



RESEARCH ARTICLE

Studies on behavior and morphological changes in freshwater fish, *Channa punctatus*, under the exposure of untreated sewage water

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Abstract

The presence of harmful chemical pollutants in freshwater ecosystems poses a significant environmental challenge due to their toxicity, persistence, and potential for bioaccumulation. This study aims to investigate the impact of untreated sewage water on the freshwater fish species *Channa punctatus*. *C. punctatus* (Bloch, 1793), class-Actinopterygii, order-Ophiocephaliformes, sub-order- Channoidae, family-Channidae. The primary objective is to elucidate the effects of exposure to this contaminated water on the behavioral patterns and physical characteristics of the fish.

The fish were kept in untreated sewage water along with normal tap water as a control for different periods, and their reactions were closely observed. The fish showed many unusual behaviors such as losing balance, gasping for air at the surface, swimming in a confused or jerky way, rapid gill movement, restlessness, jumping, becoming very slow, and in some cases, floating at the surface before dying. In addition to these behavioral alterations, there were evident physical manifestations of damage. The fish exhibited patches on their bodies, alterations in skin pigmentation, loss of scales, a mucus covering on their bodies, unusual deposits on their skin, and clumping of the gills. These results show that *C. punctatus* reacts strongly to polluted water, both in behavior and appearance. Because of this, the *C. punctatus* can be used as a good bioindicator and can also help in monitoring the water quality.

Keywords: Behavioral responses, *Channa punctatus*, Morphological changes, Physico-chemical analysis.

Introduction

Freshwater bodies such as rivers, lakes, and ponds are increasingly being polluted. These water bodies have not only become polluted but are also being used as dumping sites for domestic and industrial waste due to rapid industrialization, urbanization, agriculture, and other

developmental activities. As a result, the release of heavy metals into aquatic environments has led to serious ecotoxicological effects.

Unlike many other pollutants, metals are unique because they occur naturally and are often widespread in the environment. However, they can still cause harmful health effects. According to Kakade *et al.* (2020), heavy metals found in aquatic plants and animals are hazardous and tend to accumulate in living organisms. Although aquatic ecosystems have a natural self-purification capacity and biological cycles that can tolerate small amounts of heavy metals without harm, contamination beyond a certain threshold can lead to reduced growth, weakened immune systems, increased vulnerability to disease, and even death in fish.

Different types of waste contribute to environmental problems and increase pollution levels. Wastewater from sources such as municipal sewage, industrial discharges, and agricultural runoff is a major cause of heavy metals, i.e., zinc, mercury, nickel, cadmium, lead, chromium and copper pollution in water. These metals can build up in freshwater systems, enter the food chain, and eventually affect fish, which are top consumers in the aquatic environment

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(Afshan *et al.*, 2014; Lacerda *et al.*, 2020). Different types of waste contribute to environmental problems and increase pollution levels. Wastewater from sources such as municipal sewage, industrial discharges, and agricultural runoff is a major cause of heavy metal pollution in water. These metals can build up in freshwater systems, enter the food chain, and eventually affect fish, which are top consumers in the aquatic environment (Afshan *et al.*, 2014; Lacerda *et al.*, 2020). The study by Afshan *et al.* (2014) specifically mentions lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), zinc (Zn), nickel (Ni), and mercury (Hg) as heavy metals of concern in aquatic ecosystems. Lacerda *et al.* (2020) focus on mercury (Hg) contamination in freshwater fish, highlighting its significant impact on aquatic ecosystems and potential risks to human health through fish consumption.

These heavy metals can accumulate in fish tissues, leading to various physiological and biochemical alterations (Afshan *et al.*, 2014). For instance, exposure to lead can cause neurological damage and affect growth rates in fish, while cadmium can disrupt calcium metabolism and cause skeletal deformities. Mercury, particularly in its organic form (methylmercury), is known for its neurotoxic effects and ability to biomagnify through the food chain (Lacerda *et al.*, 2020).

