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Domestic wastewater management: Floating islands come to a rescue

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Abstract
Managing water resources is an essential dimension of sustainable development. This study aims to provide an effective practice for domestic wastewater treatment by designing eco-friendly floating-islands. The method utilizes the phytoremediation macrophytes *Vetiver zizanioides*, *Canna indica* and *Typha latifolia.* In the absence of macrophytes, water quality deteriorated, particularly in terms of dissolved oxygen (from 5.6mg/L to 2.4mg/L) and odour. *Canna* grew better at higher nutrient levels and showed greater ability for acclimatization; *Vetiver* grew more persistently, though needed acclimatization period; *Typha* was more versatile; having the three together in a single tank did not show any observed synergistic effect. Microbial community studies based on 16SrRNA, to identify wastewater remediation associated microbes, confirmed synergism, with the correspondence between the changes in the physico-chemical parameters and the changes in microbial communities. The present findings while corroborating earlier findings, open ways to further findings and more efficient applications of phytoremediation for domestic wastewater.

Keywords: phytoremediation, floating islands, domestic wastewater, plant-microbe synergism

1. Introduction

The crisis of water has reached such an overwhelming level that as further searching for sources of water would create unprecedented stress on nature; innovations to optimize the existing resources would result in greater sustainability. In this context, this study was undertaken aiming to provide an easy and eco-friendly solution for domestic wastewater treatment by means of designing floating-islands whose basic principle is phytoremediation which refers to the use of vegetation for *in situ* treatment of contaminated soils and water, by means of the uptake of organic and nutrient contaminants from soil and groundwater. The subsequent transformation by plants [1] is carried out by means of sedimentation, absorption, adsorption, degradation, volatilization, microbial uptake, etc [2]. The macrophytes besides directly increasing the quality of the water body, also provides site for birds, insects and fishes [3] and thus help balance the aquatic system [4]. Furthermore, the artificial floating islands (AFI) mimic natural wetlands and create riparian habitat [5] to help wave attenuation and landscape improvement [6]. The additional aesthetic value derived makes it even more desirable [7]. Microbial communities, being spatially heterogeneous, temporally dynamic, and structurally and functionally diverse [8] also contribute to wastewater constituent degradation.Microbes associated with the roots of the plant and from the wastewater itself, help destroy or reduce the concentration or effects of contaminant in polluted environment [9]. Thus, this implies two components: microbes and plants; they absorb, accumulate, translocate, sequester and detoxify toxic compounds to non-toxic metabolites [10].

A Floating Island could be just a mass of floating aquatic plants, mud, and peat ranging in thickness from a few inches to several feet, either staying in one place or gradually drifting with the current [11]. There are many natural floating islands, in various parts of India and of the world. Examples in India include, Loktak Lake in Manipur, described as early as 1920 by Annandale *et al*,[12] and famous *Phimudi,* a heterogeneous floating mass of soil, vegetation and organic matter [13], Prashar Lake of Himachal Pradesh, Dal, and Hokarsar and Anchar in Kashmir valley and the ones in Kuttanad, Kerala [14]. Examples in other parts of the world include: Lake Kyoga, in Uganda; Prairie Lake in the US; and Lagoon of Islands of Australia; Vlasina Lake, Serbia [15] In brief, this phenomenon exists almost every climatic condition.

The current study aims at designing eco-friendly floating-islands at the point source to compare the efficiency of removal of pollutants from wastewater treated by three species of macrophytes viz., *Vetiver zizanioides*, *Canna indica* and *Typha latifolia*, individually and in combination, by characterising water quality parameters and studying the microbial dynamics. Earlier in 1986, a systematic effort had been made to utilize *Vetiver* for soil erosion mitigation and water conservation in India under the egis of Vetiver Grass Technology (VGT) [16]. *Vetiver* besides being persistent to droughts and floods due to its being both xerophyte and hydrophyte, has leaves and roots that are aromatic and resistant to many diseases and repel rodents and other pests [17]. *Canna* a rhizomatous perennial herb with scale leaves and thick fibrous roots also manifests similar functional characteristics [18]. *Typha*, as it grows up to about 2.5m tall [19], remains a significant natural component of many wetlands providing nesting sites and desirable cover for waterfowls [20, 21]. The application of molecular microbiological methods helps us understand the community structure related to treatment and to generate a database that helps predictive analyses to estimate and improve the treatment performance [22].

