Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v46i1.61868

Combinatorial impact of physico-chemical parameters and wastewater responses on freshwater fish (*Rita rita*) of river Ganga

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ABSTRACT. This work wass designed to analyze the possibility of heavy metals inducing oxidative stress and biochemical perturbations effect on fresh water fish and their impact directly or indirectly on the human race. Due to continuous drainage of municipal waste, tanneries, and pesticides in Ganga River, lifeline of millions of people is heavily affected. The physico-chemical qualities of Ganga River were analyzed in quarterly basis during year 2018 to 2021, by taking the freshwater sample of different locations in Kanpur. While collecting the sample water the average temperature 27.6°, DO value of selected locations were fairly poor with an average of DO 6.67 mg L^{-1} and BOD ranged from 11.93 to 20.79 mg L^{-1}. On the other hand the BOD ranged from 11.93 to 20.79 mg L^{-1} which is far more than WHO standards. Additionally, levels of antioxidants in enzymatic and non-enzymatic tissues serve as proxies for fish exposure to oxidant pollutants. Higher physico-chemical responses indicate contaminants and heavy metals may be playing an important role that have to be considered. The heavy metals are affecting the antioxidant defense system particularly seen in liver and kidney of fish and this affect on kidney and liver functioning as the concentrations of heavy metals rises. The relation between enzymatic activity and the metabolite showing negatively correlation that is noted highly significant to this study ($r = -0.87$, $t = 10.14$, $p^* \leqslant 0.05$).

Keywords: oxidative stress; fish; metabolism; aquatic-pollution; heavy metals.

Received on December 15, 2021.
Accepted on October 20, 2023.

Introduction

Urban runoff, sewage waste and domestic wastes in natural water increases high probability of occurrence of heavy metals which eventually cause adverse effects on freshwater ecosystem. Though the ambient environment concentrations are low yet the aquatic species live in polluted habitat are result in the accumulation of toxic substances at high level of concentrations. At present, growing population demands for surplus increase day by day due to the load on industrial sector demand to raise the production of goods to fulfill the demand and hence it promotes the pollution. On exposure to any pollutant, the activity of the enzymes involved in oxidative metabolism and hydrolysis is shown to be changed. According to Tchounwou, Yedjou, Patlolla, and Sutton (2012), Khan and Shah (2014), and Valavanidis, Vlahogianni, Dassenakis, and Scoullos (2006), the enzymatic levels (catalase, glutamate dehydrogenase etc.) are the first indication of toxicity. The most detrimental situation is the disruption of enzyme functioning since enzymes are biological catalysts that allow the most crucial metabolic processes to be carried out in living cells. The majority of heavy metals, including aluminium, arsenic, chromium, cobalt, copper, iron, manganese, molybdenum, nickel, selenium, tin, vanadium, and zinc, have long residence times in water sediments and in the bodies of aquatic organisms because, in contrast to organic chemicals, they cannot be metabolised into less toxic compounds (Ambreen, Javed, & Batool, 2015). The industrial activities increase the effluents discharge directly without proper treatment into the Ganga River. Fish shape, physiology (growth rate, swimming speed, food consumption, intensity of breathing, productivity, survival and life cycles), biochemistry, and carcinogenic effects of heavy metals even at low concentrations such impacts may include lowered immunity, altered behavior, growth rate, and nutritional status, as well as reduced enzyme activity during digestion, reduced meal assimilation, and altered glucose metabolism (Paul, Chakraborty, & Sengupta, 2014). Aquatic Ecosystem receives number of toxic substances among which heavy metals are of much harmful elements because of their toxicity and bioaccumulation potential. Toxicants interrupt the integrity of biochemical mechanism with the alterations in antioxidants. Oxidative damage occurs due to alternations in pro-oxidants and antioxidants level. The heavy metal exposure is not only affecting the aquatic species but also endangered to humans indirectly by entering the food chain as they use fishes as their food source these heavy metals are capable of disturbing the physiology and biochemistry of fishes for example the fishes have antioxidant defense system as their guard to protect themselves from oxidative stress that can also be the result of different heavy metal exposure (Cadenas, 1989; Halliwell, 1996; Maceda-Veiga, Monroy, Navarro, Viscor, & Sostoa, 2013). The metabolic functions in the tissue are influenced by both environmental and chemical stimuli. Freshwater fish are vulnerable to water contamination, and toxins that reach their organs can seriously harm specific physiological and biochemical processes. Antioxidant defence mechanisms are present in fish tissues, especially the liver and kidney, to guard against the oxidative stress brought on by metals (Hansen, Rømma, Søfteland, Olsvik, & Andersen, 2006). Pollutants are altering the biochemical or physico-chemical parameters and disturbing the main mechanisms and their mode of action. The enzymes for example lactate dehydrogenases, succinate dehydrogenases etc. are associated with metabolic machinery of fish have their own physiological importance. The mitochondrial enzyme glutamate dehydrogenase (GDH) is responsible for catalyzing the oxidative deamination of glutamate and gives a product α-ketoglutarate that has its fate in Krebs’s cycle. For detoxification of ammonia into urine, the GDH present in extra-hepatic tissues are utilized and channel ammonia that is released during proteolysis. Hence, GDH activation may play the role as a sensitive indicator of oxidative stress. In oxidative stress condition, the oxyradicals produced are highly toxic, can damage nucleic acids and the tissues of fish. Oxy-radicals also induce the enzyme inactivation and degradation of proteins. Oxygen reactive species (ROS) are affecting the antioxidants in response to xenobiotic exposure according to previous studies. Similarly, the tissues of fish are responding on exposure of heavy metals by producing oxy-radicals. These oxy-radicals were noted to make changes in antioxidant enzymes and their actions that depend upon the degree of accumulation of toxic substances which indicates the warning signs on exposure of pollutants. Alternation in antioxidant defense system means giving birth of oxidative damages (Yamaya & Oaks, 1987). The oxidising agent, oxy-radicals, can cause protein breakdown, tissue damage, nucleic acid damage, and lipid peroxidation. It has been demonstrated in numerous studies that antioxidants are impacted by reactive oxygen species (ROS) and exhibit an adaptive response to xenobiotics that generate oxyradicals. Biomarkers of oxidative stress can provide an indicator for sign of early exposure to redox-active xenobiotics, such as changes in antioxidant enzyme activity or the degree of buildup of damaged molecules. Oxidative stress, incorporating both the antioxidant defenses as well as oxidative damage, is a common effect in organisms exposed to xenobiotics in their environment. Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST), succinate semialdehyde dehydrogenase (SAD), and other low molecular weight scavengers like glutathione (GSH) are among the enzymes that make up the antioxidant defence system (Slooff, van Kreijl, & Baars, 1983; Roberts & Oris, 2004). This study is design to emphasis on health of fresh water fish as aquatic pollutants are major concern for its habitat.

