



## Effect of physicochemical parameters and heavy metals toxicity on reproduction of Tilapia collected from different aquaculture pond of North 24 parganas, West Bengal, India

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### Abstract

Fish is one of the most important healthiest and cheapest sources of protein in third World Countries. Many waterbodies (known as bheri) situated in North 24 parganas, West Bengal, India suffer from severe environmental pollution. The source of pollution in that area comes from different sources of industrial, sewage, agricultural activities and petroleum companies which present in that area. Therefore, studying fishes for heavy metals contamination would benefit individuals living in a country surrounded by water body. The aim of this study is to determine and compare the concentration of heavy metals (Lead, Cadmium and Copper) in different water bodies and fish tissues collected from different aquaculture ponds of North 24 parganas. The present study is tried to investigate the effects of physicochemical parameters and heavy metals toxicity on reproduction of tilapia collected from different sampling sites of North 24 parganas. There were two groups, one group of samples obtained from seriously polluted waterbodies and other group of samples collected from those waterbodies that are not exposed to pollution. The former one treated as a intoxicated group and later one treated as a control group of our experiment. The results showed that there was a significant difference between the intoxicated group and the control group. The histological study of fish organs (Ovary, testes and brain) was recorded. The testis of the intoxicated group of fish showed some degenerative changes and decreased number of seminiferous tubules; the ovaries showed deformation from their normal shapes and severe lymphocytic infiltration and the brain also showed degeneration of neurons, severe loss of granular cells. So that our present study will create an awareness in societies on environmental pollution on edible aquatic animals. We recommended improving the water quality of these waterbodies and enforcement of law and legislations by the Government regarding the protection of these waterbodies.

**Keywords:** Pollution, heavy metal, physicochemical parameter, tilapia, histopathology, reproduction

### Introduction

Fish can prefer an optimal environmental condition for its growth and reproduction. Therefore, to keep the water body conducive for fish growth and reproduction, physicochemical parameters may be monitored regularly. Any adverse change in this parameter causes stress on the fish. If such a change increases arithmetically, then the stress on fish may increase geometrically. Nowadays, heavy metals pollution causes a threat to the aquatic environment and to its inhabitants when their concentrations exceed safe limits. Fish play an important role in the assessment of pollution potential risks (Lakra & Nagpure 2009; Magar & Biase 2013) <sup>[1, 2]</sup>. Fish which exposed to pollutants especially heavy metals such as (Cd, Pb, and Cu) may suffer from acute or chronic toxicity which affects all physiological processes including reproduction. The healthy reproductive process of fish is an important indicator of the fish to be able to sustain itself (Zulfahmi *et al.*, 2018) <sup>[3]</sup>. Many of the heavy metals are considered as essential nutrient elements that positively improve the growth and feed utilization of fishes (Ghazi *et al.*, 2022) <sup>[4]</sup> but upon crossing the maximum tolerable limit these metals cause not only a hazard to fish health (Jeziarska *et al.*, 2009) <sup>[5]</sup> but also to human consumers and the disruption of ecological systems (Sarkar *et al.*, 2016) <sup>[6]</sup>. Histopathological alteration or cellular changes in tissues such as brain, ovary, and testes have received much attention in assessment of the effects of environmental pollution (Blazer, 2002) <sup>[7]</sup>. Historically, there is no more attention to endocrine, neural and gonadal

histology; all systems that affect reproduction. There are many environmental chemicals that act as endocrine disruptors and affect fish reproduction, this led to an interest in assessing the reproductive health of fish (Carnevali *et al.*, 2018) <sup>[8]</sup>. Water pollution happens when pollutants discharged directly or indirectly into lakes, rivers, etc. without the removal of harmful substances. Water pollution affects fish and other organisms living in this water. The environmental pollutants especially heavy metals may increase the incidence of diseases by reduction of immune state, reproductive and developmental processes of the organism (Kaoud & El-Dahshan 2008) <sup>[9]</sup>. So, determining and recognizing the level and toxicity of these metals in waterbodies is essential to take an action and make a decision. The aim of this work is to determine and compare the concentration of heavy metals (Pb, Cd and Cu) in different water samples and fish samples collected from different zones of North 24 parganas. The present study is also to investigate the effects of physicochemical parameters and heavy metals toxicity on reproduction of tilapia collected from different sampling sites of North 24 parganas. Till today, there is no data about heavy metal pollution of some areas of North 24 pargana's waterbodies and among this sampling sites, three sites are not located within industrial belt and so it can be used as a control zone in our experiment. Last of all, the most important part of our study also highlights the fact that industrialization is the principal reason for this pollution of water and deterioration of fish health. Water samples obtained from seriously

polluted waterbodies with waterbodies that are not exposed to pollution, so that societies will have awareness on environmental pollution on edible aquatic animals. The high level of pollution caused by heavy metals and their threat they pose to consumers and public health cannot be over emphasized. So, this work will create awareness on the harmful effect of heavy metals consumption and suggest ways by which pollution by heavy metals can be reduced and it is also necessary to enforce the law and legislations regarding the protection of the aquatic environment and also to save human life.

