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IDENTIFICATION OF BIOMARKERS OF OXIDATIVE STRESS IN FRESHWATER FISH (CIRRHINUSREBA) AFTER EXPOSURE TO INDUSTRIAL WASTE IN KANPUR

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ABSTRACT: Exposure to heavy metals may modify fish behavior, metabolism, physiology, growth, and reproduction. They are also responsible for introducing genetic and teratogenic effects in fish. Now a day's in many regions; fish production is decreased due to the occurrence of diseases caused by different pathogens in aquaculture. The increasing level of trace elements is dangerous for fish growth and reproduction and for human beings. Rapid industrialization is contaminating natural freshwater by heavy metals (Fe, Zn, Co, Pb, Ni, Cr and Mn), turning it into a global problem. The effect of pollution on the aquatic ecosystem of river Ganga was monitored, and a field study was conducted by analyzing the different biomarkers enzymes. Biochemical parameters in the various tissues (muscles, liver, gills, and kidney) of the Indian carp *Cirrhinusreba* collected from the river Ganga from different study sites of Kanpur. The catalase peroxides, hydrogen peroxides, and protein content level significantly increased as compared upstream to downstream. The catalase activity in the tissue of carp fish was found to be significantly (<0.05) increased up to 4.417 ± 0.02 ($\mu\text{moles}/\text{min}/\text{mg}$ of total protein). This imbalance may lead to damage of tissues and cellular components, which in turn would trigger the induction of antioxidant defense mechanisms. The depletion of tissue protein in content under toxicity stress has already been reported. Thus, it clearly shows that oxidative damage and decline in antioxidant defense due to malathion-induced oxidative stress.

INTRODUCTION: Aquaculture is one of the important sectors contributing significantly to the Indian economy. Fish culturists are encouraged towards intensifying the culture system to increase production and profit, such as fish and shrimp farming, the disease becomes major threats.

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.

Fish have been largely used in the evaluation of the quality of aquatic systems. These organisms are often at the top of the aquatic food chain and may concentrate a large amount of metals from the surrounding waters. The concentrations of trace elements are dangerous not only to fish growth reproduction and are also for human

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beings. Because of increasing industrial practice, heavy metals enter the aquatic environment and are transferred to human beings through the food chain. Minerals perform important roles in osmoregulation, intermediary metabolism and in the formation of skeleton, healthy scales, teeth, and bones³¹. Exposure to heavy metals may modify the fish behavior, metabolism, physiology, growth and reproduction, and reproduction and are also responsible for introducing genetic and teratogenic effects in fish.

The most abundant fresh fish found in Ganga is *Cirrhinusreba* (common carp). This species is economically important and can grow to a maximum weight of 8-39 kg and the oldest recorded age of 65 years. Aquatic organisms have a specific ability to regulate essential metals up to a certain level; however, this ability is disrobed under continual exposure to initiate metals accumulation in the body organs beyond their permissible limits. Recent years have witnessed significant attention being paid to environmental pollution problems by a wide variety of chemical pollutants, including heavy metals. 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. The concentration of metals in sediments depends on the types of rocks or soils present along the watershed. Fishes are animals that cannot escape from the negative effects of these contaminants and prove as good bio-indicators of aquatic pollution²².

Fish foods are very important for human nutrition, and those from contaminated sites pose a potential risk to human health. Since fish occupy the top of the aquatic food chain, they are suitable bioindicators of metal contamination. Metals are well-known inducers of oxidative stress, and the assessment of oxidative damage and antioxidant defenses in fish can reflect the contamination of the aquatic environment¹⁸. Enzymes are necessary for normal cellular metabolism. Their activities are considered sensitive biochemicals for normal cellular metabolism. Their activities are considered sensitive biochemical of imminent hazardous effect in fish. Fish constitute an excellent model for understanding the oxidative and metabolic stress in an aquatic ecosystem. Indian major carps are of

great commercial importance and the most common freshwater fish widely consumed. For aquatic ecosystem, fish can serve as a good model to study responses to various pollutants. Gills play a multifunctional role in performing dynamic functions such as osmoregulation, acid-base balance, respiration, and excretion of nitrogenous wastes¹¹. Metals can enter into the gills by attaching with mucus layer of the gills and cause alteration in the ultrastructure and general morphology of fish gills⁵. The liver is the major place for the detoxification of toxic chemicals and also plays a significant role in the metabolism and excretion of toxic substances¹². The fish liver is the main source of antioxidant enzyme GPx and shows the higher activity of this enzyme compared to the other organs to overcome the oxidative stress caused by heavy metals.

