Annals of Plant and Soil Research 27(1): 111-117 (2025)
https://doi.org/10.47815/apsr.2025.10443

Study on seasonal heavy metal bioaccumulation and histopathological alterations in organs of selected fish living in river Gomti

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Received: December, 2024; Revised accepted: Febuary, 2025

ABSTRACT

The main source of freshwater pollution impacting fish health is heavy metal discharge. This study investigated seasonal variations in the bioaccumulation of chromium (Cr), cadmium (Cd), lead (Pb), and arsenic (As) in the gills, liver, and kidneys of Channastriatus from a polluted area near the Gomati barrage. Significant variations (P ≤ 0.05) were observed in metal bioaccumulation across seasons. In winter, Pb concentrations were highest in the liver (72.263 ± 5.02 ppm), followed by gills (9.781 ± 1.12 ppm) and kidneys (7.305 ± 1.02 ppm). In summer, the order changed to liver (12.457 ± 1.06 ppm) > gills (8.413 ± 1.04 ppm) > kidneys (8.358 ± 1.25 ppm). For Cr, the liver had the highest levels both in winter (42.186 ± 3.87 ppm) and summer (6.519 ± 0.44 ppm). All metals exceeded WHO permissible limits, indicating significant environmental contamination affecting local fish populations.

Keywords: Heavy metal, Fish tissue, Bioaccumulation, *Channa striatus*

INTRODUCTION

Freshwater contamination is a serious hazard to aquatic life and is becoming worse every day (Shaheen and Jabeen, 2015). The main reason why heavy metals build up in the different organs of freshwater fish is freshwater pollution (Afshan *et al.,* 2014). When hazardous household garbage is dumped into water bodies, the biological oxygen requirement is reduced to a lethal level. Even in low quantities, aquatic life is killed by the highly poisonous chemicals and metallic salts released into rivers by untreated industrial effluent (Jayaprakash *et al.,* 2015; Sultana *et al.,* 2017). Pb, Cr, and Cd because they are hazardous heavy metals, they can enter freshwater systems through mining, wastewater treatment facilities, gasoline combustion, soil erosion and leaching, and industrial waste (WHO, 2011). Heavy metal is defined as any metal or metalloid that has an atomic mass greater than 20, is five times heavier than water molecules, and possesses a specific gravity of more than 5 g/cm³ (Kumar *et al.,*2024).The most hazardous metals for aquatic life and ecosystems are Cr, Cd, and Pb. But quantities of Cr, Cd, and Pb over 0.05, 0.005, and 0.01 mg/g, respectively, can be dangerous (WHO, 2011). Because of their propensity to bioaccumulate, the primary cause of fatal histopathological changes in fish species' important tissues is elevated levels of heavy metals in wastewater (Gupta *et al.,* 2017). Because they are involved in the detoxification processes that remove harmful compounds, the kidney and liver were chosen as the target organs for metal accumulation detection (Tepe *et al.,* 2008). Fish serve as model animals to represent environmental changes since they are sensitive species to pollution and other environmental changes (Nikalje *et al.,* 2012). As a biomonitoring technique for aquatic pollution, fish histopathology provides information on the health risks of chemicals and pollutants to people through fish ingestion that results in cellular damage and changes (Mustafa et al, 2020). The purpose of this study was to assess the harmful effects of the metals by histopathological changes and identify a seasonal influence on changes in the bioaccumulation of heavy metals in the kidney, liver, and gills of *Channa striatus*.

MATERIALS AND METHODS

Animal collection and study site

Twenty fish samples with an average overall length of 19 cm and a wet weight of 860 gm were taken from the contaminated river.

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Fish were gathered at the Gomati barrage's entrance, a heavily polluted section of the River Gomati (Pali *et al.,* 2022). From 2020 to 2021, the representative samples were gathered. The fish specimens were carefully placed in oxygen-filled polythene bags and transported to IITR Lucknow for additional experimental analysis. Fish that were raised for food served as the control group.

Heavy metals analysis

Polystyrene water bottles with a 1.5-litre capacity were used to collect water samples. Heavy metal concentrations (Pb, Cr, Cd, and As) were measured in water samples taken from the fish harvesting region. The samples were kept in a refrigerator at 4 ºC and maintained with 55% HNO3 (Hussain *et al.,* 2016). Using the method modified by Mahboob *et al.* (2014), metals were found using an Aurora-A-I1200 Atomic Absorption Spectrophotometer.