**Materials and Methods**

**Study area:** The study was conducted in a sewage-fed aquaculture pond in Kolkata and north 24 parganas. Five sites were chosen to carry out present study. The first sampling station (Site-I) is located at Gobardanga, North 24 parganas. The second sampling station (Site-II) is located at Mochlondopur, North 24 parganas. The third sampling station (Site-III) is located at khariberi, North 24 Parganas, West Bengal. Site-I, Site-II and Site-III, these three zones are also not located within industrial area. The fourth sampling station (Site-IV) is located at Salt Lake City. This water body is 5.7 km away from Dhapa, 5.4 km away from Chemical & Petrochemical Industries, Canal S Rd, Tangra and 20.2 km away from Kolkata leather complex. The fifth sampling station (Site-V) is Dhapa Manpur, North 24 Parganas. This water body is 1 km away from Dhapa, 4.8 km away from Chemical & Petrochemical Industries, Canal S Rd, Tangra and 12.5 km away from Kolkata leather complex. Site IV and site V are aquacultural ponds under east Kolkata wetland.

**Collection of water samples**

The study was carried out for a period of one year from August 2022 to July 2023. The samples were taken fortnightly at 1st day and 15th day of a month containing 24 sampling days.

**Collection of fish samples**

Tilapias are a good source of essential amino acids, fatty acids, vitamins and minerals. They have good consumer acceptance, economically viable and are in low fat content. For this reasons we are chosen Tilapia. Fish specimens were collected a life form from five different sampling sites, then transported to the laboratory.

**Analysis of physicochemical parameters of water**

The parameters of water viz., pH, dissolved oxygen, biological oxygen demand, carbon dioxide, alkalinity, nitrite and phosphate were determined. All the parameters were analyzed following the standard methods (APHA, 1998) [10].

**Analysis of Heavy metal Toxicity of water and tissue samples**

Heavy metals (Copper, cadmium, lead) were measured using an atomic absorption spectrophotometer (APHA, 1998) [10].

**Histopathological studies**

To study the histopathology of testes, ovaries and brain, adult samples of Tilapia fish were collected from the studied area and brought alive to the laboratory. After dissecting the fish organs (testes, ovaries, and brain) were immediately

removed and fixed in formalin 20% solution for 24 h. The tissues were routinely dehydrated in an ascending series of alcohol, cleared in xylene and embedded in paraffin wax. Sections of 5-6 µm thick were cut, processed and stained with hematoxylin and eosin and then, Canada balsam was poured and covered with a cover glass. They were examined and photographed under microscope (Mohamed, 2009) [11].

**Results**

**Table 1:** Analysis of physicochemical parameters of water sample

Parameters	Study area (mean value)					Standard value IS :10500 (2012)	Stress
	Site-I	Site-II	Site-III	Site-IV	Site-V		
pH	7.6	7.4	7.1	6.8	6.4	7-9.5	<4, >11
DO (mg L <sup>-1</sup> )	6.26	6.10	5.73	4.78	4.12	3-5	<5
BOD (mg L <sup>-1</sup> )	3.21	3.68	4.83	6.45	7.13	3-6	>10
CO <sub>2</sub> (mg L <sup>-1</sup> )	3.37	3.76	4.42	6.89	8.31	0-10	>12
Nitrite (mg L <sup>-1</sup> )	0.001	0.013	0.09	0.12	0.18	0.02-2	>0.2
Phosphate (mg L <sup>-1</sup> )	0.21	0.38	1.01	2.83	2.96	0.03-2	>3

**Table 2:** Analysis of heavy metals toxicity of different waterbodies

Parameters	STUDY AREA (mean value)					Standard value IS :10500 (2012)	
	Site-I	Site-II	Site-III	Site-IV	Site-V	AL	PL
Cadmium (mg/l)	BDL	BDL	BDL	BDL	0.02	0.003	No relaxation
Copper (mg/l)	0.07	0.09	0.49	1.9	2.03	0.05	1.5
Lead (mg/l)	BDL	BDL	BDL	0.02	0.18	0.01	No relaxation

\*BDL: Below Detection level, AL: Acceptable level, PL: Permissible level

**Table 3:** Metals concentration (ppm) in brain in intoxicated group and control group. (mean ± S.E).

Group Test	Intoxicated Group	Control Group	Sig.
Cu	55.34 ± 2.977	8.968 ± 0.730	0.000 *
Pb	4.203 ± 0.911	0.390 ± 0.079	0.002 #
Cd	2.237 ± 0.402	0.039 ± 0.003	0.000 *

The mean difference is significant at the (\*: P<0.001, #: P<0.05) levels.