Material and methods

Study area

All the samples were taken from nine various locations distributed throughout 110 km stretch of river Ganga basin i.e., Mehandi Ghat or Kannauj, Nanamau, Shivrajpur, Bithoor, Ganga Barrage, Parmath, Shukla Ganj, Jajmau and Wajidpur village (respectively S_1-S_9 - Figure 1). Thirteen confluence points of sewage were found running through these locations of river Ganga.

Collection of test organism

Rita rita, a freshwater air-breathing catfish (body mass, BM = 150 g; body length, BL = 15-20 cm), was procured with the aid of neighborhood fishermen using a gill net, cast net, drag net, scoop net, hook and line, from fresh water bodies situated in the Kanpur subregion.

Estimation of physico-chemical parameters of water sample

For each site, three surface water samples were collected and subjected to the following analyses: Temperature, pH, TDS, Turbidity, EC, Hardness, chloride, Alkalinity, Dissolved Oxygen (Winkler azide method - Barnett & Hurwitz, 1939), chemical oxygen Demand (COD) were estimated. The estimation of nitrate and nitrite in sample water was done.

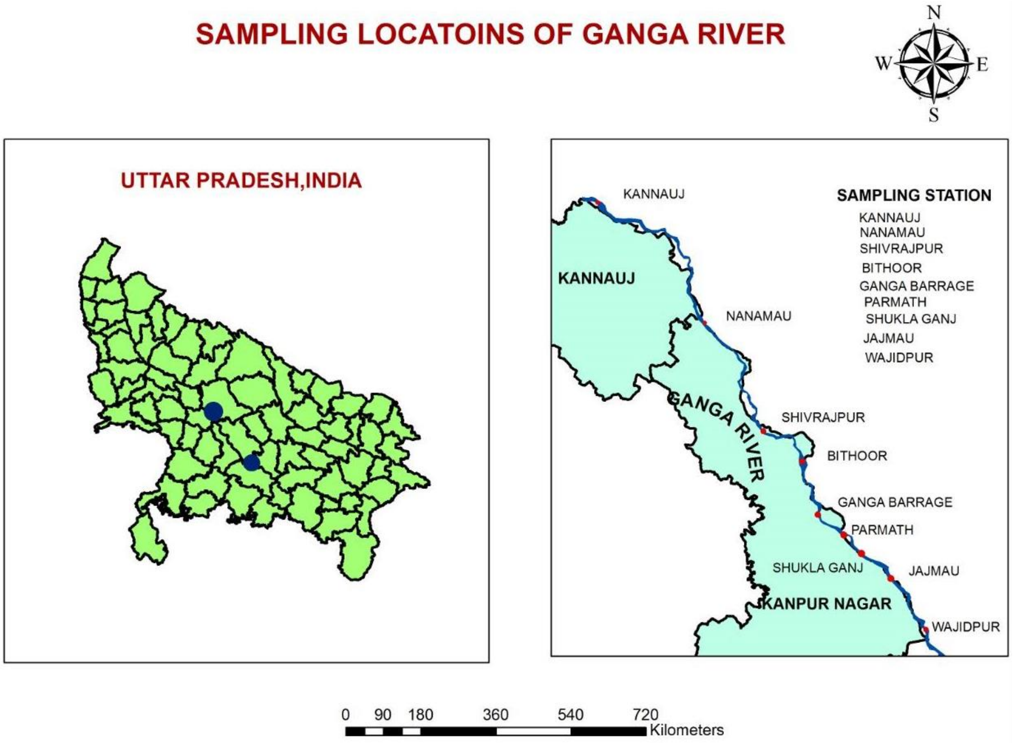

Figure 1. Geographical map of Ganga River representing sampling sites (S_1-S_9).

