Membrane and Water Treatment, *Vol. 16, No. 1 (2025) 49-59*
https://doi.org/10.12989/mwt.2025.16.1.049

Exploring the potential of rhizosphere mediated removal of pollutants in wastewater: Insights from UV and DOC Analysis

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(Received September 18, 2024, Revised December 6, 2024, Accepted December 11, 2024)

Abstract. Reed bed treatment systems utilize natural processes involving wetland vegetation, soils and their associated microbial assemblages to enhance the water quality for recycling. Reed plants are cost effective method of remediating the wastewater. In order to prove the removal mechanism from sewage and paper mill effluents, four different reed plant species, viz, *Canna indica* (Indian Shot), *Colocassia esculenta* (Taro), *Typha domingensis* (Southern Cattail) and *Xanthosoma sagittifolium* (Tannia), were compared for their removal efficacy. Up to 7 days, the screening was done with the effluents in four different reeds. The result shows that the *Canna indica* and *Colocassia esculenta* could be the better option for pollutant removal from the sewage and paper mill effluent, respectively. *Canna indica* showed the higher Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) removal by reducing the organic pollutant load due to presence of microbes near the rhizosphere which use oxygen as the source produced from the root's respiration process. This removal percentage was positively related to Radial Oxygen Loss (ROL) and microflora in the rhizosphere of reed plants. The highest ROL and the microbial population were recorded by the rhizosphere of *Canna indica* followed by *Colocasia esculenta* and *Typha domingensis*. Because of the high cost and limited effects of present physicochemical treatments in the wastewater treatment plant, this reed bed system can act as a cheaper process essential to remove the organic pollutants, thus making them suitable for agricultural and irrigation purposes. Therefore, it was concluded that *Canna indica* can be used as the best biological treatment choice for both the sewage and paper mill effluents.
Keywords: biofouling and reed bed system; pollutant removal; sewage reclamation; sustainability; wastewater treatment

1. Introduction

The water supply is anticipated to decline in many parts of the world. The water consumption for agriculture alone is predicted to rise by 20% in 2050 worldwide, and this increase would be much higher without any advancements in technology or legislative measures. Providing water for agriculture and food production is one of the most extensive demands on freshwater resources. Just 4% of the world's freshwater resources are found in India, despite the country housing almost 16% of the global population (Kaur *et al*. 2012). Per capita domestic water demand in India is expected to rise between 46 and 62 m^3/person/year by 2025 and 2050, respectively, from an estimated 31 m^3/person/year in 2000. Because of the rise in the average household water demand, there is a significant increase in the coverage of water supplies in both urban and rural areas (Amarasinghe *et al*. 2008). India's average annual freshwater availability per person has declined dramatically since 1951, from 5177 m^3 to 1869 m^3 in 2001 and 1588 m^3 in 2010, due to a growing population and a rapid loss of water resources. The anticipated reduction in water resource loss was 1341 m^3 in 2025 and 1140 m^3 in 2050. Thus, increasing the effectiveness of water use and recycling wastewater is essential for managing water resources (Ranade and Bhandari 2014). Nowadays, various industrial and commercial activities generate enormous amounts of wastewater. According to the Central Pollution Control Board (CPCB), India generated a massive 72,368 million liters of sewage daily in urban areas during 2020-21. Despite having the capacity to treat 31,841 million liters, only 26,869 million liters are currently being treated, falling far short of the sewage produced. This means that a significant portion, 72%, of urban sewage remains untreated and is dumped into water bodies like rivers, lakes, and aquifers, causing pollution and deteriorating water quality (Kelkar, 2023). India's 1,093 sewage treatment plants could only handle 26.9 billion liters of wastewater daily as of 2020/2021. Around 400 plants were either not working or still under construction. This means that only 37% of sewage was being treated, increasing the risk of diseases spread through contaminated food and water (Zandt 2024).

The pulp and paper industry ranked third in the world for intense water consumption in the production process

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ISSN: 2005-8624 (Print), 2092-7037 (Online)

after the metals and chemical industries, and it plays an integral role in the global economy. India ranks 15th worldwide in paper production, contributing about 3.7 per cent of total production. There are about 759 pulp and paper mills in India, each with an installed capacity of 12.7 million tonnes and producing 10.9 million tonnes per annum. The Indian pulp and paper industry is highly water intensive, consuming 100-250 m^3 freshwater/ton of paper production and generating the corresponding wastewater of 75-225 m^3 wastewater/ton of paper produced (Kumar and Chopra 2015). More than 80% of India's water use accounts for agriculture. However, growing demand from other uses, such as municipal and industrial, is leading to increased competition, especially near urban areas. Currently, water is polluted by anthropogenic and natural sources, so it is necessary to treat the wastewater to control water scarcity in future.

There are many technologies like the Active Sludge Process (ASP), Rotating Biological Contactor (RBC), Stabilization ponds (SP), Trickling Filter (TF) and oxidation ditch for wastewater treatment (Sayadi *et al*. 2012). Similarly, a natural wastewater treatment process is called "Constructed Wetland technology" or "Reed Bed System". Technology for treating wastewater must be suitable and long-lasting. It must also be less expensive, simple to use and maintain, and highly effective at eliminating heavy metals and organic debris. Natural therapeutic methods work better in developing nations. In warm regions especially, natural treatment solutions are thought to be among the finest available options (Duenas *et al*. 2003). Among the several natural systems employed to treat municipal wastewater with macrophytes are constructed wetlands or reed bed system technology. Commercially constructed wetland systems can treat wastewater effectively through biological means.

