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Effects of bleached wastewater released from pulp and paper mills on the Genotoxicity of freshwater fish (*Channa punctatus* (Bloch, 1793))

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ABSTRACT

Water resource pollution is a significant issue that is only becoming worse even with the appropriate laws in place. Because effluent contaminants tend to accumulate in aquatic species, genotoxic researches on these pollutants are crucial. Therefore, using aquatic organisms to measure Genotoxicity is a highly helpful environmental monitoring technique. River Ami is a significant body of water in Maghar Sant Kabir Nagar, Uttar Pradesh, India that was contaminated by many industrial sources' untreated effluents. The current study set out to assess the genotoxic effects of these effluents on *Channa punctatus* fish as well as their toxic impacts on water quality. The research of Genotoxicity (the impact of bleached effluent on fish micronuclei) was conducted using the micronuclei. Fish treated with bleached effluent showed a significant ($p<0.05$) increase in the quantity of micronuclei compared to fish that were directly collected from the Aami River. Temperature, pH, DO, BOD, COD, TSS, and TDS are among the physico-chemical characteristics of a body of water that are assessed seasonally. All of these metrics were significantly higher than the Central Pollution Control Board of India's suggested tolerance limit.

***Keywords*:** DNA deterioration, *Channa punctatus*, micronuclei, BOD, DO, Temperature

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1. INTRODUCTION

All living things are known to be susceptible to damage from pollution of the aquatic environment. Since pollution is something that man creates on his own, it is regarded as the worst transgression against humanity. The majority of aquatic contaminants originate from agriculture and industrial runoff.

As a significant source of food, the fishing industry has been highlighted for its importance. Anthropogenic contaminants are constantly entering aquatic environments, with heavy metal ions, microbial toxins, and polycyclic aromatic hydrocarbons being a significant group of concern.

The aquatic food chain is being impacted by these pollutants, which could disrupt the ecosystem as a whole. Numerous researchers have examined the interaction between physico-chemical and biological variables in a variety of aquatic habitats [1–6]. In addition to being carcinogenic, pollutants can cause cancer, atherosclerosis, cardiovascular disease, and premature aging in humans. Genotoxicity is a harmful process that alters a cell's genetic makeup and compromises its integrity.

The term "Genotoxic pollution" refers to the entry of pollutants into the primary media and resident organisms' genomes that have the potential to be mutagenic, teratogenic, or carcinogenic [7-8]. Among these pollutants are some chemical compounds, such as heavy metals [9-10] ,Polycyclic aromatic hydrocarbons (PAHs) [11-13] and microbial toxins .These genotoxicants have been shown to create strong covalent bonds with DNA, which lead to the creation of DNA adducts that obstruct correct replication and cause mutations [14].

The World Health Organization has linked pollutants that damage sperm and eggs to genetic alterations that can be passed on to offspring, which goes against the ideals of sustainable development. portraying them as important contributors to birth defects, which cause 589,000 human deaths every year.

Fish are particularly vulnerable to the genotoxic effects of contaminants, which often include heavy metals, chemicals, and agricultural waste. Since fish are a highly sensitive bio-indicator of water quality and can indicate the potential risk of new chemicals introduced in the aquatic environment, it is possible to use fish as a model in ecogenotoxicology investigations and react to toxins similarly to how they metabolize foreign substances and gather contaminants [15, 16]. The purpose of this study was to assess the genotoxic effects on fish living in the river as well as the impacts of pollutants introduced into it without treatment on water quality.

2. MATERIALS AND METHODS

2. 1. Description about the paper mill

The Aami River receives effluent from Rayana Paper Board Industries Ltd., a pulp and paper company situated in Khalilabad, an industrial area in the Sant Kabir Nagar district of India. Approximately 64 tons of paper, principally writing and craft wrapping paper are produced daily by this pulp and paper plant.

2. 2. Collection of water samples

Water samples were obtained from five different locations.

Site 1: River flowing through Dhaurahra Drain (Effluent before treatment plant) where the different types of mill effluents are dumped together in the river.

