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Phytoremediation of Heavy Metals from Mixed Domestic Sewage Through Vertical- Flow Constructed Wetland Planted with *Canna Indica* and *Acorus Calamus*

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Abstract

The removal of contaminants from sewage wastewater through constructed wetlands is becoming increasingly popular worldwide. Constructed wetland (CW) is a man-made structure for wastewater treatment that uses natural processes associated with wetland vegetation, soils, and their associated microbial combinations. This study investigated the performance of experimental vertical flow constructed wetland (VFCW) cells to remove heavy metals (HMs) from primary treated sewage. The primary treated sewage was collected from the sewage treatment plant (STP) in the campus of Indira Gandhi National Tribal University, Amarkantak, India. Sewage wastewater samples were collected from all cells of the experimental VFCW and analyzed for four heavy metals (Zn, Fe, Cu, and Cr). The plant species results show that the removal efficiency of the *Canna indica* L. for Zn, Fe, Cu, and Cr was 95%, 92%, 96%, and 93 % and *Acorus calamus* L.were 89 %, 80 %, 91 %, and 47 % respectively. These macrophytes with the substrate (gravel and sand) have presented a wide range of tolerance to all the selected metals and therefore can be used for field-scale constructed wetland removal of heavy metals from sewage wastewater.

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Domestic Sewage;
Macrophytes;
Retention Time;
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Introduction

Heavy metal contamination of water resources has become a serious concern throughout the world. Heavy metals (HMs) may pose a threat to the environment because of their highly toxic and persistent nature.[1] Effluents from household and

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industrial units are directly discharged into sewerage or drainage system thus, contaminating surface and groundwater. This mixture of domestic and industrial effluents containing HMs is highly complex in nature[2] and reaches sewage treatment plants for treatment. Numerous studies on the analysis of treated effluent from Sewage Treatment Plants (STPs) showed that the treated sewage often contained a high concentration of HMs,.[3, 4] Since most of the currently used STPs are unable to eliminate HMs from the wastewater, therefore, the treated wastewater might not be fit for any further use such as irrigation. If the treated wastewater is utilized for such purposes it can lead to the accumulation of HMs in soil and vegetation and may pose health hazards.[5, 6]

Although HMs such as zinc (Zn) and copper (Cu) are essential elements, yet their higher concentrations in crops and vegetables may be of great concern because of their toxicity to humans and animals.[5] However, irrigation with treated sewage containing HMs is practiced in India and the same has resulted in an increase in HMs in soil, plants, and groundwater. In Delhi, India; a case study was carried out to assess the long-term impact of sewage irrigation on soils, plants and groundwater. The study revealed a significant increase of 208%, 170%, 63% and 29% in the concentration of Zn, Cu, Fe, Ni, and Pb, respectively.[7] In addition, sewage pipelines of the rural and urban areas due to breakage and poor maintenance leads to leaching and polluting the surrounding areas. This leakage may cause HMs contamination of the surrounding environmental components like water, soil, and the later these heavy metals may enter into the food chain causing health risks to living beings.[8] The intake of HMs through the food chain by the human population has been reported worldwide.[9] However, excessive intake accumulation of HMs causes serious health problems and diseases, especially cardiovascular, nervous system, kidney, blood as well as bone diseases.[10]

The wastewater contaminated by HMs can be treated by several advanced methods such as reverse osmosis, electrodialysis, membrane filtration, and ion-exchange process, however, these methods are technologically complex, energy-intensive, expensive and requires trained manpower.[11, 12] The cost involved and technology needed for the complete treatment of HMs seems to be unapproachable for developing countries like India. Therefore, an alternative cost-effective, sustainable and natural principle-based technology for the treatment of HMs is required.[1] The use of constructed wetlands (CWs) for wastewater treatment can be an efficient solution. CWs are emerging as a natural, cost-effective and technologically simple solutions for the treatment of various pollutants including HMs.[13] Combination of CWs along with macrophytes can be a viable option for the treatment of HMs from various sources.[14, 15] Macrophytic plants have been proven quite successful in removing the HMs from various environmental matrices such as water and soils,.[1, 16] Macrophytes plays a major role in wastewater treatment processes, it accumulates HMs in its root and shoot systems up to 100 times higher concentration than a surrounding environment without any production of toxic symptoms.[16]

