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## Comparative performance and 16S amplicon sequencing analysis of deep and shallow cells of a full scale HFCW having sequentially decreasing depths reveals vast enhancement potential

Saurabh Singh <sup>a b</sup>, Chinmay Maithani <sup>c</sup>, Sandeep K. Malyan <sup>d</sup>, Abhishek Soti <sup>a</sup>, Niha Mohan Kulshreshtha <sup>a</sup>, Rajesh Singh <sup>e</sup> 은 쩐, Urmila Brighu <sup>a</sup>, Akhilendra Bhushan Gupta <sup>a</sup> 은 쩐, Jhalesh Kumar <sup>d</sup>, Shweta Yadav <sup>d</sup>, Omkar Singh <sup>d</sup>, V.C. Goyal <sup>d</sup>

- <sup>a</sup> Department of Civil Engineering, Malaviya National Institute of Technology, JLN Marg, Jaipur 302017, India
- <sup>b</sup> Department of Civil Engineering, Swami Keshvanand Institute of Technology, Management and Gramothan, Jaipur 302017, India
- <sup>c</sup> Department of Civil Engineering, National Institute of Technology Warangal, Warangal, Telangana, India
- <sup>d</sup> Research Management & Outreach Division, National Institute of Hydrology, Roorkee, Uttarakhand 247667, India
- <sup>e</sup> Environmental Hydrology Division, National Institute of Hydrology, Roorkee, Uttarakhand 247667, India

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#### ? What do these dates mean?



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### Highlights

- Performance data of different cells of NIH-CW with varying depth analysed.
- Removal rate coefficients (K) for BOD, NH<sub>4</sub>-N, TN and TP were calculated.
- K values of low OLR cells found to be within range of optimized K values.
- Bacterial diversity found to be different in deep vs. shallow cell.
- N and P removal corroborated by relative abundances of respective genera.

#### Abstract

The performance and <u>microbial community</u> characteristics of a full-scale horizontal flow <u>constructed</u> <u>wetland</u> (HFCW), comprising sequential cells of different depths (1.5 m to 0.8 m), treating domestic wastewater from a residential complex located at Roorkee, India was analysed. The BOD, COD, NO<sub>3</sub>-N, TN, and PO<sub>4</sub> removal efficiencies of the system were found to be 89.9%, 89.1%, 72.7%, 67.1%, and 53.3%, respectively. The areal removal rate constants k<sub>BOD</sub>, k<sub>TN</sub> and k<sub>TP</sub> for BOD, total nitrogen and total phosphorus removal were found to fit well with the optimized k values calculated using secondary data of 74 HSSF CWs. Comparative <u>16S rRNA amplicon sequencing</u> revealed widely varying <u>bacterial communities</u> in the deep and shallow cells of the wetland with several communities being exclusively present in the deep cell. Abundant heterotrophic nitrifiers, aerobic <u>denitrifiers</u>, and phosphate accumulating bacteria demonstrate the utility of such deep HFCW systems for the combined organics and nutrients removal.

### Graphical abstract



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### Introduction

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Decentralized sewage treatment systems (STPs) are gaining prominence for the efficient treatment of wastewater originating in peri-urban and rural settings (Capodaglio et al., 2017). For residential complexes located away from the town, the treatment systems like constructed wetlands (CWs) are the best suited in terms of process economics (Valipour and Ahn, 2016). In addition to reducing the cost associated with long-distance transport of sewage, such systems have practically negligible operational costs and very low maintenance costs (Stefanakis, 2019). In addition, these systems are robust, and if properly designed, they can efficiently handle a sudden increase in organic loadings. This property of CWs is majorly attributed to the presence of a large variety of microorganisms inhabiting the wetland media (Kataki et al., 2021). Depending on the media characteristics, plantation, environmental conditions, and physicochemical characteristics of the wastewater, different microorganisms are enriched and form active biofilms on the surface of the media and carry out the organics and nutrient transformation within the CWs.

Vertical flow constructed wetlands (VFCWs) are efficient in removing Biochemical oxygen demand (BOD) and Total suspended solids (TSS) and enhancing nitrification at higher Hydraulic Loading rates (HLR), but they have significant denitrification problems and performance losses, particularly in the removal of phosphorus (Singh et al., 2022b). On the other hand, nitrification-denitrification is achievable in HFCWs, and these systems are relatively reliable for the removal of BOD and TSS for secondary wastewater treatment. As a result, HFCWs are preferable to VFCWs for removing total nitrogen (N) and phosphorus (P) from wastewater (Liu et al., 2016).

