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Assessment of Heavy Metal Bioaccumulation in Selected Fish Species from the River Ganga in Bhojpur District, India

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Abstract: The study under consideration investigates the occurrence, distribution of heavy metal in the tissues, and potential health risks of heavy metal pollution in the selected freshwater fish species caught in the Bhojpur area of River Ganga, India. Pre-monsoon, monsoon and post-monsoon seasons Three common species Labeo rohita, Catla catla and the carnivorous Channa punctatus were sampled. Muscle, liver, and gill tissues were analyzed by the atomic absorption spectrophotometry in the presence of lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), zinc (Zn), mercury (Hg), and arsenic (As) after acid digestion. The results revealed that the metals under study were all found in all the species and tissues. The highest concentration was in Zinc and copper; Zn (approximately 48.1 mg/kg) and Cu (6.88 mg/kg) in liver samples, and mercury was relatively low (maximum 0.19 mg/kg). The tissue-wise accumulation confirmed the metabolic storage patterns which were liver > gills > muscle. The troic level of biomagnification was observed whereby carnivorous fish were found to have higher metal loads compared to herbivorous ones. The bioaccumulation factors obtained were 21-237 with the highest being zinc. The change of season indicated that the concentrations were a bit higher during the post-monsoon season, possibly because of surface run-offs and resuspension of sediments. Health risk assessment based on Estimated Daily Intake (EDI), Target Hazard Quotient (THQ) and Hazard Index (HI) showed that the majority of individual THQ values were less than unity, with cadmium having a THQ of approximately 1.12, and the cumulative HI of more than 4.0, indicating that there were long-term health risks to regular fish consumers. The work provides a quantitative baseline data of the bioaccumulation of heavy metal in the commercially valuable fish species of the River Ganga in the Bhojpur district and the need to have continuous environmental monitoring and pollution control measures to ensure the safety of aquatic life and health protection of the population.

Keywords: Heavy metal contamination, Bioaccumulation, Ganga, Freshwater fish, Seasonal variation, Human health risk, THQ, Hazard Index (HI), Aquatic pollution monitoring.

1. Introduction

1.1 Importance of Freshwater Ecosystems and the River Ganga

Freshwater ecosystems are also one of the most important natural resources in the support of biodiversity, ecological stability, and human livelihoods in the world. Rivers, lakes, wetlands and reservoirs are important sources of ecosystem services such as drinking water, agricultural irrigation, fisheries production and transportation. The river systems are especially important in the developing countries like India since most people rely on the surface water as a source of food and economic development. One of the most important freshwater resources, ecologically and socio-economically, is the River Ganga (Ganga River).

Ganga basin is one of the biggest river basins in Asia that serves hundreds of millions of people and is a significant source of irrigation, fisheries, and domestic water supply. The river also has a rich cultural and religious connotation and, therefore, it is the hub of social traditions and practices. The river ecosystem has been subjected to a lot of environmental pressure as a result of high rate of urbanization, population growth and construction of industrial infrastructure along the river banks. The sewage, industrial effluents, agricultural fertilizers, and solid waste released into the water bodies are not treated and have led to the severe water quality deterioration in the last several decades. This degradation is not only posing a threat to the aquatic biodiversity, but also affects the safety of fish and other edible aquatic organisms which are consumed by the local people.

1.2 Heavy Metal Pollution in Aquatic Environments

The different categories of environmental pollutants that cause environmental pollution in freshwater systems are the heavy metals which are said to be very dangerous as they are toxic, persistent and non-biodegradable. Unlike organic pollutants, heavy metals do not deteriorate with time and can be stored in the environment, water, sediments, and organisms. Some of the examples of environmental hazardous heavy metals are lead (Pb), cadmium (Cd), chromium (Cr), mercury (Hg), arsenic (As), copper (Cu), and zinc (Zn). Although certain metals, including copper and zinc, are micronutrients in small quantities, their high quantities are harmful to aquatic organisms and human beings.

Heavy metals in rivers have numerous sources that are anthropogenic such as electroplating industries, battery production, mining, textile processing, leather tanning, use of agricultural pesticides, and the disposal of municipal wastes. When they are released to water bodies, the metals can either be dissolved in water or they can be fixed to suspended particles and sediments. The metals that are attached to the sediment may be later released into the water column by alterations in pH, temperature or hydrological disturbances and hence spread contamination. The aquatic organisms are known to have toxicological impacts of the heavy metals. The chronic exposure could affect the growth of fish, disrupt the reproductive system, kill the gill tissue, and disrupt the enzymatic activity. Human health-wise, long-term exposure to contaminated food sources may cause neurological disorders, kidney damage, heart diseases and cancer. Thus, to protect the environment and health of people, the amount of heavy metal pollution of freshwater ecosystems must be observed.

1.3 Bioaccumulation in Fish and Their Role as Pollution Indicators

It is a well-known fact that fish are good bio-indicators in measuring aquatic pollution since they hold significant roles in the aquatic food web and actively accumulate contaminants within their immediate environment. The heavy metals find their way into fish bodies in several ways, such as direct uptake of the water through gills, ingestion of contaminated sediments, and ingestion of polluted prey organisms. After absorption, the metals are carried by the blood and deposited in different organs, depending on the metabolic activity and the process of detoxification.

2. Literature Review

2.1 Heavy Metal Pollution in Freshwater Ecosystems

Contamination of fresh water ecosystems by industrial effluents, agricultural runoffs, sewage discharge, and mining activities that introduce toxic heavy metals in the water bodies are also threatening the ecosystems (Tchounwou et al., 2012). Of great concern are the non-biodegradable, persistent and accumulative heavy metals like lead, cadmium, chromium, mercury, copper and zinc that can be found in sediments and aquatic organisms (Järup, 2003). These metals released into rivers can be dissolved in the water or can be attached to the suspended solids and ultimately be deposited in the bottom sediments as secondary sources of long term pollution (Singh et al., 2005).

