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Role of radial oxygen loss (ROL) and rhizosphere microflora of reed plants in treating wastepaper based paper and board mill effluent

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Abstract
The Reed bed system with five different native reed plants namely *Colocasia esculenta, Xanthosoma sagittifolium*, *Canna indica*, *Saccharum spontaneum* and *Typha domingensis* was constructed for the treatment of effluent from the wastepaper based paper and board industry. Screening was carried based on the plant biomass, plant height, shoot length and root length. The effectiveness of the five different reed plants were studied and compared for the removal of Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in effluent with 7 days of retention time. The best TSS and TDS removal was expressed by the reeds, *Colocasia esculenta* followed by *Typha domingensis* and *Canna indica.* The highest effluent treatment in terms of BOD and COD removal was observed in *Typha domingensis* and *Canna indica* respectively. Significant removal of TSS, TDS, BOD and COD was exhibited by *Colocasia esculent*a, *Typha domingensis* and *Canna indica*. The pollutant removal was also positively related to Radial Oxygen Loss (ROL) and the microbial population in the rhizosphere of reed plants. The highest ROL and the microbial population was recorded by the rhizosphere of *Colocasia esculenta* followed by *Canna indica* and *Typha domingensis.* Hence, the reed bed system with the *Colocasia esculenta*, *Typha domingensis* and *Canna indica* can be used as the best biological treatment option for the wastepaper based paper and board mill effluents.

Keywords: Reed bed system, paper and board mill effluent, native reeds, screening, (ROL)

Introduction
Asia is currently the leader in utilizing wastepaper for paper and board production. Wastepaper utilization rate (total wastepaper utilized / total paper and paperboard consumed) is more than 55%. Malaviya and Kathore, 2007 stated that the treated paper board mill effluent has dark brown to yellowish brown in colour. Elayarajan (2002) [3] reported that paper mill effluent contains dissolved solids of 650 to 787 mg L^{-1} and suspended solids ranged of 1020 to 1040 mg L^{-1}. The treated effluent of wastepaper based paper and paper board has a BOD, COD, Nitrogen, Phosphorus, Potassium, Calcium, Magnesium and Sulphate of 56-77 mg L^{-1}, 248-362 mg L^{-1}, 12-28 mg L^{-1}, 1.21-1.51 mg L^{-1}, 16.3-24.2 mg L^{-1}, 212-286 mg L^{-1}, 164 48-94 mg L^{-1}, Na 326-480 mg L^{-1} and 98-138 mg L^{-1} respectively (Suriyanarayan, 2010) [10]. Maheshwari *et al*. (2012) [8] revealed that paper board effluent is the major contributor to adsorbable organic halides in the receiving ecosystems.
Phytoremediation has merits to offer due to its environmental friendly, relatively low cost and easy maintenance (Yusuf *et al*., 2018) [15]. Leung *et al.* (2016) [6] stated that the wetland plants can be used to improve the quality of wastewater, soil or sludge through the reed bed systems. The oxygen in the rhizosphere has its importance in understanding the regulation and function of microbial communities and macrophytes. Oxygen is one of the key factors in these biological systems (Yu *et al.*, 2016) [14]. The mechanism of contaminant removal in reed bed systems are greatly influenced by the Radial Oxygen Loss (ROL). Atmospheric reoxygenation is one of the main sources of oxygen in reed bed systems. However, atmospheric reoxygenation is limited and artificial aeration is very expensive (Wang *et al.*, 2018) [13]. The distribution of microorganisms are altered by the addition of oxygen to reed bed systems and it also increases their metabolic efficiency. The removal of contaminants in wastewater could have a positive significance due to this process (Braeckevelt *et al.*, 2011) [2].

Hence, the pollutant removal efficiencies of five different reed plants and the role of oxygen released by the roots of these reeds were assessed in this study.

Materials and Methods

Sampling and characterization of effluent from wastepaper based paper and board industries

The raw effluent samples were collected from Tamil Nadu Newsprint and Paper Limited (Unit-II), Mondipatti, Trichy. The samples for Dissolved Oxygen (DO) were collected and fixed by adding with one ml of manganous sulphate and one ml of alkaline potassium iodide solution. The collected effluent samples were stored at 4ºC under refrigerated condition and used for analysis.

