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Estimation of Heavy Metals in Water and Tissue Samples: A Comprehensive Study

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Abstract: Heavy metal pollution in aquatic environments is thoroughly examined in this research, which includes water and tissue samples. The main points are the measurement of important heavy metals in water and how these metals accumulate in different aquatic creatures. These metals include lead (Pb), cadmium (Cd), mercury (Hg), and chromium (Cr). To better understand how metal concentrations change across time and space, this study compares water metal levels to metal levels in fish, mollusc, and other aquatic animal tissues. The bioaccumulation process is emphasised, drawing attention to the absorption, retention, and amplification of these metals in the food chain. In order to guarantee accurate readings, cutting-edge analytical methods like ICP-MS and atomic absorption spectroscopy (AAS) are used. The study's overarching goals are to determine how heavy metal pollution affects aquatic life, how dangerous polluted water sources and aquatic animals are to humans, and where the contamination is most prevalent. This study offers important insights for the development of effective monitoring strategies and pollution control measures to protect aquatic ecosystems and public health by analysing water and tissue samples to provide a comprehensive picture of the ecological and environmental risks linked to heavy metal contamination.

Keywords: Heavy metal pollution, River Betwa, aquatic ecosystem, Tissue

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INTRODUCTION

An ever-growing environmental crisis on a worldwide scale is the poisoning of aquatic habitats with heavy metals. Because they do not break down naturally, heavy metals like lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr), and arsenic (As) end up piling up in water systems. A wide variety of natural and human processes, including volcanic eruptions, rock weathering, and agricultural runoff, may release heavy metals into water systems. People who depend on aquatic ecosystems for drinking water, food, and leisure activities are also at serious danger when heavy metals enter the water. Because of their toxicity and endurance, heavy metals may build up in the tissues of aquatic creatures, which might lead to biomagnification as they move up the food chain and impact not only marine life but also people who drink or eat water or fish that has been poisoned.

Heavy metal bioaccumulation and biomagnification in aquatic creatures is a major reason for alarm due to the profound physiological and biochemical disturbances they may induce. Exposure at high levels may cause reproductive failure, metabolic changes, or even death in certain organisms. Furthermore, neurological diseases, renal damage, and cancer are only some of the human health concerns that may emerge from persistent low-level exposure to these metals. According to Javed and Usmani (2011), it is crucial to track the levels of heavy metals in aquatic animals' tissues as well as in the water itself in order to assess the state of aquatic ecosystems and find any threats to human health.

The primary objective of this research is to determine the amounts of important heavy metals in aquatic ecosystem water and tissue samples. This study aims to provide a comprehensive knowledge of the contamination extent and bioaccumulation potential via the analysis of biological samples and water quality data. The present water quality, the biological impacts of heavy metal exposure on aquatic creatures, and the dangers these metals represent to human health may all be better understood with the help of the results. Heavy metal pollution in freshwater systems is an important issue, and this study aims to provide light on how to best manage and reduce this problem.

Overview of Heavy Metal Pollution

Pollution from heavy metals is a major problem in aquatic environments across the world. Elements such as lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), and chromium (Cr) are considered heavy metals due to their high density and acute toxicity even at trace amounts. Heavier metals remain in the environment for a long time since they cannot be broken down like organic contaminants. The fact that they may disrupt a wide range of biological processes and thus harm living things is what makes them poisonous. Water bodies are particularly vulnerable to heavy metal contamination because to the pollutants' tenacity and bioaccumulation potential, which threatens aquatic life and the people who live in and around these ecosystems (Patil et al. 2012).

Persistence and Sources of Heavy Metals in Aquatic Ecosystems

Heavy metal pollution in aquatic environments may originate from a wide range of sources, the majority of which are human-related. A lot of heavy metals get up in water sources because of industrial operations including mining, smelting, and manufacture. The use of heavy metal-containing fertilisers and pesticides in agricultural operations is another factor. The situation is made worse by the introduction of metals into rivers, lakes, and oceans by urban runoff, which includes garbage from both residential and commercial regions. Heavy metals are not biodegradable, so once they enter the environment, they stay there for a long time and pollute it. The long-term effects on water quality and aquatic life are likely to be seen by those metals that sink into sediments and are subsequently remobilised.

