Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-023-25519-2

RESEARCH ARTICLE

Hemato-biochemical alteration in the bronze featherback *Notopterus notopterus* (Pallas, 1769) as a biomonitoring tool to assess riverine pollution and ecology: a case study from the middle and lower stretch of river Ganga

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Received: 19 July 2022 / Accepted: 19 January 2023

Abstract
Fishes are poikilothermic animals and are rapid responders to any sort of ecological alteration. The responses in the fish can be easily assessed from their hematological and biochemical responses. To study the variation in the hemato-biochemical parameters in retort to ecological alteration and ecological regime, a study was conducted at six different sampling stations of the middle and lower stretches of river Ganga. Various hematological and biochemical responses of fishes were also monitored in response to multiple ecological alterations. For the assessment of ecological alteration, various indices were calculated such as the water pollution index (WPI), National Sanitation Foundation–water quality index (NSF-WQI), and Nemerow's pollution index (NPI) has been calculated based on various water quality parameters such as dissolved oxygen (DO), pH, total dissolved solids (TDS), total alkalinity (TA), total hardness (TH), electrical conductivity (EC), biochemical oxygen demand (BOD), chlorinity (CL), total nitrogen (TN), and total phosphorus (TP). The hematological parameters such as WBC, RBC, platelet, hemoglobin, and hematocrit were monitored. The serum biochemical parameters such as SGPT, SGOT, ALP, amylase, bilirubin, glucose, triglyceride (TRIG), and cholesterol (CHOL) were investigated. The study revealed that NSF-WQI varied from 45.08 at Buxar to 110.63 at Rejinagar and showed a significantly positive correlation with SGPT, SGOT, ALP, TRIG, CHOL, and WBC, whereas a significantly negative correlation was observed between TRIG and RBC. WPI varied from 19 to 23 and showed a significant positive correlation with SGOT and a negative correlation was observed with total nitrogen. The PCA analysis illustrated the significance of both natural as well as anthropogenic factors on riverine ecology. Strong positive loading was observed with SGPT, SGOT, ALP, and platelet. The study signified the need for monitoring the hemato-biochemical responses of fishes in response to alterations in the ecological regime.

Keywords River Ganga · Hematology · Serum biochemical response · Water pollution index · NSF-WQI · NPI

Introduction

The river Ganga is the lifeline for the majority of the Indian population as it harbors a vast number of aquatic as well as terrestrial organisms (Pandey and Radhakrishnan 2022).

The river Ganga has great economic, ritualistic as well as ecological importance (Sharma and Singh 2021). Its role in rendering the aquatic ecosystem as well as providing drinking water for the citizens is remarkable. The augmented human dependency due to the multiple-fold amplified rise in population across the river bank has increased the ecological stress on the river system by the means of river pollution. The increasing riverine pollution has led, riverine as well as human health under threat due to their direct or indirect dependency (Tiwari et al. 2022b). The river Ganga is also much susceptible to any sort of ecological alteration due to diversified natural factors such as rainfall, changing weather conditions, river flow, and so on, and has

Responsible Editor: Bruno Nunes

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Published online: 01 February 2023

a significant undesirable impact on the river's ecological character (Shrestha and Kazama 2007; Zeinalzadeh and Rezaei 2017). Along with natural factors, human interferences like urbanization, industrialization, construction of dams and barrages, etc. have also adversely affected the riverine habitat (Ghosh and Maiti 2021). With the increment in the population, riverine pollution has also been amplified from various points, as well as non-point pollution sources (Taghinia Hejabi et al. 2011; Kisi and Parmar 2016). Water contamination has made food production in jeopardy and increased environmental and human health concerns (Abedin et al. 2014; Akhtar et al. 2007). River Ganga travels a path length of 2525 km and travels a large distance from its mouth at the Himalayan glacier situated at Gaumukh to its end at Gangasagar, delivering a multitude of ecosystem services.

Fish being the poikilothermal animal are extremely vulnerable and are directly exposed to all the riverine pollution modes as they feed and live in aquatic environments, from where they cannot escape from any sort of changes in the riverine ecology (Yarsan and Yipel 2013; Mahboob et al. 2014 Omar et al. 2014). For all these reasons, fish has been identified as bioindicators of environmental contamination, providing a holistic view of their surroundings across time (Plessl et al. 2017). The method of thermal acclimation in a poikilothermic animal like fish entails a slew of regulatory processes that work together to keep the metabolic rate under control by adjusting for heat influences on biochemical reactions (Das and Prosser 1967). Here, in this study, one particular fish *Notopterus notopterus* (Pallas 1769), was taken as the experimental species. *N. notopterus*, similar to any other aquatic organism, exists close to the aquatic habitat, where rapid changes are manifested as observable pathophysiological observations (Musa and Omoregie 1999; Seth and Saxena 2003). *N. notopterus* (Pallas 1769), popularly known as Bronze featherback, is a Notopterid fish from the order Osteoglossiformes found commonly in the Ganga River (Kumari and Maiti 2019). It is an omnivorous organism that consumes fish, crustaceans, insects, and plant matter (Khaing and Khaing 2020) at different trophic levels. The alteration in the water quality status and pollution effect may be more evident in this species.

For the evaluation of riverine ecology, the evaluation of physicochemical parameters including major water quality parameters is an important technique. But it is so difficult to evaluate the water quality status of the riverine ecosystem with the help of individual parameters (Matta et al. 2022). So, the globally used water quality index assessment tool, i.e., National Sanitation Foundation–Water Quality Index (NSF-WQI) has been widely applied for the assessment of water quality globally and in the river Ganga (Tripathi and Singal 2019; Kumar et al. 2021; Tiwari et al. 2022a). The National Sanitation Foundation–water quality index which has been developed in 1970 by (Brown et al. 1970) has been used for the study of water quality. Along with NSF-WQI, the evaluation of the water pollution index for the assessment of riverine water quality is also noteworthy and has been used globally for the assessment of the water quality status of the aquatic system (Suriadikusumah et al. 2021; Mohanty et al. 2022).

The health status of any organism can be determined by the examination of its hematological and biochemical parameters and are the preliminary diagnostic tool that gets changed with the alteration in the riverine ecology (Lakra et al. 2022). As fishes are poikilothermal animals and respond quickly to any sort of ecological change. So, the hematological examination of the fish can give the ultimate result of their health status and varies with various environmental constraints (Burgos-Aceves et al. 2019). Among the different hematological parameters, the major influencer parameters are red blood cell (RBC), platelet count (PLT), white blood cell (WBC), and hemoglobin (Hb) (Grant 2015; Neelima et al. 2015) and may be considered as the potential biomarker for the evaluation of fish health status in response to ecological alteration. Along with hematological changes, the serum biochemical factors are also responsible for the monitoring of fish health status by the means of different organs such as the liver, heart, and various organs of the digestive system which shows a rapid response to any sort of ecological alteration. The liver enzymes serum glutamate pyruvate transaminase (SGPT), serum glutamate aspartate transaminase (SGOT), and alkaline phosphatase (ALP) are the major liver enzyme that expresses their response via metabolic transformation caused by ecological niche (Farag et al. 2022). As the majority of liver enzymes are considered the potential biomarker for the evaluation of riverine pollution (Athira and Jaya 2018; Recabarren-Villalon et al. 2019). Similarly, other biochemical parameters such as triglyceride and cholesterol also alter with stress response and can be considered as the potential biomarker for the evaluation of fish health (Biswal et al. 2021). Fish health also changes with the change in the ecological condition, and any sort of alteration in the ecological condition can be easily monitored by evaluating these important hematological and biochemical parameters (Dinardo et al. 2021; Murthy et al. 2022).

The study was carried out in the middle and lower stretch of river Ganga where the fish *Notopterus notopterus* is well distributed at all the selected sampling sites in the river Ganga (Das et al. 2020; Sarkar et al. 2012 and Lakra et al. 2010), i.e., at Buxar, Patna, Bhagalpur, Farakka, Rejinagar, and Balagarh. The sampling sites were also selected based on demographic variability, and flow direction. The majority of the sampling sites are located on the bank of major cities.

The objective of the present study is to find out the multiple biomarkers in response to ecological variability in fish. As ecological variability can affect the hemato-biochemical parameters of aquatic organisms including fish (*N. notopterus*). So, to study the variability of hemato-biochemical parameters and find out the multiple biomarkers in response to ecological alteration various univariate as well as multivariate statistical tools were used among which the National Sanitation Foundation–water quality index, Nemerow's pollution index, and water pollution index were calculated, and were correlated with different hematological as well as biochemical parameters which can alter the fish health. The manuscript also emphasizes the individual physicochemical parameters which can alter the ecological condition of the riverine system.

Material and methodology

The flowchart for materials and methodology used for the study and analytical procedure is given in Fig. 1.

Selection of study sites

The present study was conducted at six sampling sites, namely Buxar, Patna, Bhagalpur, Rejinagar, Farakka, and Balagarh, the GPS coordinates are shown in Table 1 and Fig. 2 and are designated as middle and lower stretches of the river Ganga, encompassing a total path length of more than 900.18 km^2 (approx.). The sampling sites were selected based on the majority distribution of fish *Notopterus notopterus* in the freshwater middle and lower stretches of river Ganga.