According to Ntengwe (2005), nutrients in wastewater help microorganisms flourish by giving them an optimal environment, including food, moisture, and temperature. These microbes assist in the breakdown of organic substances into stable end products in biological wastewater. Because their rhizomes provide the ideal environment for both bacterial and physiochemical processes, macrophytes, or plants, serve as vital to the Reed bed treatment system (Chen *et al*. 2009). Reed plants transport the oxygen produced by the process of photosynthesis and atmospheric gases entered to the rhizosphere zone of the plant through the aerenchyma via pressure gradient and diffusion. Plants adapted to aquatic environments consume oxygen through their roots. A significant portion of this oxygen is released into the soil surrounding the roots, creating an oxygen-rich zone. This process, known as radial oxygen loss (ROL), which is crucial for wastewater treatment in constructed wetlands. The oxygen released by the roots establishes diverse conditions within the root zone, from aerobic to anaerobic. Wetland plants play a pivotal role in pollutant removal within wetland ecosystems. Understanding how these plants release oxygen into the root zone (radial oxygen loss or ROL) is key to enhancing the overall efficiency of pollutant cleanup. The primary sources of oxygen for this process are photosynthesis and the exchange of gases with the atmosphere. The selected four reeds were based on the physiological characteristics of plants *i.e*., high tolerance to pollutants, extensively enhanced root systems and oxygen release in the rhizosphere. Apart from these, they were widely known for wastewater treatment such as nutrient removal, pathogen removal and organic matter degradation. In this concern, these four kinds of reeds were selected because of their specific properties in the role of wastewater treatment and also the easily existing reeds grow fastly in our region. The reed, *Canna indica* has demonstrated significant abilities in absorbing nutrients, which are prevalent in wastewater. Also, this species can thrive in various soil types and water conditions, making it adaptable to different reed bed designs and grows vigorously with their root system (Deguenon *et al*. 2016). The reed, *Colocassia esculenta* (taro), well suited for waterlogged conditions and have the ability to tolerate fluctuating conditions aids in the breakdown of organic pollutants (Kumar *et al*. 2019). *Xanthosoma sagittifolium* has been well-known for its potential in phytoremediation, particularly in reoving heavy metals from contaminated water and grows rapidly. Its fast growth also helps maintain oxygen levels in the substrate through root respiration (Kumar *et al*. 2019). The reed, *Typha domingensis* has been well known for its excellent role in removing TSS, BOD and total nitrogen from wastewater. It has larger survival rate even in challenging conditions, but mostly in the sewage treatment scenarios (Hadad *et al*. 2010 and Selvakumar *et al*. 2023). This study highlights the combined use of radial oxygen loss (ROL) and UV-DOC analysis to evaluate the effectiveness of *Canna indica*, *Colocasia esculenta*, *Xanthosoma sagittifolium*, and *Typha domingensis* in pollutant removal from sewage and paper mill wastewater. High ROL from *Canna indica* enhances aerobic microbial degradation of organic pollutants, resulting in significant reductions in biochemical oxygen demand (BOD) and chemical oxygen demand (COD).

While previous research has explored the pollutant removal abilities of *Canna indica*, *Colocasia esculenta*, *Xanthosoma sagittifolium*, and *Typha domingensis* in sewage and industrial wastewater treatment, there is limited comparative analysis on their efficacy in removing pollutants from both sewage and paper mill wastewaters. Our study addresses this gap by systematically analyzing the performance of these species under varying retention times and pollutant concentrations and also proven along with the radial oxygen loss experiment and UV analysis insights. We found that the interaction between species, such as the combination of *Canna indica* and *Typha domingensis* in treating sewage and *Colocassia esculenta* in paper mill effluent, significantly enhances the removal of BOD, COD, and TDS, a finding that has not been extensively reported. Furthermore, UV-DOC analysis reveals substantial decreases in dissolved organic carbon (DOC) and UV-absorbing organic compounds, particularly aromatic substances found in paper mill effluents. These findings underscore the potential of reed bed systems utilizing these species to improve the efficiency of organic pollutant removal and offer a sustainable solution for

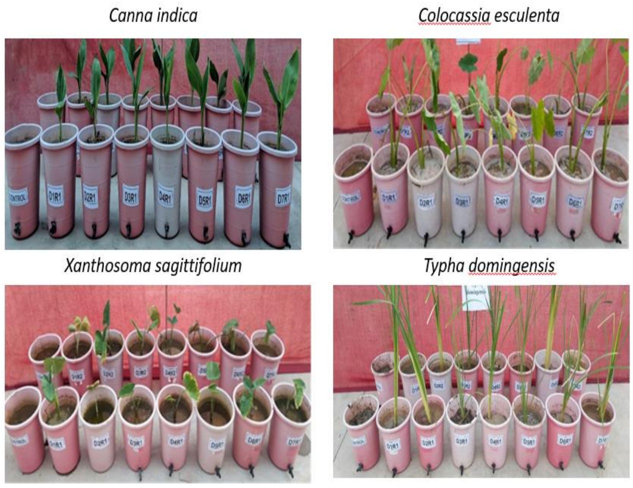

Fig. 1 Screening study setup with four reed plants

wastewater treatment. This integrates both ROL and UV-DOC into a cohesive framework, showing how these processes can be effectively utilized in reed bed systems for wastewater treatment. This contribution is crucial for optimizing the application of reed plants in treating complex industrial effluents through reed bed system, thereby advancing sustainable wastewater treatment technologies. With this prelude, this study aimed to identify the pollutant removal efficacy of four different reed plants for treating sewage and paper mill effluent along with their unique role of radial oxygen loss and rhizosphere microflora.

