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Original article

Water quality index, *Labeo rohita*, and *Eichhornia crassipes*: Suitable bio-indicators of river water pollution

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ABSTRACT

The present study investigated the water quality index (WQI) of the Kshipra river at Dewas, Madhya Pradesh, India, using native fish *Labeo rohita,* and plant *Eichhornia crassipes*. The temperature, pH, dissolved oxygen, alkalinity, turbidity, and dissolved solids were found to be within the prescribed limits. However, heavy metals concentration exceeded the limit except for Cu and Zn. Their occurrence in river water was as follows: Ni > Fe > Cd > Cr > Mn > Zn > Cu. Among these heavy metals, Cd was found to be highly bioavailable, whereas Zn was the least bioavailable metal. Based on WQI, the water was found to be unfit for drinking, and the high WQI value was due to the presence of Cr and Cd. In fish tissues (muscle, liver, gut, gills, and kidney), the highest and lowest metal pollution index was found in gills (45.03) and kidneys (12.21), respectively. Bioaccumulation of these metals resulted in significant depletion of energy reserves (protein, glucose, and glycogen) and also altered hematological parameters. Moreover, liver function tests showed hepatic damage in the exposed fish. In-plant, both the bioaccumulation and mobility factor exceeded 1 for all these metals. On the other hand, the translocation factor was found to be beyond 1 for Fe, Ni, and Zn. These high values make this plant fit for phytoextraction of Mn, Fe, Cu, Zn, and Cd and phytostabilization of Cr in water. Moreover, consumption of *L. rohita* from the Kshipra River does not pose a non-cancer risk as the target hazard quotient was below 1, but it may pose cancer risk because of the presence of Cr in the range of 1.402×10^{-3} to 1.599×10^{-3}.

1. Introduction

In developed and developing countries, the water quality is incredibly uneven, reflecting their social, economic, and physiological factors. These countries do not have water shortage issues, but they face deteriorated water quality (Gupta et al., 2014). Industrialization, urbanization, improper agricultural activities, and the expanding population have destroyed freshwater resources (Tabrez et al., 2011). These issues can be minimized by regular monitoring and assessing the water quality status of the water bodies. We took the present study to measure the degrading quality of the Kshipra river at Dewas district (22.89°N75.98°E), Madhya Pradesh, India. Monitoring of river Kshipra is of utmost importance because it merged into the major riverine system of the Narmada River. In this region, the river receives waste from leather, textile, iron, and steel industries and dyes from the currency printing industry (Ganasan and Hughes, 1998). Some studies reported the worse water quality of the Kshipra river in the Ujjain region (Kumawat and Sharma, 2015; Pawar and Bhatia, 2016; Gangwar et al., 2017). However, to our knowledge, no study was conducted at the Dewas segment. The core pollutants among such wastes are heavy metals which are persistent and non-biodegradable (Tabrez et al., 2021). Their presence in freshwater alters the overall water quality and makes it unfit for drinking, resulting in economic loss, and restricts upgrading of native community living conditions. There is a shortage of clean drinking water in Dewas, so the native people rely partly/fully on natural resources highlighting the importance of water quality. Therefore, it is mandatory to take organized steps to monitor water quality. The water quality index (WQI) combined complex data and produced a score that describes water quality status (Reza and Singh, 2010; Batabyal and

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Chakraborty, 2015). Besides WQI, living organisms can also be used as bio-indicators to assess the effect of pollution. Since fishes are a prominent part of the freshwater ecosystem and their health status reflects water quality. Heavy metals result in the production of reactive oxygen species (ROS), which cause stress in the fish. To cope up with the stress, fish use energy sources like glucose, protein, and glycogen. In addition, aquatic plants also bioaccumulate heavy metals by absorbing them through roots and shoots (Matache et al., 2013; Singh et al., 2017). Translocation to shoots is restrained, but heavy metals can bio-magnify in roots and reach as high as 100,000 times more than the ambient aquatic environment (Mishra et al., 2008; Singh et al., 2017). Due to the bioaccumulation property of aquatic plants, they are also commonly used to monitor the pollution status of water bodies (Souza et al., 2013; Singh et al., 2017). *Eichhornia crassipes* is a freshwater tropical and subtropical plant, grown for ornamentation purposes and proliferates in a suitable environment (https://www.cabi.org/isc/datasheet/20544#tosummaryOfInvasiveness).

In this study, the area under investigation was rural, where locals depend on natural water resources for drinking and domestic purposes. We aim to evaluate the suitability of river water for consumption by humans based on the physicochemical parameters and WQI. Besides this, bioaccumulation in endemic fish *Labeo rohita* and plant *E. crassipes* were also considered bio-indicators of Kshipra River pollution. Furthermore, metal pollution index and the adverse effects of heavy metals on energy reserves like glucose, glycogen and protein were determined along with hematological parameters such as hemoglobin content, blood cell count, and differential leucocyte count (DLC) oxygen-carrying capacity in *L. rohita*. In addition, pathological marker enzymes were also assayed to determine the liver function of the fish. *L. rohita* is a herbivore fish and feeds mainly on phytoplankton and vegetation. In *E. crassipes,* bioaccumulation factor (BAF), translocation factor (TF), and mobility factor (MF) were assessed. Since the native residents of this area also catch fish from the Kshipra river and consume them as food, therefore, human health risk assessment parameters, such as target hazard quotient (THQ), hazard index (HI), and target cancer risk (TR), were also evaluated.