Site 2: Part of river present near Sarya village (Effluent after treatment plant) India glycol limited IGL, Gorakhpur industrial development authority, GIDA, Sahjanwa Gorakhpur.

Site 3: The sugar mill situated in Rudhauli Satnariya Basti(Entry point of effluent treatment plant) discharge the treated and untreated mill effluent in river Ami,

Site 4:200 m away from upstream of entry point of effluent treatment plant.(Rudhauli sugar mill Satnariya Basti)

Site 5: 200 m away from downstream of entry point of effluent treatment plant.(Rudauli sugar mill Satnariya Basti).

Measurements of physico-chemical parameters, including chemical oxygen demand (COD), total solids (TDS), suspended solids (TSS), and biological oxygen demand (BOD), were made using the methods of Trivedi & Goel [13]. The pH was determined at the sampling location using the Water Analysis Kit, and the temperature was also recorded at that time using a thermometer.

2. 3. Collection of Experimental fish (*Channa punctatus*)

Fish *Channa punctatus,* measuring 15.5 ±1.20 cm in length and 27.21 ±1.83 grammes in weight, was obtained as a test subject from the Aami River in the Khalilabad, Sant Kabir Nagar region of India. To assist the fish acclimate to laboratory conditions, they were housed in glass tanks with 100 liters of dechlorinated tap water for a week. The water in Aquaria was constantly aerated. The deceased and injured organisms were removed from the tanks in order to avoid any possible contamination.

2. 4. Toxicity Experiment

In toxicology experimentssix litters of dechlorinated tap water were kept in glass aquariums containing ten *Channa punctatus* (experimental animals). For 24, 48, 72, and 96 hours, fish were exposed to bleached effluent that was collected from the Aami River at four different concentrations; control animals were kept in the same settings but were not given any treatment. Six runs of each set of experiments were conducted. Every 24 hours during the 96-hour observation period, mortality was recorded. To prevent the decomposition of their corpses, dead animals were removed from the experimental tank.

2. 5. Genotoxicity experiment

An investigation on Genotoxicity was carried out using the Das and Nanda approach . In toxicity experiment, common freshwater fish (*Channa punctatus*) were caught and treated to sublethal doses (40% and 80% of LC50 after 24 hours) of toxicant. The next day, the fish were used for the micronuclei test. Blood smear slides were made using Das and Nanda's technique [17]. Samples of peripheral blood were taken from the fish's caudal vein. Every assessment involved the analysis of 2500 cells or fish, with 7500 erythrocytes per slide. After the slide was air dried, it was fixed for ten to fifteen minutes in 100% methanol and stained for one to two hours in Geimsa (pH 7.0). For every slide, the average frequencies of anomalies were calculated and are shown in Table 2 as MN frequencies.

$$MN\% = \frac{\text{Number of cells containing micronucleus}}{\text{Total number of cells counted}} \times 1000$$

The each set of experiment mean ± standard error was determined. The control group and treated group of fish were compared using the Student's t-test .

3. RESULTS

3. 1. Physico-chemical study

Water is a necessary component for aquatic life to exist, and because of its unique qualities as a cultural medium, lakes' productivity naturally depends greatly on it. The over-influence of human activity is having a significant impact on freshwater systems. Any aquatic system's biological inhabitants are determined by the interplay of its physical and chemical factors. Table 1, presents the findings from the physico-chemical examination of water samples collected from specific locations during various seasons.

Table 1. physicochemical characteristics of sample water collected from different sites of sampling station of river Aami of seasonally (summer, rainy and winter season).