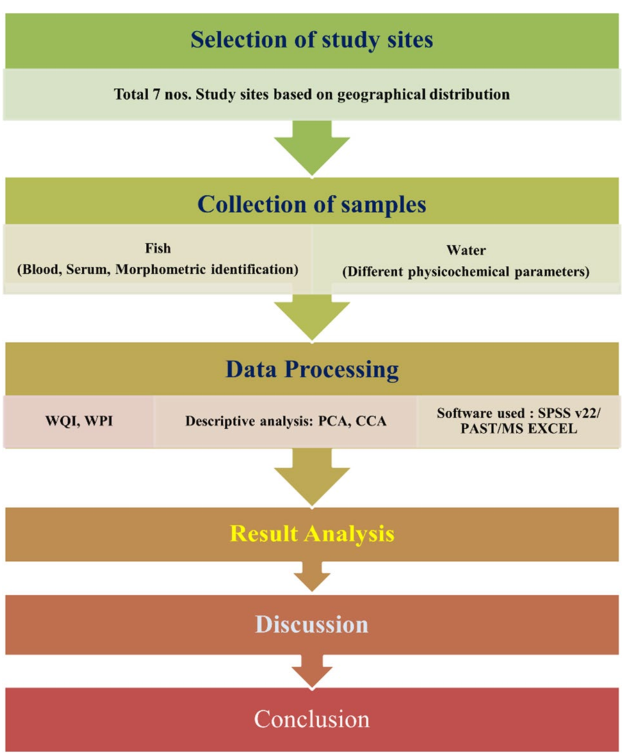

Fig. 1 Methodological flowchart of the study

Table 1 GPS coordinates of the sampling and study sites

Site	X (Long)	Y (Lat)
Buxar	83.9777482	25.5647103
Kanpur	80.3318736	26.449923
Patna	85.1375645	25.5940947
Bhagalpur	86.9824288	25.3478004
Farakka	87.9089623	24.8006687
Rejinagar	88.2679264	24.0988265
Balagarh	88.4646107	23.118857

Selection of species

The species *Notopterus notopterus* (Pallas 1769) belongs to the order Osteoglossiformes and the family Notopteridae. This species was selected for this study due to its wide distribution throughout the freshwater stretch of the river Ganga. It has been reported to the wide range of the river system i.e., from Bijnor, Uttar Pradesh to Tribeni, West Bengal (Das et al. 2020). Due to the bottom-feeding habit of *Notopterus notopterus* (Kumari and Maiti 2019), the species is much more prone to decomposed organic and inorganic materials in the river bed. So, for this purpose, *Notopterus notopterus* fish having an average weight of 66.97 ± 3.12 g and an average length of 23.13 ± 1.06 cm were collected from these six sampling sites, for the purpose thirty fishes (30) were collected, and sampled from every sampling point, using specialized gill net.

Prior to the study, ethical approval has been taken from the Institute ethical committee of ICAR-CIFRI, Barrackpore Kolkata regarding the study, and the experiment was carried out following the standard protocols and ethical guidelines of CPCSEA 2021; Ministry of Fisheries, Animal Husbandry and Dairying, Government of India (CPCSEA 2021) and Institute ethical committee, ICAR-CIFRI, Barrackpore, Kolkata.

Collection and analysis of samples

Hematological and biochemical sample

For the assessment of hematological and biochemical parameters, the fish were anesthetized by dipping in 50 μL^{-1} clove oil–water solution, and then blood samples were collected by caudal vein puncture using a 2-mL sterile syringe and kept in 2 mL EDTA coated vials and were preserved at 4 ℃ till analysis. For different biochemical analyses, the collected blood was stored in autoclaved sterile Eppendorf

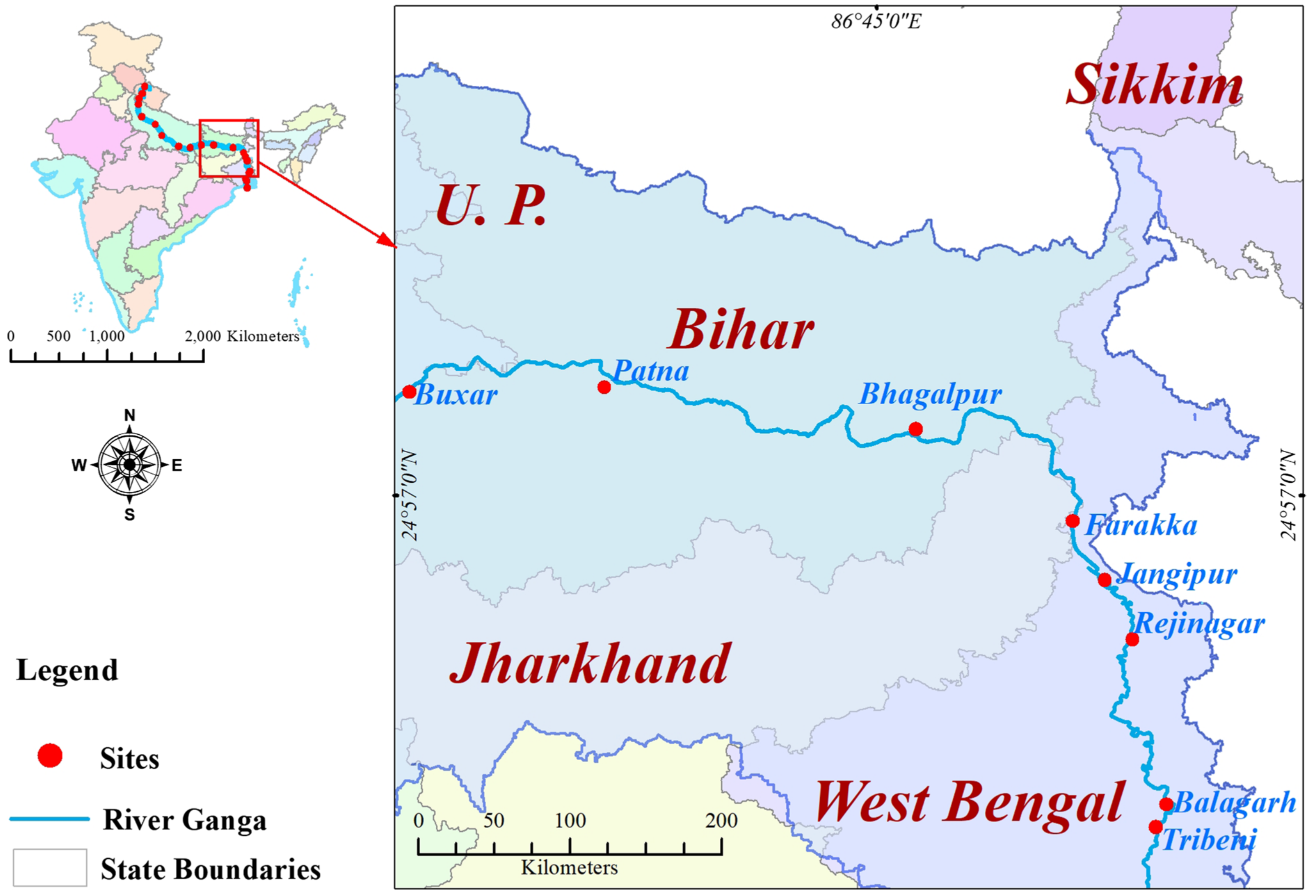

Fig. 2 Study area map of the sampling sites

tubes and was kept undisturbed for 1 h, then the blood was centrifuged at 3500 rpm for 10 min for serum separation. The straw-colored supernatant was collected by micropipette and was kept in a − 80 ℃ freezer for further analysis. From the sampling sites, all the collected samples were transported immediately in the ice pack to the laboratory. The Sysmex XP-100 a 3-part differential hematological analyzer was used to examine several hematological parameters such as RBC, WBC, Hemoglobin, Hematocrit, and Platelets (Sysmex Corporation, Kobe, Japan). Subsequently, biochemical parameters such as SGPT, SGOT, ALP, amylase, bilirubin, glucose, triglyceride, and cholesterol were analyzed using a fully automated blood biochemistry analyzer (Model – EM-200, ERBA-Transasia, Mumbai).

Water sample collection and analysis

The sampling was done in 2021 during the post-monsoon season, following the APHA 2017 standards (APHA 2017). The sampling took place between the hours of 9 AM and 10 AM. Triplicate sampling was done, with samples taken from both the river's banks and the middle. The water samples for nutrient analysis were collected in 1000 mL high-density polyethylene bottles (Make- TARSON®). Before each sampling, the sampling bottles were autoclaved and rinsed with diluted HNO_3, and double-distilled water. At the time of sample collection, the bottles were rinsed with the river water before collection of samples and after collection, the cap of the bottles was tightly closed. All the bottles were sealed with PARAFILM and kept in the Ice-box and brought to the laboratory for further analysis.

Some of the physicochemical parameters like dissolved oxygen (DO), free CO_2, and alkalinity were analyzed in the field by using the titrimetric method following APHA 2017. Total hardness, magnesium, and calcium were analyzed by EDTA Titrimetric Method (APHA 2017). Other parameters like pH, turbidity, TDS, electrical conductivity, and salinity were evaluated by using the multi-parameter water quality analysis equipment (YSI PRO DSS 4-Port Digital Sampling System, Made in the USA). The riverine flow was measured by a digital flow meter (Global Instrument FP-111®). The water depth was measured using a depth sounder (HONDEX PS-7®). A Secchi disk was used to measure the transparency of the water following the standard methods followed by (Tiwari et al. 2022a). For analysis of biochemical oxygen demand (BOD), the samples were collected in 300-mL glass BOD bottles and brought to the laboratory and kept in a BOD incubator for three days at 37 ℃, and then the analysis was done as per the standard methods following APHA 2017, the nutrient parameters like silicate, total phosphorus, and total nitrogen were analyzed.

Quality control

During the analytical procedures, the instruments were calibrated by using the standard solution and recommended protocol by the manufacturer. The electrodes of YSI Pro DSS were washed with double distilled water after every use. At the time of analysis, the electrodes were pre-stabilized till the data displayed was stable. At the time of laboratory analysis, to reduce the error during spectroscopic reading the readings were taken in triplicate, and standard along with blank sample readings was taken during the analysis. In every analysis, the glassware used was class "A" NABL certified and manufactured by Borosil®. During the analysis, the used chemicals were MERCK®.

Statistical analysis

The statistical analyses were carried out from IBM 126 SPSS 22, Microsoft Excel 2007, and Past 4.02 applications were used. Microsoft Excel 2007 software was applied for the calculation of WQI.

National sanitation foundation-water quality index (NSF-WQI)

The NSF-WQI was calculated for the assessment of water quality status. The method used for the calculation was of same as that (Deshmukh and Aher 2016). For the assessment, the selected parameters were pH, dissolved oxygen (DO), temperature, turbidity, total dissolved solids (TDS), phosphate, nitrate, and BOD.

Step 1

In step 1, each of the selected parameters was given a specified assigned weight (Wa) in the range of 1 to 5, where 1 signifies the least importance and 5 signifies the higher significance of the parameter. After assigning the weight to an individual parameter, the relative weight was estimated using the formula:

$$\mathrm{Wr} = \mathrm{Wa} \div \sum_{\mathrm{i}=1}^{\mathrm{n}} \mathrm{Wa}$$

In step 2, the quality rating scale (q_i) was assessed using the formula.

