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Molecular Biomarkers as Key Factors to Evaluate the Extent of Industrial Pollution Exposure

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Abstract
Over recent decades, environmental pollution is rapidly increasing because of the anthropogenic activities of uncontrolled development, for example, industry, transport, agriculture, and urbanization, which generates harmful contaminants for living organisms including humans. These contaminants get accumulated in the organism via different routes and get bioaccumulated in different tissues exerting detrimental effects at different levels (molecular, cellular and physiological levels). The measurement of biological assays in sentinel species to access quality and changes of the environment is known as environmental biomonitoring which provides an indication of environmental stress. Several biomarkers evaluate the nature and extent of the exposure and evaluation of adverse biological responses to pollutants in a biomonitoring program of the aquatic environment. These include behavioral response, genotoxicity (comet assay and micronucleus assay) and oxidative stress. Here we present the importance of these biomarkers.

Keywords: Environmental pollution; Industry; Agriculture; Oxidative stress; Genotoxicity

Introduction
Contaminants present in their environment might act as stressors, disturbing the metabolism of fishes leading to the activation of compensating and adaptive responses that readjust metabolic processes in order to minimize the effects of contaminants, moreover, they respond to toxic agents similar to higher vertebrates and that allow the assessment of substances that are potentially hazardous to humans. Fish have great sensitivity and response towards changes in the aquatic environment and any undesirable change might be reflected in the biochemical, physiological as well as histological parameters of fish. The toxic impact of textile waste imposes a negative impact on fish both directly as well as indirectly. The accumulation of such xenobiotics and the alterations in physical parameters including color, temperature and total dissolved solids makes light a limiting factor that alters the food chain of fish. This section includes a detailed review of the literature highlighting the importance of gene-cytotoxicity and oxidative stress analysis to study the effect of different xenobiotics.

Oxidative stress
Oxidative stress biomarkers are becoming increasingly important in the field of eco-toxicology. The high number of pollutants can disturb the equilibrium between ROS and the antioxidant defense system. It has been suggested that they could also be used in environmental monitoring programs. A plethora of metals, pesticides, pulp mill effluents and dyes are some of the pollutants that have been reported to elicit oxidative stress in aquatic organisms. Oxidative stress primarily occurs through the generation of reactive oxygen species (ROS) and can damage DNA, lipids, and proteins. As a result, it may contribute to the loss of enzymatic activity and structural integrity of enzymes and activate inflammatory responses. Oxidative stress in biological systems instigates as a consequence of a discrepancy between the production of oxidizing species and cellular antioxidant defenses. Elucidation of alterations in the levels of GST, CAT, and SOD has been considered effective to assess the overall antioxidant status of an organism. It depicts the overall health of an organism, as any decrease in its activity indicates poor detoxification capacity. The extent of MDA production is determined by the balance between the removal and scavenging of those oxidants by antioxidants. Malondialdehyde (MDA) is an oxidation byproduct of peroxidized polyunsaturated fatty acids and an increase in MDA content is an imperative marker of LPO.

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Glutathione-S-transferase (GST) is the detoxifying enzyme that catalyzes the conjugation of a variety of electrophilic substrates to the thiol group producing lesser toxic forms and reducing the formation of lipid peroxides. Similarly, superoxide dismutase (SOD) and catalase (CAT) form the most important defense line against the free radicals generated and alleviate the toxic effects of ROS by catalyzing the conversion of superoxide radicals into the water.

Glutathione is probably the most abundant natural low molecular weight thiol that is detected in virtually all living cells of the vertebrates even in milliliter concentration. It involves in a series of critical cell functions including, transport of amino acid across the cell membrane, catalyzing di-sulfide exchange reactions, serving as coenzyme for certain enzymes, maintaining thiol group in proteins and detoxifying peroxides and free radicals as well as reactive toxic intermediates free radicals. Glutathione – S – transferase (GST) is a superfamily of mainly cytosolic conjugated enzymes which use the tri-peptide glutathione, instead of glucuronic acid. It was also reported that glutathione – S – transferase is a superfamily of enzymes that can express detoxification activity through some of these isozymes; glutathione peroxidase, towards lipid hydroperoxides generated by pollutants or organic contaminants. A significant decrease in the levels of glutathione exposed to hexachlorobenzene and elevated activity of glutathione reductase and GST was observed in the brain of common carp (Song et al., 2006).