2. Materials and Methods

The process used in this study was sub-divided into (a) designing pilot scale artificial floating islands; (b) wastewater treatment with *ex situ* growing of the plants with artificial floating islands; (c) qualitative and quantitative analyses of wastewater;(d) a comparative study of the macrophytes to test their efficiency in pollutant removal; and (e) a brief study of microbial community associated with wastewater remediation.

2.1. Experimental Setup

Vetiver zizanioides, *Canna indica* and *Typha latifolia* were grown on floating mats in tubs containing wastewater. The floating mat was made of thermocol and green (house) cloth; these two materials are relatively non-biodegradable, and so would interfere little with the study conducted. Each mat was provided with three apertures, each containing two saplings of a particular variety of macrophytes. While the thermocol gave the buoyance required, the green cloth prevented overexpansion of the aperture in the thermocol, thus providing extra strength. The allocation of the five water tubs and of the plants was done as: Tub-L for Control with no plants; Tub-V with 6 saplings of *Vetiver zizanioides;* Tub-C with 6 saplings of *Canna indica* var. *sanctae-rosae;* Tub-T with 6 saplings of *Typha latifolia;* Tub-M with a combination of all the three (mixed system). The experimental design is mentioned in Figure 1.

2.2. Sampling and Physico-chemical Analysis

Wastewater (25L) was collected from Aniket Canteen located in the campus of Savitribai Phule Pune University (SPPU), Maharashtra and further analyses were done under laboratory set up. Exactly 25 L of well-mixed sample filtered with 0.2mm sieve was transferred to each of the five tubs as mentioned above. The sample was subject to lab analysis at three stages: Pre-treatment at day 0; Mid-treatment: at day 25; and Post-treatment Stage at day 50. The physico-chemical parameters analysed were: pH, Turbidity (NTU), Electrical Conductivity (mS/cm), Dissolved Oxygen (mg/L), Biochemical Oxygen Demand (mg/L), Chemical Oxygen Demand (mg/L), Oil (mg/L), Total Dissolved Solids (mg/L), Total Suspended Solids (mg/L), Total Solids (mg/L), Calcium Hardness(mg/L), Magnesium Hardness(mg/L), Total Hardness(mg/L), Nitrate (NO_3^-)(mg/L), Phosphate (PO_4^{3-})(mg/L) and volume of water (L) as per the standard guidelines of APHA.

2.3 Microbial Community Analyses

The BioEra Genomic DNA extraction kit (BioEra, India) was used to extract total community DNA from environmental samples as per the manufacturer's protocol. The quality of the genomic DNA extracts was evaluated by electrophoresis at 120 V for 45 min on a 2% w/v agarose gel. 16S rDNA genes were amplified from extracted genomic DNA by PCR using a PCR ThermoCycler version 2.2 (Applied Biosystem, India). The V3 region of the 16S rDNA gene from members of the domain *Bacteria* were amplified using the 314 F primer (5' CGCCCGCCGCGCGCGGCGGGCGGGGCGGGG – 3') with incorporation of a 40–bp GC–clamp in the 5' primer and the 518 R primer (5'- ATTACCGCGGCTGCTGG-3'). The PCR protocol included a 5 min initial denaturation at 95°C, 4 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s followed by 25 cycles of 92°C for 30 s, 55°C for 30 s, 72°C for 30 s of denaturation, annealing and extension followed by 10 min of final extension at 72°C. Amplified PCR products were purified using the PCR clean-up system (BioEra, India) as per the manufacturer's instructions, and were sent to the GeneBiome Facility (Banglore, India) for sequencing on the automated sequencer AB3730× l . Sequencing reactions were carried out using 1 µl of 6.4 pmol µl $^{-1}$ primer 314 F and 518 R for 5 ng of purified Muyzer primers amplified 200 bp sized PCR products. For archaea, amplified 200 bp sized PCR products of 15 ng concentration, the sequencing reaction was carried out with 1 µl of 6.4 pmol µl $^{-1}$ primer A934F and 1390R GC. The sequencing reactions were run at 96°C for 1 min, 96°C for 10 s, 50°C for 5 s, 60°C for 4 min for 30 cycles and then held at 10°C. Nucleotide sequences were analysed using SEQUENCHER software (Sequencher Version 4.1.4) and homology searches were completed with the BLAST server of the National Centre for Biotechnology Information (NCBI) using a BLAST algorithm (http://www.ncbi.nlm.gov.library.vu.edu.au/BLAST/) for the comparison of a nucleotide query sequence against a nucleotide sequence database (blastn). All 16S rRNA gene sequences were manually checked for chimeric artefacts using the CHIMERA_CHECK program ver.2.7 of the Ribosomal database Project II (RDP- II).