The bioaccumulation of these metals in fish not only poses a threat to aquatic biodiversity but also raises concerns about food safety for human consumers, especially in regions where fish is a significant part of the diet (Afshan *et al.*, 2014; Lacerda *et al.*, 2020).

As a result, fish are particularly vulnerable to toxic substances in water. However, due to their high protein content, low levels of saturated fat, and rich omega fatty acid content, fish remain an important part of the human diet. This makes it essential to study the contamination of different fish species by heavy metals (Sivaperumal *et al.*, 2007).

Toxicity testing is a vital tool for understanding the effects of pollutants on aquatic ecosystems. It helps in setting water quality standards and identifying suitable organisms as bio-indicators. The goal of toxicity testing is to detect abnormalities caused by pollutants and to determine the toxicity level of various chemicals (Absunullah *et al.*, 1981). Fish toxicity results from a combination of physical, chemical, and biological processes. Fish species exhibit heightened susceptibility to aquatic toxins due to their continuous exposure through gill respiration, osmoregulation, and feeding behaviors (Van der Oost *et al.*, 2003). Despite this vulnerability, fish remains a crucial component of human nutrition, providing high-quality protein, essential omega-3 fatty acids, and various micronutrients (Mozaffarian and Rimm, 2006). This nutritional importance underscores the necessity of comprehensive studies on heavy metal contamination across diverse fish species, considering

their potential for bioaccumulation and biomagnification in aquatic food webs (Sivaperumal *et al.*, 2007).

Toxicity testing serves as a fundamental approach in ecotoxicology, offering insights into the impacts of pollutants on aquatic ecosystems and individual organisms. These assessments play a pivotal role in establishing water quality criteria, identifying suitable bio-indicator species, and evaluating the ecological risk of various contaminants (Chapman, 2000). The primary objectives of toxicity testing encompass the detection of physiological, biochemical, and behavioral abnormalities induced by pollutants, as well as the determination of toxicity thresholds for different chemical compounds (Absunullah *et al.*, 1981).

The toxicity of pollutants in fish results from a complex interplay of physical, chemical, and biological factors. Physical factors may include temperature and pH, which can influence the bioavailability and toxicity of contaminants. Chemical factors involve the properties of the toxicant itself, such as its solubility, persistence, and reactivity. Biological factors encompass species-specific characteristics, including metabolic rates, detoxification mechanisms, and life-stage sensitivities (Di Giulio and Hinton, 2008).

The snake-headed, air-breathing fish *C. punctatus* is a purely freshwater species with a wide distribution across Asia, ranging from China to India (Kaushal and Mishra, 2013). It has long been regarded as an important food fish in many Asian countries (Laovitthayanggoon, 2006) and serves as a key source of income for many small-scale fish farmers.

In India, *C. punctatus* has received considerable attention in fishery statistics and inland water management policies. When living in contaminated water, this species often shows clear behavioral and morphological changes, which can be used as biomarkers of pollution. Behavior helps organisms adapt to changing environments, and these changes may result in noticeable physical effects. The use of such abnormalities as biomarkers has become increasingly common in recent years, as they can serve as reliable indicators of environmental conditions (Sabullah *et al.*, 2015).

These changes serve as important diagnostic signs when studying the effects of polluted water on fish. *C. punctatus* has the potential to be a good bio-indicator species for detecting chemical and heavy metal contamination in water. Therefore, it is important to assess the concentration and bioaccumulation of heavy metals and their toxic effects on fish in large aquatic ecosystems. The snake-headed, air-breathing fish *C. punctatus*, widely distributed across Asia from China to India, has garnered significant attention in fishery research and aquatic ecology (Kaushal and Mishra, 2013). This species holds considerable importance not only as a food source but also as a valuable bio-indicator for environmental monitoring (Laovitthayanggoon, 2006). Recent studies have focused on the physiological and behavioral responses of *C. punctatus* to various

environmental stressors, particularly water pollution and heavy metal contamination.

