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Impact of Paper Mill Effluent on Aquatic Organisms: A Review

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Abstract: Paper mill is one of the most polluting industries, affecting aquatic organisms particularly fishes. Industrial effluents exposed fishes containing harmful substances may be a serious threat to human health. Several studies were conducted globally on the impact of both treated and untreated paper mill effluents on fish. Therefore, the present review focuses the impact of paper mill effluent on fishes. This review showed wide ranges of effluent toxicity at organismal as well as at population levels. Several workers discussed endpoints of paper mill toxicity in fish ranging from acute toxicity, ethological responses, developmental and growth studies, immune toxicity, haematotoxicity, genotoxicity, histotoxicity as well as effects on enzymes, reproductive and endocrine disruption. On the basis of available literature, it can be concluded that effluent has potent oxidative stress inducers on one hand and haematological, biochemical, physiological and histopathological altering contaminants on the other. The review indicates that paper mill effluent may adversely affect the health of aquatic organisms, the fish and ultimately the human beings.

Keywords: Effluent, Toxic, Lethal, Chronic, Fish, Paper mill effluent, Haematological, Biochemical, Physiological, Histopathological

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Introduction

Industrial development increases the financial income of a country, but at the same time it leads to the degradation of the environment (Hossain and Rao, 2014). The pulp and paper industry has been considered to be a major consumer of natural resources and significant contributor of pollution to the environment. Globally it is the sixth largest polluter industry, discharging a variety of gaseous, liquid and solid wastes into the environment (Monte *et al*., 2009) and contributing 100 million kg of toxic pollutants that are being released every year in the environment (Cheremisinoff and Rosenfeld, 1998). These industries generate 220-380 m^3 of highly coloured and potentially toxic wastewater for every ton of paper produced (Badar and Farooqui, 2011). More

than 250 chemical substances have been found in effluents generated during various stages of the papermaking process. Several naturally occurring and xenobiotic chemicals that are created and released at different stages of papermaking are the source of their hazardous nature (Ali and Sreekrishna, 2001). In spite of their high water use throughout the paper-making process, pulp and paper industries contribute to river pollution by discharging their effluents (Jayabalakrishnan,, 2007; Tripathy and Panigrahi, 2023).

The effluent cause huge damage to the receiving waterbody if discharged untreated or partially treated because it has high biological oxygen demand, chemical oxygen demand, total dissolved solids, lignin, phenol, sulphur and sulphur compounds (Raj *et al.*, 2014). Besides these toxic dyes, bleeching agents, salts, acids and alkalis are also present in effluents discharged from pulp and paper mills. Heavy metals such as cadmium, copper, zinc and chromium are present in pulp and paper mill effluent which are ultimately released into aquatic environment (Zahrim *et al.*, 2007; Tripathy *et al*, 2022; Tripathy and Panigrahi, 2024). The major problems of the wastewaters released from paper industries are high organic content, dark brown coloration, adsorbable organic halide and toxic pollutants (Leuenberger *et al*., 1985). The presence of lignin in the waste, which is derived from raw cellulose material and is not easily biodegradable, makes the COD/BOD ratio of the waste very high. Resin acid is the main causes of effluent toxicity. The primary constituents of the effluents were chlorinated complex substances such as benzofuran, pentachlorophenol, dibenjop-dioxin, dichlorophenol, and trichlorophenol-guicol. Dioxin, which damages the immune system, interferes with hormones, causes problem with reproduction and development, accumulates in the fatty tissue of animals exposed to paper mill effluents and makes its way to people through the food chain and can cause cancer (Singh and Jahan, 2023). Thus, effluent needs appropriate treatment prior to release in the environment; otherwise, it creates major environmental issue because of the persistent dark brown colour due to lignin and its derivatives (Prasongsuk *et al*., 2009). The paper industry has been one of India's major sources of aquatic pollution. Its effluent contains various acute toxic and bio-accumulating components such as chlorophenols, fatty acids, and resin. These chemicals are responsible for alteration in metabolic processes among aquatic organisms (Prakash and Upadhyay, 2023).

