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A review on assessment of oxidative stress in freshwater fish tilapia exposed to sugar mill effluent

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Abstract
In the context of industrialization and human activities, water bodies gradually get destroyed, polluted and transformed in a way that adversely affects aquatic ecosystems and human health. Sugar industrial effluents are a significant source of water pollution as they discharge heavy metals and nutrients into the ecosystems; they are also high in BOD and suspended solids which create a greater OS in water. This review discusses the oxidative stress measured in freshwater fish associated with sugar mill effluent discharges. Such industrial effluents, including those from sugar mills, contain a high load of organic and inorganic contaminants that pose a threat to the aquatic environment. As a hardy freshwater fish species, Tilapia is important because it is a useful pollution bio-indicator due to its ecological and economic value. The review summarizes biochemical and molecular responses of oxidative stress in organisms found nearby effluents discharge areas and specially focused on the changes of antioxidant enzyme activities, lipid peroxidation as well as protein oxidation. In this review we also find out the composition of effluent and its deleterious effects on oxidative biomarkers, and their relationship with fish health. This assessment also underlines the demand for eco-toxicological assessment and measures to control the negative impacts of industrial effluents on fish populations and freshwater ecosystem.

Keywords: Oxidative stress, biological oxygen demand, sugar mill effluent, reactive oxygen species, toxicity, fish tilapia

Introduction

Industrialization and human activities have severe negative impacts on water bodies including pollution, habitat destruction and disruption of aquatic ecosystems, which have direct or indirect effects on human health. There are many sources of water pollution, the most polluting of them are the industrial wastes discharged into the rivers (Sudhira & Kumar, 2000) [39]. Factories discharge pollutants like heavy metals (e.g., mercury, lead), toxic chemicals, solvents, and dyes into rivers, lakes, and oceans. Environmental fluctuations in the aquatic environment are mainly caused by abiotic stress resulting from extremes in pH, salinity, temperature, dissolved oxygen (DO), acidity, pollution load, etc. (Masson *et al*., 2002; Moon *et al*., 2003; Wu *et al*., 2018; Bernhardt *et al*., 2020; Song and Choi, 2021; Booth *et al*., 2023) [5, 8, 20, 21, 37, 44]. The sugar industry in India is one of the country's most significant agro-based industries. India is one of the largest producers of sugar in the world, and the industry plays a crucial role in the nation's rural economy. A large amount of wastewater is generated during sugarcane crushing. This wastewater is dumped into nearby water bodies and used for irrigation. The release of this waste into water bodies or soil is causing a serious problem of water pollution, resulting in severe damage to flora and fauna and degradation of the environment (Pande, 2005) [27]. Sugar mill effluent is a byproduct of sugar production and contains various organic and inorganic substances, including high levels of sugars, suspended solids, organic acids, phenolic compounds, and chemicals used in processing. Sugar mills are associated with effluent characterized by biological oxygen demand and suspended solids, the effluent is high in ammonium content (Prakash, & Capoor, 2019) [30]. Contaminants in the effluent, such as heavy metals and organic pollutants, can induce the formation of ROS in fish tissues. Oxygen plays a dual role in the body. While it is essential for life, being a critical component in cellular respiration and energy production, it is also involved in the production of reactive oxygen species (ROS), which can lead to oxidative stress. While ROS is a normal by-product of cellular respiration, when produced in excess or when the body's antioxidant defences are overwhelmed, they can cause oxidative stress. Free radicals are highly reactive molecules that can damage cells, proteins and DNA, leading to various health issues. Reactive Oxygen Species (ROS) are highly reactive molecules containing oxygen. They are a byproduct of normal cellular metabolism, particularly during the process of aerobic respiration, but can also be generated through exposure to environmental factors such as radiation, pollution, and certain chemicals. Elevated synthesis of ROS can cause oxidation of proteins and lipids, alterations in gene expression, and changes in cell redox status (Livingstone, 2003) [19]. Atmospheric O_2 has two unpaired electrons (in its ground state), prevents it from readily interact with organic molecules unless activated, partial reduction in oxygen leads to the synthesis of ROS such as superoxide radical (O_2•-) singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical (HO•) (Asada & Takahashi, 1987; Cadenas, 1989; Fridovich, 1998; Halliwell & Gutteridge, 2015) [2, 9, 12, 14]. The free radical has a different degree of reactivity and half-life, but most of them are very unstable and can promote the non-enzymatic oxidation of biomolecules (proteins, carbohydrates, lipids and nucleic acids) (Halliwell 2009, Buonocore *et al*. 2010, Hamanaka and Chandel 2010) [9, 10, 13]. The sources of ROS generation can be endogenous or exogenous. Endogenous sources of ROS are mitochondria (electron transport chain), peroxisomes, lysosomes, ER and enzymatic sources (such as NADPH oxidases (NOX enzymes), Xanthine oxidase, Cyclooxygenases (COX), Lipoxygenases, cytochrome p450 oxidase) (S. Noori, 2012) [33]. The sources of exogenous ROS may be Ultraviolet (UV) light, ionizing radiation, and other forms of high-energy radiation, Pollutants and Toxins (such as chemicals, tobacco smoke, and heavy metals), Drugs and Xenobiotics. ROS also produced by immune cells like neutrophils and macrophage during inflammation, which is needed short term to help the body fight off infections (Biller, & Takahashi, 2018) [6]. Reactive oxygen species are intermediates of the univalent reduction of oxygen. To minimize the damaging effects of ROS, aerobic organisms evolved both nonenzymatic and enzymatic defences. The molecules participating in elimination of ROS is known as antioxidants. Antioxidants are substances that significantly delay or prevent the oxidation of an oxidizable substrate (Franco and Martínez-Pinilla 2017, Sorg 2004, Wang *et al*. 2013). Enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzymes. Non-enzymatic Defences are Vitamins C, E, Glutathione, Carotenoid, Ubiquinone.

