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Enzymes as Biomarkers of Pollution Stress in *Channa punctatus* (Bloch 1793) collected from Sawan nallaha, Balrampur, U.P.

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ABSTRACT

Oxidative stress, incorporating both antioxidant defences as well as oxidative damage, is a common effect in organisms exposed to xenobiotics in their environment. The studies included in this investigation evaluate the effects of industrial effluent on oxidative stress biomarkers in teleost fishes. So the effect of industrial pollution on the aquatic ecosystem of Sawan nallaha was monitored, by analyzing the different biomarkers antioxidant enzymes. Biochemical parameters in the various tissues (Liver, Kidney, Gills and muscles) of the freshwater snake headed fish, *Channa punctatus* collected from the upstream (non polluted site) and downstream (polluted site) of Sawan nallaha Balrampur. The antioxidant enzymes, Superoxide dismutase (SOD) , Catalase (COD) and Peroxidase (POD) activities in liver, kidney, gills and muscles was found to be significantly increased in fishes inhabiting in polluted site of Sawan nallaha. The results of the present study revealed that the fish like other animals, undergo oxidative stress and respond by changes in activity of the antioxidant enzymes.

Keywords: Biomarker, Pollutants, Oxidative stress, Antioxidant enzymes, *Channa punctatus.*

INTRODUCTION:

Aquaculture is one of the important sectors contributing significantly to the Indian economy. Fish are one of the most important food resources and are considered as economy source of primary protein. The freshwater fishes are key and the nutritive value of fishes are depends on the amount and quality of protein present in muscles of fishes. The amount and quality of nutritive substances gradually degrading due to pollutants present in water bodies. The quality and quantity of nutritive substance in fish tissue is directly depending upon quantity of pollutants present in water. The major fresh water bodies of world are getting polluted due to addition of different polluting effluents. The disease is one of the most important constraints of fish production both in the culture system and in wild conditions. As economically, the production is decreased due to the occurrence of diseases in fishes which are caused by various pathogens in aquaculture.

In the past century the problem of pollution is increased due to pace of the industrialization, use of advanced technologies and modern life style products. The aquatic environment is badly polluted by the wastewater released from industries or factories constructed near the water bodies which deteriorate not only the quality of water but also harm the aquatic animals. (Javed and Usmani, 2013). The increasing level of industrial effluent in aquatic ecosystem is dangerous for fish growth and reproduction and for human beings. Rapid industrialization is contaminating natural freshwaters by the heavy metal use of metals mainly iron, zinc, copper, lead, nickel, and manganese, turning it into a global problem. Heavy metals can show high toxicity even in low concentration producing cumulative deleterious effects in an aquatic ecosystem (Aisha *et al*, 2022). The concentration and composition of industrial effluents brings physiological and behavioral changes in the fishes living in the environment. When the

living animals are exposed to polluted water it causes to damage the internal organs too.

In the aquatic environment, toxicants present in minimal levels also become part of various food chains through biomagnification and their concentration increases to such a level that may prove to be hazardous to both humans and other living organisms (Prakash, 2020). Heavy metals considered as the most toxic pollutants in aquatic system. These are important inducers of oxidative stress in aquatic animals, promoting the formation of reactive oxygen species (ROS) which ultimately leads to tissue damage and oxidation of biomolecules. Aquatic organisms possess antioxidant system to cope with the oxidative stress (Shaukat *et al*., 2018).

The main intake routes of chemicals in fish are the gills, skin and the gut (via the food ingestion). In case of absorption through gills and skin, chemicals go directly into the bloodstream and to the most of the organs, while ingested chemicals first undergo initial intestinal metabolism, absorption and hepatic biotransformation (Dina Tenji *et al*, 2021). Fish, top consumer of the aquatic food chain have been largely used in the evaluation of the quality of aquatic systems. These may concentrate a large amount of toxicants from the surrounding waters which are dangerous not only to fish growth and reproduction and are also their consumer like human beings. Because of increasing industrial practice, various chemicals and metals enter the waterbodies and are enters into human beings via food chain (Vutukuru *et al*., 2007).