MATERIALS AND METHODS

Study Area: The study of Ganga river water quality was undertaken during April 2019 at Kannauj and Kanpur, India. The water sample was collected from Mehandi Ghat (Kannauj), Nanamau, Shivrajpur, Bithoor, Gangabairaj, Parmath, Shuklaganj, Jajmau, and Wajidpur at Kanpur region. In contrast, fish health studies were undertaken during the same periods in different sampling points such as Bithoor, Gangabairaj, Parmath, Shuklaganj, Jajmau, and Wajidpur Kanpur region.

Collection of Test Organism: The freshwater air-breathing white fish, *Cirrhinusreba* (Body mass, BM = 20.250 gm; Body length, BL = 17 cm) was collected with the help of local fisherman with the help of dragnet, gill net, cast net, scoop net and hook and line from water bodies located in the sub-region of Kanpur. These fishes were carried to the laboratory in large mouthed plastic containers in natural water to avoid stresses and injuries as far as possible.

Estimation of Sample Water: The water quality of river Ganga is being monitoring at various locations and assessed based on the physico-chemical parameters. These surface water sample per site was taken and analyzed for the following parameters: TDS, Turbidity, temperature, EC, pH were analyzed by the digital Kit HANNA (HI98129) and Globe Instruments (ISO9001),

hardness, chloride, Alkalinity, Dissolved oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand Demand (COD) was estimated⁴. The water sample was an estimation of Nitrate by methods of Kit of AQUA Check (WT013) and Nitrate estimated by methods of Kit of AQUA Check (WT007C).

Biochemical Analysis: In the freshwater, *Cirrhinusreba* was exposed to various sites of river Ganga in the Kanpur region. The impact of heavy

metal with reference to biochemical enzymes analyzed by different methods such are antioxidative catalase 2, 21, H₂O₂³⁰, peroxidizes¹⁰, and protein¹⁹.

Statistical Analysis: The biochemical biomarkers data were expressed as mean \pm SD (n= no. of the sample). The data were analyzed by the student t-Test using the SPSS- 10 statistical programs; significance is noted as *, p < 0.05 or **p < 0.01.

TABLE 1: PHYSICO-CHEMICAL CHARACTERISTICS OF RIVER WATER FROM UPSTREAM (KANNAUJ) TO DOWNSTREAM (WAJIDPUR) DURING APRIL 2019