**Table 4:** Metals concentration (ppm) in ovary in intoxicated group and control group. (mean ± S.E).

Group Test	Intoxicated Group	Control Group	Sig.
Cu	21.349 ± 2.336	10.378 ± 1.435	0.020 #
Pb	2.826 ± 0.276	1.395 ± 0.221	0.029 #
Cd	0.505 ± 0.101	0.175 ± 0.040	0.039 #

The mean difference is significant at the (\*: P<0.001, #: P<0.05) levels.

**Table 5:** Metals concentration (ppm) in testis in intoxicated group and control group. (mean ± S.E).

Group Test	Intoxicated Group	Control Group	Sig.
Cu	10.683 ± 1.865	6.103 ± 1.019	0.064
Pb	2.956 ± 0.246	1.465 ± 0.253	0.045 *
Cd	1.737 ± 0.262	0.620 ± 0.223	0.110

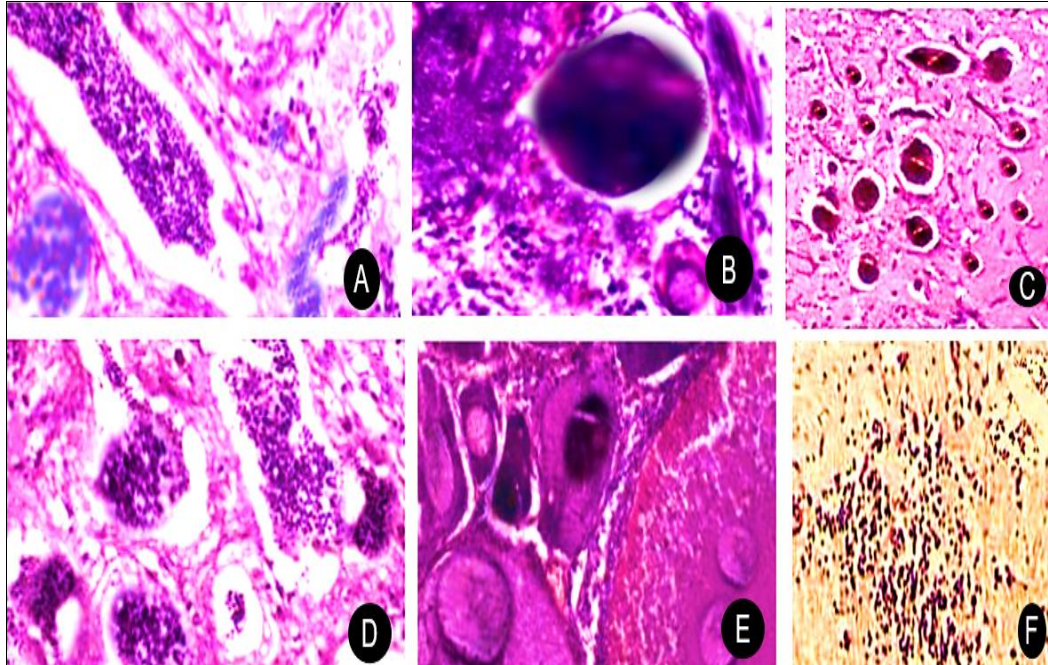
The mean difference is significant at the (\*: P<0.05) level.

### The histopathological analysis of selected organs as the following

**a. Testis:** Histopathological examination of the testis of the polluted group demonstrated that the testis of *Tilapia* showed loss of its ideal shape of seminiferous tubule and has residuals of primary and secondary spermatocytes. Testes display severe congestion in interstitial capillaries in polluted areas.

**b. Ovary:** Microscopically, the ovary of *Tilapia* showed severe lymphocytic infiltration. According to our study, the gonads of *Tilapia* are obviously suffering from deformation from their ideal shapes.

**c. Brain:** The histological section in the brain illustrated severe congestion and glial cell proliferation.



**Fig 3:** Normal tissues of *Tilapia* from non-polluted water bodies (control group); testis (A), ovaries (B), brain (C) and tissues of intoxicated *Tilapia* from polluted water bodies; testis (D), ovaries (E), brain (F)

### Discussion

#### Effect of physicochemical parameters of waterbodies on fish health

Fish have an average blood pH of 7.4 and it is conducive to fish life. pH between 7 to 8.5 is ideal for biological productivity, fishes can become stressed in water with a pH ranging from 4.0 to 6.5 and 9.0 to 11.0. In the present study, pH value of Site I, II, III are 7.6, 7.4 and 7.1 respectively. These values are ideal for fish growth and reproduction. But in Site IV and V, pH value is 6.8 and 6.4 respectively. This indicates poor pond productivity and reduced fish growth.