2. Materials and methods

2.1. Collection of samples

Fish *L. rohita* (n = 10), plant *E. crassipes* (n = 8), and water samples (n = 3) were collected from different nearby locations of the River Kshipra at Dewas, Madhya Pradesh. The fish's body length from snout to the tip of caudal fin was measured 15.6 ± 0.5 cm, and their weight was 155.62 ± 0.39 g. The selection of fish was made carefully to minimize the errors due to size and age. The reference fish was also collected (n = 10) from the culture pond whose length from snout to the tip of caudal fin was 18 ± 0.4 cm, and weight was 195 ± 0.86 g. The fishes were euthanized, and different organs like the liver, kidney, gills, and gut were removed, washed, and digested in $HClO_4$ and HNO_3 (1:4 v/v) for heavy metals analysis by atomic absorption spectrophotometer (Javed et al. 2018). Furthermore, the metal pollution index (MPI) was calculated by the following equation:

$$MPI = (Cf_1 \times Cf_2 \times \ldots Cf_n)^{1/n}$$

where Cf_1, Cf_2 ….. up to Cf_n is the metal 'n' concentration in the sample (Javed et al., 2016a).

The *E. crassipes* plant was scant in number and found only a few places. These plants do not grow in clean water and are mainly found in polluted waters; that is why we couldn't use reference plants.

The temperature, turbidity, total dissolved solids (TDS), pH, dissolved oxygen (DO), and alkalinity ($CaCO_3$) of sample water were measured as per the recommendation by APHA (2005).

3. Bioavailability of heavy metals

The bioavailability of heavy metals (%) was calculated by using the following formula:

Bioavailability (%) = Metal content in filtered water sample/ Metal content in unfiltered water sample × 100

4. Water quality index (WQI)

WQI was calculated by using the Indian standards for drinking water (BIS, 1991). It was estimated in 3 steps as described below:

1. Weight (wi) was assigned between 1 and 5 to each of the investigated water quality parameters (temperature, pH, DO, alkalinity, TDS, turbidity, Cr, Mn, Fe, Ni, Cu, Zn, and Cd) based on their relative significance in the overall water quality for drinking purposes. The maximum weight 5 was given to Cr and Cd due to their significant effects in water quality evaluation, while minimum weight of 1 was given to Zn due to its less importance.
2. Calculation of the relative weight (Wi) of each parameter was done as follows:

$$Wi = \frac{wi}{\sum_{i=1}^{n} wi}$$

where,

Wi is the relative weight of ith parameter
wi is the assigned weight of each parameter
n is the total number of studied water quality parameters

3. Calculation of quality rating scale (qi) for every parameter was done as follows:

$$qi = Ci/Si \times 100$$

where, qi is the quality rating

Ci is the concentration of every studied water quality parameter in the sample water.
Si is the Indian permissible limit for drinking water for every parameter.

For calculation of WQI, the sub-index (SI) was computed for each studied water quality parameter, as per the below-mentioned equation:

$$SIi = Wi \times qi$$

$$WQI = \sum SI_{i-n}$$

where SIi is the sub-index for ith parameter;

Wi is the relative weight of ith parameter;
qi is the rating of the ith parameter, n is the number of studied water quality parameters.

5. Hematological parameters

Collection of blood was done through cardiac puncture using disposable syringes. Total RBC count (tRBC) (10^6 mm^{-3}) and total leucocyte count (TLC) (10^3 mm^{-3}) were quantitated by hemocytometer (Rohem, India). A blood smear was prepared with Giemsa stain for differential leukocyte count (DLC) and recorded in percentage. Hemoglobin concentration (Hb) (gdl^{-1}) was estimated with the help of DIAGNOVA test kit (Ranbaxy, India). On the other hand, oxygen-carrying capacity (O_2 CC) was calculated as per the method described by Johansen (1970).

6. Biochemical parameters:

6.1. Glucose, glycogen, and protein assay

Blood samples were centrifuged at 3500g for 10 min to collect the serum. The glucose concentration was determined by utilizing the Eco-Pak glucose kit (Accurex Biomedical Pvt. Ltd., India), and OD was measured at 505 nm on a UV–Vis spectrophotometer (Systronics, 118). Glycogen concentration was measured by the Anthrone reagent method (Carrol et al., 1956). In addition, the total protein concentration was estimated by Bradford's (1976) method.

6.2. Enzyme assay for liver function test

The activities of enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured with the help of a specific test kit (RANDOX Laboratories Ltd., Crumlin, UK) by following the manufacturer's instructions.