Digestion of water sample

Placed a 100 ml sample of preserved water in a conical flask, heated to a capacity of 20 milliliters on a hot plate. After letting it cool for a while, add 5 milliliters of 55% HNO3 and 10 milliliters of 70% HClO4. Under the fume hood, heat this mixture until the yellow fumes give way to white vapours. Then, add up to 100 millilitres of distilled water to bring it to room temperature. This combination was filtered using 0.45 mm-pore Whatman filter paper, and the filtrate was examined for specific heavy metals.

Digestion of fish tissue samples

After weighing and transferring one gram of fresh sample tissue into a glass beaker, it was digested using hydrogen peroxide (>35% H2O2) and nitric acid (65% HNO3). The beaker was then put on a hotplate beneath a fume hood until the yellowish vapours became white. The sample was allowed to cool at room temperature before being filtered using Whatman filter paper with 0.45 mm pores and 100 cc of distilled water. Using an Atomic Absorption Spectrophotometer, the filtrate's Pb, Cr, Cd, and as concentrations were measured by APHA (1998) (Authman *et al.,* 2015).

Histopathology

For morphometric measures, fish specimens were documented. The procedure used by Sultana *et al.* (2016) was followed in order to harvest liver and renal tissues. After being chopped into sections that were around 5 mm thick, the tissue specimens were preserved for three to four hours at room temperature in sera (60 % ethanol, 10% acetic acid, and 30% formalin) (Jabeen and Chaudhry, 2013). Sections of fixed tissue were embedded in molten paraffin after being dehydrated at room temperature using increasing ethanol grades and cleaned with xylene. A Histo-line MR 258 microtome was used to cut tissue sections that were 3 mm thick. After selecting a tissue slice ribbon from the water's surface and placing it on a slide that had been coated with albumen/glycerin as an adhesive, the slide was placed in an oven set at 37 °C and then moved three times each for three minutes into xylene. Slides were stained for three to five minutes in hematoxylin after being submerged in decreasing ethanol grade for one to two minutes each. Mounting was done in Canadian balsam, and it was photographed and examined under a light microscope. By comparing with control and contemporary literature, histopathological alterations were noted.

Statistical analysis

The heavy metal data were examined using a one-way ANOVA. The mean and standard error are displayed in the findings.

RESULTS AND DISCUSSION

The mean Pb value in drain water was at its highest during the summer (0.94 ± 0.29), at its lowest during the monsoon (0.303 ± 0.11), and at its lowest during the winter (0.38 ± 0.07). In river water, the mean value of Cr was at its highest in the summer (0.77 ± 0.26), at its lowest in the monsoon (0.356 ± 0.19), and at its lowest in the winter (0.70 ± 0.19 ppm) (Table 1). Winter showed the highest mean value of Cd (1.7 ± 0.05) in drain water, while the monsoon season saw the lowest (0.068 ± 0.03), and summertime saw levels of 0.13 ± 0.03. In river water, the mean value of As was at its highest in winter (0.01 ± 0.03), at its lowest in monsoon (0.006 ± 0.03), and at its lowest in summer (0.0116 ± 0.001) ppm (Table 1).

Table 1: Heavy metals concentration (ppm) in River Gomati water at selected sites

Heavy metals	Seasons	Khadra Pakka Pul	Hanuman Setu	Gomti barrage	WHO, 2011
Cr	Monsoon	0.28 ± 0.13	0.24 ± 0.11	0.55 ± 0.35	0.05
	Winter	0.46 ± 0.14	0.59 ± 0.14	1.05 ± 0.29	
	Summer	0.48 ± 0.11	0.46 ± 0.21	1.39 ± 0.47	
Cd	Monsoon	0.06 ± 0.01	0.014 ± 0.03	0.13 ± 0.07	0.005
	Winter	0.12 ± 0.05	0.14 ± 0.04	0.25 ± 0.06	
	Summer	0.09 ± 0.02	0.12 ± 0.06	0.19 ± 0.03	
Pb	Monsoon	0.15 ± 0.04	0.08 ± 0.02	0.68 ± 0.24	0.01
	Winter	0.19 ± 0.06	0.21 ± 0.09	0.34 ± 0.13	
	Summer	0.49 ± 0.13	1.61 ± 0.48	0.74 ± 0.19	
As	Monsoon	0.0007 ± 0.001	0.0015 ± 0.001	0.0043 ± 0.002	0.001
	Winter	0.006 ± 0.001	0.01 ± 0.001	0.014 ± 0.003	
	Summer	0.007± 0.001	0.0198 ± 0.001	0.008 ± 0.001	