Our earlier work on the secondary data of a large number of Horizontal flow (HF) and vertical flow (VF) CWs has indicated that the area of constructed wetlands can be reduced without affecting the CW performance by increasing the depth of the wetlands (Soti et al., 2022; Singh et al., 2022, Singh et al., 2022; Verma et al., 2022). The deep stretches of the wetlands can potentially develop anoxic/anaerobic/low redox conditions conducive to the development of microorganisms of significantly different metabolic potential than the microorganism growing in the upper stretches where the conditions would be largely aerobic or the environment would be at a higher redox potential (Rampuria et al., 2020). Thus, a comparative microbial analysis of microbial communities in shallow vs. deep wetlands operating in similar conditions would be able to give us an insight into the interrelation between the physico-chemical conditions and the depth of the wetland.

The metagenomic analysis of microbial communities is preferred over the culture based method as in the latter, the microbial growth is limited by (1) the type of nutrients provided by the culture media and (2) the incubation conditions provided for microbial growth. On the other hand, the metagenomic analysis can detect both culturable and non-culturable bacteria present in the given environmental conditions and would act as a better indicator of the microbial communities present in the system (Chen et al., 2022). The metagenomic analysis of full-scale deep CWs is limited (Rampuria et al., 2020). The analysis of a full-scale system would be important as the microbial communities developing in the lab-scale wetlands working on artificial/simulated water acclimatized for a limited period would be very different from full-scale STPs that are functional for many years.

The removal of pollutants (BOD, Total nitrogen (TN), Total Kjeldahl nitrogen (TKN), and total phosphorous (TP)) from wastewater is challenging and increases the overall treatment cost considerably. The efficacy of HFCWs depends on the K values for different pollutants. The

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effectiveness of pollutant removal in wetlands systems increases with increasing K values. The current study intends to examine the impact of depth on the makeup of the microbial community in the full-scale HFCWs and its relationship with the organic and nutrient transformations taking place in the system. The studies on domestic field scale HFCWs with depth ~ 1.5 m are limited. Thus, this manuscript gives us a chance to understand the microbiology of each type of system. By extending the depth of the system where necessary, it would also help optimize the wetland area. In this study, the performance of HFCW cells with sequentially decreasing depths was evaluated by determining the K values for different pollutants removal which were compared with optimized values. The present study also aims to analyze the effect of depth on the microbial community composition in the full-scale HFCW and its correlation with the organics and nutrient transformations occurring in the system. This study would aid in understanding the microbiology of deep vs. shallow systems and would aid in utilizing these systems to their full capacities.

#### Section snippets

### Description of the CW

A schematic diagram of the horizontal subsurface flow constructed wetland located in the residential complex NIH-staff colony (NIH-CW) is shown in Fig. 1. This CW is established and maintained by National Institute of Hydrology, Roorkee, India. The location and dimensions of the wetland are given in Table 1.

For comparison of areal removal rate coefficients and microbial communities, data from two fullscale deep HFCWs (CW1 and CW2) were used. CW1 treats domestic wastewater whereas CW2 treats ...

### Physico-chemical analysis

The average values of physicochemical parameters of wastewater samples collected from NIH HFCWs over a period of one year are shown in Table 3. The inlet BOD load of the wetland  $(98.7 \pm 9.1 \text{ mg/L})$  is in the medium range. This might be due to partial secondary treatment in the septic tank preceding the wetland. The effluent reaches the BOD discharge norms of 20 mg/L after cell 3.

The major removal of BOD, TN, TKN, and TP was found to occur in Cell 1 which led to a marked reduction in the values of ...

### Conclusions

The full-scale horizontal sub-surface flow constructed wetland utilizing sequential shallower depths was found to carry out efficient removal of organics and nutrients. The areal removal rate coefficients of different cells of this system were found to be within range for organics, nitrogen and phosphate when compared with the optimized removal rate coefficients calculated using secondary data barring few cases (K<sub>BOD</sub> and K<sub>TP</sub> for cell1). The metagenomic analysis corroborates the removal of ...

## CRediT authorship contribution statement

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SS: Data analysis, Writing; CM: Sampling, Analysis; SKM: Sampling, Data curation, Writing; AS: Writing- Reviewing and Editing, NMK: Data analysis, Writing- Original draft; RS: Supervision, Resources, Conceptualization, Writing- Reviewing and Editing; UB: Writing- Reviewing and Editing; ABG: Conceptualization, Supervision, Writing- Reviewing and Editing; JK: Sampling, analysis; SY: Data curation, Writing- Reviewing and Editing; OS: Writing- Reviewing and Editing; VCG: Project Administration, ...

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. ...

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...Constructed wetlands (CWs) are integrated ecosystems for efficiently removing pollutants via the synergetic processes of plant uptake, microbial degradation and substrate adsorption (Wu et al., 2015). Owing to their advantages of environmental friendliness, low cost-efficiency, convenient management, and aesthetic value, CWs have been successfully utilized to various types of wastewater, including saline wastewater (Singh et al., 2023). Gao et al. (2021) reported NH4+-N removal rates as high as 88.3–100 % for CWs with 0.4–2.4 % salinity....

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