Catalase, the primary antioxidant defense component, eliminates hydrogen peroxide a non–radical reactive oxygen species which can penetrate through all biological membranes and directly inactivate the enzymes. Various responses of catalase activity have been observed in animals exposed to organic or metallic contaminants. The catalase activity gets altered by metals depending on the dose, the species, or the route of administration. Oxidative stress has gained considerable interest in the field of eco-toxicology. Therefore, catalase activity is also considered a sensitive biomarker of oxidative stress that occurs in fish. Atli et al. (2006) reported a decrease in the activity of catalase in the gill of *Oreochromis niloticus* after chromium exposure whereas silver elevated the catalase activity, however, after exposure to chromium, silver, cadmium and zinc caused a significant decrease in the catalase activity in the intestine of *Oreochromis niloticus*

Similarly, superoxide dismutase (SOD) forms the most important defense line against the toxic effects of oxygen metabolism and alleviates the toxic effects of ROS by catalyzing the conversion of superoxide radicals into water. The impact of water contamination due to industrial effluents was evaluated in three different cichlid species through assessment of catalase, glutathione peroxidase and superoxide dismutase in the blood. Results reported by Mansour et al. (2009) and Stara et al. (2013) in freshwater fish treated with profenofos and prometryne respectively also showed the effect of free radicals generated due to xenobiotics on activities of enzymes involved in defense mechanism. The increase in free radicals resulting in an imbalance in antioxidant enzyme status also leads to ROS interaction with biomolecules such as DNA, resulting in the formation of DNA adducts which prevents replication accurately. As DNA carries genetic information, its stability and integrity are crucial for life sustainability. So, the damage associated with DNA caused by xenobiotics needs scientific approaches to combat the problems. Among several biomarkers used to access genetic toxicity, the micronucleus test and comet assay are known to be the most reliable and convenient methods for measuring DNA damage. These have been widely used for evaluating the genotoxicity of industrial chemicals, agrochemicals, biocides, food additives and pharmaceuticals. One test is not sufficient to evaluate a hypothesis as each test carries few constraints and error rates. Therefore, a combination of these tests should be used to evaluate the genotoxic potential of contaminants.

Micronucleus assay

Micronuclei are formed from chromosomal fragments or whole chromosomes with damaged or no centromere that delay, in relation to others in their migration to the poles of the cell in anaphase. They can also be formed by apoptosis, inactivation of the spindle formation and chromosome damage beyond the action of the physical agent. Their assessment with the help of micronucleus/nucleo-cellular abnormality assay is useful to detect even the slightest damage to

DNA in a variety of tissues and in a short time. Thus, nowadays, the analysis of micronuclei (MN) and nucleo-cellular abnormalities in RBCs of fish is used to evaluate the cytogenetic damage. Dyes and other xenobiotics cause irreversible structural modification in the DNA of fish and appear even at those levels of toxins that are otherwise safe for survival. Therefore, its results are being used nowadays as biomarkers for environmental biomonitoring and for developing control strategies and preventive measures.

Parmar and Shah (2019) reported cytogenotoxicity of sublethal concentrations (0.35, 0.7, 3.5 mg/L) of Reactive Red 120 for 10, 20 and 30 days on *Catla catla* using DNA damage in gill cells and blood cells as sensitive biomarkers and observed nuclear and cytoplasmic deformities. Sub-lethal concentrations of RR120 increased the micronuclei frequencies in both tissues in a dose- and time-dependent manner, but higher micronuclei frequencies in gill cells than in erythrocytes cells was observed may be due to direct contact of gills with the test chemical solution. The results clearly revealed the potential of dye as a genotoxic agent at all doses tested in a dose-dependent manner. Likewise, Alimba et al. (2017) elucidated abnormalities in the nucleus in *Clarias gariepinus* (Burchell, 1822) after exposure to hospital effluent at sub-lethal concentrations (0.08, 0.16, 0.33, 0.65, and 1.30%) 96 h LC_{50} value, of the effluent for 7 days. There is a varying fold increase in induced micronuclei in comparison to control fish. They also exposed fishes to the 96h LC_{50} (1.30%) of the effluent along with fifty and hundred mg/kg of dietary ascorbic acid for 7 days and illustrated a reduction in micronuclei as well as nuclear abnormalities in comparison to the control. Likewise, genetic damage was illustrated in peripheral erythrocytes of *Anabas testudineus* when exposed to a sublethal concentration of acid orange 7 (Rishin et al., 2019). The formation of micronucleus along with other nuclear abnormalities such as irregular, notched and blebbed nuclei were observed. DNA damage was elucidated after 24 h of acid orange 7 exposures and at 72 and 96 h, the grade of DNA damage was increased to grade 2 and 3, respectively.