Sampling Sites	Physico- Chemical Parameters											
	Temp.	Turbidity NTU	TDS (ppm)	pH	EC mS/cm	TH (mg/l)	TA (mg/l)	DO mg/l	BOD (mg/l)	COD	Nitrate (ppm)	Nitrite (ppm)
WHO Standard	25 °C	Up to 10	Up to 100	6.5-8.5	250	50-300	80-200	5-14.7	2-8	<250	0.01-4.00	0-1
Mehndighat	27.8 °C	25.466 ± 0.35	165.333 ± 3.05	8.233 ± 0.25	324.666 ± 5.85	124.000 ± 17.77	199.666 ± 1.52	6.276 ± 0.31	20.366 ± 0.39	68.333 ± 5.77	46.666 ± 2.88	2.833 ± 0.28
Nanamau	27.4 °C	20.066 ± 0.15	141.000 ± 2.64	8.940 ± 0.06	299.666 ± 1.52	137.000 ± 17.05	210.333 ± 2.51	6.966 ± 0.14	18.300 ± 0.66	58.666 ± 1.15	10.000 ± 0.00	1.266 ± 0.46
Shivrajpur	27.0 °C	16.666 ± 1.15	134.666 ± 3.05	9.206 ± 0.07	275.000 ± 3.60	156.333 ± 19.08	219.333 ± 3.78	7.046 ± 0.13	14.826 ± 0.36	26.000 ± 1.73	10.000 ± 0.00	0.850 ± 0.13
Bithoor	28.1 °C	12.433 ± 0.35	128.333 ± 1.15	9.533 ± 0.05	257.000 ± 1.00	200.000 ± 27.71	286.666 ± 5.77	7.400 ± 0.39	12.300 ± 0.32	24.100 ± 0.36	08.333 ± 2.88	0.766 ± 0.25
Ganga Bairaj	27.8 °C	10.466 ± 0.30	144.666 ± 3.05	8.870 ± 0.12	284.000 ± 2.64	214.666 ± 8.08	260.000 ± 0.00	7.486 ± 0.46	12.226 ± 0.65	24.000 ± 0.40	10.000 ± 0.00	0.333 ± 0.14
Permath	27.8 °C	14.233 ± 0.41	146.000 ± 2.64	8.800 ± 0.60	289.666 ± 3.51	220.000 ± 0.00	265.000 ± 30.41	7.433 ± 0.70	13.546 ± 0.38	28.066 ± 0.11	10.000 ± 0.00	1.866 ± 1.55
Shuklaganj	28.0 °C	11.766 ± 0.55	156.333 ± 8.08	9.400 ± 0.10	301.666 ± 8.02	208.000 ± 2.00	235.000 ± 8.66	7.006 ± 0.03	16.140 ± 0.42	64.000 ± 0.40	10.000 ± 0.00	0.250 ± 0.00
Jajamau	27.5 °C	13.033 ± 1.44	170.000 ± 4.58	9.433 ± 0.11	335.333 ± 3.51	234.000 ± 3.46	238.333 ± 2.88	6.790 ± 0.20	18.823 ± 0.92	47.833 ± 0.53	19.666 ± 10.11	1.800 ± 0.00
Wajidpur	27.1 °C	11.366 ± 0.55	191.000 ± 6.24	9.766 ± 0.20	362.000 ± 25.53	203.333 ± 4.16	230.000 ± 0.00	6.013 ± 0.03	21.073 ± 0.43	80.213 ± 1.11	10.000 ± 0.00	2.000 ± 0.00

TABLE -2: BIOCHEMICAL ACTIVITY IN FRESHWATER CARP FISH (CIRRHINUSREBA) IN GANGA RIVER AT KANPUR REGION.

Sampling sites	Organ	Enzymatic activity			
		Catalase ($\mu\text{moles/min/mg}$)	Peroxidase (Unit/ml)	Hydrogen peroxide ($\mu\text{g g}^{-1}$ f. wt.)	Protein ($\mu\text{g g}^{-1}$ f. wt.)
		Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
SITE 1	Kidney	1.800 \pm 0.11	0.095 \pm 0.01	0.083 \pm 0.01	099.346 \pm 0.41
	Gills	4.243 \pm 0.20	0.089 \pm 0.02	0.119 \pm 0.02	043.300 \pm 5.56
	Muscle	2.433 \pm 0.19	0.052 \pm 0.02	0.038 \pm 0.01	146.016 \pm 0.19
	Liver	2.636 \pm 0.11	0.113 \pm 0.01	0.077 \pm 0.01	131.756 \pm 5.56
SITE 2	Kidney	2.393 \pm 0.04	0.098 \pm 0.02	0.084 \pm 0.01	106.936 \pm 2.37
	Gills	4.556 \pm 0.15	0.092 \pm 0.01	0.129 \pm 0.01	047.786 \pm 1.21
	Muscle	2.713 \pm 0.07	0.059 \pm 0.01	0.043 \pm 0.01	159.746 \pm 0.91
	Liver	3.220 \pm 0.11	0.118 \pm 0.02	0.081 \pm 0.00	197.863 \pm 1.83
SITE 3	Kidney	3.046 \pm 0.08	0.107 \pm 0.01	0.088 \pm 0.01	116.970 \pm 1.99
	Gills	5.140 \pm 0.09	0.099 \pm 0.02	0.133 \pm 0.01	064.156 \pm 1.58
	Muscle	3.656 \pm 0.15	0.065 \pm 0.03	0.046 \pm 0.01	184.303 \pm 10.19
	Liver	3.556 \pm 0.04	0.124 \pm 0.02	0.084 \pm 0.01	154.993 \pm 2.54
SITE 4	Kidney	3.613 \pm 0.08	0.112 \pm 0.01	0.094 \pm 0.02	127.266 \pm 1.20
	Gills	5.733 \pm 0.06	0.109 \pm 0.02	0.133 \pm 0.01	075.776 \pm 2.78
	Muscle	4.063 \pm 0.13	0.074 \pm 0.02	0.051 \pm 0.01	197.506 \pm 7.81
	Liver	3.773 \pm 0.25	0.132 \pm 0.05	0.087 \pm 0.01	166.080 \pm 2.28