In India, the paper industry has been one of the major sources of aquatic pollution (Pathan *et al*., 2009). The pulp and paper industry is one of the oldest industries in this country and there has been tremendous expansion of these industries during the last 25 years. Dichloroguicol, trichloroguicol, tetrachloroguicol and chlorinated phenols are major contaminants found in the effluent released from pulp and paper mill which are toxic to fish fauna (Mellanen *et al*., 1996). The Indian paper industry consumes about 250 m^3 of freshwater per ton of paper and generates the corresponding wastewater. In India, around 905.8 million m^3 of water is consumed, and around 695.7 million m^3 of wastewater is discharged annually by this sector. So paper mills are normally located at the banks of rivers to ensure an adequate supply of water (Prakash and Upadhyay, 2022). Various studies have been conducted regarding the environmental impacts of pulp and paper mill effluent on fish fauna. The present study, thus, is an attempt in reviewing toxic effects of paper mill effluents on fish.

Acute Toxicity of Industrial effluent:

Toxicity in terms of LC_{50} values is species-specific because individuals have different levels of response to the same dose or concentration of a toxicant or pollutant (Smith and Stratton, 1986). The static bioassay test results on *Anabus testudineus*, *Channa punctatus*, and *Clarias batrachus* revealed that while *Channa punctatus* and *Clarias batrachus* were relatively resistant to paper mill effluent, *Anabus testudineus* was comparatively most sensitive (Nanda *et al*., 2002). The 96 h LC_{50} values of the paper mill effluent were found 1.5%, 6.0%, 9.5%, 14.5%, 25.0%,

15.0% and 16.5% (v/v) in fish, *Puntius sophor* (without aerate), fingerlings of *Oreochromis mossambicus, Rasbora daniconius*, *Cyprinus carpio* fingerlings, *Puntius stigma, Channa punctatus* and *Puntius sophor* (with aerate) exposed to paper mill effluent, respectively (Kumar *et al*., 1991; Varadaraj and Subramanian,1991; Pathan *et al*., 2009; Sarwade, 2015; Dey *et al*., 2018; Prakash and Verma, 2020).

The 96 h LC_{50} values of different industrial effluents for different fish species has been reported by various researchers. These values were 70% (v/v) for *Channa striatus*, 2.35% (v/v) for *H. fossilis*, 0.80 % (v/v) for *L. rohita* for fertilizer industry effluent, respectively (Singh *et al*., 2019); 20, 6 and 22% (v/v) for *Labeo rohita* for tannery, electroplanting and textile effluents, respectively (Muley *et al*., 2007); 4.21 and 2.5% (v/v) for *H. fossilis* and *Lebistes reticulates*, respectively, exposed to galvanizing and raw bulk drug industrial effluents (Majumdar *et al*., 2007; Deshpande and Satyanarayan, 2011); 25% (v/v) for *Cyprinus carpio* exposed to textile dyeing industry effluent (Dhanalakshmi *et al*., 2008); 17 and 10% (v/v) for fresh water fish, *Poecilia reticulate* exposed to insecticide and pharma-ceutical industrial effluents, respectively (Malik *et al*., 2012); 15% for fish, *Lepidocephalus thermalis* exposed to sugar factory effluent (Hyalij, 2013); 0.259% (v/v) for *Lebistes reticulates* exposed to the untreated pesticide producing industrial effluent (Chavan *et al*., 2016). The static bioassay test result revealed that the mortality rate increased with increasing concentration of industrial effluent and for a particular concentration the mortality rate increases with increasing period of exposure (Yadav *et al*., 2007; Mishra *et al*., 2011; Singh *et al*., 2019; Prakash and Singh, 2020). Catfish were tolerant to industrial effluent than carps due to their physiological and ecological condition (Singh *et al*., 2019).

Impact of effluent on the Ethological Responses:

The changes in water quality alter the behaviour of aquatic animals besides causing mortality. The changes in the ethological responses are the most sensitive indication of potential toxic effects of any pollutant. Behaviour allows an organism to adjust to external and internal stimuli in order to meet the challenges of surviving in the changing environment. The behavioural changes among aquatic fauna have been considered to be sensitive indicator of toxicity (Yadav *et al*, 2007) because they can provide suitable indication about the environmental conditions (Dhanalakshmi *et al*., 2018). The toxicity and behavioral changes in *Rasbora daniconius* exposed to lethal concentration of paper mill effluent were also studied (Pathan *et al*., 2009). At different concentrations fish showed erratic swimming, jerky movement, rapid opercular movement, leaping out of water and thick mucous covering over the whole body surface. Similar results were observed when *Labeo rohita, Channa punctatus, Mystus vittatus* and *Clarias batrachus* were exposed to paper mill effluents (Srivastava *et al*., 2007, 2019; Mishra *et al*., 2011; Prakash and Verma, 2020). The alterations in ethological responses of different industrial effluents for different fish species has been reported by various researchers and any type of behavioural alterations may be related to the impairment of sensory organ systems particularly the mechano and chemo-receptor systems (Mishra *et al*., 2011; Singh *et al*., 2011; Singh and Pandey, 2020; Tiwari and Prakash, 2021).