Properties	**Season**	**Chosen Sites**					**References value set by WHO (world health organization)**
		Site-1	Site-2	Site-3	Site -4	Site-5	
Temperature	summer	29.8±0.33	30.2±0.40	30.4±0.43	29.5±0.30	29.5±0.28	Should not exceed 5 °C than receiving water temperature
	winter	19.8±0.37	19.6±0.25	19.7±0.25	19.4±0.21	19.48±0.28	
	Rainy	29±0.35	29±0.26	30.0±0.25	28.1±0.78	28.4±0.63	
pH	Summer	8.56±0.09	8.42± 0.12	8.46±0.10	8.4±0.10	8.3±0.08	6.5-8.5
	winter	8.5±0.11	8.34±0.09	8.5±0.12	8.46±0.11	8.34±0.08	
	Rainy	8.16±0.23	8.2±0.20	8.6±0.19	7.96±0.12	7.94±0.16	
TSS (total suspended solids) (mg/l)	summer	853.6±112	579.1±4.63	800.4±116.4	815±111.3	816±0.08	100 m/l
	winter	703.4±81.5	677.4±89.98	700.4±83.38	705.8±81.4	705.6±81.02	
	Rainy	1243.4±17	1256.4±171	1228±177.8	1245.6±173.5	1244.8±173.2	
TDS(total dissolved solids) (mg/l)	summer	588.1±0.21	524.4±1.36	597.4± 6.06	584.8±0.97	583.4±1.20	500 -1500 mg/l
	winter	592.3 ±0.1	591.6±0.65	590.6±0.53	592.1±0.42	571.3±80.34	
	Rainy	598.4±0.48	595.4 ±0.77	593.1 ± 1.6	593.4±1.46	593.4±2.01	

DO (dissolved oxygen) (mg/l)	summer	2.08 ±0.23	2.04 ±0.20	1.68 ± 0.19	2.16±0.22	2.18±0.27	4 mg/l
	winter	2.3 ± 0.70	2.28 ±0.03	2.32 ± 0.06	2.5±0.07	2.5±0.1	
	Rainy	2.52 ± 0.09	2.4 ±0.08	2.42 ±0.14	2.48±0.11	2.42±0.01	
BOD (biochemical oxygen demand) (mg/l)	summer	811.4±25.9	811 .6±26.4	802.8±22.5	799.4±22.44	801±22.1	30 mg/l
	winter	1180.4±58	1177.4±54.9	1166.2±55.1	1109±59.58	1108.2±59.4	
	Rainy	685.2±57.2	685.4±55.3	747±60.7	703.8±50.63	702.8±50.61	
COD (chemical oxygen demand) (mg/l)	summer	1049.6±61	1003.9±42.6	1012.3±51.6	1037.2±65.07	1037±64.55	250 mg/l
	winter	1009.6±62	951.16±85.6	1039.6±40.7	1019±81.35	1017±81.8	
	Rainy	1093.6±21	1084.6±24.7	1092.4±20.2	1094.8±20.17	1094±20.59	

Seasons had an impact on the temperature of the water samples. Summertime records for temperature were made, with winter time records for the lowest. At site-3, the summer time temperature reached its maximum of 30-30.4 °C, while at site-2, the winter time temperature dropped to 19-20 °C. One crucial environmental component is the pH of the water. One of the most reliable ways to determine how acidic or alkaline water is to measure its pH value. pH values ranged from 7.94 ±0.16 to 7.96 ±0.12, with sites 4 and 5 having the lowest pH values and all three seasons having the highest pH values, from 8.5 ±0.08 to 8.56 ±0.06. The results of the Dissolved oxygen experiment showed that the lowest DO (2.08 ±0.23 to 1.68 ±0.19) were recorded in site -3, while the greatest DO (2.48 ±0.11 to 2.52 ±0.09. Biological Oxygen Demand (BOD) is consistently higher than the standard amount throughout the site-1, which measures 1180.4 ±58 to 1108 ±52 in the winter, and site-3, which measures 702.8 ±50.6 - 685.2 ±57.2 in the rainy season, have the lowest BOD values correspondingly. The quantity of oxygen needed to oxidize all organic matter, including biodegradable and non-biodegradable, is known as thechemical oxygen demand. Maximum COD level recorded at site-1 during rainy season is 1004.8 ±20.1 .While the lowest COD level at site-2 in winter season, is 951.1 ±85.6respectively.

The total suspended solid (TSS) The maximum TSS was recorded at site-1 during the rainy season, measuring 1243.4 ±17.2, and the lowest TSS was recorded at site-2 during the winter, measuring,677.4 ±89.98.

