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# EFFECT OF PAPER MILL EFFLUENT ON ANTIOXIDANT ENZYME IN THE LIVER OF FISH, *Channa punctatus* (BLOCH, 1793)

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## ABSTRACT

**In the present investigation the fish, *Channa punctatus* was exposed to three sublethal concentrations (4, 8 and 12% v/v) of paper mill effluent and the activity of antioxidant enzymes, peroxidase and superoxide dismutase in the liver were investigated. A significant increased in peroxdase and superoxide dismutase enzymes activities in the liver of fish *Channa punctatus* exposed to sublethal concentrations as compared to control. The effect was more pronounced as the concentration of effluent and duration of exposure increased. The present results suggest that the activities and expression levels of antioxidant enzymes and oxidative stress can be used as biomarker to evaluate the impact of industrial effluent on the biochemical pathways and enzymatic function in the fish.**

**KEYWORDS**: Antioxidant Enzyme, COD, SOD, Paper Mill Effluent

Industrial effluents are the main culprit for undesirable changes in physicochemical properties of water of fresh waterbodies. The industrial effluent is one of the major sources of environmental pollution in developing countries like India. The industrial effluents contain highly toxic chemicals that may pollute the aquatic environment so the safe disposal of waste water discharges from various industries becomes a serious problem worldwide (Tiwari and Prakash, 2021a). In India, about more than two tones industrial wastewater is discharged waterbodies annually and pollutes surface water resources (Verma and Prakash, 2021).

The environmental pollutants can induce changes in metabolic reactions or processes occurring in the body of aquatic organism that inhibits its growth (Prakash and Verma, 2019 & 2020). Alteration in enzymes activities in the exposed fish is one of the major biomarkers indicating the level of changes consequent upon exposure to toxicant / xenobiotics / pollutant in fish that can be recognized and associated with established health impaired processes (Singh and Prakash, 2021). Untreated or partially treated effluents of various industries have toxic substances in the form of inorganic and organic compounds and heavy metals which are protein bound or lipophilic so accumulate in fatty tissues of aquatic animals like fish (Prakash, 2021). Fish is affected by even the smallest changes in physico-chemical condition of water because it always lives in direct contact of water; therefore, these are considered to be the most important bio-indicators in aquatic ecosystems for the estimation of pollutants or toxicants level. Liver, Kidney and Gills of fishes are the pivotal organs involved in osmoregulation, detoxification, biotransformation and excretion of xenobiotics (Vesey, 2010). The pollutants cause disturbance in the physiological state of the fish, affect enzyme activities that causes distortions in the cell organelles and lead to the elevation of various harmful products (Vinodhini and Narayanan, 2009). Impact of toxicant on aquatic animals can be estimated by measuring the changes in histopathological and biochemical parameters of liver, kidney and gills of the fish that respond specifically to the degree and type of pollutants (Srivastava and Prakash, 2019).

Among various water pollutants, industrial wastewater is one of the major contaminants of aquatic environments. The major sources of wastewater are the untreated or semi treated effluents discharging from various industries like chemical, pesticides, fertilizer, pulp and paper, sugar and distillery etc. (Tiwari and Prakash, 2021b). The xenobiotics present in these effluents enter into the fish body through different routes, *i.e.* skin, gills and digestive tract. After entering these xenobiotics distributed and accumulate in various tissues and organs like gills, muscles, liver, kidney, heart and gonads as well as in bloods of fish. At higher concentrations, these xenobiotics induce oxidative damage that may directly affect the cell membrane. The liver in fish is an organ that performs various functions associated with the metabolism of xenobiotics. Hepatocytes like other cells are dependent on antioxidant enzymes for the protection against reactive oxygen

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species produced during the biotransfermation of xenobiotics (Nahed, 2011)

A biomarker is an any biological response to an environmental chemical at the individual level or below demonstrating a departure from the normal status; therefore they are the decisive indicators of the toxic effect (Van der Oost *et al*., 2003). Biochemical markers like protein and enzymes are frequently used as an indicator of the general state of health and early warning of stress in fish under stressful conditions (Eleyele *et al*., 2017). Oxidative stress is often used as a biomarker of the effects of exposure to environmental pollution in aquatic environments. Antioxidants are defined as compounds that can inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Many pollutants (or their metabolites) may exert toxicity related to oxidative stress. Several classes of pollutants, including trace metals and organic compounds, are known to enhance the formation of ROS resulting from xenobiotic redox cycling (Amal *et al*., 2020).

