

## Role of Marine Pollutants in Impairment of DNA Integrity

Subhodeep Sarker<sup>1\*</sup> and Sarkar A<sup>2,3</sup>

<sup>1</sup>Clinical Division of Fish Medicine, University of Veterinary Medicine, Veterinaerplatz, Vienna, Austria

<sup>2</sup>CSIR-National Institute of Oceanography, Dona Paula, Goa, India

<sup>3</sup>Global Enviro-Care, Caranzalem, Goa, India

\*Corresponding author: Sarker S, Clinical Division of Fish Medicine, University of Veterinary Medicine, Veterinaerplatz, Vienna, Austria, Tel: 00436604597069; +91-9422639034; Email: [ssarker007k@gmail.com](mailto:ssarker007k@gmail.com); [asarkar52@gmail.com](mailto:asarkar52@gmail.com)

Received date: Feb 23, 2015; Accepted date: Apr 25, 2015, Published date: Apr 30, 2015

Copyright: © 2015 Sarker S, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

### Abstract

In this article, we present an overview on the role of marine pollutants in impairment of DNA integrity in marine gastropods exposed to xenobiotics released from various sources into the coastal ecosystem. We provide an insight into the impact of various types of genotoxic compounds on the physiologic status of marine organisms, with specific focus on DNA integrity. DNA damage in marine gastropods was found to be highly correlated to the level of contaminants prevalent in the marine environment. DNA integrity in marine organisms was significantly affected upon exposure to genotoxicants such as polycyclic aromatic hydrocarbons (PAH), persistent organic pollutants, cadmium, lead, manganese, chromium, copper, etc. The prevalence of persistent xenobiotic contaminants in the marine environment coupled to their deleterious impact on marine organisms, exemplifies the measurement of DNA integrity as a prognostic tool for marine pollution assessment, control and policy-making.

**Keywords:** DNA impairment; DNA integrity; Biomarker; Marine gastropods; Genotoxic contaminants

### Abbreviations

au-DNA: Alkaline Unwinding DNA; B[a]P: Benzo[a]Pyrene; DDT: Dichloro Diphenyl Trichloro ethane; DSB: Double-Strand Breaks; ds-DNA: Double-stranded DNA; HCH: Hexachloro Cyclo Hexane; OC: Organochlorine; OCP: Organochlorine Pesticides; PAH: Polycyclic Aromatic Hydrocarbons; PCBs: Polychlorinated Biphenyls; PHAH: Planar Halogenated Aromatic Hydrocarbons; POPs: Persistent Organic Pollutants; ROS: Reactive Oxygen Species; ss-DNA: Single-stranded DNA

### Introduction

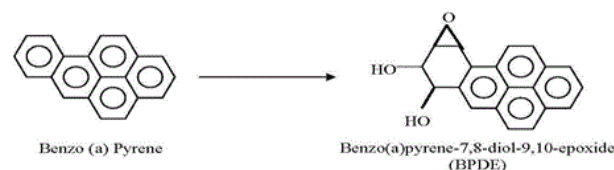
The DNA integrity in marine organisms is significantly affected owing to their exposure to genotoxic contaminants like heavy metals, polycyclic aromatic hydrocarbons (PAH) which are prevalent in the marine ecosystem. They inflict detrimental damage to the biologic integrity and physiologic functions of individual organisms. A part of the potentially toxic material is readily accumulated within the tissues of resident organisms from surrounding environment and thereby cause severe damage into their physiological status [1-8].

The detection of the presence of genotoxic contaminants in the marine environment can be ascertained in molecular terms by studying the responses of organisms exposed to such genotoxicants [9,10]. The damage caused to organisms thereof is reflected as impairment to physiologic and biologic processes fundamental to their survival and proliferation. The resulting genetic alteration in marine organisms due to their exposure to xenobiotics of various kinds can [9] be used as a tool to investigate the genotoxic potency of environmental pollutants. However, the induction of single-strand breaks by xenobiotic agents or genotoxicants is mediated for example,

by chemical induction during excision repair, interaction with DNA-intercalating agents, degradation due to autolysis or disruption, formation of alkali labile sites [9,11,12]. In particular, significant DNA damage has been observed by alkylating agents which can interact with DNA at multiple sites leading to single-strand breaks [7,13].