Various organs of *Channa striatus* that were tested from various Gomati River locations throughout various seasons of the year showed concentrations of the chosen metals. Certain heavy metal concentrations varied with the season. Pb concentrations in the gills (9.781 ± 1.12 ppm), kidney (7.305 ± 1.02 ppm), and liver (72.263 ± 5.02 ppm) were measured in the winter, although the concentration distribution in the gills, liver, and kidney was in declining order. From liver (12.457 ± 1.06 ppm) to gills (8.413 ± 1.04 ppm) to kidney (8.358 ± 1.25 ppm), the concentration of lead decreased in the summer. The winter months had the greatest Pb levels, whereas the summer months had the lowest levels. The concentration of Cr in winter was 42.186 ± 3.87 ppm in the liver, 2.607 ± 1.23 in the gills, and 2.362 ± 0.87 in the kidney. In contrast, the concentration of Cr in the summer was found to be 6.519 ± 0.44 ppm in the liver, 4.766 ± 0.98 in the kidney, and 3.133 ± 0.87 in the gills (Table 2).

Table 2: Seasonal variation of metals concentration (ppm) in different organs of *Channa striatus* collected from River Gomati (means ± SE)

Heavy Metal	Fish Tissues	Seasons			Mean
		Monsoon	Winter	Summer	
Cr	Liver	9. 833 ± 1.22	42.186 ± 3.87	6.519 ± 0.44	19.512 ± 5.55
	Kidney	2.295 ± 0.67	2.362 ± 0.87	4.766 ± 0.98	3.141 ± 0.77
	Gills	7.486 ± 1.34	2.607 ± 1.23	3.133 ± 0.87	4.408 ± 1.14
Cd	Liver	4.315± 0.98	50.205 ± 6.78	1.301 ± 0.98	18.607 ± 2.91
	Kidney	2.673 ± 0.78	1.391 ± 0.34	1.768 ± 0.56	1.944 ± 0.56
	Gills	9.646 ± 1.16	2.838 ± 0.99	2.566 ± 0.54	5.016 ± 0.89
Pb	Liver	31.585 ± 2.21	72.263 ± 5.02	12.457 ± 1.06	38.768 ± 2.76
	Kidney	7.396 ± 1.09	7.305 ± 1.02	8.358 ± 1.25	7.686 ± 1.12
	Gills	32.485 ± 4.32	9.781 ± 1.12	8.413 ± 1.04	16.893 ± 2.16
As	Liver	0.024 ± 0.003	0.171 ± 0.004	0.013 ± 0.001	0.0693 ± 0.002
	Kidney	0.016 ± 0.001	0.008 ± 0.001	0.006 ± 0.001	0.01 ± 0.001
	Gills	0.042 ± 0.001	0.251 ± 0.001	0.031 ± 0.001	0.108 ± 0.001

Cd levels were also measured in the winter, and they were as follows: liver (50.205 ± 6.78 ppm) > gills (2.838 ± 0.99 ppm) > kidney (1.391 ± 0.34 ppm). The concentrations of Cd in the gills (9.646 ± 1.16), liver (4.315 ± 0.98), and kidney (2.673 ± 0.78 ppm) were in decreasing order over the monsoon season. The concentration of Cd was lowest during the monsoon season and greatest throughout the winter (Table 2). The concentrations were also noted in the winter, and they were as follows: gills (0.251 ± 0.001) > liver (0.171 ± 0.004) > kidney (0.008 ± 0.001) times the concentration. The concentration of Cd during the monsoon season was 0.042 ± 0.001 ppm in the gills, 0.024 ± 0.003 in the liver, and 0.016 ± 0.001 in the kidney. The concentration of Cd was lowest during the monsoon season and greatest

throughout the winter (Table 2). In the current study, hepatocytes obtained during the winter showed an aberrant structure in the liver of fish taken from the upstream river location. In the winter, fish gathered from certain downstream locations of the River Gomati showed vacuolar and hepatocyte deterioration. During the summer and monsoon seasons, necrosis and dilatation in sinusoids in hepatic tissues were seen, along with blood cell infiltration and cytoplasmic vacuolation (Table 3; Fig. 1).