SITE 5	Kidney	4.266 ± 0.13	0.118 ± 0.01	0.098 ± 0.01	135.186 ± 4.83
	Gills	6.203 ± 0.13	0.114 ± 0.02	0.138 ± 0.01	083.696 ± 3.20
	Muscle	4.543 ± 0.09	0.083 ± 0.01	0.054 ± 0.01	210.706 ± 4.19
	Liver	4.266 ± 0.04	0.136 ± 0.01	0.088 ± 0.03	173.740 ± 1.21
SITE 6	Kidney	4.340 ± 0.08	0.119 ± 0.01	0.097 ± 0.01	134.660 ± 4.40
	Gills	6.113 ± 0.09	0.113 ± 0.01	0.137 ± 0.01	083.696 ± 3.20
	Muscle	4.500 ± 0.06	0.082 ± 0.01	0.055 ± 0.00	213.346 ± 2.54
	Liver	4.283 ± 0.02	0.136 ± 0.01	0.087 ± 0.02	173.740 ± 1.21

Result: The result of physicochemical qualities of Ganga river freshwater samples were determined after the sampling of nine different sites like Mehdighat, Nanamau, Shivrajpur, Bithoor, Ganga Bairaj, Parmath, Shuklaganj, Jajmau, and Wajidpur at Kanpur region. The river water temperature was found in different site ranges between 27.1- 28.1 °C with an average temperature of 27.0 °C ± 0.6 °C. The estimated turbidity values at different sites were varying from 10.46 to 25.46 NTU and increase seen in Mehdighat while decreasing as seen in Ganga Bairaj. The TDS value in Mehdighat was 165.3 ± 3.05 mg/l, while in Jajmau 170.0 ± 4.58 mg/l was observed. This shows a decrease in TDS as compared to Ganga Bairaj and Wajidpur. Observations revealed that the value of pH was lowest at Mehdighat, while the highest pH was recorded in Wajidpur. However, the pH range gradually increases from Mehdighat to Wajidpur, but a slight decrease is seen in Ganga Bairaj and Parmath sampling point. The values of TA, TH, DO, COD, BOD, Nitrate, and Nitrite are also shown in **Table 1**.

Biochemical Analysis: In the freshwater of River Ganga carpfish, *Cirrhinusreba* was exposed to various sites of Ganga river at Kanpur region the alteration of heavy metals (present at Ganga river water) in biochemical parameters for protein and enzymes such as catalases, peroxidase, hydrogen peroxide and protein. In the present investigation, Indian surface freshwater carp *Cirrhinusreba* was exposed to the Ganga river. The alteration of organic and inorganic components in carp fish influenced of biomarkers catalase activity, peroxide activity, hydrogen peroxide activity, and protein content. During the study period, catalase activity in the tissue of kidney was found to be significantly (<0.05) increased Bithoor -0.281 ± 0.06 (µmoles/min/mg of total protein) to Wajidpur-4.417 ± 0.02 and also increased in liver- 1.147 ± 0.02 to 3.037 ± 0.06 (µmoles/min/mg of total protein) and muscles -3.473 ± 0.06 to 5.333 ± 0.06