The concentration of xenobiotic contaminants in the tissues of marine organisms increases with their increasing bioavailability, lipophilicity/hydrophobicity and rate of their uptake from sediments, water column and other biota. The accumulation patterns of contaminants differ for different organisms and depend upon the balance between the rates of their uptake, metabolism and elimination from the animals [14].

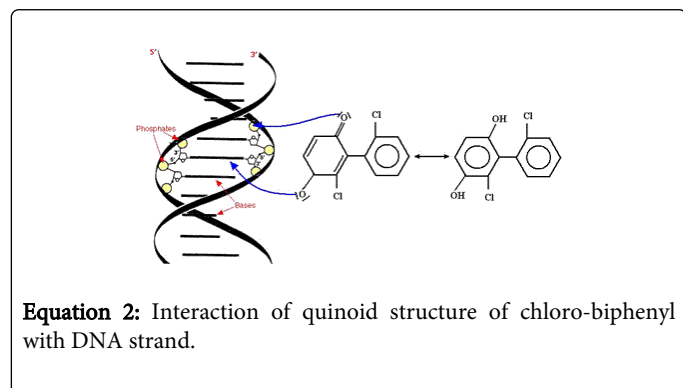
Many of these chemical pollutants are potent carcinogens and mutagens with the ability to inflict DNA damage. In this regard, polycyclic aromatic hydrocarbons compounds are an important class of chemicals prevalent in the environment [15]. The cellular conversion of benzo(a)pyrene, a representative PAH to chemically reactive Benzo(a)pyrene diol epoxide (BaPDE) and its subsequent interaction with DNA forming stable DNA adduct leading to DNA strand breaks, is a classic example of the impact of PAH on DNA integrity [14] (Equation 1).



**Equation 1:** Conversion of Benzo(a)pyrene to Benzo(a)pyrene-7,8-diol-9,10-epoxide.

Similarly, the bio-transformation of 3,3'-dichlorobiphenyls, a representative PCB into its metabolite, 2',5'-dihydroxy-3,3', dichlorobiphenyl, the chemically reactive quinoid form of it (reactive oxygen species) while interacting with DNA can form stable DNA adduct (scheme-2) which is responsible for DNA strand breaks.

Pfeiffer Petra (1998) showed the mutagenic potential of environmental contaminants on DNA double-strand breaks repair. Of all DNA lesions known so far, double-strand breaks (DSB) are the most fatal because they disrupt the continuity of the DNA template essential for DNA replication and transcription (Equation 2).



In order to assess the effect of genotoxic agents on the integrity of DNA, various techniques have been developed over the years to determine the level of DNA double strandedness under defined conditions of pH and temperature over a period of specified time. For example, the measurement of DNA integrity in the pyloric caeca of seastar, *Asterias rubens* from the North Sea demonstrated the impact of persistent organic pollutants in different regions of the North Sea [10]. In this article, we review the literature focusing on the significance of the study of the impairment of DNA from different species of marine organisms and its potential use as a valuable tool in marine pollution monitoring.

### Marine Pollution and its relevance to DNA damage

The marine environment has been under constant stress of xenobiotic pollutants such as heavy metals, pesticides and hydrocarbons through direct disposal of untreated and/or semi-treated municipal wastes, industrial effluents, riverine runoff besides extensive shipping activities along the coastal regions.

Polycyclic aromatic hydrocarbons are identified as an important class of chemicals because of their widespread occurrence in the environment. They appear to be responsible for the etiologic factors associated with the high frequency of certain tumors and cancers in aquatic species. Polycyclic aromatic hydrocarbons are natural

constituents of crude oil. They account for about 20% of the total hydrocarbons in crude oil and are biologically the most toxic of all petroleum compounds.

The contamination of the marine environment is a global concern because of the bio-accumulation of contaminant residues within tissues of marine organisms. This bio-accumulation has the potential to lead to deleterious effects on the health of the marine ecosystem. The concentrations of these pollutants are biomagnified across the food chain to higher trophic levels. This enhances their concentrations manifold in the body tissues of higher organisms. Chlorinated organic compounds (or organochlorines) are organic chemicals that contain bound chlorine. They include pesticides such as DDT (dichloro-diphenyl-trichloroethane), lindane ( $\gamma$ -hexachlorocyclohexane) and polychlorinated biphenyls (PCBs) which are used extensively in a wide range of industrial applications. Organochlorines have been implicated in teratogenic, reproductive and immunological abnormalities.