The adaptability of *C. punctatus* to diverse aquatic environments has made it a subject of interest in ecotoxicological research. When exposed to polluted water, this species exhibits distinct behavioral and morphological alterations, which serve as potential biomarkers for environmental contamination (Sabullah *et al.*, 2015). These changes may include alterations in swimming patterns, feeding behavior, and reproductive activities, as well as visible physical abnormalities such as skin lesions or fin erosion.

The use of behavioral and morphological biomarkers in *C. punctatus* has gained traction in recent years due to their sensitivity and reliability as indicators of water quality. Researchers have observed that these biomarkers can provide early warning signs of environmental stress, often manifesting before more severe physiological impacts occur. For instance, studies have shown that exposure to heavy metals like lead and cadmium can induce significant changes in the opercular movement and swimming behavior of *C. punctatus*, indicating respiratory distress and neurotoxicity (Kumar *et al.*, 2019).

Furthermore, the ability of *C. punctatus* to bioaccumulate heavy metals in its tissues makes it an excellent model for studying the long-term effects of aquatic pollution. Investigations into the bioaccumulation patterns of various heavy metals in different organs of *C. punctatus* have revealed organ-specific accumulation tendencies, with the liver and gills often showing the highest metal concentrations (Javed and Usmani, 2019). This information is crucial for understanding the potential transfer of contaminants through the food chain and assessing the ecological risks associated with water pollution.

The potential of *C. punctatus* as a bio-indicator species extends beyond heavy metal contamination to include other forms of chemical pollution. Recent research has explored its sensitivity to pesticides, industrial effluents, and emerging contaminants such as microplastics (Singh *et al.*, 2020). These studies have highlighted the species' utility in comprehensive environmental monitoring programs and

its potential role in developing early warning systems for aquatic ecosystem health.

Given the increasing concerns about water quality in many Asian countries where *C. punctatus* is native, there is a pressing need for further research into the species' responses to various pollutants. Such studies would not only enhance our understanding of the ecological impacts of water pollution but also contribute to the development of more effective environmental management strategies and conservation policies for freshwater ecosystems.

Material and Methods

Collection of sewage water samples

The sewage water samples were collected from the sewage treatment plant (Figure 1) Integral University, Lucknow. The samples were collected in 2.5-liter capacity high-density polyethylene (HDPE) bottles that were pre-washed (rinsed 4–5 times with distilled water) and dried before use.

All the samples were immediately transported to the laboratory and were stored at 4°C till analysis. During the sampling procedure, precautions were taken as per the standard guidelines of APHA (2012) to avoid the possibility of any contamination.

Analytical procedure of sewage water and control water sample

The pH and total dissolved solids (TDS) in sewage water samples were determined using a microprocessor-based digital water and soil analysis kit (ESICO, Model-1160). The temperature was recorded using a digital thermometer (Maxtech, multi-thermometer). Other parameters like dissolved oxygen (DO), biochemical oxygen demand (BOD), total hardness and alkalinity were calculated by titrimetric analysis. The analysis of the above-mentioned parameters was done in triplicate following the standard methods of APHA (2012).

Fish collection and maintenance

Healthy *C. punctatus* fish were collected from the local fish market (Behta and Paikramau Village) in Lucknow, Uttar Pradesh and brought to the laboratory. Only healthy, uninjured and uninfected fish specimens (Length: 11–14 cm, Weight: 50–60 g) were taken for the experiment.

Fishes were acclimatized in glass aquaria containing tap water for 15 days in the laboratory. The fish were fed with fish food and water in the aquaria was changed every 24 hours, leaving no fecal matter, unconsumed food, or dead fish. Proper aeration was maintained in test as well as control aquaria by aerators throughout the experiments.

Experimental design

About 10 fish were exposed to untreated sewage water to know the acute toxicity at different exposure periods, i.e., 24, 48, 72 and 96 hours. For studying the behavioral and



Figure 1: Photographs of sewage water sampling site at Integral University, Lucknow, Uttar Pradesh, INDIA (Lat: 26.958215° Long: 81.006316°).

morphological changes, fish were divided into two groups: the control and experimental group as shown in Figure 2.

