Comparative Analysis of *Ranunculus muricatus* and *Typha latifolia* as Wetland Plants Applied for Domestic Wastewater Treatment in a Mesocosm Scale Study

S. Aziz, M. Ali, S. Asghar, S. Ahmed

***Abstract*—**Comparing other methods of waste water treatment, constructed wetlands are one of the most fascinating practices because being a natural process they are eco-friendly have low construction and maintenance cost and have considerable capability of wastewater treatment. The current research was focused mainly on comparison of *Ranunculus muricatus* and *Typha latifolia* as wetland plants for domestic wastewater treatment by designing and constructing efficient pilot scale horizontal subsurface flow mesocosms. Parameters like chemical oxygen demand, biological oxygen demand, phosphates, sulphates, nitrites, nitrates, and pathogenic indicator microbes were studied continuously with successive treatments. Treatment efficiency of the system increases with passage of time and with increase in temperature. Efficiency of *T. latifolia* planted setups in open environment was fairly good for parameters like COD and BOD_5 which was showing reduction up to 82.5% for COD and 82.6% for BOD_5 while DO was increased up to 125%. Efficiency of *R. muricatus* vegetated setup was also good but lowers than that of *T. latifolia* planted showing 80.95% removal of COD and BOD_5. *Ranunculus muricatus* was found effective in reducing bacterial count in wastewater. Both macrophytes were found promising in wastewater treatment.

***Keywords*—**Biological oxygen demand, chemical oxygen demand, horizontal subsurface flow, Total suspended solids, Wetland.

I. Introduction

THERE is global stress on sustainable use and reuse of water and wastewaters. During the last century, the increasing demands for freshwater along with environmental concerns about the discharge of wastewater into ecosystems and the high cost and technology requirements of wastewater treatment have impelled processes in water reclamation and reuse [1].

Ever since man realized that discharging wastes into surface water cause many environmental and health problems dilemma of treating wastewater has beleaguered him [2]. For treatment, plant designed must be in such a way that it confine the treatment processes in restricted, controlled environments which provide favorable conditions for physical and biochemical reactions taking place during treatment process and it can remove as much contaminants as possible so that the treated water can be disposed off or can be utilized for agriculture purposes without any threat.

Different practices used to treat wastewater include sludge processing, rotating biological contactor, stabilization ponds and micro-algae techniques etc. but energy consumption, economic problems, requirement of land at large area, intricate construction and operation, sensitivity to temperature and too much sludge tribulations are some problems which limit their usage [3], [4]. So there is dire need for designing of simple, safe, cost-effective and green technology. For successful designing of a sustainable treatment process for wastewater, features which should be considered are energy consumption, mechanical technology requirements, capital, operation and management, and user costs [5]. For purification of water in controlled way, various natural biological systems like ponds, land treatment and wetlands can potentially be used [2]. Because of their design, performance, operation and maintenance these systems show efficiency. Constructed wetlands are a half-way world between terrestrial and aquatic ecosystems displaying some of the features of both ecosystems [6]. Countries as England, in some states in the U.S., Canada and Australia where the climate is either arid or semiarid this technology is in practice, for renovation of wastewaters [7], [8]. In Pakistan approximately 9.7 per cent of the total surface area is occupied by lakes and wetlands. From sea coast in the south to high mountains in the north almost 225 significant wetlands are located [9].

Constructed wetlands are basically attached growth bioreactor [10] in which microbial attachment is on media material and on roots, stems, leaves, and litter of wetland vegetation [11]. Hydrology and macrophytes categorize constructed wetlands [12], [13]. Constructed wetlands for wastewater treatment may be classified according to the life form of the dominating macrophyte, into systems with free-floating, floating leaved, rooted emergent and submerged macrophytes [12]. Further division could be made according to the wetland hydrology (free water surface and subsurface systems) and subsurface flow CWs could be classified according to the flow direction (horizontal and vertical) [13].

Planted HSSF wetland not only has high redox potential in rhizosphere region comparing unplanted one [4] but also have high microbial density, activity, and diversity [14]-[16], this concludes that plants enhance the establishment of

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microorganisms liable for removing pollutants by transferring oxygen to their roots and release a part of this oxygen into the rhizosphere [17]. Thus by forming a mixture of strong redox gradients the rhizosphere facilitates the formation of a lot of ecological slots that encourage a number of microbial processes. Rhizospheres of constructed wetlands have microorganisms which exhibit elevated aerobic respiration rate potential in comparison to unplanted wetlands [15], [16] showing that microorganisms are affected by oxygen released from roots. Root oxygen release depends on plant species [17], [18] and it was found that *Typha latifolia* shows highest oxygen release rates.