Some of the highly persistent organic pollutants (POPs) which are so biomagnified are organochlorine (OC) pesticides (e.g. DDT, HCHs, aldrin, dieldrin, endrin, hepox, heptaclor etc.); polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons, planar halogenated aromatic hydrocarbons (PHAH), heavy metals (e.g. Pb, Hg, Cd, Cu, Mn, Fe, etc.). It has previously been observed that some higher order marine organisms at the top of the food chain in the marine environment viz., seals and whales can accumulate the highest levels of such pollutants [16]. Thus, the contamination of seafood with such toxic contaminants poses a serious threat to human health thereof. Persistent organic pollutants have been detected in different segments of the marine environment such as water, sediments and biota [5,17-20]. The natural capacity of coastal zones to disperse and to assimilate pollutants varies according to the tidal zones and the seasonal variation in the physico-chemical characteristics of coastal zones. The marine pollutants once having entered the sea interact with various types of materials and undergo many transitions between different compartments such as water, suspended matter, sediments and organisms. Copepods for example, can accumulate organochlorine contaminants directly by sorption from seawater, from the particulate matter in the seawater or by feeding on contaminated prey. Table-1 shows the concentration of various contaminants such as total DDT, total PCBs and total HCHs in different types of marine organisms. Table-2 shows the mean concentration of PCBs, t-HCH,  $\gamma$ -HCH and dieldrin into the coastal ecosystems of Mediterranean Sea, East China Sea, Atlantic Ocean, Aegean Sea [3]. North Sea [13], East Java Sea [21], Bay of Bengal [18], Arabian Sea [19,22] and Indian ocean (along the Australian coast) [4]. The riverine input of organochlorine contaminants into the Mediterranean Sea along the Egyptian coast was prominent through the river Nile. It contributes considerable amount of PCBs (19.3 ng/L) into the Mediterranean Sea [3].

Contaminants	Ringed blubber <sup>e</sup>	Seal	Crustacean <sup>d</sup> (crabs, shrimp)	Deep sea Fish <sup>c</sup> ( <i>Mora moro</i> ) (Muscle tissue)	Edible clams <sup>a</sup>	Seabird <sup>d</sup> (Herring gull)
Total DDT (ng/g)	34.8-904		18-24	7.4-12.6	0.6-3.4	0.2-18.8
Total PAHs ( $\mu$ g/g wet wt)	65.-140.7		98-180	0.2-0.6	2.1-24.5	0-333
Total PCBs (ng/g wet wt)	501-6010		6.1-14	13.8-24.0	1.6-15.4	0.4-340

<sup>a</sup>[26] <sup>b</sup>[37] <sup>c</sup>(Sole et al.) <sup>d</sup>[38] <sup>e</sup>[31,32]

**Table 1:** Concentrations of some persistent organic pollutants in marine organisms.

Heavy metals constitute a major source of concern for the health of the marine environment. Heavy metals are natural constituents of rocks and soils; they enter the marine environment as a result of weathering and erosion. Many of these metals are biologically

essential, but have the potential to be toxic to biota above certain threshold concentrations. Because of rapid industrial growth, unusual quantities of metals such as Cd, Cu, Hg, Pb, Ni and Zn have been released into the aquatic contaminants is an urgent need of the hour.

	t-DDT River mouth	t-DDT Coast	γ-HCH River mouth	γ-HCH Coast	Dieldrin River mouth	Dieldrin Coast	PCBs River mouth	PCBs Coast
Mediterranean Sea (s)	2.7	9.7	0.1	4.4	nd	nd	19.3	8.9
Mediterranean Sea (b,l)	12	nd	nd	nd	0.9	nd	65	nd
Aegean Sea (w)	0.4	0.6	0.02	0.03	0.3	0.2	2.1	1.8
Bay of Bengal (b, l)	103	209	51	46	126	145	0.95	0.98
East China Sea (b)	nd	nd	nd	nd	nd	nd	9.3	4.9
North Sea (b,l)	440	145	100	43	183	27	5069	13500
East Java Sea (b l)	156	143	34	70	Nd	Nd	192	200
Atlantic (s)	15.2	14.3	nd	nd	nd	nd	7.4	5.8
Indian Ocean	0.3	nd	nd	nd	nd	Nd	9.02	21.1
Australian coast (w)								