A Brief Review of The Work Already Done in The Field

Turinayo, (2017) [42] studied the physico-chemical properties and its impact of sugar industry and molasses-based distillery wastewater on the water quality of Musamya River in Uganda. The analysis was carried out between February and April 2010 using standard methods for testing water and wastewater. The results showed that the pollutant concentrations (4.3 pH, 9104mg TDS/l, 48mg TN/l, 16 mg TP/l, 2256mg BOD/l, 8064mg COD/l) in the wastewater (2,523±728 m3/day) were above the acceptable limits (6.0 - 8.0 pH, 1200mg TDS/l, 10mg TN/l, 10mg TP/l, 50mg BOD/l, 100mg COD/l) by NEMA. This caused a significant change in water quality in the downstream of the river after discharge ($P < 0.05$). The pH, TDS, T-Fe, Na+, Ca2+, TN, TP, turbidity, BOD, COD of the upstream and downstream river after wastewater discharge varied from 7.1 to 5.6, 88 to 1007mg/l, 3.2 to 10.5mg/l, 5.5 to 8.0mg/l, 8.5 to 25mg/l, 1.4 to 6.8mg/l, 0.8 to 2.7mg/l, 49 to 616NTU, 3.8 to 184mg/l, 13 to 675mg/l, respectively, and the difference was significant ($P < 0.05$).

Kumar, *et al*., (2018) [17] studied the physico-chemical characteristics of sugar mill effluent. The parameters studied are colour, odour, pH (6.1), temperature (39°C), total dissolved solids (2653.1 mg/l), total suspended solids (401.3 mg/l), total solids (3054.4 mg/l), D.O (2.83 mg/l), BOD (86 mg/l), COD (325 mg/l), chloride (262.31 mg/l), and sulphate (641.52 mg/l). According to the limits suggested by Bureau of Indian Standards, almost all the parameters have been found to be very high and well above the permissible limits.