Table 3: Summary of histological abnormalities observed in the kidney, gills and liver of *Channa striatus*

S. No	Parameters of kidney/Gills damage	*Channa striatus*
1	Necrosis	+++
2	Tubular haemorrhage	+++
3	Congestion	+++
4	Fusion of lamellae	+++
5	Lamellar breakage	+++
6	Lamellar hyperplasia	+++
7	Clubbed lamellae	+++
8	Cellular inflammation	+++
	Parameters of liver damage	
1	Cytoplasmic disorganization	+++
2	Cytoplasmic vacuolation	+++
3	Dilation of sinusoids	+++
4	Necrosis	+++
5	Congestion	+++
6	Edema	+++
7	Degenerated and split elements	+++

(+++) Severe

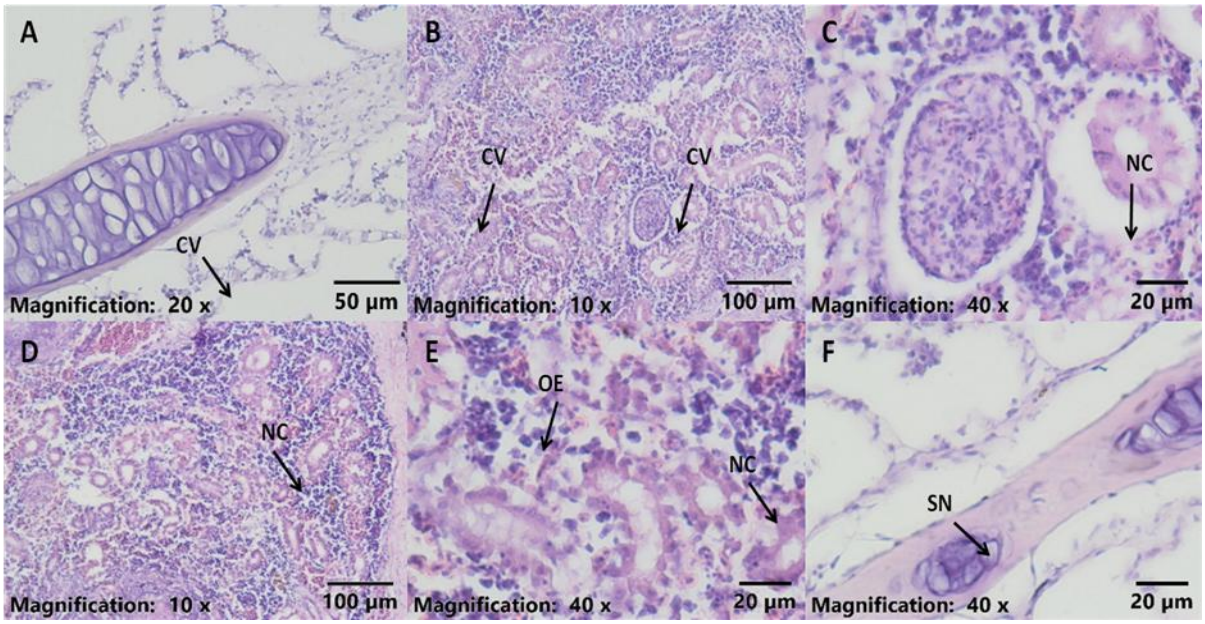

Fig. 1: The histological photomicrographs of C. *striatus* taken from the contaminated locations of the Gomati River (H and E staining, 10x, 20x, 40x) show histological abnormalities such as cytoplasmic vacuolation (CV), necrosis (NC), oedema (OE), and sinusoid dilatation (SD) (A-F)

Normal renal corpuscles were visible in kidney photomicrographs, as were the glomerulus, tubules, and epithelial cells with round, conspicuous nuclei that define the proximal segment in healthy farm fish. After receiving drain water, the experimental fish (*C. striatus*) specimens were recovered downstream, where they displayed more

damage as a result of increased pollution (Fig. 2). Regarding the condition of fish gills that were taken over various seasons from the Gomati River's upstream and downstream. These findings revealed a number of alterations in fish histology. Fish gills collected in the winter showed a variety of gill pathologies, including hypertrophy and congestion of the primary and secondary lamellae. The secondary lamellae were seen to break, shorten, and fuse during the monsoon season. Summertime observations of damaged epithelium, lamellar hyperplasia, and many primary gill lamellae were accompanied by the deposition of numerous inflammatory cells, particularly at the base of the primary gill lamellae (Table 2; Fig. 3).

