Prepublished copy

Nature Environment & Pollution Technology

This is a peer-reviewed prepublished version of the paper

Original Research

Oxidative stress and associated neurotoxicological impact in *Cirrhinus reba* from the River Mahananda, Malda: An ecotoxicological assessment

Mayukh Hore[1], Shubham Bhattacharyya[1], Samir Barai[1] and Subhrajyoti Roy[1†]

[1] Immunopharmacology and Molecular Cell Biology Laboratory, Department of Zoology, University of Gour Banga, Malda – 732103, West Bengal, India

[†]Corresponding author: Dr. Subhrajyoti Roy; subhrajyoti_roy@rediffmail.com

ORCID: 0000-0002-2285-8185

Abstract: (1) Background: The water quality of the River Mahananda has continuously deteriorated, due to increased exposure of untreated wastewater from the urban areas, increasing the concentration of anthropogenic toxicants in aquatic environments that might enhance the cellular oxidative stress induced physiological imbalance on the aquatic biota; (2) Methods: In the present study, we have assessed the water quality of the River Mahananda and evaluate its detrimental effects on the oxidative stress parameters and neurotoxic biomarker of *Cirrhinus reba*; (3) Results**:** The principal component analysis revealed significant impact of zinc, copper, fluoride, and ammonia on the pollution status of the River Mahananda. A significant decrease in the activity of superoxide dismutase, catalase and glutathione reductase was observed in the liver, while significantly increased ($p<0.001$) concentrations of TBARS in the liver, kidney, brain and gill of *C. reba* were found at the polluted sites. An organ-specific significant decrease ($p<0.001$) in the acetylcholinesterase activity was noted in the brain tissue of *C. reba* at the polluted sites (S2<S3<S4) compared to the control; (4) Conclusions: The result of our study indicates the noxious impact of anthropogenic pollutants on the physiological metabolisms of *Cirrhinus reba*, an alternative model for ecotoxicological study.

Key Words	Anthropogenic pollution, River Mahananda, Oxidative stress, neurotoxicity, ecotoxicology
DOI	https://doi.org/10.46488/NEPT.2025.v24i03.B4292 (DOI will be active only after the final publication of the paper)
Citation of the Paper	Mayukh Hore, Shubham Bhattacharyya, Samir Barai and Subhrajyoti Roy, 2025. Oxidative stress and associated neurotoxicological impact in Cirrhinus reba from the River Mahananda, Malda: An ecotoxicological assessment. *Nature Environment and Pollution Technology*, 24(3), B4292. https://doi.org/10.46488/NEPT.2025.v24i03.B4292

1. INTRODUCTION

Freshwater pollution is a major global concern, contributed by rapid industrialization, agricultural activities, population growth, and urbanization (Qin et al. 2020). Anthropogenic pollution significantly contributes to the degradation of river water quality, particularly in urban regions where water quality monitoring is severely hindered by inadequate testing facilities and capabilities (Chen et al. 2022). Nowadays, the issue of anthropogenic pollution and its effects on the aquatic ecosystem has gained immense attention. Anthropo-genic pollutants *viz.,* heavy metals, pesticides, microplastics etc. affect aquatic animals by generating oxidative stress mediated by reactive oxygen species (ROS) like superoxide radicals ($O_2^{\bullet-}$) in their cells and hampers cellular metabolism (Melegari et al. 2013; Rahman et al., 2024). The gradual accumulation of these ROS collapses the antioxidant machinery, which in turn, generates a number of free radicals viz. $NO_2^{\bullet}$, $OH^{\bullet}$, $ONOO^{-}$ and others (Tharmalingam et al. 2017). The over accumulation of these free radicals causes lipid peroxidation, protein denaturation, DNA damage, alteration in the tis-sue architectures and neurotoxicity via modulation of the en-zyme acetylcholinesterase (AChE) (Hore et al. 2023; Sharma et al., 2023). The alteration in AChE activity is responsible for several essential physiological functions in fishes including locomotion, prey detection and con-sumption, social communication and others. The modulation in AChE activity may be attributed to several anthropogenic activities, viz. agricultural runoff, gardening, and biomedical waste, etc. (Massei et al. 2023).

The River Mahananda, a transboundary Himalayan River, flowing through the Malda district of West Ben-gal, India, is presently suffering from anthropogenic crisis and its water quality along with-it biota is continu-ously deteriorating (Hore et al. 2023). As the population along the river bed continues to grow, untreated wastewater as well as agricultural runoff are discharged increasingly into the river (Mondal & Sinha 2020). Some regions of the river bed are utilized as dumping sites, where all the sewages from urban areas and farms cause the water flow to cease. These anthropogenic disturbances not only deteriorate the physico-chemical qual-ities of the river water but also have deleterious effects on its aquatic biota, and due to which numerous indige-nous fish species have declined in this river, causing a major economical seatback to the local fishermen.

To assess the effects of anthropogenic toxicants, fishes have been considered as potential bio-indicators (Ali et al. 2020). *Cirrhinus reba* is one of the most popular Gangetic edible fish species in this region with high nutritional values that play a significant role in the local economy. In recent years, a notable decline has observed in *C. reba* population in the River Mahananda, which may be attributed to overfishing, habitat degradation, and other man-made ecological disturbances. Therefore, the present study was aimed to investigate the effects of the anthropogenic pollutants on (a) the water physico-chemical properties of the River Mahananda, and (b) to evaluate its detrimental effects on the enzymatic- (superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) and non-enzymatic (reduced and oxidized glutathione) antioxidants, cellular lipid perox-idation, and acetylcholinesterase activity.