(µmoles/min/mg of total protein) found significantly. The maximum catalase level in Carp *Cirrhinusreba* recorded during the sampling site Bithoor to Wajidpur is shown in **Table 2**. The peroxidase enzyme activities were also increase in significant under the stress condition and recorded to be Kidney 0.100 ± 0.00 to 0.132 ± 0.01 (Unit/ml), gills 0.090 ± 0.01 to 0.128 ± 0.02 (U/ml), muscle 0.049 ± 0.02 to 0.077 ± 0.02 (U/ml), liver 0.119 ± 0.00 to 0.149 ± 0.01 (U/ml) significant. The maximum peroxidase activity level in carp *Cirrhinusreba* were recorded during the sampling site Wajidpur shown in **Table 2**.

The hydrogen peroxide enzyme activity was also increased in under the stress condition of presented heavy metal and recorded to be kidney 0.140 ± 0.00 to 0.159 ± 0.00 (µmol g⁻¹ f. wt.), gills 0.079 ± 0.00 to 0.095 ± 0.00 (µmol g⁻¹ f. wt.), muscles 0.098 ± 0.00 to 0.113 ± 0.00, liver 0.103 ± 0.00 to 0.121 ± 0.00 (µmol g⁻¹ f. wt.), significant. The maximum hydrogen activity level in carp *Cirrhinusreba* were recorded during the sampling site Bithoor to Wajidpur shown in **Table 2**. The protein content was also increased in under the stress condition and recorded as significant in kidney 184.56 ± 2.09 to 237.908 ± 1.21 (µg g⁻¹ f. wt.), gills 128.06 ± 1.20 to 163.182 ± 3.16 (µg g⁻¹ f. wt.), muscle 144.69 ± 1.20 to 177.17 ± 1.20 (µg g⁻¹ f. wt.), liver 127.65 ± 2.19 to 167.935 ± 1.58 (µg g⁻¹ f. wt.). The maximum protein content level in carp *Cirrhinusreba* was recorded at the sampling site Wajidpur shown in **Table 2**.

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. Various studies about the water quality of the Ganga river are available, although complete middle stretch data from Kannauj to Wajidpur are not available, which is the most important concern to fisheries and human life. The physicochemical parameters of the Ganga river

water for this region revealed the adverse impact of pollution. Surface water temperature varies considerably during the post-monsoon period, a full record of water temperature may also be attributed to low macrophytic production and the highest load of suspended matter. Temperature and pH are important aspects of the aquatic ecosystem as they play a key role in determining other parameters such as conductivity, saturation stage of gases, and different forms of alkalinity²⁷.

The turbidity value varying from 25.4 ± 0.35 NTU to 10.4 ± 0.30 NTU increase seen in Mehndighat and decrease seen in Ganga Bairaj and inhibited other sampling sites as shown in **Table 1**. Turbidity of water is an essential parameter, which manipulates the light break through inside water and thus affects the aquatic life. The reading of turbidity ranged between 10.46 to 25.46 NTU. The maximum and minimum turbidity was found at 10.48 NTU and 25.46 at Ganga Bairaj. Excessive turbidity in water can cause water purification processes such as flocculation and filtration, which may increase treatment costs. High turbid waters are associated with microbial contamination. Again turbidity causes a decrease in the photosynthesis process since turbidity precludes deep penetration of the light of water.

The electric conductivity (EC) varies from 257.0 to 362.0 $\mu\text{S}/\text{cm}$ values from Mehndighat to Wajidpur. During the sampling dates for the reason that domestic sewage as well as industrial influents direct release in the Ganga. On the other hand, the leaching of chemical fertilizers spread on agricultural lands by rainwater also causes high water conductivity²⁵. A Study reported that EC values between 990 to 1285 mg/l were observed in Yamuna river at Agra 13, which are much higher than those of the present study. High EC values are indicating the presence of a higher amount of dissolved inorganic substance in ionized form. pH is one of the important tools to measure acidity or alkalinity in water. Aquatic organisms are sensitive for pH change due to any change in the pH cause changes in the structure of the aquatic system. pH of river Ganga varied from 8.233 ± 0.25 to 9.766 . According to a study, the lowest pH at Mehndighat and highest pH in Wajidpur showed that the pH of the water was higher mostly during the monsoon period. However, an acceptable pH for drinking

water is specified as 6.5-8.51. The pH of different aquatic ecosystems reveals the health and biological characteristics of those systems. The pH value of the Ganga river waterfalls between slightly acidic to moderately alkaline and has a relationship with the solubility and accumulation of heavy metal in river water and sediment.