Total dissolve solids (TDS) The rainy season of site-1 had the greatest TDS value, which is 598.4 ±0.48 mg/l and minimum value of TDS were noted in winter at site-5 which were 524.4 ±1.36 respectively .

3. 2. Genotoxicity Observation

The fish that were treated with a different dose of LC50 had significantly more micronuclei than the control group. Different kinds of aberrations, such as MN, NN, LN, NB, and so forth, were identified in the MN test from both treated and untreated groups of fish in both laboratory and river conditions. Figure 1 and Table 2 display the findings of nuclear and micronucleus abnormalities in *Channa punctatus* peripheral erythrocytes. Every experiment revealed that, in comparison to the control group, the frequency of MN and other nuclear abnormalities were significantly greater ($p<0.05$).

Table 2. Showing frequency of micronuclei (MN) induced by pulp and paper mill effluents after ETP at different concentration in *Channa punctatus* fish treated with 40% and 80% of LC $_{50}$ (24h) of bleached effluent after 24 h and 96 h.

Exposure concentration	Time of exposure	Total no of cells scored	Total no of MN	% Micronuclei frequencies
Control	24 h	2117	00	00
	96 h	2057	00	00
40% of 24 h LC_{50}	24h	2102	04	03 ±0.73*
	96 h	2245	05	4.8 ±1.07*
80% of 24h LC_{50}	24 h	2165	06	6.3 ±1.33*
	96 h	2212	11	11.5 ±0.76*

Values are mean ± SE of six replicates. Data were analyzed through student t test .*, significant ($P<0.05$),when treated groups were compared with control.