2. Materials and methods

2.1 Collection of reeds and characterization of effluents

The effluents were collected from sewage treatment plant (STP) and paper mill effluent from nearby Coimbatore. This study was carried out in Tamil Nadu Agricultural University (11.0122° N and 76.9354° E) in Coimbatore District, Tamil Nadu, India. This site usually experienced a maximum temperature of 38°C and a minimum of 19°C, with an average precipitation of 800 mm. Sampling was done thrice, and the average was considered the value of the initial characteristics.

The native reed plants were obtained from lakes and sewage entering areas in the Coimbatore district and the nearby irrigated areas of paper mill effluent. Botanical Survey of India (BSI), TNAU, Coimbatore verified and identified the reed as *Canna indica* (Indian Shot) near lakes, *Colocasia esculenta* (Taro) from paper mill areas, *Typha domingensis* (Southern Cattail) nearby lake and *Xanthosoma sagittifolium* (Tannia) near the sides of the polluted sites.

The sewage effluent and paper mill effluent characterized for the physio chemical parameters like pH, EC, TDS, TSS, TS, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), carbonates, bicarbonates, calcium, magnesium, chloride, sulphate, potassium content, total nitrogen and total phosphorus (Jackson 1973). After the samples were collected, analysis was completed immediately, and they were carefully preserved. The effluents were kept in polyethene plastic bottles, carried to the lab the same day, and kept cold and dark at 4°C till the experiment was conducted (Cavusoglu *et al*. 2010).

2.2 Characterization of substrates used for screening

The substrates used in this screening study were soil, sand and gravel. The physical and chemical properties of the substrates used were characterized by following the standard methods. The standard method determined the porosity of soil and sand (Chopra and kanwar, 1982). The soil mass was measured by weighing a balanced instrument and recorded in kg. The substrates' pH, electrical conductivity and redox potential are also characterized (Jackson 1973).

2.3 Screening of native reed plants

To identify the native reed plants suited to treat sewage and paper mill effluent, a pot culture experiment using a reed bed system was conducted at Tamil Nadu Agricultural University, Coimbatore. After thoroughly washing, the reeds were set aside for a month to develop steadily. In this investigation, pots of the same size that have a feature to collect treated effluent samples from the bottom were employed. The planting medium comprised 8 cm of gravel, coarse sand, and garden soil. The plants were layered in a sandwich pattern, with gravel at the bottom and coarse sand and garden soil on top. The reeds were evenly planted in the pots once their biomass was ascertained.

The parameters such as plant biomass, plant height, shoot height and root height were measured in native reeds, namely *Colocasia esculenta*, *Xanthosoma sagittifolium*, *Canna indica* and *Typha domingensis*, to determine the ability to grow with effluents and suitability for treating the sewage and paper mill effluents. The raw effluent of 7 litres was poured into each of the pots. The treated effluent from the pot culture experiment was collected for seven days with an interval of 24 hours, and they were analyzed by following the standard analytical procedures mentioned earlier. For screening, the treated effluents were extracted and collected daily using a valve opening from the bottom of the pots. The screening study setup is given in Fig. 1. The pollutant removal efficiency was calculated by influent concentration – (treated) effluent concentration / influent concentration × 100 (Yasar *et al*. 2018).

$$Efficiency\ of\ pollutant\ removal = \frac{Input\ Conc.* - Output\ Conc.}{Input\ Conc.} \times 100$$

**Conc. – Concentration*

2.4 Plant biometric observation

Plant height was recorded and the root length of the plants was measured using a meter scale and was recorded in cm. The distance between the tip of the shoot and its junction with the root was measured using a meter scale, and the mean was recorded as shoot length in cm. Plant

Fig. 2 Experimental setup for estimating the radial oxygen loss (ROL)

biomass was measured using a weighing balance instrument and recorded in gm. These biometric parameters were measured and compared between the reeds during the initial and final stages of the screening experiment.

2.5 Measurement of radial oxygen loss in rhizosphere of reed plants

According to Wang *et al*. (2015), the ROL in the rhizosphere of a few selected reed plants was determined using the titanium citrate method. The oxygen release rate from plant roots was measured by a colorimetric technique using titanium citrate solution. When combined with sodium citrate, titanium (III), a potent reducing agent, generates titanium citrate complex. By means of a first-order reaction, this complex will remove oxygen from the solution. Hoagland nutrition solution and titanium citrate solution were added to a two-liter container. 5 mL of titanium citrate were added to 100 mL of Hoagland solution. In order to remove the dissolved oxygen percent, the container was emptied and nitrogen gas was bubbled through for 20 minutes prior to adding titanium citrate solution.

Deionized water was used to gently and carefully wash one plant sample per flask in order to remove any extraneous objects. After covering the root base with parafilm up to a height of 6 cm above the collar, the roots were carefully placed into the flask, making sure they were fully submerged in the nutritional solution. The purpose of the parafilm coating was to stop shoot exposure to the parafilm oil or wax layer on the solution's surface as well as to stop any potential O_2 leaks. Immediately after overlaying the solution surface, 5 ml of titanium citrate was injected into the container. Following a 4-hour period, the container was gently shaken, and samples were extracted using a syringe through rubber tubing that had been inserted next to the roots and placed into solution. The samples are fed in the spectrophotometer and the absorbance was measured at 527 nm. Fig. 2 illustrate the system for measuring ROL estimating set up in a laboratory scale. The formula for calculating the ROL was given below (Tanaka *et al*. 2007):

Net rate of ROL was calculated as:

ROL = [c(y-z)]

Where, ROL= radial oxygen loss, µmol O_2 plant^{-1} d^{-1}

c = initial volume of Ti^{3+} citrate added to each test tube, L;

y = concentration of Ti^{3+} in solution of control (without plants), µmol Ti^{3+} L and

z = concentration of Ti^{3+} in solution after 4h treatment with plants µmol Ti^{3+} in solution (plant^{-1} L^{-1})

2.6 Microbial population from rhizosphere of reeds

The microbial population in reeds rhizosphere soil was determined by the standard methods as nutrient agar medium for bacteria and Martin's rose Bengal agar medium for fungi (Waksman and Fred 1922).

