

**The Study of Bacterial Communities and Organic  
Pollutants of Pulp and Paper Industries and  
Their Effects on Aquatic Ecosystem**

**SUMMARY OF THESIS**

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Babasaheb Bhimrao Ambedkar University  
(A Central University)**



**FOR THE DEGREE OF  
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**IN  
ENVIRONMENTAL MICROBIOLOGY**

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## SUMMARY

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The study of the thesis has been revealed that persistent organic pollutants are discharged as wastewater even after the secondary treatment process of the industry. These pollutants are persistent in nature and causes toxicity in aquatic ecosystem. The most common gaseous pollutants are methyl mercaptan, sulfur dioxide, sodium sulfide, hydrogen sulfide, sulfur, nitrogen, nitrogen dioxide, sulfur oxide, hydrogen peroxide, and chlorine dioxide. These are the main source of occupational health hazards, while the discharged wastewater contains lignocellulosic and chlorolignine compounds. The major reported organic pollutants are Chlorophenols, chloroguaiacols, chlorosyringols, chlorocatechols, octacosane, pentane, silane,  $\beta$ -sitosterol, octadecenoic acid amide, octylphenol, chlorovanillins, terpenes, nonylphenol, trimethylsilyl ether, and estradiol etc. During the bleaching and papermaking processes, a variety of metallic and non-metallic contaminants such as nickel, iron, chromium, lead, sulphur, phosphorus, zinc, and arsenic are released. PPI wastewater is also harmful to aquatic flora and wildlife because to additional refractory substances such as resin acids, fatty acids, dioxins, furans, and biocides. These chemicals are hazardous to aquatic flora and fauna directly. These contaminants have been shown to have estrogenic and androgenic effects in a variety of aquatic creatures. The induction of coliform bacteria in rivers, ponds, and aquatic resources as a result of PPI wastewater contamination has been recorded, posing health risks. Female fishes have been observed to be masculinized, with smaller gonads and other secondary sexual traits.

All the above study report of the thesis has been systematically described in the chapter first as introduction. Subsequently, the second chapter of the thesis has mentioned the objectives. Further, chapter three of the thesis is review of literature on the topic that has been described about the pulp and paper mill wastewater and their toxic profile of the organic compounds present in the effluent and role of bacterial communities found in the contaminated site. According to several studies, PPI is one of the most polluting industries due to the release of complicated hazardous gaseous and aquatic contaminants that are currently poorly understood. Methyl mercaptan, sulphur dioxide, sodium sulphide, hydrogen sulphide, sulphur, nitrogen, nitrogen dioxide, sulphur oxide, hydrogen peroxide, and chlorine dioxide are the most frequent gaseous pollutants. The discharged wastewater contains lignocellulosic and chlorolignine chemicals, are the main source of occupational health problems. Chlorphenols, chloroguaiacols, chlorosyringols, chlorocatechols,

octacosane, pentane, silane, -sitosterol, octadecenoic acid amide, octylphenol, chlorovanillins, terpenes, nonylphenol, trimethylsilyl ether, and estradiol are the most commonly reported organic. Some of these substances are screened under the USEPA's 2012 list of EDCs. During the bleaching and papermaking processes, a variety of metallic and non-metallic contaminants such as nickel, iron, chromium, lead, sulphur, phosphorus, zinc, and arsenic are released. PPI wastewater is also harmful to aquatic flora and wildlife due to presence of various refractory substances such as resin acids, fatty acids, dioxins, furans, and biocides. These chemicals are hazardous to aquatic plants and fauna directly. These contaminants have been shown to have estrogenic and androgenic effects in a variety of aquatic creatures. The induction of coliform bacteria in rivers, ponds, and aquatic resources as a result of PPI wastewater contamination has been recorded, posing health risks. Female fishes have been observed to be masculinized, with smaller gonads and other secondary sexual traits. Even after secondary treatment of PPI wastewater, the presence of mutagenic and androgenic chemicals as residual contaminants draws the attention to tertiary treatment for pollution protection to the aquatic ecosystem and environment.

The chapter four has been described about the detected toxic organic pollutants of the pulp paper industry. The discharged effluent contains different chemicals that have endocrine-disrupting properties. This is the first report on the detection of persistent organic pollutants and their toxicity in agricultural crop and aquatic ecosystems. However, due to the presence of organic chemical compounds in the affluent, there is an increase in the BOD, COD and TDS level in the aquatic resources and the aquatic organisms (*Tubifex-tubifex*) get affected in terms of their reproduction and other metabolic activities were stopped and they may be lost or died. Besides, these chemicals may also increase the toxicity level in the receiving water sources. The crop plants (*T. aestivum* and *B. campestris*) are also affected due to the excessive use of sludge and effluent in the crop field as irrigated water in undeveloped areas where there is no proper irrigation system available. The affected crop has been destroyed by their root length, shoot length and seed quality. The chemical pollutants of the effluent may also reach the body of higher animals through the food chain. The effect of effluent was noticed and found roots of the plants were reduced and tubifex worms cell wall became whitened and burst in above 25% concentration of effluent and sludge. Hence, this research article is very useful for the researchers for the understanding of the pollutants of paper mill effluent and their toxicity in the environment.