7. Estimation of bioaccumulation factor (BAF), translocation factor (TF), and mobility factor (MF)

The indices BAF, TF, and MF, were calculated using the below-mentioned equations:

BAF = mean metal quantity (mg/kg) in shoot (root + stem + le aves)/metal quantity (mg/kg) in water

TF = mean metal quantity (mg/kg) in shoot (root + stem + lea ves) / metal quantity (mg/kg) in root

MF = metal quantity (mg/kg) in receiving level / metal quantity (mg/kg) in source level.

8. Health risk assessment

8.1. Target hazard quotient (THQ)

It depicts non-carcinogenic risk and has no dimensions. It was calculated using USEPA region III risk-based concentration table (USEPA, 2011):

$$THQ = \frac{Mc \times IR \times 10^{-3} \times EF \times ED}{RfD \times Bw \times ATn}$$

where Mc is the metal concentration (mg/kg dry weight), IR is ingestion rate (19.5×10^{-3} kg/day), Bw is bodyweight 57 kg and 50 kg (average) for human adult males and females, respectively (Shukla et al., 2002); EF is exposure frequency which is 365 days/year, ED is exposure duration which is 67 years (life expectancy of Indian males and females is around 65 and 68 years respectively). Therefore, their average is used for calculation (https://countryeconomy.com/demography/life-expectancy /India). RfD is reference dose of metals (mg/kg/day) (USEPA, 2012). ATn is an average time for non-carcinogens exposure [365 days/year $\times$ ED] (USEPA, 2011).

8.2. Hazard index (HI)

It is the sum of all THQs (USEPA, 2011)

$$HI = THQCr + THQMn + THQFe + THQNi + THQCu + THQZn + THQCd$$

8.3. Target cancer risk (TR)

It represents the carcinogenic risk, and has no dimensions, and was calculated using the USEPA region III risk-based concentration table (USEPA, 2011).

$$TR = \frac{Mc \times IR \times 10^{-3} \times CPSo \times EF \times ED}{Bw \times ATc}$$

where CPSo is carcinogenic potency slope for oral dose in mg/kg bw-day^{-1}. ATc is the average time for carcinogens exposure (365 days/year $\times$ 67 years) because of the average life expectancy for Indians (already described earlier).

Heavy metals like Mn, Fe, Cu, and Zn are not carcinogens, so their CPSo has not yet been reported (USEPA, 2012). Therefore, TR for Cr was calculated only. While Ni and Cd were not detected in the fish fillet.

Assumptions during the calculation of THQ and TR for human health risk are as follows:

(a) Ingested dose of pollutant is equal to the absorbed dose (USEPA, 1989).
(b) Cooking does not affect pollutants concentration (Forti et al., 2011).

9. Statistical analysis

The analyzed parameters were assayed in duplicates, and values were reported as mean ± SEM (standard error of the mean). Statistical analysis was done with the help of the Student's *t*-test, two-way ANOVA, and Duncan's multiple range test using SPSS software (version 18).

10. Results

10.1. Physicochemical parameters and water quality index

The studied water quality parameters of the Kshipra river are given in Table 1. The temperature, pH, DO, alkalinity, turbidity, and TDS were found to be within the permissible limits of Indian standards (BIS, 1991) and WHO (2006, data obtained from UNEPGEMS). The heavy metals were present in the following order Ni > Fe > Cd > Cr > Mn > Zn > Cu. Among the heavy metals Cr, Mn, Fe, Ni, and Cd were found to be above the set limits. They all have minor differences in their concentrations but the variable difference in their percent bioavailability. Bioavailability is the fraction of metal that is available to the organisms for uptake. Moreover, their bioavailability was in the following order Cd (79.14%) > Ni (66.83%) > Cr (56.56%) > Fe (53.30%) > Cu (37.27%) > Mn (31.36%) > Zn (22.3%).

10.2. Bioaccumulation and metal pollution index in L. rohita

The significant amounts of heavy metals was accumulated in *L. rohita* muscle, gills, liver, kidney, and gut (Table 2). In muscle, the highest accumulated metal was Fe (131.5 mg/kg), and Mn was found to be lowest in concentration (3.7 mg/kg). At the same time, Ni and Cd were not detected at all. Likewise, in gills, liver, and kidney, Fe was found to have accumulated highest in the order of 1440 mg/kg, 255 mg/kg, and 95 mg/kg, respectively. Moreover, the accumulation of Ni was found to be lowest in gills and kidneys, 1.9 mg/kg and 2.6 mg/kg, respectively. While in the liver, Cr accumulation was found to be lowest (7.5 mg/kg), Ni and Cd were not detected. Moreover, Fe and Cd concentrations were highest and lowest 261.2 mg/kg 6.6 mg/kg in the gut. As far as MPI is

Table 1
Water quality parameters of Kshipra river.