2. MATERIALS AND METHODS

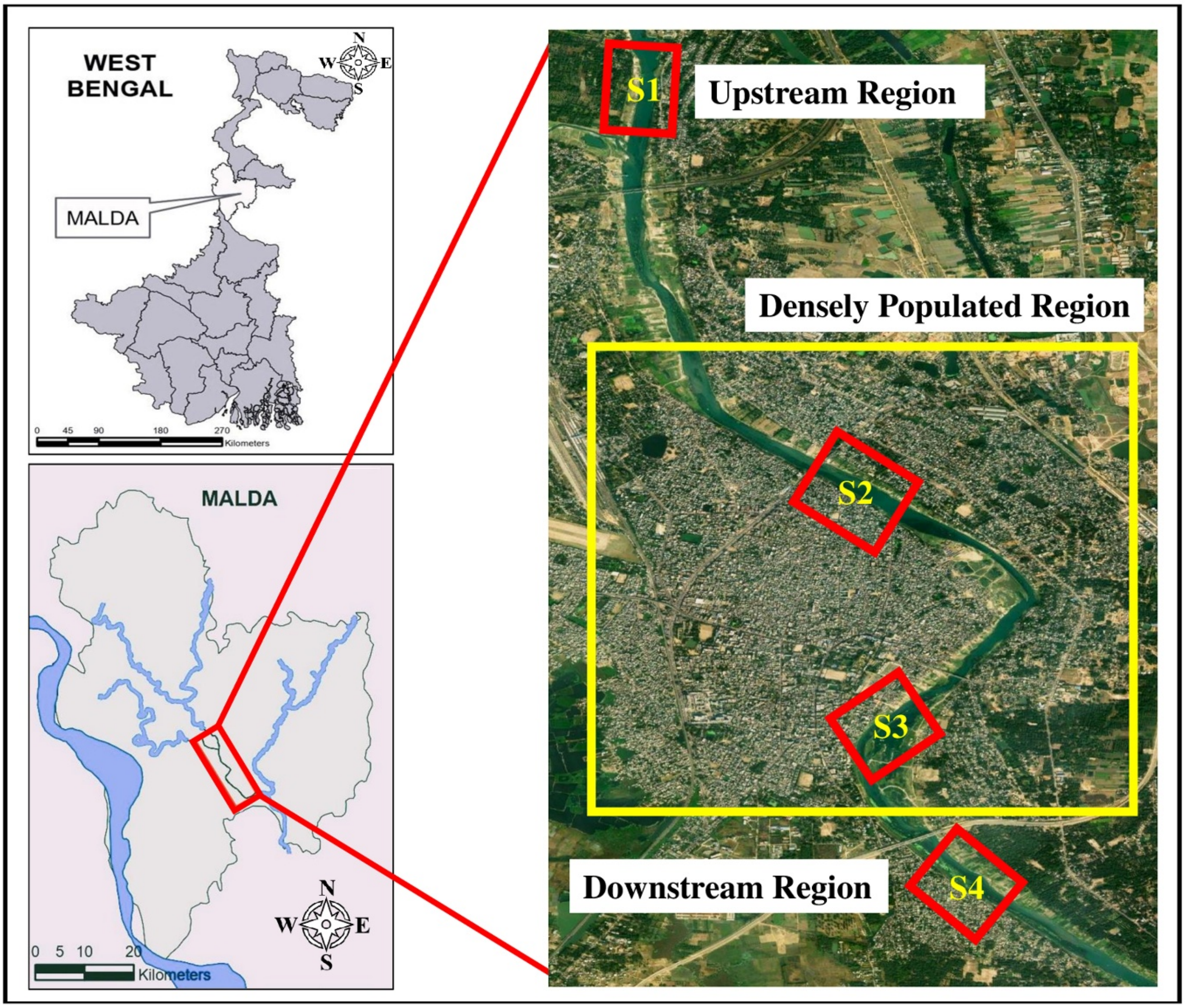
2.1. Description of the sampling sites

The study area was divided into four sampling sites, S1, S2, S3 and S4 (Fig. 1). S1, the upstream sampling site (25°03'39.1"N, 88°07'50.5"E to 25°03'00.2"N, 88°08'01.6"E) exhibits low population density and minimal anthropogenic activities, followed by the S2 (25°01'01.8"N, 88°08'24.6"E to 25°00'28.7"N, 88°09'12.9"E) and S3 (24°59'43.2"N, 88°08'59.3"E to 24°58'57.1"N, 88°09'05.4"E) sampling sites, characterized by high popula-tion density and potential anthropogenic activities. The S4 sampling site of the river is the downstream region (24°58'21.7"N, 88°09'46.7"E to 24°57'51.0"N, 88°10'28.1"E), exhibited lower anthropogenic activity compare to the S2 and S3 sampling sites.

2.2. Collection of samples (water and fish)

Samples (water and *Cirrhinus reba*) were collected from different sampling sites twice a month in March, April, May and June, 2024, as per the standard method (Central Pollution Control Board, 2007). *Cirrhinus reba* (six in number) (body weight: 80±15 g, each) were sampled from each site using fish nets, and brought alive into the laboratory for further evaluation.

Fig. 1: Location map of the study area in the River Mahananda, Malda.



2.3. Experimental methods

2.3.1. Estimation of the water physico-chemical parameters and evaluation of the water pollution load

In order to assess the physico-chemical quality of the River Mahananda, physical parameters, including temperature, total dissolved solids (TDS) and electrical conductivity (EC); and chemical parameters such as pH, dissolved carbon dioxide, dissolved oxygen, total alkalinity, total hardness, calcium hardness, ammonia, free chlorine, chloride, nitrate, nitrite and fluoride were measured. Fe(total), Zn^{2+}, Cu^{2+} were also estimated to determine the load of heavy metals in the sampled water. Quantification of all the physico-chemical parameters was performed using “HANNA instruments” and "HIMEDIA AQUA Check^{TM} water analysis system".

To evaluate the pollution load in the river, Water Pollution Index (WPI) was computed as per the standard method developed by Hossain & Patra (2020), considering the combined effect of all estimated water physico-chemical parameters including heavy metals, based on their upper permissible limits (Bureau of Indian Standards, 2012). According to the authors, it is an alternative approach that disregards the issues of opacity, weighting, and aggregation related to the other conventional methods and easy to compute, flexible and allows incorporation of several parameters as per requirement. WPI is considered as a precise comprehensive method for illustrating surface water quality in relation to pollution. The result of WPI scores were categorized into "excellent" (WPI <0.5), "good" (WPI 0.5–0.75), "moderately polluted" (WPI = 0.75–1), and "highly polluted" (WPI >1) to classify the water quality or pollution load.

2.3.2. Preparation of tissue homogenate and biochemical analysis of oxidative stress parameters

Liver, kidney, brain and gill tissues were isolated from *C. reba* immediately after sampling to prepare tissue homogenate. Tissue homogenates were prepared using 0.1M Na/K phosphate buffer; pH 8, 0.1% Triton X-100 and 0.6% sulfosalicylic acid in potassium phosphate buffer and 1% Triton X-100 (1:9, w/v) and centrifuged at 3500g at 4ºC for 30 min. Following centrifugation, the supernatant was collected and stored at -20 ºC for further assessment.

2.3.3. Estimation of intracellular enzymatic- and non-enzymatic antioxidants

The relative enzymatic activity of superoxide dismutase (SOD) has been measured using a standard protocol with slight modifications and defined as U/mg protein, where a single unit (U) signifies 50% inhibition of the formation of the tetrazolium salt (Kumari et al. 2014). Briefly, 0.025 ml of liver homogenate was mixed subsequently with 0.975 ml phosphate buffer (0.1 M, pH 7.5), followed by 24 µM riboflavin, 840 µM nitro blue tetrazolium (NBT), 1.2 mM Na2-EDTA, and 150 mM methionine. Following an incubation period of half an hour, the absorbance change in the reaction mixture was measured at 560 nm. The CAT activity was determined according to the conventional method which measures the decreasing concentration rate of H_2O_2, spectrophotometrically at 240 nm and expressed by *k*/ml where, *k* is the rate constant of first-order reaction (Aebi, 1984). GPx activity was measured based on the Box-Behnken design (BBD) (Ahmed et al. 2021). The enzyme activity was determined via spectrophotometrically measuring the rate of reduction of Cu (II)-neocuproine complex (Cu $(Nc)_2^{2+}$) to strongly colored Cu(I)-neocuproine complex (Cu $(Nc)_2^{+}$) at 450 nm. GR activity was measured according to a standard method, which involved spectrophotometric analysis of NADPH oxidation at 340 nm, coupled with the reduction of GSSG (Mannervik, 1999). Briefly, 0.2 M EDTA-Phosphate buffer (pH 7.0) was mixed with 20 mM oxidized glutathione followed by 2 mM NADPH solution and tissue homogenate in a ratio of 10:1:1:0.2, well mixed and then measured at 340 nm. Intracellular GSH and GSSG concentration was determined spectrophotometrically, where the absorbance of yellow-colored 5′-thio-2-nitrobenzoic acid was measured at 412 nm (Rahman et al. 2006).

2.3.4. Estimation of cellular lipid peroxidation

Cellular lipid peroxidation level was determined spectrophotometrically by quantifying the level of thiobarbituric acid reactive substances (TBARS) at 535 nm and expressed as nmoles per mg protein (extinction coefficient, 1.56×10^{-5} $M^{-1}cm^{-1}$) (Dubovskiy et al., 2008). Briefly, the liver, kidney, gill and brain tissue homogenates were mixed with 20% trichloroacetic acid (TCA) and 0.8% thiobarbituric acid (TBA) followed by an incubation period of 1 hour in a boiling water bath. After the incubation, the reaction mixtures were cooled, and centrifuged at 15000g for 10 min and the supernatant was separated to measure the concentration of TBARS.

2.3.5. Estimation of acetylcholinesterase activity

The activity of acetylcholinesterase (AChE) was determined according to a standard method (Ellman et al., 1961). Briefly, the reaction mixtures of tissue homogenate (liver and brain) and phosphate buffer (pH 8, 0.1M) were mixed with 25 µl DTNB (0.01M) and measured the absorbance at 412nm till the OD was stabilized. After that, acetylthiocholine iodide was added to the reaction mixture. The rate of change of absorbance was measured at 412 nm in a plate reader (Bio-Rad, Hercules, USA).