Screening of native reeds for treating wastepaper based paper and board mill effluent

Collection of native reeds

The native reed plants were collected from noyyal riverbasin and lakes in the Coimbatore district. They were authenticated and identified as *Canna indica* (Indian Shot), *Colocasia esculenta* (Taro), *Saccharum spontaneum* (Wild Sugarcane), *Typha domingensis* (Southern cattail) and *Xanthosoma sagittifolium* (Tania) by Botanical Survey of India (BSI), TNAU, Coimbatore.

Screening of native reed plants for wastepaper based paper and board mill effluent

Pot culture experiment with reed bed system for screening the native reed plants suitable for wastepaper based paper and board mill effluent was conducted at Tamil Nadu Agricultural University, Coimbatore. The collected reeds were washed thoroughly and allowed for stabilized growth. The pots of uniform size with a facility to collect samples of the treated effluent from the bottom were used in this study. Gravel, coarse sand and garden soil (each of 8 cm) were used as a media for the experimental study and they were arranged in a sandwich manner with gravel at the bottom followed by coarse sand and garden soil. The biomass of the reeds were determined and they were planted uniformly in the pots.

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*, *Saccharum spontaneum* and *Typha domingensis* to determine the suitability for treating the wastepaper based paper and board mill effluent. The raw effluent of 7 litres was poured into each of the pots. The treated effluent from the pot culture experiment was collected for 7 days with an interval of 24 hours and they were analysed by following the standard analytical procedures (Jackson, 1973; Gupta, 2002; Anon, 1989) [5, 4, 1].

Plant parameters of reed plants

Plant height

Plant height is measured from the base of the root to the tip of primary leaf were recorded. The plant height was measured in cm.

Root length

Root length of the plants was measured using a meter scale and are recorded in cm.

Shoot length

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

Plant biomass

Biomass of plants was measured by weighing balance instrument and recorded in gm.

Radial Oxygen Loss (ROL) measurement in the rhizosphere of reed plants

The ROL in the rhizosphere of selected reed plants was measured by Titanium Citrate method as suggested by Wang *et al*. (2015) [12]. A colorimetric method involving titanium citrate solution was used for measurement of oxygen release rate from plant roots. Titanium (III) is a strong reducing agent, that when complexed with sodium citrate, forms titanium citrate complex. This complex will eliminate oxygen from the solution by first order reaction. Two litre container was filled up with hoagland nutrient solution along titanium citrate solution. For 100 ml of hoagland solution 5 ml of titanium citrate was added.

Before adding titanium citrate solution, container was evacuated and nitrogen gas was bubbled through for 20 min to remove the dissolved oxygen percent. Plants samples (one per flask) were gently and carefully washed to remove foreign materials with deionized water. The root base (above the collar upto 6cm) were coated with parafilm and then inserted into flask while ensuring that roots were completely immersed in the nutrient solution. The parafilm coating was done to prevent shoot exposure to parafilm oil or wax layer on the surface of solution and also to eliminate to prevent any possible O_2 leakage. Five ml of Titanium citrate was then injected to container immediately after layering the solution surface. After 4h, the container was gently shaken and samples were taken with syringe through rubber tubing that has introduced into solution alongside the roots. The samples are fed in the spectrophotometer and the absorbance was measured at 527 nm.

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}.

Fig 1: Experimental setup for ROL measurement

Determination of microbial population from rhizosphere of reeds

The microbial population in reeds rhizosphere soil was estimated by the standard methods as shown in Table 1.

Table 1: Standard methods for the population of microbes

1	Bacteria	Nutrient Agar medium	(Waksman and Fred, 1922)
2	Fungi	Martin's Rose Bengal Agar medium	

Experimental design

Design	FCRD
Factor 1	Reeds
Factor 2	Retention time
Replication	Two
Duration	7 days
Location	Department of Environmental Sciences, TNAU.