Exposure to water pollutants may modify fish behavior, metabolism, physiology, growth, and reproduction. Accumulation of industrial, sewage and domestic wastewater in water bodies retards the self regulatory capabilities of aquatic organisms including fishes. They are also responsible for introducing genetic and teratogenic effects in fish. Thus fishes cannot escape from the negative effects of these contaminants or pollutants and prove as good bio-indicators of aquatic pollution (Pandey and Madhuri, 2014).

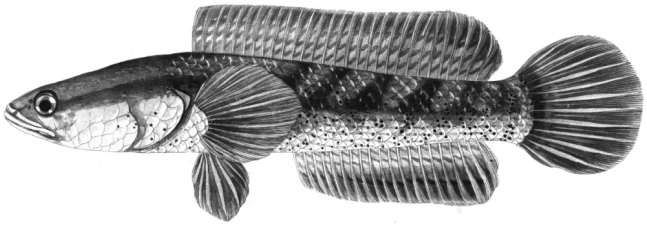
Fish as inhabitant of aquatic system cannot avoid the inimical effects of the pollutants. A set of biomarkers is generally used to evaluate the biological effects of pollutants. Such biomarkers act as an early warning of a specific detrimental biological endpoint. Oxidative stress and histopathologic biomarkers are used in ecotoxicology (Faheem and Line, 2017). In biological systems, oxidative stress has become an area of significant interest for aquatic toxicological studies. The problems associated with pollutants exposure indicate the biochemical stress in the body by generating imbalance between reactive oxygen species (ROS) and antioxidants thereby inducing oxidative stress (Yadav *et al*., 2015). Enzymatic and non-enzymatic antioxidants are important defense mechanism of organisms which provide protection against environmental pro-oxidants by countering the impact of reactive oxygen species (Tabrez, Ahmad, 2009). Therefore, antioxidant parameters and oxidative stress indices are considered potential biomarkers and are frequently used as screening tools to assess the impacts of environmental stress. Biomarkers should primarily be used as a tool for predicting the state of biota under stochastic environmental conditions (Dina Tenji *et al*, 2020).

About 75% of aquatic pollution is due to directly discharged of untreated or partially treated industrial effluents, sewage and domestic waste. The water and soil of such waterbodies containing oxidzable and fermentable matters causes depletion of dissolve oxygen in the receiving water bodies affecting the aquatic flora and fauna severely. Though, almost all lotic and lentic waterbodies of Balrampur district of the Terai region, of eastern U.P. are free from the industrial pollution and have unique capacity of purification through self regulatory mechanism, yet there is one small lotic freshwater body called Sawan nallaha is a highly polluted river. This river receives wastewater from sugar mills, distillery, domestic sewage and other chemical effluents along with the agricultural runoff and domestic sewage and after traveling about 7 km join to the River Rapti.

Fish tissue, generally the liver, kidney and gill, have antioxidant defense mechanisms consisting of catalase, superoxide dismutase and peroxidase etc. to protect them from the oxidative effect of heavy metals (Basha and Rani, 2003). The antioxidant enzymes are capable to protect the tissues by stabilizing, neutralizing and or deactivating the effects of free radicals (oxidative damage) (Srivastava and Reddy, 2020). These enzymes provide the first line of defense against oxygen-derived free radicals and protect the fish tissues from oxidative stress. Antioxidant enzymes can be used as biomarkers of exposure to aquatic contamination (Ahmad *et al*., 2000). However, antioxidant enzymes exhibit different activities between the cells, tissues and organs of saltwater fish and freshwater fish depending upon feeding habitat, environmental conditions and other ecological conditions.

Freshwater snakeheaded fish, *Channa punctatus* (Order: Ophicephaliformes; Family: Channidae) is an air breathing hardy fish and can survive in polluted fresh waterbodies. The availability of data regarding antioxidant enzymes with reference to heavy metal stress on this species is very limited. So, present study was designed to assess the activity of various antioxidant enzymes in

Channa punctatus inhabiting in the highly polluted Sawan nallaha, a small lotic fresh waterbody.