The fact that higher plants have bactericidal effect on pathogens which varied depending on plant species used was first revealed by [19] when she practically used this aspect for wetlands construction in Germany. *Phragmites* and *Typha* (35–91%) caused increased removal of *E. coli* in the rhizosphere in comparison to unplanted controlled microcosm experiments. However no noticeable disparity was found in removal efficiency between these two plant species. A decline in elimination was observed in winter comparing summer months in a pilot scale study [20]. References [21] and [22] reported antibacterial properties of *R. muricatus*. The present study was designed to compare the activities of two wetland plants in pilot scale systems.

II. Materials and Methods

A. Design of Horizontal Subsurface Flow Constructed Wetland Setup

The present study is focused on the analysis of *Typha latifolia* and *Ranunculus muricatus* as wetland macrophytes by constructing HSSF mesocosms effective treatment of domestic wastewater from residential colony, Quaid-i-Azam University, Islamabad, as this wastewater ultimately discharged into Rawal Lake and pollutes it. In this study pilot scale three experimental setups of horizontal subsurface flow mesocosms were constructed and run in parallel for treatment of domestic wastewater sampled from residential colony of QAU.These three experimental setups were run in open environment near field scale constructed wetland at University residential colony. Out of three mesocosms built, two were planted with buds of *Typha latifolia* and third one was planted with *Ranunculus muricatus*. In each setup four concrete pots were used with approximately 0.37x0.37x0.37 m (length x width x height).The four pots were (Gravel filter + HSSF + HSSF + Sand filter) and arranged sequentially by making interconnections with polyvinylchloride pipes having diameter of 1 inch and were placed with decreasing heights to assist the natural flow of water under gravitational pull. The distance between each pot was 1 foot and the distance between first and last pot was 9 feet. First pot was at height of 10 feet with relative to last pot. Inlet for water in each pot was 2 inch below the top of the pot while outlet was 2 inch above the bottom of pot. First pot in which water was entering from septic tank was filled with washed and air dried gravel of size of 0.6 inches. 2nd and 3rd units of each setup were filled in such a way that lower most 2 inch layer at bottom is of 2-4 inch size stones. Upper 4 inch layer is of gravel having size of 0.6 inch and uppermost 7 inch layer is of 1:1 soil and sand mixture while 4th unit was sand filter (Figs. 1 and 2). Performance efficiency of each setup was studied and for two setups vegetated with *Typha* average efficiency was recorded and compared with *R. muricatus.* Set ups were operated from starting of March to end of May 2014. Temperature was continuously monitored during the study by using thermometer and was found to be in the range of 13–41°C.

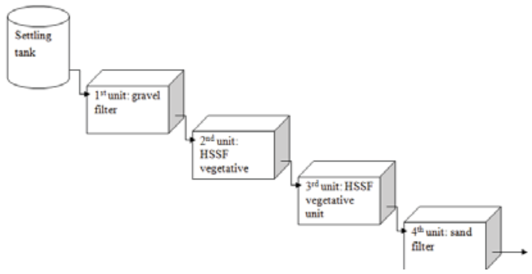

Fig. 1 Schematic representation of HSSF mesocosms

Fig. 2 Mesocosms set up of constructed wetland built in open field environment

B. Sampling of Municipal Wastewater

The standard procedures [23] were performed for the characterization of wastewater during the research period. Sterile plastic bottles of 250 ml were utilized for collecting and transporting the sample for microbiological and physicochemical analysis. Parameters that demanded immediate characterization like microbiology and DO were accomplished within a period of 24 hours. While for other Physico-chemical analysis wastewater samples were stored at 4°C.

C. Characterization of Microbial Community of Constructed Horizontal Subsurface Flow Constructed Wetland

Soil was taken from the rhizosphere of macrophytes (*T. latifolia* and *R. muricatus*) grown in pots. 1 gm of soil was added in a flask having 99 ml of autoclaved distilled water. Ten dilutions starting from 10^{-1} to 10^{-10} were prepared. 0.1 ml of sample was taken from each of 10^{-3}, 10^{-5}, 10^{-7} dilutions by micropipette and was inoculated on nutrient agar plates by spread plate method.

Various colonies were appeared on nutrient agar plate following incubation. These colonies were differentiated according to their morphological characteristics. For obtaining pure culture different colonies were then further sub-cultured on differential and selective media i.e., Mannitol Salt Agar, Pseudomonas Cetrimide Agar, Salmonella-Shigella Agar, Eosin methylene blue agar and MacConkey's agar media and plates were then incubated for 24 hours at 37C. After incubation, identification of sub-cultured microbes was done on the basis of morphology, microscopy and biochemical tests.

D. Treatment of Wastewater in HSSF CW with Continuous Flow

The wastewater was treated using a subsurface flow constructed wetland system in which the water flowed in a continuous manner through first unit filled with gravel which causes sedimentation of any large particle and adsorption of solids to gravel. After leaving first unit water entered second vegetative unit which was designed to have horizontal subsurface flow and then water entered the third unit which was also vegetative and having a horizontal subsurface flow. After that water finally entered fourth unit which was sand filter to provide maximum sedimentation and adsorption of any remaining solids to sand particles. Through all units water flowing mode was horizontal subsurface and continuous.