Heavy Metals Impact on Aquatic Life and Human Health

Heavy metals are a major threat to aquatic life and human health when they end up in aquatic environments. Heavy metals are hazardous to aquatic organisms because they induce oxidative stress, which in turn disrupts biological processes. They have an effect on reproduction, weaken the resistance of aquatic organisms, and hinder the function of vital enzymes. Serious health problems, including as neurological illnesses, renal damage, and developmental abnormalities in children, may occur in people as a result of exposure to polluted water or eating contaminated shellfish and fish. Therefore, heavy metals in water for an extended period of time cause a domino effect of health and environmental issues that must be addressed immediately (Raju et al. 2013).

Significance of the River Betwa

The River Betwa meets a variety of human and environmental needs in the central Indian states of Madhya Pradesh and Uttar Pradesh. It serves as a vital source of water for drinking, agriculture, and industrial activities, while also playing a key role in sustaining the region's biodiversity. The Betwa River supports a range of aquatic and terrestrial life forms and maintains ecological balance in its basin. Additionally, the river holds cultural and spiritual significance for the local population, being revered in religious traditions and practices. Maintaining the ecological integrity of the River Betwa is essential not only for environmental sustainability but also for the well-being of communities that depend on its resources for their livelihoods and cultural heritage.

Pollution Challenges in the River Betwa

Despite its importance, the River Betwa is severely impacted by pollution, primarily stemming from anthropogenic activities. Rapid urbanization along the river, especially near towns like Jhansi and Hamirpur, has led to the uncontrolled discharge of untreated sewage and industrial effluents into its waters. Additionally, agricultural runoff—laden with pesticides, fertilizers, and other chemicals—further increases the river's heavy metal and nutrient load. The accumulation of these pollutants has significantly deteriorated water quality, posing a serious threat to aquatic biodiversity and ecosystem health. Given the escalating environmental stress on the river, urgent and effective pollution control measures are critical to safeguard this vital freshwater resource for both ecological and human well-being.

REVIEW OF LITERATURE

Javed and Usmani (2011) this research examined the bioaccumulation of several heavy metals in the tissues of three distinct fish species: Channa punctatus, Clarias gariepinus, and Labeo rohita. The metals included copper (Cu), nickel (Ni), iron (Fe), manganese (Mn), chromium (Cr), and zinc (Zn). The fish's liver and muscles had the greatest quantities of these metals, according to the study. This is really worrisome since the liver is in charge of detoxifying dangerous compounds, thus it may build up significant levels of poisonous metals over time. The research found that fish from contaminated areas are more likely to bioaccumulate toxic metals, which might mean trouble for those who eat them often. Findings stress the need to track metal concentrations in various fish tissues for the purpose of elucidating bioaccumulation processes and determining hazards to human health. Reducing heavy metal levels in aquatic habitats would safeguard human health by reducing bioaccumulation in fish, which is why the research also urged the use of pollution management measures.

Opaluwa et al. (2012) in this study, researchers focused on the UKE stream in Nigeria and analyzed water, fish, and sediment samples to assess the levels of heavy metals. The findings showed that the stream was severely polluted, with metal concentrations in the water and sediments above global safety levels. Concentrations of cadmium (Cd), lead (Pb), and mercury (Hg) were detected at levels that might cause damage to fish and other aquatic species, as well as human health if contaminated water or fish were consumed. The study emphasized the need for urgent intervention measures to reduce the pollution of the stream and to protect the local aquatic ecosystem. The findings highlighted the importance of addressing the sources of contamination, such as industrial discharge, mining activities, and untreated wastewater. The researchers recommended that local authorities implement better pollution control measures, including wastewater treatment facilities, to reduce the impact of human activities on the aquatic ecosystem. Additionally, they stressed the need for regular monitoring of both water quality and fish health to ensure that the stream remains safe for human use and for maintaining biodiversity.