The hardness of water is not a pollution indicator parameter but an indicator of water quality, mainly in terms of Ca^{2+} and Mg^{2+} bicarbonate, sulphate, chloride, and nitrates. Total hardness value in Ganga river water varied from 124.0 ± 1.77 mg/l to 234.0 ± 3.46 mg/l and increased to 203.3 ± 4.16 mg/l in Wajidpur, respectively. This indicates that water with less than 75 mg^{-1} of CaCO_3 is considered soft and above 75 mg^{-1} of CaCO_3 as hard⁹. The hardness of water is an important criterion for determining the usability of water for domestic, drinking, and various industrial purposes²⁰. It is an important Total hardness due to carbonate and bio carbonate ranged from 124.0 ± 1.77 mg/l to 234.0 ± 3.46 mg/l in Mehndighat & Wajidpur. These observed values were slightly higher than the prescribed limit of WHO and ISI (150 and 200 mg/l). Although alkalinity has little public health significance, highly alkaline waters are unpalatable and are not used for domestic water supply¹³. Water hardness refers to the concentration of divalent calcium, magnesium, strontium, ferrous, and manganese ions. It is derived from soil and rock erosion.

Dissolved Oxygen is the essential parameter in water quality assessment. Its presence is essential to maintain a variety of life forms in the water, and the effect of waste discharge in a water body is largely determined by the discharge of the oxygen demanding waste. Inorganic reducing agents such as H_2S , ammonia, nitrite, ferrous iron, and certain oxidizable material also decrease dissolved oxygen in the water. In the present study, the overall lowest and highest value of DO was observed 6.013 ± 0.03 mg/l and maximum DO 7.486 ± 0.03 mg/l was found at the Ganga Bairaj site, while the minimum DO 6.013 ± 0.03 mg/l was found at Wajidpur site. The dissolved oxygen (DO) appears to be due to its greater solubility, reduced microbial decomposition of dead organic matter and low organism respiratory demand at low temperature, and increased progressive growth of submerged

macrophytes. The biochemical Oxygen demand ranged between 12.3 ± 0.32 mg/l to 21.0 ± 0.43 mg/l, the higher DO in the surface water due to the depth of mixing of air with water temperature. The minimum BOD 12.3 ± 0.32 mg/l was found at Bithoor and Ganga Bairaj, while the maximum BOD 21.0 ± 0.43 mg/l. was originate at Wajidpur for the reason that the higher BOD in water due to direct release of influence into the water bodies.

The biological oxygen demand (BOD) value varies from 2.2 to 13.5 in pre-monsoon and 4.5 to 150 post-monsoon seasons. The BOD values clearly indicated pollution, which may be attributed to the maximum biological activity. Ray and David noticed that oxygen and high BOD went hand in hand at the sight in the river Ganga where sewage pollution was taking place ²⁴. A study observed that the BOD was ranged from 66.2 ± 3.4 to 87.5 ± 2.9 mg/l at Rajghat (Varanasi) ²⁶, while BOD range was from 97 ± 42 to 265 ± 78 mg/l in the year 2005 at the same site ²³, however minimum BOD 1.38 mg/l during winter and maximum value 6.51 mg/l in monsoon season records the value of 3.9 mg/l BOD at Ganga Kashi and 2.1 mg/l at Lucknow in Gomti River⁸. Another study provides a mean BOD value 2.8 ± 0.4 in summer and 3.5 ± 0.4 mg/l in winter at Kanpur from the Ganga River ¹⁵. BOD values are thus used fully in the evaluation of the self-purification capacity of a water body and for possible control measures of pollution.