Parameters	[a]Values in Kshipra water	[b]Indian standards/WHO	wi	Wi	qi	SI
Temperature °C	24.3 ± 0.001	20–30	4	0.083	81	6.723
pH	7.5 ± 0.001	6.5–8.5	4	0.083	88.2	7.320
DO (mg/L)	7.6 ± 0.01	6–8	5	0.104	95	9.88
Alkalinity ($CaCO_3$) (mg/L)	450 ± 0.23	200–600	2	0.041	75	3.075
Turbidity (NTU)	5 ± 0.11	10	2	0.041	50	2.05
TDS (mg/L)	730 ± 1.5	500–2000	4	0.083	36.5	3.029
Cr (mg/L)	0.815 ± 0.001	0.05	5	0.104	1630	169.52
Mn (mg/L)	0.475 ± 0.001	0.1–0.3	4	0.083	158.33	13.141
Fe (mg/L)	1.105 ± 0.001	0.3–1.0	4	0.083	110.5	9.171
Ni (mg/L)	1.224 ± 0.001	0.02	5	0.104	61.20	636.48
Cu (mg/L)	0.421 ± 0.001	1.5	3	0.062	28.06	1.739
Zn (mg/L)	0.450 ± 0.001	5–15	1	0.020	3	0.06
Cd (mg/L)	0.911 ± 0.001	0.003	5	0.104	30366.66	3158.126
			Σwi = 48			WQI = 4020.28

[a] All values are given as mean ± SEM (n = 3) collected from three different locations; b Values are in the range where lower limit indicates desirable standard.

Table 2
Heavy metal concentrations in *Labeo rohita* tissues (mg/kg. dw).

Organs	Cr	Mn	Fe	Ni	Cu	Zn	Cd
Muscle	$_{c}8.2 \pm 0.001^{d}$	$_{e}3.7 \pm 0.001^{e}$	$_{d}131.5 \pm 2^{a}$	–	$_{d}15.8 \pm 0.001^{c}$	$_{d}99.3 \pm 0.7^{b}$	–
Gills	$_{a}13 \pm 0.12^{e}$	$_{a}74 \pm 0.11^{c}$	$_{a}1440 \pm 1.2^{a}$	$_{a}1.9 \pm 0.3^{g}$	$_{a}30.9 \pm 0.1^{d}$	$_{a}641.6 \pm 0.96^{b}$	$_{a}7.2 \pm 0.01^{f}$
Liver	$_{cd}7.5 \pm 0.01^{d}$	$_{c}8.6 \pm 0.31^{d}$	$_{c}255 \pm 1.8^{a}$	–	$_{cb}23.5 \pm 0.21^{c}$	$_{c}242 \pm 0.32^{b}$	–
Kidney	$_{b}11 \pm 0.01^{cd}$	$_{d}6 \pm 0.01^{e}$	$_{e}95 \pm 0.89^{a}$	$_{a}2.6 \pm 0.001^{a}$	$_{e}13 \pm 0.001^{c}$	$_{e}48 \pm 0.01^{b}$	$_{c}4 \pm 0.001^{f}$
Gut	$_{c}8.4 \pm 0.01^{e}$	$_{b}13.4 \pm 0.01^{d}$	$_{b}261.2 \pm 1.6^{b}$	–	$_{b}25.6 \pm 0.02^{c}$	$_{b}369.7 \pm 1.89^{a}$	$_{ab}6.6 \pm 0.001^{e}$

All values are given as mean ± SEM (n = 10) collected from three different nearby locations; Superscripts shows significant differences along the row while subscripts indicate significant differences along the column; Significance was established at $p < 0.05$

concerned, the total metal burden was as follows gills > liver > gut > muscle > kidney (Table 3).

10.3. Hematological parameters and oxygen-carrying capacity

The effect of these bioaccumulated heavy metals in different hematological parameters like hemoglobin content (Hb), total RBC count, total leucocyte count (TLC), and oxygen-carrying capacity of blood (Fig. 1a-e), differential leucocytes count, serum glucose, glycogen, and protein content (Fig. 2) and liver function test enzymes were estimated (Fig. 3). The present study showed that hematological parameters and other biochemical assays could serve as a suitable tool for investigating fish health status. There was a decline in Hb, tRBC, and O_2 carrying capacity by 37.79%, 48.24%, and 37.84%, respectively, in exposed *L. rohita* over reference fish. However, TLC showed elevation in its level by 38.22%. The percentage DLC, neutrophils, and monocytes were found to be increased by19.71% and 26.9%, respectively, and lymphocytes and eosinophils percentage showed a decline by 37.5% and 17.5% over reference was observed.

10.4. Serum biochemical assays

There was a decline in serum glucose and protein by 35.13% and 28.19%, respectively. On the other hand, a rapid rise in serum glycogen by 160% was observed (Fig. 2).