Table 2 BIS standards used for the calculation of water quality index (WQI)

Water quality parameter	Standard permissible limits, BIS 2012
Total dissolved solid (ppm)	< 2000
Dissolved oxygen (ppm)	> 6
Specific conductance (µS/cm)	< 300
Nitrate (ppm)	< 45
Biochemical oxygen demand (ppm)	< 5
Total alkalinity	< 300
pH	6.5–8.5

Table 3 Reference range of WQI and water quality status given by Yadav et al. (2010)

Range of WQI	Water quality status
0–25	Excellent
26–50	Good
51–75	Poor
76–100	Very poor
> 100	Unsuitable

$$qi = \left(\frac{Ci}{Si}\right)100$$

where Ci is the observed concentration of the individual parameter.

Si is the ideal value as given by (BIS 2012) (Table 2).

Then the sub-index value (SIi) was calculated using the formula

$$\mathrm{SIi} = \mathrm{W_i q_i}$$

And finally, the WQI was calculated using the formula given below

$$WQI = \sum_{i=1}^{n} SIi$$

And after calculation, the values were compared with the reference range given by (Yadav et al. 2010) (Table 3).

Nemerow's pollution index

The Nemerow's pollution index (NPI) is the featured statistical calculation tool used for the categorization of the river system

(Xiong et al. 2019). The NPI was calculated using a mathematical expression used by Xiong et al. (2019) and Haque et al. (2020). The NPI is calculated using the formula:

$$NI = \sqrt{\left(\frac{\left[\left(1/_n\right)\sum\left(Ci/_{Si}\right)\right]^2 + \left[max\ Ci/_{Si}\right]^2}{2}\right)}$$

And later the values were compared (Subagiyo et al. 2019).

Principal component analysis (PCA)

Principal component analysis (PCA) is the multivariate statistical tool used for the classification and categorization of different ecological niches using multiple water quality parameters (Tripathi and Singal 2019). So, using IBM SPSS 22 software, PCA was carried out to highlight the anomaly and improve the viability of the datasets for the study of the different physicochemical parameters of the Ganga River water. The Kolmogorov- Smirnov tests and Shapiro- Wilk tests, were also carried out to ensure the normality of the datasets. Earlier to that, the Kaiser–Meyer–Oklin and Barlet sphericity tests were used to assess the result's reliability. The PCs having eigenvalue more than were extracted using KMO–Barlett's test and scree plot. To reduce the data variability the VARIMAX rotation was carried out.

Water pollution index (WPI)

The water pollution index was assessed to evaluate and characterize the various study sites based on their chemical health. It was developed and modified in the USA namely the nutrient pollution index and was developed by Dodds et al. (1998) and later (Lee and An 2009) in South Korea and newly improved by Atique and An (2019). Total nitrogen/total phosphorus ratio was used to analyze the nutrient load caused by different nutrients such as total nitrogen and total phosphorus. BOD was assessed for the assessment of the decomposed organic matter. The total suspended solids (TSS) and electrical conductivity were used to calculate the ionic contents and solids present in river water. The concentration of chlorophyll in the water was used to estimate the primary production (Wang et al. 2017). Scores were used to compare and grade the derived values, i.e., excellent (31–35), good (25–29), fair (19–23), poor (13–17), and very poor (7–11).

Cluster analysis

Cluster analysis is carried out to identify the relationship between the different sampling stations based on the similarities and dissimilarities among the different biotic and abiotic parameters (Maity et al. 2022). For this purpose, cluster analysis was carried out using PAST 4.03, using all the water quality, hematological, and serum biochemical parameters.

Result and discussion

The amplified blasting human population of the Earth has ultimately led to an increase in the unemployment status for the majority of the population, which encourages the pollution load in the aquatic ecosystem. There are several sources of pollution including construction of industries, wastes from farmland, discharge of sewerage from industries, and domestic households, etc. on the river banks (Roy and Shamim 2020). The increased pollution load in the aquatic ecosystem has led to environmental alteration including changes in the water quality parameters, which can adversely affect the health of aquatic organisms including hematological and serum biochemical parameters of the fish. So, the present study was carried out to know the impact of the alteration in the water quality parameters which may have resulted in the change in the hematological and serum biochemical parameters of the fish *Notopterus notopterus*.

Table 4 Observed mean (± SE) of physicochemical parameters at the sampling sites during the study period

Station	Buxar	Patna	Bhagalpur	Farakka	Rejinagar	Balagarh
pH	7.09 ± 1.82	7.6 ± 1.34	7.96 ± 1.41	8.2 ± 0.8	8.3 ± 1.6	8.2 ± 1.11
DO (ppm)	7.4 ± 1.42	6 ± 0.86	6.4 ± 0.28	7.2 ± 1.25	4.8 ± 1.2	5.07 ± 1.21
TDS(ppm)	83.2 ± 10.25	124.4 ± 12.54	123.2 ± 14.62	376.4 ± 12.85	382.4 ± 12.65	185.2 ± 13.82
TH (ppm)	150 ± 20.54	132 ± 21.62	120 ± 12.66	110 ± 10.28	90 ± 12.3	102 ± 18.49
TA (ppm)	126 ± 12.4	156 ± 13.8	136 ± 14.6	140 ± 14.8	106 ± 11.22	100 ± 13.6
EC (µS/cm)	317 ± 24.6	275 ± 22.5	260 ± 13.5	195 ± 30.2	211 ± 21.4	245.3 ± 23.6
CL (ppt)	1.68 ± 0.25	0.89 ± 0.21	1.58 ± 0.12	2.67 ± 0.31	1.01 ± 0.61	1.63 ± 0.12
BOD (ppm)	1.2 ± 0.2	1.47 ± 0.41	2 ± 0.55	2.4 ± 0.62	4.8 ± 1.28	5 ± 0.54
TN (ppm)	0.12 ± 0.1	0.07 ± 0.00	0.12 ± 0.01	0.04 ± 0.0	0.03 ± 0.00	0.07 ± 0.00
TP (ppm)	0.06 ± 0.0	0.08 ± 0.0	0.09 ± 0.0	0.15 ± 0.0	0.1 ± 0.0	0.07 ± 0.0

Assessment of physicochemical parameters

The recorded mean physicochemical parameters have been displayed in the (Table 4). The result showed that the majority of physicochemical parameters have influenced the ecological health of the river system. During the analysis at all the sampling sites, the pH ranged in the optimum range values provided by (WHO 1993; BIS 2012). In the entire studied sites, it ranged from 7.09 to 8.3. During the study, the highest pH was recorded at Rejinagar (8.3 ± 1.6) followed by Balagarh (8.2 ± 1.11), Farakka (8.2 ± 0.8), Bhagalpur (7.96 ± 1.41), and Patna (7.6 ± 0.8) and the lowest pH was observed at Buxar (7.09 ± 1.82). The trend of pH values in the entire studied stretch was similar to that of earlier studied reports in the stretch (Tiwari et al. 2022b). The dissolved oxygen in the entire stretch was also in the optimum range as per guidelines and the prescribed standard range of (BIS 2012), i.e., > 5 ppm, except at Rejinagar where the oxygen level is < 5 ppm. The site, Rejinagar is closer to Berhampore city of West Bengal, India which is having relatively higher population density which might have affected the oxygen values in the stretch(Mohanty et al. 2022; Tiwari et al. 2022a). Similar to dissolved oxygen, the TDS, electrical conductivity, and biochemical oxygen demand were also found relatively higher at Rejinagar signifying the pollution and anthropogenic activities in the stretch. The higher electrical conductivity was also recorded at Farakka signifying the impact of the Farakka barrage, which might have hindered the riverine flow and contributed to the increased pollution pressure at the studied site and the results were similar to the reports observed by (Sinha et al. 1996; Thakur et al. 2012; Sonkar and Gaurav 2020) in which the impact of Farakka barrage on the alteration of the ecological habitat of aquatic organisms was mentioned by the researchers. The total hardness and total alkalinity in the stretch were also in the optimum ranges provided by BIS (2012). The chlorine value was also found higher at the Farakka signifying the impact of the barrage. Relatively higher BOD values were also observed in the lowermost stretch, i.e., at Farakka, Rejinagar, and Balagarh. Among the nutrient parameters, the total nitrogen at all the sampling sites was observed in the acceptable range of BIS (2012). However, a relatively higher total nitrogen value was observed at Buxar. The total phosphorus was found relatively highest at Farakka. The observed data on water quality parameters at the sampling sites does not signify the eutrophication status of the river and confers relatively better water quality status of the river (Wang et al. 2021; Zhang et al. 2023).

Water pollution index (WPI)

The water pollution index is the statistical method used to distribute and classify the river system into differing ecological zones, relying on multiple physicochemical parameters and riverine health (Lee and An 2009). The calculated index is majorly based on nutrient loading in the aquatic system which has been developed by Atique and An (2019). From the analysis, it has been observed that all the sampling stations have a similar kind of WPI and the values of WPI lies in the Fair category, i.e., 19–23. The higher value of WPI signifies a relatively less polluted status and vice versa. So, as per observation at all the sampling stations, the value of WPI varied from 19 to 23. The highest value of WPI (23) was observed at the majority of sampling stations. Patna, Farakka, Rejinagar, and Balagarh are having the highest value of WPI, i.e., 23, whereas the lowest WPI value was observed at Buxar (19), followed by Bhagalpur (21)