Factors

Factor 1: Plant species	Factor 2: Retention time
Colocasia esculenta	D1: 1st day of retention time
Xanthosoma sagittifolium	D2: 2nd day of retention time
Canna indica	D3: 3rd day of retention time
Saccharum spontaneum	D4: 4th day of retention time
Typha domingensis	D5: 5th day of retention time
	D6: 6th day of retention time
	D7: 7th day of retention time

Fig 2: Pot culture experiment for screening to paper and board mill effluent with different native reed plants a. *Canna indica,* b. *Colocasia esculenta,* c. *Xanthosoma sagittifolium,* d. *Saccharum spontaneum* and e. *Typha domingensis*

Results and Discussion

Screening of reed plants

The characteristics of the paper and board mill effluent (raw) used for the study is shown in Table 2. The quality of the effluent was assessed after treatment by the reed bed system through five different reed plants with the retention time of 7 days. The morphological parameters of different reed plants growing in paper mill effluent are prescribed in Table 3. The highest increase in plant biomass, plant height, shoot height and root height was observed in *Canna indica* with the recordings of 25.23g, 7cm, 4.3cm and 2.7 cm respectively. The growth of plants in wastepaper based paper and board mill effluent were observed in the order of *Canna indica* > *Colocasia esculenta* > *Typha domingensis* > *Saccharum spontaneum* > *Xanthosoma sagittifolium*.

Table 2: Characteristics of raw paper and board mill effluent used for the study

Parameters	Raw effluent
pH	7.2
Total Dissolved Solids (mg L^{-1})	2020
Total Suspended Solids (mg L^{-1})	1560
Biological Oxygen Demand (mg L^{-1})	180
Chemical Oxygen Demand (mg L^{-1})	534

Table 3: Morphological characteristics of reed plants

Plants	Plant Biomass (g)		Plant Height (cm)		Shoot Length (cm)		Root Length (cm)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Canna indica	99.78	123.52	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
Saccharum spontaneum	77.18	79.35	145.3	146.0	135.3	135.4	10	10.6
Typha domingensis	103.54	106.23	135.4	138.2	128.5	130.4	6.9	7.8

Removal of TSS and TDS in effluent by reeds

In the present investigation, the reed plants and retention time had a significant influence on reduction of total suspended solids and total dissolved solids in the paper and board mill effluent. They had shown a decreasing trend with respect to increase in the retention time from day 1 to day 7 as shown in the Figure 3 and Figure 4. The TSS and TDS of the samples collected at different retention times ranged from 800 to 1500 mg L^{-1} and 940 to 2020 mg L^{-1} respectively. *Colocasia esculenta* has recorded the highest percentage removal of total suspended solids and total dissolved solids with 45.2% and 51% respectively followed by *Typha domingensis* with 42.2% and 50.5% in the effluent respectively with a retention time of 7 days. *Canna indica* showed no significant difference when compared to *Colocasia esculenta* and *Typha domingensis* in the removal of TSS and TDS. This might be due to the physical action which involves filtration and settlement by the substrates used in the reed bed system. A review by Wang *et al.* (2018) [13] stated that the gravitational interception of solid objects between sand and stone particles and the blocking effect of macrophyte roots plays a major role in the removal of suspended solids in the reed bed system. He also stated that the ionized contaminants could be removed by adsorption occurring on the macrophyte root surface.