Analysis of enzymatic activity

Biochemical analysis and homogenate preparation

Rita rita was exposed to numerous sites in Kanpur while swimming in freshwater of river Ganga. The effect of heavy metals on biological enzymes such as anti-oxidative catalase, peroxide, hydrogen peroxide and protein content were analyzed. The gills, liver kidney and muscles were separately homogenized in 1:4 (w v^{-1}) ice-cold chilled buffer of Tris-HCl (pH 7.4 & 0.05 M,) using a homogenizer. A refrigerated centrifuge (CPR-40) was used to centrifuge the sample tissues for 30 min. at 2000 rpm at 4°C, and the supernatant was used for biochemical assays of catalase, peroxidase, and protein estimations.

Determination of catalase (EC: 1.11.1.6)

The method of Aebi (Aebi & Lester, 1984) was used to measure catalase activity. The 2 mL of 100 mM phosphate buffer solution of pH 7.0 then add 0.5 mL 30 mM H_2O_2 and 0.5 mL extract was taken and made volume 3 mL, shake well and waited for 3 min. to determine OD at 240 nm (Double Beam Microprocessor UV-Vis Spectrophotometer LI-2700).

Determination of H_2O_2

Using the Velikova method (Velikova & Loreto, 2005), fresh tissue (100 mg) was extracted with 5 mL of 0.1% trichloro acetic acid (TCA), then centrifuged at 12000 RPM on room temperature for 15 min. to assess the amount of H_2O_2 present. Then, after stirring for three minutes, 0.5 mL of supernatant was combined with 1 mL of 1 M potassium iodide and phosphate buffer solution of (pH 7.0). The absorbance was determined at 390 nm.

Determination of peroxidase activity

By combining 3 mL of buffer solution [750 mL guaiacol, 47 mL phosphate buffer (pH 6.5), and 0.3 mL H_2O_2 (30%)] with 0.06 ml of enzyme extract, the peroxidase enzyme activity was measured using spectrophotometry at a wavelength of 470 nm (Maehly & Chance, 1954).

Estimation of protein content

The Lowry technique was used to measure the protein content. The 100 µl supernatant then add 1 mL reagent C and stand for 10 min. then add 0.1 mL Follin's reagent (follin sociocultural). Shake well and stand for 30 min. then taken) OD at 240 nm.

Ethical statement

The safety, care and methods on experimental animal complied with 'Institutional Animal Ethical Committee (IAEC)' animal welfare laws, guidelines and policies as approved by IAEC, Ref No. 1589/GO/Re/S/2012/CPCSEA.

Results

Physico-chemical characteristics of the River Ganga at several sites from 2018 to 2021

After samples were taken from nine different locations, including Mehandi Ghat, Nanamau, Shivrajpur, Bithoor, Ganga Barrage, Parmath, Shukla Ganj, Jajmau, and Wajidpur, the physico-chemical characteristics of the freshwater in the Ganga River were determined. The results obtained were shown in Table 1. The temperature of the water found in different sampling site range between 27.1-28.1°C with an average temperature was 27.6°C. Turbidity values also estimated in different sites. The value of the turbidity ranged from 10.64 to 24.79 NTU, with an increase in Mehandi Ghat and a reduction in Ganga Barrage. The TDS values were also recorded of various location. TDS values were found decreasing from Mehandi Ghat to Bithoor and then increase is seen from Ganga barrage to Wajidpur. The values of EC were from 236.13 to 341.84 values varies from Mehandi Ghat to Wajidpur. The pH range gradually increase from Nanamau to Wajidpur, but a slight decrease is seen in Mehandi Ghat. Mehandi Ghat had the lowest pH, and Wajidpur had the highest pH. Total hardness value varies from 125.8 to 234.5 mg L^{-1}. There was increase seen in Mehandi Ghat to Parmath and decrease was seen in Shukla Ganj and Wajidpur. The alkalinity values were ranging from 199.04 to 286.79 from Mehandi Ghat to Wajidpur. DO values were ranging between 5.90 to 7.35 and Ganga Barrage had maximum and Wajidpur had lower value seen. The average DO level of the river Ganga at the selected site was a reasonably low 7.01 mg L^{-1}. Values of COD range from 18.3 to 74.27 mg L^{-1}. In addition, Wajidpur has abnormally grown. The maximum COD observed was in Wajidpur, while the smallest was at Ganga Barrage. The range of the BOD value is 11.93 to 20.79 mg L^{-1}. BOD decrease was seen from Mehandi Ghat to Ganga Barrage and increase had beenrecorded from Parmath to Wajidpur. Nitrite value varies from 0.25 to 2.83 ppm. Nitrite decrease was seen from Mehandi Ghat to Ganga Barrage and then increases in Parmath and then decrease was seen in Shukla Ganj and then further increase was seen in Jajmau and Wajidpur and Nitrate value varies from 46.24 to 8.27 ppm. Mehandi Ghat has the maximum values with 46.26 and Bithoor has minimum values with 8.27 ppm (Table 1).