Table 3
MPI values in *Labeo rohita.*

Tissues	MPI
Muscle	22.87
Gills	45.03
Liver	39.28
Kidney	12.21
Gut	34.99

10.5. Uptake of heavy metals, bioaccumulation factor, transfer factor, and mobility factor in E. crassipes

Bioaccumulation of heavy metals in leaves, stalk, and root of *E. crassipes* is listed in Table 4. Cd showed the highest accumulation of 48 mg/kg.dw and 53.9 mg/kg in leaves and stalk.dw respectively. On the other hand, the accumulation of Cr (1.2 mg/kg.dw and 1.6 mg/kg.dw in leaves and stalk respectively) was found to be the lowest. While in roots Fe (57.4 mg/kg.dw) was found to be highest, and Cr (2 mg/kg.dw) was the lowest concentrations. On average, the high metal burden was found in *E. crassipes* roots because they are in direct contact with the surrounding environment. The bioaccumulation factor, transfer factor, and mobility factor of *E. crassipes* is summarized in Table 5. BAF in plant was found to be as follows: Mn > Zn > Cd > Cu > Fe > Ni > Cr. The highest TF was for Fe (102.4) and the lowest for Cu (0.66), while other remaining heavy metals were found to have comparable TF mostly below 1 except Ni and Zn. Furthermore, the trend of MF showed as mentioned from root to stalk and from stalk to leaves were: Mn > Cu > Cd > Zn > Fe > Ni > Cr; and Zn > Mn > Cd > Cu > Fe > Ni > Cr respectively.

10.6. Human health risk assessment

Heavy metals' pollution of freshwater ecosystems is of great concern worldwide because of water quality issues and seafood contamination. The present study investigated the possible health hazard in the form of THQ, HI, and TR on human health posed by fish consumption from the Kshipra River (Table 6). THQ represents a non-cancer risk, and in the current study, only Cr posed the highest risk in adult males (9.3×10^{-4}) and females (10.66×10^{-4}). The THQ for Cr, Cu, and Zn was found to be above 1. The value of THQ should not go beyond 1; if it is higher than 1, then there are chances that the exposed population may have some non-carcinogenic risk sometime in their lifetime. Furthermore, the HI value is the total of all THQs, and it was found to be

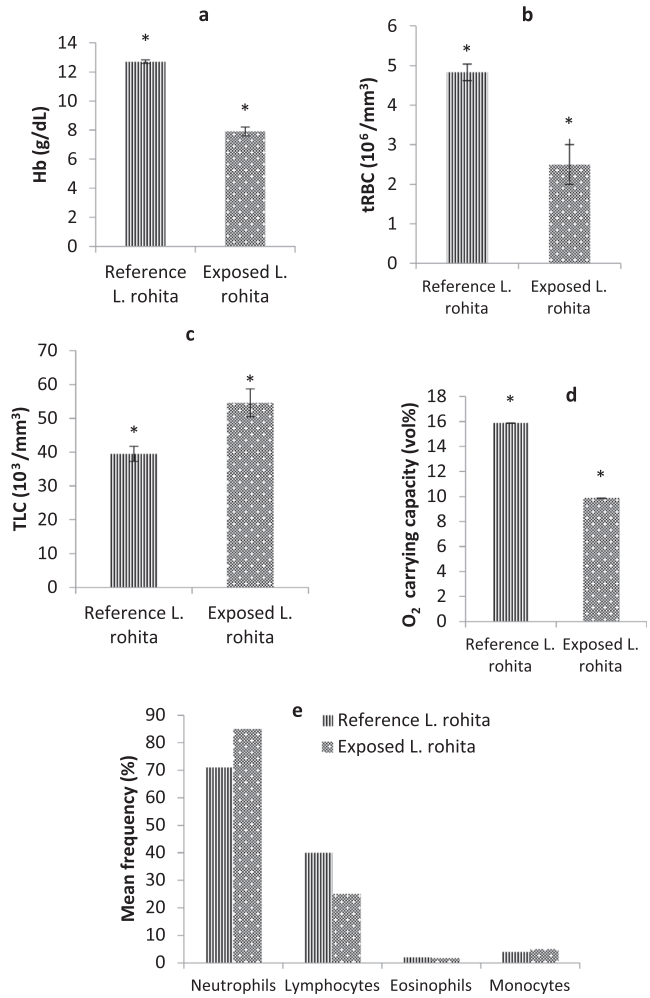

Fig. 1. a, b, c, d, and e shows hematological parameters of reference and exposed *L. rohita* fish; significant difference was found to be at $p < 0.05$.

12.51×10^{-4} and 14.32×10^{-4} for males and females, respectively. The higher HI values in females imply more risk to females than their male counterparts of the same age group. This difference could be due to their weight and lifespan because THQ calculation depends on these factors. In addition to this, TR values were also found to be high in females (1.59×10^{-3}) compared to males (1.402×10^{-3}).