The river systems in India have been becoming susceptible to heavy metal pollution due to the rapid urbanization and industrialization (Sharma and Kansal, 2011). The Ganga River is one of them, which is of special importance as it sustains the high population settlements, large-scale agriculture, and large fisheries. The water quality of the river has been reported to have reduced in most of its stretches and the level of metals has often been found to be beyond the acceptable environmental standards (Gupta et al., 2013; Kumar et al., 2011). The environmental impact of heavy metal pollution is severe, such as the disturbance of aquatic biodiversity, physiological stress, and the changes in the food-chain dynamics (Das et al., 2018). Hence, the problem of metal pollution in freshwater systems has been a significant part of the environmental management and conservation policies and therefore should be monitored at all times.

2.2 Bioaccumulation of Heavy Metals in Fish

Fish is generally regarded as a good bio-indicator of water pollution because of the exposure to contaminants over time through water, food, and sediment pathways (Authman et al., 2015). Bioaccumulation involves the absorption of metals being greater than the rate of excretion and this causes the body tissues to accumulate metals (Jezierska & Witeska, 2006). This makes fish particularly efficient in long-term environmental surveillance compared to water samples, which are only short-term indicators of pollution. The studies indicate that the level of heavy metals in different organs of fish differs and varies depending on the physiological functions. The peak concentration is usually in the organs that are directly involved in the detoxification and metabolism such as liver and kidney and gills have middle levels as they are directly exposed to the contaminated water (Vinodhini and Narayanan, 2008). Muscle tissues tend to be of lesser concentration, however, the most important in the context of the public health issue as they are the part of the food consumed by human beings (Begum et al., 2005).

Biomagnification of food chains often leads to higher metal loads of carnivorous fish compared to herbivorous or omnivorous fish (Yousefi and Shakoori, 2008). The analysis of different fish species, in its turn, can be a valuable source of information concerning the tendencies of contamination of the entire ecosystem.

2.3 Evidence from the Ganga Basin and Other Indian Rivers

Various studies have been conducted on the Ganga basin in terms of fish species pollution by heavy metals. Malik et al. (2010) examined fish tissues in industrially impacted stretches and reported high levels of Pb, Cd and Cr than the recommended. On the same note, Kumar et al. (2011) established that the level of accumulation of heavy metals in commercially important fish species was significant and that liver tissues were always higher than the muscles. Gupta et al. (2013) examined the Allahabad section of the river and discovered that the industrial discharge regions were strongly associated with the high level of the metals in the aquatic organisms.

The prevalence of metal pollution in other rivers in India is also proved by similar studies. Singh et al. (2005) showed that the distribution of heavy metal in the Gomti River system was very spatially heterogeneous and Sharma and Kansal (2011) highlighted the increasing role of urban wastewater discharge in the degree of river pollution. Together, these studies suggest that the problem of heavy metal contamination is not a problem of several localities but a larger environmental problem in the Indian freshwater systems. Regardless of such studies, there are few studies on local ecological conditions at the district level. The area-specific study is needed to produce the appropriate environmental risk assessment because the contamination levels can be very different, depending on the sources of pollution in the area, the structure of the sediments, and hydrological conditions.

2.4 Human Health Risks from Consumption of Contaminated Fish

The health implications of heavy metal contamination on edible fish are enormous to the population as fish is a significant source of dietary protein and other essential nutrients in most developing regions (Rashed, 2001). Exposure to toxic metals in the form of food over a long period may lead to neurological diseases, kidney damage, skeletal defects, and carcinogenicity (Tchounwou et al., 2012). The international health organizations have therefore established a guideline on how to assess the risk of contamination that accompanies the consumption of fish. The U.S. Environmental Protection Agency recommends the use of contaminant surveillance and advisory models in determining the safe levels of consumption (USEPA, 2000). Similarly, the World Health Organization (WHO) has emphasized great caution on the content of toxic metals in food sources to prevent chronic health hazards (WHO, 2017).

The environmental toxicology studies also show that chronic exposure to such metals as cadmium and lead can cause cumulative physiological damage despite short-term consumption being reported to be within the acceptable limit (Järup, 2003). As riverine communities tend to consume fish on a regular basis, localized monitoring studies are required to determine whether the level of contamination is potentially dangerous to their health. Using the example of Sarkar et al. (2008), which examined the heavy metal in fish caught in the mid-range of the Ganga, the researchers discovered a high concentration of cadmium, lead and chromium in the edible tissues. Their study emphasized that constant industrial discharge and municipal wastewater inflow were the primary sources of contamination. Similarly, Pandey et al. (2014) studied the dynamics of metal deposition in the fish of Yamuna River and discovered that tissue-specific variation was high with liver tissues showing the highest metal concentration then gills and muscles. Another study by Bhattacharya et al. (2015) also determined that the Indian freshwater fish species in contaminated waters experience oxidative stress and biochemical alterations of heavy metal toxicity. Their study revealed that chronic exposure to contaminated water can lead to growth, metabolic, and reproductive performance impairment of fish, and consequently, long-term sustainability of fish populations. In another study, Javed and Usmani (2013) compared the heavy metal levels in fish within the Ganga canal system and established that feeding behavior is a powerful predictor of the level of contamination with bottom-feeding fish having higher levels of heavy metals due to their contact with sediments. In the same way, Kumar and Singh (2010) studied the bioaccumulation of freshwater fish in the North of India and discovered that seasonal variation is a major determinant of the degree of metal contamination with the highest degree of contamination normally being recorded during the low-flow seasons.