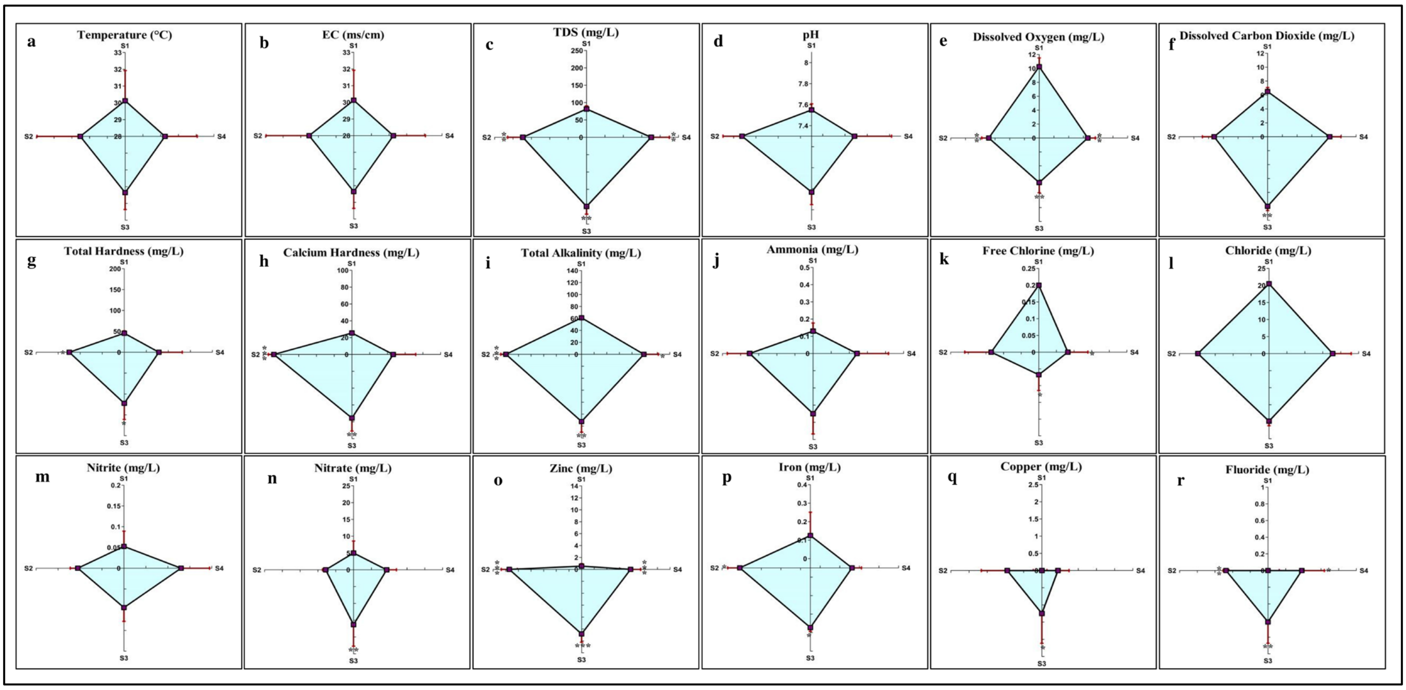
2.4. Statistical analysis

Shapiro-Wilk test was performed to determine the normality of the data set. Differences in mean ± SD (n = 6) between the groups [comparing S2, S3, S4 with S1 (as control)] were evaluated employing one-way ANOVA, followed by Dunnett's test. $p < 0.05$ was considered as the level of significance. Standardized Principal Component Analysis (PCA) was performed to identify the predominant contributors of the water quality deterioration in the river. Pearson's correlation (r) test was also performed to assess the correlations between the contributors of water pollution and oxidative stress biomarkers. All statistical analyses were performed using XLSTAT 2016 and KyPlot 6.0.

3. RESULTS

3.1. Determination of water pollution level and identification of the major contributors of pollution

The general physico-chemical nature and quality of the river water are depicted in Fig. 2, which clearly demonstrates that the sampling sites S2, S3 and S4 of the River Mahananda possess a varying degree of pollution load. In our present study. It was noted that, among the physical parameters, TDS was significantly increased ($p \leq 0.01$) at the S2, S3 and S4 sampling sites of the River Mahananda, when compared to control site S1. The highest value of dissolved oxygen was recorded at S1 while, an average value of 6.61±0.17 at the downstream sites, very close to the lower permissible limit (Bureau of Indian Standards, 2012). Total hardness, calcium hardness and total alkalinity were found to be significantly higher at S2 sampling sites followed by S3>S4>S1 (control site) respectively. The highest concentration of ammonia was recorded at the S2 and S3 (0.35±0.11 mg/L) sampling sites, approximately three times higher than the S1 (0.13±0.04 mg/L) sampling site along with the measured anionic concentrations sequenced in order of $Cl^{-} > NO_3^{-} > NO_2^{-}$ at the polluted sites compare to control. Zn^{2+} and Cu^{2+} were both found to be significantly higher at S2 site with the highest value of 11.44±1.10



mg/L and 0.95±0.62 mg/L respectively, exceeding the value of the upper permissible limit (Bureau of Indian Standards, 2012). The sampling site S1 showed "Excellent" water quality as indicated by WPI (0.309), maybe due to low population density and fewer anthropogenic disturbances. In contrast, the WPI of the S2 and S3 were found to be 1.3 and 1.5, respectively, representing "highly polluted" water quality, mainly attributed to the exposure and overaccumulation of anthropogenic toxicants in these regions of the River Mahananda. The S4 sampling site exhibited a WPI of 0.76. Although the population load and anthropogenic disturbances around the S4 sampling site were negligible, the pollutants from the upstream region (S2 and S3) tend to accumulate in this region making it "moderately polluted".

Fig. 2: Water physico-chemical parameters of different sampling sites in the River Mahananda, Malda.

To reduce the dimensionality of the dataset and define the principal contributors to water pollution, principal component analysis (PCA) was performed using the physico-chemical parameters of the water. In the principal component analysis of the physicochemical properties of water, the principal components PC1, PC2, and PC3 can account for 100% of the variation in the data set; of these, PC1 and PC2 explain 76.87% and 13.88% of the total variance (Fig. 3). From the outcomes of PCA, it was confirmed that heavy metals like zinc and copper, fluoride, and ammonia, were the principal contributors to polluted water quality, in association with pH, electric conductivity, TDS, total hardness, calcium hardness, total alkalinity and chloride were the principal descriptors that had a great impact on the water quality of the River Mahananda, determining the pollution levels.

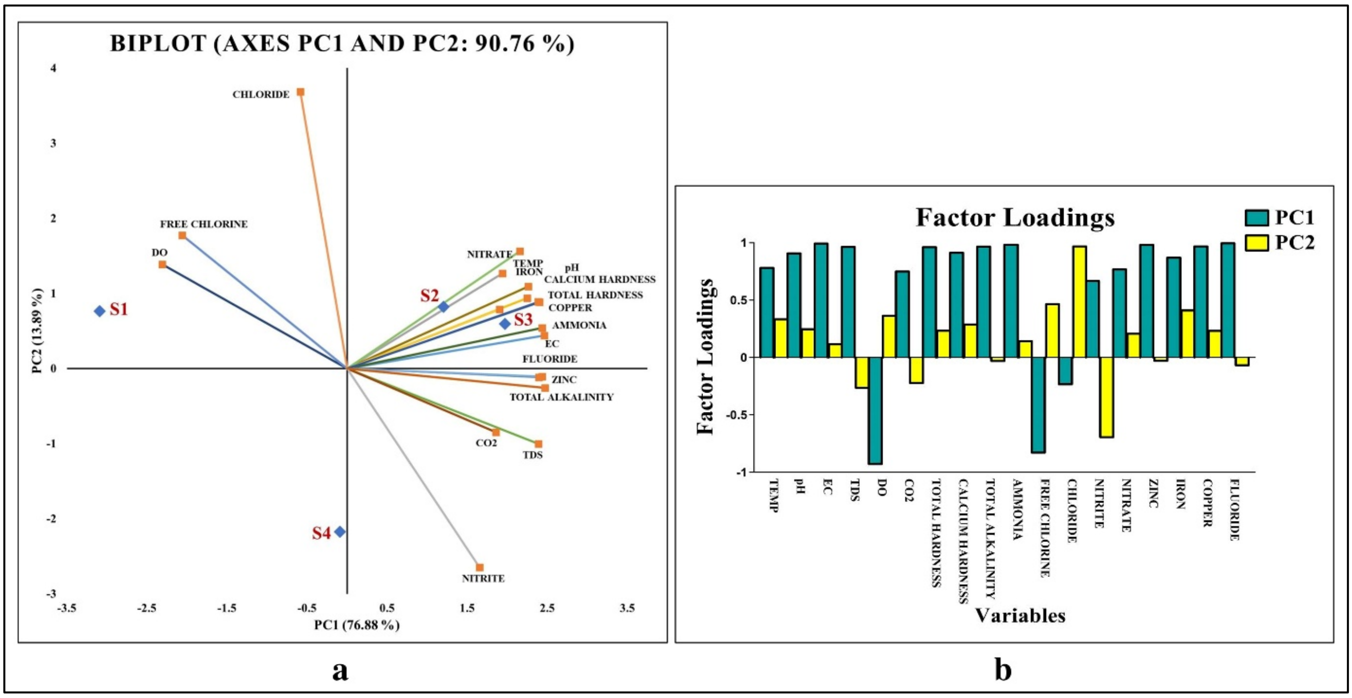


Fig. 3: (a) The PCA biplot and (b) factor loading of water physico-chemical parameters of the River Mahananda, Malda.