W: Water (ng/L), s: Sediment (ng/g); b (l): Biota (ng/g, lipid basis)

**Table 2:** Mean concentrations of chlorinated hydrocarbons in different coastal environment.

Such diagnostic and prognostic early-warning tests can be applied to a wide range of organisms for bio-monitoring purposes.

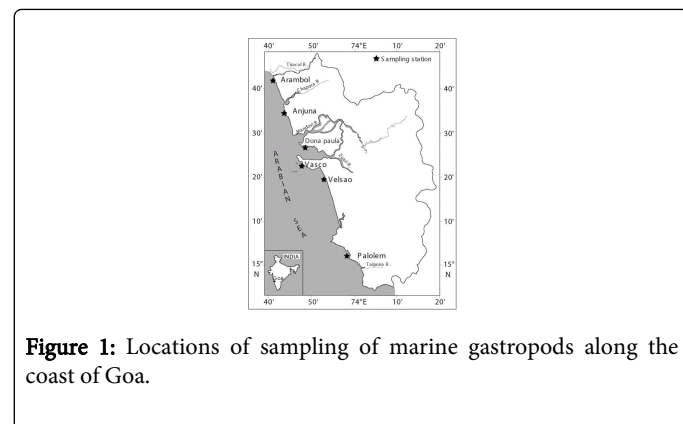
**Impairment of DNA integrity in marine gastropod, Cronia contracta along the West coast of India around Goa – A case study**

The impairment of DNA in different species of marine molluscs was evaluated in terms of the variation of DNA integrity as a function of the degree of contamination of the sites along the coast. For this purpose a large number of marine gastropods, Cronia contracta was collected randomly irrespective of their sex from selected sites along the coast of Goa (Figure 1).

**Measurement of DNA integrity:** The samples were brought into the laboratory immediately after collection for dissection and the muscle tissues were removed softly from the shells. Five individual species were taken for determination of the DNA integrity. The muscle tissues were removed from the individual species of the marine molluscs and homogenized with the help of a clean stainless steel spatula to make composite samples and subdivided into three parts to make triplicate samples for DNA isolation. The isolation of DNA from the samples was accomplished by homogenizing about 250-350 mg (wet wt.) muscle tissues of the composite samples in 1N NH4OH/0.2% Triton-X-100 and further purified by extraction with CIP mixture (Chloroform:isoamyl alcohol:phenol 24:1:25 v/v) followed by

fractionation through a molecular sieve column (Sephadex G-50). After isolation of DNA all the measurements were conducted with a spectrofluorometer (Dyna Quant DNA analyzer).

DNA integrity in different species of marine molluscs were determined following the technique of partial alkaline unwinding assay [11,23] in which three parameters were measured: the percentage of double-stranded DNA (ds-DNA), single-stranded DNA (ss-DNA) and the fraction of double-stranded DNA after alkaline unwinding (au-DNA) after a specified time.



**Figure 1:** Locations of sampling of marine gastropods along the coast of Goa.

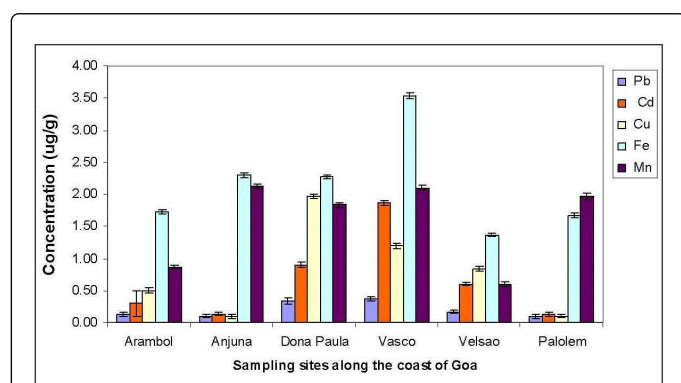
The integrity of DNA is expressed as 'I', which was calculated using the following expression:  $I = (X_{au-DNA} - X_{ss-DNA}) / (X_{ds-DNA} - X_{ss-DNA})$ , Where X stands for observed fluorescence, au-DNA, alkaline unwinded DNA, ss-DNA, single stranded DNA and ds-DNA, double stranded DNA.