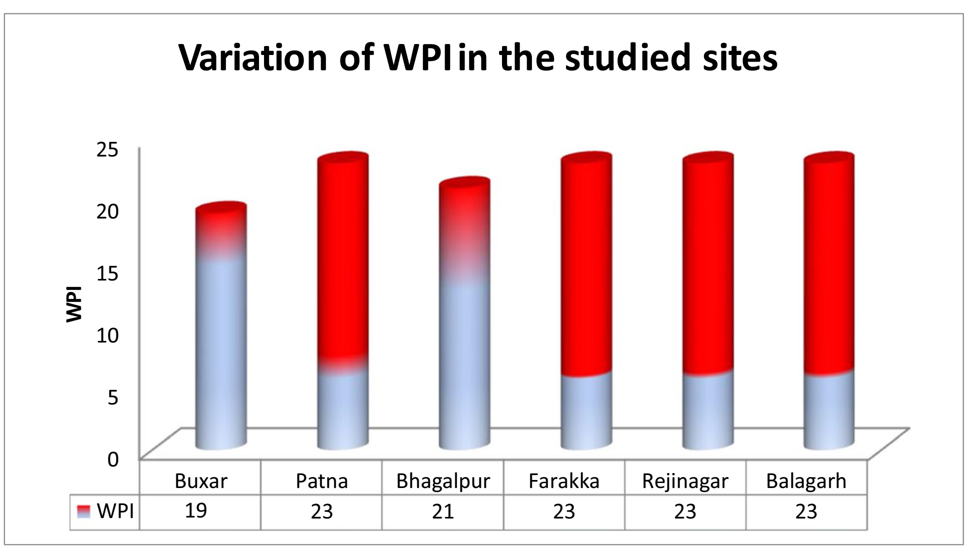

Fig. 3 Calculated water pollution index at different studied sites

(Fig. 3). The probable reason for the higher WPI values at Patna and Rejinagar is the city sewage discharge which has impacted the riverine ecology by altering the water quality status of the river. Both the sites are having various points and non-point sources of pollution which has altered the riverine ecology over the decades (Dutta et al. 2020; Singh and Jha 2021). The riverine flow at Farakka is also affected by the construction of the Farakka Barrage which might have resulted in the reduction of riverine flow in the river system. The reduction in the riverine flow might have incorporated the higher nutrient load which leads to an amplified rate of pollution load in the river system (Dalu et al. 2019). The sampling station Balagarh is surrounded by a vast agricultural area in which various inorganic fertilizers are used. The wastes and runoff from the agricultural farms have led to the accumulation of nutrients in the riverine system which has also been reported in the Ramganga river (Pathak et al. 2018). The sampling site Bhagalpur is also having pollution stress incorporated with the agricultural, industrial and domestic discharge (Leena et al. 2011). The site Buxar is having less polluted status as it has relatively less population load from the river bank. As at the sampling site, Buxar, the river flows between Buxar, Bihar, and Baliya district of Uttar Pradesh. The major pollution source in the area is domestic sewage which incorporates relatively less pollution load in the region (Singh et al. 2013) as compared to other industrial pollution sources.

National sanitation foundation–water quality index (NSF-WQI)

The NSF-WQI has been used to classify the water quality status at the studied sites based on their feasibility as drinking water. The calculations were based on BIS (2012) and standard limits provided by CPCB and WHO. In the study, it has been observed that Sampling site Buxar is having a poor category of water quality. The sampling site Patna is having a very poor category of water quality, whereas the majority of sampling sites, i.e., Bhagalpur, Farakka, Rejinagar, and Balagarh, are having an unsuitable category of riverine water quality (Fig. 4). In the study, it has also been observed that in the majority of cases moving towards a downward direction, the quality of water in the studied sites is deteriorating. The most probable reason for the increment in the NSF-WQI toward the downstream is the increased population load in the region and reduced riverine flow, which helped in the amplification of eutrophication in the region. The present studied sites are having relatively higher population stress than that of the upper stretch of the Ganga River system (Dimri et al. 2021).

Nemerow's pollution index

Nemerow's pollution index is the universally used pollution index calculation tool used for the classification of the surface and groundwater based on their pollution characteristics

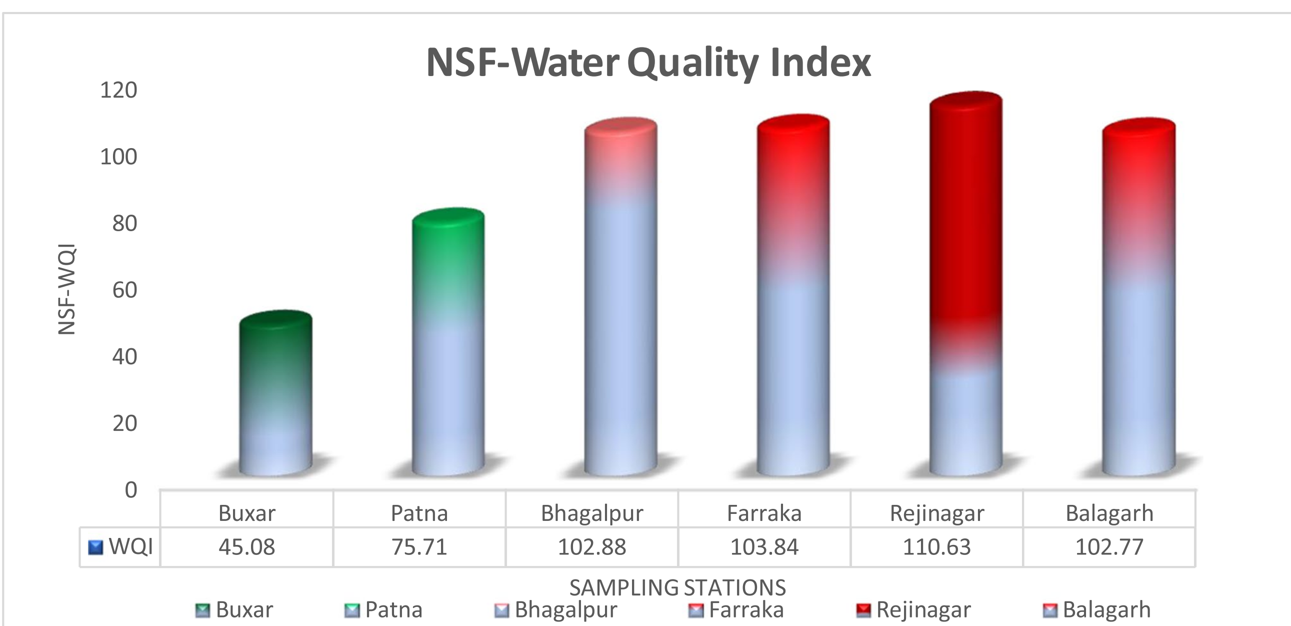

Fig. 4 Calculated NSF-WQI at studied sites

Table 5 Calculated Nemerow's pollution index at the studied sampling sites

Buxar	0.86
Patna	0.86
Bhagalpur	0.85
Farakka	0.83
Rejinagar	0.82
Balagarh	0.83

(Nemerow 1991; Ji et al. 2016). The index has been calculated by using 7 different ecologically significant parameters which can affect the pollution status of the river (Effendi 2016; Wan and Wang 2021). The selected parameters for the study of Nemerow's pollution index in the river were pH, electrical conductivity, calcium, total alkalinity, total hardness, magnesium, and sulfate. At all the sampling sites, the calculated Nemerow's pollution index ranged from 0.82 to 0.86 (Table 5) which signifies the slightly polluted status of the river at all the sampling sites. Later, the observed values were compared with the prescribed ideal values provided by Nemerow (1991).

Variation in the hematological parameters

Hematological parameters are a well-known health status evaluation tool that has been traditionally used for the assessment of fish health status in response to various biotic as well as abiotic changes (Burgos-Aceves et al. 2019). Fishes being poikilothermal animals can respond quickly to any sort of environmental and geographical changes and signifies in the context of hematological alteration (Gaber et al. 2013). In the present study, also the alteration in the various hematological parameters has been observed at all the sampling sites (Fig. 5).

The WBC count varied significantly among all the sampling stations ($P < 0.05$). The highest value of WBC was observed at Rejinagar (216.9 ± 27) followed by Farakka (193.2 ± 25), Balagarh (181.3 ± 31), Bhagalpur (178.45 ± 23), and Patna (150.62 ± 75.71), and the least WBC count was observed at Buxar (145.8 ± 21) (Fig. 5). Increased pollution load in the river system enhances the biological stress on the fish which has already been reported from various rivers across the world, and an increase in the WBC count in various fish including *Wallago attu* in the riverine system signifies infection or damage to the body cells due to ecological disbalance or eutrophication (Singh and Tandon 2009; Fazio 2019). In many studies, it has been observed that in the river Ganga also the leukocyte count plays a significant role in the estimation of fish health status (Ahmad and Jamaluddin 2021). The present study shows a similar result as of earlier studied reports in the polluted stretch of the river Ganga, where a higher WBC count has also been observed in *Labeo rohita* (Vaseem and Banarjee 2013). The higher WBC count especially in the selected lower freshwater zone of the studied sites of the river signifies the deteriorated water quality status on the studied