3.2. Estimation of oxidative stress biomarkers

Oxidative stress biomarkers, including intracellular antioxidant enzymes (SOD, CAT, GPx, and GR), non-enzymatic antioxidants (GSH and GSSG), lipid peroxidation, and acetylcholinesterase activity, are widely utilized biomarkers to assess the vulnerability and adverse effects of aquatic pollution on fish (Varó et al., 2002). The results of estimated oxidative stress biomarkers in *C. reba* are shown in Table 1, which demonstrates the significant alterations in antioxidant enzymes and other oxidative parameters attributed to anthropogenic stresses.

Table 1. Oxidative stress biomarkers of *Cirrhinus reba* collected from the River Mahananda

¶ all values are mean ± SD of six observations.

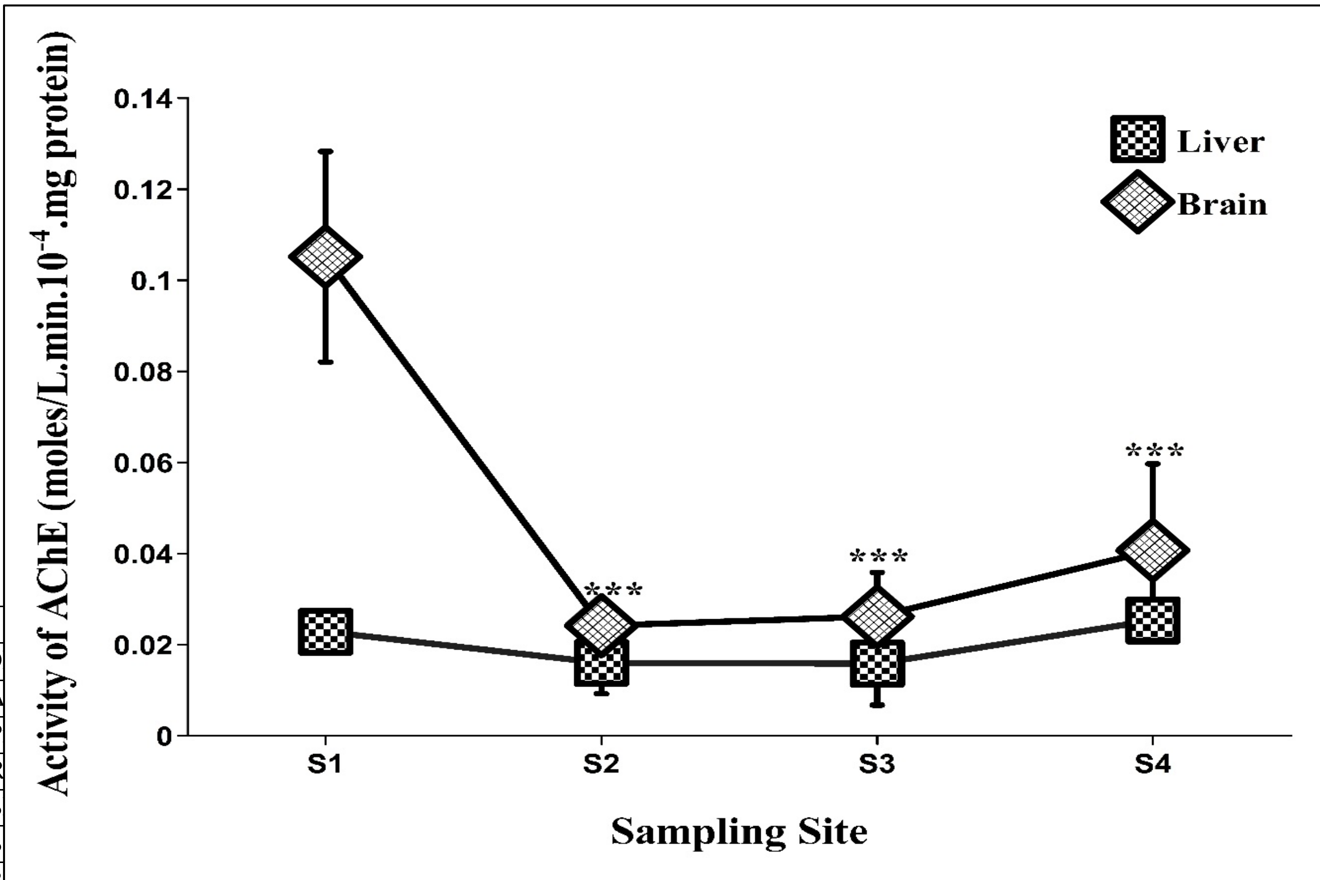
*$p < 0.05$ when compared with S1 (control) (significantly different).

**$p < 0.01$ when compared with S1 (control) (significantly different).

***$p < 0.001$ when compared with S1 (control) (significantly different).

In our present study, it was clearly observed that among the intracellular antioxidant enzymes, only the activity of SOD and CAT were significantly decreased (p<0.001) in the liver tiver tissue of *C. reba* collected from the polluted sites (S2 and S3), and S4 of river Mahananda, compared to control S1 site. A significant decrease (p<0.001) in the AChE activity was noted only in the brain tissue of *C. reba* at the polluted sites (S2<S3<S4) compared to the control (Fig. 4). On the other hand, the concentration of reduced glutathione (GSH) was found to be significantly lower (p<0.001 and p<0.01), while higher (p<0.01 and p<0.05) concentration of GSSG was found in the liver of *C. reba* in the polluted sites, compared to control. Notable alterations in the amount of TBARS concentrations were observed in the case of all organs (liver, kidney, brain and gill) of *C. reba* which depicted increased levels of cellular lipid peroxidation in the organism.

Fig. 4: The activity of acetylcholinesterase of *Cirrhinus reba* collected from the River Mahananda, Malda.



SO[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CA[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
GP[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
GR[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
GS[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
GS[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TBARS (L) (nmoles/l. min ×10^{-6} mg protein)	[illegible]	[illegible]	[illegible]	[illegible]
TBARS (K) (nmoles/l. min ×10^{-6} mg protein)	76.240±14.30	360.258±44.33***	374.502±80.15***	204.460±55.77**
TBARS (B) (nmoles/l. min ×10^{-6} mg protein)	67.038±4.22	377.9±74.08***	313.973±57.33***	233.347±68.27***
TBARS (G) (nmoles/l. min ×10^{-6} mg protein)	89.6±10.74	443.555±130.28***	374.928±37.70***	278.017±89.82**