Channa punctatus (Bloch 1793)

MATERIAL AND METHODS:

Collection of fish: The live adult *Channa punctatus* (8.5±0.5cm;12.5±0.32g) were caught from two different sampling sites of Sawan Nallaha (27º24'41.2″N and 82º11'06.1″E) by means of a cast net and with the help of local fisherman during summer season. The unpolluted site was located at upstream about one km before and another polluted site was located about one km away from the point where Sawan nallaha, receive industrial waste water from both industries and domestic sewage water of Balrampur city. The collected fishes were placed in two separate containers (Upstream and downstream) with Sawan river and transported immediately to the laboratory for oxidative stress examinations.

Biochemical assays of Antioxidant enzymes:

The collected live fishes were washed and anaesthetized by 0.1 g/L of benzocaine than liver, kidney, gill and muscles tissues were dissected out for the study of enzymatic antioxidants. For enzyme analysis, fish was dissected and the organs viz. liver, kidney, gills and muscles were collected. Tissue samples to be processed for the determination of enzyme activities were homogenized (1:4, w/v) in phosphate buffer (pH 6.5). Homogenate samples were centrifuged at 10,000 rpm for 15 min. at 4ºC. A supernatant fraction was used to check the activity of various enzymes.

Superoxide dismutase: The activity of Superoxide dismutase (SOD) in liver, kidney, gills and muscles tissue was determined by an indirect method given by Worthington (1988). The technique is based on the ability of superoxide dismutase (SOD) to inhibit the photoreduction of nitroblue tetrazolium. The absorbance was noted on a digital spectrophotometer (Model AN-10-03; Zenith Engineers, Agra) at 560 nm and expressed as µ/mg protein.

Catalase: Catalase (COD) is a universally known antioxidant enzyme that degrades the hydrogen peroxide (H_2O_2) into water and oxygen. The activity of catalase was calculated by determined by measuring its ability to reduce the hydrogen peroxide concentration at 240 nm Aebi (1984). The reaction mixture (2 mL) contained 1.95 mL of buffered substrate solution and when 0.05 mL enzyme extract was added, the reaction started in the cuvette and absorbance was recorded at 240 nm on the digital spectrophotometer within 3 minutes.

Peroxidase: The activity of Peroxidase (POD) was determined by measuring its capacity to decrease the hydrogen peroxide concentration at 470 nm (Zia *et al*., 2011). The spectrophotometer was set at 470 nm wavelength, after inserting the blank solution. Then 0.02 mL of crude extract was added in 1 mL of buffered substrate solution and the absorbance was noted after 3 minutes.

Statistical Analysis: The biochemical biomarkers data were expressed as mean ± SD (N= Number of sample). The data were analyzed by the student t-test using the SPSS software.

RESULTS AND DISCUSSION:

Water pollution of river in India has now been reaching to a point of predicament due to unplanned urbanization and rapid growth of industrialization (Katiyar *et al*., 2021). Water quality plays a vital role in regulating various metabolic and physiological processes occurring in the body of aquatic animals including fishes. The activities of antioxidant enzymes in the liver, kidney, gills and muscles of *Channa punctatus* caught from two different sampling sites of Sawan Nallaha are presented in Table1. Results clearly indicates that the SOD, CAT and POD activities were significantly increased in liver, kidney, gills and muscles tissues of fish collected from the downstream ($P<0.05$ & $P<0.01$). The SOD activity increases in liver, kidney, gills and muscles from 10.52 to 20.81unit/mg, 23.81 to 45.24 unit/mg, 29.32 to 52.45 unit/mg and 15.71 to 26.23 unit/mg, respectively. The COD activity increases in liver, kidney, gills and muscles from 2.61 to 4.56 n mole/min/mg, 2.11 to 4.02 n mole/min/mg, 4.57 to 7.89 n mole/min/mg and 2.43 to 4.32 n mole/min/mg, respectively. The POD activity increases in liver, kidney, gills and muscles from 0.098 to 0.130 unit/mg, 0.092 to 0.111 unit/mg, 0.86 to 0.105 unit/mg and 0.055 to 0.072 unit/mg, respectively. Increased levels of the enzymes activity indicate the oxidative stress. On exposure to industrial waste water, fish would enhance the production of reactive oxygen species (ROS) in response to which activity of antioxidant enzymes increases (Prakash, 2021). Many xenobiotics use general toxicity mechanism by causing oxidative stress in fish which is a pathological process related to increase production of ROS (Nazish, *et. al*., 2018).

