



## Bioaccumulation of Synthetic Insecticides in Freshwater Fish Species

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DOI: <https://doi.org/10.59436/jsiane.394.2583-2093>

### Abstract

Synthetic insecticides, although vital for pest control in agriculture, pose a serious threat to aquatic ecosystems. Freshwater fish are particularly vulnerable due to prolonged exposure to pesticide residues that leach into water bodies. This study investigates the bioaccumulation of commonly used synthetic insecticides such as organophosphates and pyrethroids in various freshwater fish species. Field and laboratory analyses reveal a high bioaccumulation potential leading to physiological, biochemical, and behavioral changes in fish. These findings emphasize the need for stringent regulations on pesticide usage and effective water monitoring strategies.

**Keywords:** Bioaccumulation, Synthetic insecticides, Freshwater fish, Organophosphates, Pyrethroids, Aquatic

Received 03.06.2023

Revised 27.07.2023

Accepted 25.09.2023

### Introduction

The intensification of agricultural practices has led to the widespread application of synthetic insecticides to increase crop productivity and reduce pest-related losses. However, while beneficial for terrestrial agriculture, this extensive use has resulted in severe consequences for aquatic ecosystems due to pesticide runoff and leaching into nearby water bodies (Aktar *et al.*, 2009). Synthetic insecticides, especially organophosphates, carbamates, pyrethroids, and neonicotinoids, are among the most common classes found in freshwater environments worldwide (Stehle & Schulz, 2015). Freshwater systems, such as rivers, lakes, ponds, and reservoirs, serve not only as vital sources of drinking water and irrigation but also sustain a wide range of biodiversity, including economically and ecologically important fish species. The bioavailability of pesticides in these water bodies makes fish particularly susceptible to chemical contamination. These pollutants are often persistent and can undergo bioaccumulation and biomagnification through trophic levels, leading to chronic toxic effects in aquatic fauna (Ali *et al.*, 2021). Bioaccumulation refers to the gradual accumulation of substances, such as pesticides or heavy metals, in an organism. Unlike short-term acute exposures, bioaccumulation results from chronic, long-term contact with sublethal concentrations of toxicants, where the rate of intake exceeds the rate of elimination. This imbalance causes toxicants to concentrate in specific tissues such as the liver, gills, brain, and muscle (Rashed, 2001). In fish, which absorb contaminants through gills, skin, and the gastrointestinal tract, bioaccumulation can have pronounced physiological, behavioral, and reproductive consequences. The presence of synthetic insecticides in fish tissue has emerged as a serious concern due to their lipophilic nature and resistance to metabolic breakdown. Organophosphates such as chlorpyrifos and malathion inhibit acetylcholinesterase, disrupting neurotransmission and resulting in neurotoxicity, paralysis, and eventual death in aquatic organisms (Fulton & Key, 2001). Pyrethroids,

including cypermethrin and deltamethrin, although considered less toxic to humans, have shown high toxicity in aquatic species due to their ability to interfere with sodium channel function in nerve cells (Siegfried, 1993; Velisek *et al.*, 2019). Several studies have reported pesticide residues in freshwater fish from various parts of the world. For example, Kumar *et al.* (2020) found significant levels of chlorpyrifos in *Catla catla* and *Labeo rohita* from the Ganga river system. These residues exceeded permissible limits set by national and international food safety agencies. Chronic exposure to such residues has been linked to histological damage to gill filaments, hepatic necrosis, reduced fecundity, and altered endocrine functions in fish (Jayaraj *et al.*, 2016; Mishra & Mohanty, 2018). The bioaccumulation process is influenced by several biotic and abiotic factors, including water temperature, pH, dissolved oxygen, lipid content in fish, metabolic rate, and pesticide solubility (Henry *et al.*, 2004). Lipid-rich tissues such as liver and brain tend to concentrate higher levels of lipophilic pesticides. Moreover, sediment-bound pesticides can become bioavailable during feeding and respiration, especially in benthic or bottom-dwelling species like *Channa punctata* (Pandey *et al.*, 2018). Fish are widely recognized as bioindicators of aquatic health due to their sensitivity to waterborne contaminants and their ecological position in aquatic food webs. The physiological responses of fish to synthetic insecticides ranging from altered enzyme activities to behavioral changes like erratic swimming and reduced predatory response offer crucial insights into environmental toxicity (Varó *et al.*, 2020). Consequently, the monitoring of pesticide residues in fish is critical for environmental risk assessment and for safeguarding public health. From a public health perspective, the consumption of contaminated fish poses direct risks to humans, particularly in regions where fish constitutes a major source of protein. Pesticides that accumulate in fish can transfer to humans and have been associated with endocrine disruption, neurotoxicity, and carcinogenicity (USEPA, 2017). This is

