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DNA damage in marine rock oyster (*Saccostrea Cucullata)* exposed to environmentally available PAHs and heavy metals along the Arabian Sea coast

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ABSTRACT

Molecular biomarkers are used world wide for quick assessment of the immediate effect of environmental pollution on marine ecosystems. Recently, we evaluated oxidative stress responses of marine rock oyster, *Saccostrea cucullata* impacted due to polycyclic aromatic hydrocarbons (PAHs) accumulated in their tissues at a few sampling sites along the coast of Goa around the region of the Arabian sea coast, India (Sarkar et al., 2017). Using a combination of partial alkaline unwinding and comet assays, we now report a comprehensive study on the impairment of DNA integrity (DI) in *S. cucullata* due to exposure to environmentally available PAHs and also heavy metals (Pb, Cd, Cu, Fe and Mn) along the Arabian Sea coast, Goa, India exclusively around the entire coast of Goa. First, we determined significant correlation between DI in *S. cucullata* and the extent of exposure to and bioaccumulation of different PAH compounds including 2–3 aromatic ring PAHs (R^2, 0.95), 4–6 aromatic ring PAHs (R^2, 0.85), oxygenated-PAHs (oxy-PAHs, R^2, 0.84) and total PAHs (t-PAHs, R^2, 0.98). Second, we observed dose-dependent decrease in DI in *S. cucullata* with increasing concentrations of different PAH components in oyster tissues. We substantiated our field observations with appropriate laboratory controls using benzo[*a*] pyrene (BaP). Third, we performed stepwise multiple regression analyses of different water quality parameters including pH, salinity, temperature, dissolved oxygen (DO), biochemical oxygen demand (BOD), nitrite (NO_2), nitrate (NO_3), phosphate (PO_4), turbidity and also t-PAH-biota, t-PAH-water with DI as the dependent variable. Among all these parameters, only four parameters such as t-PAH-biota in combination with DO, BOD and NO_2 showed significant correlation ($\overline{R}^2$ = 0.95) with loss in DI in *S. cucullata*. Based on these results, we created a map indicating the percentage of DNA damage in *S. cucullata* exposed to PAHs and heavy metals at each sampling location along the west coast of India around Goa, India.

1. Introduction

Environmentally available xenobiotics such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and heavy metals etc. are likely to cause severe hazards to the health and sustenance of marine ecosystems (Richardson et al., 2008; Mirza et al., 2012; Diamond et al., 2000; Sarkar et al., 2008, 1997; Sarkar and Everaarts, 1998; Sarkar, 1994). PAHs are the predominant constituents of oil contaminants and highly toxic in nature (Chen and White, 2004; Grasshoff et al., 1999a, 1999b; Lemiere et al., 2005; Pavlidou et al., 2014; Perez-Cadahia et al., 2004; Woo et al., 2006; Rekadwad and Khobragade, 2015; Roose and Brinkman, 2005; Shirani et al., 2012; Siddens et al., 2012; Wang et al., 2016). Anthropogenic activities are the primary causes of pollution of the aquatic ecosystem with PAHs which include urban runoff, shipping activities, use of smelters and fossil fuels, etc. Moreover, indiscriminate dumping of waste materials, incomplete combustion of fossil fuels, effluent discharges from various chemical industries and fortuitous spillage or leakage of oil and their by-products into adjoining land or aquatic system contribute PAH pollution of the marine ecosystem (Lan et al., 2015; Sarkar et al., 2008; Ingole et al., 2006). The marine organisms can easily take up the contaminants like PAHs from water, sediments and food into their body tissues. (Rocher et al., 2006)

The toxicological potency and adverse effects of persistent organic

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pollutants like PAHs on various species of marine organisms including (but not limited to) *vitellogenesis* in rainbow trout, *Oncorhynchus mykiss* (Navas and Segner, 2000), in sea urchins, *Paracentrotus lividus*, oyster, *Crassostrea gigas* (Mat et al., 2013; Qian et al., 2008; Geffard et al., 2001) and marine gastropod, *Nerita chaemeleon* (Bhagat et al., 2017) are well documented. In addition, PAHs like naphthalene, benzo[*a*]pyrene (BaP), etc. have been reported to disrupt physiologic functions in fish species including *Fundulus heteroclitus* (Bates et al., 1997). Bioaccumulation of sediment-associated PAHs in C. gigas caused abnormalities during larval growth (Geffard et al., 2001). PAHs tend to bioaccumulate in fatty tissues owing to their lipophilicity (Sarkar et al., 2008). Once within the body, PAHs induce the detoxification enzyme systems *viz.,* phase-I followed by phase-II enzyme activities which tend to form reactive oxygen species (ROS) as intermediates (Canty et al., 2009; Jha, 2004). The elevated levels of ROS in tissues has been shown to induce antioxidant enzyme activities in marine edible rock oyster, *Saccostrea cucullata* (Niyogi et al., 2001a, 2001b). Importantly, ROS intermediates have high affinity to guanine residues which forms covalent linkages with DNA strands that result into irreversible and stable DNA adducts with carcinogenic potential (Phillips et al., 2006). Thus enhanced level of different polycyclic organic contaminants and heavy metals in the marine ecosystem pose severe threat to aquatic organisms as well as to humans who are at risk due to consumption of such contaminated fish, oyster and other aquatic organisms (Yesudhason et al., 2013; Sarker et al., 2006; Chavan et al., 2016; D'Costa et al., 2017). Thus, biological markers are appropriate bio-monitoring tools for immediate measurement of the impact of such pollutants on the health of affected marine flora and fauna.