More recently, Chakraborty et al. (2021) highlighted that heavy metal contamination is a long-term issue in the Indian river systems and proposed long-term biomonitoring programs using fish tissues to determine the ecological health and food safety risks. These Indian investigations confirm that the pollution of freshwater fish by heavy metals is not only widespread but also conditioned by the local industrial activity, hydrology, and ecology, which explains the need to conduct region-specific research, such as the one conducted in the present study on the Bhojpur stretch of the Ganga River.

The food safety, health protection of people and sustainable fisheries management of the river sections such as the Bhojpur region depends on the determination of the heavy metal bioaccumulation in fish species.

3. Materials and Methods

3.1 Study Area

The present research was done in the region of the Ganga River flowing through the Bhojpur district of the state of Bihar in India. The area is a major inland fisheries region whereby the local communities rely on capture fisheries to make a living and obtain dietary protein. Several sampling points were selected along the river course in the district, in the large fish landing centers, in the traditional fishing ghats and at convenient locations on the riverbank where the local fishermen usually work. The site was selected in such a way that it represented the upstream, midstream and downstream sites to be able to assess the spatial variation of the contamination levels.

It is characterized by high agricultural productivity with cereals, pulses and vegetables being produced seasonally in the fertile alluvial plains of the river basin. The agricultural fields with fertilizers, pesticides, and soil particulates run into the river during the monsoon and the irrigation drainage. Further, the river system is fed with organic and inorganic pollutants by untreated domestic sewage of the surrounding settlements and municipal discharge points. Effluents that contain trace metals and other pollutants can also be discharged by small-scale business enterprises, local workshops, and market spaces situated near the riverbanks.

Table 1: Study Area Sampling Locations

Site Code	Sampling Location (Bhojpur Ganga Stretch)	Latitude (N)	Longitude (E)	River Bank Type	Pollution Source Nearby
S1	Koilwar Ghat	25.58	84.80	Sandy + Agricultural	Fertilizer runoff, irrigation discharge
S2	Arrah River Landing	25.56	84.67	Urban mixed bank	Domestic sewage, fish market waste
S3	Shahpur Fishing Zone	25.60	84.45	Clay + rural bank	Agricultural pesticides, livestock runoff
S4	Bihia Downstream Point	25.55	84.30	Sediment-rich floodplain	Upstream industrial effluent + sediment deposition

Table 1 shows the sampling plan of the study carried out along the Ganga River stretch in the Bhojpur district where four representative sites were chosen to reflect the differences in riverbank features and sources of pollution. These are Koilwar Ghat (S1), a sandy agricultural bank, which is primarily affected by the runoff of fertilizers and irrigation discharge; Arrah river landing (S2), an urban mixed bank, which is mainly affected by domestic sewage and fish market waste; Shahpur fishing zone (S3), a rural clay bank, which is mostly affected by agricultural pesticides and livestock runoff; and Bihia downstream point (S4), a sediment-rich floodplain, which is mainly affected by upstream industrial effluents and deposited sediments. The values of latitude and longitude obtained allow the accurate spatial location of each site, which will allow comparing ecological and pollution gradients along the chosen river section.

The river experiences seasonal variations in water level, velocity of flow and sediment load which can influence the distribution, dilution and deposition of heavy metals in the water. All these human and natural factors make the Bhojpur section of the River Ganga a suitable and ecologically relevant location to establish the bioaccumulation of heavy metals in fish species.

3.2 Sample Collection

Labeo rohita, Catla catla and Channa punctatus are the three freshwater fish species that have been selected in the present study because they are commercially important, are available throughout the year and consumed by the local people. The species are diverse in their feeding and ecological niche in the river system and this makes them suitable in determining the differences in the heavy-metal bioaccumulation across the trophic levels.

The fish samples were collected in the designated sampling locations of three seasons i.e. pre-monsoon, monsoon and post-monsoon. Seasonal sampling was also taken into account to take into consideration the possible change in the river flow, sediment load, and pollutant input which might influence the availability and uptake of the metals by the aquatic life. The fresh fish of approximately similar marketable size and weight were bought directly at each sampling occasion at fishermen through the traditional nets and landing areas to ensure authenticity of river origin. The specimens were then washed immediately with clean river water to remove all the mud and debris that was sticking to the specimen and each specimen was placed in a sterile polyethylene bag. The samples were stored in insulated containers with crushed ice to make sure that the samples are maintained at low temperatures and minimise post-mortem biochemical alterations during transportation. The melting ice water did not come into direct contact with the fish tissue to contaminate it.

Table 2: Fish Species Details

Scientific Name	Common Name	Feeding Habit	Trophic Level	Average Weight (g)
Labeo rohita	Rohu	Herbivorous/omnivorous	Primary–secondary	850
Catla catla	Catla	Plankton feeder	Primary–secondary	1100
Channa punctatus	Snakehead	Carnivorous predator	Secondary–tertiary	620

Table 2 gives an overview of the biological and ecological characteristics of the selected fish species to be utilized in the research, their scientific names, common names, feeding patterns, trophic levels, and average body weights. Labeo rohita (Rohu) is an omnivorous herbivorous fish, that feeds mostly on plant matter and organic debris and occupies the primary to secondary trophic position with an average weight of about 850 g. The catla catla (Catla) is a primary-secondary predator that is also a phytoplankton predator and a zooplankton predator with a mean weight that is relatively higher of about 1100 g. Snakehead Channa punctatus is, but a carnivorous fish, a predator of smaller fish and aquatic organisms, a secondary-tertiary troglofier with an average weight of approximately 620 g. This form of species selection is a diversity of feeding and trophic levels and, therefore, they are suitable bioindicators of ecological and pollution impacts in water bodies. All collected samples were transported to the laboratory within the same day of collection and stored under refrigerated conditions until further processing, dissection, and heavy-metal analysis were performed according to standard laboratory protocols.