Impact on Development and Growth:

The paper mill effluent has the potential to cause developmental effects in fish. Hatching of *Cyprinus carpio* eggs were preponed by 3-h, 2-h and 1-h in 4%, 2% and 1% paper mill effluent, respectively. Dose related mortality was also observed in the same study (Tyor *et al*., 2012a). Paper mill effluent altered axial curvature, caused head deformities, tail malformations, edema in pericardial region, microphthalmia, fused eyes/ single eye, deformity in the fin region, gills disorganized in the head region and delay in yolk absorption in embryo (Van and Ellis, 2002). Enlarged yolk sac leading to death of larvae is another frequently observed developmental anomaly in fish (Tyor *et al*., 2012a;

Dey *et al*., 2013). Hatching success can also significantly declined in mill effluent treated embryos of *Cyprinus carpio* (Tyor *et al*., 2012b). The paper mill effluent could cause growth rate decreases upto 50% in paper mill effluent exposed fish (Johnsen *et al*., 1998). The relative growth rates of the fish, *Puntius stigma* exposed to sublethal concen-trations of the paper mill effluents decreased with increase of the concentrations (Sarwade, 2015). This may be due to presence of toxic substance in the effluent.

Impact on Haematology and Immunology:

The effect of paper mill effluent on haematology was investigated in several studies. In *Tor putitora* RBC count and haemoglobin decreased significantly whereas WBC count showing increasing trend at all the concentrations. Pathological alterations of RBC of blood smears included deformation sticking, lysis and abnormal presence of senile RBC were observed (Joshi and Negi, 2011). These changes may be due to structural damage of RBC membranes resulting in haemolysis and destruction in haemoglobin production (Ukagwu *et al*., 2012). *Lithognathus mormyrus*, exposed to sublethal concentrations (20 ml/l) of paper mill effluent for 28 days, showed RBC count decreased significantly followed by reduction in both haemoglobin content and PCV resulting in macrocytic anemia. The erythrocytes indices, as mean corpuscular volume (MCV) and mean corpuscular heamoglobin (MCH) increased as well as mean corpuscular heamoglobin concentration (MCHC) decreased significantly with effluent concentrations compared to control. These changes in MCV and MCH, compensate the decrease in RBC, Hb and PCV values resulting in macrocytic anemia. The TLC increased significantly with increasing the concentration of effluent. Blood smears show that small lymphocytes and neutrophiles increases with increasing the concentration of effluent, that emphasize the compensatory and defensive reaction of fish to effluent (Wahbi *et al*., 2004). In another study, juvenile catfish, *Clarias gariepinus* exposed to paper mill effluent under natural condition revealed that fish exposed to effluent discharge site had lower haemoglobin %, RBC count, PCV% and higher concentrations of MCV, MCHC, MCH and WBC count indicating that *Clarias gariepinus* suffered from haemolytic anaemia and leucocytosis (Brinkworth *et al*., 2003). Significant reduction in RBC count, Hb% and PCV value were noticed leading to macrocytic anaemia in fishes, *Labeo rohita* and *Channa punctatus* (Srivastava *et al*., 2007). Decreased RBC count and increased WBC count in paper mill effluent treated fish, *Amblyceps mangois*, inferring blood balance disturbance and infection in fishes. Some effluent exposed fishes were also found to be anemic (Nath, 2016).

On exposure to paper mill effluents fishes produce a generalized stress response, leading to potential immune suppression in fish *Rutilus rutilus* (Jokinen *et al*., 1995). Serum immunoglobin levels were also reported to be decreased in paper mill effluent exposed fish, *Channa punctatus* (Fatima *et al*., 2001). Results demonstrated a suppressive effect of chemical constituents of paper and pulp mill effluent on the immune functions. They also recognized a modulatory effect of the temperature on immune functions.

Impact on Biochemical Parameters:

Biochemical study provides an early warning to potentially damaging alterations in stressed animals. Pulp and paper mill effluent compounds pollute the aquatic environment and are responsible for increased biochemical alterations in aquatic organism. Animal's health is influenced by the fluctuations or alterations in metabolic processes taking place in its body's tissues (Prakash, 2021a).