4. DISCUSSION

The health of aquatic ecosystems is significantly influenced by water quality, which is determined by various physico-chemical and biochemical parameters. Consistent evaluation of these water quality parameters is becoming a more crucial component in managing and protecting aquatic ecosystems (Boussaha et al., 2024). In our present study, we have observed a higher degree of pollution load of the River Mahananda in the regions which are in proximity to the densely populated area, attributed to the deteriorating water quality. The anthropogenic interferences in and around these areas influence the amount, duration of exposure, and chemical composition of the contaminants that may hamper the water resources, all along the sedimentation process. Hence, it is reasonable to assume that the deterioration in water quality is a major issue that is influenced by urbanization and its noxious intervention with the water resources (Umwali et al., 2021). Dey (2022) found that the deterioration of these water quality mainly influenced by the increased population burden in an around the river bank of the River Mahananda, Malda. As per our findings, notable alterations in DO, EC, TDS, hardness of the water, alkalinity, ammonia and presence of the heavy metals *viz.*, zinc, copper, and iron may be responsible for deteriorating water quality. The concentration of dissolved oxygen at the polluted sites was found to be decreased by approximately 40% compared to the control, while the concentration of dissolved CO_2 was increased by 50% (approx.) at the S3 sampling site. Decreased levels of dissolved oxygen are most frequent in surface water due to the deposition of untreated organic Wastewater and biogenic and humus fecal materials (Aristarkhova et al., 2021). Reduced concentrations of DO in river water can profoundly affect aquatic ecosystems, water quality, and environmental health (Rajesh & Rehana, 2022). Total hardness, calcium hardness and total alkalinity were found highest at S2 sampling sites in an order of S3>S4>S1 respectively. Calcium-based compounds can be found in a variety of solid wastes, including building and demolition waste, lime dewatering sludge, household garbage, and municipal solid waste incineration residue. The disposal of these untreated pollutant sources may increase water hardness and alkalinity (Li et al., 2021). Increased concentration of TDS by several folds in the river water is also impacted by over-urbanization as the surface impermeability of urban areas allows several anthropogenic dissolved solids from buildings, construction sites, roads and other urban infrastructures to flow into rivers (Adjovu et al., 2023). Similar kind of results were also recorded in previous studies conducting the water quality of River Mahananda, Siliguri, West Bengal and it was found that electric conductivity, TDS, dissolved oxygen, and chloride were highly influenced by the dense population and associated anthropogenic activities in this area, altering the water quality (Parween et al. 2022; Shil et al. 2019). It was also found that the river flow was ceased in some downstream regions due to the accumulation and dumping of urban wastes. According to our previous study, the urban anthropogenic sources can alter the water physico-chemical parameters, affecting the water quality of the river (Hore et al., 2023). Similarly, the current pollution status of the Upper Awash River Basin, Ethiopia is an example of anthropogenic stressors induced riverine pollution, attributed to the presence of excessive amounts of heavy metals coming from various industrial sources (Dessie et al., 2022). In this present study, we have also detected heavy metals *i.e.,* zinc, iron, copper and fluoride, exceeding the upper permissible limits, indicating metallic contamination in the river water. According to Shil & Singh (2019), this metalloid contamination in the River Mahananda may arise from household and municipal wastes, not industrialization, an indication of anthropogenic pollution. This increased concentration of Zn^{2+}, Cu^{2+}, Fe (total) and F^{-} at the downstream sites of the River Mahananda may be due to the improper disposal of urban solid wastes *viz.*, rubber garbage, battery trash, discarded electronic gadgets, and other e-wastes (Ishchenko, 2019).

Pollutants may act as an exogenous factor that elevates the physiological and cellular oxidative stress via dysregulating ROS generation and the cellular protection mechanism against it (Song et al., 2023). An imbalance between the production and neutralization of reactive oxygen species (ROS) leads to oxidative stress. Excessive oxidative stress can hamper cells and tissues via DNA hydroxylation, protein denaturation, lipid peroxidation, and cell death. In the present study, it was noted that among the oxidative stress parameters, the highest fall in the enzymatic activity from the control was observed in the case of catalase (75.72%) followed by SOD (41.04%) in the liver, while, acetylcholinesterase (71.24%) in the brain of *C. reba* collected from the polluted sites. An excessive amount of $O_2^{\bullet-}$ may lower SOD activity, which could hasten the influx of $O_2^{\bullet-}$ and cause the catalase enzyme to become inactive, which may activate certain redox-sensitive pathways (Kono & Fridovich, 1982). The level of cellular lipid peroxidation was found to be increased almost six times (compared to the control) in the brain tissue followed by gill, kidney and liver *C. reba* collected from polluted sites of the River Mahananda. In our previous study, anthropogenic stress induced oxidative damage was noted in *Puntius sarana* collected from the River Mahananda, West Bengal (Hore et al., 2023). Other scientific evidences also indicated that excessive cellular ROS generation can cease the activity of antioxidant enzymes like SOD and CAT in fishes (Azevedo et al., 2021; Carvalho et al., 2012; Naz et al., 2023). It was reported that heavy metals decrease enzymatic antioxidant activity while induce lipid peroxidation in several organs of *Labio rohita*, collected from the River Yamuna (Mahamood et al., 2021). A somewhat similar organ-specificity of AChE has been seen in *Anabas testudineus*, exposed to naphthalene (Nayak & Patnaik, 2021). Inhibition of acetylcholinesterase activity in the brain tissue of fish exerts severe necrotic changes such as condensed chromatin and neuronal shrinkage along with altered biochemical parameters (Jindal & Sharma, 2019). The Pearson's correlation matrix revealed that zinc was positively correlated with TBARS (p<0.05), while negatively correlated with SOD (p<0.05, r=-0.979), CAT (p<0.05, r=0.947), GR (p<0.05, r=0.957), GSH (p<0.05, r=0.965) and AChE (p<0.01, r=0.991) (Fig. 5). A strong positive correlation (p<0.01) was found between concentration of copper and lipid peroxidation level. Similarly, a positive correlation was also recorded among copper, zinc and cellular lipid peroxidation levels of *Leporinus obtusidens*, previously studied by Gioda et al. (2007). Heavy metals are non-biodegradable and can harm aquatic biota at low concentrations. Prolonged exposure to these hazardous substances can cause oxidative stress in fish and alter cellular metabolism (Rani et al., 2022). According to Abdel-Tawwab et al. (2015), exposure to heavy metals can deteriorate the health status of fish. It has been demonstrated that elevated amounts of copper may seriously harm cells by generating an excessive amount of reactive oxygen species (Pourahmad & O'Brien, 2000). The bioaccumulation of copper and zinc in *Pavlova viridis* to varying concentrations resulted in increased cellular lipid peroxidation, indicating oxidative damage (M. Li et al., 2006). Exposure to 120 μg/L Cu for 48 h significantly increased the lipid peroxidation in the liver whereas significantly decreased the catalase activity in large yellow croaker (Pan et al., 2020). Waterborne Zinc at a dose of 8 mg/L for a week also considerably reduced the GSH levels in the liver and gill of *Paralichthys olivaceus* (Kim et al., 2019). Fluoride in urban waste comes from a variety of places, including industrial effluents, agricultural runoff, domestic garbage, and sloppy trash disposal (Bahukhandi et al., 2020). Because of its high electronegativity, fluoride may inhibit enzyme activity through the generation of