**Figure 2:** Marine gastropod, *Cronia contracta* collected from the West coast of India around Goa for measurement DNA integrity.

## Results and Discussion

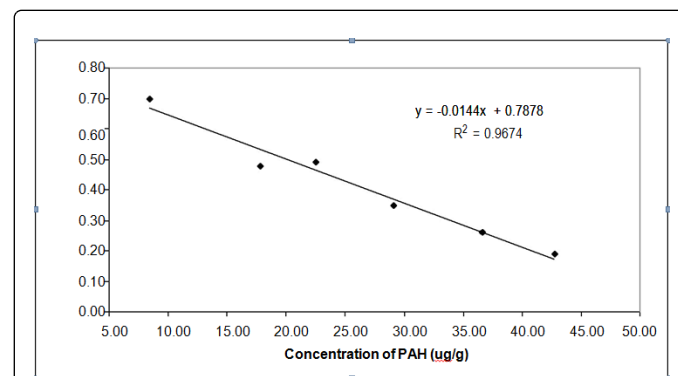
Figure 2 shows the typical picture of a marine gastropod, *Cronia contracta* used for the molecular biomarker studies. The marine gastropods were abundantly distributed all along the coast of Goa, India. They were exposed to the toxic contaminants prevalent in the coastal ecosystem of Goa. The contamination of the coastal environment of Goa can be attributed to the extensive shipping activities besides indiscriminate dumping of waste materials, industrial discharges, dispersion of powdered ore materials into the coastal water during the transport of the extracted ore materials from the mines to the Mormugao harbour through barges leading to contamination of the coastal region by heavy metals. Figure 3 shows the accumulation pattern of different metals such as Pb, Cd, Cu, Fe, Mn in the tissues of the marine gastropod along the coast of Goa [8]. It clearly shows that the mean concentration of Pb, Cd and Cu were least at Palolem whereas that of Fe and Mn at Arambol which is situated at the extreme North of Goa.



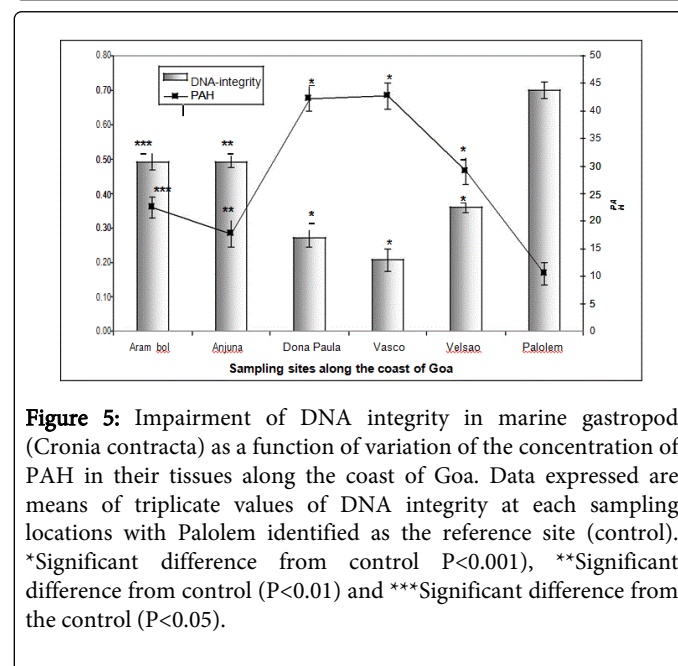
**Figure 3:** Variation in concentration of metals in the tissues of marine gastropod (*Cronia contracta*) along the coast of Goa.