During normal metabolic processes, reactive oxygen species (ROS) are continuously produced at lower concentrations while over-production of ROS is one of the initial responses against oxidative stress in biological systems (Raza *et al*., 2016). Fish as all other aerobic organisms generate endogenous reactive oxygen species (ROS) and other oxidants during aerobic metabolisms and energy production in the mitochondria. Like all aerobic organisms, fish are also susceptible to the toxic effects of ROS that lead to the oxidation of lipids, proteins and nucleic acids (Rigoulet, 2001). The antioxidant enzymes like peroxidase, superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase are reduces the harmful effect of ROS by converting it into less or non harmful products (Cossu *et al.,* 2000). Antioxidant enzymes are considered as sensitive biomarkers for determining the environmental stress prior to the onset of adverse effect on the fish. Among antioxidant enzymes, peroxidase and superoxide dismutase, comprise the antioxidant defence system and are responsible for the removal of hydrogen peroxide which is metabolized to oxygen and water (Van der Oost *et al*., 2003) and also protect the tissues from damage caused by toxicants or pollutants. Fish responded have been used as biomarkers of water pollution. It is important to study the toxic effects of paper mill effluent on fish since they constitute an important link in the food chain. The freshwater snake headed air breathing fish, *Channa punctatus* are widely distributed freshwater fish that can persist in a highly polluted habitat and is possible to use as a potential bioindicator for water pollution. Therefore, the present investigation is aimed to assess the alterations in antioxidant enzyme, peroxidase in the liver of paper mill effluent exposed fish, *Channa punctatus*.

## MATERIALS AND METHODS

The snake headed fish, *Channa punctatus* (45±5 g & 12±5 cm) were collected with the help of fisherman from local fresh waterbodies and then kept in 1% potassium permanganate ($KMnO_4$) for one hour to remove any dermal infection. After this healthy fishes were kept in plastic jar containing 50L of clean tap water and acclimatized for 15 days to the laboratory conditions, during that period they were fed on boiled egg yolk and commercial fish food.

For the present study, the treated paper mill effluent was collected from Yes paper mill Ltd. Darshan Nagar, Ayodhya in polyethylene container. The percent concentration of test solution has been calculated by using the formula: Volume % = $V_E$ / ($V_E$ + $V_{DW}$) X 100; Where, $V_E$ = Volume of effluent , $V_{DW}$ = Volume of dilution water.

The 96h $LC_{50}$ of *Channa punctatus* for treated paper mill effluent was 15% (Prakash and Verma,2020) so, the fishes were exposed to 5% concentrations for three weeks. A control group in dechlorinated water was also maintained in the same environment for same duration. Three replicates for each treatment with ten fish of equal size were maintained for 30 days along with control. The fishes of both groups were regularly fed with commercial food and the medium was exchanged daily.

The fishes of both groups were sacrificed at the end of 21 days than the fish were dissected and their liver was isolated carefully and rinsed with ice cold saline (0.7 NaCl). The tissue, liver was homogenized in 0.25 M sucrose buffer at pH 7.4 and then centrifuged at 10,000 rpm for 15. After centrifugation, the clear supernatant was preserved at - $4^0$C for the enzyme assay. The activity of peroxidase (POD) and superoxide dismutase (SOD) enzymes in these tissues was determined spectrophotometrically at wave length 480 nm by following the method as described by Civello *et al*., (1995) and Misra (1972), respectively. The data in this paper have been presented with mean ± standard error and the statistical significance of difference between control and experimental group was calculated by student's t- test.

## RESULTS AND DISCUSSION

The changes in activity of peroxidase and superoxide dismutase enzymes in liver of *Channa punctatus* after exposure of 21 days to sublethal

concentrations of paper mill effluent as well as in control group were presented in Table 1.