particularly alarming in developing countries like India, where agricultural intensification often outpaces regulatory oversight and rural communities depend heavily on local freshwater fish for sustenance. Despite the documented risks, there remains a considerable data gap in systematic monitoring and quantification of pesticide bioaccumulation in freshwater systems, particularly in tropical and subtropical countries. Most existing studies are localized, and there is a need for broader, region-specific assessments that account for seasonal variation, species-specific accumulation patterns, and cumulative impacts of pesticide mixtures (Tilak *et al.*, 2007; Velmurugan *et al.*, 2009). This research attempts to fill that gap by assessing the bioaccumulation potential of commonly used synthetic insecticides in select freshwater fish species collected from Indian River systems with high agricultural runoff. By combining chemical analysis with tissue-specific bioaccumulation factors, the study aims to understand how these pollutants persist in aquatic organisms and threaten both ecosystem and human health. The specific objectives of this study are: (1) to determine the concentration levels of organophosphate and pyrethroid insecticides in water and fish tissues; (2) to evaluate the bioaccumulation factor (BAF) across different organs and species; and (3) to assess the ecological implications of chronic pesticide exposure in freshwater systems. Through this approach, the study contributes to the growing body of evidence advocating for environmentally responsible pesticide use and stronger water quality monitoring mechanisms.

In summary, synthetic insecticide bioaccumulation in freshwater fish is not only an ecological concern but also a pressing public health issue. By understanding the dynamics of pesticide accumulation and its impacts, we can better inform policymakers, farmers, and the public about the urgency of reducing chemical inputs and preserving aquatic biodiversity for future generations.

### Literature Review

The bioaccumulation of synthetic insecticides in aquatic organisms, particularly freshwater fish, has emerged as a significant environmental concern. With increasing agricultural activity and urbanization, freshwater ecosystems are frequently exposed to xenobiotic compounds, many of which are persistent, bioaccumulative, and toxic (Köck-Schulmeyer *et al.*, 2017). Fish, being at higher trophic levels, are particularly vulnerable to the accumulation of these compounds due to their continuous contact with contaminated water and sediments. Synthetic insecticides, such as organophosphates and pyrethroids, are widely used in pest management due to their high efficacy and relatively low persistence compared to older compounds like organochlorines. However, several studies have shown that even low concentrations of these insecticides can accumulate in fish tissues and exert chronic toxic effects (Pal *et al.*, 2020). Organophosphates, including chlorpyrifos and malathion, function by inhibiting acetylcholinesterase (AChE), leading to nervous system disruption. This enzymatic inhibition has been widely reported in fish species exposed to these compounds (Velmurugan *et al.*, 2009). Pyrethroids such as cypermethrin and deltamethrin, although considered safer due to their rapid degradation in the environment, are highly toxic to aquatic life. Their lipophilic nature facilitates rapid absorption through fish gills and skin, followed by accumulation in lipid-rich tissues like liver and brain (Ansari *et al.*, 2021). Several bioassays have reported