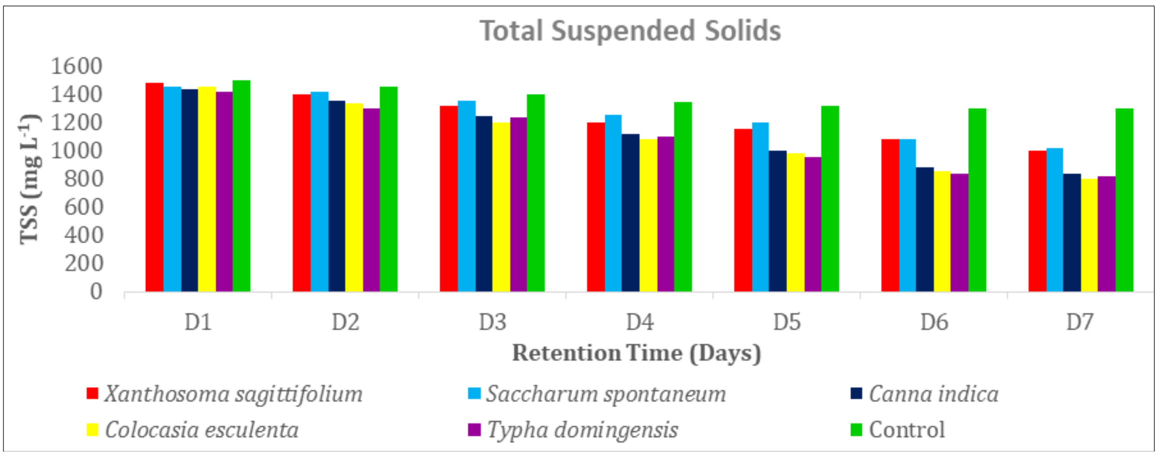

Fig 3: Removal of total suspended solids by the reeds

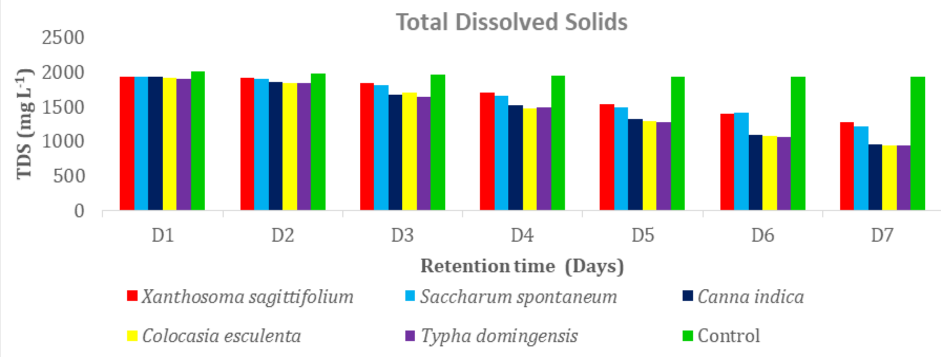

Fig 4: Removal of total dissolved solids in effluent by the reeds

Removal of BOD and COD in effluent

The results showed a gradual decrease in BOD with increase in retention time as shown in Figure 5. The BOD of the effluent ranged from 40 (D7 of *Typha domingensis*) to 176 mg L^{-1} (D1 of *Xanthosoma sagittifolium* and Control). The highest percentage removal of BOD in the effluent were recorded as 75.3% by *Typha domingensis* and then by *Colocasia esculenta* with 73.7 % at the retention time of 7 days (Day 1 to Day 7). This type of reduction in the organic matter present in the effluent might be due to hydrolysis which converts them into the soluble form so that they could get penetrated into the media and then gets attached to biofilm for the decomposition. A study by Rehman *et al.* (2017) [9] revealed that the release of oxygen through the roots of plants enhances the aerobic microbes in the biofilm which in turn supports organic pollutant degradation. A review by Wang *et al.* (2018) [13] stated that the degradation of contaminants into more unstable compounds were the outcome of redox reactions.

By the observation from the Figure 6, the COD from the effluent was reduced by the reed bed system during the time of 7 days *i.e.*, from D1 to D7. The COD ranged from 272 to 532 mg L^{-1} in treated effluent with respective of retention time. This could be due to the decomposition of aerobic and anaerobic microbes combined with the physical processes of filtration and sedimentation. *Canna indica* had reduced the BOD from 170 mg L^{-1} to 48 mg L^{-1} from D1 to D7. The best percentage of COD removal in the effluent was possessed by *Canna indica* as 47.6% followed by *Colocasia esculenta* with 46.8% during the retention time of 7 days (Day 1 to Day 7) which had no significant difference with *Typha domingensis*. The coupling effects between plants and microorganisms, and the decomposition of microorganisms causes the overall decline in the organic and inorganic matter (Wang *et al.*, 2018) [13].