Chapter five of the thesis has mentioned the profiling of bacterial communities present at different sites of paper industry and their relationship between the toxic compounds detected from the same site. Sludge samples (PA1, PA2, and PA3) showed different physiochemical properties of each site. Toxic residual organic and inorganic POPs are found at each sampling site, the majority of which have been classified as endocrine disrupting chemicals. Furthermore, the ability of bacterial communities to detoxify pulp paper mill pollutants and mineralize metallic and non-metallic organic residual pollutants at different stages of mixing domestic waste and river water was revealed with the luxuriant growth of bacterial communities in polluted sludge at different stages. The bacterial communities' phylum *proteobacteria*, *firmicutes*, *chloroflexi*, and *bacteroidetes* are dominant in each sample. Due to the high content of pulp paper mill pollutants that represent metabolic activity and the mechanism of action in the bacterial communities, the bacterial communities have stress tolerance mechanisms that allow them to survive in extreme environments. These communities also decrease pollutant toxicity by using them as nutrient sources, detoxification processes, and remediation processes. The relationship between POPs in pulp paper mill sludge and bacterial populations is extremely useful for future study on paper industry contaminants and their toxicity assessment using bacterial communities.

The indicator bacterial populations of the pulp paper mill wastewater are discussed in Chapter six of the thesis. The identification of bacterial cultures was performed by the use of high chrome specific media and biochemical tests. The bacterial strain was isolated and biochemically characterized. Following morphological analysis of the bacterial strain, 16s RNA sequencing has been used to confirm the presence of bacterial communities in the effluent. Seed germination tests were performed to determine the effluent's toxicity. In this experiment, we found that increasing effluent concentration from 0 percent to 25 percent, 50 percent, 75 percent, and 100 percent reduced seed germination percentage. In addition, rising effluent concentration has reduced root and shoot length. It occurred as a result of the presence of hazardous organic contaminants in paper mill effluent. As a result, the overall findings of the study have been focused on the effluent toxicity profile as well as the isolation and characterization of bacterial communities. However, because bacterial isolates are pathogenic, they may be hazardous to human health. This pathogen may also percolate to the ground water and causes toxic effects on grounds water and ground water may not be used as drinking water. Further toxicity tests revealed that developing countries lacked

suitable irrigation techniques and that the wastewater of was used directly to irrigate crop areas. It also has an impact on crop plant growth. As a result, this research is extremely beneficial in determining the toxic profile of paper mill effluent and indicating that the effluent is extremely harmful to crops and humans. Before being released from the industry, it must be properly treated.

The toxicity of organic pollutants found in pulp and paper mill wastewater and sludge samples was assessed in Chapter 7. The wastewater from the pulp paper mill was extremely toxic, making it unsafe for both the aquatic environment and human health. Some of the identified organometallic contaminants had mutagenic, endocrine-disrupting chemical (EDC), and carcinogenic characteristics. Chemicals utilized in the papermaking process produce recalcitrant residuals that do not degrade completely. Toxicity test with *Heteropneustes fossilis* and *Tubifex-tubifex* provided strong evidence that wastewater pollution has a harmful impact on aquatic life. It can be inferred that physicochemical waste characterization revealed elevated pollution-loaded parameters that exceeded the permissible limit. Furthermore, wastewater from paper mills is not suitable for direct environmental disposal and must be cleaned and monitored before to discharge. The study has concluded that pulp paper mill wastewater contains several toxic pollutants which are toxic to freshwater *Heteropneustes fossilis* and *Tubifex-tubifex* even after its secondary treatment in the mill.

The paper industry released residual organic pollutants containing wastewater, which are detrimental to the terrestrial and aquatic ecosystems, as discussed in Chapter 8. Some of detected residual organic pollutants were found endocrine-disrupting chemical (EDCs) properties in different RT (N-(1,2,2,2-tetrachloroethyl) trimethylacetamide, octadecane, 1-iodo, oleic acid, trimethylsilyl ester, eicosane; hexadecane, 2-methyl-; octadecane, docosane; tricosane), these compounds are not removed during secondary treatment. In addition to these residual organic pollutants along with heavy metals (copper, chromium, cadmium, iron, lead, zinc and nickel) and beyond the permissible limits may also induce pathogenic bacteria and accumulate in the food chain. Toxicity testing revealed that waste water from the paper mill was extremely hazardous, making it unsuitable for plant growth, aquatic life, or human health. The toxicity test results on *T. aestivum* and *Tubifex-tubifex* indicates the toxic effects, which affected directly or indirectly all living organisms. In addition, crop (*T. aestivum*) is affected due to the excessive use of industrial

wastewater as irrigation water. However, wastewater contains large amount residues of plant extracts and pollutant that's why crop has been destroyed. The *Tubifex-tubifex* worms activity i.e their growth, reproduction were reduced and may be lost or died due to wastewater. As a result, this study found that it is highly valuable for researchers in understanding the contaminants in paper industry wastewater and their toxicity, as well as the need for tertiary treatment prior to wastewater disposal in the environment.

### **Conclusion**

As a result, this study found that it is highly valuable for researchers in understanding the contaminants in paper industry wastewater and their toxicity, as well as the need for tertiary treatment prior to waste disposal in the environment. According to the identified organic compounds from the different industries, the most common compounds are hexadecanoic acids, pentachlorophenol, decane, dodecane and octadecane. These detected compounds are highly toxic in nature and the toxicity of these compounds were also discussed in each chapter. However, the conclusion of the thesis study of each chapter is written at the end of the chapters. The presence of mutagenic and androgenic compounds as residual pollutants even after secondary treatment of PPI wastewater draws the attention to tertiary treatment for pollution prevention to the aquatic ecosystem and environment.

Based on the research work, seven original research papers have been published in high impact journals and two research papers are under consideration. While three conference papers were presented at national and international conferences. Besides, one book chapter is published in firm of reputed publishers.