Temperature was constantly monitored throughout the working phase of the horizontal subsurface flow CW (Table I). Water samples were collected from each processing unit. These samples were then analyzed through different physico-chemical and microbiological tests.

TABLE I
AVERAGE TEMPERATURE NOTED IN MARCH, APRIL AND MAY

Months	Average temperature
March	13-28°C.
April	20-39°C.
May	24-41°C

Setups vegetated with *T. latifolia* and *R. muricatus* were given labels as ST1, ST2 and SR. As it is mentioned above that two setups were planted with *T. latifolia* and both were operating under similar conditions in open environment, so during study we checked average efficiency for both setups and compare it with setup planted with *R. muricatus*. Finally treated samples collected after each treatment were designated according to their respective set ups i.e. samples collected from final unit of a single setup were labeled as ST1 and ST2 for *T. latifolia* and SR for *R. muricatus* respectively. Influent sample was labeled as S0. Samples from each setup were specified in this manner and are illustrated in Table II.

TABLE II
NAME AND ABBREVIATIONS FOR SETUPS AND SAMPLES

Setups vegetated with	Sample title
T. latifolia	ST1
T. latifolia	ST2
R. muricatus	SR

E. Physicochemical Analysis

Physicochemical analysis of wastewater was carried out by determining different parameters. The pH (Sartorius pH meter) and DO (CRISON OXI 45 +) were determined by their respective digital meters. Biochemical oxygen demand (BOD) was estimated by 5-day BOD test (5210 B standard method) and chemical oxygen demand (COD) by Closed Reflux, Titrimetric Method 5220 C. Standard methods 2540 C, 2540D were used to estimate total dissolved solids (TDS) and total suspended solids (TSS) in water samples, respectively. Standard method 4500-P, was used to measure orthophosphates and 0375 Barium chrometery was employed to determine sulfates in water samples. 4500 NO_3-N and 4500 NO_2- N standard methods were practiced to determine nitrates, and nitrites, respectively, in water sample.

F. Microbiological Analysis

Microbiological analysis of samples was carried out by colony forming unit (CFU/mL) and most probable number method (MPN index) before and after treatment through constructed mesocosms.

Determination of Total Bacterial Count by Plate Count Method

For the investigation and enumeration of fecal coliforms, *E. coli* and other *Enterobacteraceae*, technique applied was serial dilution and plate count method. For untreated and treated water samples, a series of 10 dilution tubes was setup, containing 9 ml of pre-autoclaved saline in each tube to ensure plating success. These wastewater samples were serially diluted in sterile water up to 10^{-7}. Then dilutions were inoculated on nutrient agar plates by employing spread plate method. The plates were incubated at 37°C for 24 hrs. The colonies appeared on plates were enumerated by colony counter and the CFU of each colony was then determined by:

$$\text{CFU/mL} = \text{number of colonies} \times \text{dilution factor/inoculum size}$$

Determination of Most Probable Number Using Multiple Tube Tests

For the examination and enumeration of fecal coliforms, pathogens and other coliforms in untreated, and treated wastewater samples were incubated at 37°C for 24–48 hrs in Lactose fermentation broth using multiple tube technique having inverted Durham tubes. Number of tubes that are positive for gas production (contain bubble in Durham tube) are measured against standard dilution table.

III. RESULTS AND DISCUSSION

A. Bacteriological Analysis of Rhizosphere

Typha latifolia is an "obligate wetland" species, meaning that it is always found in or near water. The species generally grows in flooded areas where the water depth does not exceed 2.6 feet (0.8 meters). However, it has also been reported growing in floating mats in slightly deeper water. Typha was selected because of its extensive rhizome growth that serves as an important reservoir for accumulation of various microorganisms and thus helps in wastewater clarification, allowing good microbial growth. *Ranunculus muricatus* grows in wetlands, swamps, winter-wet depressions, and waterways. It is known to possess antimicrobial activities.

On the basis of microscopic examination, cultural and biochemical characterization bacterial strains isolated from *T. latifolia* rhizosphere were identified as *Escherichia coli, Proteus vulgaris, Enterobacter aerogenes, Salmonella typhimurium, Klebsiella pneumoniae, Shigella dysntriae, Bacillus cereus, Alcaligenes faecalis, Micrococcus luteus* and *Staphylococcus aureus* while isolates obtained from rhizosphere of *R. muricatus* included *Staphylococcus aureus, Corynebacterium xerosis, Micrococcus luteus, Bacillus cereus, Enterobacter aerogenes, Streptococcus lactis* and *Alcaligene faecialis*.