Interestingly, The mean concentrations of all the above metals were quite high at Anjuna, Dona Paula, Vasco and Velsao. Apart from the heavy metals the coastal environment of Goa was also contaminated by petroleum hydrocarbons of which PAHs were the major constituents. In fact, the extent of DNA damage in marine gastropods

was caused due to their exposure to PAH being spread over the surface of the coastal water of Goa due to extensive shipping activities as well as other tourist activities all along the Goa coast. Figure 4 shows the variation in DNA integrity as a function of the concentration of PAH in the tissues of the marine gastropods (*Cronia contracta*). The impact of such genotoxic contaminants on the marine organisms can be expected to be quite predominant. The variation in DNA integrity in the marine gastropods along the Goa coast clearly indicated that it decreased drastically from Arambol to Vasco and then increased gradually to Palolem (reference site) (Figure 5) [8].



**Figure 4:** Variation of DNA integrity in the marine gastropod as a function of the concentration of PAH in their tissues along the coast of Goa, India.



**Figure 5:** Impairment of DNA integrity in marine gastropod (*Cronia contracta*) as a function of variation of the concentration of PAH in their tissues along the coast of Goa. Data expressed are means of triplicate values of DNA integrity at each sampling locations with Palolem identified as the reference site (control). \*Significant difference from control ( $P < 0.001$ ), \*\*Significant difference from control ( $P < 0.01$ ) and \*\*\*Significant difference from the control ( $P < 0.05$ ).

The DNA integrity was found to be quite low at Dona Paula and Vasco which can be attributed to the prevalence of genotoxic contaminants like PAH. Extensive shipping activities coupled with accidental oil spills from the ship-wrecks of the grounded ship, River Princess [24-30] as well as tourism activities along the coast contributed largely towards the contamination of the sites leading to DNA strand breaks in marine organisms exposed to such enhanced levels of contamination by PAHs. Besides, accumulation of heavy

metals in the tissues of marine gastropods might also inflict such impairment of DNA integrity in the marine organisms. Apart from PAHs, the Goan coastal environment was also reported to be contaminated with PCBs [3,6] the residues of which might be accumulated in the tissues of the marine gastropods and thus caused severe DNA damage. Moreover, the discharge of industrial effluents from the adjoining industries situated along the Goa coast (e.g. Zuari Agrochemicals) might be a cause of concern for DNA damage in the marine gastropod exposed to such contaminants [31-33].

## Conclusion

Marine pollution around the world poses a serious threat to the marine ecosystem. The contamination of the marine environment by various types of genotoxic compounds is indeed of great concern in view of rapid development of industrialization along coastal belts. It has caused a serious global concern as most of them discharge their effluents into the coastal ecosystem. It has been observed that the various species of marine organisms were greatly affected due to the discharge of industrial effluents as well as indiscriminate dumping of waste materials from various sources, extensive shipping activities, municipal sewage discharge etc. In order to assess the contamination of coastal environment, the use of molecular biomarkers is a viable option especially to identify the hot spots of pollution. In this regard, the measurement of DNA integrity in various species of marine organisms exposed to such enhanced levels of genotoxic contaminants can provide an insight into prevalence of genotoxic contaminants in the regions and their impacts on the coastal ecosystem. It has been substantiated by earlier studies on the impairments of DNA in marine gastropods (such as *Planaxis sulcatus* and *Morula granulata*) [16,34-39] that the DNA integrity in marine gastropods are greatly affected by the genotoxic contaminants. Based on the evaluation of impairment of DNA in different species of marine organisms the measurement of DNA integrity in marine organisms can be used as a useful tool for bio-monitoring of genotoxic pollution in the marine environment.

## Acknowledgement

The authors are thankful to the Director, CSIR-National Institute of Oceanography, Goa, India for his keen interest and profound encouragement. They thank the Director General CSIR, New Delhi, India for providing infrastructural facilities. They are also thankful to Prof. Mansour El-Matbouli for his constant inspiration and kind cooperation. They are grateful to Dr. Lee R Shugart, Editor-in-Chief, Ecotoxicology, for his valuable guidance in measurement of DNA integrity.