The behavioral and morphological changes were recorded simultaneously at different exposure periods. The experimental sewage water in the aquaria was changed every 24 hours during the acute exposure and for chronic exposure, water was changed twice a week. Fishes were regularly noticed for any variation in behavior and external morphology.

Statistical Analysis

The observed data was subjected to statistical analysis for the mean, standard deviation (SD) and Karl Pearson Correlation matrix for the characteristic correlations of different physico-chemical parameters (Table 1). All these parameters were calculated using MS Excel 2007.



Figure 2: A photograph of experimental design

Table 1: Physico-chemical analysis of sewage water sample

S.N.	Parameters	Specifications
1.	pH	7.9 ± 0.5
2.	Temperature (°C)	$30.5 \pm 2.0^\circ\text{C}$
3.	Total dissolved solid	$280 \pm 10.0 \text{ mg/l}$
4.	Dissolved oxygen	$2.3 \pm 0.2 \text{ mg/l}$
5.	Alkalinity	$200 \pm 0.5 \text{ mg/l}$
6.	Total hardness	$260 \pm 0.8 \text{ mg/l}$

Results

Behavioral Study

In the present study, exposure of *C. punctatus* to untreated sewage water resulted in increased mortality along with various behavioral (Table 2) and morphological changes (Table 3). The behavior and condition of the fish were observed throughout the experiment in both the control and test aquaria. Fishes exposed to sub-acute concentrations of sewage water exhibited marked behavioral alterations, whereas no such changes were observed in the control group. Immediately after introduction into the test aquaria, the fishes displayed symptoms of impaired swimming, including uncontrolled, irregular, erratic, and darting movements; restlessness; loss of equilibrium; drowning; frequent collisions with the aquarium walls; and attempts to jump out of the aquaria, presumably to escape the toxic environment. These avoidance reactions may be associated with changes in the sensitivity of chemoreceptors.

Morphological Study

During the course of the study, several morphological changes were documented in fishes exposed to untreated sewage water over different periods. These changes included skin discoloration, the formation of a thin chemical layer on the skin, reduced mucus secretion (likely contributing to a thin chemical layer observed at the bottom of the aquarium), and shedding of scales (Table 3; Plate 1).

Discussion

Due to increasing anthropogenic activities, fish are among the first vertebrate groups to exhibit physiological and behavioral responses when aquatic ecosystems are contaminated with pollutants (Sehonova *et al.*, 2017; Gupta *et al.*, 2018). Fish serve as sensitive indicators of water quality because they remain in constant contact with the aquatic environment for respiration and nutrition, making them highly vulnerable to environmental changes.

Table 2: Effect of sewage water exposure on behavioral responses of freshwater fish, *Channa punctatus*

S.N.	Behavioral changes	Control	Exposure (Untreated Sewage water) period					
			Acute test				Chronic test	
			24 hours	48 hours	72 hours	96 hours	15 day	30 day
1.	Erratic Swimming	-	++	+++	++++	++++	++	++
2.	Jumping	-	++++	++++	++++	++++	+++	++
3.	Gulping air at the surface	-	+++	+++	++++	++++	+++	++
4.	Opercular movements	-	++	+++	+++	++++	++	++
5.	Sluggishness	-	++	++	+++	++++	+++	++
6.	Restlessness	-	++++	+++	++++	++++	+++	++
7.	Loss of equilibrium	-	++	++++	++++	++++	+++	++

Note: (-) Normal, (0) Nil, (++) Less Change, (+++) Moderate Change and (+++++) Prominent Change.