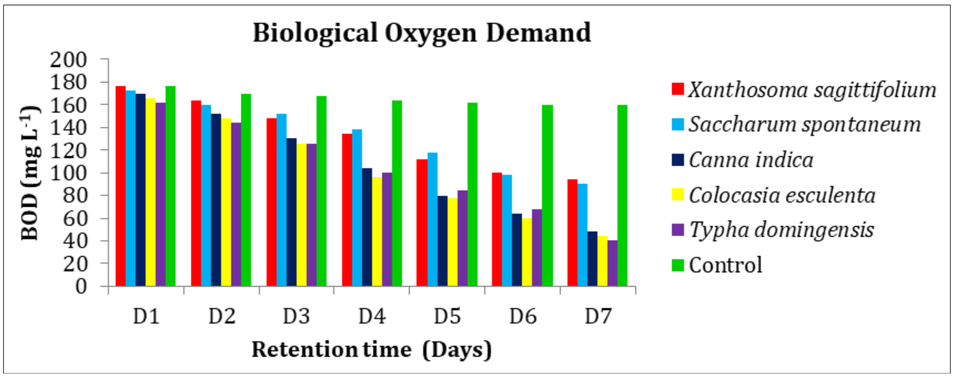

Fig 5: Removal of BOD in effluent by the reeds

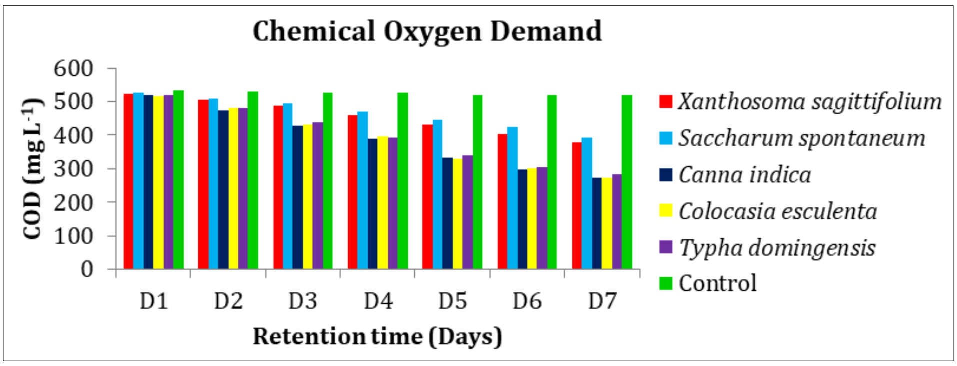

Fig 6: Removal of COD in effluent by the reeds

Radial Oxygen Loss (ROL) in the rhizosphere of reeds

The Radial Oxygen Loss (ROL) by the reeds used in the reed bed system was measured and presented in Table 4. The values of ROL were expressed as the amount of oxygen released from plant per day per root biomass at the temperature of 30 degree Celcius and 1100 lumens of light intensity.

Table 4: ROL of different reed plants

Plant species	Root oxygen release ($\mu mol\ O_2\ d^{-1}\ g^{-1}\ DW_{root}$)
Colocasia esculenta	55.2
Canna indica	51.5
Typha domingensis	45.4
Xanthosoma sagittifolium	29.5
Saccharum spontaneum	27.5

Table 5: Microbial population in the rhizosphere soil

Plant species	Bacteria (CFU / g of soil)	Fungi (CFU / g of soil)
Colocasia esculenta	175 x 10^6	47 x 10^4
Canna indica	142 x 10^6	43 x 10^4
Typha domingensis	124 x 10^6	40 x 10^4
Xanthosoma sagittifolium	102 x 10^6	30 x 10^4
Saccharum spontaneum	78 x 10^6	21 x 10^4