3. Results and Discussion

Since the parameters have got varied mode of measurement, all the results of physico-chemical analyses have been converted into percentage scale, as presented in Fig.1 to Fig.5 which could serve as a common scale for the comparison of the results. Leaving out the first two columns which are common to all, there are five sets of three columns each, for the Control, *Vetiver*, *Canna*, *Typha* and Combination. The three columns in each set, in order, stand for percentage difference in the value of the parameters (i) between the pre-treatment and mid-treatment; (ii) between the mid-treatment and post-treatment and; (iii) between the pre-treatment and post-treatment.

3.1. Overall: Control vs. the Rest

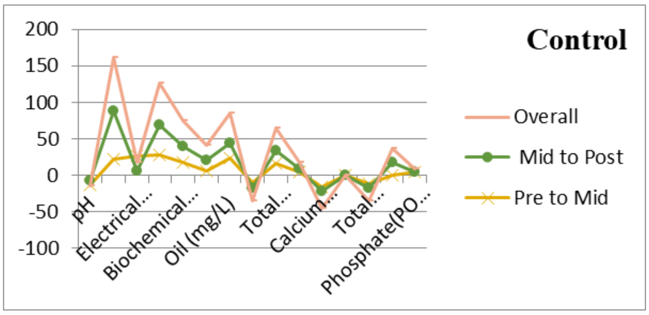

Fig 1: Control Treatment

A significant difference between the results of the analysis of the sample from the Control and those from the tubs with macrophytes was observed. The Control also showed the desirable change with respect to certain parameters, but at much lower rate than the rest. The difference in the desirable change by the rest (compared to the Control) was double in some cases, while it was even higher in other cases, like as high as nineteen times in the case of phosphate reduction. However, the Control showed undesirable change with respect to DO and odour; gradual fall of DO level (5.5mg/L to 2.4mg/L) and development of offensive odour simply means that the wastewater left to its own, develops more anaerobic condition which is an indication of deterioration of the water quality.