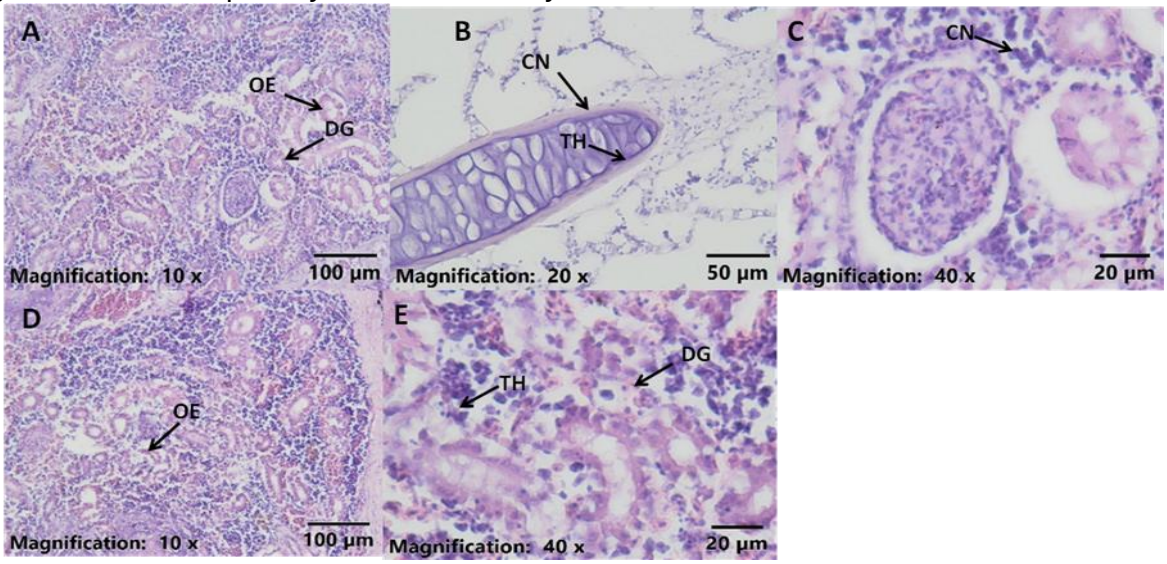

Fig. 2: Photomicrographs of *C. striatus's* control kidney tissue. Necrosis, tubular bleeding, oedema, damaged glomerulus, and collecting duct damage and congestion were seen in fish tissues taken from specific locations in the River Gomati (A-E). Blood cell congestion (CN) and Bowman capsule space dilatation (DL). Tubular haemorrhage (TH), glomerular degeneration (DG), and necrosis (NC) (H and E staining 10x, 20x, 40x)