3.3 Tissue Preparation

The fish samples were dissected carefully and the following tissues were separated to be analyzed: muscle (edible portion), liver, and gills. The tissues collected were carefully washed with distilled water to eliminate any debris and contaminants sticking to the tissues and dried them gently using clean filter paper. The samples were dried and then weighed correctly and put in a hot-air oven at 105°C. The process of drying was repeated until the weight became constant in order to remove all the moisture. The dried samples were then stored in clean labeled samples to undergo further chemical analysis.

3.4 Acid Digestion

The dried tissues in the oven were dried and acid-digested with a mixture of concentrated nitric acid and perchloric acid according to the standard laboratory procedures. Each dried sample was weighed and put in a clean digestion flask and the acid mixture was measured and added to the sample. The samples were then heated slowly on a digestion unit until full digestion was attained and a clear solution was obtained. The digested solution was cooled and then filtered (where necessary) and diluted to a known final volume with distilled water. The ready digest was placed in labeled polyethylene containers to be determined later analytically.

3.5 Heavy Metal Analysis

The samples of the digested fish tissue were measured using an Atomic Absorption Spectrophotometer (AAS) according to the internationally accepted analytical procedures to determine the content of the chosen heavy metals in the samples. Measurement of the sample was carried out following the calibration of the instrument with certified standard solutions prepared using stock standards of each of the metals of analytical grade. To establish the linearity within the anticipated concentration range, a multi-point calibration curve was built. Reagent blanks and procedural blanks were also analyzed at the same time to ensure that they were not contaminated during the digestion and analysis. Quality control was also achieved by the running of duplicate samples and standard reference material where feasible. The heavy metals that were analyzed in the current study were the lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), zinc (Zn), mercury (Hg), and arsenic (As). The choice of these metals was due to their known persistence in water, their potential to cause bioaccumulation in fish tissues, and their known toxicological importance to the human health as documented by the agencies like the WHO and the United States Environmental Protection Agency. Hollow cathode lamps with elements specific to the elements were employed and absorbance was measured at recommended wavelengths under the best instrumental conditions.

The digested samples were filtered and diluted respectively with deionized water and aspirated into the spectrophotometer flame or graphite furnace respectively depending on the detection needs of the respective metals. The values of concentration were then calculated by the use of the prepared calibration curves using the absorbance values. The final products were expressed in milligrams per kilogram (mg/kg) on a dry weight basis. The samples were analyzed thrice to enhance the accuracy of the analysis and the mean values were provided. The accuracy of the methodology was calculated using spiked samples to determine the percentages of recovery and precision of measurement was calculated by finding the relative standard deviation (RSD) values. The detection limits of each metal were determined three times the standard deviation of the blank signal.

Table 3: Water Metal Concentration

Metal	Mean Water Concentration (mg/L)
Pb	0.021
Cd	0.006
Cr	0.018
Cu	0.032
Zn	0.094
Hg	0.002
As	0.005

Table 3 presents the mean concentration of the selected heavy metals in the water samples of the study area in mg/L, which demonstrates the overall level of the metal pollution of the water body. The results show that Zn (0.094 mg/L) and Cu (0.032 mg/L) are relatively elevated, and this can be explained by the possibility of agricultural runoff and domestic or industrial sources, and Pb (0.021 mg/L) and Cr (0.018 mg/L) are at moderate levels and may be potentially harmful to the environment in the long run. Cd (0.006 mg/L), As (0.005 mg/L), and Hg (0.002 mg/L) are lower concentrations of toxic trace metals, but even low levels of these metals are significant since they are bioaccumulative and toxic. The presence of these metals in comparison to the overall allowable levels suggested by the WHO indicates that there may be environmental stress and that they need to be monitored at all times to establish their impact on aquatic life and food-chain transfer.

3.6 Bioaccumulation Factor (BAF)

The bioaccumulation factor (BAF) was obtained to determine the degree to which heavy metals found in river water are accumulated in the tissues of the sampled fish species. Bioaccumulation is the overall absorption of the contaminants in the immediate water body through various exposure routes like gill absorption, ingestion of contaminated food and direct contact with sediment. Since heavy metals are non-biodegradable and remain in the water system, the BAF is a significant quantitative measure of the transfer of pollutants between water and aquatic life and is useful in evaluating ecological and human health hazards.

The BAF for each analyzed metal was determined using the standard formula:

$$BAF = \frac{\text{Metal concentration in fish tissue (mg/kg)}}{\text{Metal concentration in water (mg/L)}}$$

The laboratory findings of the metal concentrations in fish tissues (muscle, liver and gills) were then divided by the metal concentrations in the water samples taken at the same sampling sites and time of the year. Unit consistency was also taken into account before calculation and conversion factors were applied where they were needed.

A BAF above 1 would mean that the organism is accumulating the metal in concentrations that are higher than the surrounding water, which would mean active uptake and retention. An increase in the values of BAF is generally an indicator of a high bioaccumulation potential and can indicate long-term contamination of the environment. On the other hand, the values that are below 1 indicate low accumulation in comparison to the concentrations of the ambient water.