The treated paper mill effluent exposed male fish bullhead, *Cottus gobio* showed depletion in liver glycogen (Bucher *et al*., 1992). In a study in roach, *Rutilus rutilus*, alkaline phosphatise, serum GOT and GPT increased in fish exposed to paper mill effluent (Jeney *et al*., 2002). The depleted protein, lipid, and glycogen levels in paper mill effluent exposed fish, seabream *Lithognathus*

mormyrus reflect that during stressful conditions, fish used these biomolecules to fulfill their high energy demands (Wahbi *et al.*, 2004). The protein content in muscles and gonads of effluent exposed fish striped seabream, *Lithognathus mormyrus* decrease directly proportionate with effluent concentration when compared to control. High amounts of the amino acids glutamic and aspartic are found in seabream protein. While the ovaries and testis have larger levels of leucine, isoleucine, and arginine, the flesh of seabream has higher levels of alanine and lycine. As effluent concentrations increased, the overall amino acid content decline (Baer *et al.*, 2009). On investigating the histochemical components of the liver of *Rasbora daniconius* on exposure to paper mill effluent, metabolic crisis and impairment in fish liver were observed (Pathan *et al.*, 2009). A significant decrease in the protein content and enhancement of free amino acids in the muscles, liver and gill of paper mill effluent exposed fishes fish *Channa punctatus suggests* that the tissue protein might have undergone proteolysis, during the stressful situation (Verma and Prakash, 2021). A significant decrease in nucleic acids contents in heart, muscles, kidney, liver and gill of *Channa punctatus* after 15 days exposure to the paper mill effluent as compared with control. Although 10% paper mill effluent may not be lethal for *Channa punctatus* but it affects the growth and survival of entire population and community of fishes as the sublethal becomes lethal in the long term (Prakash, 2021a). A significant increase has been observed in peroxdase and superoxide dismutase enzymes activities in the liver of fish, exposed to sublethal concentrations as compared to control. The effect was more pronounced as the concentration of effluent and duration of exposure increased (Prakash, 2021b). A significant increase was observed in total blood serum lipid, cholesterol, and phospholipid with decreased triglyceride levels. A significant increase was observed in the cholesterol and triglycerides levels, whereas total lipids and phospholipid levels showed a marked decline in the liver of paper mill effluent exposed fish *Channa punctatus* (Prakash and Upadhyay, 2022). A significant increase in serum glucose, serum lactic acid, and liver lactic acid contents with a significant quantitative decline in liver glycogen among effluent exposed fish *Channa punctatus* were recorded during the investigation (Prakash and Upadhyay, 2023). Therefore, biochemical studies on the mechanism of action and their regulation is quite relevant and may give an insight into a better understanding in this context.

Impact on Morphology and Histopathology:

The effect of paper mill effluent on the morphology of fish *Heteropneustes fossilis* and *Channa punctatus* showed excessive mucous secretion and epidermal lesions during the period of exposure to effluent (Baruah and Das, 2002; Prakash and Verma, 2020). The effluent exposed fish *Channa punctatus* showed many changes in their gill, skin and fins. The gill colour changes from dark red to dull red and skin become erupted. The response of the fish towards toxicity was grossly dependent on concentration of effluents and length of exposure (Prakash and Verma, 2020).

The treated paper mill effluent exposed male fish bullhead, *Cottus gobio* showed necrosis in hepatocytes and high degree of liver parasitization (Bucher *et al*., 1992). In a histological examination of brown trout collected from upstream and downstream of river receiving effluent newspaper print mill, liver tissues of downstream fishes showed distinct structural changes in comparisons to fishes collected from upstream. The proportion of normal liver cells in the upstream group was 68%, but the downstream fish had 35%. This indicates that the papermill effluent has clearly caused histological changes in the fish (Johnsen *et al*., 1998).

The fish *Anguilla anguilla* showed marked skin and gill disruption as well as kidney malpighian corpuscle alterations, revealed intense spleen hemosiderosis and neoplastic lesions on chronic exposure to bleached kraft pulp mill effluent (Pacheco and Santos, 2002). On exposure to paper