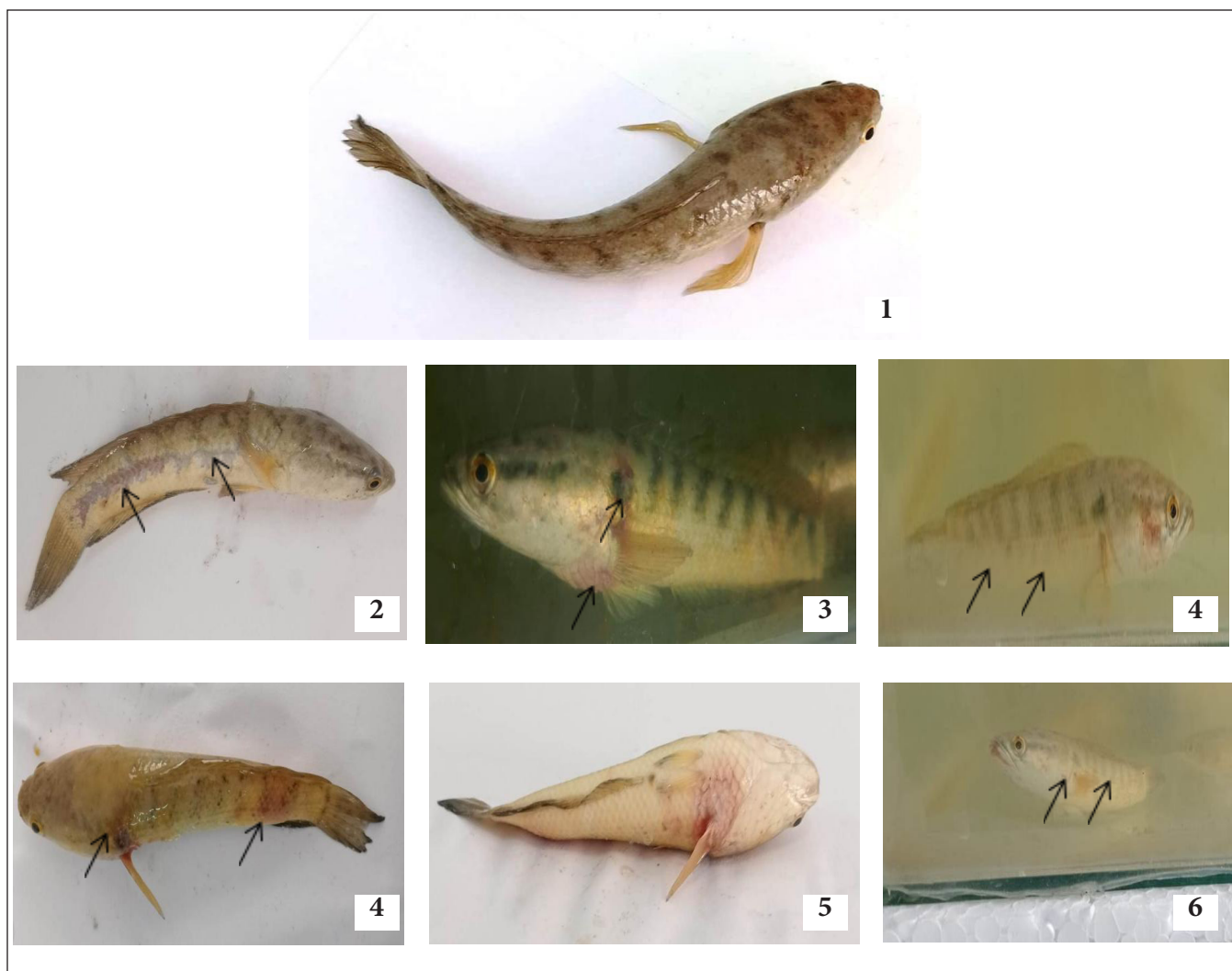


Plate 1: Explanation of Figures: Fig. 1. A photograph of freshwater fish *C. punctatus* (control group) Figs. 2-7. Photographs of freshwater fish *C. punctatus* (exposed to untreated sewage water): Figs. 2. shows shedding of scale; 3. shows patches on body; 4. mucus secretion; 5. sedimentation of chemical on body; 6. clumping of gills and 7. discoloration of skin.