Table 1. Physico-chemical parameters (value in Mean ± SD) of river water in the years 2018 to 2021, from S_1-S_9.

Sampling Sites	Physio- Chemical Parameters											
	Temp.	Turbidity (NTU)	TDS (ppm)	pH	EC (µS cm^{-1})	TH (mg L^{-1})	TA (mg L^{-1})	DO (mg L^{-1})	BOD (mg L^{-1})	COD (mg L^{-1})	Nitrate (ppm)	Nitrite (ppm)
WHO Standard	25ºC	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
Mehandi Ghat (S_1)	26.9ºC	24.79±0.554	162.69±0.718	8.07±0.106	325.00±0.617	125.85±0.865	199.04±0.647	6.13±0.114	20.43±0.417	67.90±0.333	46.24±0.890	2.85±0.432
Nanamau (S_2)	27.4ºC	20.83±0.648	142.12±0.727	8.50±0.319	300.56±0.828	137.68±0.50	210.30±0.430	6.10±0.541	17.86±0.404	57.79±0.614	9.92±0.869	1.24±0.348
Shivrajpur (S_3)	27.0ºC	17.19±0.485	135.93±0.79	8.79±0.369	275.86±0.729	156.85±0.828	219.13±0.441	6.96±0.089	13.9±0.634	25.8±0.543	10.07±0.903	0.75±0.095
Bithoor (S_4)	28.1ºC	12.365±0.383	129.15±0.607	8.87±0.511	257.13±0.758	200.93±0.795	286.08±0.794	6.94±0.450	11.93±0.366	24.97±0.637	8.27±0.454	0.635±0.093
Ganga Barrage (S_5)	27.8ºC	10.64±0.376	145.59±0.695	8.26±0.362	284.35±0.759	214.10±0.955	259.94±0.714	7.35±0.160	11.95±0.235	24.32±0.465	10.67±0.903	0.28±0.032
Parmath (S_6)	27.8ºC	14.385±0.329	147.09±0.727	8.42±0.280	289.02±0.791	220.6±0.646	264.89±0.685	7.21±0.203	12.88±0.506	27.95±0.717	8.72±1.16	1.30±0.324
Shukla Ganj (S_7)	28.0ºC	11.802±0.691	155.87±0.686	8.78±0.499	302.11±0.362	209.66±0.968	235.36±0.451	7.08±0.247	15.84±0.571	63.82±0.664	9.9±0.728	0.30±0.033
Jajmau (S_8)	27.5ºC	12.435±0.713	170.60±0.794	8.77±0.462	335.97±0.793	234.58±0.731	237.98±0.595	6.38±0.316	18.33±0.302	47.33±0.611	19.86±0.648	1.87±0.465
Wajidpur (S_9)	27.1ºC	11.532±0.646	191.54±0.762	8.99±0.512	362.84±0.733	204.11±0.889	230.12±0.722	5.90±0.251	20.79±0.600	80.47±0.495	9.87±0.739	1.7±0.587

Level of heavy metals found in sampling locations

Level of heavy metals depend upon the means of contamination (Table 2) in a particular area. The results are found that mainly five study areas (S_1, S_6, S_7, S_8 & S_9) have higher occurrence of heavy metals than the rest (Figure 2).

Table 2. Occurrence of heavy metals in drained waste water in various locations of River Ganga.

Elements	Sewage/ Municipal waste	Industrial waste/ Tanneries effluent	Agriculture/ Pesticides waste	Detergents
Chromium	+	+	-	+
Cadmium	+	+	+	+
Lead	+	+	+	-
Copper	+	+	+	-
Nickel	+	+	-	+
Arsenic	-	+	-	-
Zinc	+	+	+	-
Cobalt	-	-	+	-
Selenium	-	-	-	-
Calcium	-	-	-	+
Magnesium	-	-	-	+