3.2. Primary remediation: Initial to Mid-treatment

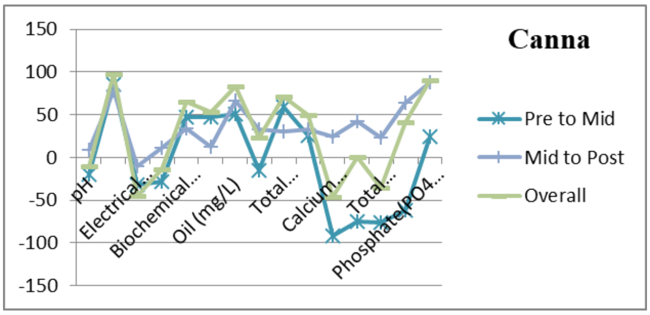

Fig 2: *Canna* Treatment

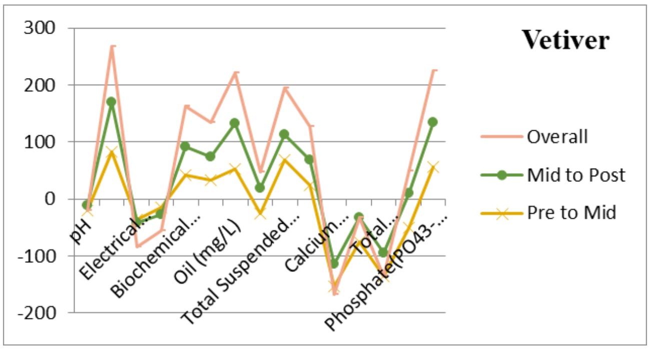

Fig 3: *Vetiver* Treatment

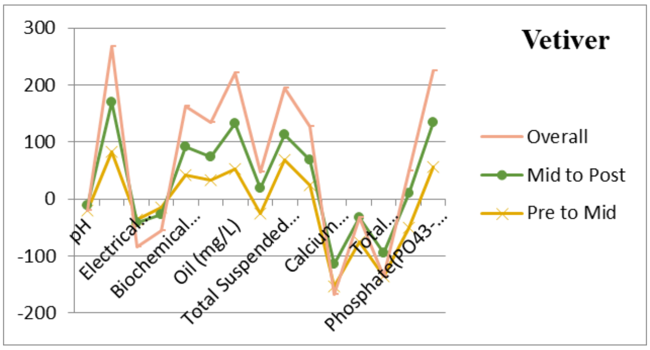

Fig 4: *Typha* Treatment

Canna had the best reduction rate of COD (600mg/L to 280mg/L), which demonstrates its ability to take in more persistent substances as shown in an earlier study [23]. *Canna* showed superior treatment efficiency at the initial stage (pre to mid) with respect to most of the parameters, except turbidity, TS and EC and phosphate (which is much less effective).*Vetiver* showed overall greater efficiency in dealing with TS (3125.7mg/L to 1316.7mg/L); this was illustrated during the process by the increase of the TDS (1433.3mg/L to 1803.3mg/L) with a drastic decrease of TSS (1693.3mg/L to 543.3mg/L). Except for the solid related criteria, *Vetiver* showed the lowest efficiency during the Pre-Mid Interval. The overall increase in the TDS, in all except the Control, and the overall reduction in TSS and TS, could be attributed to the microbial activity and to the root exudates that are capable of dissolving the nutrients in order to increase root uptake [24]. So it can be concluded that *Canna* acclimatizes and begins its normal growth almost immediately and *Vetiver* takes the longest time for the same while the combination has only a cumulative average effect, and not the desired synergistically enhanced effect.

3.3. Subsequent remediation: Mid to Post-treatment

Canna showed relatively lower efficiency during the second half of the treatment. *Canna*'s percentage difference for the interval between Mid-treatment analysis and Post-treatment analysis was least. This change might be due to the deterioration of the nutrient supply in the water; as there were more nutrients at the initial stage when the plant grew rapidly. However, when the nutrient level dropped, the growth also dropped correspondingly. On the other hand, it could also mean that *Vetiver* and *Typha* took longer acclimatization period; but after the initial lag, once acclimatized, they were able to thrive, a characteristic that makes it highly tolerant to severe disturbances in its substratum [25]. During the latter half of the treatment, *Vetiver* showed the maximum efficiency with respect to all the parameters, except in the case of hardness related parameters and of BOD (where it was the same as in others). So by considering the difference in efficiency in the initial remediation and the subsequent remediation, it can be concluded that,

1. *Canna* with its rapid acclimatizing ability was the more efficient at the earlier stages of the treatment;
2. *Vetiver* with its greater tolerance was found to be more efficient at the latter stages of the treatment and;
3. Typha with its intermediate acclimatizing ability and tolerance could be used on either of the stages.