## References

1. Sethi PK, Bhattacharya AK, Sarkar A (1999) Current trend of some somorganochlorinated pesticides in Yamuna river sediments around Delhi. Environmental Pollution Control Journal 40-43.
2. Sen Gupta R, Sarkar A, Kureshey TW (1996) PCBs and organochlorine pesticides in krill, birds and water from Antarctica. Deep Sea Research 43: 119-126.
3. Sarkar A, Everaarts JM (1998) Riverine input of chlorinated hydrocarbons in the coastal pollution. The Pennsylvania Acad of Sci 400-422.
4. Sarkar A (1994) Occurrence and distribution of persistent chlorinated hydrocarbons in the seas around India. The Pennsylvania Academy of Science 445-459.
5. Sarkar A, Singbal SYS, Fondekar SP (1994) Pesticide residues in the sediments from the lakes of Schirmacher Oasis, Antarctica. Polar Record 30: 33-38.
6. Sarkar A, Ray D, Shrivastava AN, Sarker S (2006) Molecular Biomarkers: their significance and application in marine pollution monitoring. Ecotoxicology 15: 333-340.
7. Sarkar A, Gaitonde DC, Sarkar A, Vashistha D, D'Silva C, et al. (2008) Evaluation of impairment of DNA integrity in marine gastropods (*Cronia contracta*) as a biomarker of genotoxic contaminants in coastal water around Goa, West coast of India. Ecotoxicol Environ Saf 71: 473-482.
8. Deasi SR, Verlecar XN, Ansari ZA, Jagtap TG, Sarkar A, et al. (2010) Evaluation of Genotoxic Responses of *Chaetoceros tenuissimus* and *Skeletonema costatum* to Water Accommodated Fraction of Petroleum Hydrocarbons as Biomarker of Exposure. Wat Res 44: 2235-2244.
9. Shgart LR, Bickiaam J, Jackim G, McMahon G, Ridley W, et al. (1992) DNA alterations. In: Biomarkers: biochemical, physiological and histological markers of anthropogenic stress, pp.125-153.
10. Everaarts JM, Sarkar A (1996) DNA damage as a biomarker of marine pollution: strand breaks in sea stars (*Asterias rubens*) from the North Sea. Wat Sci Tech 14: 157-162.
11. Kohn KW (1983) The significance of DNA-damage assays in toxicity and carcinogenicity assessment. Ann N Y Acad Sci 407: 106-118.
12. (1987) Biological markers in environmental health research. Committee on Biological Markers of the National Research Council. Environ Health Perspect 74: 3-9.
13. Everaarts JM, Den Besten PJ, Hillebrand M Th J, Halbrook RS, et al. (1997) DNA strand breaks, cytochrome P-450- dependent monooxygenase system activity and levels of chlorinated biphenyls congeners in the pyloric caeca of the seastars (*Asterias rubens*) from the North Sea. Ecotoxicology 7: 69-79.
14. Livingstone DR (1993) Biotechnology and pollution monitoring: Use of molecular biomarkers in the aquatic environment. J Chem Tech Biotechnol 57: 195-211.
15. Sarkar A, Bhagat J, Sarker S (2014) Evaluation of impairment of DNA in marine gastropod, *Morula granulata* as a biomarker of marine pollution. Ecotoxicol Environ Saf 106: 253-261.
16. Noël M, Barrett-Lennard L, Guinet C, Dangerfield N, Ross Peter S (2009) Persistent organic pollutants (POPs) in killer whales (*Orcinus orca*) from the Crozet Archipelago, southern Indian Ocean. Marine Environmental Research 68: 196-202
17. Sarkar A, Sen Gupta R (1988a) Chlorinated pesticide in marine sediment. Mar Pollut Bull 19: 35-37.
18. Sarkar A, Sen Gupta R (1988) DDT residues in sediments from the Bay of Bengal. Bull Environ Contam Toxicol 41: 664-669.
19. Sarkar A, Sen Gupta R (1991) Pesticide residues in sediments from the west coast of India. Mar Pollut Bull 22: 42-45.
20. Sarkar A, (1992) Evaluation of the toxicity of organic matter in marine sediments. Water Science and Technology 25: 255-257.
21. Boon JP, Everaarts JM, Kastoro WW, Razak H, Sumanta I, et al. (1989) Cyclic organochlorines in epibenthic organisms from coastal waters around East Java Neth. J of Sea Res 23: 427-439.
22. Sarkar A, Nagarajan R, Singbal SYS, Chhapadkar S, Pal S (1997) Contamination of organochlorine pesticides in sediments from the Arabian Sea along the west coast of India. Water Research 31: 195-200.
23. Everaarts JM, Shgart LR, Gustin MK, Hawkins WE, Walker WW (1993) Biological markers in fish DNA integrity, hematological parameters and liver somatic index. Mar Env Res 35: 101-107.
24. Rao SS, Neheli TA, Carey JH (1996) DNA alkaline unwinding assay for monitoring the impact of environmental genotoxins. Environmental toxicology and Water Quality 11: 351-354.
25. Ingole B, Sivasdas S, Goltekar R, Clemente S, Nanajkar M, et al. (2006) Ecotoxicological effect of grounded MV River Princess on the intertidal benthic organisms off Goa. Environ Int 32: 284-291.

