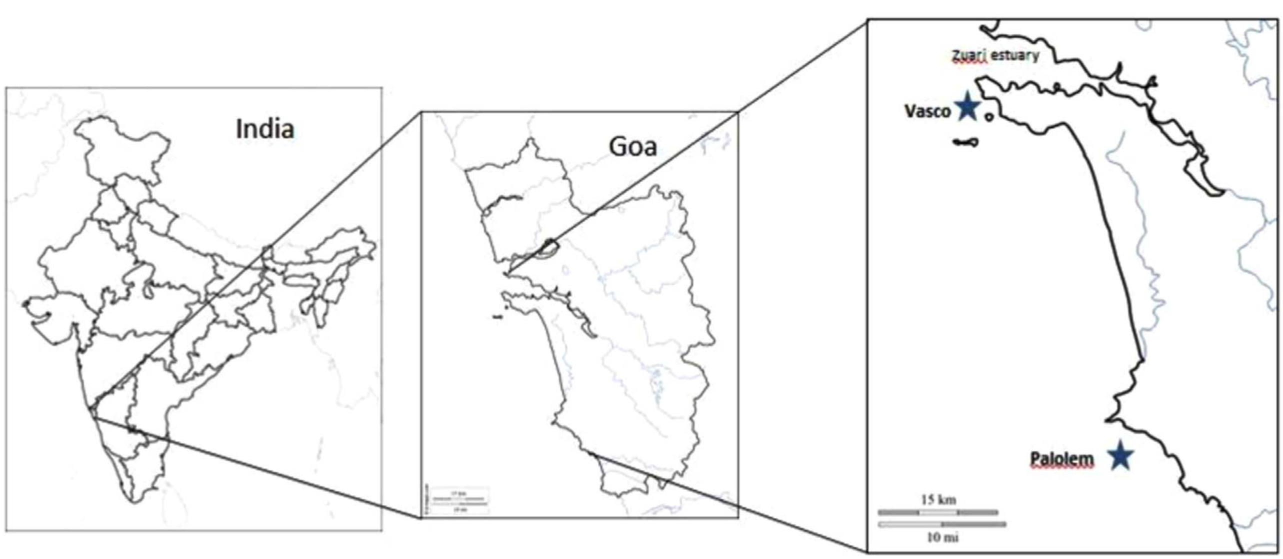

Fig. 1. Sampling sites at Vasco and Palolem along the coast of Goa, India.