2.7 Measurements of Dissolved Organic Carbon (DOC) and UV_{254}

The samples were then placed in a 4C refrigerator to be further examined. The Shimadzu TOC-5000 Total Organic Carbon Analyser (catalyzed combustion) was used to measure the DOC. Samples were acidified to pH < 2 using 2 N HCl and CO_2 was removed by purging with oxygen for 4 minutes before to the analysis of DOC. Using a computer-controlled CARY 50 Bio UV-Visible Spectro-photometer, the absorbance at 254 nm (UV_{254}) was measured in a quartz cuvette measuring 1.0 cm. The relationship between optical density and optical length (1.0 cm) is used to express absorbance (cm^{-1}). The effluents were subjected to record the UV_{254} spectroscopic absorbance value. Thus, gives the quick measurement of organic matter in water body. The absorbance at wavelength 254nm was recorded and compared with untreated wastewater. According to Weishaar *et al*. (2003), SUVA values (specific UV absorbance; l mg^{-1} m^{-1}), which indicate the proportion of aromatic compounds in the total DOM, were computed as (UV_{254}/DOC) × 100 (Sayadi *et al*. 2006).

2.8 Statistical analysis

Using a 95% confidence level, the nutrient removal performance was optimized and assessed using Design Expert - 13 (D-optimal design). The significance of the variation in the amount of pollutant removed between the four reeds of treatment conditions with control (no plant) was ascertained using statistical analysis using a two-way (2 factor interaction) ANOVA (analysis of variance).

3. Results and discussion

Characterization of wastewater results were given in table 1 which indicated that the pH of sewage and paper mill raw effluent were 7.84 and 7.20, respectively. The electrical conductivity of both the samples was 2.41 and 3.03 dSm^{-1}. BOD and COD were recorded as 256 and 180 mg L^{-1}. 469 and 534 mg L^{-1} respectively.

The initial values of TDS, TSS and TS for sewage were 1880 mg L^{-1}, 810 mg L^{-1} and 2690 mg L^{-1}, whereas for

Table 1 Characteristics of Wastewater employed for the study

Parameters	Raw Sewage Effluent	Raw Paper mill Effluent
pH	7.84	7.20
Electrical conductivity (dS m^{-1})	2.62	3.03
Total Solids (mg L^{-1})	2690	3580
Total Dissolved Solids (mg L^{-1})	1880	2020
Total Suspended Solids (mg L^{-1})	810	1560
Biological Oxygen Demand (mg L^{-1})	256	180
Chemical Oxygen Demand (mg L^{-1})	469	534
Total Nitrogen (%)	0.27	0.28
Total Phosphorus (%)	0.34	0.37

Table 2 Characteristics of the substrates used for the reed bed system

Parameters	Soil	Sand	Gravel
Porosity (%)	31	48	-
pH	7.81	7.32	-
EC (dS m^{-1})	1.03	0.18	-
Redox potential (mV)	-694	-272	-
Mass (kg)	3.5	2	2.50

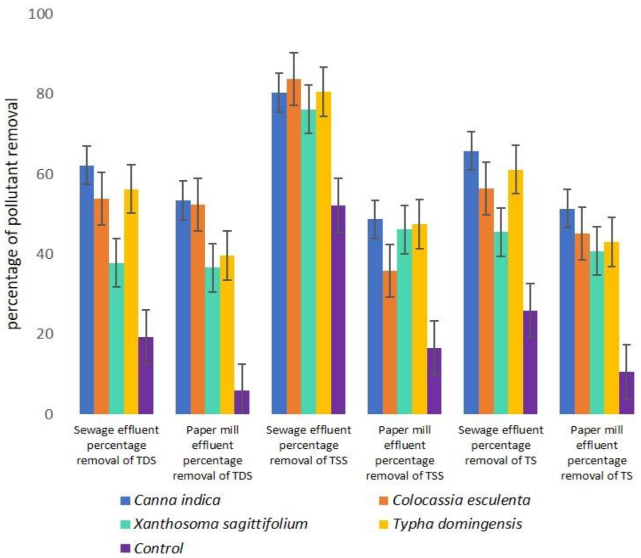

Fig. 3 Pollutant removal trend by different reeds

paper mill raw effluent, were 2020 mg L^{-1}, 1560 mg L^{-1} and 3580 mg L^{-1}, respectively. The anion and cations of sewage and paper mill effluents are given in Table 1. The sewage samples recorded as 0.27% and 0.34%, whereas, the paper mill effluent recorded as 0.28 and 0.37% of total nitrogen and phosphorus respectively. The characteristics of the substrates used for the reed bed system are furnished in Table 2. The soil used for the study was categorized as black soil, which had a porosity of 48 % with a pH, EC, Redox potential and mass of 7.81, 1.03 dS m^{-1}, -694 mV and 3.5 kg, respectively.

Porosity, pH, EC, redox potential and mass of the fine sand used for the reed bed system are 34 %, 7.32, 0.18 dS m^{-1}, -272 mV and 2 kg, respectively. The total mass of the gravel used in the reed bed system is 2.50 kg. The difference in biometric parameters of different reeds are given in Table 3. The highest increase in plant biomass, plant height, shoot height and root height was observed in *Canna indica*, which recorded 25.23g, 7cm, 4.3cm and 2.7 cm, respectively. The plant biometric observation was compared for four reeds after seven days. The growth of reeds was observed and increased in the order of *Canna indica* > *Colocasia esculenta* > *Typha domingensis* > *Xanthosoma sagittifolium*.