Yadav, (2014) [45] carried out physico-chemical analysis of sugar mill effluent collected in three different months (January, March and May). Parameters like colour, temperature, pH, alkalinity, total dissolved solids, total suspended solids, total solids, biological oxygen demand, chemical oxygen demand etc. were determined using standard method. The effluent samples were compared with the prescribed limit of effluent discharge on irrigation lands by BIS. The results of this analysis show that some of the parameters like alkalinity, TDS, BOD and COD of sugar industry are more than the permissible limit.

Bishnoi, (2019) [7] studied the water quality of untreated sugar mill wastewater in Sri Ganganagar city and analyzed various physico-chemical parameters like colour, odour, pH, total hardness, chloride, alkalinity, TS, TDS, dissolved oxygen, BOD and free CO2. Untreated sugar mill effluent contains high, BOD, TDS, TS and low amount of DO which is toxic to plants. Hence, it is not allowed for irrigation. But diluted distillery wastewater can be used for irrigation purpose.

Kaur and Braich (2022) [16] studied the effect of industrial effluents on physico-chemical parameters of water and fatty acid profile of fish, *Labeo rohita* (Hamilton) collected from Ramsar sites of Punjab, India, they found poor quality water: Harike wetland WQI = 56.68: good quality water WQI (water quality index) = 39.54 in Nangal wetland. Heavy metal pollution index (HPI = 144.9) and metal index (MI = 4.76) were much higher in Harike wetland in contrast to Nangal wetland (HPI = 3.12, MI = 0.22). Polyunsaturated fatty acids (PUFAs): Decreased in liver and intestine from Nangal samples, except in winter season.

Samuel *et al*., (2014) [34] studied the physico-chemical and heavy metal analysis of sugar mill wastewater. They detected the presence of heavy metals like arsenic, cadmium, copper, lead, mercury, zinc, chromium in all the samples analysed, same result also seen by Laxmi *et al*., (2015).

Javed and Usmani (2013) [43] Study of water bodies affected by sugar mill effluents and health status of prevalent fish Chana punctatus. Sugar mill effluents are tested for the presence of heavy metals such as Cu, Ni, Co and Cr. He observed the order in which heavy metals are present in water is Ni>Cr>Cu>Co. It is observed that Cu is within the limit while Ni and Cr are out of the recommended limits of UNEPGEMS. Only Cu and Cr were accumulated in fish tissues such as gills, muscles and dermal sheath (integument) while Ni and Co could not be detected. Highest concentration of Cu and Cr accumulated in gills and lowest in dermal sheath.

Tiwari (2016) [41] carried out an analytical study on the impact of industrial effluents on Kharun River, Raipur, Chhattisgarh. Five water samples collected from the study area during February-March 2015 were analysed. High concentrations of alkalinity (209 mg/l) and hardness (220 mg/l) indicate the critical condition of the river water due to the discharge of industrial effluents. The concentration of heavy metals or toxic metals was found to be negligible, whereas sample no. 1 had concentrations of Pb (0.811 mg/l), Cr (0.642 mg/l), Fe (0.498 mg/l), Zn (0.326 mg/l) and Mn (0.3 mg/l). The concentrations of some metals were found to be below the detectable limit in other locations, although iron appeared in small quantities.

Bhatt and Verma (2018) [4] studied the physico-chemical characteristics of sugar mill effluent from Bhoramdev

Sahakari Sugar Mill, Kabirdham. Various parameters including colour, odour, temperature, pH, total hardness, turbidity, iron, chloride, sulphate, fluoride ions, total dissolved solids (TDS), biological oxygen demand (BOD), chemical oxygen demand (COD), calcium and magnesium were analysed. The analysis revealed that the concentration levels of some parameters were higher than the permissible limits prescribed by the Bureau of Indian Standards (BIS).

Kaur *et al*. (2013) [46] studied the behavioural and morphological changes in the fish *Cirrhinus mrigala* induced by dyeing industry waste. Various behavioural responses were observed such as erratic movements, gulping air at the surface or jumping out of water, opercular movements, loss of balance, hitting the wall, restlessness, lethargy, fish lying on the water surface before dying and loosening of scales, redness in eyes, increased mucous secretion, bleeding from gills, swelling and upward movement of abdomen, pigment patches on abdomen.