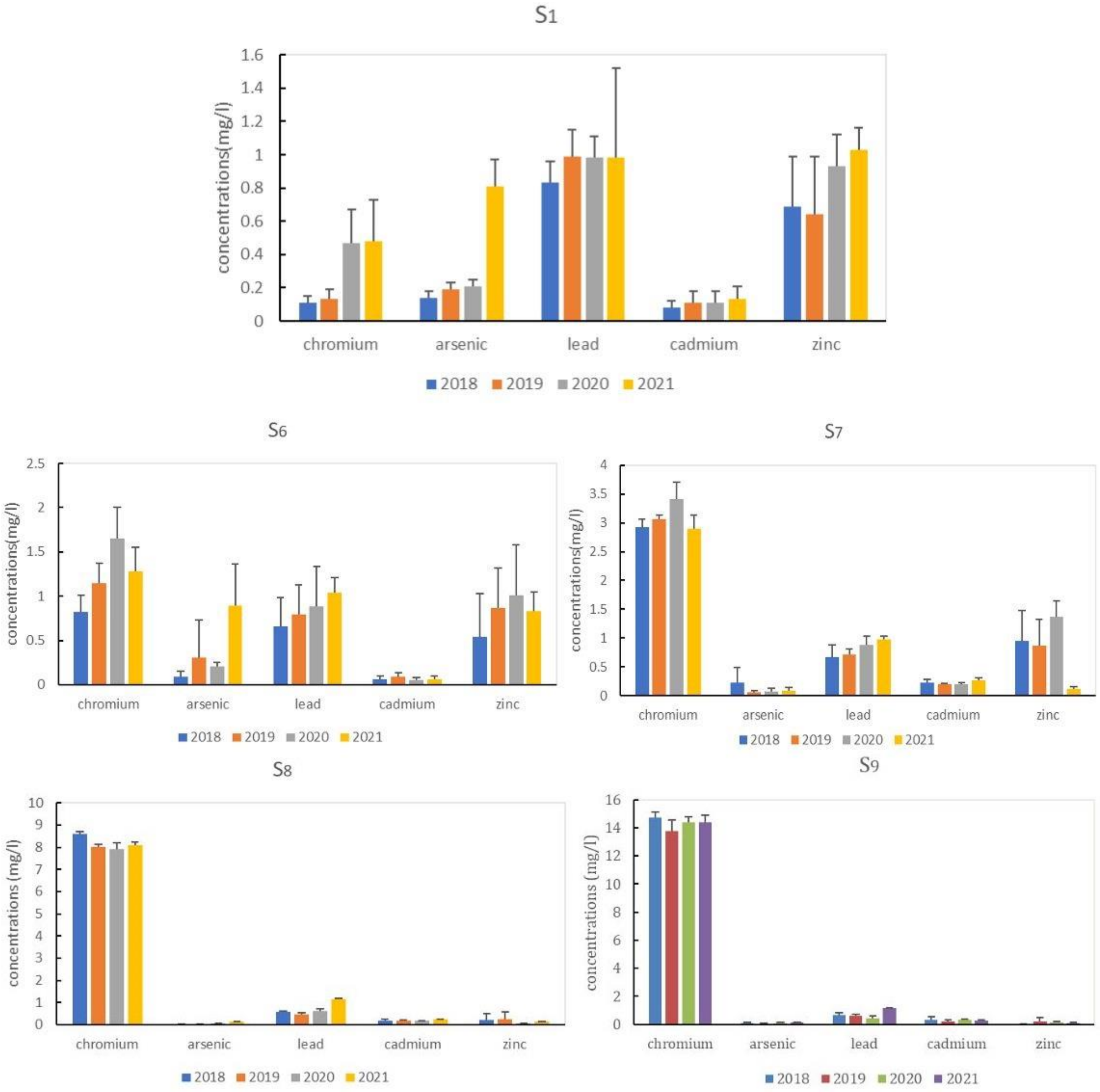

Figure 2. The concentration of heavy metals found in sampling sites (a) S_1, and S_6, to S_9.

Metabolic profile

Biomarkers such as level of catalase, peroxidase, hydrogen peroxide, and protein content are affected by the modification of organic and inorganic components in catfish (Table 3). According to a study, fish tissues with decreased catalase and peroxidase activity were observed in areas with high concentrations of heavy metals, such as Wajidpur, Jajmau, Shukla Ganj, and Mehandi Ghat. Catalase activity in the different tissues of catfish found in Wajidpur were reported lesser as compared to others (in kidney 0.23±0.07, gills 1.40±0.60, muscles 0.80±0.22 & liver 0.48±0.23) as having presence of high number of heavy metals, mainly chromium that was at its peak. The deterioration of catalytic activity was seen in liver in every year of sampling (Figure 3).

Significant correlation between catalase activity and concentration of hydrogen peroxide

Heavy metals were found affecting the different tissues of fishes that is leading to decrease in enzymatic activity and increase in metabolites concentration. After exposure of heavy metals, the correlation analysis between catalase activity and the protein content of in kidney was observed negatively significant ($r = -0.87$, $t = 10.14$, $p \leqslant 0.05$) (Figure 4).

Table 3. Impact of combinatorial response of physicochemical and heavy metals with reference to metabolic enzymes on catfish, *Rita rita* in Ganga River.