3.1 Efficacy of reed plants in the removal of TDS, TSS, and TS

Variation in pH and electrical conductivity were observed in every stage of the crop growth in both effluents. The reduction is due to the plant's ability to uptake the soluble ions through roots (Vymazal 2011).

With a retention period of seven days, *Canna indica* had the highest percentage removal of total dissolved solids (62.18%), and *Colocasia esculenta* had the high potential to remove total suspended solids (83.67 percent) in sewage effluent. In contrast, *Canna indica* showed highest removal of TDS and TSS in paper mill effluent. Fig. 3 depicts the trend of the pollutant removal percentage in both the effluents. This reduction in the pollutant removal in terms of TSS and TDS may be due to the physical action of substrates utilized in the reed bed system, such as filtration and settlement. Filtration/interception and flocculation/ sedimentation are the primary approaches used by the FWS wetland to remove suspended particles. This reed plants have an active coating of biofilm termed periphyton covering their surfaces, which allows them to absorb soluble and colloidal materials. Following their metabolization, these solids could be transformed into gasses or biomass. The water column may then be rented by the substance (Wang *et al*. 2018).

According to a review by Wang *et al*. (2018), the gravitational interception of solid items between sand and stone particles, as well as the blocking impact of macrophyte roots, contribute significantly to the removal of suspended solids in reed beds. He suggested that ionized pollutants can be eliminated through adsorption on macrophyte root surfaces. Therefore, the reduction in suspended solids removal may be due to the gravity settling, straining and adsorption onto gravel and plant media.

Canna indica has a fibrous root system (rhizome), which is effective in trapping suspended solids and facilitating the absorption of dissolved nutrients, thereby contributing to lower TDS levels in both the wastewaters. Similarly, *Typha domingensis* has extensive rhizome network which improves the nutrient uptake and supports microbial activity, which aids in TDS reduction through adsorption and absorption mechanisms. Also, these reed plants were able to thrive in water logged conditions, because of this condition, it influences the microbial diversity in the rhizosphere regions. Both *Colocasia esculenta* and *Xanthosoma sagittifolium* are valuable for TDS removal in wastewater treatment systems due to their robust growth characteristics, support for microbial

Table 3 Biometric observation of reed plants

Reeds	Plant Biomass (g)		Plant Height (cm)		Shoot Length (cm)		Root Length (cm)	
	Initial (D1)	Final (D7)	Initial (D1)	Final (D7)	Initial (D1)	Final (D7)	Initial (D1)	Final (D7)
Canna indica	99.78	113.52	64.2	71.2	43.2	47.5	21.00	23.7
Colocasia esculenta	139.12	156.35	105.2	108.3	78.2	79.1	27.00	29.2
Xanthosoma sagittifolium	123.56	124.62	60.2	61.1	50.1	50.2	10.1	11.1
Typha domingensis	103.54	106.23	135.4	138.2	128.5	130.4	6.9	7.8

Table 4 Results of Two Factor Interaction ANOVA comparing the effects of reeds, retention time and combination of reeds × retention time on removal rate of TDS, TSS, TS, BOD and COD in the wastewater treatment

Factor	TDS		TSS		TS		BOD		COD	
	F ratio	p-value	F ratio	p-value	F ratio	p-value	F ratio	p-value	F ratio	p-value
Retention time	1044.3	<0.0001	847.10	<0.0001	1834.7	<0.0001	1949.8	<0.0001	854.2	<0.0001
Reeds	394.4	<0.0001	139.8	<0.0001	583.9	<0.0001	377.2	<0.0001	109.03	<0.0001
Retention time × Reeds	38.00	<0.0001	32.68	<0.0001	66.34	<0.0001	75.93	<0.0001	38.96	<0.0001
CV* (%)	2.53		1.94		2.09		2.92		2.25	

(CV* – Coefficient of variation)

communities, and adaptability to polluted environments. Their combined use can enhance the efficiency of reed bed system or other phytoremediation systems treating paper mill effluents or similar wastewaters (Peng *et al*. 2020). Apart from this, the retention time also play a vital role in pollutant removal. Longer retention time (day 7) allows the water and reed roots, lowering the TDS levels.

3.2 Efficacy of reeds in removal of BOD and COD of effluents

After a seven-day retention period, *Colocasia esculenta* and *Xanthosoma sagittifolium* showed the highest percentage elimination of BOD (77.22 and 76.67%) in the paper mill effluent. In contrast, in sewage effluent, *Canna indica* (75.39%) and *Typha domingensis* (67.19%) showed a higher reduction in the BOD level (Figure 4). This kind of decrease in the effluent's organic matter may result from hydrolysis, which changes the organic matter into a soluble state so that it can enter the media and attach itself to biofilm to decompose. Periphyton are affixed to the leaves and stems of rooted plants. Plankton are organisms that accompany the flow of water. Neuston are aquatic organisms that are at rest on the water's surface.

Nektons are organisms that can move through the water on their own action. Potentially the most significant players in the breakdown of organic materials are fungi, and bacteria.

Macrophytes are aquatic plants that live on the water's surface and are crucial to the water's ability to produce oxygen (Norton 2014). According to a study by Rehman *et al*. (2017), oxygen released through a plant's roots improves the aerobic bacteria in the biofilm, promoting the breakdown of organic pollutants. Redox processes result in the breakdown of pollutants into more unstable chemicals, according to a review by Wang *et al*. (2018). An increase in organic load causes a greater BOD concentration in the effluent. The elimination of organic materials by both attached and free-living bacteria may cause the BOD reduction. The aquatic plants' root systems provide more surface area for microorganisms, speeding up the breakdown of organic materials because the organic material was made up of about 50% carbon.