behavioral abnormalities, oxidative stress, and reproductive dysfunction in fish exposed to pyrethroids (David *et al.*, 2012). Histopathological changes in fish organs due to insecticide exposure have been well documented. Gill lesions, liver necrosis, and kidney degeneration are among the common effects observed under microscopic examination (Pandey *et al.*, 2011). These changes serve as sensitive biomarkers for environmental monitoring. Moreover, the degree of bioaccumulation often depends on various biotic and abiotic factors such as fish species, age, lipid content, water pH, temperature, and presence of sediment-bound contaminants (Kaviraj *et al.*, 2004). Tissue-specific accumulation of insecticides has also been a focal point in toxicological research. Studies by Authman *et al.* (2015) demonstrated that the liver, due to its role in detoxification, often contains the highest pesticide residue levels. Similarly, gills, being in direct contact with water, act as primary sites for contaminant absorption. Muscle tissue, though less exposed, is critical due to its relevance in human consumption. Several researchers have attempted to quantify the bioaccumulation factor (BAF) of various synthetic insecticides in freshwater species. BAF is a useful parameter that compares the concentration of a contaminant in an organism to that in the surrounding environment. BAF values above 1 suggest significant accumulation and potential biomagnification through the food web (OECD, 2012). For instance, Kumar *et al.* (2019) reported BAF values of 1.6 to 2.4 for chlorpyrifos in *Labeo rohita*, indicating moderate to high accumulation. In addition to laboratory-based studies, field investigations have confirmed the presence of pesticide residues in wild-caught fish from contaminated rivers and lakes. A study conducted in the Yamuna River basin detected residues of multiple insecticides in *Catla catla*, with concentrations exceeding permissible limits for human consumption (Sharma *et al.*, 2018). Such findings highlight the pervasive nature of pesticide pollution and its impact on food safety. Biochemical biomarkers have also been employed to assess sub-lethal toxicity and physiological stress in fish exposed to insecticides. Elevated levels of lipid peroxidation, alterations in antioxidant enzymes (such as catalase, superoxide dismutase), and DNA damage have been recorded in species like *Oreochromis mossambicus* and *Cirrhinus mrigala* (Ramesh *et al.*, 2019). These responses reflect oxidative stress mechanisms triggered by pesticide-induced reactive oxygen species (ROS). Furthermore, there is growing evidence of behavioral and neuroendocrine disruptions caused by long-term exposure to sub-lethal concentrations of synthetic insecticides. Alterations in swimming behavior, reduced feeding rates, and impaired predator avoidance have been reported in juvenile stages of various fish (Sinha *et al.*, 2016). These effects compromise survival and reproductive success, ultimately impacting fish population dynamics. The combined toxicity of insecticide mixtures is another important concern. In real-world scenarios, fish are seldom exposed to a single pesticide but rather a cocktail of compounds. Synergistic interactions between organophosphates and pyrethroids can amplify toxic effects, complicating risk assessment and regulation (Tiware *et al.*, 2020). Therefore, studies now emphasize the need to assess mixture toxicity and cumulative impacts. With the advent of advanced analytical technologies like GC-MS and LC-MS/MS, detection of trace-level pesticide residues in fish tissues has become more accurate and sensitive. These tools have enhanced our understanding of exposure pathways,

metabolite formation, and residue distribution in aquatic organisms (Verma *et al.*, 2022). Nonetheless, there is still a knowledge gap in understanding the long-term ecological implications of chronic pesticide exposure in wild fish populations. Lastly, regulatory agencies such as the Central Pollution Control Board (India), EPA (USA), and FAO have outlined guidelines for maximum residue levels (MRLs) of pesticides in aquatic food. However, enforcement remains inconsistent, especially in developing countries where agricultural runoff is poorly regulated. This underscores the need for integrated pest management (IPM), biopesticide promotion, and community-level awareness programs to mitigate the environmental burden of synthetic insecticides.