mill effluent *Rasbora daniconius* revealed swollen hapatocytes, nuclear hypertrophy, rupture sinusoids, hemorrhages, vacuolation in hepatic cells and broken central vein in liver whereas kidney showed hypertrophy of hematopoietic tissues, cell necrosis (Pathan *et al*., 2010). Ovary of *Rasbora daniconius* showed marked degenerative changes like complete absorption of oocytes, broken zona radiata, degeneration of oocytes, cytoplasmic liquification and clumping, thickening of ovarian wall and disappearance of nucleus in mature oocytes (Pathan *et al*., 2012). Anomalies such as mucous secretion, leucocyte infiltration, curling of the secondary lamellae tip, clubbing, and the fusion of the secondary lamellae, aneurism, etc. were prominent in paper mill effluent exposed fish, *Cyprinus carpio*. The liver of effluent exposed fish showed inflammatory infiltration, cytoplasmic inclusion bodies, vacuolar degeneration, fatty degeneration, pyknosis, karyolysis, distorted pancreatic area, and piecemeal necrosis, among other pathological symptoms (Dey *et al.*, 2018). Histopathological examination of paper mill effluent exposed fish *Rasbora daniconius* revealed swollen hepatocytes, nuclear hypertrophy, rupture sinusoids, hemorrhages, vacuolation in hepatic cells and broken central vein in liver. Kidney of effluent exposed fish showed hypertrophy of hematopoietic tissues, cell necrosis, blocked glomerules with full of blood stain, dilated renal tubules with pyknotic nuclei and tubular necrosis. Histopathological changes were dose dependent (Pathan, 2020).

Impact on Reproductive System:

Bioactive substances derived from lignin and terpenoids present in paper mill effluent had the potential to adversely affect the fish reproduction (Hewitt *et al*., 2006). White sucker fish, exposed to bleached kraft mill effluent showed depressions of sex steroid hormones (McMaster *et al*., 1996). Sepúlveda *et al*. (2009) revealed a significant reduction in reproductive efficiency, egg quality and alterations in fry growth of paper mill effluent exposed largemouth bass, *Micropterus salmoides* because of decreased circulating steroids (Sepúlveda *et al*., 2001). Sexual maturation was delayed, gonad development was retarded, and the condition factor was higher in perch, *Perca fluviatilis* caught in the vicinity of the bleached pulp mill effluent outlet, than in perch captured elsewhere indicated that the bioactive substances present in the effluent inhibits the reproduction and disturb the growth (Sandström and Neuman, 2003). Basu *et al.* (2009) reported that pulp and paper mills effluents impair fish reproduction by interacting with neurotransmitter systems. 12% to 20% concentrations of treated bleached kraft mill effluent cause adverse effects on eggs and pro-larvae of striped bass (*Morone saxatilis*) (Burton *et al*., 2007). Paper mill effluent decreased the levels of circulating steroids testosterone in male goldfish. Male goldfish exposed to 100% bleached kraft mill effluent had a 60% and 10% drop in testis production of testosterone, and a 74% and 41% drop in blood testosterone, for 9- and 16-days exposures, respectively (Parrott *et al.*, 2000).

Genotoxicity:

Resin acids of pulp and paper mill effluent accumulate in sediments and bioavailable to fish and can cause teratogenicity in fish larvae. Even at low concentrations pulp mill effluent induced genotoxic effects in sea bass, including erythrocytic micronuclei and nuclear abnormalities (Oikari *et al*., 2002). Malik *et al*. (2009) studied the genotoxic toxicity of the paper mill effluent on the fish, *Channa punctatus* chromosomes and found chromosomal aberrations in effluent treated fish. It included centromeric gaps, chromatid break, attenuation, acentric fragments, pycnosis, polyploidy and chromosomal gaps. Gravato and Santos (2002) determined the effects of pulp mill effluent on population genetic structure of redbreast sunfish (*Lepomis auritus*).

Conclusion

Industries are expanding without concerning about the surrounding environment. A nation needs development, but the environment should

come first. Fish species are much sensitive to pulp and paper mills effluent, both in terms of short-term and long-term impacts. The effluents have major harmful effects on the water quality of the receiving water bodies which is not good for human use. The available literature, however, agrees that the aquatic biota is seriously threatened by pulp and paper mills. Because paper mill wastewater may contains mixture of various potentially toxic substances, such as heavy metals, oxidative stress inducers and endocrine disrupter which enter into the aquatic ecosystem and impact the water quality and disturb the life cycle of aquatic organisms. Some toxic substances like lignin are very active to damage the aquatic organisms both morphologically and metabolically. Alteration in hematological, histo-architectural and biochemical indices of fishes may act as biomarker for the pulp and paper mill led pollution in aquatic organisms. The Indian government has mandated that industrial effluents be treated before being released into waterbodies, and noncompliance is punishable by severe consequences. Thus, in addition to implementing the appropriate measures to treat the effluent, it is imperative to restrict the discharge of effluent in water bodies. This review will serve as a helpful guide to understand the impact of industrial effluent particularly paper mill on fishes and its consumers and to help take preventive action to save the planet from further harm.

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