11. Discussion

The variable concentration of heavy metals in water bodies highlights their possible role in deteriorating water quality and health hazards. To know the overall water quality, the WQI is computed, which explains the combined effect of different physico-chemical parameters on water (Batabyal and Chakraborty, 2015). It combines the complex data and produces a score that expresses the status of water quality (Reza and Singh, 2010; Batabyal and Chakraborty, 2015). The calculated values of WQI can be ranked into five categories: WQI value < 50 represents excellent quality; WQI = 50–100, good quality; WQI = 100–200, reflects poor quality; WQI = 200–300 indicate inferior quality; and if WQI exceeds 300 then it is unsuitable for drinking (Batabyal and Chakraborty, 2015). In the present study, the WQI was found to be 4020.28 (Table 1), a way beyond the set WQI for drinking purposes. This clearly indicates that the Kshipra river water in the studied rural area was unfit for drinking and other domestic uses. The very high level of WQI could be due to the presence of Cr and Cd. These two metals seem to be the main culprit because of their very high qi values 1630 and 30366.66, respectively (Table 1), which corresponds to high SI, ultimately resulting in high WQI. The high content of these metals may be due to the effluents from the iron and steel industries, dyes from currency printing, and other untreated waste from domestic sources. Giao et al. (2021) reported poor water quality in a low-lying area of the Vietnamese Mekong Delta. The rural regions of the Columbian Caribbean have been lower than the international and national water quality guidelines (Galezzo and Susa, 2021). Olayinka-Olagunju et al. (2021) reported bioaccumulation factors in water, pelagic, and benthic fishes in Nigeria's Ogbese river. In another study, Ghannam (2021) also used the water pollution index and bioaccumulation factor in fish to determine the water quality and health status of the Nile River, Cairo. The poor quality of water bodies has also been reported from India's other natural freshwater resources (Khan et al., 2020; Mahamood et al., 2021). Such water quality studies become stronger and more meaningful when adverse effects are assessed on the health outcomes of bio-indicator organisms dwelling in their environment. Therefore, endemic fish *L. rohita* and plant *E. crassipes* were selected for our investigations.

The bioaccumulation study observed gills as the target organs of heavy metals as they always remain in direct contact with the surrounding medium. Also, because of their thinness, delicateness, and rich blood supply, they instantly absorb toxicants and heavy metals along with dissolved respiratory gases from water. The gills could not excrete out heavy metals fully because after their absorption in the blood, they may bind with the enzymes and other

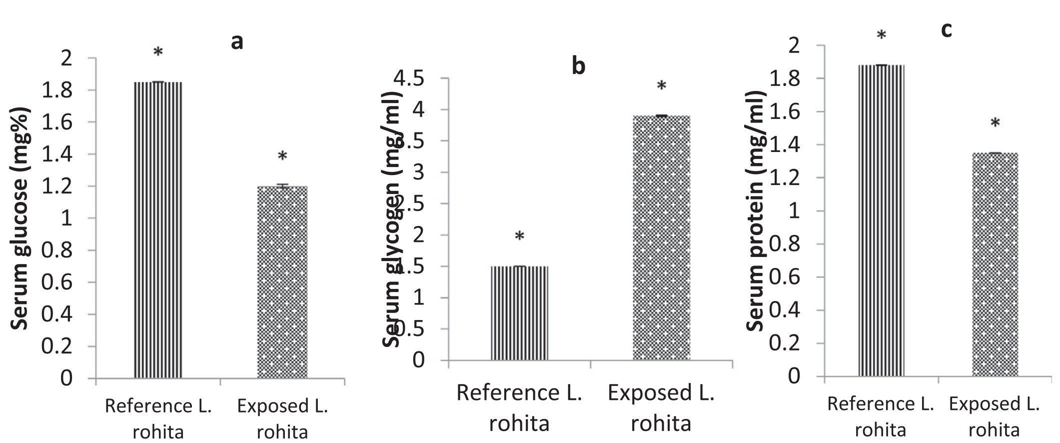

Fig. 2. a, b, and c shows serum glucose, glycogen, and protein levels in reference and exposed *L. rohita*; significant difference was found to be at $p < 0.05$.

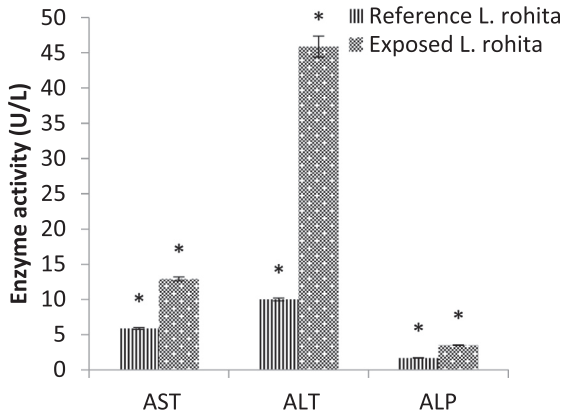

Fig. 3. The activity of serum pathology marker enzymes of reference and exposed *L. rohita* for liver function test; significant difference was found to be at $p < 0.05$.

macromolecules in the fish body. The accumulation and MPI values were comparable in the fish liver and gut, which could be attributed to the absorption of nutrients and other substances in the gut. In the liver, they get metabolized, and part of it binds with the myoglobin and stays in the muscle tissue. The low burden of heavy metals in the kidney indicates that it is working efficiently to remove them. Corroborating results to the present study were also reported by Khan et al. (2020) and Mahamood et al. (2021) in *Oreochromis niloticus* and *L. rohita* inhabiting the polluted Yamuna river. We recently reported gills and liver as the target organs of heavy metals in the *Mystus vittatus* and *Mystus tengara* fish (Tabrez et al., 2021).