Sarvade, (2015) studied the toxicity and behavioral changes in freshwater fish, Puntius stigma, exposed to paper mill effluent. During the experiments, severe effects such as erratic swimming, jerky movement and rapid opercular movements with leaping out of water and excess mucous secretion were observed.

Devarajan, *et al*., (2015) [11] studied the behaviour of freshwater fish *Tilapia mossambica* induced by sugar mill waste and found that the LC50 values of the prepared concentrations for 24, 48, 72 and 96 hours were found to be 11, 10.5, 10.1 and 9.5%, respectively. At this concentration, erratic swimming, jerky movements, rapid opercular movements with jumping out of water and thick mucus coating over the entire body surface were observed during the experiments.

Leese *et al*., (2021) [47] studied the effects of wastewater treatment plant effluent in a receiving stream on reproductive behavior of fathead minnows (*Pimephales promelas*). These effluents contained endocrine-disrupting chemicals (EDCs) that exhibited estrogenic activity, including 17β-estradiol, 17α-ethinyl estradiol, and nonylphenol, which could influence vertebrate behavior and physiology. Juvenile and adult male minnows were exposed upstream and downstream of the waste site for 21 days. Estrogenicity levels were significantly higher downstream, though vitellogenin production was not related to estrogenicity, and reproductive behavior was significantly reduced in downstream males.

Muley *et al*., (2007) [22] studied the impact of industrial effluents on the biochemical composition of fish Labeo rohita and found that, electroplating effluent was more toxic than tannery and textile mill wastes. After acute toxicity experiments for different industrial effluents, various tissue's *viz*. gill, liver, muscle and kidney were obtained separately from control, LC_0 and LC_{50} groups. These tissues were used for biochemical estimations. The glycogen content in all the tissues decreased considerably upon acute toxicity of three industrial effluents except muscle in LC50 group of tannery effluent and kidney in LC50 group of textile mill effluent, when compared to control group. The total protein content decreased in all tissues in three effluents except gills in LC50 group of tannery effluent, kidney in LC50 group of electroplating effluent and kidney in LC50 group of textile mill effluent.

Baskar *et al*., (2016) [3] investigated the effect of sub-lethal concentrations of untreated sugarcane mill wastewater on the biochemical and histological integrity of Tilapia mossambica. Histological observations showed that, in the experimental group, there were ruptured nerve bundles and scattered glial cells, severe inflammation, mucous secretion and bleeding due to capillary damage, proliferation of liver cells, hepatic necrosis and severe damage, renal tubular damage and glomerular edema, testicular swelling, interstitial cell shrinkage and tubular vacuole, atretic follicles, yolk granule degeneration, disintegration and scattering of oocytes.

Rajkumar *et al*., (2016) [32] studied toxicity evaluation on haematology, biochemical and histopathological changes of freshwater fish Labeo rohita exposed to silver nanoparticles. Antioxidant responses were studied in three major tissues of L. rohita such as gill, liver and muscle. The results of this investigation showed that increasing concentration of AgNPs led to bioaccumulation of AgNPs in major tissues. The treated fish showed significant changes in hematological parameters. Histological changes caused by chemically synthesized AgNPs demonstrated damage in tissues, primary lamella and blood vessels of L. rohita. Histological study also demonstrated vacuole formation in liver and muscle compared to untreated tissues (control) of L. rohita.

Nath (2016) [24] investigated the effect of industrial effluent on the morphology and hematological indices of fish *Amlyceps mangois* and found that fish exposed to industrial pollutants showed abnormal swimming patterns, opercular movements and other physiological responses.

Noorjahan and Rohini (2015) [25] studied the effect of flavour effluent on growth and biochemical assessment of fish, *Tilapia mossambica*. Their investigation has been carried out to study the parameters of untreated and treated flavour effluent and its effect on growth (60 days) and biochemical components (different tissues) of fish, *Tilapia mossambica*. The results of physicochemical parameters of untreated flavour effluent showed that pH was alkaline and other parameters such as electrical conductivity, total suspended solids, total dissolved solids, biological oxygen demand and chemical oxygen demand were found beyond the acceptable limits of CPCB (1995).