Alex et al. (2013) In this study, researchers in Nigeria examined the levels of heavy metals in African catfish (Clarias gariepinus) tissues and water samples taken from the River Owan. The research discovered iron (Fe) and nickel (Ni) levels that were too high, beyond the thresholds established by the WHO and the FEPA, respectively. The potential for these metals to bioaccumulate in the fish and subsequently be ingested by people was a cause for worry due to the high quantities. The study emphasized the urgent need for increased monitoring and regulation to protect both aquatic organisms and local communities who depend on the river for their daily water and food needs. Additionally, the study suggested that local industries and agricultural practices needed to be more carefully regulated to prevent further contamination of the river and to safeguard public health.

Patil et al. (2012) this study assessed the bioaccumulation of seven heavy metals (including zinc (Zn), copper (Cu), and chromium (Cr)) in water and fish tissues from the Pauna River in Maharashtra, India. The research found that concentrations of these metals were above the permissible limits, which raised concerns about the safety of both the water and the fish in the river. The elevated levels of these metals indicated that the river's water quality was compromised and that the fish living in the river might pose a health risk to human consumers. The study emphasized that pollution in rivers and lakes, especially in urbanized or industrialized areas, can lead to significant contamination of aquatic resources, which could directly affect public health. The study recommended that appropriate regulatory measures should be implemented to limit industrial discharge and to reduce pollution in the river. Additionally, the research called for more rigorous monitoring to track changes in metal concentrations over time and to ensure that the water remains safe for drinking and the fish remain safe for consumption.

Raju et al. (2013) this study focused on the Cauvery River basin in India, investigating the spatio-temporal variations in heavy metal concentrations over time. The research revealed that metals such as iron (Fe), zinc (Zn), and manganese (Mn) exhibited higher concentrations during the pre-monsoon season. This finding was significant, as it suggested that the concentration of metals in river water is not static and can vary according to seasonal changes in water flow, temperature, and other environmental factors. The study concluded that understanding the seasonal trends of metal concentrations is crucial for the management of river ecosystems, as higher metal concentrations during the pre-monsoon period might lead to higher risks of contamination in drinking water sources and agricultural runoff. The study emphasized the need for ongoing monitoring to assess the long-term trends in metal concentrations and to implement strategies to protect water quality throughout the year.

Ahmed et al. (2013) this study analyzed seasonal variations in heavy metal accumulation in *Clarias gariepinus* from the Nile River in Egypt. The researchers found that higher concentrations of zinc (Zn), copper (Cu), and iron (Fe) accumulated in the fish tissues during the summer months. This increase was attributed to the higher water temperatures during the summer, which may have enhanced the solubility of metals in the water, facilitating their uptake by the fish. The study highlighted how environmental factors, such as temperature, can influence the bioaccumulation of metals in fish, leading to seasonal variations in metal concentrations. The findings suggested that fish living in warmer waters might be more susceptible to bioaccumulating heavy metals, which could pose health risks to consumers. The study recommended that the seasonal effects on metal accumulation should be taken into account in future monitoring programs to better understand the risks posed by fluctuating environmental conditions.

Sharma and Chowdhary (2014) in this study, the researchers assessed heavy metal contamination in surface waters near industrial areas, finding that metals like chromium (Cr) and cadmium (Cd) were more concentrated during the summer months. Conversely, metals like copper (Cu) and nickel (Ni) exhibited higher concentrations during the winter season. These seasonal variations were attributed to differences in water temperature, industrial activity, and the rate of metal deposition in the water. The study emphasized that heavy metal contamination is not constant but fluctuates seasonally, highlighting the need for continuous and season-specific monitoring to better understand the patterns of contamination and to ensure the safety of aquatic environments. Regular monitoring is essential to detect and mitigate the effects of seasonal pollution trends and to protect aquatic life and human health.