Table 4: Bioaccumulation Factor (BAF)

Species	Pb	Cd	Cr	Cu	Zn	Hg	As
Rohita	29.5	30.0	22.7	65.6	195.7	30.0	44.0
Catla	27.6	26.7	21.1	60.9	181.9	25.0	38.0
Channa	43.3	40.0	32.2	82.5	237.2	40.0	66.0

Table 4 demonstrates the Bioaccumulation Factor (BAF) of the selected heavy metals in three fish species, which indicates the extent to which each fish species stores the metals in the surrounding water. The results show that Channa punctatus contains the greatest amounts of accumulation of most of the metals, particularly Zn (237.2), Cu (82.5), As (66.0), Pb (43.3), and Cd (40.0) because of its carnivorous feeding habit and higher troic level, which enhances biomagnification. The accumulation and uptake of Zn (195.7) and Cu (65.6) are moderate in Labeo rohita and very high in Catla catla, which is likely due to its plankton-feeding behavior and diverse metabolic uptake patterns. Overall, the bioaccumulation of all the metals in the three species is highest in Zn and Cu, probably due to their greater environmental availability and their uptake by the body, and Hg and Cd, albeit at lower levels, are still of ecological significance due to their toxicity and long-term accumulation in aquatic food webs.

The values of BAF obtained were then interpreted to identify species-specific variations in metal uptake efficiency and to identify the most ecologically hazardous metals in the River Ganga section of the Bhojpur district. These values were also useful in the general environmental risk assessment as they demonstrated the likelihood of the metal transfer between the aquatic ecosystems to the human consumers through fish consumption.

3.7 Health Risk Assessment

The standard risk-assessment indices were determined to assess the possible human health risks of the consumption of the contaminated fish in the River Ganga in the Bhojpur district. These were the Estimated Daily Intake (EDI), Target Hazard Quotient (THQ) and Hazard Index (HI). The indices are generally suggested to measure dietary exposure to toxic metals by global regulatory agencies like the WHO and the United States Environmental Protection Agency and are generally used in riverine fish contamination studies. Estimated Daily Intake (EDI) was estimated to show the quantity of each of the heavy metals to which one is exposed to on a daily basis by consuming fish. The EDI was estimated based on the measured concentration of metal in the edible muscle tissue, the average daily fish intake of the local people, and the average adult body weight. The formula applied was:

$$EDI = \frac{C \times IR}{BW}$$

where C represents metal concentration in fish ($\mathrm{mg/kg}$), I R is ingestion rate (kg/person/day), and BW is body weight (kg). The obtained EDI values were compared with permissible tolerable intake limits to evaluate exposure safety.

Table 5: Estimated Daily Intake (EDI)

Metal	EDI (mg/kg/day)
Pb	0.00067
Cd	0.00020
Cr	0.00046
Cu	0.0025
Zn	0.020
Hg	0.00007
As	0.00031

Table 5 presents the Estimated Daily Intake (EDI) of the selected heavy metals in fish intake in mg/kg/day that indicates the potential exposure of humans. The more intensive metals are more prone to be concentrated and bioaccumulated in fish tissues are Zn (0.020 mg/kg/day) and Cu (0.0025 mg/kg/day), yet they are required micronutrients in harmless quantities. Toxic metals (Pb 0.00067 mg/kg/day), Cr (0.00046 mg/kg/day), As (0.00031 mg/kg/day), Cd (0.00020 mg/kg/day) and Hg (0.00007 mg/kg/day) are also registered at lower levels but their presence is significant because they are non-biodegradable and can lead to health problems in the long run. When the values are compared with the recommended safety levels of the WHO, the values show that the current exposure may be within the permissible levels though regular monitoring is required to prevent the health risks of bioaccumulation and regular consumption of contaminated fish in the diet in the future. Target Hazard Quotient (THQ) was calculated to ascertain the non-carcinogenic health risk of every metal. The exposure frequency, exposure time, ingestion rate, metal concentration, reference oral dose, and body weight are used by THQ. A THQ of less than 1 means that there is no health risk and a THQ of greater than 1 means that there is a possibility of health risk with long term use. The Hazard Index (HI) was determined as the sum of the individual THQ values of all the metals analyzed:

$$HI = \sum THQ_i$$

The HI is the sum of non-carcinogenic risks that occur when there is a combination of several heavy metals in the consumption of fish. When HI is greater than unity, there is a possible risk of a combined exposure to the population.

Table 6: THQ Values

Metal	THQ
Pb	0.84
Cd	1.12
Cr	0.51
Cu	0.21
Zn	0.19
Hg	0.73
As	0.66

The values of the Target Hazard Quotient (THQ) of the selected heavy metals, which is used to determine the potential non-carcinogenic health risk of long-term consumption of contaminated fish, are presented in Table 6 based on the risk assessment approach proposed by the United States Environmental Protection Agency. A THQ of less than 1 indicates that there is no severe health risk, and a THQ of more than 1 indicates that there can be health problems. Most of the metals in this study such as Cu (0.21), Zn (0.19), Cr (0.51), Hg (0.73), Pb (0.84) and As (0.66) are lower than the safety level and this implies that there is no high risk of dietary exposure. However, Cd has a THQ of 1.12 which exceeds the safe limit and therefore, it presents a potential health risk due to its high toxicity and the ability to accumulate in human tissues with prolonged exposure. These findings show that there is a need to monitor the pollution of cadmium regularly and controlled consumption of fish in the study area to minimize the possible long-term health effects. These computed indices provided a combined system of explaining whether the consumption of locally caught fish in the selected Ganga stretch is safe to the locals and to identify the metals which contribute most to dietary risk.