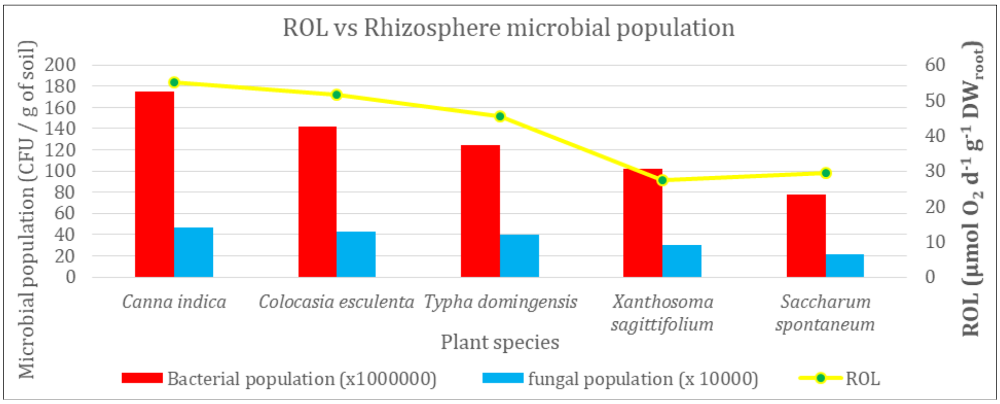

Fig 7: Relationship between ROL and rhizosphere microbial population

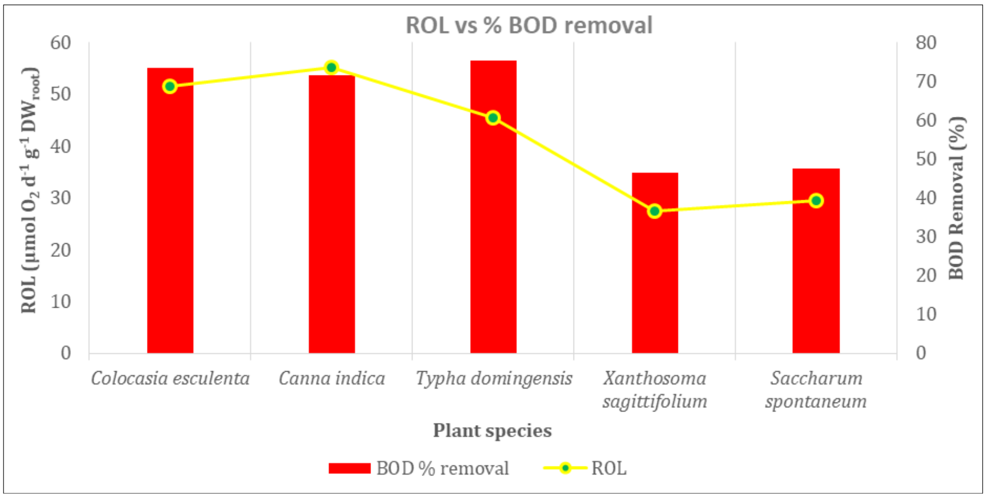

Fig 8: Relationship between ROL and % BOD removal

By the measurement of ROL, it is determined that the ROL produced by the reeds were in the order of *Colocasia esculenta* > *Canna indica* > *Typha domingensis* > *Xanthosoma sagittifolium* > *Saccharum spontaneum* as shown in the Table 4. The rhizospheric microbial population of different reeds is presented in Table 5. As per Figure 7, the bacterial and fungal population in the rhizosphere is directly proportional to ROL. The highest bacterial and fungal population is found in the rhizosphere of *Colocasia esculenta* with 175 x 10^6 (CFU / g of soil) and 47 x 10^4 (CFU / g of soil). This might be due to ROL which provides different aerobic environments for diverse microorganisms in the root system and this process is highly essential for the removal of contaminants as stated by Wang *et al.*, (2018) [13].

Per cent BOD removal is directly proportional to the ROL by the reed plants as shown in Figure 8. As Rehman *et al.* (2017) [9] reported that the degradation of organic matter by the mineralization and transformation carried out by the microbes were influenced by the rhizospheric oxygen, the above results of ROL might be the reason for the best organic matter removal by *Typha domingensis, Colocasia esculenta* and *Canna indica*.

Conclusion

From the above results, it is concluded that the reed bed system with *Colocasia esculenta*, *Typha domingensis* and *Canna indica* have proved to be effective in the removal of the TSS, TDS, BOD and COD of wastepaper based paper and board mill effluent. The gradual decrease of the pollutant load was observed with sincrease in the retention time from 1st to 7th day and it could be revealed that the Radial Oxygen Loss (ROL) also has its priority for the detoxification of the contaminants. The best pollution removal efficiency were found to be possessed by *Colocasia esculenta*, *Typha domingensis* and *Canna indica* and the performance of these reeds were found to be superior than *Saccharum spontaneum* and *Xanthosoma sagittifolium.* Therefore, these native reeds can be used for the biological treatment of wastepaper based paper and board mill effluent through the reed bed systems.

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