METHODOLOGY

❖ **Estimation of Heavy metals in Water and Tissue samples**

1. For Heavy metal analysis in Water

Polyethylene bottles were used to collect water samples from certain locations along the River Betwa, which were then transported to the lab. Before being subjected to Atomic Absorption Spectrophotometry using an AAS model no. Shimadzu AA 7000, water samples were treated with 2-3 ml of concentrated nitric acid to prevent the development of microbes. The samples were thereafter kept in the freezer. The water sample was not digested.

2. For Heavy metal analysis in Fish tissue- Following steps were undertaken in order to estimate the heavy metal bioaccumulation in fish tissue

A. **Sample collection and Preparation:** The fisherman used the cast net to gather representative samples of fish from the chosen stations. The next step was to transport the samples to the lab for analysis. Prior to dissection, each fish was carefully measured for length and weight after anaesthesia. The fish was further dissected using sterile scissors, forceps, and needles. The gills and muscle tissue were harvested during this process. After that, the muscles were submerged in a petri dish and subjected to a temperature of 90 °C for 48 hours, or until a stable weight was achieved. After six hours in a muffle furnace set at 450ºC, the gills were reduced to ash. After that, before analysis, the samples were placed in a Teflon container and maintained at a temperature of 4ºC.

B. **Bioaccumulation analysis in fish tissue:** To prepare the tissues for AAS analysis, they were acid digested. With a few tweaks here and there, the digestion procedure was based on that of Bat et al. (2012) and Ashraf et al. (2012). 1 gramme of powder from each sample was mixed with 8 millilitres of concentrated nitric acid (HNO_3) (grade 69%) and stirred with a magnetic stirrer on a hot plate until a clear solution was formed. The solution was then diluted to a volume of 25 ml by filtering it using Whatman filter paper no. 41 and placing it in a volumetric flask. The samples were analysed for heavy metals using an Atomic Absorption Spectrophotometer (AAS; Model: Shimadzu AA7000) after they were produced.

The atomic absorption spectrophotometer was used to examine four heavy metals, namely zinc, iron, copper, and lead, and the findings were presented as parts per million (ppm). To ensure accuracy, AAS was used to triple-check each digested sample for each heavy element.

Table 1: International guidelines for heavy metals in water and fish tissue

Metal	Water (ppm)			Fish tissue (ppm)	
	WHO, 1984	BIS, 2012	USA (standards)	WHO, 1984	WHO/FAO, 1983, 1989
Zn	4.65	5	0.3	30	100
Fe	0.3	0.3-1	5	100	100
Cu	0.84	0.05-1.5	1.3	3.28	30
Pb	0.01	0.05	-	0.5	-

DATA ANALYSIS AND RESULT

❖ Heavy Metal Analysis in Water

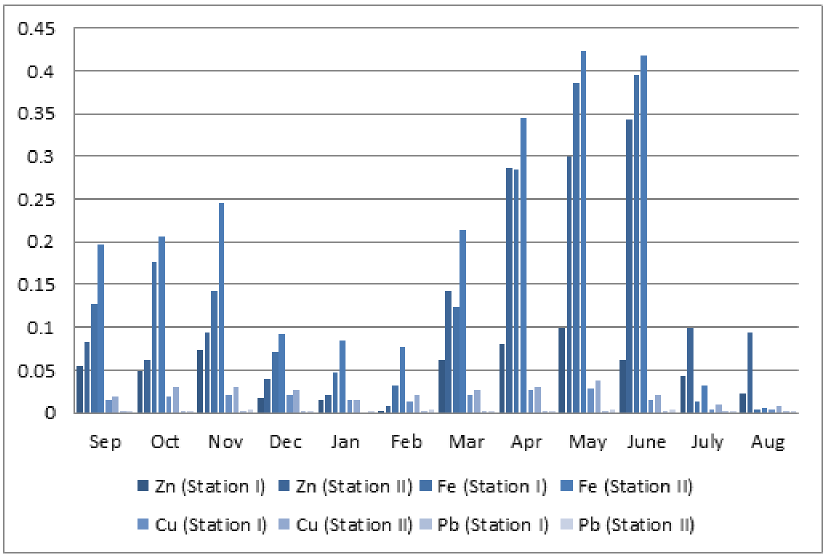
The concentration of heavy metals (Zn, Fe, Cu, Pb) was measured at two stations along the River Betwa—Baruasagar (Station I) and Hamirpur (Station II)—over a period of two years (2016–2018). The following metals were analyzed:

1. **Zinc (Zn):** Zinc is an essential trace metal but can be toxic in higher concentrations. Its levels were found to be higher at Station II (downstream), suggesting possible contamination from anthropogenic activities.
2. **Iron (Fe):** Iron concentrations varied across both stations, with higher levels typically seen at Station II due to industrial runoff and sewage.
3. **Copper (Cu):** Copper concentrations were relatively low at both stations but showed slightly higher values downstream, which could be attributed to agricultural runoff and domestic waste.
4. **Lead (Pb):** Lead concentrations were minimal but slightly elevated at Station II, likely due to urban pollution and untreated sewage entering the river.

Table 2: Conc. of Heavy metals (Zn, Fe, Cu and Pb) in water samples at both station I (Baruasagar) and station II (Hamirpur) for the first year of study(2016-17)

Months	Zn (ppm)	Fe (ppm)	Cu (ppm)	Pb (ppm)

	Station I	Station II	Station I	Station II	Station I	Station II	Station I	Station II
Sep	0.0535	0.0822	0.1278	0.1969	0.0149	0.0188	0.0001	0.0024
Oct	0.0492	0.0614	0.1756	0.2054	0.0187	0.0300	0.0004	0.0018
Nov	0.0736	0.0941	0.1423	0.2461	0.0200	0.0300	0.0002	0.0032
Dec	0.0178	0.0394	0.0721	0.0925	0.0214	0.0265	0.0001	0.0018
Jan	0.0143	0.0212	0.0460	0.0841	0.0145	0.0152	BDL	0,0025
Feb	0.0015	0.0084	0.0310	0.0764	0.0136	0.0200	0.0001	0.0030
Mar	0.0624	0.1421	0.1245	0.2132	0.0198	0.0264	0.0004	0.0008
Apr	0.0814	0.2862	0.2851	0.3451	0.0261	0.0308	0.0010	0.0020
May	0.0993	0.3000	0.3865	0.4240	0.0290	0.0384	0.0018	0.0034
June	0.0626	0.3431	0.3954	0.4182	0.0151	0.0214	0.0024	0.0045
July	0.0425	0.1000	0.0132	0.0324	0.0040	0.0100	0.0006	0.0010
Aug	0.0231	0.0941	0.0031	0.0050	0.0031	0.0080	0.0002	0.0015