The decline in Hb and tRBC levels in exposed fish indicates anemia, impaired RBC production, and mild hypoxic conditions. On the other hand, the reduction in blood oxygen content might be due to a decrease in Hb content as it is the only molecule that transports oxygen to different parts of the body. A similar finding was reported due to LC50 and LC85 exposure of Cr, Cu, and Pb in *Ctenopharyngodon idella* (Shah et al., 2020). Moreover, the higher TLC value could be due to the general defense mechanism of the fish against heavy metals. The leukocytosis can be due to the rise in neutrophils and monocytes. In the present study, neutrophils and monocytes seem to be actively involved in the phagocytosis of infectious agents and other cell debris. The role of lymphocytes (B-cells, NK cells, and T-cells) is to target the antigen and produce antibodies against it. The exposure to heavy metals may have affected the subspecies of lymphocytes, causing lymphocytopenia. However, their decrease may not have a significant effect on TLC (msdmanuals.com/en-in/home/blood-disorders/white-blood-cell-disorders/lymphocytopenia). Low eosinophil percentage could also be associated with some blood infections. In one study, Shah et al. (2016) reported that lymphocytopenia and low eosinophils could indicate cardiovascular disorders. The different leucocytes are commonly present in the fish blood except for basophils which are either rarely present or absent in healthy conditions (Mahamood et al., 2021). We also didn't observe any basophils in a smear of both reference and exposed fish. Recent studies reported changes in hematological parameters such as hemoglobin, blood cell count, oxygen-carrying capacity, etc., in fish resulting from water pollution (Samim and Vaseem, 2021).

It is well-known that heavy metals affect the normal physiological processes resulting in stress. The carbohydrate, namely glucose serves as an instant energy source during stress; the decrease in its levels indicates its utilization in various metabolic processes. When there is a shortage of glucose in the body, a non-carbohydrate product could also convert into glucose. In addition, gluconeogenesis could occur in which protein first converts into glycogen and later to glucose to meet the energy demand. We observed higher glycogen levels and reduced glucose and protein levels in exposed fish (Fig. 2). In an early study, Bhilave et al. (2008) observed the decline in glucose, glycogen, and protein level under chronic heavy metals exposure. Recently, Tewari et al. (2019) reported a decrease in glycogen concentrations in gill, liver, kidney, and muscle at 4, 7, 15, and 30 days' exposure to $CdCl_2$. The exposure of As_2O_3 and $PbCl_2$ has also been reported to disturb carbohydrate metabolism in *Heteropneustes fossilis* (Tariang et al., 2019).

The AST, ALT, and ALP are found in the liver, and the high concentration in serum indicates liver damage, resulting in leakage of these enzymes in serum (Javed et al., 2016b). Similar findings were observed in response to Zn and Cu exposure in *O. niloticus*, *Tilapia zilli*, and *Clarias gariepinus* (Abdel-Khalek et al., 2015; Zaghloul et al., 2006). Recently, Samim and Vaseem (2021) reported elevated ALT, AST, and ALP enzyme levels in *H. fossilis* due to chronic NiO exposure.

On average, the high metal burden was found in *E. crassipes* roots because they are in direct contact with the surrounding environment. Similar result was also observed by Singh et al. (2017) in aquatic plants namely *P. antidotale*, *L. camara,* and *A. conyzoids*. The indices BAF, TF, and MF are used to check anthropogenic pollution in plants and the media (Singh et al., 2017; Caunii et al., 2015). BAF indicates the amount of heavy metals uptake by plants from the water. The BAF value > 1 observed in our study, showed hyperaccumulation of heavy metals in *E. crassipes*. On the contrary, the TF is the ability of plant to translocate the accumulated metals to other parts rather than only roots. The root of *E. crassipes* was found to have *a* maximum capacity to translocate Fe to other regions (Table 5). However, further verification is required because in BAF, Fe stands at fifth place. TF > 1, also reflects high accumula-

Table 5
Bioaccumulation factor (BAF), translocation factor (TF), and mobility factor (MF) in *E. crassipes* plant.

Heavy metal	BAF	TF	MF	
			root-stalk	Stalk-leaves
Cr	1.96	0.80	2.40	1.96
Mn	79.85	0.76	103.57	70.31
Fe	40.72	102.40	51.94	38.55
Ni	9.60	1.186	8.16	16.17
Cu	49.07	0.66	73.63	50.59
Zn	69.24	1.07	64.44	85.55
Cd	59.60	0.89	66.95	59.16

Table 4
Heavy metal concentrations in *Eichhornia crassipes* (mg/kg. dw).