CWs are the engineered structure used worldwide to improve water quality.[17] These systems include a complex combination of design, water flow, an arrangement of filter media, overlying plants and microbial consortia for removing nutrients, organic matter, HMs and other pollutants from wastewater,.[18, 19] Vertical flow constructed wetlands have been used for the sewage treatment previously in many studies[20-22] however relatively a few studies have been conducted regarding the treatment of HMs from wastewater.[23] It has been noticed that the application of macrophytes species with suitable media and hydraulics retention time can effectively eliminate HMs from the wastewater, however, to get the maximum benefits of this process some more studies are required.[24, 25] Therefore, we hypothesized that using VFCW cells can simultaneously remove HMs, organic materials, and nutrients from the wastewater. The presence of HMs does not affect the capacity in the removal of other pollutants.[18] In studies of various plant species, such as *T. Latifolia, P. Australis, C. indica,* and *A. calamus* were shown to accumulate appreciable amounts of Zn, Mn, Cu, Cr, and Fe.[26, 27] The objective of this study was to investigate the removal efficiency of two plant species (*Canna indica* and *Acorus calamus*) in the designed experimental pot of VFCWs setups for the removal of four heavy metal i.e. Cu, Cr, Fe, and Zn from primary treated sewage.

Materials and methods

Experimental Wetland Plants

Two local wetland macrophytes *C. indica* (Indian shot) and *A. calamus* (Bach), were selected to assess their capacities to remove four HMs (Cr, Cu, Fe, and Zn) from primary treated sewage through experimental VFCW cells. These plant species were selected due to their large biomass, extensive root system, fast growth, resistance to pore-clogging and HMs tolerance.[28, 18] Plant species of *C. indica* and *A. calamus* was planted in simulated experimental VFCW cells. Primary treated sewage was obtained from the STP of IGNTU, Amarkantak, India.[13] This primary treated sewage was analyzed for physicochemical parameters prior to its application as influent in the experimental VFCWs cell. After an incubation period, the plants gained stability in the experimental VFCW cells. The different wetland sets were irrigated with sewage mixed with HMs and the physicochemical characteristics of influent and effluent were observed continuously at five times (in hours) 24 h, 48 h, 72 h, 96 h, and 144 h. The physicochemical characteristics of treated sewage and its metal concentration from each pot setup were analysed regularly and the entire experiment was conducted for six months.

Preperation of Sewage Mixed with Heavy metals

Stock solutions of four heavy metals viz. Cr, Cu, Fe, and Zn were prepared from potassium dichromate (K_2Cr_2O7), copper sulphate pentahydrate ($CuSO_4.5H_2O$), ferric chloride anhydrous ($FeCl_3$), and zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) respectively in distilled water. Further, it was mixed in primary treated sewage to get the desired contamination level of 5 mg L^{-1}. Certified AR grade chemicals were used.

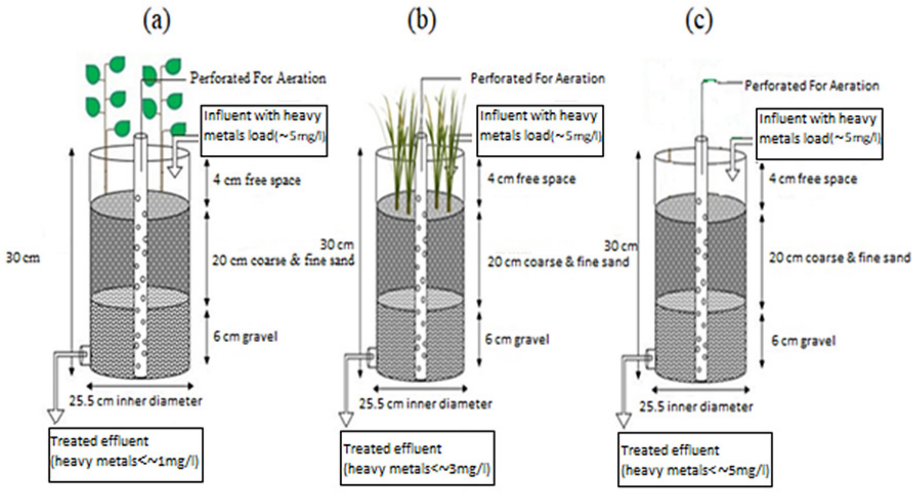

Fig. 1: Experimental vertical flow constructed wetland cells designed for this study. (a) *Canna indica* (b) *Acorus calamus* (c) Unplanted