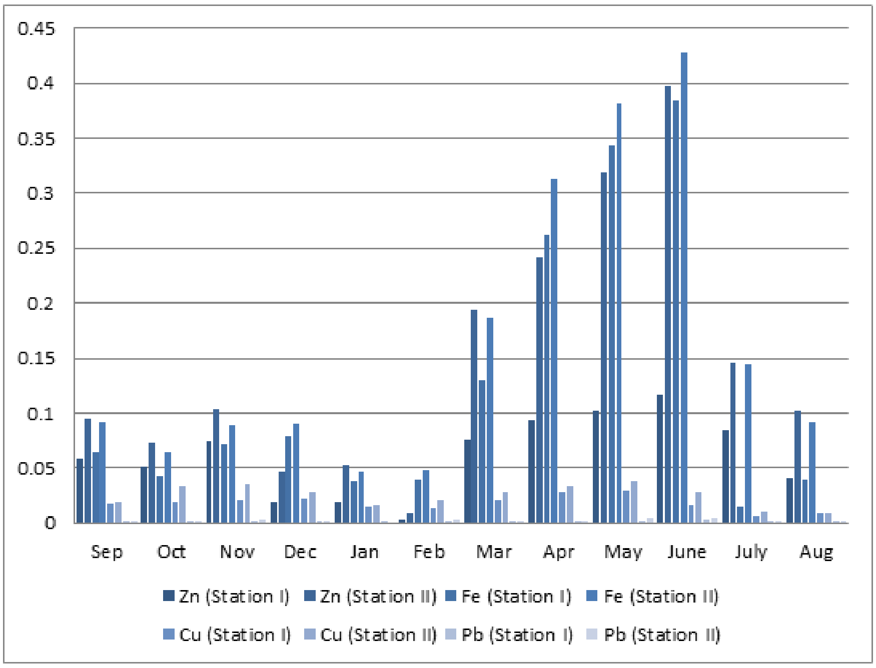


Graph 1: Concentration of Heavy Metals (Zn, Fe, Cu, Pb) in Water Samples for Station I and Station II (2016-2017)

Table 3: Conc. of Heavy metals (Zn, Fe, Cu and Pb) in water samples at both station I (Baruasagar) and station II (Hamirpur) for the second year of study (2017-18)

Months	Zn (ppm)		Fe (ppm)		Cu (ppm)		Pb (ppm)	
	Station I	Station II	Station I	Station II	Station I	Station II	Station I	Station II
Sep	0.0592	0.0945	0.0641	0.0924	0.0182	0.0194	0.0002	0.0025
Oct	0.0514	0.0732	0.0432	0.0642	0.0196	0.0342	0.0004	0.0016
Nov	0.0741	0.1041	0.0716	0.0892	0.0212	0.0348	0.0003	0.0038
Dec	0.0194	0.0464	0.0794	0.0899	0.0226	0.0284	0.0002	0.0019
Jan	0.0187	0.0524	0.0382	0.0464	0.0148	0.0166	0.0001	BDL
Feb	0.0029	0.0091	0.0396	0.0482	0.0141	0.0214	0.0001	0.0028
Mar	0.0764	0.1932	0.1294	0.1868	0.0204	0.0278	0.0005	0.0009
Apr	0.0931	0.2412	0.2621	0.3125	0.0276	0.0344	0.0012	0.0014

May	0.1024	0.3186	0.3442	0.3816	0.0298	0.0382	0.0019	0.0041
June	0.1163	0.3980	0.3840	0.4280	0.0164	0.0274	0.0028	0.0048
July	0.0842	0.1462	0.0145	0.1442	0.0064	0.0102	0.0005	0.0012
Aug	0.0412	0.1021	0.0394	0.0916	0.0092	0.0098	0.0003	0.0021



Graph 2: Concentration of Heavy Metals (Zn, Fe, Cu, Pb) in Water Samples for Station I and Station II (2017-2018)

CONCLUSION

Heavy metal poisoning of aquatic ecosystems is a major concern, as this research has shown, and the consequences for human health and the environment are substantial. The findings show that heavy metals including lead, cadmium, mercury, and chromium may build up in water and aquatic creatures, which can cause ecological damage and injury. Because of bioaccumulation, which occurs when contaminants enter food chains, these dangers are amplified, endangering not just aquatic species but also the human populations who depend on these ecosystems for sustenance. In order to reduce heavy metal contamination, the results highlight the critical need for better monitoring methods, stronger pollution control policies, and long-term environmental management campaigns. To ensure the long-term ecological restoration of aquatic ecosystems and the people who rely on them, as well as the development of more sophisticated technologies for tracking and eliminating these contaminants, should be the goals of future study.

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