Sampling sites	Organ	Enzymatic activity			
		Catalase (μmoles min.$^{-1}$ mg^{-1}	Peroxidase (Unit mL^{-1})	Hydrogen peroxide (μg g^{-1} f. wt.)	Protein (μg g^{-1} f. wt.)
Mehandi Ghat	Kidney	0.39±0.04	0.23±0.077	2.91±0.24	142.07±0.9
	gills	1.99±0.46	1.23±0.65	3.99±0.47	112.86±0.75
	muscles	1.86±0.40	1.09±0.18	3.68±0.68	125.25±0.63
	liver	1.18±0.53	2.7±0.44	4.8±0.17	157.7±0.47
Nanamau	kidney	3.97±0.06	1.70±0.51	2.01±0.42	88.97±1.07
	Gills	3.59±0.59	3.86±0.82	1.02±0.12	97.46±0.83
	muscles	2.49±0.79	3.10±0.80	1.60±0.42	108.71±0.73
	Liver	2.53±0.50	2.60±0.45	2.03±0.45	121.68±0.62
Shivrajpur	kidney	2.3±0.10	4.01±0.65	0.85±0.17	109.35±0.47
	Gills	1.82±0.57	4.64±0.43	1.07±0.36	86.11±0.90
	muscles	2.25±0.22	3.90±0.49	1.33±0.54	95.81±0.32
	Liver	0.76±0.28	2.9±0.66	0.52±0.30	98.10±0.71
Bithoor	Kidney	2.10±0.15	1.90±0.46	2.15±0.34	111.5±0.91
	Gills	4.03±0.73	3.040.86	1.85±0.66	74.88±0.31
	Muscle	2.18±0.54	3.05±0.38	2.4±0.50	86.03±0.42
	Liver	2.91±0.35	2.71±0.61	0.92±0.49	103.25±0.47
Ganga Barrage	Kidney	1.64±0.09	2.12±0.48	1.42±0.66	100.75±1.8
	Gills	3.01±0.56	4.53±0.42	1.90±0.42	96.9±0.37
	Muscle	2.9±0.83	3.50±0.70	2.96±0.36	120.97±0.60
	Liver	3.90±0.26	3.20±0.21	2.63±0.40	97.64±0.39
Parmath	Kidney	1.18±0.15	3.61±0.44	0.83±0.51	103.14±1.3
	Gills	3.13±0.51	4.42±0.58	2.91±0.30	82.44±0.76
	Muscle	2.2±0.91	3.20±0.21	2.93±0.53	58.91±0.48
	Liver	3.86±0.58	3.61±0.611	3.11±0.47	64.25±0.81
Shukla Ganj	Kidney	0.38±0.02	0.14±0.044	3.14±0.23	144.7±1.15
	Gills	2.9±0.68	1±0.29	2.93±0.17	135.3±0.68
	Muscle	1.04±0.52	1.9±0.73	3.76±0.22	161.5±0.77
	Liver	0.42±0.17	1.4±0.44	3.52±0.53	163.02±0.65
Jajmau	Kidney	0.40±0.03	0.30±0.04	2.96±0.61	142.8±1.94
	Gills	2.03±0.55	0.72±0.37	2.76±0.72	144.61±0.63
	Muscle	0.30±0.05	0.83±0.39	2.80±0.24	174.06±0.18
	Liver	0.62±0.18	0.74±0.20	4.83±0.52	161.10±0.23
Wajidpur	Kidney	0.23±0.07	0.13±0.04	2.99±0.43	144.5±0.52
	Gills	1.40±0.60	0.25±1.44	3.12±0.39	131.53±0.39
	Muscle	0.80±0.22	0.59±0.19	3.09±0.63	154.61±0.80
	Liver	0.48±0.23	0.59±0.51	4.5±0.51	149.13±0.33

Justification: same as for Table 1.

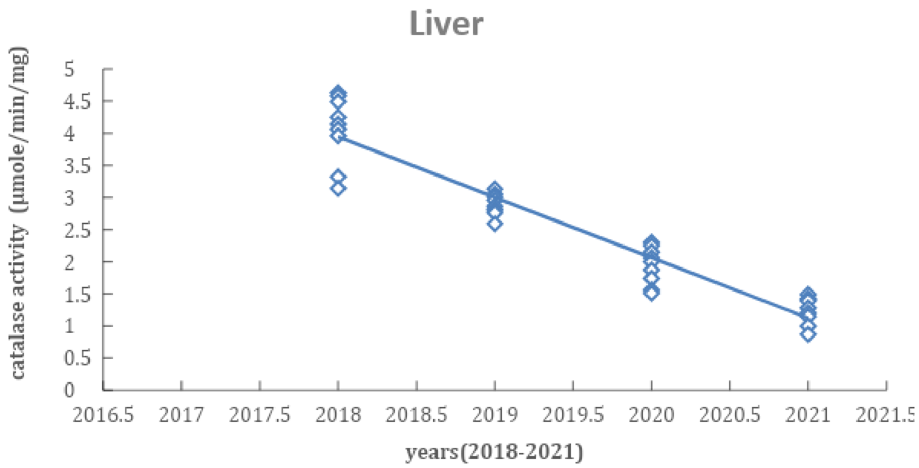

Figure 3. Deterioration in catalytic activity in four-year data analysis.