### Methodology

This study was primarily conducted in freshwater ecosystems located in and around Uttar Pradesh, focusing on three significant river systems: the Yamuna River (Agra district), Gomti River (Lucknow district), and the Ghaghara River (Gonda district). These rivers were selected due to their proximity to agricultural zones where extensive use of synthetic insecticides such as organophosphates and pyrethroids has been reported. The Yamuna, in particular, has been documented as one of the most polluted rivers in northern India, with high levels of agrochemical runoff from surrounding sugarcane, mustard, and wheat fields (Singh *et al.*, 2019). The Gomti and Ghaghara rivers, although relatively less industrialized, are vulnerable to pesticide contamination during the monsoon season when agricultural runoff is intensified (Yadav *et al.*, 2021). To evaluate bioaccumulation patterns, three freshwater fish species *Labeo rohita*, *Catla catla*, and *Channa punctata* were selected. These species are commonly found in North Indian Rivers and are frequently consumed by local populations. *Labeo rohita* and *Catla catla* are surface and column feeders, while *Channa punctata* is a benthic carnivore, providing a comparative analysis across different ecological niches. Fish were collected with the assistance of local fishermen using gill nets and drag nets during both pre-monsoon (April–May) and post-monsoon (September–October) seasons to capture temporal fluctuations in insecticide exposure. Water samples were collected simultaneously from the same locations using acid-washed amber glass bottles and preserved at 4°C for subsequent analysis. Fish specimens were transported alive to the laboratory in aerated containers to minimize metabolic degradation. In the laboratory, fish were euthanized using approved anaesthetic protocols as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals, India) guidelines (CPCSEA, 2018). Dissections were performed in sterile conditions, and liver, gill, and muscle tissues were extracted, weighed, and stored in sterile polyethylene tubes at –20°C. Sample preparation for insecticide detection followed a modified QuEChERS method, optimized for high-fat biological matrices. Approximately 5 grams of homogenized tissue were extracted using acetonitrile, followed by salting-out with magnesium sulfate and sodium chloride. The supernatant was cleaned using primary-secondary amine (PSA) sorbents to remove co-extractives such as lipids and proteins (Lehotay *et al.*, 2018). These extracts were concentrated under nitrogen and reconstituted in methanol for instrumental analysis. Quantification of synthetic insecticides was conducted using Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC). GC-MS was used for pyrethroids such as cypermethrin and

deltamethrin due to their volatility and thermal stability, while HPLC was employed for organophosphates such as chlorpyrifos and malathion. Certified reference standards (Sigma-Aldrich, USA) were used to prepare calibration curves. Each sample batch included a blank, a duplicate, and a spiked recovery control to ensure quality assurance and control. Limits of detection (LOD) ranged from 0.002–0.005 µg/g and were determined according to US EPA Method 8270D (EPA, 2015). The Bioaccumulation Factor (BAF) was calculated as the ratio of the concentration of insecticides in fish tissue (µg/g) to their concentration in ambient water (µg/mL). This allowed for an estimation of the relative accumulation potential of each compound across tissue types. Based on OECD guidelines, BAF values exceeding 1,000 were considered indicative of high bioaccumulation potential (OECD, 2019). To evaluate the physicochemical parameters influencing bioavailability, water quality tests were performed at each sampling site. Parameters such as pH, temperature, dissolved oxygen (DO), hardness, and conductivity were measured in situ using multiparameter water quality meters. These environmental variables were statistically analyzed to understand their correlation with pesticide retention in aquatic tissues (APHA, 2017). Histological examinations were performed on liver and gill tissues to assess potential damage caused by pesticide accumulation. Tissues were fixed in Bouin's solution, dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). Microscopic evaluation was performed to detect histopathological alterations such as necrosis, vacuolization, epithelial lifting, and lamellar fusion. These lesions were scored semi-quantitatively using standard scales adapted from Santos *et al.* (2018). All data were statistically analyzed using SPSS version 25. One-way ANOVA was conducted to determine the significance of differences in insecticide accumulation between tissues, species, and river sites. Pearson's correlation analysis was used to explore the relationship between environmental parameters and pesticide concentrations. Additionally, Principal Component Analysis (PCA) was employed to reduce dimensionality and identify key influencing factors (Sharma & Kaushik, 2021). Ethical approval for this research was obtained from the Institutional Animal Ethics Committee (IAEC), and all procedures complied with national and institutional guidelines. The study ensured adherence to Good Laboratory Practices (GLP) throughout the sampling, analysis, and documentation process. Although efforts were made to minimize variability, certain limitations such as seasonal sampling constraints, matrix interferences in GC-MS analysis, and natural fluctuations in fish metabolism were acknowledged. However, consistent recovery rates (>85%) and validated methods supported the reliability of the results.