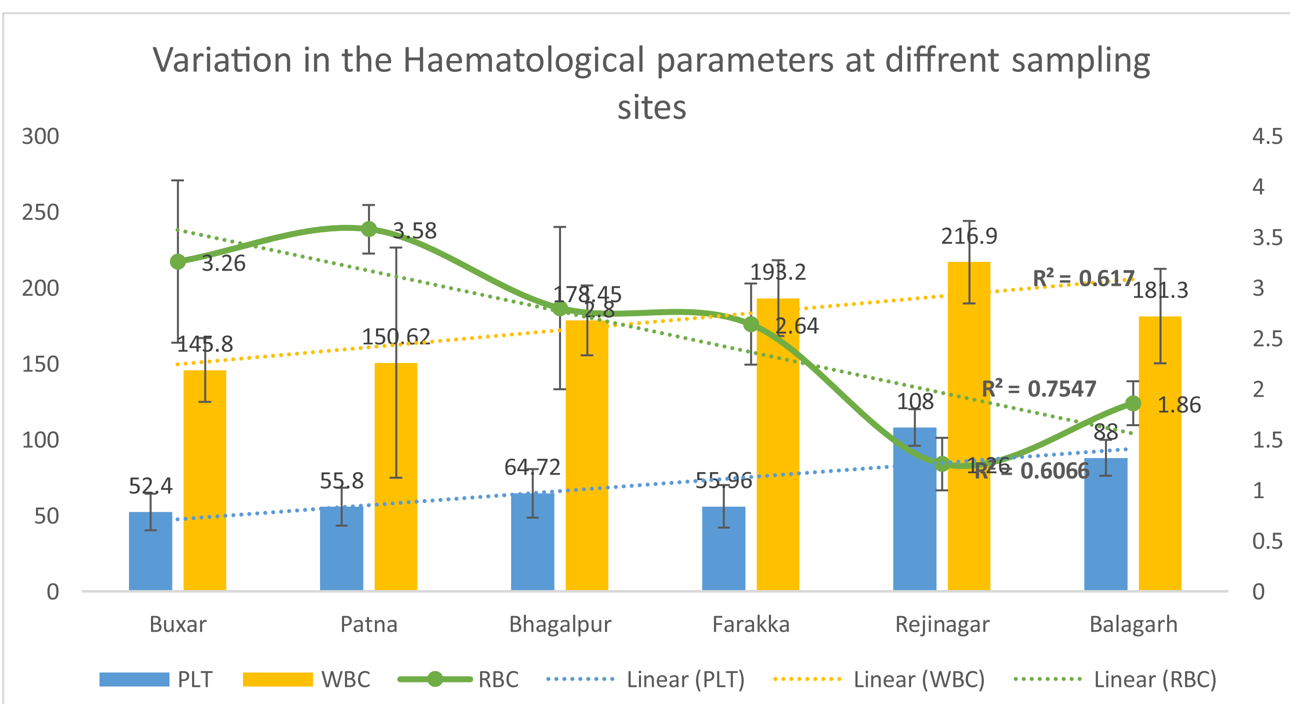

Fig. 5 Variation in the hematological parameters at different sampling sites

riverine stretch, which may have contributed to the increased WBC count of the fish. Similar to WBC, the RBC count also varied significantly among the sampling stations ($P < 0.05$). The RBC plays a remarkable role in the transport of oxygen and other essential micronutrients to the target cells, so it is much more important to study the RBC count of the fish which may indicate the status of the ecological niche (Namdee et al. 2015). In the present study, highest value of RBC was observed at Patna (3.58 ± 0.24), followed by Buxar (3.26 ± 0.8), Bhagalpur (2.8 ± 0.38), Farakka (2.64 ± 0.40), and Balagarh (1.86 ± 0.22), and the lowest RBC count was observed at Rejinagar (1.26 ± 0.26). The study report signifies that the majority of the study sites where relatively higher WBC count was recorded are having lower RBC counts which may be due to the reduced metabolic rate and other essential biological activities caused by increased pollution load in the river system. A the similar study on the fish *S. niger* in the river Jhelum, India, has also been reported by Ahmed and Sheikh (2019). The relatively higher RBC count also signifies the higher oxygen-carrying capacity and the lower RBC count signifies the polluted stretch of the river; the study was similar to the *O. niloticus* in the river Ogun of Nigeria (Ugokwe and Awobode 2015). The platelet has an important function in the regulation of body metabolism, and its counts also varied significantly among the different sampling sites; the highest platelet count was observed at Rejinagar (108 ± 12) followed by Balagarh (88 ± 11.8), Bhagalpur (64.72 ± 16), Farakka (55.96 ± 14), and Patna (55.8 ± 12.4), and the lowest platelet count was observed at Buxar (52.4 ± 12). A similar study on the river Ganga in the *Labeo rohita* has also been made and signified similar results (Vaseem and Banerjee 2013). In the present study, it has been evident that hematological parameters alter at different sampling sites in response to changes in the physicochemical parameters and pollution status of the river.

Variation in the serum biochemical parameters

The serum biochemical parameters are the well-known bioindicative assessment tool used for the assessment of fish health status altered by the effect of various intrinsic as well as extrinsic factors resulting in the enhanced stress level in the fish (Prakash and Verma 2020). For this purpose, various serum biochemical parameters such as serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), triglyceride (TRIG), cholesterol (CHOL), lipase (LIP), and amylase (AMY) were analyzed. The analyzed serum biochemical parameters mainly comprised the tests related to the liver function test (LFT), the cardiac function test (CFT), and two of the major digestive enzymes (amylase and lipase). The enzymes responsible for the proper functioning of the liver are SGPT and SGOT, varied significantly ($P < 0.05$). During the study, the minimum value of SGPT was recorded at Buxar (17.80 ± 2.84 U/I), followed by Patna (23 ± 1.82 U/I), Bhagalpur (24.80 ± 3.82 U/I), Farakka (29.40 ± 2.2 U/I), and Balagarh (32.1 ± 10.78 U/I) and was observed maximum at Rejinagar (33.80 ± 6.78 U/I). The SGOT values also varied significantly among all the sampling

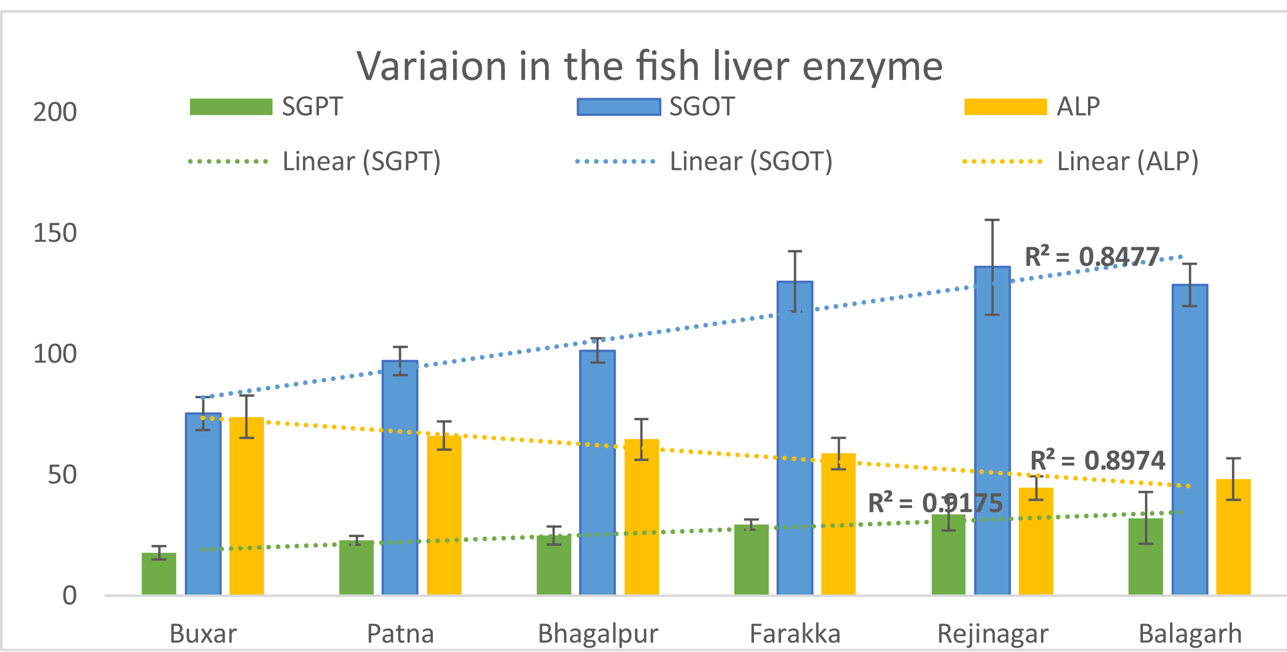

Fig. 6 Variation in the serum liver enzyme at different sampling stations

stations from 75.4 ± 6.82 U/I at Buxar to 136.00 ± 19.62 U/I at Rajnagar. Similar to major liver enzymes SGOT and SGPT, the ALP also varied from 44.60 ± 1.82 U/I at Rejinagar to 74 ± 29.82 U/I at Buxar (Fig. 6). The liver is an important organ well known for its multifarious function such as excretory function, circulatory function, metabolic function, hematological function, defensive, and detoxification functions (Bruslé and Anadon 1996). For the assessment of liver function, the important enzymes are SGPT, SGOT, and ALP, and among them, the values for SGPT and SGOT increase with the impact of riverine pollution resulting in liver cirrhosis and hepatic inflammation. In the present study, also the higher values of SGPT and SGOT were recorded in the region, signifying the increased pollution status in the concerned regions such as Rejinagar, Balagarh, and Farakka. The higher value of the released enzymes, i.e., SGPT and SGOT, are responsible for chronic liver cirrhosis and increased metabolic activities induced by environmental stressors are observed in relatively higher concentrations. As it is already evident that with the increased pollution, the fish encounters more metabolic pressure, which results in the release of a higher amount of SGPT and SGOT, the two important liver biomarker enzymes (Akbary et al. 2018). The present study's result is similar to the observations made in the fish *L. rohita* in the river Kshipra of Dewas, Madhya Pradesh, India, where the influencing liver enzymes altered with the impact of riverine pollution load. Dissimilar to SGPT and SGOT, the concentration of ALP was recorded in decreasing trend as the higher values were recorded at Buxar (74 ± 8.68 U/I) followed by Patna (66.20 ± 5.78 U/I), Bhagalpur (64.70 ± 8.43 U/I), Farakka (58.92 ± 6.6 U/I), and Balagarh (48.40 ± 8.6 U/I), and the least ALP concentration was recorded at Rejinagar (44.60 ± 4.88 U/I). The relatively lower concentration of the liver enzyme ALP signifies the cellular damage by the impact of riverine pollution (Palaniappan and Vijayasundaram 2009; Akbary et al. 2018), which is also evident in the present study. A similar impact has also been reported in the *O. niloticus* in the polluted stretch of the river Yamuna at Agra, India, where riverine pollution has impacted the ALP concentration (Khan et al. 2020). Triglyceride is the major chronic stress indicator in the fish which gets altered in the fish serum with the impact of stress, and in the present study also the ecological condition of the river is diversified with relation to physio-chemical changes (Eldridge et al. 2015). In the present study, the variation in triglyceride has been observed among the sampling sites. The lowest triglyceride was observed 62 ± 8.82 mg/dL at Rejinagar followed by 76.80 ± 8.55 mg/dL at Farakka, 77.00 ± 9.75 mg/dL at Balagarh, 80.12 ± 6.68 mg/dL at Bhagalpur, and 82.54 ± 12.6 mg/dL at Patna, and the highest triglyceride was recorded 93.20 ± 18.86 mg/dL at Buxar (Fig. 7). As triglyceride is the form of lipid found in the blood, it provides energy and is an important serum biomarker for the assessment of fish health. The lower triglyceride was observed in the relatively ecologically stressed stretch