Experimental Setup of VFCW Cell

The experimental vertical flow constructed wetland cell was made by using a PVC (polyvinyl chloride) bucket with a 30-cm length and 25. 5 cm inner diameter with a faucet opening at the bottom (Fig 1). A perforated PVC pipe was also inserted in the cell for aeration. The cells were first filled with a layer of 6 cm of coarse gravel followed by a 20 cm layer of coarse and fine sand, leaving about 4 cm of free space at the top. Three sets of each plant species were maintained for a wastewater treatment experiment as well as plant control and a metal control was also established in the similar setup. The wetland plants were collected from the local area and were provided four weeks incubation period in the laboratory, thereafter they were transferred into experimental wetland sets.

Sample Analysis

The primary treated sewage was collected from the sewage treatment plant of the Campus of Indira Gandhi National Tribal University, Amarkantak, and transported to the Laboratory of Department of Environmental Science of the Univesity, and was supplied to experimental VFCW cells. The physicochemical analysis of the primary treated sewage and treated wastewater collected from experimental VFCWs after the treatment were analyzed for the following parameters: temperature, pH, conductivity, alkalinity, total dissolved solids (TDS), NO_3-, PO_4^{3-}, and TKN (Total Kjeldahl Nitrogen), BOD (Biochemical Oxygen Demand), and dissolved oxygen (DO) on the same day. Whereas, for HMs, samples were collected at different retention time (HRT) and preserved with 2-3 drops of concentrated HNO_3 at 5^0C for heavy metals until for the analysis.

Some of the parameters like temperature, pH, conductivity, and TDS were measured on-site using a calibrated digital probe PCS Tester 35TM Series Multi-parameter kit (Hanna). All the analyses were carried out as per standard methods prescribed in the American Water Works Association- Americal Public Health Association.[29] Briefly, biochemical oxygen demand (BOD_5) was measured using the Winkler method. Nitrate (NO_3-N) was estimated by ultraviolet (UV) spectrophotometric screening method and phosphate (PO_4^{3-}) was measured by the stannous chloride method. Total nitrogen was estimated by the Kjeldahl method. The primary treated sewage was mixed with four HMs (Cr, Cu, Fe, Zn) each was maintained with a concentration of 5 mg L^{-1} and this mixture was given to the experimental VFCWs cells and consequently treated effluent from these wetlands was sampled on different time intervals (in hours) at 24 h, 48 h, 72 h, 96 h, 120 h, and 144 h. Further analysis beyond this point revealed almost no further removal therefore the experiment was performed up to 144 h only in subsequent analyses. A hundred milliliters of treated wastewater from each VFCW cell were preserved for analysis of heavy metals. Concentrations of Cr, Cu, Zn and Fe were detected by Microwave Plasma-Atomic Emission Spectroscopy (Agilent 4210 MP-AES).

Calculation and Statistical Analysis

The removal efficiency of the constructed wetland was evaluated in terms of the percent removal of heavy metals in specified period. The removal efficiency (removal %) was calculated as the following:

Removal efficiency=(Ci-Co)/Ci×100

Where, C_i and C_o = inlet and outlet concentrations, respectively (mg L^{-1}).

CWs Inlet and outlet physico-chemical parameters and heavy metal concentration of treated effluent to calculate average (mean), standard deviation, and removal of HMs in the different retention time to plot the percentage error bars in the graphs were used Microsoft excel 2013.

Results and discussion

Quality of Treated Sewage Used for the Study

The inlet primary treated sewage wastewater had an average temperature of 28.6°C, and the average concentration of its general physicochemical parameters such as pH was 7.33, conductivity was 931 (µS/cm), alkalinity was 265.6 mg L^{-1}, BOD (5 days) was 93.33 mg L^{-1}, phosphate was 5.42 mg L^{-1}, and total Kjeldahl nitrogen was 46.33 mg L^{-1} during the experimental period (Table 1).