**Table 1: Changes in Peroxdase and Superoxide dismutase enzymes activity in the Liver of *Channa punctatus* after 21 days exposure to sublethal concentrations of Paper mill effluent**

| Conc. of Effluent | POD (U/mL) | | | SOD (unit/g wet wt.) | | |
|---|---|---|---|---|---|---|
| | Exposure Period (Days) | | | Exposure Period (Days) | | |
| | 7 days | 14 days | 21 days | 7 days | 14 days | 21 days |
| Control | 0. 091±0.012 | 0. 092±0.011 | 0. 091±0.014 | 247.0±1.54 | 249.0±1.74 | 251.0±1.84 |
| 4% | 0.108±0.021 (+18.68%) | 0.111±0.021 (+20.65%) | 0.127±0.021 (+39.56%) | 262.0±1.52 (+6.07%) | 295.0±1.44 (+18.47%) | 328.0±1.51 (+30.67%) |
| 8% | 0.142±0.011 (+56.04%) | 0.187±0.24 (+68.46%) | 0.219±0.022* (+140.65%) | 298.0±1.55 (+20.64) | 322.0±1.33 (+29.31%) | 354.0±1.28* (+41.03%) |
| 12% | 0.198±0.021* (+117.58%) | 0.258±0.017* (+180.43) | 0.321±0.018** (+252.74%) | 331.0±1.25* (+34.00%) | 372.0±1.35* (+49.39%) | 411.0±1.25** (+63.74%) |

Significant at *p<0.05; **p<0.001

In aquatic animals, xenobiotics are known to induce oxidative stress by reducing the activities of antioxidant enzymes which results in the enhanced production of reactive oxygen species (ROS) at cellular level that leads to oxidation of bio-molecules (Raza *et al*., 2016). To overcome the toxic effect of ROS, all the aquatic organisms possess antioxidant defence system (Livingstone, 2001). In the present study a non significant increase was in POD and SOD enzymes after 21 days of exposure at 4% concentrations and after 14 days of exposure at 8% concentrations of paper mill effluent. However after 21 days exposure at 8% and after 7, 14 and 21 days of exposure at 12% concentration of paper mill effluent the activities of POD and SOD enzymes were increased significantly. In the control group, lowered values of enzymes, peroxidase and superoxidase dismutase activities may be due to the lower production of ROS. The result of the present study revealed that on exposure to industrial effluent, fish would enhance the production of ROS in response to which activity of antioxidant enzymes increases. The present results are in-conformity with the earlier studies on the fish exposed to different xenobiotics (Vinodhini and Narayanan, 2009; Nahed, 2011;Vinay and Yadav,2014; Raza *et al*., 2016 ).The activity of antioxidant may be increased or inhibited under chemical stress depending on the intensity or concentration and duration of stress applied as well as susceptibility of exposure species (Nahed, 2011). Peroxidase can act as a scavenger to reduce the harmful effects of ROS and converts the hydrogen peroxide into water and oxygen (Aruljothi and Samipillai, 2014). Superoxidase dismutase help in the removal of hydrogen peroxide which is metabolized to oxygen and water (Van der Oost *et al*., 2003). Therefore, after industrial effluent exposure, activities of both enzymes were significantly increased to overcome the severe effects of ROS on biomolecules.

In the present study, increased activities of POD and SOD in the liver of effluent exposed fishes may be attributed to the fact that many metabolic processes as well biotransfermation reactions are occurs in liver. During these processes or reactions reactive oxygen species (ROS) are formed. Fish liver is the main source of antioxidant enzymes to detoxify the environmental pollutants. Therefore, it has been used as an indicator of environmental pollution (Siscar *et al*., 2014). In the present study, the increased activity of peroxidase and superoxidase dismutase in liver of industrial effluent exposed fish, *Channa punctatus* may be explained that liver is an important organ for the production of antioxidant enzymes and therefore protects the fish from oxidative stress. The similar findings have been also observed by various workers (Nahed, 2011;Vinay and Yadav,2014; Raza *et al*., 2016) who reported that the liver is a major organ for the production of antioxidant enzymes and therefore protects organisms from oxidative stress.

## CONCLUSION

Antioxidant enzymes, Peroxidase (POD) and superoxide dismutase (SOD) activity in liver was found to be significantly increased in the paper mill effluent exposed fish as compared to the control. From the results it can be concluded that the activities and expression levels of antioxidant enzymes and oxidative stress can be used as biomarker to evaluate the impact of paper mill effluent on the biochemical pathways and enzymatic function in the fish, *Channa punctatus* so it can be used as a bio-indicator to monitor unacceptable levels of environmental pollution. Human and fish exhibit similar toxicological and adaptive responses to oxidative stress; therefore, this study will be used for ongoing future understanding of mechanisms underlying the oxidative stress response.

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