B. Wastewater Evaluation before Treatment

Before treatment wastewater was evaluated for factors like pH, DO, BOD_5, COD etc. During study BOD_5, COD and pH showed variation in a broad range. For COD range observed was 147-943 mg/mL, for BOD_5 it was 99-632 mg/mL and for pH range was 7.2-8.21. For other parameters i.e., NO_2 (0.20-1.8 mg/ml), NO_3 (38.96-76.23), SO_4 (55.28-75.82 mg/ml), PO_4 (6.15- 18.62 mg/ml), TSS, TDS, range was within limits. Considering physical parameters domestic wastewater was turbid with abhorrent smell.

C. Analysis of Wastewater after Successive Treatments

Odor and pH

According to WHO standards, the clean water should not have any odor. Abundant microbial, algal and plant growth in polluted waters with high concentration of nutrients is the main reason of foul odor. In the degradation process of these plants and algae, bacteria produces a wide variety of unpleasant odor such as methane, rancid, sulfur etc. [24]. In the present study of HSSF treatment system the odor of water was efficiently removed by 100%. Removal of organic substances and microbes during treatments in the HSSF treatment system cause elimination of odor.

Another parameter to estimate water quality was pH. Although no direct effect of pH on aquatic and terrestrial life is reported but if it deviates from 6.5-8.5 prescribed by WHO it can effect microbial growth. This parameter also controls various sorts of chemical transformations in living organisms [25]. In the present study pH of water lied in range of 7.01-7.65 and during study no any prominent effect on pH of water after treatment was observed. (Fig. 3)

Removal of Solids

The values of TSS, and TDS were found to be 51-130, and 110-400 mg/L, respectively, in the untreated samples of colony wastewater. According to [17], the prescribed value of EC, TSS, and TDS in drinking water is 25–80mg/L and <1000mg/L, respectively. In water samples treated by *Typha latifolia* planted HSSF mesocosms 57.5% reduction in TSS level was recorded for sample analyzed in March. 62.7% reduction was recorded in April and 78% reduction was recorded in TSS level in May. Treatment by *Ranunculus muricatus* planted setup showed 53% removal of total suspended solids in March and 68.6% reduction with TSS of 51 mg/L and 16 mg/L for untreated and treated effluents in April. By the end of May TSS values for untreated influent noted was 94 mg/L and for treated effluents this value was reduced to 22 mg/L indicating 76.5% TSS removal. While considering TDS after treatment by open environment *Typha latifolia* planted HSSF mesocosms decline observed in TDS in treated effluent was of 59% in March. On analysis of sample in April TDS value was noted to reduce to 134.5 mg/L while untreated sample had 400 mg/L of TDS. After further treatments in May TDS level was further reduced by 74%. Effluent analysis by *Ranunculus muricatus* planted HSSF mesocosms in March revealed 59.2% reduction in TDS level. In April removal efficiency was increased up to 67.7% with TDS concentration of 400 mg/l and 129 mg/lfor untreated and treated effluents respectively. By the end of May TDS level for untreated influent noted was 340 and for treated effluents this value was reduced to 88 mg/l indicating 74.11% TDS reduction (Figs. 4, 5).

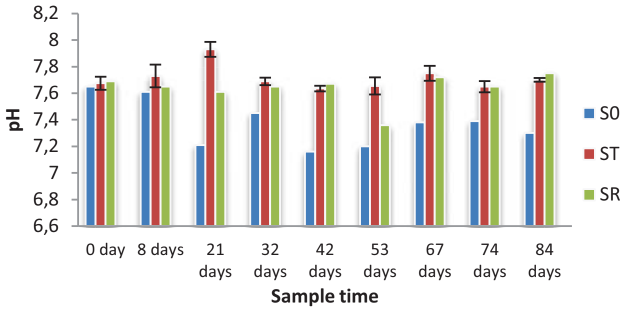

Fig. 3 Changes in pH during treatment by HSSF mesocosms planted with *Typha* and *Ranunculus*

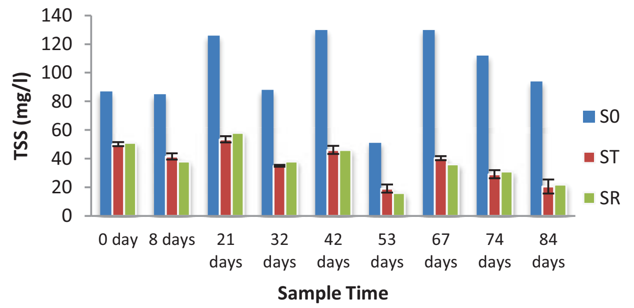

Fig. 4 Change in TSS during treatment by HSSF mesocosms planted with *Typha* and *Ranunculus*