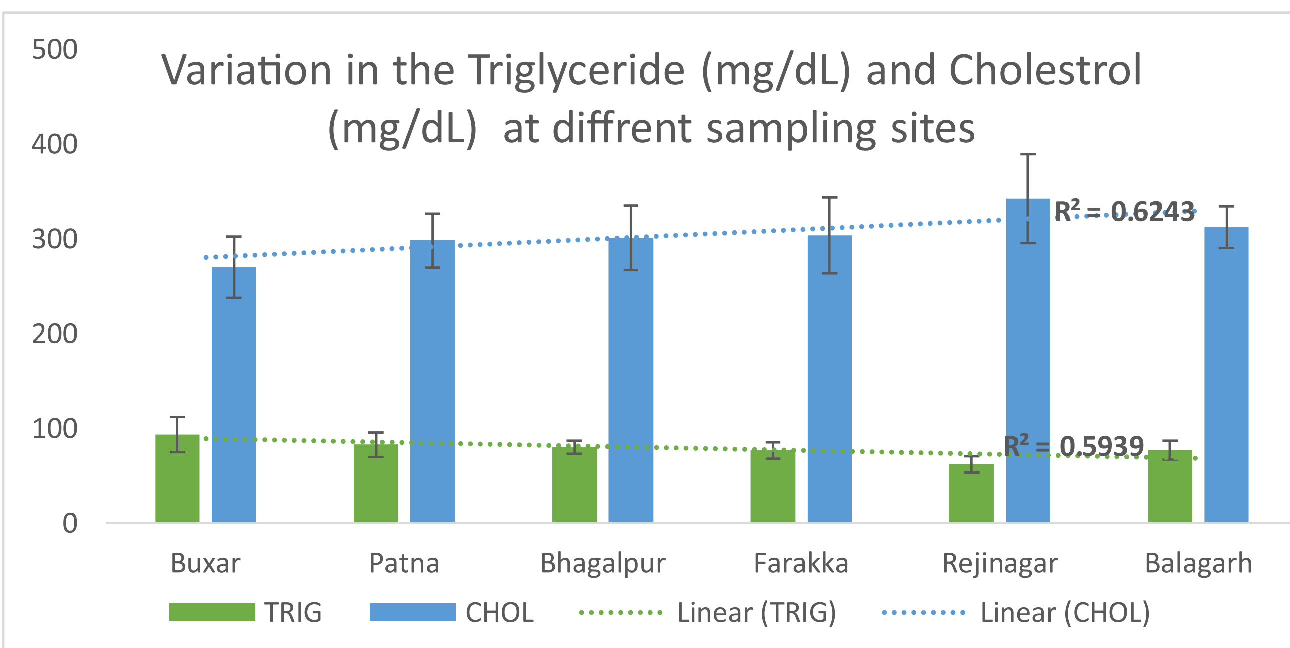

Fig. 7 Variation in the serum triglyceride and cholesterol at different sampling stations

signifying the use of more energy to cope with the metabolic stress (Lu et al. 2022). Similar observations have been observed in the *C. punctata* exposed by the thermal power plant effluents (Javed and Usmani 2015). Similar to triglyceride, cholesterol is also a significant stress indicator help to analyze the health status of fish (Lee et al. 2022). In the present study, the cholesterol concentration varied from 270 ± 32.55 mg/dL at Buxar followed by 298.44 ± 28.4 mg/dL at Patna, 301.44 ± 33.98 mg/dL at Bhagalpur, 303.40 ± 40.2 mg/dL at Farakka, and 312.44 ± 22 mg/dL at Balagarh and was found a maximum of 342.20 ± 46.88 mg/dL at Rejinagar (Fig. 7). The ecological alteration and the changes in the physicochemical parameters alter the process of digestion, by the means of alteration in the digestive enzymes (Jiang et al. 2022). In the present study, two important digestive enzymes, i.e., amylase and lipase, also varied among the sampling sites (Fig. 8). The amylase content is responsible for the digestion of polysaccharides such as glycogen content in the fish (Champasri et al. 2021). The amylase content in the present study varied at different sampling sites, i.e., was recorded 28.15 ± 2.62 U/I at Balagarh, followed by 28.62 ± 2.98 U/I at Rejinagar, 29 ± 2.88 U/I at Buxar, 31 ± 4.2 U/I at Bhagalpur, 31.6 ± 4.8 U/I at Farakka, and 32.40 ± 6.66 U/I at Patna. Similar to amylase, lipase is also an important digestive enzyme responsible for lipolysis (Cao et al. 2023). In the present study, the lipase content varied from 22.80 ± 2.87 U/I at Balagarh, followed by 24.2 ± 3.3 U/I at Rejinagar, 25.17 ± 3.1 U/I at Buxar, 30.4 ± 3.41 U/I at Patna, and 30.8 ± 3.46 U/I at Bhagalpur, and the highest was recorded 31.40 ± 4.8 U/I at Farakka (Fig. 8). The lower content of the enzymes is recorded in the lower stretch of the river, signifying that the enzyme content gets reduced in the relatively polluted stretch of the river (Abdel-Latif et al. 2022).

Variation in the serum glucose

Serum glucose is the significant stress variable used for the evaluation of stress via osmoregulatory alterations (Makaras et al. 2022). As glucose is an important monosaccharide that delivers immediate energy to the cells and is a potent bioindicator for the assessment of the ecological condition of the aquatic environment (Wei et al. 2022). In the present study, it has been found that the highest average serum glucose was observed at Balagarh 218.4 ± 6.8 mg/dL followed by 194.2 ± mg/dL at Rejinagar, 161.64 ± 5.87 mg/dL at Farakka, 153.23 ± 5.66 mg/dL at Bhagalpur, and 146.23 ± 2.86 mg/dL at Patna, and the lowest serum glucose was observed 133.6 ± 4.6 mg/dL at Buxar (Fig. 9). The higher serum glucose observed at Balagarh signifies the fish stress in the relatively polluted stretch, as in the pollution stretch with the increased metabolic activities the level of serum glucose gets increased (Biswal et al. 2021). The present result was similar to the findings of Sunardi et al. (2021) in the *O. niloticus* in the Citarum river.

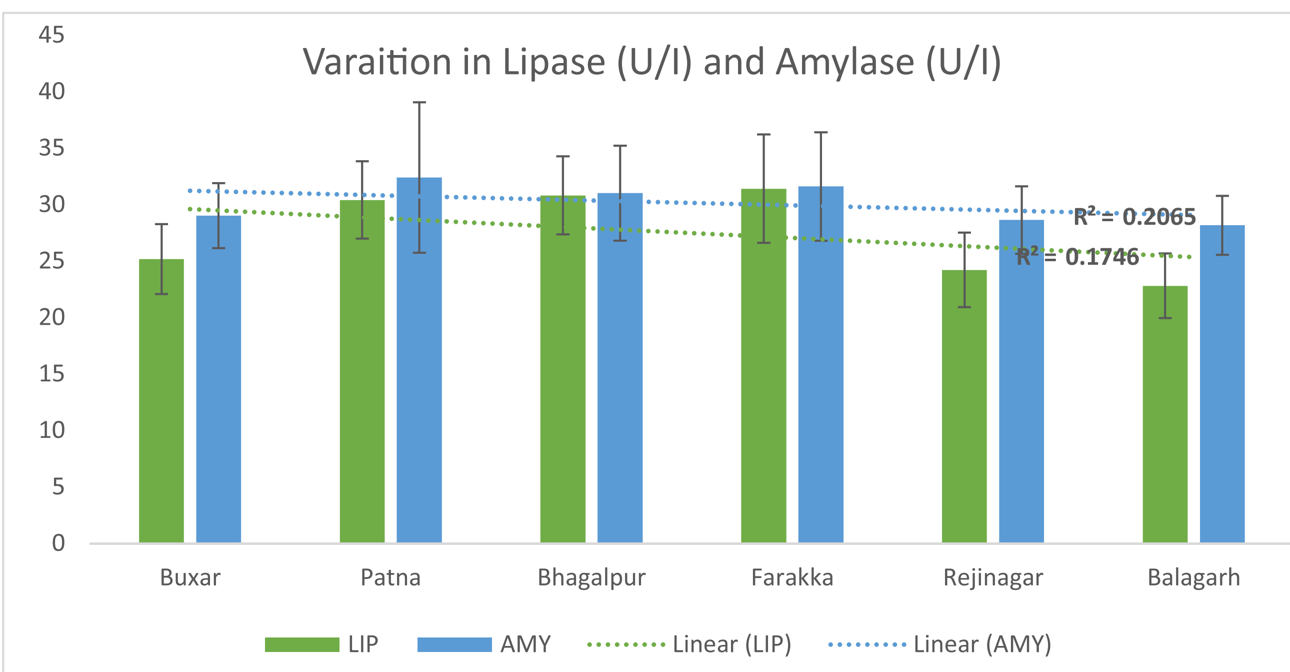

Fig. 8 Variation in serum lipase and amylase at different sampling stations

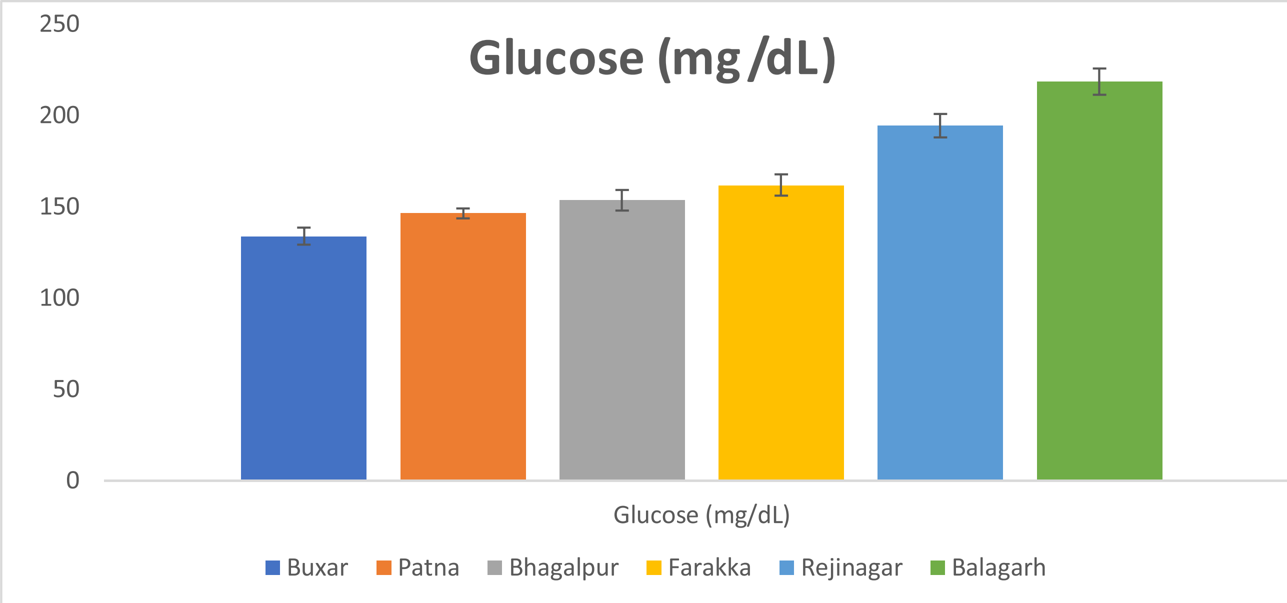

Fig. 9 Variation in the serum glucose (mg/dL) at different sampling stations

Correlation between different abiotic and biotic components influencing hemato-biochemical parameters