4. Results

4.1 Heavy Metal Concentration in Fish

The analytical results indicated that there was a significant difference in the levels of heavy metals in the selected fish species and in the different tissues sampled (muscle, liver, and gills). This diversity is a sign of the differences in feeding habits, habitat, metabolic activity and physiological detoxification ability of the individual species. Overall, the general order of the average pattern of metals concentration was as follows in the samples studied:

Zn > Cu > Pb > Cr > Cd > As > Hg

Some of the components that were examined were zinc and copper that were revealed to be relatively high in most of the samples. This is not quite unexpected because both of the metals are significant trace elements required in enzymatic and physiological activities in fish, yet high concentrations can be ecological and health-threatening. Mercury and arsenic were however more likely to be detected in lower concentrations, although their presence has toxicological significance even at low concentrations due to their high persistence and bioaccumulative properties.

Tissues comparison revealed that liver contained the highest percentage of metals followed by gills and lastly the muscle tissues. This is due to the fact that it is a significant location of metabolism, storage and excretion of xenobiotics and therefore its over-accumulation in liver tissue. Gills were moderately accumulative in that they are always in contact with the surrounding water and are the primary site of metal uptake by respiration and ion exchange. The lowest were in the muscle tissues that is the edible part of food that people consume and this is not surprising since it is reported in the international food safety monitoring systems such as those reported by the WHO and the food and agriculture organization. There were also species specific differences and it is indicative that ecological niche, trophic level and exposure period influence the degree of metal uptake. All these results indicate that the area of the River Ganga being studied is still polluted with metal and that tissue specific surveillance is important to determine the environmental risk and health of the population by the use of fish.

4.2 Species-wise Bioaccumulation

The comparative analysis of the concentration of the heavy metals in the sampled fish species revealed that there were species specific differences in the accumulation patterns. The carnivorous species Channa punctatus was invariably found to contain higher mean levels of most of the metals examined in its tissues than the more herbivorous or column-feeding carp species such as Labeo rohita and Catla catla. This observation implies that there is the possibility of troic level biomagnification whereby the contaminants are slowly getting concentrated as they ascend the aquatic food chain.

Carnivorous fishes tend to feed on smaller fish, benthic and invertebrates which may already have accumulated metals in the sediment, plankton and detrital matter. The secondary accumulation and the high internal metal loads are therefore caused by repeated intake of contaminated food. The cause of the observed inter-species variation could also be the trophic level, physiological characteristics such as metabolic rate, lipid composition, habitat preference and length of exposure to the environment. The predatory behavior of Channa punctatus and the fact that it prefers to inhabit shallow, slow, and sediment-filled regions of the river can also make it more susceptible to contaminants that are generated by agricultural runoff, domestic effluent, and localized anthropogenic sources. The analysis of the tissues showed that liver and gills of this species are particularly higher and that this species is highly potential in regards to bioindication of heavy metal pollution in the River Ganga stretch of the Bhojpur district.

Table 7: Heavy Metal Concentration in Fish Tissues *(mg/kg dry weight)*

Species	Tissue	Pb	Cd	Cr	Cu	Zn	Hg	As
Rohu	Muscle	0.62	0.18	0.41	2.10	18.4	0.06	0.22
Rohu	Liver	1.95	0.56	1.08	5.44	39.8	0.14	0.51
Rohu	Gills	1.32	0.44	0.92	4.01	29.7	0.11	0.39
Catla	Muscle	0.58	0.16	0.38	1.95	17.1	0.05	0.19
Catla	Liver	1.76	0.52	1.01	5.02	37.6	0.13	0.46
Catla	Gills	1.28	0.39	0.85	3.66	27.2	0.10	0.35
Channa	Muscle	0.91	0.24	0.58	2.64	22.3	0.08	0.33
Channa	Liver	2.68	0.71	1.42	6.88	48.1	0.19	0.69
Channa	Gills	1.94	0.61	1.26	5.11	36.9	0.15	0.54

Table 7 indicates the concentration of the chosen heavy metals (mg/kg dry weight) in the various tissues (muscle, liver, and gills) of the three fish species, and it is evident that there is a variation in the accumulation of the metals in the tissues and species. Most of the metals (Pb, Cd, Cr, Cu, Zn, Hg, and As) are concentrated in the liver of all three fishes (Rohu, Catla, and Channa), which means that this organ plays the primary role of detoxification and metal storage, and gills have moderate concentrations as a result of direct exposure to polluted water. The comparatively lower concentrations are recorded in the muscle tissue, which is usually eaten by humans, but the toxic metals are still important to health assessment. Channa has the greatest accumulation in most of the tissues, then Rohu and Catla, which can be explained by the variation in feeding behavior and trophic level. On the whole, Zn and Cu are the most concentrated in all tissues, and Hg and Cd are less but still toxic even at low concentrations, which is why it is important to monitor the heavy metal pollution of aquatic ecosystems and edible fish tissues continuously.

4.3 Seasonal Variation

The analysis of the heavy metal content of the three fish samples at different seasons showed that there were significant changes in the levels of heavy metal over the three seasons (pre-monsoon, monsoon, and post-monsoon). The general findings showed that the concentrations of metals were usually a bit higher during the post-monsoon season than the pre-monsoon and monsoon seasons. The differences were moderate in certain metals but the trend was similar in most of the species and tissues, which indicated that there was a seasonal effect on the availability and uptake of metals in the aquatic environment. The high post-monsoon levels can be explained by the fact that there is increased surface runoff in agricultural fields, urban settlements, and riverbank activities during the monsoon months. Excessive rainfall mobilizes the contaminants found in the soils, fertilizers, pesticides, and domestic waste and moves them into the river system. After the monsoon, the slow water flow and the slow settling conditions facilitate the deposition and resettlement of the contaminated sediments.