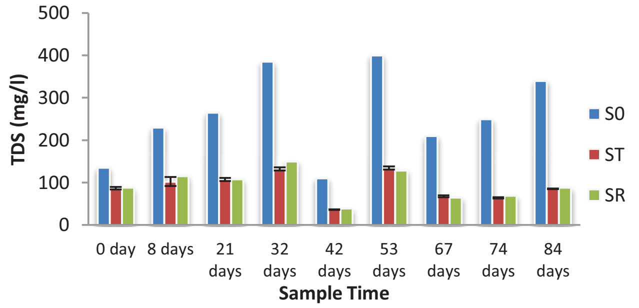

Fig. 5 Change in TDS during treatment by HSSF mesocosms planted with *Typha* and *Ranunculus*

Removal of Organic Contaminants

Organic pollutants include biological and chemical oxygen demand and are interrelated with the amount of dissolved oxygen, which is another crucial parameter to check the efficiency of treatment system and quality of water. It is dependent on oxygen uptake and consumption for respiration and break down of carbon compounds by macrophytes planted in vegetative units. In current study DO of wastewater lied in range of 2.35-3.56 mg/L. and according to WHO, the prescribed limit of DO for drinking water is 6–8 mg/L. By treatment values of DO increased subsequently. For open environment setups planted with *T. latifolia* average highest value of DO recorded was 7.105 mg/L after treatment. *R. muricatus* planted setup gave 109.6% increase in value of DO at the end of May. Over all % efficiency for DO increase was highest for *T. latifolia*. More oxygen is released in root zones of plants which have greater amount of aerenchyma tissue like *T. latifolia* [26] and vegetation known to possess extensive root systems show greater removal efficiency related to rhizosphere [27]. Oxygen release depends on plant species. References [17], [18] reported that *T. latifolia* shows highest oxygen release rates [28] (Fig. 6).

Range of 8-10 mg/l COD was prescribed by EPA. In current study COD of wastewater collected from colony was in the very high range of 147-943mg/l, which might be due to very high content of organic compounds in wastewater. A factor of temperature is important in this regard as temperature rises in summer season it causes increase in chemical reactions in septic tank which lead to reduction in COD value before treatment.

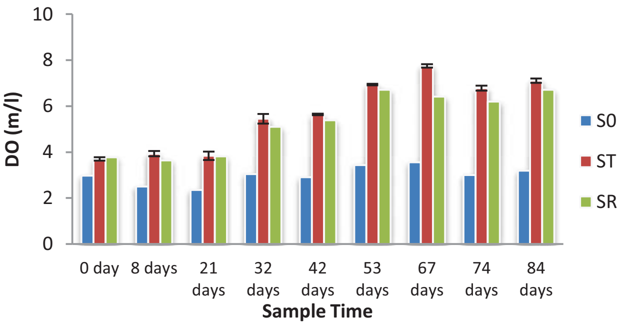

Fig. 6 Change in DO during treatment by HSSF mesocosms planted with *Typha* and *Ranunculus*

In this study high value of COD for untreated influent was noted in time periods when temperature was low. With the passage of time temperature started increasing which aided in reducing COD value in untreated influent. Following treatment average % reduction in COD values was 82.55% for both *T. latifolia* vegetated setups. While percentage reduction for *R. muricatus* planted set up was 80.95%. These results revealed higher efficiency of *T. latifolia* planted setup for COD removal in open environment. Also it was observed that with increase in temperature efficiency of setups increased suggesting impact of temperature and weather conditions on removal efficiency of treatment systems. Reference [29] reported 50% reduction in COD values in a four week study. COD removal by subsurface flow CW system is because of good interaction between physical and microbial processes. The former separates the organic solids thus allowing better hydrolysis for biodegradation while the gravel media allow the accumulation of immense amounts of attached bacteria, which help in rapidly catalyzing chemical reactions [30].Overall lower efficiency in COD removal of setups was its mode of operation, which was continuous. Comparing batch flow feed, continuous flow feeding is less efficient in COD removal (Fig. 7).

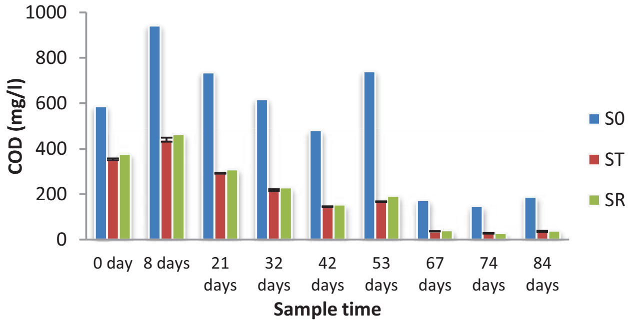

Fig. 7 Change in COD during treatment by HSSF mesocosms planted with *Typha*and *Ranunculus*

Environmental protection agency reported standard range of BOD_5 present in drinking water should lie in range of 5-8mg/l. BOD_5 in wastewater collected from colony had BOD_5 value which lied in wide range of 98.49-633mg/L. High values of BOD_5 were because of excessive amount of organic compounds present in wastewater. In this study average BOD_5 removal efficiency obtained for both *T. latifolia* planted setups was 82.55% while for *R. muricatus* it was 80.95%. In this case over all removal efficiency observed was greater for *T. latifolia*. One reason is that horizontal subsurface flow systems give greater efficiency in terms of COD and BOD removal. Reference [31] reported average treatment efficiency of 86.6% for HSSF CW. Reference [29] reported 56.72% reduction in BOD_5 from wastewater using *T. latifolia* as vegetation in duration of one month study (Fig. 8).