Comet assay

Comet assay is considered the reliable method for determining DNA damage. It has been widely used for evaluating the genotoxicity of industrial chemicals, agrochemicals, biocides, food additives and pharmaceuticals. One test is not sufficient to evaluate a hypothesis as each test carries few constraints and error rates. The comet assay is used for the detection of DNA single and double-strand breaks, alkali-labile sites, and incomplete excision repair events in individual cells. In this, any cell type can be used for genotoxicity testing. The comet assay has been used in different fields ranging from genetic, environmental and occupational toxicology to biomonitoring and human epidemiology using different animal models. Many studies have been performed which involve the use of this technique to evaluate DNA damage in samples when exposed to different environmental contaminants.

Ateeq et al. (2005) revealed the genotoxic effect of 2,4-dichlorophenoxyacetic acid and butachlor on erythrocytes of C. *batrachus* by using the comet assay. In this study, tail length was used as a measure of DNA damage and the highest damage at the highest concentration and highest duration of exposure was observed. The study performed by Osterauer et al. (2011) includes a survey of the continual entry of platinum into the aquatic environment by road runoff and hospital sewage and raised concerns about its toxicity to organisms. Genotoxicity of platinum was tested at 0, 0.1, 1, 10, 50, 100, and 200µg/l in *D. rerio* and *Marisa cornuarietis* using the comet assay. The increased level of genotoxicity was illustrated in M. *cornuarietis* at 1 µg/l and beyond. Mohanty et al. (2011) revealed a similar trend in fish L. *rohita* after exposure to organophosphate pesticide in blood and gill tissue. Likewise, Sharma et al. (2018) illustrated DNA damage after exposure to tetra-bromo-bisphenol A in C. *punctatus*. The dose-dependent symbolic hike was also elucidated in the genotoxicity C. *punctatus* after exposure to profenofos (Pandey et al., 2011). Gill tissue showed a concentration-dependent increase in the DNA damage when exposed to sublethal concentrations (0.58ppb, 1.16ppb, and 1.47ppb) for a period of 24, 48, 72 and 96 hours. The data determined by one biomarker individually provides limited information therefore as there is a considerable likelihood of misinterpretation. Therefore, combining the information elucidated

from the plethora of biological levels, such as metabolism, genotoxicity expression, and histopathological effects levels, might lead to a substantial improvement in the knowledge of integrated fish toxic response. Disturbance of living processes at the molecular and subcellular levels of the biological organization by xenobiotics frequently leads to cell injury resulting in degenerative and neoplastic diseases in target organs.

Conclusion

Water is conducive to life sustainability and development. However, increasing anthropogenic activities have devastated the earth's natural resource i.e., water. Textile industries being a major contributor of xenobiotics primarily including synthetic dyes and intermediates have attained the top position in pollution-causing industries. A perusal of the literature revealed the toxic potential reported for all the contaminants released as industrial discharge mainly dyes. These are illustrated to have oxidative stress-inducing and cytotoxic and genotoxic abilities against aquatic organisms. As the potential health hazard to the aquatic organism is highlighted thus this study entails the augmentation and adoption of pertinent policies regarding the management of such toxic intermediates. The study also demands the generation of a plethora of bioremediation methods to curb the deteriorating effects of such toxicants by reducing them to a non-toxic hydrogenated form.

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SM and PC conceived the concept, wrote and approved the manuscript.

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