Table1. Enzymatic activities in freshwater fish, *Channa punctatus* in Sawan nallaha, Balrampur

(Values are Mean ± SD of four individual fish)

Tissues Sites	Superoxide dismutase (Unit/mg)		Catalase (n mole/min/mg)		Peroxidase (Unit/mg)	
	Upstream (Non Polluted Site)	Downstream (Polluted Site)	Upstream (Non Polluted Site)	Downstream (Polluted Site)	Upstream (Non Polluted Site)	Downstream (Polluted Site)
Liver	10.52±0.7	20.81±1.2** (+97.81%)	2.61±0.5	4.56±2.3* (+74.71%)	0.098±0.01	0.130±0.03* (+32.65%)
Kidney	23.81±2.1	45.24±3.5** (+90.00%)	2.11±0.3	4.02±0.7** (+90.52%)	0.092±0.02	0.111±0.01* (+20.65%)
Gills	29.32±3.2	52.45±4.1* (+78.89%)	4.57±0.2	7.89±0.5* (+72.65%)	0.086±0.03	0.105±0.05* (+22.09%)
Muscles	15.71±2.7	26.23±1.9* (+66.96%)	2.43±0.5	4.32±0.7* (+77.78%)	0.055±0.2	0.072±0.4* (+30.90%)

**Significant at 5% level (P<0.05); ** Significant at 1% level (P<0.01)*

The order of the percentage of SOD increase in fish tissues was Liver> Kidney>Gills > Muscles; percentage of COD increase Kidney>Muscles>Liver>Gills and the percentage of POD increase was Liver>Muscles>Gills>Kidney. The results also indicate that the percentage of enzymatic activities of different antioxidant enzymes increases differently in different tissues. Samanta *et al.,* (2018) and Srivastava & Reddy (2020) also confirmed increased activities of antioxidant enzymes in freshwater fishes collected from different rivers. The activities of antioxidant enzymes SOD, COD and POD in the hepatic tissues of paper mill effluent exposed fish *Channa punctatus* was significantly increased as compared to control (Prakash, 2021). In contrast the Kim and Jung (2016) has shown a significant decrease in the enzymatic activities of SOD, COD and POD in the liver of fish, *Zacco platypus* caught from downstream. The activity of antioxidant enzyme 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).

CONCLUSION:

The antioxidant enzymes, Superoxide dismutase (SOD), Catalase (COD) and Peroxidase (POD) activities in liver, kidney, gills and muscles was found to be significantly increased in the Sugar factory and its distillery unit effluent exposed fishes collected from down streams. Increased levels of the enzymes activity indicate the oxidative stress. For this reason, fishes can be used as model animal and /or bioindicator of oxidative stress induced by aquatic pollutants.

The results of the present study clearly indicate the threat posed by continuous discharge of treated effluents in the river may impair the fish health by inducing oxidative stress. Thus 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 industrial waste water.

Fishes serve as a bio-indicator to assess the water pollution level caused by anthropogenic activities in an aquatic ecosystem and also predict hazardous effects of pollutants on aquatic animals and their consumers. Since fish are rich source of protein and lipids so its health is very important for humans. Therefore, the protection of aquatic waterbodies and their water quality will be possible only with the judicious and rationalized applications of chemicals and metals.

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Declaration: *We also declare that all ethical guidelines have been followed during this work and there is no conflict of interest among authors.*

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