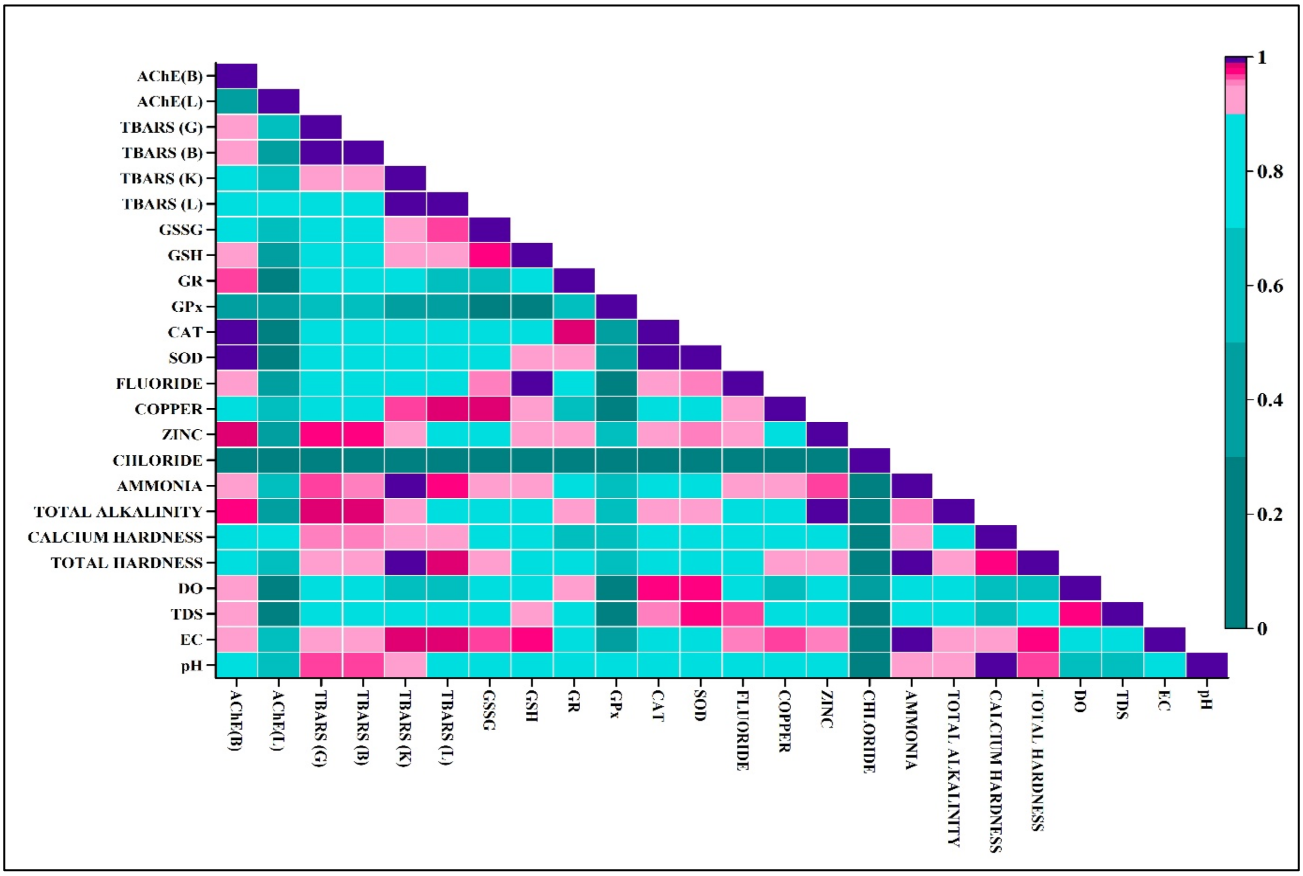


Fig. 5: Heat map of the Pearson's correlation matrix between water physicochemical parameters and oxidative stress biomarkers of *Cirrhinus reba*.

ions (Barbier et al. 2010). In our study, it seemed that fluoride was negatively correlated with SOD (p<0.05, r=-0.978), CAT (p<0.05, r=-0.953), GSH (p<0.01, r=-0.998) and AChE (p<0.05, r=-0.965). Chronic exposure to sodium fluoride in zebrafish can significantly decrease the activity of acetylcholinesterase, superoxide dismutase and catalase (Dondossola et al., 2022). On the other hand, increased lipid peroxidation was recorded in the liver of *Heteropneustis fossilis* exposed to fluoride at 35 and 70 mg/L (Yadav et al., 2015). Although there was no statistically significant change in SOD levels, fluoride at the dose of dosages of 10-50 mg/L was able to fortify TBARS associated with lipid peroxidation while decreasing CAT activity in blood. Moreover, due to alterations in enzyme metabolism and disruption of redox equilibrium, high fluoride exposure is linked to harm to cellular functions in several tissue types (Miranda et al., 2018). In aquaculture environments, ammonia is the most prevalent stressor and pollutant, and it has the potential to result in significant fish mortality through the production of oxidative stress (Parvathy et al., 2023). The oxidative stress biomarkers such as reduced glutathione and TBARS were found significantly correlated (p<0.05 & p<0.01) with the ammonia concentration of the River Mahananda. Similar findings were observed in various studies, where ammonia exposure at different concentrations can increase cellular lipid peroxidation whereas GSH as well as some other antioxidants were decreased in freshwater fishes (Elshopakey et al., 2023; Long et al., 2023; Zhang et al., 2023).

4. CONCLUSIONS

The degradation of surface water in developing nations is mostly attributed to pollution associated with urban populations, along with agricultural and industrial sources. The water quality of the River Mahananda is deteriorating due to anthropogenic contamination; this condition can be made worse by the high intensity of the exposure of anthropogenic toxicants into the river bed without proper environmental management. As per our knowledge, this is the first study to evaluate the toxic effects of anthropogenic pollutants on the oxidative stress level and associated neurotoxicity in *cirrhinus reba*. Our findings showed that fluoride, copper, zinc, and ammonia were the principal toxicants, which not only influenced the quality of river water but also induced cellular

ROS generation that caused multiple physiological impacts in the fish. This may be due to the over-accumulation of heavy metals and other toxicants. Consequently, the activity of intracellular enzymes, including SOD, CAT, and GR, was significantly decreased at polluted sites of the river may also be due to the presence of heavy metals such as, zinc and copper. The level of lipid peroxidation in the organs of *C. reba* collected from the polluted sites was several folds higher than that of the control. The deteriorating water quality and the presence of heavy metals also modulate the acetylcholinesterase activity via inhibition of its active catalytic site. Additionally, in this investigation, we tried to identify potential pathways within a single chain that may explain the mechanism of toxicant-induced oxidative stress in fish. Future research should focus on utilizing analytical techniques such as atomic absorption spectrophotometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS) to accurately measure the concentration of heavy metals in the river water and confirm their bioaccumulation within the fish species. High-performance liquid chromatography (HPLC), Raman Spectroscopy, etc. should be also utilized to quantify the potential presence of other toxic substances viz., microplastics, pesticides, organic pollutants, etc. that could negatively impact the health of aquatic animals, particularly fish. In conclusion, monitoring of water quality is an essential metric for achieving the "Sustainable Development Goals" and "Namami Gange Programme" pertaining to clean river water and sanitation. Our study aims to offer critical insights for policy formulation and wastewater management development by monitoring and analyzing water quality data, thereby mitigating anthropogenic activities to address this issue and protect natural resources, ensuring universal access to safe drinking water for the local people and enhanced sanitation. In order to address the growing problem of urban run-off and its impact on water quality, our research suggests that local administration should implement new strategies for wastewater collection and treatment. One possible approach should be the use of adsorption and phytoremediation technologies to recycle wastewater and effectively remove heavy metals. Other strategies include aeration, bio-film, and microbial preparation and microorganism dosing technique. Moreover, our research will facilitate the cultivation and propagation of this commercially significant fish species, as well as educate the local population regarding the hazards and impact of contaminants in the water.

Author Contributions: "Conceptualization, M.H. and S.R.; methodology, M.H., S.BH. and S.BA.; software, M.H.; validation, M.H. and S.R.; formal analysis, M.H.; investigation, M.H.; data curation, M.H., S.BH. and S.BA.; writing—original draft preparation, M.H., S.BH. and S.BA.; writing—review and editing, S.R.; visualization, S.R.; supervision, S.R.; All authors have read and agreed to the published version of the manuscript."

Funding: This research received no external funding.

Institutional Review Board Statement: "Ethical review and approval were waived for this study as *Cirrhinus reba* is currently classified under the "Least Concern" (LC) category in the IUCN Red List, 2022 and widely consumed as one of the most popular commercial edible fish species.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

REFERENCES

Abdel-Tawwab, M., Sharafeldin, K.M., Mosaad, M.N.M., Ismaiel, N.E.M., 2015. Coffee bean in common carp, *Cyprinus carpio* L. diets: Effect on growth performance, biochemical status, and resistance to waterborne zinc toxicity. *Aquac.,* 448, pp.207–213. https://doi.org/10.1016/j.aquaculture.2015.06.010

Adjovu, G.E., Stephen, H., James, D., Ahmad, S., 2023. Measurement of Total Dissolved Solids and Total Suspended Solids in Water Systems: A Review of the Issues, Conventional, and Remote Sensing Techniques. *Remote Sens (Basel),* 15, pp.3534. https://doi.org/10.3390/rs15143534

Aebi, H., 1984. Catalase in vitro, in: Methods in Enzymology. *AP*, pp. 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

Ahmed, A.Y., Aowda, S.A., Hadwan, M.H., 2021. A validated method to assess glutathione peroxidase enzyme activity. *Chem. Pap.,* 75, 6625–6637. https://doi.org/10.1007/S11696-021-01826-1/METRICS

Ali, D., Almarzoug, M.H.A., Al Ali, H., Samdani, M.S., Hussain, S.A., Alarifi, S., 2020. Fish as bio indicators to determine the effects of pollution in river by using the micronucleus and alkaline single cell gel electrophoresis assay. *J. King Saud Univ. Sci.,* 32, pp.2880–2885. https://doi.org/10.1016/j.jksus.2020.07.012

Aristarkhova, E.O., Fedoniuk, T.P., Romanchuk, L.D., Latushynskyi, S. V., Kot, I. V., 2021. Features of the surface water oxygen regime in the Ukrainian Polesie Region. *J. Water Land Dev.,* 49, pp.104–110. https://doi.org/10.24425/jwld.2021.137102