Plant parts	Cr	Mn	Fe	Ni	Cu	Zn	Cd
Leaves	$_{b}1.2 \pm 0.001^{g}$	$_{c}30 \pm 0.1^{c}$	$_{c}35 \pm 0.7^{b}$	$_{c}5.8 \pm 0.001^{f}$	$_{c}9.7 \pm 0.01^{e}$	$_{b}26 \pm 0.1^{cd}$	$_{b}48 \pm 0.24^{a}$
Stalk	$_{b}1.6 \pm 0.001^{g}$	$_{b}33.4 \pm 0.11^{cd}$	$_{b}42.6 \pm 1.2^{b}$	$_{a}19.8 \pm 0.03^{ef}$	$_{b}21.3 \pm 0.8^{e}$	$_{a}38.5 \pm 0.66^{c}$	$_{a}53.9 \pm 1.3^{a}$
Root	$_{a}2 \pm 0.001^{d}$	$_{a}49.2 \pm 0.01^{c}$	$_{a}57.4 \pm 1.8^{a}$	$_{b}10 \pm 0.02^{f}$	$_{a}31 \pm 0.21^{d}$	$_{b}29 \pm 0.02^{de}$	$_{a}54.3 \pm 0.72^{ab}$

All values are given as mean ± SEM (n = 8) collected from three different locations; Superscripts shows significant differences along the row while subscripts indicate significant differences along the column; Significance was established at $p < 0.05$

Table 6
Health risk assessment parameters THQ, HI, and TR.

Heavy metals	THQ		HI		TR	
	Adult male	Adult female	Adult male	Adult female	Adult male	Adult female
Cr	9.3×10^{-4}	10.66×10^{-4}	12.51×10^{-4}	14.32×10^{-4}	1.402×10^{-3}	1.59×10^{-3}
Mn	0.0904×10^{-4}	0.1031×10^{-4}				
Fe	0.642×10^{-4}	0.732×10^{-4}				
Ni	–	–				
Cu	1.351×10^{-4}	1.540×10^{-4}				
Zn	1.132×10^{-4}	1.291×10^{-4}				
Cd	–	–				

tion efficiency; hence this plant is included in the hyperaccumulation category (Majid et al., 2014). Whereas TF < 1, for Cr, Mn, Cu and Cd indicate the non-hyper-accumulating behavior of this plant's roots for these heavy metals. Furthermore, the highest recorded MF value from root to stalk for Mn (103.57) and lowest for Cr. Likewise, Zn (85.55) had the highest MF and Cr (1.96) had the lowest. These values imply that this plant is suitable for phytoextraction of Mn, Zn, Fe, Cu, and Cd. Although the BAF, TF, and MF of Cr are low in the present study, it indicates that roots are restricting Cr, and this plant can be used as a photo stabilizer for Cr. Moreover, plants having BAF $\leq$ 1.00 reflect absorption capacity only rather than accumulation efficiency (Sulaiman and Hamzah, 2018). If BAF, TF, and MF are greater than 1, the plants can be used for phytoextraction. On the other hand, BAF > 1 and TF < 1, indicate that the plant is a good photo stabilizer (Majid et al., 2014; Caunii et al., 2015).

Only Cr, Ni, and Cd are listed in the established carcinogens category among the studied metals. Ni and Cd were not detected in the fish fillet; hence, TR was calculated only for Cr. Qureshi and Mahmood (2010) described that Cr and Cd interfere with the steroid and thyroid hormone metabolism and cause thyrotoxicosis. Likewise, in the present study, gender differences in the toxicity of heavy metals were also observed (Tchounwou et al., 2012; Balali-Mood et al., 2021). Heavy metal toxicity damages different body parts and can cause various acute and chronic disorders such as gastrointestinal, renal malfunction, lesions in skin and vessels, immune and nervous system disorders, congenital disabilities, and cancer. There are also reports of synergistic effects resulting from the concomitant exposure to several metals (Tabrez et al., 2014; Costa, 2019; Gazwi et al., 2020).

12. Conclusion

The present study observed a high concentration of heavy metals in Kshipra river water. Among heavy metals, Cr and Cd were considered the main culprits responsible for the poor water quality of this river. These metals bioaccumulate in aquatic plants and fish and affect their health. Also, the users of this river water may have carcinogenic risks, sometimes in their life as shown by health risk assessment parameters. We also observed gender-specific differences in the health risk assessment parameters. The study highlights the absence of water quality surveillance systems in rural areas that need to be looked at by regulatory bodies because of the overreliance of residents on these riverine systems.

Availability of data and material

Data will be available upon request to the corresponding author.

Consent to participate

Not applicable.

Consent to publish

All authors have given their consent to publish this research article.

Author contributions

ST and MJ conceived, designed, and executed the work. TAZ did statistical analysis and also made first draft of this article. All authors reviewed the final draft of manuscript.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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