3.4. Overall

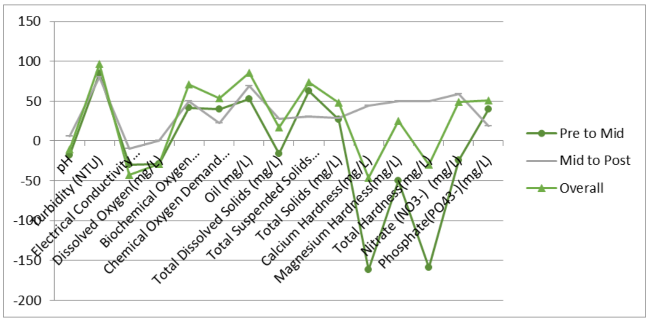

Fig 5: Combination Treatment

Coming to overall performance of the treatment, *Vetiver* and *Canna* showed the best efficiency (from 12.5mg/L to 1.2mg/L and 2.8mg/L respectively) with regard to phosphate. However, for the rest, the overall performance of *Canna*, despite its initial lushly growth was below the efficiency level of *Vetiver* and *Typha*, and slightly above that of the combination in a few cases (hardness and solid related parameters).*Canna* showed least activity with respect to hardness –in terms of the increasing during the initial stage and in terms of utilization during the latter stage – compared to *Vetiver* and *Typha*. This could be attested by the fact of low level of calcium in *Canna sp* [26]. Between *Typha* and *Vetiver*, the former showed greater overall efficiency only with respect to nitrate and hardness related parameters. The initial increase and the gradual decrease in TDS, total hardness and nitrate, in all cases but the control, are an indication of the plant's ability first to dissolve the substances and then eventually to utilize the dissolved nutrients.

Although *Vetiver* showed the best ability to reduce turbidity (0.4NTU) in this study, the ability of *Typha* (quite close with 2.2NTU) to reduce turbidity has been demonstrated in other instances. For example, a study by Govindwar and Khandare (2015)on phytoremediation of textile dyes and effluents, demonstrated that *Typha angustifolia* was able to perform decolourization of the effluent with Reactive Blue 19, up to 70% in just 144hrs.In fact, *Typha* even in its natural condition is known for maintaining low turbidity [27].

In the remaining parameters, *Vetiver* showed the greatest overall efficiency. *Vetiver* is quite robust in its extraction of nutrients. Its efficiency in reducing COD is quite clear; it was much more efficient (with 60% from 600mg/L to 240mg/L) compared to the rest (the Control just 20% and the rest around 53%). This ability of *Vetiver* in degrading even persistent substances has been demonstrated in earlier cases as in remediation of trichloroethylene (up to 98%) [28] and heavy metals like aluminium, iron, zinc and lead [29].

3.5. Plant Growth and Evapotranspiration

Canna showed exuberant shoot-growth initially, though subsequently it declined slightly probably owing to the decline in the availability of ready nutrients. Indeed *Canna* is known for its capacity to grow rapidly into dense colonies [30], and this makes it an ideal choice if the end purpose is high biomass production. *Vetiver* showed a slow growth in the beginning but thrived better in the latter stage. However, *Typha* showed a good growth in the earlier stage and better growth in the latter; in fact, other studies involving longer observation period have also demonstrated high biomass production and nutrient accumulation (266kcal/100g) and evapotranspiration rates in *Typha* [31, 32]. In each case, increasing growth i.e. Increase in the biomass was directly related with the decline in the volume of the water in the tub. *Vetiver* seemed to have shown maximum remediation; however, since the volume of wastewater in the tub with *Vetiver*, at the end, was comparatively larger, it is possible that its apparent higher efficiency was due to the lower evapotranspiration, which could mean that there was more dilution due to more water left. On the other hand, the less loss of water in the tubs with *Vetiver* and *Canna* than the one with *Typha*, also means that if the purpose of the treatment is reuse of the treated water, then use of *Vetiver* and *Canna* may be the best option.

Plants that are grown directly on floating islands have advantage over soil grown plants, for while in the case of soil, the nutrients may be diluted, however in the case of hydroponics, the nutrients are more easily available [33]. This is because not only maximum root system is made available for remediation, but also nutrients are more mobile and by means of simple diffusion can drift towards the region of the roots, thus making hydroponics a better option for remediation. Besides being able to remove the pollutants to a greater extent, the hydroponics is able to do it at a much higher rate as well [34], and the plants with capacity for more luxuriant growth with respect to shoot and root, have greater efficiency for phytoremediation [35].