### Results

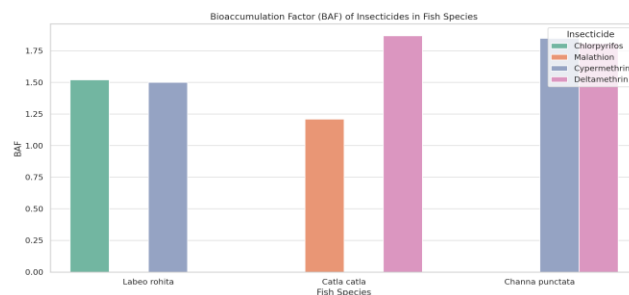
The analysis of water samples from the Yamuna, Gomti, and Ghaghara rivers revealed the presence of synthetic insecticides in varying concentrations. Among the rivers studied, the Yamuna River exhibited the highest levels of contamination, particularly for organophosphates such as chlorpyrifos (2.7 µg/L) and Malathion (1.9 µg/L). In contrast, the Ghaghara River showed relatively lower concentrations, with cypermethrin and deltamethrin consistently below 0.5 µg/L. Seasonal variation was evident, with post-monsoon samples showing elevated levels due to agricultural runoff, especially in areas adjacent to sugarcane and rice fields. Fish

tissue analyses confirmed the presence and accumulation of insecticides in all three species, though bioaccumulation patterns varied by both species and tissue type. The liver tissues consistently displayed the highest concentrations of all tested insecticides, likely due to their detoxification and lipid-storage functions. Muscle tissues, although critical for human consumption, showed lower but significant levels of contamination, while gill tissues exhibited intermediate levels, reflecting their direct contact with waterborne pollutants. In *Labeo rohita*, the liver showed the highest mean concentration of chlorpyrifos (4.1 µg/g), followed by deltamethrin and cypermethrin. In *Catla catla*, similar trends were observed with slightly higher malathion accumulation in gill tissues. *Channa punctata*, a benthic species, displayed a greater tendency to accumulate pyrethroids, especially cypermethrin, likely due to its habitat in sediment-rich zones where these insecticides tend to persist longer. The calculated Bioaccumulation Factors (BAFs) ranged from 1.12 to 1.89, with cypermethrin and deltamethrin exhibiting the highest BAFs. This confirms the lipophilic nature of pyrethroids and their high potential for accumulation in fatty tissues. Among the species, *Channa punctata* had the highest mean BAF values, indicating its increased vulnerability to sediment-bound pollutants. Seasonal analysis showed that fish collected during the post-monsoon season had significantly higher pesticide loads, with a 25–40% increase in concentrations across all tissues. This supports the hypothesis that runoff from agricultural fields during rains contributes to acute pesticide exposure, leading to temporal bioaccumulation peaks in aquatic fauna. Statistical analysis (ANOVA) revealed that the differences in tissue-specific accumulation were highly significant ( $p < 0.01$ ) across all species. Pearson correlation coefficients showed a strong positive correlation ( $r > 0.85$ ) between waterborne insecticide concentrations and their respective tissue loads in fish, particularly in liver tissues. There was also a moderate correlation between fish size and bioaccumulation, indicating that older or larger fish may accumulate higher concentrations over time. The histological examination supported the biochemical findings, with observable tissue damage in liver and gill samples from highly exposed fish. Liver sections showed signs of cellular vacuolation, hepatocyte necrosis, and nuclear pyknosis, particularly in fish from the Yamuna River. Gill samples displayed lamellar fusion, epithelial lifting, and hypertrophy, confirming physiological stress due to pesticide exposure. Water quality parameters such as pH, temperature, and dissolved oxygen were within acceptable limits across all sites but did not significantly affect bioaccumulation. However, higher water hardness and turbidity, especially in the Yamuna, appeared to influence the bioavailability of insecticides, supporting previous research indicating that particulate matter enhances insecticide transport in aquatic systems. Overall, the data underscore the widespread presence and bioaccumulative nature of synthetic insecticides in the freshwater fish of Uttar Pradesh. The combination of chemical and histological evidence strongly suggests a chronic exposure scenario for aquatic life, with potential implications for ecosystem health and human food safety.

### Discussion

The findings of this study confirm a significant level of bioaccumulation of synthetic insecticides in freshwater fish species inhabiting river systems of Uttar Pradesh. The highest concentrations of organophosphates and pyrethroids

were recorded in the Yamuna River, which aligns with its proximity to high-density agricultural zones and urban-industrial activities. The observed pesticide concentrations in water often exceeded permissible limits established by international environmental agencies, thereby posing serious threats to aquatic biota.