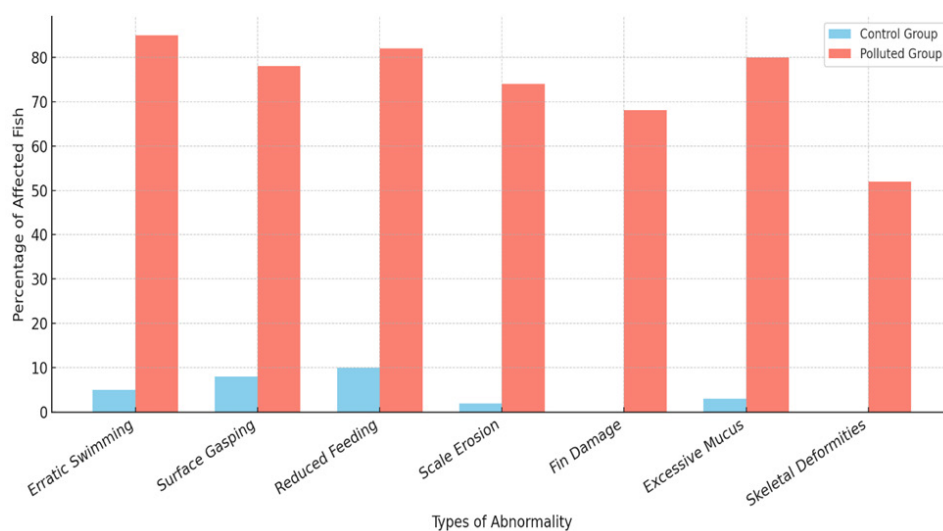


Figure 3: Comparative analysis of behavioral and morphological abnormalities.

Table 3: Effect of sewage water exposure on Morphological changes of freshwater fish, *C. punctatus*.

S.N.	Morphological changes	Control	Exposure (Untreated Sewage water) period					
			Acute test				Chronic test	
			24 hours	48 hours	72 hours	96 hours	15 day	30 day
1.	Mucus secretion	-	++	++	++	+++	+++	++++
2.	Shedding of scale	-	++	++	++	++	+++	++++
3.	Discoloration of skin	-	++	++	+++	+++	+++	++++
4.	Clumping of Gills	-	++	++++	++++	++++	+++	+++
5.	Patches on body	-	++	++	++	+++	++++	++++
6.	Sedimentation of chemical on body	-	++	++	++	+++	+++	++++

Note: (-) Normal, (0) Nil, (++) Less Change, (+++) Moderate Change and (++++) Prominent Change.

Heavy metals are persistent pollutants that do not degrade in the environment and can bio-accumulate in fish tissues over prolonged periods, even at low concentrations (Jaishankar *et al.*, 2014; Wang *et al.*, 2018; Markowicz *et al.*, 2019). Since fish are a significant source of dietary protein, consumption of contaminated fish may lead to bio-accumulation of heavy metals in humans, posing potential health risks.

Behavioral Changes

In the present study, exposure of *C. punctatus* to untreated sewage water resulted in increased mortality, along with distinct behavioral and morphological alterations (Table 2). Both control and experimental groups were monitored throughout the exposure period.

Fish exposed to sub-lethal concentrations of sewage water exhibited noticeable behavioral deviations compared to the control group. Immediately after exposure, fish demonstrated swimming abnormalities such as erratic, darting movements, restlessness, loss of equilibrium, frequent collision with the aquarium walls, and attempts to escape the toxic environment by jumping. These avoidance behaviors are likely linked to chemoreceptor sensitivity (Svecevieus, 2001; Agarwal, 1991).

The observed loss of balance during swimming may indicate neurological impairment within the central nervous system (Kawade & Khillare, 2014). Other symptoms included rapid opercular movements, increased surface activity, and frequent air gulping—behaviors that suggest respiratory distress and heightened oxygen demand (Katja *et al.*, 2005). Previous studies (Maruthanayagam *et al.*, 2002; Laovitthayangoon, 2006) also reported similar behavioral alterations in *C. punctatus* upon exposure to cadmium compounds. Additional signs included hyperactivity, disrupted schooling and shoaling behavior, decreased feeding activity, and lethargy after prolonged exposure. The progression to sluggishness likely results from energy depletion due to excessive locomotor activity.