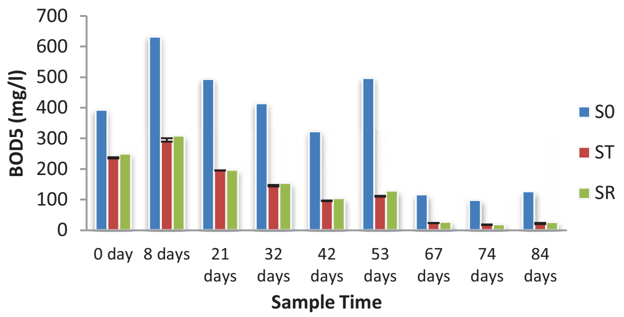

Fig. 8 Change in BOD5 during treatment by HSSF mesocosms planted with *Typha* and *Ranunculus*

Nutrient Removal

Efficiency of treatment systems having different macrophytes with passage of time for nutrient removal was also checked. Safe level of sulfates in water recommended by WHO is 250 mg/L. In the present study sulfates content ranges from 55-113 mg/L. With the passage of time ultimate removal percentage for both *T. latifolia* setups in average was 66.28%, while for *R. muricatus* percentage removal reached up to 60.07%. Over all percentage removal efficiency for sulfates was greater for *T. latifolia* comparing *R. muricatus*. There are many factors, some of which are carbon availability, the presence of more energetically favorable elements and redox conditions, which have direct effect on sulphur cycling [28]. The relative concentration of sulfate to other electron acceptor compounds will determine whether sulfate reduction will occur or not. For example, denitrification is more energetically favorable, so sulfate reduction will occur only after all the nitrates has been consumed [32]. Reference [33] found that sulfates removal is better in winter and in higher redox conditions. But results of most of the studies, have demonstrated better sulfate removal at warmer temperatures and lower redox potentials. This might be due to the conditions developed which become suitable for sulfur reducing bacteria thus leading to better sulfate removal [34]-[36]. *Typha latifolia* spp because of its ability to maintain lowest redox conditions irrespective of the season make it the desired species for constant year round sulfate removal [34], [37] (Fig. 9).

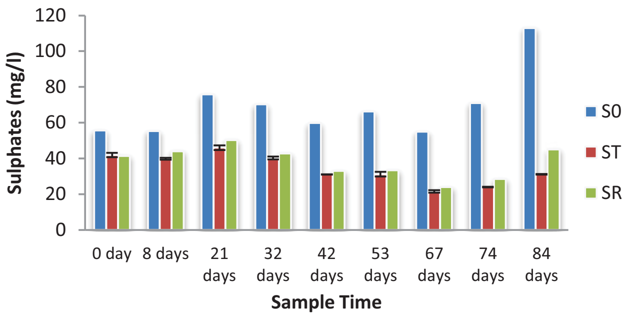

Fig. 9 Change in sulphates concentration during treatment by HSSF mesocosms planted with *Typha* and *Ranunculus*

Orthophosphate is inorganic form of phosphates which is soluble in water and is available for uptake by plants and microorganisms [38]. In wetlands phosphate removal chiefly occurs by physical process like sedimentation and chemical process like adsorption and biological transformations [39]. Reference [40] reported that principally phosphorous removal in constructed wetland systems occur by substratum, litter and Al/Fe component, while plant uptake has minimum role in phosphate removal. Standard limit prescribed by EPA for quantity of phosphorus in drinking water is 0.05 mg/L. In wastewater collected from colony the amount of phosphates ranged from 6.15-18.62 mg/L. This much high level of phosphates is responsible of eutrophication in water bodies. Percent removal obtained for *R.muticatus* was 78.56% and average % reduction for *T. latifolia* was 80.58%. Efficiency of *R. muricatus* is lower than that of *T. latifolia*. Reference [22] reported that efficiency of horizontal subsurface continuous flow treatment system observed for TRP, and SRP removal was 86.2, and 90.0%, respectively. Phosphorus removal efficiencies are generally difficult to predict and it has been reported that substrate selected to be used within a treatment system effect the removal efficiency of P [41]. It is generally accepted that aerobic conditions are more favorable for P sorption and co-precipitation [42] (Fig. 10).