It has been proved that the reed plants had the potential to reduce the COD in both the wastewater. This might be the result of physical processes like filtration and sedimentation working in tandem with the breakdown of aerobic and anaerobic microorganisms. Within seven days of retention time, *Colocasia esculenta* (50.56%) showed the most significant COD removal in the effluent, followed by *Canna indica* (49.25%) in treated paper mill effluent. Similarly, in the sewage effluent, *Canna indica* (52.72%) and *Xanthosoma sagittifolium* (49.45%) showed the highest percentage elimination of COD compared to paper mill effluent. The total decrease in organic and inorganic materials is caused by the coupling effects between microbes and plants, as well as the breakdown of microorganisms (Wang *et al*. 2018). Two Factor Interaction (2 FI) ANOVA showed that the activity of the pollutant removal rate by the reeds differed statistically according to the retention time, reeds and combined effect of reeds × retention time. The coefficient of variation shows that the level of dispersion in the pollutant removal rate around the mean value for the parameters of TDS, TSS, TS, BOD and COD (Table 4, $p<0.0001$). The table interprets that each reed shows a significant effect on all the parameters (*eg*., F-ration of 394.4 for TDS), with p-value less than 0.0001, confirming a strong influence of reeds in pollutant removal.

Retention time (*i.e*. day 7) has a significant effect on all the parameters (TDS, TSS, TS, BOD and COD) with very

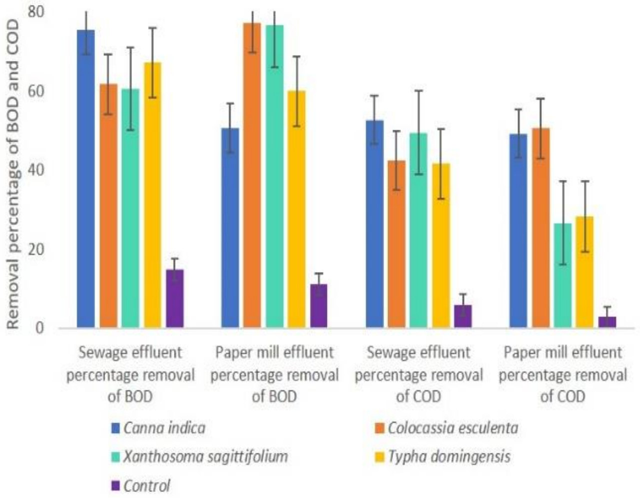

Fig. 4 Removal percentage of BOD and COD in the effluents with various reeds

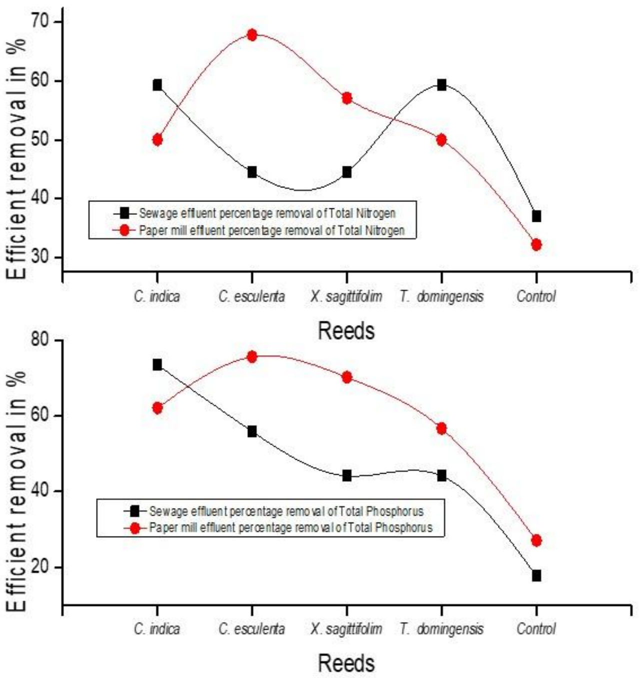

Fig. 5 Nutrient removal pattern with various reeds

high F-values. Based on the retention time, high significant reduction was observed in BOD. By combining the factor reeds and retention time that shows significant effect for all parameter by the effect of longer the accumulation of effluent near the rhizosphere region of the reeds, greater the removal of pollutants from wastewater.

3.3 Efficacy of reeds in removal of nutrient from effluents

Fig. 5 illustrates the nutrient removal efficiency of reed plants used in the study. *Canna indica* and *Typha domingensis* showed a greater nitrogen reduction (59.26%) among the reeds over a seven-day retention period in treated sewage effluent. Alternatively, the declining trend of total nitrogen was more in *Colocassia esculenta* (67.86%) for paper mill effluent. After seven days of retention, the total nitrogen concentration was at its lowest, measuring 0.09 percent. The nitrogen content decreased as the retention period extended from the first to the seventh day. This elimination might be brought about by ammonification, volatilization, denitrification, fixation, adsorption of ammonia, ANAMMOX and plant absorption (Norton 2014) or microbial metabolism in the rhizosphere zone (Vymazal 2020). Anaerobic heterotrophic microbial denitrification is frequently regulated by the availability of labile carbon substrates and oxygen (O_2). Ong *et al*. (2011) stated that nitrification is an anaerobic chemoautotrophic process and in wastewater, nitrification and denitrification are the primary methods to remove nitrogen (Sudarsan *et al*. 2015).