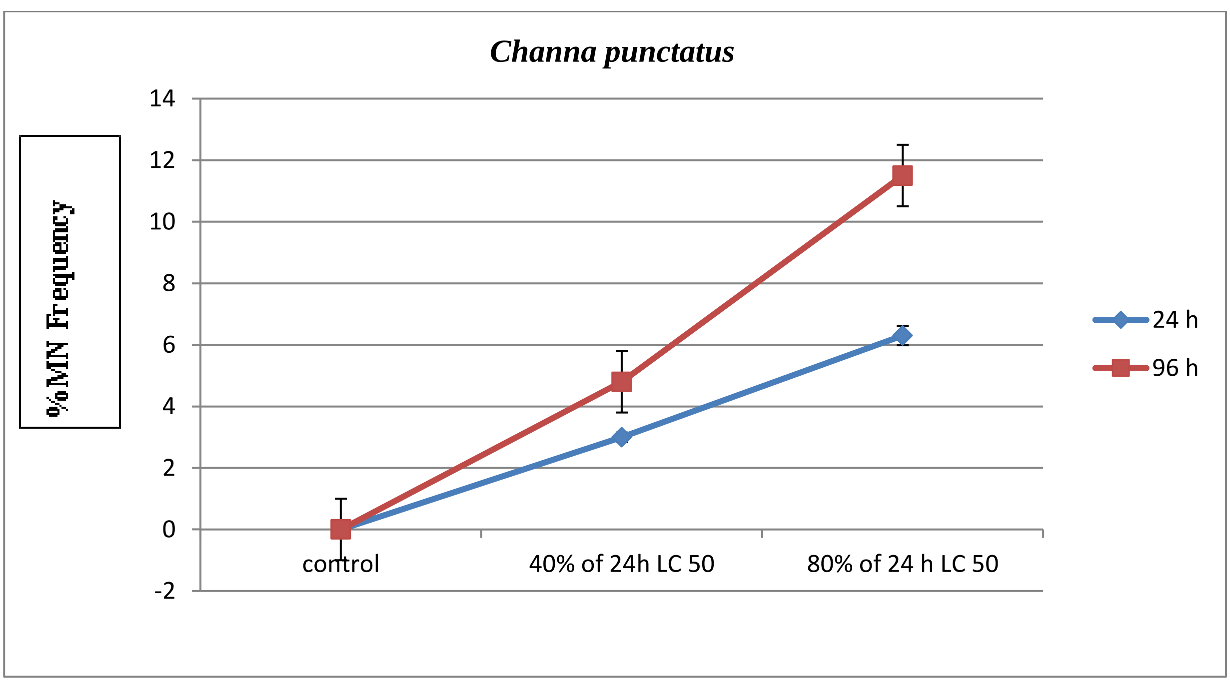

Figure 2. Bar diagram showing frequency of micronuclei of (MN) induced by pulp and paper mill effluent (after site-1)in erythrocytes of fish *Channa punctatus* after 24 and 96 h of exposure to 40% and 80% of LC_{50} (24 h) in laboratory conditio.

4. DISCUSSION

Because it affects the chemical and biological activity of organisms in aquatic media, temperature is a critical factor. Waste water typically has a high temperature due to the inclusion of warm water from industrial processes [18]. The rate of microbial activity also increases with temperature. Elevations in temperature have the potential to impede fish migration, hence having a detrimental impact on species reproduction. Changes in species composition, chemical reactions, and biological processes are all associated with pH fluctuations. It is one of the reliable methods for determining how acidic and alkaline the water is.

The ideal pH range that the WHO and the Central Pollution Control Board of India propose .One crucial factor in determining the quality of water is the amount of dissolved oxygen. Its presence is very beneficial to the aquatic ecosystem's biological life maintenance. The photosynthetic activity of aquatic plants and certain species of algae at the upper level of the water body may also be the cause of higher DO throughout the winter. The elevated DO concentration resulting from the phytoplankton bloom's prolific development [19]. Dissolved oxygen (DO) assessment is a crucial component of all ecological investigations connected to pollution. An increase in BOD may result from the excessive release of household materials, domestic was, animal and crop waste, and industrial waste water effluents.

Higher types of aquatic life may access oxygen more quickly the higher the BOD. Both BOD and COD quantify the amount of organic chemicals in water, which is a comparable purpose. It calculates the organic matter's carbonaceous factor. COD is a valuable indicator of water quality because it shows the mass of oxygen utilized and the amount of organic contaminants present in surface water. The quantity of solid present in both dissolved and suspended form is known as the total dissolved solid. The Central Pollution Control Board of India has advised a desirable limit of total solids of 20–1000 mg/L and 500–1500 mg/L, respectively. Particles of diverse sorts colloidal particles of different organic complexes make up suspended matter. For fish with a relatively high number of chromosomes, the MN test is one of the finest methods for determining the Genotoxicity of contaminants. Reactive oxygen species (ROS) are produced when the quantity of nitrogenous metabolites in blood rises, which has an impact on fish enzymatic systems.

Fish blood contains DNA stains due to the production of highly hazardous reactive intermediates, such as ROS [20]. Each anomaly is a sign of cytogenetic damage in fish, which can lead to failures in cell division or the process of cell death with mutagenity. DNA lesions can vary in size and shape, such as bud forms (attach to the main nucleus), lobed forms (envagination of nuclear membrane), or bridge forms. When amplified DNA is removed from the nucleus as a result of gene amplification via the breakage-fusion-bridge cycle, nuclear abnormalities may arise Binucleation, which can result in genetic imbalance in the blood, can also be caused by abnormal cell division brought on by the inhibition of cytokinesis [20].

5. CONCLUSION

It is clear that the Aami River becomes progressively contaminated as a result of sewage being dumped into bodies of water without first undergoing adequate treatment. The results of the water quality experiment showed that, with the exception of DO, all physico-chemical parameters temperature, pH, BOD, COD, TDS, and TSS values were higher than the levels suggested by the Central Pollution Control Board of India. Additionally, in the event of Genotoxicity, the nucleus was significantly damaged, resulting in the formation of micronuclei and other nuclear abnormalities.