The Karl-Pearson correlation was carried out to evaluate the relationship between different hematological and biochemical parameters along with environmental parameters (Table 6). In the analysis, it has been found that NSF-WQI showed a significantly positive correlation ($P < 0.05$) with WPI ($r = 0.817$), SGPT ($r = 0.954$), SGOT ($r = 0.961$), CHOL ($r = 0.876$), WBC ($r = 0.889$), and pH ($r = 0.998$) (Fig. 10 and Table 6). The significantly positive correlation with WPI shows that WQI and WPI have direct relation as the higher value of WQI and WPI both signifies the deterioration in the water quality. The values for WPI and WQI rise parallelly with increased pollution load in the river; the observations were similar to the Tyśmienica River Basin of Poland (Grzywna and Sender 2021). The significantly negative correlation with NSF-WQI was found with ALP ($r = -0.940$), TRIG ($r = -0.889$), TH ($r = -0.963$), EC ($r = -0.922$), and NPI ($r = -0.873$). The ALP is mainly secreted from the liver and the values for which reduce response to alteration in the ecological niche by the means of enhanced body metabolism, so a significantly negative correlation is observed with NSF-WQI (Bharti and Rasool 2021). Similarly, the value for TRIG also gets reduced with the impact of pollution stress in the river as has been reported in other many studies (Ling et al. 2019). The TH, EC, and NPI also showed a significantly negative correlation as their increased values negatively impact the riverine water quality (Matta et al. 2018). The WPI showed a significantly positive correlation with SGOT ($r = 0.822$) and a significantly negative correlation was observed with total nitrogen ($r = -0.825$). The significantly positive correlation with SGOT signifies the importance of major liver enzyme which is released excessively during liver damage and are interdependent on the ecological habitat (Singh et al. 2016; Ghosh et al. 2018). The total nitrogen which gets raised in the condition of eutrophication and is a major pollution biomarker for riverine ecology also has a significant role in the alteration of riverine water quality (Kamboj and Kamboj 2020). The NPI showed a significantly positive correlation ($P < 0.05$) with TRIG ($r = 0.862$), RBC ($r = 0.921$), total hardness ($r = 0.940$), and EC ($r = 0.896$), while a significant negative correlation was observed with SGPT ($r = -0.950$), SGOT ($r = -0.952$), ALP ($r = -0.968$), CHOL ($r = -0.832$), WBC ($r = -0.937$), TDS ($r = -0.871$), and BOD ($r = -0.866$). Dissimilar to NSF-WQI, the NPI showed an inverse relationship with the parameters as the lower value of NPI signifies deteriorated water quality and vice versa (Vega et al. 2022).

Principal component analysis

The principal component analysis is the multivariate analytical tool used for the assessment of multiple factors responsible for the determination of the riverine ecology without altering the principal information (Dimri et al. 2021) (Fig. 11). In the analysis, the datasets were analyzed for the suitability of the PCA via Barlett's test of sphericity, which was measured 0.00. In the analysis, three PCs were extracted where the eigenvalue is more than 1.

Table 6 Correlation between different water quality parameters and various hematological and biochemical parameters

	WQI	WPI	NPI	SGPT	SGOT	ALP	TRIG	CHOL	LIP	AMY	RBC	PLT	WBC	PH	DO	TDS	TH	TA	EC	CL	BOD	TN	TP
WQI	1																						
WPI	.817*	1																					
NPI	−.873*	−.625	1																				
SGPT	.954**	.792	−.950**	1																			
SGOT	.961**	.822*	−.952**	.983**	1																		
ALP	−.878*	.646	−.968**	.955**	.951**	1																	
TRIG	−.889*	−.725	.862*	−.910*	−.883*	−.923**	1																
CHOL	.876*	.741	−.832*	.910*	.864*	.892*	−.991**	1															
LIP	−.019	.078	.361	−.299	−.174	−.213	.195	−.256	1														
AMY	−.080	.188	.440	−.327	−.210	−.316	.217	−.254	.954**	1													
RBC	−.758	−.446	.921**	−.884*	−.817*	−.901*	.845*	−.848*	.604	.679	1												
PLT	.666	.459	−.777	.809	.702	.777	−.844*	.882*	−.661	−.662	−.939**	1											
WBC	.889*	.567	−.937**	.897*	.896*	.984**	−.932**	.889*	−.172	−.291	−.882*	.766	1										
PH	.998**	.786	−.884*	.954**	.960**	.946**	−.871*	.857*	−.039	−.114	−.771	.664	.892*	1									
DO	−.619	−.617	.562	−.728	−.607	−.588	.743	−.825*	.543	.443	.726	−.890*	−.534	−.601	1								
TDS	.763	.623	−.871*	.780	.853*	.853*	−.806	.730	−.017	−.079	−.674	.514	.876*	.754	−.278	1							
TH	−.963**	−.754	.940**	−.991**	−.966**	−.974**	.944**	−.941**	.264	.313	.898*	−.833*	−.933**	−.962**	.736	−.778	1						
TA	.327	.551	.113	.083	.167	.044	−.164	.151	.836*	.892*	.335	−.296	.033	.289	.032	.127	−.100	1					
EC	−.922**	−.769	.869*	−.866*	−.935**	−.904*	.825*	−.766	−.135	−.059	.647	−.482	−.884*	−.917*	.348	−.925**	.865*	−.354	1				
CL	.122	−.078	−.218	.045	.179	.156	.191	−.287	.302	.122	.056	−.394	.123	.164	.615	.306	−.002	−.006	−.348	1			
BOD	.756	.577	−.866*	.903*	.823*	.820*	−.778	.815*	−.666	−.670	−.942**	.923**	.749	.766	−.837*	.547	−.884*	−.281	−.581	−.132	1		
TN	−.730	−.825*	.797	−.795	−.852*	−.742	.790	−.756	.117	.048	.606	−.545	−.723	−.704	.473	−.899*	.757	−.206	.838*	−.066	−.609	1	
TP	.591	.490	−.499	.435	.582	.552	−.438	.333	.579	.466	−.167	−.059	.574	.585	.208	.774	−.440	.538	−.826*	.617	.035	−.597	1

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

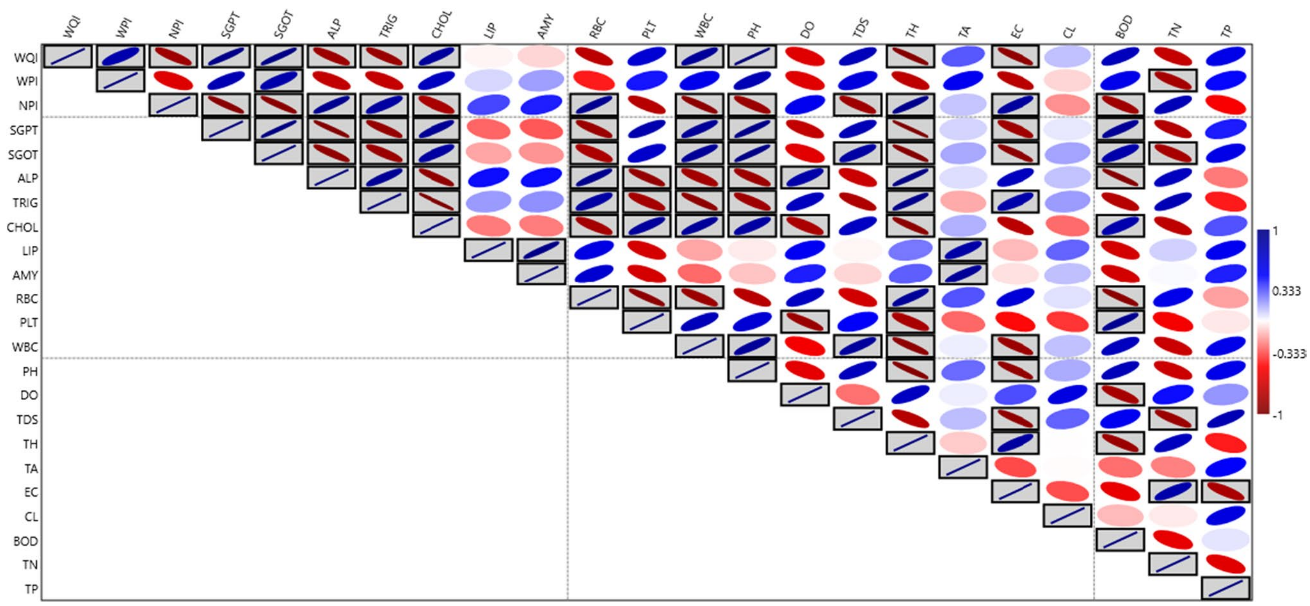

Fig. 10 Correlation of WQI, NPI, and WPI with different abiotic and biotic factors