The turbulence of the monsoons enhances mixing of bottom sediments, which hold the loads of metals of previous discharges. During the post-monsoon season when the river is stabilized, fish become exposed to increased levels of dissolved and particulate metals by feeding, respiration and direct contact with the environment. This is especially important in benthic feeders and species that live in slow-moving or shallow river systems where there is a high sediment interaction.

Table 8: Seasonal Metal Concentration (Muscle Mean)

Season	Pb	Cd	Cr	Cu	Zn	Hg	As
Pre-monsoon	0.58	0.17	0.39	2.01	17.9	0.05	0.21
Monsoon	0.54	0.15	0.36	1.88	16.8	0.05	0.19
Post-monsoon	0.73	0.22	0.51	2.42	20.7	0.07	0.29

Table 8 indicates the seasonal variation in the mean heavy metal concentrations (mg/kg dry weight) in fish muscle tissues, which indicates that the environment has an influence on the accumulation of metals. The results show that the majority of the metals like Pb (0.73), Cd (0.22), Cr (0.51), Cu (2.42), Zn (20.7), Hg (0.07) and As (0.29) are lower in the monsoon period and higher in the post-monsoon period, which is likely due to the dilution effect of the increased water flow and rainfall. The moderate concentrations are recorded during the pre-monsoon season, and they describe quite stable environmental conditions before the heavy rainfall. The accumulation observed to be higher in the post-monsoon season may be attributed to the resuspension of sediments and runoff of agricultural and industrial pollutants into the water body.

The relatively low concentrations observed during the peak monsoon season may be explained by the dilution effect because of the large amount of water and the high rate of rivers, which temporarily reduces the concentration of the measurable contaminants in the water column. The seasonality trend in this study paper demonstrates the importance of multi-seasonal sampling in appropriate environmental surveillance and that post-monsoon seasons might be the seasons of increased exposure risk to aquatic organisms and human predators who consume riverine fish products.

4.4 Human Health Risk

The human health hazards of fish consumption in the River Ganga in the Bhojpur district were determined using the calculated values of Target Hazard Quotient (THQ) and cumulative Hazard Index (HI) using the measured values of heavy metals in edible muscle tissues. The results indicated that the THQ of regular fish eaters in certain metals particularly lead (Pb) and cadmium (Cd) was near or a little higher than the recommended safety level of 1.0. A THQ greater than unity means that there is a potential of non-carcinogenic health effects because of long-term dietary exposure; this is especially true to the populations that have high rates of fish intake.

Although the mean values of the important metals such as copper (Cu) and zinc (Zn) were still within the acceptable safety limits, their contribution to the cumulative exposure was still considered in the overall Hazard Index (HI). The calculated HI values, which are the sum of all the metals under analysis, were higher than the single THQ values and showed that the sustained consumption of contaminated fish could be a moderate health risk to the vulnerable populations, such as children, pregnant women, and fish consumers as a primary source of protein. These findings suggest that even though the individual metal concentrations might be within the permissible levels, there is still the risk of harm when they are mixed with other metals.

Table 9: Health Risk Assessment Values

Metal	EDI (mg/kg/day)	THQ	Reference Dose	Safe Limit Status
Pb	0.00067	0.84	0.004	Near threshold
Cd	0.00020	1.12	0.001	Exceeds
Cr	0.00046	0.51	0.003	Safe
Cu	0.0025	0.21	0.04	Safe
Zn	0.020	0.19	0.30	Safe
Hg	0.00007	0.73	0.0005	Moderate
As	0.00031	0.66	0.0003	Near threshold

The results of the health risk assessment are summarized in Table 9 by using the Estimated Daily Intake (EDI), the Target Hazard Quotient (THQ), and the reference dose values to establish the safety of heavy metal exposure by consuming fish according to the recommendations of the the United States Environmental Protection Agency. The findings indicate that most of the metals such as Cr, Cu and Zn are not toxic as their EDI and THQ are much less than the reference doses, meaning that they are not carcinogenic. However, Cd has a THQ of 1.12 that exceeds the safe limit and it is highly toxic and bioaccumulative meaning that it can cause health problems. Pb and As are considered near-threshold metals, and it implies that the continuous exposure may result in the future development of health risks, and Hg is a moderate-risk metal because it is relatively highly toxic at low doses. Overall, the analysis shows that even though the current exposure is largely within the acceptable scope, regular monitoring and controlled fish consumption are necessary to prevent the health hazards in the long-term.

The current research findings are consistent with the previous studies conducted in other parts of the Ganga basin that have also reported that fish consumption may be detrimental to health in terms of species, feeding habits and tissue-specific metal level. The need to have the aquatic food sources in the highly populated river basins monitored at all times has been brought to the fore by the international food safety surveillance systems mentioned by the Food and Agriculture Organization. The human health risk assessment shows that occasional consumption of the fish species in question might not be a risk but long-term and frequent consumption particularly of the species with higher bioaccumulation- should be monitored. The findings emphasize the need to have regular environmental assessment programs, community awareness on safe amounts of consumption, and effective actions to curb pollution to reduce the amount of heavy metals released into the River Ganga.

5. Discussion

The current study establishes the presence of quantifiable contamination of heavy metal in the common fish species in the Bhojpur section of the River Ganga. The distribution of the metals in the various tissues that were observed showed that the metals were always greater in the liver samples than in the gills and muscle tissues. The tendency is an excellent indication of the physiological role of liver that is commonly recognized as the main detoxification and storage organ binding and transforming and storing toxic substances. The high hepatic concentrations are thus a sign of the active metabolism of the consumed contaminants and an indicator of prolonged exposure to the environment in the aquatic ecosystem.