The chemical Oxygen Demand (COD) value varies from 24.1 to 80.21 mg/l during the study period. Wajidpur has maximum COD seen and Bithoor has a minimum, and incidentally, Shuklaganj has abnormal increase while Shivrajpur has abnormal decreases. Higher mean values of COD 520 ± 180 mg/l were recorded at Varanasi ²⁹ as compared to the present findings. The value of 22 mg/l at Ganga Kashi and 14 mg/l at Lucknow from the Gomti river was also evident ⁸, lower compared to the present observation. However, a study indicates that lower mean value of COD compared to present findings 14 ± 0.8 in summer and 19.7 ± 0.9 mg/l in winter season at Kanpur region of river Ganga ¹⁵. The lower value of COD indicates a high amount of contamination through domestic sewage containing decaying organic matter and another effluent. The mean value of NO_3 was 1.10 ± 0.40 mg/l at Varanasi and observed NO_3 ranged

0.85 ± 0.31 mg/l to 1.30 ± 0.22 mg/l in 2005 at Rajghat (Varanasi) in the river Ganga ²⁹. In surface waters, nitrogen may exist as particulates or dissolved forms. Organic nitrogen decays to produce ammonia *via* the anaerobic bacteria decaying process. Ammonia converted to nitrites and readily took up dissolved inorganic nitrogen, consisting of ammonia and nitrate with a typical preference for ammonia. Nitrates added in to the river water mainly by agriculture runoff.

Long-term exposure to organic and inorganic contents in river Ganga induces physiological disturbance, behavioral dysfunctions, his to pathological alteration, biochemical changes, immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities, and cancer. Fishes serve as important bio-indicators for aquatic contamination to access the changes caused by human activities effectively and reliable monitoring bio-system to recognize and predict hazardous effects of pollutants. Since fish are rich sources and lipids, their health is very important for humans. Dominant species in an area are the most important indicators as they receive the full impact of the habitat for longer periods. Therefore, the protection of the aquatic ecosystem and water quality will be possible only with the judicious and rationalized applications of organic and inorganic contents.

River Ganga, lifeline of millions of people, got heavily polluted due to uncontrolled anthropogenic activity. To monitor the effect of pollution of the river on its aquatic life, a field study was conducted by analyzing the different biomarker enzymes, and biochemical parameters in the various tissues (muscles, liver, gills, and kidney) of the Indian carp *Cirrhinusreba* collected from the river Ganga from different study sites of Kanpur. The activity of catalase peroxides and hydrogen peroxide significantly increased as compared to upstream to the downstream group. Similar observations have also been recorded in gill, liver and kidney of other and same species of fishes such as *Carassius auratus* ¹⁷. Cu and Cd exposures to freshwater teleost *Oreochromis mossambicustilapia* ^{6, 7}. Therefore, it might be due to an increase in peroxidase enzyme activity, which uses H_2O_2 same as CAT. The heavy metals (Cr, Mn, Fe, Co, Ni, Cu, and Zn) accumulated in these tissues are all

potentially redox-active, suggesting an imbalance between the production of ROS and their neutralization, and bearing fish is said to be under the influence of oxidative stress^{16, 3}. This imbalance may lead to damage of tissues and cellular components, which in turn would trigger induction of antioxidant defense mechanisms^{14, 28}. Proteins are the most fundamental and abundant biochemical constituent present in fishes. Proteins are the most important energy source to spare during the chronic period of stress³².

CONCLUSION: Fish have proven useful as sentinel organisms that display measurable biological responses that vary in proportion to the extent of exposure to contaminants. Significant efforts are invested in field studies using fish, and it is important to optimize the number of organisms collected to evaluate the possible impacts of contamination. Biomarkers are not the only response assessed to evaluate the health status of fish populations.

However, each biomarker may demonstrate a unique variability and require a different number of specimens to establish inter-site differences. The present study represents the first comprehensive investigation that set oxidative stress responses in fishes from freshwaters of the river Ganga at Kanpur region, essential information for a proper interpretation of future ecotoxicological data and environmental monitoring programs in this region.

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