Chlorides are natural content of water and in domestic wastewater they are more probably due to waste released from kitchen and laundry. According to World health organization, it must not exceed the concentration of 250 mg/L in water. In this study chloride contents of wastewater lied in acceptable range of 23-27.8 mg/L and after treatment in case of *T. latifolia* percentage reduction observed was 54.13% and 55.75% removal was noted by *R. muricatus*. Due to continuous flowing mode least HRT is available for chlorides to come into contact with plant roots and biofilm in rhizosphere.

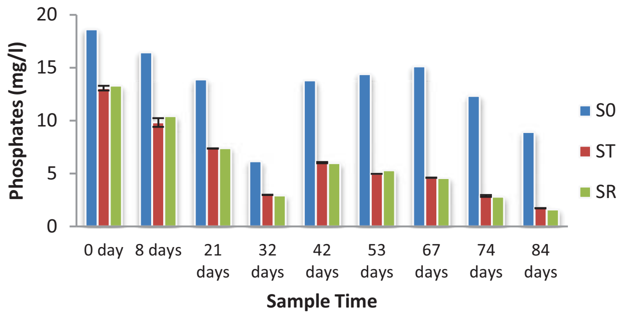

Fig. 10 Change in Phosphates concentration during treatment by HSSF mesocosms planted with *Typha* and *Ranunculus*

A decrease of 8.86% in the levels of chlorides with HRT of 10 hours in a horizontal subsurface CW indicating direct effect of HRT on removal of chlorides [43] (Fig. 11).

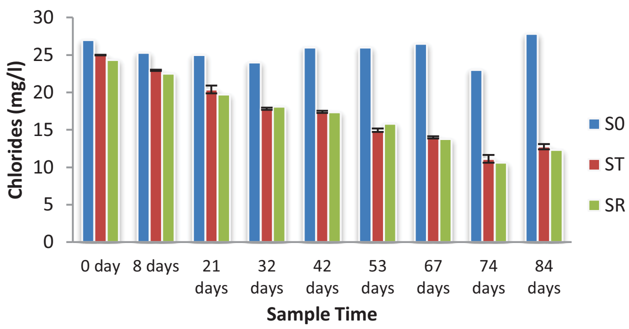

Fig. 11 Change in Chlorine concentration during treatment by HSSF mesocosms planted with *Typha* and *Ranunculus*

Nitrites and nitrates are also important parameters to determine water quality. In domestic wastewater after carbon the second most abundant element is nitrogen whose forms vary e.g. organic N, ammonia, urea, and nitrate, which depend on source [28]. The permissible value provided by WHO for nitrate is 50 mg/L and for nitrite is 3 mg/L in drinking water respectively. In current study nitrites value in wastewater lied in range of 0.2-1.8 mg/L while for nitrates, value varies from 38.96-76.23 mg/L. Initially values of nitrites and nitrates increased then they start lowering down. Final reduction obtained was 79% and 76% by *T. latifolia* and *R. muricatus* respectively in case of nitrites removal. For nitrate average % reduction observed for setups vegetated with *T. latifolia* was 70.03% while for setup planted with *R. muricatus* it was 67.42%. Ammonia removal requires aerobic conditions while nitrates removal can proceed in anaerobic environment (Figs. 12, 13).

Reasons for satisfactorily removal of nitrite comparing nitrate could be the factor that initially around root zones of planted macrophytes due to oxygen release more ammonia get converted into nitrite and finally nitrates. But as far as nitrate is concerned denitrification is an anaerobic process, in HSSF system due to short HRT and because of continuous flow mode proper aerobic conditions are not available for nitrate to get converted into nitrogen gas as major product of denitrification is N_2 [44]. Denitrifying bacteria are facultative anaerobic chemoheterotrophs using organic compounds as electron donors and as a cellular carbon source and nitrogen oxides as terminal electron acceptors [45]. Anoxygenic conditions, availability of organic matter and nitrate, appropriate redox potential, temperature, pH, soil type, and degree of moisture saturation influence denitrification [46].

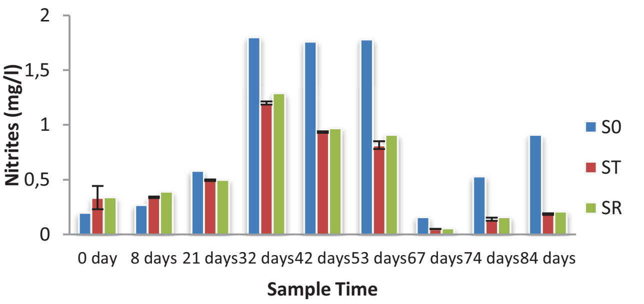

Fig. 12 Change in Nitrites concentration during treatment by HSSF mesocosms planted with *Typha* and *Ranunculus*