Behavioral abnormalities persisted up to 96 hours, after which fish exhibited signs of acclimatization. Daily observations indicated a gradual reduction in activity, and after 20 days, partial adaptation to the polluted environment was noted. Interestingly, temporary recovery in behavior was observed for a brief period following water replacement, indicating that the fish remained responsive to changes in water quality. These behavioral changes often precede physiological and genetic alterations. They are widely recognized as early biomarkers of chemically induced stress in aquatic organisms (Suedel *et al.*, 1997; Remyla *et al.*, 2008; Hesni *et al.*, 2011; Aziz *et al.*, 2015). Increased locomotion raises metabolic demands and oxygen requirements. Such behavioral indicators reflect disruptions in enzyme activity, neurotransmission, and overall metabolic function.

Morphological Changes

The study also revealed various morphological abnormalities in *C. punctatus* associated with different exposure durations (Table 3; Plate.1; Figure 3). These included skin discoloration, visible chemical deposits, reduced mucus secretion, scale shedding, and lesions on the epidermis. Similar effects were documented in fish exposed to mercury by Gupta and Dua, 2015 and Aziz *et al.*, 2015.

Additional deformities included gill clumping, fin necrosis, hyperextension and splitting of fins, eye abnormalities, and muscular tetany. Copious mucus secretion and its coagulation were noted at higher pollutant concentrations, with changes becoming more prominent after 10-30 days of exposure. The severity and frequency of these deformities correlated with increased exposure duration (Halappa & David, 2009; Anita *et al.*, 2010). Maruthanayagam *et al.* (2002) reported that cadmium toxicity in *C. punctatus* primarily affects gill morphology, leading to impaired oxygen uptake and eventual hypoxia. Prolonged exposure also caused loss of natural pigmentation, with fish turning pale yellow a response similarly observed by Braich and Kaur (2015).

Profuse mucus secretion is considered a defensive response aimed at minimizing toxicant absorption. This mucous layer may bind with contaminants and reduce their cutaneous penetration (Santha *et al.*, 2000; Sivakumar *et al.*, 2006; Bisht and Agarwal, 2007; Shallangwa, 2011). However, mucus deposition on gills can obstruct gas exchange, leading to oxygen deficiency (Maina, 1997). Ultimately, such internal damage can result in mortality.

Morphological abnormalities serve as indicators of stress and tissue injury caused by metal toxicity (Kaur *et al.*, 2013; Dhara *et al.*, 2014). Toxicological studies using these parameters offer insights into pollutant impact and help establish safe water quality standards.

Conclusion

The elevated physicochemical parameters observed at the sampling sites indicate significant pollution levels compared to the control. This is likely due to the influx of domestic sewage, vehicle wash runoff, and industrial waste into drainage systems.

Our study shows that *C. punctatus*, when exposed to acute and sub-acute concentrations of untreated sewage water, exhibits significant behavioral and morphological alterations. Mortality increased with prolonged exposure, and stress responses became more evident over time. These findings highlight the detrimental impact of untreated sewage on fish health and aquatic biodiversity. Such studies are essential for identifying safe contaminant levels that prevent ecological harm.

Significance of the Study

This study emphasizes the impact of sewage discharge from domestic, municipal, and industrial sources on aquatic ecosystems, particularly in relation to pollution. The results provide valuable insights into how sewage effluents affect fish, which are crucial components of aquatic food webs. As sensitive organisms, fish like *C. punctatus* can serve as effective bio-indicators of water pollution. By assessing behavioral and morphological changes, this study contributes to the calculation of safe heavy metal concentrations in aquatic environments. These findings hold academic as well as applied significance for environmental monitoring and conservation.

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Conflict of Interest

None

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