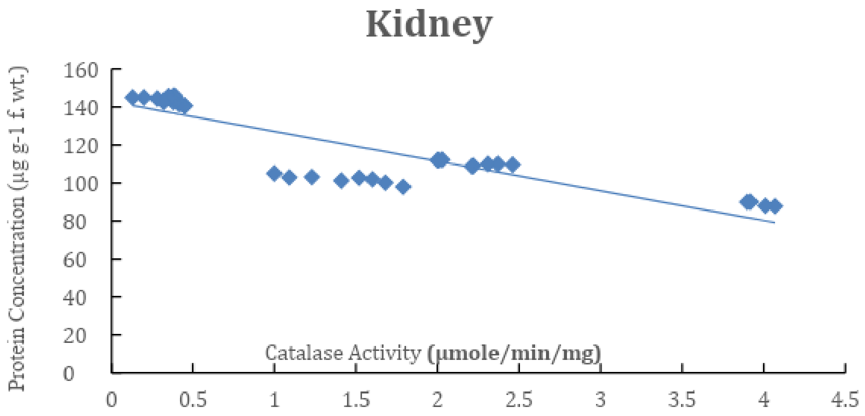

Figure 4. Correlation analysis between catalytic activity and protein accumulation in kidney ($r = -0.87$, $t = 10.14$ and $p \leq 0.05$).

Discussion

Due to urban runoff, drainage of tanneries, rapidly growing industrialization, sewage waste, pesticides exposure, the quality of water in Ganga River have shown in deterioration. The results are explaining that how the health of fishes is affecting the human life indirectly. Water temperatures were recorded and showed variations according to the seasons. In the post monsoon period, the most changes have been recorded in surface temperatures in this period the species in water have heavily suspended matters. Temperature is playing crucial role in the determination of alkalinity, conductivity and other physico-chemical characteristics (Arnell, Bates, Lang, Magnuson, & Mulholland, 2016).

The turbidity value ranged from 10.640.37 to 24.790.55 NTU, with Mehandi Ghat showing a rise and Ganga Barrage showing a reduction. Turbidity of water may be the indicator of some external factors are present that can also be heavy metals that are making water quality poor (Bilotta & Brazier, 2008). The minimum and maximum readings for turbidity were 10.64 and 24.79 NTU, respectively. The more turbid water means the more difficult to perform purification processes like flocculation and filtration techniques. Microbes are the main reason of highly turbid water. Turbidity is making the barrier for light to penetrate deeply (Gros, Petrović, & Barceló, 2007).

The EC varies from Mehandi Ghat to Wajidpur, with values ranging from 236.13 to 341.84 S cm^{-1}. The nearby industrial effluents are running into the river and also found sewage confluence point to River Ganga. The rainwater causes leaching of substances that can be toxic like pesticides results in high EC. In the Yamuna River in Agra, EC values between 990 and 1285 mg L^{-1} were reported (Gupta, Yadav, Kumar, & Singh, 2013), which are significantly higher than those in the current study. According to Chandra et al. (2012) high EC values suggested the presence of more inorganic matter than dissolved inorganic compounds in ionised form.

One of the most crucial instruments for determining how acidic or alkaline water is is pH. Aquatic species are sensitive to pH changes because these changes affect the way the aquatic system is structured. The Ganga River had a pH range of 8.07 to 8.99. Mehandi Ghat had the lowest pH, and Wajidpur had the highest pH. The high pH was found during monsoon period. The pH range for potable water is between 6.5 to 8.5, analysis of pH is a important for ecosystem's health and other prospects. The accumulations of heavy metals are found related to pH as it is falling between slightly acidic to moderately alkaline (Shakirat & Akinpelu, 2013; Soceanu et al., 2021).

The measurement of hardness in water is not a indicator of pollution but reflects the change in parameter, mainly in terms of chloride, Mg^{2+} and bicarbonate, Ca^{2+}, sulphate and nitrites. Total hardness values in Ganga River water varies from 125.8±0.86 to 234.50±0.73 mg L^{-1} and decreased to 204.11±0.88 mg L^{-1} in Wajidpur. Less than 75 mg L^{-1} of CaCO3 is regarded as soft water, and more than 75 mg L^{-1} as hard water. It is a crucial factor in figuring out whether water is suitable for drinking, household use, and a variety of industrial applications. Total hardness due to carbonate and bicarbonate that is 234.5±0.73 mg L^{-1} in Jajmau. TH readings in the Yamuna River at Agra ranged from 252 to 304 mg L^{-1} (Gupta et al., 2013). The detected values were marginally greater than the 150 and 200 mg L^{-1} WHO and ISA recommended limits. Highly alkaline water is unappealing and cannot be used for residential water supply, despite the fact that alkalinity has no bearing on public health (Jothivel & Paul, 2014). Water hardness means the presence of several ions for e.g., manganese ions, magnesium, calcium, strontium and ferrous, basically originated from rock and soil erosion.

The most crucial factor to analyze the condition of the water is the dissolved oxygen. Oxygen balance is very necessary requirement in water that maintains the various forms of life running under the water. Discharge of oxygen demanding wastes in Ganga River rapidly showed decreasing level of DO. Ammonia, nitrite and several other inorganic reducing agents were found responsible to decrease DO level. The DO level in water is determined by both aerobic and anaerobic processes. The minimal DO of 5.90±0.25 mg L^{-1} was discovered at the Wajidpur site, while the maximum of 7.35±0.16 was observed at the Ganga Barrage site. The increased solubility of DO, decreased microbial breakdown of dead organic waste, low respiratory demand of organisms at low temperatures, and the progressive growth of buried macrophytes all seem to be contributing factors. The amount of DO controls whether anaerobic or aerobic processes are occurring in the water. In order to support healthy growth of fish and other aquatic species, DO is often more than 6 ppm and in sufficient quantities (Wetzel, 1990; Francis-Floyd, Watson, Petty, Pouder, 2009).