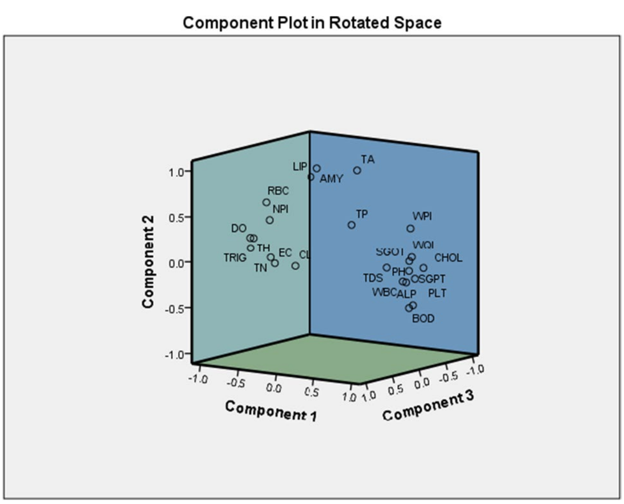

Fig. 11 Principal component analysis of different variables

Table 7 Extracted three principal components

Component transformation matrix			
Component	1	2	3
1	.984	−.143	−.106
2	.176	.877	.448
3	−.028	.459	−.888

Extraction method: principal component analysis. Rotation method: varimax with Kaiser normalization

The cumulative variance for all the 3 extracted PCs was 94.832% (Tables 7, 8, and 9). The datasets were compared with the reference range given by Liu et al. (2003) used for the determination of the loading values. The analysis reveals that the 1st Pc has an eigenvalue of 15.171, in which the variance percentage was 65.963. The 1st PC shows strong positive loading with WQI ($r=0.967$), WPI ($r=0.797$), SGPT ($r=0.968$), SGOT ($r=0.986$), ALP ($r=0.972$), CHOL ($r=0.906$), WBC ($r=0.943$), pH ($r=0.962$), TDS ($r=0.889$), and BOD ($r=0.781$). The PC shows moderate positive loading with PLT ($r=0.715$) and TP ($r=0.626$). The strong negative loading was observed with NPI ($r=-0.945$), TRIG ($r=-0.934$), RBC ($r=-0.819$), TH ($r=-0.971$), and TN ($r=-0.851$), while moderate negative loading was observed with DO ($r=-0.596$). The PC shows the maximum variance, i.e., 65.963%. The strong loading with WQI and WPI was observed which is the assessment method for the total water quality status. The water quality in the present study sites was also determined by using two of the water quality assessment tools which can be a determining tool for the evaluation of riverine water quality (Tripathi and Singal 2019; Dimri et al. 2021). The PC also showed significant positive loading with SGPT, SGOT ALP, and CHOL which is the important liver enzyme that shows its impact on any sort of ecological alteration by the means of biochemical changes in these three important liver enzymes (Kang et al. 2010; Oyeniran et al. 2021; Soulivongsa et al. 2021). Cholesterol is an important parameter responsible

Table 8 Extracted total variance of different principal components

Total variance explained									
Component	Initial eigenvalues			Extraction sums of squared loadings			Rotation sums of squared loadings		
	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
1	15.171	65.963	65.963	15.171	65.963	65.963	14.835	64.499	64.499
2	4.619	20.084	86.047	4.619	20.084	86.047	4.288	18.643	83.141
3	2.021	8.785	94.832	2.021	8.785	94.832	2.689	11.690	94.832

Extraction method: principal component analysis

Table 9 Rotated component matrix of principal components

Rotated component matrix[a]			
	Component		
	1	2	3
WQI	.967	.093	−.068
WPI	.797	.364	−.287
NPI	−.945	.305	−.113
SGPT	.968	−.154	−.126
SGOT	.986	−.047	.011
ALP	.972	−.169	.045
TRIG	−.934	.050	.261
CHOL	.906	−.077	−.375
LIP	−.110	.920	.297
AMY	−.161	.980	.105
RBC	−.819	.546	.128
PLT	.715	−.515	−.451
WBC	.943	−.158	.067
PH	.962	.054	−.030
DO	−.596	.269	.750
TDS	.889	.021	.291
TH	−.971	.141	.150
TA	.209	.962	−.125
EC	−.955	−.192	−.225
CL	.134	.058	.945
BOD	.781	−.515	−.283
TN	−.851	−.084	.002
TP	.626	.510	.575

Extraction method: principal component analysis. Rotation method: varimax with Kaiser normalization

[a]Rotation converged in 5 iterations

for the proper functioning of the body's metabolism, and the value for which rises during the stress, the value for which rises in the riverine and other aquatic conditions (Kim et al. 2021). The WBC also shows the preliminary defense response for the ecological alteration as has also been reported in several studies (Shekarabi et al. 2022; Witeska et al. 2022). The 2nd PC has an eigenvalue of 4.619 and a variance percentage of 20.084. The 2nd PC has strong positive loading with LIP ($r = 0.920$), AMY ($r = 0.980$), and TA ($r = 0.962$), while moderate positive loading was observed with TP ($r = 0.510$). The PC shows moderate negative loading with PLT ($r = -0.515$) and BOD ($r = -0.515$). The PC has a strong positive loading with LIP and AMY, which are the two major digestive enzymes responsible for the digestion of fat and carbohydrate sources which also get altered with the change in the riverine ecology (Palaniappan and Vijayasundaram 2009; Zhao et al. 2022), while moderate negative loading with PLT and BOD also signifies the importance of BOD in determining the riverine water quality (Lung 2022). In the 3rd PC, the observed eigenvalue was 2.021 and the variance percentage was 8.785, in which the strong loading was observed with DO ($r = 0.750$), CL ($r = 0.945$), and TP ($r = 0.575$). The PC does not show any strong or moderate negative loading. The strong loading with DO, CL, and TP shows the impact and importance of anthropogenic activities in the riverine condition which is similar to the observations of the upper stretch of the river Ganga (Dimri et al. 2021).

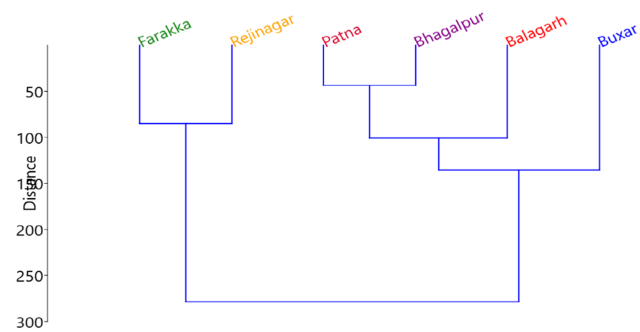

Fig. 12 Cluster analysis between different sampling sites

Cluster analysis

The cluster analysis is the superlative analytical method used for the classification of the sampling sites based on the similarities among the different stations. In the present study also a classical cluster analysis was performed via paired group (UPGMA) algorithm, by using the Euclidean similarity index. In the analysis, the observed cophentic correlation was 0.92, which signifies the suitability for cluster analysis. In the analysis, it has been observed that based on different abiotic and biotic components the 3 major cluster groups were formed. The analysis revealed that based on all the analyzed parameters the Buxar and Farakka are distantly related and show the least similarity. A close association was observed between Farakka and Rejinagar. Patna and Bhagalpur show a close relationship with Balagarh, while these three sampling sites, viz., Patna, Bhagalpur, and Balagarh, showed a close relationship with Buxar and Rejinagar (Fig. 12).

Conclusion

The present study deals with the study of the interaction between abiotic and biotic components of the freshwater zone of middle and lower zone of the river Ganga, which is unique of its kind in the large river system. The study explains the new form of biomarker approach in which the physiological changes in the fish with response to alteration in the physicochemical parameters leading to amplification in the pollution status of the river have been monitored. For the assessment of fish physiological health various hematological as well as serum biochemical parameters of the important omnivorous food fish (*Notopterus notopterus*) have been studied and later compared with different physicochemical parameters and water quality indices such as NSF-water quality index, water pollution index, and Nemerow's pollution index, by using various univariate as well as multivariate statistical techniques. From the study, it can be concluded that the major influencing water quality parameters, which have contributed to the rise of riverine pollution status and altered the water quality indices, are total dissolved solid, total hardness, electrical conductivity, biochemical oxygen demand, and nitrate. Whereas, among the biotic components, the hemato-biochemical parameters, which play a significant role as a biomarker and are altered with response to changes in the various water quality indices, are serum glutamic pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), triglyceride, cholesterol, and white blood cells (WBCs). From the study, it is also known that only the assessment of abiotic components by the means of physicochemical parameters is not sufficient for the proper management and comprehensive management of the aquatic ecosystem. Along with abiotic components, the new biomarker approach by the use of biotic components in the form of assessment of hemato-biochemical parameters of the fish in aquatic ecosystems such as rivers, ponds, wetlands, etc. will be much more helpful.

Acknowledgements The authors acknowledge the support provided by the local audience and fishermen of each sampling site during the sampling procedure. The authors are thankful to Ms. Manisha Bohr for the preparation of the study area map. The authors are also thankful to Shri. Loknath Chakrovarty, Shri. Subhendu Mandal, Shri Kausik Mondal, Shri Umasankar Ram, of ICAR-CIFRI, and Shri Amarnath Prasad, retd. staff of ICAR-CIFRI, for their help during the sampling and analytical procedure. Thanks are also due to NMCG field staff members, especially Mr. Arindam Biswas, Mr. Ayon Roy, Mr. Chittranjan Dhal, Mr. Bhola Das, Mr. Saikat Sardar, and Mr. Samir Biswas for their help during the sampling procedure.

Author contribution Nitish Kumar Tiwari, Trupti Rani Mohanty, and Subhadeep Das Gupta: sampling, data analysis, laboratory analysis, and MS preparation; Shreya Roy: sampling, Himanshu Sekhar Swain, Raju Baitha, and Mitesh Hiradas Ramteke: data analysis, monitoring, and guidance; Basanta Kumar Das: conceptualization, investigation, fund acquisition, and overall guidance.

Funding The study has been financially supported by the National Mission for Clean Ganga, the Ministry of Jal Shakti, Government of India.

Data availability The data for the above mentioned work may be made available, based on a reasonable request to the corresponding author.

Declarations

Ethics approval All the work has been carried out following the standard operating protocol and guidelines provided by the Institute ethical committee of ICAR-CIFRI.

Consent to participate Not applicable.

Consent to publish All the author's provided consent to publish the study work.

Conflict of interest The authors declare no competing interests.

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CPCSEA for Experimentation on Fishes Government of India Ministry of Fisheries, Animal Husbandry and Dairying Department of Animal Husbandry and Dairying Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) 2021 *** CONTENTS

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