Dissolved oxygen is absolutely essential for growth and survival of fish. DO is essential to sustain fish for long period and 5.0 mg L<sup>-1</sup> are adequate in fishponds. The present study revealed that DO was remained slightly disturbed in Site IV and V during our study. But in other three water bodies viz Site I, II, III, DO level can exceed the desirable limit.

On the other hand, under low DO content, high CO<sub>2</sub> hinders oxygen uptake by fish, causing respiratory problem and stress. In our present study, especially Site V where DO level is low, CO<sub>2</sub> content is too high and this is harmful for fish growth.

BOD of fish ponds ranged from 3.0 - 6.0 mg/l in all ponds. Biochemical oxygen demand (BOD) is a measure of oxygen required by microbes to degrade the organic matter under aerobic condition. The excess entry of cattle and domestic sewage from the non-point sources and similarly increase in phosphate in the village ponds may be attributed to high organic load in these ponds thus causing higher level of

BOD. In the study, BOD level of Site IV and V can exceed the desirable limit. High BOD depletes the oxygen level to a critical condition thus indicating the pollution status of water.

In most natural waterbodies, nitrite content is generally low. But if the water body is contaminated with high organic pollution and has low DO, the nitrite content may increase to toxic level. In our study, in case of Site V, nitrite level can reach up to 0.18 mg/l and 0.12 mg/l for Site IV, it is sublethal to fishes. When fish absorbs nitrite, it reacts with hemoglobin to form met hemoglobin. Since, met hemoglobin is not effective as an oxygen carrier continued absorption of nitrite lead to hypoxia and cyanosis.

The phosphate level is tremendously high in Site V and Site IV. High levels of nitrates phosphates in water bodies indicate high levels of eutrophication in these two water bodies. These phosphates prove to be the key to highly algal blooms in water bodies, resulting in increased levels of contamination. The main sources of phosphate are detergents and soaps used for house cleaning, bathing and clothes cleaning, while nitrates are found in the discharge of treated domestic sewage water.

#### Effect of heavy metal on fish reproduction

Water pollution has a serious inhibitory effect in fish reproduction. The different pollutants such as heavy metals, industrial wastes, pesticides, and agricultural wastes, have histopathological effects on the reproductive tissues of fish gonads. The maximum acceptable levels of lead and cadmium in surface water were 0.01 mg/l and 0.003 mg/l

respectively according to IS:10500 (2012). On comparing our results, we can find that the levels of cadmium, lead, and copper in a water sample are higher than the acceptable limits in Site V. In IV, there is no trace of cadmium but lead and copper in water sample can exceed the acceptable limits. On the other hand, in Site I, II; there is no trace of Cd, Cu, and Pb. These two waterbodies are free from heavy metal pollution. In case of Site III, only copper can exceed the desirable limit.

Therefore, gonads deformation of Tilapia from their ideal shapes was found to indicate the effect of the heavy metal pollution on the Site IV and V. The deformations of testis and ovary at the studied site were obviously noticed. This is may be due to the presence of different kinds of pollutants and because of these two fisheries located near to Dhapa, Chemical & Petrochemical Industries, and Kolkata leather complex. In this study, the results of gonads histological examination sharply proved that the pollution especially by heavy metals had their great effect on the gonads of Tilapia and also the reproduction. Its effect appeared as a gonadal development disruption. The different pollutants such as heavy metals, industrial wastes have histopathological effects on the fish gonads. These effects may upset the development of germ cells and may decrease the ability of the fish to reproduce.

### Conclusion

There was strong evidence of a correlation between physicochemical parameter variation with heavy metal pollution of waterbodies and fish reproduction. The accumulation of metals in fish tissues depends on numerous factors, such as environmental conditions (pH, alkalinity, DO, CO<sub>2</sub>), exposure duration and species-specific living and feeding habits. In aquatic system, industrial wastes are potential source of heavy metal pollution in aquatic environment. Nowadays, the anthropogenic pollution of aquatic ecosystems increased the need for studies to identify the impact of heavy metals on the species living there. Monitoring programs for bioaccumulation measurements serve as a biomarker for fish from contaminated places and provide information about the environmental conditions. Histological changes provide a better assessment technique of fish health and to the effects of pollution on each biochemical parameter. Metal pollution may damage aquatic organisms at the cellular level and possibly affect the ecological balance. The present study documented that increasing water quality control and periodically environmental monitoring are needful for Site V and Site IV. Both of them are aquacultural pond under East Kolkata wetland and their fishes are brought to different fish markets of Kolkata. Therefore, it is necessary for enforcement of law and legislations by the Government regarding the protection of these two aquatic environments, thus save human life. Because heavy metal contamination in the body of aquatic organisms can biomagnified and persist in the food chain, results in transfer to the human body and today's

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