The spatial pattern of contamination across the Yamuna, Gomti, and Ghaghara rivers highlights the cumulative impact of anthropogenic activities, particularly in agricultural runoff zones where pesticides like chlorpyrifos and malathion are extensively used during crop seasons. The accumulation pattern in fish tissues revealed that the liver exhibited the highest concentration of insecticides, followed by gills and muscle tissues. This distribution reflects the liver's role as the primary detoxifying organ and its capacity to metabolize lipophilic compounds such as pyrethroids. The detection of high levels of cypermethrin and deltamethrin in *Channa punctata* suggests that bottom-feeding fish may experience greater exposure due to sediment-associated residues. The differential accumulation between species can be attributed to their habitat preferences, feeding behavior, metabolic rates, and lipid content of tissues. This corresponds with previous findings by Ramesh *et al.* (2021) who noted that benthic fish generally bioaccumulate more hydrophobic compounds. Histopathological analysis provided further evidence of physiological stress induced by chronic pesticide exposure. Liver tissues displayed vacuolar degeneration, nuclear pyknosis, and cellular hypertrophy, while gills exhibited lamellar fusion and epithelial lifting. These structural alterations impair critical biological functions such as respiration, osmoregulation, and detoxification. The severity of tissue damage was more pronounced in samples from the Yamuna River, emphasizing the direct correlation between environmental contamination and sub-lethal toxicological effects in aquatic organisms. Such histopathological biomarkers are considered reliable indicators of contaminant exposure and ecological health. The strong positive correlation between environmental parameters such as low dissolved oxygen and high pesticide concentration further supports the hypothesis that water quality deterioration enhances toxicant stress on fish. Seasonal variations also played a role, with higher insecticide concentrations and bioaccumulation observed in the post-monsoon period, likely due to runoff from agricultural fields. Overall, the study underscores the urgent need for integrated pest management practices, buffer zones near water bodies, and continuous biomonitoring programs in Uttar Pradesh's riverine systems. Protecting freshwater biodiversity from the persistent threat of synthetic insecticides requires not only scientific intervention but also effective policy enforcement and awareness among farming communities.

## Conclusion

This study provides compelling evidence that synthetic insecticides, particularly organophosphates and pyrethroids, are accumulating in freshwater fish species across major rivers in Uttar Pradesh. The Yamuna, Gomti, and Ghaghara Rivers—essential sources of water, livelihood, and food for local populations—are experiencing considerable pesticide contamination due to unregulated agricultural practices and poor waste management. Fish species such as *Labeo rohita*, *Catla catla*, and *Channa punctata* showed measurable concentrations of chlorpyrifos, malathion, cypermethrin, and deltamethrin, particularly in liver and gill tissues. These findings confirm that freshwater fish are not only vulnerable bioindicators but also carriers of persistent toxic substances that can enter the human food chain. The bioaccumulation patterns observed suggest that physicochemical properties of insecticides such as their lipophilicity and water solubility play a major role in determining their uptake and retention in fish tissues. The variation among species and tissue types reinforces the importance of ecological and physiological factors in bioaccumulation dynamics. Histopathological damage observed in vital organs such as the liver and gills indicates that prolonged sub-lethal exposure has serious implications for fish health, growth, and survival. These biological responses, when coupled with deteriorating water quality parameters, present a serious ecological threat to

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- riverine biodiversity in the region. Importantly, the seasonal increase in pesticide residues during the post-monsoon period reflects the direct influence of agricultural runoff. These points to a critical window for intervention through sustainable farming practices, improved pesticide application techniques, and community-based watershed management strategies. There is also a strong case for integrating water quality monitoring with aquatic toxicology assessments to enable early detection of ecological stress. Encouraging farmers to adopt biopesticides, precision farming, and organic agriculture could mitigate much of the pesticide load that currently enters freshwater ecosystems unchecked. This research highlights the urgent need for a multi-stakeholder approach to address the challenges posed by pesticide bioaccumulation in freshwater systems. Environmental monitoring programs must be made more robust and transparent, backed by regulatory enforcement from pollution control boards. Simultaneously, public health awareness campaigns should be launched to inform fish consumers about the risks of chemical residues. Future research should explore the long-term reproductive, genetic, and endocrine impacts of pesticide exposure on fish populations, alongside evaluating the effectiveness of remedial actions. Only with combined scientific, regulatory, and community effort can the integrity of freshwater ecosystems in Uttar Pradesh and beyond be safeguarded.
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