The assessment of environmental quality and the use of molecular biomarkers are one of the main focal points of genetic ecotoxicology research (Barranger et al., 2014; Mat et al., 2013; Qian et al., 2008; Shugart et al., 1992; Sarkar et al., 2006; Deasi et al., 2010; Sarkar, 2006a, 2006b; Sarker et al., 2016). These biomarkers are characterized by biochemical responses of organisms exposed to enhanced levels of contaminants or are specific biochemical assays which can provide clear indication of the prevalence of different types of toxic contaminants in the ecosystem. Measurement of DNA integrity is extensively used as a biomarker with respect to the effects of exposure to different types of genotoxic compounds such as POPs and heavy metals (Sarker et al., 2016; Sarkar et al., 2014, 2013; Sarkar et al., 2016; Shugart et al., 1992). In this regard, a wide-ranging studies on biomarker have been performed using various species of aquatic organisms, however, the data is lacking with respect to DNA damage in *S. cucullata* (Krishna Kumari and Nair, 1989). Interestingly, marine rock oysters like *S. cucullata* are widely consumed as delicious marine food by human communities living along the Indian coast (Arabian sea). Pollutants accumulated in oyster tissues pose a serious risk of being transmitted to humans. The impact of enhanced levels of detrimental pollutants on marine biota is thus of prime concern. The main focus of this study, is to assess the genotoxic responses of *S. cucullata* exposed to various environmentally available contaminants prevalent along the coast of Goa around the Arabian coastal region, India and their effect on DNA integrity (DI).

2. Materials and methods

2.1. Sampling

S. cucullata are primarily brackish water species inhabiting nearshore region, especially available on the rocky shore along the coastal belt. Among the various types of oysters available along the Arabian Sea coast, rock oyster, *S. cucullata* (shown in Fig. 1) is most popular with locals and tourists who refer to it by its local name, *Kalwa*. A large number of resident rock oysters, *S. cucullata* were collected thrice during the period (October to December 2011) from 10 selected sites (viz. Tirakol, Arambol, Anjuna, Sinquerim, Dona Paula, Bogmalo,

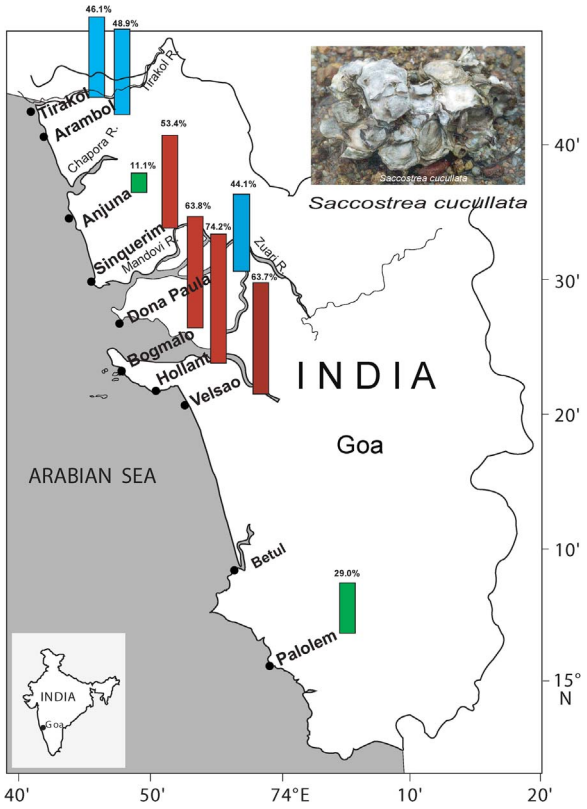

Fig. 1. Sampling locations along the Arabian Sea coast around Goa with the ecotoxicological significance. The bars at each sampling locations indicate the extent of DNA damage (% loss of DNA integrity) in *S. cucullata*. The picture of typical rock oyster, *Saccostrea cucullata* found along the Goa coast is embedded in the map.

Hollant, Velsao, Palolem and Betul) (Fig. 1) from Goa coastal region along the Arabian Sea. The rock oysters are of different types and forms. They appeared to be roughly oval shaped with uneven outline. The mature adult oysters were found to have grown up to 4–6 cm along the Arabian Sea coast. The valves of the rock oyster were found to be quite thick and mostly solid. The rock oysters were found to be purple-brown. The sampling site around Betul beach, located in southern Goa was considered as the reference site because of its immaculate environment and low anthropogenic activities, Dona Paula is a prime tourist spot and a focal point for recreational activities including water sports in the region. However, Dona Paula is situated opposite the Mormugao port and receives massive oil spills from a large numbers of ships and trawlers anchored around Mormugao port. Velsao beach is situated right atop the hill around Verna Industrial estate, Goa and receives industrial discharges from the surrounding agrochemical industries. This has resulted in Velsao being one of the most polluted beaches in Goa. Tiracol situated at the extreme North of Goa is a very small beach, surrounded by the Tiracol fort. It comprises mostly rocky shores having large numbers of oysters adhering to the rock surface. Thin films of oils were detected on the surface of the seawater around Tiracol beach during sampling. This was possibly be due to leakage of oil from the ferryboats sailing regularly across the Tiracol river. Arambol beach is a major attraction among visiting tourists. In addition, due to extensive tourism-related activities, the coastal waters along the Arambol beach appear to be greatly contaminated with various toxic substances. Overall, a large number of restaurants and hotels built along beaches which contribute significantly towards contamination of the Goan beaches. Besides, shipping activities often along the beaches might also contaminate the coastal water to a great extent. Anjuna beach, which is also a major tourist attraction is directly exposed to oil spills from various ships sailing along this route. Tourism-related activities is also a suspected cause of contamination of the site. Sinquerim beach is