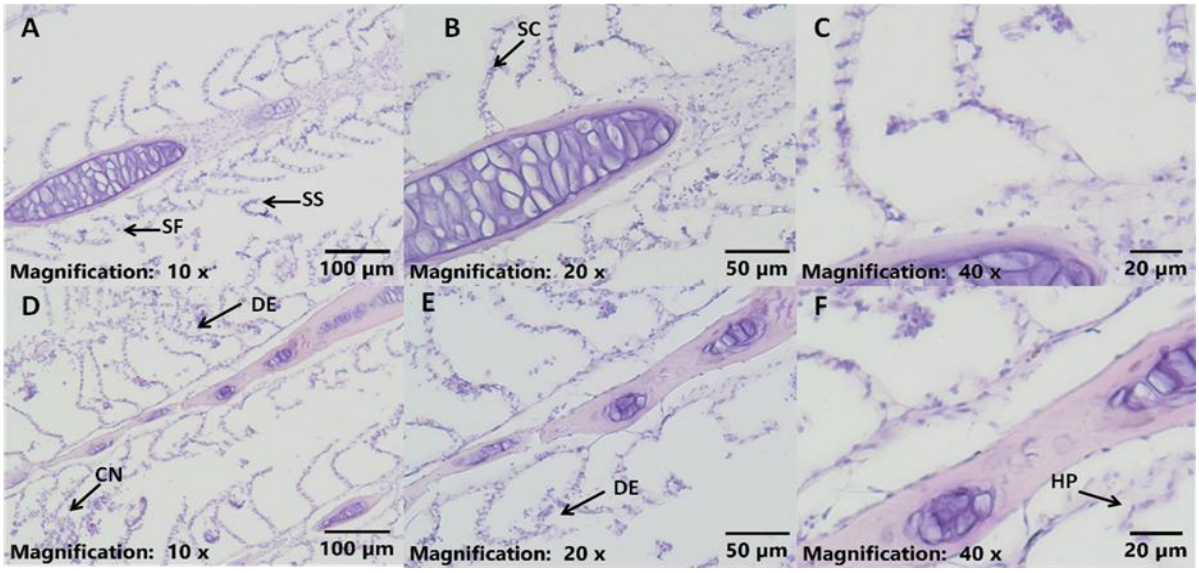

Fig. 3: During several seasons when fish gills were collected, photomicrographs of *C. striatus* gill tissues revealed a variety of gill abnormalities, including primary and secondary lamellae congestion (SC) and hypertrophy (HY). Secondary lamellae breaking (SB), shortening and fusion of sec. lamellae (SS, SF), and congestion (CN) occur during the monsoon season. During the summer, there was an accumulation of many inflammatory cells, particularly at the base of the main gill lamellae, together with damaged epithelium (DE), lamellar hyperplasia (LH), and some primary gill lamellae were clubbed (CB)

According to the current study, some metals are more abundant in the kidneys, gills, and liver. All of the targeted organs showed the highest concentration burden of these chosen metals during the winter months. Iqbal and Shah also reported comparable results (Iqbal and Shah, 2014); they also noted that *Cyprinus carpio* from Rawal Lake had the highest metal levels in the summer and the lowest levels in the winter. According to this study, the liver and kidney had the highest metal contents, whereas the muscle had the lowest. The results of this investigation supported those of Mohammadi *et al.* (2011) about the accumulation of heavy metals in fish. Toxic chemicals are eliminated from the circulation by the liver and kidneys, which also carry out detoxification (Kent, 1998). In vertebrates, the liver is an essential organ with a substantial metabolism (Liu *et al.,* 2012). Aquatic life and public water sources are now seriously threatened by the buildup of metalintoxicants (Jamdade and Gawande, 2017). Cytoplasmic vacuolation, cytoplasmic disarray, hepatic necrosis, oedema, blood cell congestion, and sinusoidal dilatation were among the histological changes found in the liver. These results were consistent with *Labeorohita* findings by Bantu *et al.* (2017). According to Faheem *et al.* (2016), these liver tissue changes include tissue degradation, inflammation, hepatocyte necrosis, and central vein congestion.

Liebel *et al.* (2013) observed comparable changes in the liver's histology. Paul *et al.* (2014) supported these findings by seeing necrosis, hepatocyte deterioration, vacuolation of cells, enlarged sinusoids with pyknotic nuclei, and blood vessel degeneration. Similar histological alterations in the liver (*Catlacatla, Labeorohita,* and *Cirrhinusmrigala*) were observed by Rana *et al.* (2017). Oedema, damaged glomerulus, collecting duct injury, tubular bleeding, necrosis of renal tubular cells, and blood congestion were all seen in kidney sections of experimental fish used in this investigation. The results of this investigation are consistent with those of Latif *et al.* (2012), who reported kidney abnormalities include glomerular tissue necrosis and degeneration, as well as blockage in the tubular lumen. The results of the current study were comparable to those of significant carp studies conducted by Mustafa *et al.* (2017) and Rana *et al.* (2017).

The liver, kidney, and gills of fish living in the natural water system were tested for heavy metals such as Cr, Cd, As, and Pb. The impacted fish poses a risk to the health of his final customer. Histopathological alterations in *Channa staitus* essential organs are useful indicators of xenobiotic toxicity in water. The current study's findings are consistent with evaluating the ecotoxicological impacts of freshwater contaminants that eventually reach people through the food chain (e.g., eating fish). The primary source of imbalance in the food chain is anthropogenic activities. Advanced and more effective methods that produce less or no heavy metal pollution can help decrease aquatic loss and imbalance.

CONCLUSIONS

The River Gomati receives a significant quantity of pollutants from the Gomati barrage. Fish and water taken from the river were found to be significantly contaminated with heavy metals. Fish histopathology revealed massive tissue deterioration in the kidney, liver, and gills.

ACKNOWLEDGEMENTS

The author would like to extend heartfelt appreciation to the Department of Zoology at Maharishi University of Information Technology in Dubagga, Lucknow (Uttar Pradesh), India, for their invaluable guidance and unwavering support throughout the process of completing this article. Their expertise has significantly enriched the quality of this work.

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