The level of BOD ranged from 11.93±0.36 to 20.79±0.60 mg L^{-1}. Due to mixing of air in depth BOD is higher in surface water. The lowest BOD of 11.93±0.036 mg L^{-1} was discovered in Bithoor and the Ganga Barrage, whereas the highest BOD was discovered in water during the discharge of effluents into water bodies. The amount of oxygen needed by microorganisms to use or stabilise organic materials in water over a five-day period is known as BOD. It is a crucial indicator of a water body's level of organic contamination. The BOD values of industrial waste streams have climbed to 25000 mg L^{-1}, when normal unpolluted water has BOD values of 3 mg L^{-1} or less. In general, BOD readings indicate the amount of organic matter that can be oxidized and is consequently, BOD measures the level of water pollution. BOD values play crucial role in determining the capacity of self-purifying ability of waterbodies that is essential to control the pollution (Wen, Schoups, & van de Giesen, 2017).

Throughout the study period, the COD value ranges from 24.3 to 80.47 mg L^{-1}. The highest observed COD is at Wajidpur, while the lowest is inadvertently in the Ganga Barrage. The COD level is abnormally higher in Shukla Ganj and abnormally lower in Shivrajpur. decrease in the COD level. High COD in water indicating the water is contaminated. The observed NO_3 ranged 8.27 to 46.24 ppm in the river Ganga. As particulate, nitrogen is abundant in surface water. Bacterial aerobic and anaerobic processes decay the organic nitrogen into ammonia then this ammonia in turns into nitrites and ultimately nitrification process turns nitrites to nitrates. Nitrates are added due to agricultural runoff mainly (Abdel-Raouf, Al-Homaidan, & Ibraheem, 2012; Edokpayi, Odiyo, & Durowoju, 2017).

The current study examined how the Ganga River's *Rita rita* catfish responded to oxidative stress in order to assess the value of a multi-biomarker approach in environmental monitoring instruments and methods in the Kanpur area. This species also exhibits a spatially varied antioxidant response to counteract the natural pro-oxidant elements found in these freshwater environments.

A lot of people’s lives are on stake due to contaminated through uncontrolled anthropogenic activities in Ganga River water. In order to track the impact of river pollution on aquatic life, a field study was carried out in which various biomarker enzymes and biochemical parameters from the muscles, liver, gills and kidneys of Indian catfish Rita rita collected from the River Ganga at various Kanpur locations were analysed quarterly from 2018 to 2021 (Katiyar, Gupta, Katiyar, & Sharma, 2021). The level of catalase and peroxidase activity significantly were decreasing as the concentrations of heavy metals increases. In a similar way, data from earlier research have also been documented in the gill, liver, and kidney of other fish species, including Carassius auratus (Khan, Liu, & Shah, 2014), Cu and Cd exposures to freshwater teleost *Oreochromis noloticus* in gills and liver tissue of genetically improved form tilapia (*O. niloticus* - Parthasarathy & Joseph, 2011). The accumulation of heavy metals in the tissues leads in misbalancing showed that the experimented catfish were under the influence of oxidative stress. The most basic and prevalent biochemical component found in fishes is protein. Decreasing enzymatic level affects the metabolism negatively and this causes protein accumulation in tissues more than the required amount resulting the formation of free radicals that are toxic. Remarkable increase or decrease in protein and hydrogen peroxide content were reported in response to concentrations of heavy metals leading impair breakdown of both content (Basha & Rani, 2003; Bhatkar, Vankhede, & Dhande, 2004; Khan et al., 2015).

After analyzing four-year data, in mainly five places (Wajidpur, Shukla Ganj, Jajmau, Mehandi Ghat and Parmath) and reported the continuous deterioration of river Ganga in every year. In these areas, the concentrations of heavy metals that are studied in this time period was continuously getting worse that is needed to take a quick action to remove these contaminants.

Conclusion

The sentinel organism that are the fishes show the variable biological responses that vary to the time of exposure and the concentration of contaminants. The findings are basically based on the combinatorial study of physicochemical parameters on fresh water fish metabolism with special reference to drainage of pesticides, tanneries, and industrial waste. The health state of fish populations is determined using a variety of responses in addition to biomarkers. To establish differences in different places, however, a varied number of specimens is needed due to the inherent variability displayed by each biomarker. Controlling the anthropological activities may contribute to not increase pollutants. The study that is being presented is the first in-depth examination of the combinatorial analysis of physicochemical parameters and wastewater responses at sampling locations. This information is essential for correctly interpreting eco-toxicological data in the future and for environmental monitoring programmes in the area.

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