Azevedo, A.C.B., Bozza, D.A., Doria, H.B., Osório, F.H.T., Corcini, C.D., Pereira, F.A., Junior, A.S.V., Esquivel, L., Silva, C.P., Campos, S.X., Randi, M.A.F., Ribeiro, C.A.O., 2021. Low levels of inorganic copper impair reproduction parameters in *Oreochromis niloticus* after chronic exposure. *Aquac.,* 545, pp.737186. https://doi.org/10.1016/j.aquaculture.2021.737186

Bahukhandi, K.D., Siddiqui, N.A., Bartarya, S.K., Shefali, A., 2020. Assessment of Heavy Metal and Physiochemical Parameter in Surface and Groundwater Quality of Dehradun District of Uttarakhand. In: Siddiqui, N., Tauseef, S., Dobhal, R. (eds) *Advances in Water Pollution Monitoring and Control*, pp 151–161. https://doi.org/10.1007/978-981-32-9956-6_16

Barbier, O., Arreola-Mendoza, L., Del Razo, L.M., 2010. Molecular mechanisms of fluoride toxicity. *Chem. Biol. Interact.*, 188, 319–333. https://doi.org/10.1016/j.cbi.2010.07.011

Boussaha, A., Bezzalla, A., Zebsa, R., Amari, H., Houhamdi, M., Chenchouni, H., 2024. Monitoring and assessment of spatial and seasonal variability in water quality at Lake of Birds (Algeria) using physicochemical parameters and bacterial quality indicators. *Environ. Nanotechnol. Monit. Manag.*, 22, pp.100955. https://doi.org/10.1016/j.enmm.2024.100955

Bureau of Indian Standards, 2012. *DRINKING WATER — SPECIFICATION*. URL http://cgwb.gov.in/Documents/WQ-standards.pdf. Accessed 10 November 2024.

Carvalho, C. dos S., Bernusso, V.A., Araújo, H.S.S. de, Espíndola, E.L.G., Fernandes, M.N., 2012. Biomarker responses as indication of contaminant effects in *Oreochromis niloticus*. *Chemosphere*, 89, pp.60–69. https://doi.org/10.1016/j.chemosphere.2012.04.013

Central Pollution Control Board (2007). *Guidelines for Water Quality Monitoring*. URL https://www.mpcb.gov.in/sites/default/files/water-quality/standards-protocols/GuidelinesforWQMonitoring%5B1%5D.pdf. Accessed 26 June 2024.

Chen, S.S., Kimirei, I.A., Yu, C., Shen, Q., Gao, Q., 2022. Assessment of urban river water pollution with urbanization in East Africa. *ESPR.*, 29, pp.40812–40825. https://doi.org/10.1007/s11356-021-18082-1

Dessie, B.K., Tessema, B., Asegide, E., Tibebe, D., Alamirew, T., Walsh, C.L., Zeleke, G., 2022. Physicochemical characterization and heavy metals analysis from industrial discharges in Upper Awash River Basin, Ethiopia. *Toxicol. Rep.,* 9, pp.1297–1307. https://doi.org/10.1016/j.toxrep.2022.06.002

Dey, S. K., 2022. A Statistical Assessment of Mahananda River Water Quality Parameters. *JETIR.*, 9(8), pp.549–551. www.jetir.org

Dondossola, E.R., Pacheco, S.D., Visentin, S.C., Mendes, N.V., Baldin, S.L., Bernardo, H.T., Scussel, R., Rico, E.P., 2022. Prolonged fluoride exposure alters neurotransmission and oxidative stress in the zebrafish brain. *Neurotoxicology*, 89, pp.92–98. https://doi.org/10.1016/j.neuro.2022.01.008

Dubovskiy, I.M., Martemyanov, V.V., Vorontsova, Y.L., Rantala, M.J., Gryzanova, E.V., Glupov, V.V., 2008. Effect of bacterial infection on antioxidant activity and lipid peroxidation in the midgut of *Galleria mellonella* L. larvae (Lepidoptera, Pyralidae). *CBPC*, 148, pp.1–5. https://doi.org/10.1016/j.cbpc.2008.02.003

Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.,* 7, pp.88–95. https://doi.org/10.1016/0006-2952(61)90145-9

Elshopakey, G.E., Mahboub, H.H., Sheraiba, N.I., Abduljabbar, M.H., Mahmoud, Y.K., Abomughaid, M.M., Ismail, A.K., 2023. Ammonia toxicity in Nile tilapia: Potential role of dietary baicalin on biochemical profile, antioxidant status and inflammatory gene expression. *AQUACULT REP.,* 28, pp.101434. https://doi.org/10.1016/j.aqrep.2022.101434

Gioda, C.R., Lissner, L.A., Pretto, A., da Rocha, J.B.T., Schetinger, M.R.C., Neto, J.R., Morsch, V.M., Loro, V.L., 2007. Exposure to sublethal concentrations of Zn(II) and Cu(II) changes biochemical parameters in *Leporinus obtusidens*. *Chemosphere*, 69, pp.170–175. https://doi.org/10.1016/j.chemosphere.2007.04.008

Hore, M., Saha, R., Bhaskar, S., Mandal, S., Bhattacharyya, S., Roy, S., 2023. Oxidative stress responses in *Puntius sarana* collected from some environmentally contaminated areas of River Mahananda, Malda, West Bengal. *Ecotoxicol.*, 32, pp.211–222. https://doi.org/10.1007/s10646-023-02630-1

Hossain, M., & Patra, P. K., 2020. Water pollution index – A new integrated approach to rank water quality. *Ecol. Indic.*, 117, pp.106668. https://doi.org/10.1016/j.ecolind.2020.106668

Ishchenko, V., 2019. Heavy metals in municipal waste: the content and leaching ability by waste fraction. *J. Environ. Sci. Health A.,* 54, pp.1448–1456. https://doi.org/10.1080/10934529.2019.1655369

Jindal, R., Sharma, R., 2019. Neurotoxic responses in brain of *catla catla* exposed to cypermethrin: A semiquantitative multibiomarker evaluation. *Ecol. Indic.,* 106, pp.105485. https://doi.org/10.1016/j.ecolind.2019.105485

Kim, J.-H., Choi, H., Sung, G., Seo, S.-A., Kim, K. Il, Kang, Y.J., Kang, J.-C., 2019. Toxic effects on hematological parameters and oxidative stress in juvenile olive flounder, *Paralichthys olivaceus* exposed to waterborne zinc. *Aquaculture Reports,* 15, 100225. https://doi.org/10.1016/j.aqrep.2019.100225

Kono, Y., Fridovich, I., 1982. Superoxide radical inhibits catalase. *JBC.*, 257, pp.5751–5754. https://doi.org/10.1016/S0021-9258(19)83842-5

Kumari, K., Khare, A., Dange, S., 2014. The Applicability of Oxidative Stress Biomarkers in Assessing Chromium Induced Toxicity in the Fish *Labeo rohita*. *Biomed Res. Int.*, 2014, pp.1–11. https://doi.org/10.1155/2014/782493

Li, M., Hu, C., Zhu, Q., Chen, L., Kong, Z., Liu, Z., 2006. Copper and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in the microalga *Pavlova viridis* (Prymnesiophyceae). *Chemosphere*, 62, pp.565–572. https://doi.org/10.1016/j.chemosphere.2005.06.029

Li, Yuqian, Ma, J., Ren, Y., Li, Yijia, Yue, D., 2021. Calcium leaching characteristics in landfill leachate collection systems from bottom ash of municipal solid waste incineration. *JEM.,* 280, pp.111729. https://doi.org/10.1016/J.JENVMAN.2020.111729

Long, X., He, K., Zhang, M., Jiang, H., Dong, X., Wang, C., Shao, J., Gan, L., Hu, X., Li, M., 2023. Ferroptosis preceded the onset of oxidative stress under acute ammonia exposure and quercetin relieved ammonia-induced ferroptosis of yellow catfish (*Pelteobagrus fulvidraco*). *AQUACULT REP.,* 33, pp.101766. https://doi.org/10.1016/j.aqrep.2023.101766

Mahamood, M., Javed, M., Alhewairini, S. S., Zahir, F., Sah, A. K., & Ahmad, Md. I., 2021. *Labeo rohita*, a bioindicator for water quality and associated biomarkers of heavy metal toxicity. *Npj Clean Water*, 4(1), pp.17. https://doi.org/10.1038/s41545-021-00107-4