Sumi, and Chitra, (2017) [40] studied the oxidative stress induced by fullerenes and found that exposure to fullerenes at 0.1 mg. L-1 concentrations for 24, 48, 72 and 96 h indicated that nanoparticles have potential roles in inducing oxidative stress in muscle tissue of fish. The activities of antioxidant enzymes such as superoxide dismutase, catalase and glutathione reductase were significantly decreased ($P<0.05$) after 72 and 96 h of exposure to toxins. However, the levels of hydrogen peroxide and lipid peroxidation were significantly increased ($P<0.05$) after 72 and 96 h of treatment in muscle tissue. Their findings provide good evidence that fullerene nanoparticles induced oxidative stress in muscle tissue of fishes. Moreover, the changes in phosphatase enzymes indicate that fullerenes also altered metabolic processes in muscle tissue of fishes.

Parveen *et al*., (2017) [28] studied the effect of tannery waste on haematological parameters of freshwater fish, *Channa punctatus*. The effect of tannery waste on various haematological parameters was evaluated by exposing freshwater fish, *C. punctatus* to tannery waste at different concentrations i.e. [control, 5% tannery waste (TE), 10% TE and 20% TE]. Fish exposed to tannery waste showed significant decrease in haemoglobin (Hb) content (9.16 ± 0.08), red blood cells (3.32 ± 0.12), packed cell volume (34.66 ± 0.33) and mean corpuscular haemoglobin (MCH) values, while white blood cells (WBC), erythrocyte sedimentation rate (ESR) and clotting time were significantly increased with increase in exposure period compared to control. The Hb, RBC and MCHC values showed fluctuating results. The blood parameters decreased from the 15th day of exposure period to the 45th day of exposure period. The decrease in blood parameters clearly indicates that the fish suffered from anaemia due to exposure to tannery waste.

Afolabi (2022) [1] studied the histological effects of local palm oil mill waste on gill, liver, kidney, heart and muscle histology and its hematological effects on African catfish, *Clarias gariepinus*. Histopathological examination of the liver after 14 days of exposure revealed lesions ranging from necrosis, vacuolation and diffuse agglomeration of cells, which worsened with increasing POME waste concentrations. Total white blood count recorded an initial significant increase compared to control, but at higher concentrations of 0.09 mgl-1 and 0.07mgl-1, the count declined.

Moschini *et al*., (2023) [23] studied the biological mechanisms of cell oxidative stress and death during short-term exposure to nano CuO. It is well known that copper oxide nanoparticles (CuO NPs) are highly toxic in in*vitro* systems. The results show that CuO NPs induced oxidative changes immediately after 1 hour of contact, as revealed by increased protein carbonylation and reduced-protein-thiol oxidation. In parallel, cell viability decreased significantly, as shown by the MTT assay.

Prakash & Upadhyay (2022) [31], studied on snake-headed fish, *Channa sp. Bloch* (Actinoptrygii: Channidae) is an important component of inland fishery in the Indian subcontinent and one of the fashionable commercial fish. The industrialized effluents' accomplishment to the aquatic ecosystem is one of the foremost causes of environmental pollution, and its hazardous influence on the exposed edible fishes may be a serious threat to human health. The investigation has been designed to study the effects of sublethal concentrations of paper mill effluent on the serum glucose, serum lactic acid, liver glycogen, and liver lactic acid contents of *Channa punctatus* (Bloch) after in *vitro* exposure for 96 hours. The investigation recorded a significant increase in serum glucose, serum lactic acid, and liver lactic acid contents with a significant quantitative decline in liver glycogen among effluent exposed fishes were recorded during the investigation.