3.6. Contribution of Microbial Communities

From the point of plant-microbe synergism, microorganisms function synergistically with macrophytes in dissolving, degrading and utilizing the substances in water, which otherwise would persist as water pollutants [36]. the occurrence of dominant microbial communities in the samples in pre-treatment analysis with specification of the appearance of new species at subsequent stages; and the numbers in the parentheses indicate their accession numbers. The disappearance of the organisms in the observations need not mean that the organisms completely died out of the system, rather they reached a much lower detectable-level than the dominant organisms; conversely, appearance of new organisms could simply mean that the below-detectable-level organisms present in water, or in roots, or introduced from air, reached detectable levels. In fact, the dynamics of microbial communities depends on environmental parameters [37], which in our case are a product of absorption of nutrients and release of exudates by the macrophytes.

To come to the specifics, the microorganisms present in control were also present in the sample with *Canna*, throughout the treatment phase: *Uncultured bacterium*, *Pedobacterglucosidilyticus*, *Uncultured Acidovorax* and *Uncultured bacterium*. This conservation is in harmony with the preceding observation that *Canna* gets acclimatized easily and so apparently interferes little in altering its surrounding including the microbial community. In the sample with *Typha*, while three earlier species disappeared, there were two additional microbes, *Hydrogenophaga sp.* and *Uncultured Pseudomonas*that appeared in mid-treatment phase and remained till the end. In case of *Vetiver*, three completely new species appeared during the mid-treatment analysis: *Uncultured Bacteria*, *Uncultured Bacteroidetes* and *Uncultured Desulfosporosinus*. Among these only *Uncultured Desulfosporosinus* persisted till the end of the treatment phase, with the appearance of two additional bacteria, *Uncultured bacteria* and *Denitrifying bacteria*, where the latter must have evolved due to rhizospheric activities [38], Denitrifying bacteria not only have the potential to remediate nitrogenous wastes, but also play their role in sediment based aquaculture bioremediation systems [39]. In the sample with macrophytes in combination, the mid-treatment complete change in the community with the appearance of *Enterobacter sp*, *Pseudomonas sp*, *Uncultured pseudomonas* and *Uncultured Desulfosporosinus* which persisted till the end of the treatment. *Pseudomonas* sp. had been found in many of the wastewater treatment practices since it is very efficient in reduction of COD, TS, MLSS etc [40].

The samples with the macrophytes showed initial increase and gradual decrease in TDS and total hardness, demonstrating plants ability initially to dissolve the substances and then to utilize the dissolved nutrients, in line with earlier observations [41]. Interestingly the Control showed much slower increase in TDS and hardness, and there was no eventual decrease. This decrease and the slowness of the decrease in the Control, demonstrates that microorganisms in the absence of macrophytes, still contribute to dissolution of the nutrients, and dissolution is not prerogative of root exudes alone. However, microorganisms can affect also the enzyme activities of the phytoremediating [42] thus, the microorganisms not only make nutrients better available, but also influence their physiological functions. Studies have also shown that an additional inoculum of microbial communities (bioaugmentation) would speed up the remediation even further [43].

3.7. Prolongation
Remediation through plants in association with microbial communities is far superior to physical and chemical methods.Further study on these macrophytes can be done inquiring the preferences with respect to the composition of the wastewater being treated and their combination with others; other indigenous macrophytes serving the same purpose at a higher rate; and their secondary benefits.Keeping in mind the varying characteristics of the wastewater from place to place and from season to season, there could be further studies focusing on the dimensions of the treatment facilities; suitability for the given type of a wastewater source, considering its acceptability in terms of health, sanitation and aesthetics. With regard to floating mat, studies could be conducted investigating aspects such as feasibility of using woody materials, plastic wastes, e-waste, agricultural and domestic waste which may contain pollutants and, in the long run, can themselves get remediated along with the wastewater being remediated. There are other players that have complementary and supplementary roles to play, so we could study further study role of bio-stimulation and bio-augmentation and impact of aquatic flora and fauna throughout the process of wastewater remediation.

4. Conclusions
In summary, this study has reaffirmed the efficiency of *Vetiver*, *Canna* and *Typha* in bioremediation of domestic wastewater. Furthermore, it opens way to further research and practical applications of AFI using variety of macrophytes, to treat different types of wastewater. This strategy has potential to be applied for in houses, societies, canteens, malls etc. with self-sufficient water management systems for both small and large scale properties. Floating islands thus emerge as an efficient eco-friendly solution for the on-going water crisis.

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