26. Binelli A, Ricciardi F, Riva C, Provini A (2006) New evidences for old biomarkers: effects of several xenobiotics on EROD and AChE activities in Zebra mussel (*Dreissena polymorpha*). *Chemosphere* 62: 510-519.
27. Burmeister B, Schwerdtle T, Poser I, Hoffmann E, Hartwig A, et al. (2004) Effects of asbestos on initiation of DNA damage, induction of DNA-strand breaks, P53-expression and apoptosis in primary, SV40-transformed and malignant human mesothelial cells. *Mutat Res* 558: 81-92.
28. den Besten PJ, Valk S, van Weerlee E, Nolting RF, Postma JF, et al. (2001) Bioaccumulation and biomarkers in the sea star *Asterias rubens* (Echinodermata: Asteroidea): a North Sea field study. *Mar Environ Res* 51: 365-387.
29. Everaarts JM (1995) DNA integrity as a biomarker of marine pollution: Strand breaks in seastar (*Asterias rubens*) and dab (*Limanda limanda*). *Marine Pollution Bulletin* 31: 431-438.
30. Frenzilli G, Scarcelli V, Del Barga I, Nigro M, Förlin L, et al. (2004) DNA damage in eelpout (*Zoarces viviparus*) from Göteborg harbour. *Mutat Res* 552: 187-195.
31. Holsbeek Ludo J, Claude R, Debacker V, Ali IB, Roose Patrick N, (1999) Heavy Metals, Organochlorines and Polycyclic Aromatic Hydrocarbons in Sperm Whales Stranded in the Southern North Sea During the 1994/1995 Winter *Mar Pollut Bull* 38: 304-313
32. Kucklick JR, Struntz WDJ, Becker PR, York GW, O'Hara TM (2001) Persistent organochlorine pollutants in ringed seals and polar bears collected from northern Alaska. *Sci of Total Environ* 287: 45-59.
33. Kurelec B, Garg A, Krca S, Chacko M, Gupta RC (1989) Natural environment surpasses polluted environment in inducing DNA damage in fish. *Carcinogenesis* 10: 1337-1339.
34. Pfeiffer P (1998) The mutagenic potential of DNA double-strand break repair. *Toxicol Lett* 96-97: 119-29.
35. Sarkar A, Deepti V, Nitin G, Kirti M, Dipak G (2011) Measurement of DNA Integrity in Marine Gastropods as Biomarker of Genotoxicity 108-112.
36. Sarkar A, Bhagat J, Ingole BS, Rao DP, Markad VL (2015) Genotoxicity of cadmium chloride in the marine gastropod *Nerita chamaeleon* using comet assay and alkaline unwinding assay. *Environ Toxicol* 30: 177-187.
37. Sericano JL, Brooks JM, Champ MA, Kennicutt MC 2nd, Makeyev VV (2001) Trace contaminant concentrations in the Kara Sea and its adjacent rivers, Russia. *Mar Pollut Bull* 42: 1017-1030.
38. Shore RF, Wright J, Horne JA, Sparks TH (1999) Polycyclic Aromatic Hydrocarbon (PAH) Residues in the Eggs of Coastal-Nesting Birds from Britain. *Mar Pollut Bull* 38: 509-513.
39. Shugart LR (1999) Structural damage to DNA in response to toxicant exposure. In: Forbes VE (ed.) *Genetics and Ecotoxicology*, pp. 151-168.