Canna indica (73.53%) outperformed well in reducing total phosphorus in sewage effluent after seven days of retention when compared with other reeds. Apart from *Canna indica*, *Typha domingensis* and *Colocasia esculenta* have been found to remove 44.12% and 55.88% of the total phosphorus, respectively. In treating the paper mill effluent, the highest removal efficiency was found in *Colocassia esculenta* (75.68%). The order of phosphorus removal from paper mill effluent was found to be *Colocassia esculenta* > *Xanthosoma sagittifolium* > *Canna indica* > *Typha domingensis*. In the process of eutrophicating lakes or any other body of water, phosphorus plays a significant part. It is, therefore, essential to treat wastewater to remove phosphorus. According to the experimental study, the total phosphorus declined steadily from the first to the seventh day. This is because most phosphorus exists as an organophosphate, which the macrophytes utilized in the experiment could readily absorb. Similar findings are corroborated by Sayadi *et al*. (2012), who found that phosphorus removal effectiveness in a hybrid reed bed system treating wastewater with *Cyperus alternifolices* and *Canna indica* was 76%.

Table 5 Radial Oxygen Loss of different reed plants

	Sewage	Paper mill effluent
	ROL (µmol O_2 d^{-1} g^{-1} DW_{root})	ROL (µmol O_2 d^{-1} g^{-1} DW_{root})
Canna indica	33.4±0.306	32.7±0.655
Colocassia esculenta	29.2±0.137	33.9± 0.617
Xanthosoma sagittifolium	18.1± 0.452	28.6± 0.283
Typha domingensis	26.7± 0.125	25.4± 0.079
SED*	0.23	0.26
CD* (0.05)	0.45	0.35

(SED* – Standard error of deviation; CD* – Critical Difference)

3.4 Radial Oxygen Loss and rhizosphere microflora of reeds

The radial oxygen loss by the reeds used in the reed bed system was measured and presented in Table 5. The values of ROL were expressed as the amount of oxygen released from plant per day per root biomass at the temperature of 30° C and 1100 lumens of light intensity.

Table 6 Microbial population of rhizosphere soil

	Canna indica	*Colocassia esculenta*	*Xanthosoma sagittifolium*	*Typha domingensis*
Sewage Effluent				
Bacteria (x10^6 CFU g^{-1})	54.7	39.6	34.3	47.9
Fungi (x10^4 CFU g^{-1})	24.2	26.1	23.6	26.5
Actinomycetes (x10^2 CFU g^{-1})	3.7	1.4	1.2	2.9
Paper mill effluent				
Bacteria (x10^6 CFU g^{-1})	44.3	41.6	42.7	39.6
Fungi (x10^4CFU g^{-1})	24.2	23.5	21.4	25.3
Actinomycetes (x10^2 CFU g^{-1})	3.4	2.6	1.2	1.8

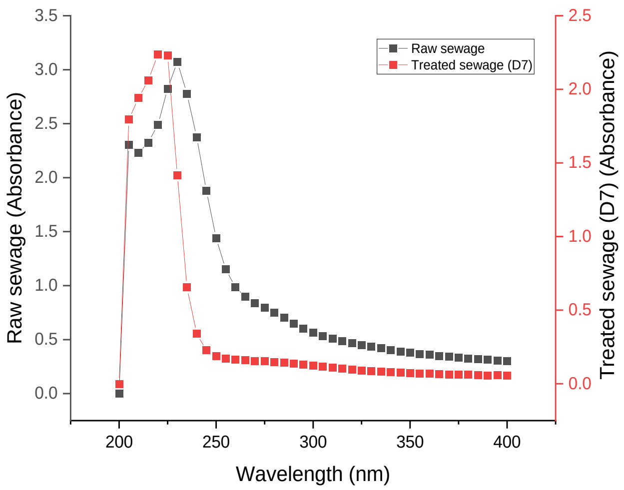

Fig. 6 Absorbance trend in sewage effluent

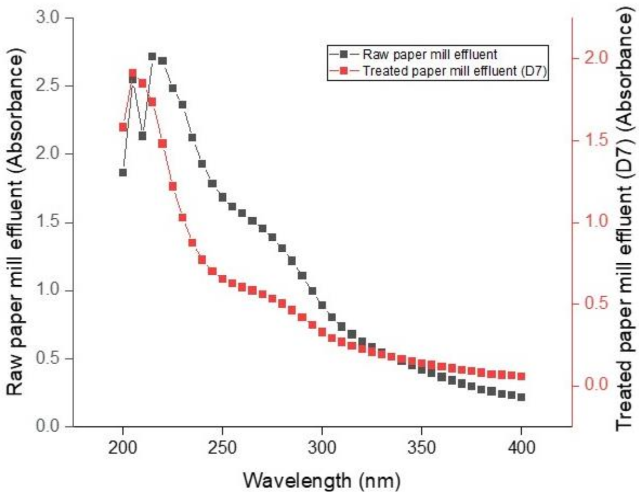

Fig. 7 Absorbance trend in paper mill effluent

By the measurement of ROL, it is determined that the ROL produced by the reeds were in the order of *Canna indica* > *Colocassia esculenta* > *Typha domingensis* > *Xanthosoma sagittifolium*. Reed plants have well-developed tissues that transport oxygen to their roots, enabling them to survive in flooded and partial- flooded conditions. In CWs, oxygen comes from the atmosphere, root oxygen release (ROL), and the incoming water. The oxygen released by macrophyte roots influences the distribution of dissolved oxygen and microorganisms in the root zone (Wang *et al*. 2018). This process benefits the removal of pollutants from the wastewater.