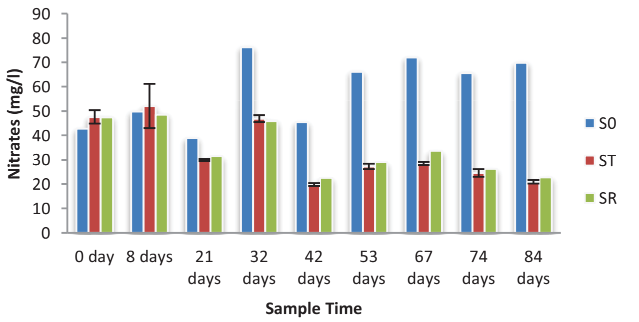

Fig. 13 Change in Nitrates concentration during treatment by HSSF mesocosms planted with *Typha* and *Ranunculus*

Pathogen Removal

Bacteriological analysis was crucial to estimate water quality. Untreated colony wastewater showed the bacterial count ranging from $1.05x10^7$-$4.35x10^7$ chiefly because of high amount of nutrients in domestic wastewater. Comparing all four systems high efficiency for microbial and pathogen removal was obtained for setup vegetated with *R. muricatus*. For setups planted with *T. latifolia* in open environment efficiency was good but lower than that of *R. muricatus* planted setup. For *T. latifolia* planted setups 82.41% reduction of bacterial count and for *R. muricatus* 84.71% (Fig. 14). Lowest HRT might be the reason for incomplete removal. Because all setups were run in continuous flow so least time was available for interaction of pathogens present in wastewater with that of plant roots and biofilm developed in rhizosphere. Higher efficiency of *R. muricatus* setup in pathogen removal was due to some antibacterial properties of this plant reported by [21], [22]. However, the main mechanisms involved in bacterial removal in wetlands include sedimentation, aggregation, oxidation, filtration, solar irradiation, antibiosis, predation, and competition [47].

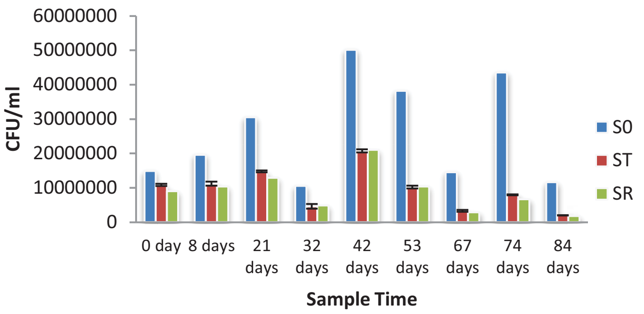

Fig. 14 Change in CFU/ml during treatment by HSSF mesocosms planted with *Typha* and *Ranunculus*

The most probable number (MPN) technique was used for determination of the presence or absence of fecal coliforms in wastewater samples. Media used for this test was lactose broth. All the untreated colony wastewater samples gave MPN results which lied in the range of 1100 MPN index/100 ml (probably more than 150-4800/100 mL). For setups planted with *T. latifolia* MPN index was >1100 /100 mL after 1st treatment at 0 day while *R. muricatus* planted setup give 1100 MPN index/100 mL. Sample analysis after 8 days revealed that MPN index/100ml for *T. latifolia* was 1100 while for *R. muricatus* it was 960. After 21 days MPN index values for *T. latifolia* and *R. muricatus* were 795 and 460 respectively. With passage of time after successive treatments MPN index kept on decreasing after passing through all units of built up setups till the final value of MPN index obtained was 195 and 107 respectively for *T. latifolia* and *R. muricatus* (Fig. 15).

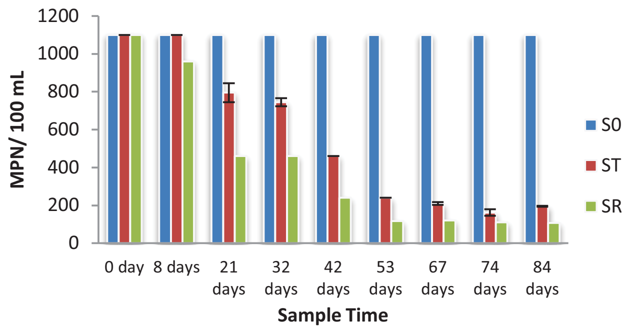

Fig. 15 Decrease in MPN index during treatment by HSSF mesocosms planted with *Typha* and *Ranunculus*

IV. Conclusion

Current study was conducted for comparison of two macrophytes to check their water treatment efficiency and results showed that *Typha* and *R. muricatus* both proved to be effective in improving wastewater quality. But on comparison, efficiency of *Typha* recorded for parameters like COD, BOD_5, DO was higher than that of *Ranunculus*. For MPN, CFU and removal, efficiency of *R. muricatus* was better. Bacterial community colonizing roots of the plants showed that *T. latifolia* was colonized by both Gram positive and Gram negative bacteria while most strains isolated from *R. muricatus* were Gram positive bacteria. High efficiency was observed at high temperature of summer months indicating seasonal effects on treatment efficiency of the system.

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