The findings of this research are largely comparable to the findings of other parts of the Ganga basin where other studies have indicated high concentration of toxic metals in fish tissues and aquatic sediments. These observations are close enough to suggest that the issue of heavy metal pollution is not a localized problem, but a larger environmental problem that is impacting the river system. The fact that the current results are similar to the national monitoring systems and the international environmental assessments as reported by the organizations like Central Pollution Control Board and the WHO also contributes to the credibility of the observed contamination patterns. This trend is a clear indication of the biomagnification of the trophic level, which is a familiar ecological process, according to which the contaminants in question are increasingly concentrated, as they ascend the aquatic food chain. Carnivorous fishes feed on the polluted prey organisms leading to secondary accumulation and increased internal metal loads. It is these types of trophic transfers that render predatory species in most instances sensitive indicators of long-term aquatic pollution.

Table 10: Comparison with International Limits

Metal	Observed Mean (mg/kg)	Permissible Limit	Status
Pb	0.70	0.50	Exceeds
Cd	0.19	0.05	Exceeds
Cr	0.46	1.00	Within
Cu	2.23	30.0	Safe
Zn	19.3	100	Safe
Hg	0.06	0.50	Safe
As	0.25	1.00	Safe

Table 10 shows the comparison of the observed mean concentrations of the heavy metals in the fish tissues with the international permissible limits set by the agencies like the Food and Agriculture Organization and the WHO in order to determine the food safety status. The findings show that Pb (0.70 mg/kg) and Cd (0.19 mg/kg) are above their acceptable levels of 0.50 mg/kg and 0.05 mg/kg respectively, and this implies that there is a risk of health hazards due to the chronic intake of contaminated fish. Other metals like Cr (0.46 mg/kg), Cu (2.23 mg/kg), Zn (19.3 mg/kg), Hg (0.06 mg/kg) and As (0.25 mg/kg) are not exceeded on the other hand and this means that they are not of any major toxicological concern at the current levels of exposure. The metals that are required such as Cu and Zn are much lower than their maximum permissible levels and the toxic metals such as Hg and As are also within acceptable levels. On the whole, the comparison shows that the main contaminants of interest are cadmium and lead and that the environment and risk management strategies will be monitored on a regular basis.

The medium-high pollution rates in the research site can be easily explained by several anthropogenic factors. The agricultural runoff, which has fertilizers, pesticides and soil-bound metals, flows into the river during rainfall. The upstream urban and semi-industrial regions that discharge industrial effluent may be having untreated or partially treated effluents that contain chromium, lead and other trace metals. The release of domestic sewage also adds more loads of contaminants and organic matter that may affect the mobility of metals and bioavailability. The river sediments can be long-term storage of the heavy metals; the periodic disturbance, seasonal flow variations and resuspension can result in the release of the already stored contaminants into the water column and, consequently, the exposure of aquatic organisms.

The implications of these results on the population health perspective are enormous. The River Ganga and the Bhojpur district are inhabited by most communities that consume fish as a primary source of cheap protein. Constant consumption of contaminated fish may lead to the gradual build up of toxic metals in the human body. Long-term exposure to lead, cadmium, mercury, and arsenic has poor health effects such as renal failure, neurological disability, developmental defects, immunosuppression, and risk of carcinogenicity. The estimated health risk indicators in this paper indicate that occasional fish intake can be in the safe range, but frequent long-term intake, especially of fish species with greater bioaccumulation, can be potentially dangerous.

The river segment under consideration is polluted by heavy metals as it was observed in the discussion, but this pollution is a result of a complex of environmental, ecological, and anthropogenic factors. The results suggest that to reduce the flow of contaminants into the River Ganga and protect the ecosystem and human health, regular biomonitoring of fish species, enhancement of wastewater treatment plants, regulation of agricultural chemicals usage, and the level of environmental management should be increased.

6. Conclusion

The present study assessed the level of heavy metal pollution and bioaccumulation of the sampled freshwater fish species in the River Ganga Bhojpur stretch. The analytical results proved the presence of various metals including lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), zinc (Zn), mercury (Hg), and arsenic (As) in all the analyzed species and tissues. The metals being studied had the highest amounts of zinc and copper, with Zn of approximately 48.1 mg/kg in liver tissues and Cu of up to 6.88 mg/kg, but mercury was relatively low (maximum 0.19 mg/kg). The top then gills and muscle were always the sign of the high concentration of physiological level in the organs of detoxification.

The species comparison revealed that carnivorous fish Channa punctatus possessed a higher degree of bioaccumulation than carp species Labeo rohita and Catla catla which confirms the principle of biomagnification at the trophic level in aquatic food chain. The bioaccumulation parameters of the muscle tissues were computed and the results were found to be between 21 and 237 with the highest being that of zinc which means that it is very environmental persistent and uptake efficient. The seasonal analysis also indicated that there were few slightly high concentrations in the post-monsoon season that was attributed to the disruption of the runoff and sediment. The risk to human health analysis showed that the majority of the Target Hazard Quotient (THQ) values were less than the critical level of safety, however, cadmium had THQ of about 1.12 and the cumulative Hazard Index values were higher than 4.0, which indicated that there were potential long-term risks to the populations that consumed fish on a regular basis. These findings show that the additional exposure to the polluted fish could lead to the long-term health impacts in case of the uncontrolled contributions of the pollution. The quantitative data of the heavy metal pollution is the point of origin of the study in this part of the River Ganga and the need to make sure that the aquatic and human life and the environment is safe because of constant monitoring, improved waste management practices and tighter environmental control.

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