The rhizospheric microbial population of various reeds, as shown in Table 6, indicates that bacteria, fungal and actinomycetes populations in the rhizosphere are directly proportional to ROL. *Canna indica* exhibited the highest bacterial and fungal populations, with a count of 54.7 x 10^6 (CFU/g of soil) and 24.2 x 10^4 (CFU/g of soil), respectively. This may be attributed to ROL, which creates diverse aerobic environments within the root system, facilitating the growth of various microorganisms - a process crucial for contaminant removal, as highlighted by Wang *et al*. (2018). According to Rehman *et al*. (2017), the degradation of organic matter through mineralization and transformation by microbes is influenced by rhizospheric oxygen.

This suggests that the high ROL observed in C*anna indica*, *Typha domingensis and Colocasia esculenta* and may account for their superior organic matter removal efficiency from wastewater. ANOVA and correlation were determined and given in the table 7. In sewage wastewater treatment, the correlation between bacteria and ROL showsthat strong positive correlation (0.826) which indicates that higher radial oxygen loss enhances the presence of bacteria, likely because the additional oxygen promotes aerobic microbial activity.

Radial Oxygen Loss content has strong positive correlation (0.983) with actinomycetes population, which are important in breaking down organic material, also thrive in the presence of oxygen released by reed roots. ROL significantly boosts bacterial and actinomycetes growth, supporting aerobic and microbials process. This can help in optimizing reed bed systems by understanding how reed selection and oxygen dynamics influence microbial populations responsible for pollutant degradation. From the ANOVA results, the F-value of *Canna indica* and *Typha domingensis* shows significant effect on content of ROL and microflora population in the rhizosphere for sewage wastewater treatment. Whereas, all the reeds show significant impact on ROL and microbial population which was maintained in the paper mill effluent.

3.5 Measurement of UV and DOC analysis

The trend of absorbance at 254 nm for all samples are

Table 7 Results of ANOVA for comparing the effects of radial oxygen loss and rhizosphere microflora population of different reeds

Sewage	F- value	p- value	Correlation coefficient (reeds vs ROL)
Canna indica	3.78	<0.05	0.827
Colocassia esculenta	1.96	NS	0.459
Xanthosoma sagittifolium	1.86	NS	0.298
Typha domingensis	70.84	<0.05	0.712
Paper mill			
Canna indica	4.52	<0.05	0.846
Colocassia esculenta	9.37	<0.05	0.927
Xanthosoma sagittifolium	3.84	<0.05	0.715
Typha domingensis	56.78	<0.05	0.636

Table 8 UV parameters of Sewage and Paper mill effluents

Parameters	RSE	TSE (day 7)	RPME	TPME (day 7)
Dissolved organic carbon (DOC, mg l^{-1})	19.3	4.6	15.6	6.3
UV absorbance at 254 nm (UV_{254}; cm^{-1})	1.152	0.195	1.628	0.624
Specific UV absorbance ($SUVA_{254}$; 1 mg^{-1} m^{-1})	5.969	4.239	10.436	9.905
Specific UV absorbance ($SUVA_{280}$; 1 mg^{-1} m^{-1})	3.896	3.543	8.372	8.095
Specific UV absorbance ($SUVA_{400}$; 1 mg^{-1} m^{-1})	1.595	1.217	1.371	1.095

(RSE – Raw Sewage Effluent; TSE – Treated Sewage Effluent; RPME – Raw Paper Mill Effluent; TPME – Treated Paper Mill Effluent)

given in the Figs. 6 and 7. The untreated and treated wastewater of sewage and paper mill effluents were compared for their absorbance value at 254 nm.

The decreasing rate of the absorbance value surely depicting the deletion of aromatic rings, which is present in the effluents after treating them in reed bed system. During 7 days of retention period, the DOC was reduced by 76.17% in sewage and 59.62% in paper mill effluent. Absorbance at 254 nm decreased in both the effluents *i.e*., sewage and paper mill effluent by 83.04% and 61.67%, respectively. In both experiments, reduction in DOM was associated with increase in SUVA values. The $SUVA_{254nm}$, $SUVA_{280nm}$ and $SUVA_{400nm}$ also gives the strong evidence for the occurrence of biodegradation of pollutant from effluents (Table 8). As the retention time for the reed bed system increases, the degradation of organic matters (humic substances) occurs. This led to the variation in SUVA values (Jin *et al*. 2011). Thereby, rhizosphere of reed plants aids in the increased pollutant removal mechanism in both the effluents (Rodríguez *et al*. 2016).

4. Conclusions

The reed bed system has the greater potential to reduce the concentration of the organic pollutants from both the sewage and paper mill effluents. All the reed plants in this experimental study performed better in reducing pollutant load. In particular, the study on screening of the reed plant shows that *Canna indica* and *Colocassia esculenta* perform well in reducing the pollutant load due to the combined action of rhizosphere and microbes present in the root zone.

- The difference in efficiency between the species may be due to the lower tolerance level and the rhizosphere's natural microbial processes capable of reducing the pollutants.
- Incoming high nutrients and other compounds in the effluents aid in plants' growth, which supports rhizosphere microorganism, forming a chain.
- These microbes play a vital role in the biochemical transformation of pollutants, adsorptions, precipitation and plant uptake.
- Reed plants provide oxygen through root hairs, which proliferate the microbes to break down organic substances.

Therefore, it is necessary to have a more profound study of rhizosphere microbial diversity and the interaction between the macrophytes and effluents in reed bed system technology using pilot scale set-up.

Acknowledgement

Financial assistance for this study was provided by DST SERB Scheme and the study were carried out in the Department of Environmental Sciences at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

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