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References

[1] Sukumaran PK and Das AK. Plankton abundance in relation to physico-chemical features in a peninsular manmade lake. *Environment and Ecology* 1999; 20(4): 873-879

[2] Pandey BN, Hussain S, Jha AK and Shyamanand. Seasonal fluctuation of zooplankton community in relation to certain parameters of river Ramzan of Kishanganj, Bihar. *Nature, Environment and Pollution Technology* 2004; 3(3): 325-330

[3] Chandrasekhar SVA. Cladoceran Fauna of Malda district, West Bengal. *Journal of Bombay Natural History Society* 2002; 99(3): 467-470

[4] Kumar A and Tripathi S. Zooplanktonic diversity in relation to aquaculture in some ponds of Durg-Bhillai city, Chhattisgarh state. *Nature, Environment and Pollution Technology 2004*; 3(2): 175-178

[5] Srivastava N, Harit G and Srivastava R. A study of physico-chemical characteristics of lakes around Jaipur, India, Journal of environmental biology. 2009; 30 (5): 889-894.

[6] Sharma LL and Sarang N. Physico-chemical limnology and productivity of Jaisamand Lake, Udaipur, Rajasthan/ *Poll. Research.* 2004; 23(1): 87-92.

[7] Fagr A, El-Shehawi AM and seehy MA. Micronuclease test in fish genom: A sensitive monitor for aquatic pollution. African journal of Biotechnology. 2008; 7(5): 606-612

[8] Badr EA and El-Dib SE. Effects of water pollution on the cell division cycle and chromosome behavior in Tillapia spp. *Egypt Journal. Genet. Cytol.* 1978: 7:193-200

[9] Matsumoto ST, Janaina R, Mario SM and Maria AM. Evaluation of the genotoxic potential due to the action of an effluent contaminated with chromium, by the Comet assay in CHO-Ki Cultures. *Caryologia* 2005; 58 (1): 40-46

[10] Pruski AM and Dixon DR. Effects of cadmium on nuclear integrity and DNA repair efficiency in the gill cells of Mytilus edulis L. *Aqua. Toxicol.* 2002; 57: 127-137

[11] IARC Polynuclear Aromatic Compounds, Part 1, Chemical, Environment and Experimental Data. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans.* 1983; 32: 33-91

[12] SantodonatoJ, Haward P and Basu D. Health and Ecological assessment of polynuclear aromatic hydrocarbons. *J. Environ. Pathol. Toxico* 1981; 5: 1-36

[13] Black JA, Birge WJ, Westerman AG and Francis PC. Comparative aquatic Toxicology of aromatic hydrocarbons. *Fund. Appl. Toxicol.* 1983; 3: 353-358

[14] Hartwell LH, Hood L, Goldberg ML, Reynold AE, Silver LM and Veres RC. Genetics: from genes to genomes. McGraw Hill Higher Education 2000; ISBN 0-07- 540923-2.p. 70-98 144-169, 179-182, 341-351

[15] Bailey E, Crespo S, Nonnotte C, Colino A, Leray L and Aubree A. Morphological and function alterations induced in trout intestine by dietary cadmium and lead. *Fish Biology* 1992; 28: 69-80

[16] Grisolia CK and Corderio CMT. Variability in micronucleus induction with different mutagens applied to several species of fish. *Gene. T Nol. Bios* 2000; 23: 235-239

[17] Das RK and Nanda NK. Induction of Micronuclei in peripheral erythrocytes of fish, Hetropneustes fossils by mitimycin C and paper mill effluents. *Mutatants Research Lett.* 1986; 175: 67-71

[18] Mohan V, Raj S, Padmavathy and Sivakumar S. Water quality parameters and it influences in the Ennore estuary and near Coastal Environment with respect to Industrial and Domestic sewage. *International Research Journal of Environment Sciences* 2013; 2(7): 20-25

[19] Swarnalatha N. Seasonal Rythms of various physio-chemical characteristic of lake. *Journal Mendel.* 1994; II (1): 65-66

[20] Afroz Z and Singh A. Toxic effects of pulp and paper mill effluents on Physico-chemical parameters of River Aami, Gorakhpur, Uttar Pradesh, India. *The Journal of Toxicology and Health. Photon.* 2013; 103: 234-243