Mannervik, B., 1999. Measurement of Glutathione Reductase Activity. *Curr. Protoc. Toxicol.,* 00. https://doi.org/10.1002/0471140856.tx0702s00

Massei, R., Brack, W., Seidensticker, S., Hollert, H., Muz, M., Schulze, T., Krauss, M., Küster, E., 2023. Neurotoxicity in complex environmental mixtures—a case-study at River Danube in Novi Sad (Serbia) using zebrafish embryos. *ESPR.*, 30, pp.96138–96146. https://doi.org/10.1007/S11356-023-29186-1/FIGURES/3

Melegari, S.P., Perreault, F., Costa, R.H.R., Popovic, R., Matias, W.G., 2013. Evaluation of toxicity and oxidative stress induced by copper oxide nanoparticles in the green alga *Chlamydomonas reinhardtii*. *Aquat. Toxicol.,* 142–143, pp.431-440. https://doi.org/10.1016/j.aquatox.2013.09.015

Miranda, G.H.N., Gomes, B.A.Q., Bittencourt, L.O., Aragão, W.A.B., Nogueira, L.S., Dionizio, A.S., Buzalaf, M.A.R., Monteiro, M.C., Lima, R.R., 2018. Chronic Exposure to Sodium Fluoride Triggers Oxidative Biochemistry Misbalance in Mice: Effects on Peripheral Blood Circulation. *Oxid Med Cell Longev.*, 2018, pp.1-8. https://doi.org/10.1155/2018/8379123

Mohan, S.V., Nithila, P., Reddy, S.J., 1996. Estimation of heavy metals in drinking water and development of heavy metal pollution index. *J.Environ. Sci. HealthATox. HazardSubst. Environ. Eng.*, 31, pp.283–289. https://doi.org/10.1080/10934529609376357

Mondal, A., Sinha, N., 2020. Water quality monitoring of the river Mahananda near a sewage disposal point at Malda, West Bengal, India. *Int. res. j. biol. sci,* 9, pp.7–13. https://doi.org/10.1007/S1135

Nayak, S., Patnaik, L., 2021. Acetylcholinesterase, as a potential biomarker of naphthalene toxicity in different tissues of freshwater teleost, *Anabas testudineus*. *JEELM.* 29, pp.403–409. https://doi.org/10.3846/JEELM.2021.15808

Naz, S., Hussain, R., Guangbin, Z., Chatha, A.M.M., Rehman, Z.U., Jahan, S., Liaquat, M., Khan, A., 2023. Copper sulfate induces clinico-hematological, oxidative stress, serum biochemical and histopathological changes in freshwater fish rohu (*Labeo rohita*). *Front Vet Sci.,* 10, pp.1142042. https://doi.org/10.3389/FVETS.2023.1142042

Pan, Y., Ai, C.X., Zeng, L., Liu, C., Li, W.C., 2020. Modulation of copper-induced antioxidant defense, Cu transport, and mitophagy by hypoxia in the large yellow croaker (*Larimichthys crocea*). *Fish Physiol. Biochem.*, 46, pp.997–1010. https://doi.org/10.1007/S10695-020-00765-0/METRICS

Parvathy, A.J., Das, B.C., Jifiriya, M.J., Varghese, T., Pillai, D., Rejish Kumar, V.J., 2023. Ammonia induced toxico-physiological responses in fish and management interventions. *Reviews in Aquac.*, 15, pp.452–479. https://doi.org/10.1111/raq.12730

Parween, S., Siddique, N.A., Mahammad Diganta, M.T., Olbert, A.I., Uddin, M.G., 2022. Assessment of urban river water quality using modified NSF water quality index model at Siliguri city, West Bengal, India. *Environ. Sustain.*, 16, pp.100202. https://doi.org/10.1016/j.indic.2022.100202

Pourahmad, J., O'Brien, P.J., 2000. A comparison of hepatocyte cytotoxic mechanisms for Cu^{2+} and Cd^{2+}. *Toxicol.*, 143, pp.263–273. https://doi.org/10.1016/S0300-483X(99)00178-X

Qin, G., Liu, J., Xu, S., Wang, T., 2020. Water quality assessment and pollution source apportionment in a highly regulated river of Northeast China. *Environ. Monit. Assess.*, 192, pp.446. https://doi.org/10.1007/s10661-020-08404-0

Rahman, Abd. G., Samawi, M. F., and Werorilangi, S., 2024. Characteristics, Abundance and Polymer Type of Microplastics in *Anadara granosa* (Blood Clam) from Coastal Area of Palopo City. *Nat. environ. pollut. Technol.*, 23(3), pp.1589–1596. https://doi.org/10.46488/NEPT.2024.v23i03.028

Rahman, I., Kode, A., Biswas, S.K., 2006. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nat. Protoc.,* 1, pp.3159–3165. https://doi.org/10.1038/nprot.2006.378

Rajesh, M., Rehana, S., 2022. Impact of climate change on river water temperature and dissolved oxygen: Indian riverine thermal regimes. *Sci. Rep.,* 12, pp.9222. https://doi.org/10.1038/s41598-022-12996-7

Rani, L., Srivastav, A.L., Kaushal, J., Grewal, A.S., Madhav, S., 2022. Heavy metal contamination in the river ecosystem, In: S. Madhav, S. Kanhaiya, A. Srivastav, V. Singh, P. Singh (eds.) *Ecological Significance of River Ecosystems*. Elsevier, pp. 37–50. https://doi.org/10.1016/B978-0-323-85045-2.00016-9

Sharma, A., Gupta, S., and Kaur, M., 2023. Postnatal Exposure to A Low Dose of Imidacloprid: Oxidative Stress in Brain Without Affecting Learning and Behavior in Swiss Albino Mice. *Nat. environ. pollut. Technol*, 22(3), pp.1591–1598. https://doi.org/10.46488/NEPT.2023.v22i03.045

Shil, S., Singh, U.K., 2019. Health risk assessment and spatial variations of dissolved heavy metals and metalloids in a tropical river basin system. *Ecol. Indic.*, 106, pp.105455. https://doi.org/10.1016/j.ecolind.2019.105455

Shil, S., Singh, U.K., Mehta, P., 2019. Water quality assessment of a tropical river using water quality index (WQI), multivariate statistical techniques and GIS. *Appl. Water Sci.*, 9, pp.1–21. https://doi.org/10.1007/S13201-019-1045-2/TABLES/7

Song, C., Sun, C., Liu, B., Xu, P., 2023. Oxidative Stress in Aquatic Organisms. *Antioxid.,* 12, pp.1223. https://doi.org/10.3390/antiox12061223

Tharmalingam, S., Alhasawi, A., Appanna, V.P., Lemire, J., Appanna, V.D., 2017. Reactive nitrogen species (RNS)-resistant microbes: adaptation and medical implications. *Biochem.*, 398, pp.1193–1208. https://doi.org/10.1515/hsz-2017-0152

Umwali, E.D., Kurban, A., Isabwe, A., Mind'je, R., Azadi, H., Guo, Z., Udahogora, M., Nyirarwasa, A., Umuhoza, J., Nzabarinda, V., Gasirabo, A., Sabirhazi, G., 2021. Spatio-seasonal variation of water quality influenced by land use and land cover in Lake Muhazi. *Sci. Rep.,* 11, pp.17376. https://doi.org/10.1038/s41598-021-96633-9

Varó, I., Navarro, J.C., Amat, F., Guilhermino, L., 2002. Characterisation of cholinesterases and evaluation of the inhibitory potential of chlorpyrifos and dichlorvos to *Artemia salina* and *Artemia parthenogenetica*. *Chemosphere*, 48, pp.563–569. https://doi.org/10.1016/S0045-6535(02)00075-9

Yadav, S., Kumar, R., Khare, P., Tripathi, M., 2015. Oxidative stress biomarkers in the freshwater fish, *Heteropneustes fossilis* (bloch) exposed to sodium fluoride: Antioxidant Defense and role of ascorbic acid. *Toxicol. Int.*, 22, pp.71. https://doi.org/10.4103/0971-6580.172261

Zhang, C., Ma, J., Qi, Q., Xu, M., Xu, R., 2023. Effects of ammonia exposure on anxiety behavior, oxidative stress and inflammation in guppy (*Poecilia reticulate*). *CBPC.,* 265, pp.109539. https://doi.org/10.1016/j.cbpc.2022.109539