Prakash (2021) [29] studied the effect of paper mill wastewater on antioxidant enzymes in the liver of fish, Channa punctatus. In their investigation, fish, Channa punctatus was exposed to three sub-lethal concentrations (4, 8 and 12% v/v) of paper mill wastewater and the activity of antioxidant enzymes, peroxidase and superoxide dismutase in the liver was examined. The activities of peroxidase and superoxide dismutase enzymes were significantly increased in the liver of fish Channa punctatus exposed to sub-lethal concentrations compared to control. As the concentration of wastewater and duration of exposure increased, the effect became more pronounced.

The presence of ROS in cells activates biochemical reactions that result in a decrease in cellular function due to oxidative damage to proteins, carbohydrates and lipids, which can lead to apoptosis and the accumulation of oxidized molecular aggregates. According to Scandelios (2005), Canizzo *et al*., (2011), aged cells decrease the production of antioxidant enzymes such as SOD, CAT and GPx and increase the accumulation of FR; macrophages, for example, show a decrease in the Mn-SOD enzyme when aged and become sensitive to apoptosis induced by lipid peroxidation.

The hydroxyl radical ($HO^{\bullet}$) produced during phagocytosis can react with amino acids and proteins of microorganisms, damaging biochemical bonds and, consequently, inactivating enzymatic activity, altering active transport through cell membranes, causing cytolysis and cellular destruction, however, HO• can react with the superoxide anion radical and produce singlet oxygen ($^{-1}O_2$), the most harmful form of oxygen. According to Barreiros *et al*. (2006), Biller-Takahashi *et al*. (2013), the $O_2^{\bullet -}$ produced can participate in reactions with other molecules such as NO and produce peroxynitrite, chloramines and consequently hypochlorous acid or hypochlorite (catalyzed by myeloperoxidase, an enzyme present mainly in cells of the innate immune system.

Owolabi and Abdulkareem, (2021) [26] studied the activities of SOD, GPx and MDA levels in blood, gill and liver of Heterobranchus longifilis when exposed to different ZnO-NPs concentrations for 96 hours. They found that Heterobranchus longifilis (mean length ± SD, 10.28 ± 1.34) was exposed to lethal concentrations (0.00, 60.00, 80.00, 100.00 and 120.00 mg/l) and sub-lethal concentrations (0.00, 6.00, 8.00, 10.00 and 12.00 mg/l) of ZnO-NPs for 96 hours and 45 days, respectively. The results showed that during lethal exposure, SOD and GPx activities in the tissues of ZnO-NPs-exposed fish showed slight changes ($p > 0.05$) compared to control, except in liver where GPx showed significant changes ($p < 0.05$). MDA levels in blood and liver increased significantly, while no such changes were observed in gill.

Kumar, *et al*., (2024) [18] studied the effects of mancozeb exposure in fish C. punctatus and found that exposure of test fish to mancozeb for 96 hours caused oxidative stress due to high levels of ROS species. Histopathological abnormalities were observed in the vital organs of the fish of the treated group. DNA damage in erythrocytes in the form of micronuclei (MN) was also observed in fish exposed to the toxic substance. The study clearly shows that the fish, C. punctatus is highly affected when exposed to MZB-contaminated aqueous medium.

Saranraj and Stella (2012) [35] studied the bioremediation of sugar mill wastewater by immobilized bacterial consortium

(Bacillus subtilis + Serratia marcescens + Enterobacter asburiae). Sugar mill effluent to be bio remedied was collected and the physicochemical properties of sugar mill effluent were analyzed. The bacterial consortium was immobilized in the form of beads and used for bioremediation of collected sugar mill effluent. The collected sugar mill effluent was inoculated with immobilized beads (5% inoculum) containing >105 cfu/ml bacterial consortium, and air was continuously passed using an aerator. After 3 and 6 months, the samples were filtered under aseptic condition and physicochemical parameters were estimated. The physicochemical properties of raw sugar mill effluent showed high BOD, COD, TSS, TDS, heavy metals (iron, zinc, copper, lead and manganese). The immobilized bacterial consortium used for bioremediation of effluent showed significant reduction in the levels of COD, TSS, TDS, heavy metals and other physical properties after six months of treatment.

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