Performance of VFCW Cells through different Macrophytes

The primary treated sewage wastewater spiked with four HMs (Cr, Cu, Fe, Zn) having a concentration of 5 mg L-1 was used as an influent source in VFCW cell. The analysis of physicochemical characteristics and HM content of the influent and effluent was at five different time intervals (i.e. 24 h, 48 h, 72 h, 96 h and 144 h) revealed a continuous and significant decrease in the metal content of treated sewage. The result of the analysis with respect to sewage quality parameters suggested an improvement in the quality of sewage with increasing time. The parameters like BOD, nutrients were removed significantly in 144 h duration. An analysis after this point revealed no significant change in the sewage quality and metal concentration of the treated effluent.

The Removal Efficiency of BOD, Phosphate and TKN

BOD was present with an average value of 93 mg L^{-1} in influent wastewater after 144 h of retention time it was reduced to ~23 mg L^{-1} and ~26.6 mg L^{-1} in cells planted with *C. indica* and *A. calamus* respectively. Maximum BOD of about 75 % was removed by wetland planted with *C. indica*. BOD removal was higher in planted wetland when compared to unplanted cells, probably due to presence of microbial consortia which is present in the root zone of plants.[30] The influent concentration of phosphate was about 5.43 mg L^{-1} when treated through VFCW cells (with 144 h retention time), its concentration was decreased to 1.49 mg L^{-1} in cell planted with *Canna indica* and 2.09 mg L^{-1} in *A. calamus*. Cell planted with *C. indica* revealed maximum removal efficiency i.e., 72.5% for removal of phosphate.

TKN (Total Kjeldahl Nitrogen) decreased from an initial average value of 46.33 mg L^{-1} to ~20 mg L^{-1} after 144 h retention period in wetland planted with *C. indica* and ~33.33 mg L^{-1} in cell planted with *A. calamus* respectively. Since the effluent remained within a subsurface level in the experimental constructed wetland, the TKN might have been transformed and removed from the system through biological processes i.e., ammonification. In ammonification, ammonia (NH_3) is released when biochemical processes oxidise nitrogenous compounds present in wastewater. As the DO (Dissolved Oxygen) level in both the planted cells has increased (Table 1), subsequently promoted the ammonification process.[31, 20] In general, the percent removal of BOD, PO_4^{3-}, and TKN of all VFCW cells were in the following order: BOD>PO_4^{3-}>TKN. The higher removal efficiency of BOD through plant species other than PO_4^{3-}, TN might have resulted from high adsorption capacity of PO_4^{3-} by the *C. indica*.

Table 1: Quality of inlet (untreated) and outlet (treated) wastewater

Parameter	Initial concentration (mean ± standard deviation in mg L^{-1})	Final concentration (mean ± standard deviation in mg L^{-1})		
	Primary Treated Sewage	Unplanted	Canna indica	Acorus calamus
pH	7.33±0.06	7.13±0.05	6.92±0.01	6.96±0.02
Temp.°C	28.60±0.10	27.37±0.11	26.33±0.15	26.40±0.10
Conductivity [µS cm^{-1}]	931.00±2.00	941.67±1.15	945.00±2.00	944.33±1.15
Alkalinity [mg L^{-1}]	265.67±2.08	245.67±2.08	164.67±1.15	167.00±1.00
BOD_5 days [mg L^{-1}]	93.33±5.77	83.33±5.77	23.33±5.77	26.67±5.77
DO [mg L^{-1}]	1.73±0.12	1.87±0.11	4.53±0.12	4.37±0.06
PO_4^{3-} [mg L^{-1}]	5.42±0.32	5.02±0.57	1.49±0.13	2.09±0.30
Total nitrogen [mg L^{-1}]	46.33±1.53	43.33±1.15	20.00±2.00	33.33±1.15

Removal of HMs

The elimination of toxic HMs i.e., Zn, Fe, Cu and Cr from primary treated sewage using two different experimental VFCW cells were appraised under controlled lab-scale experimental setup during 144 h retention time and is presented in Table 2. The VFCW cells planted with *C. indica* had shown, removal efficiencies of 96%, 95%, 93%, and 91% for Cu, Zn, Cr, and Fe respectively. The performance of this experimental VFCW cells (Fig 2), was better in comparison to the wetland planted with *A. calamus*. The performance of two plant species of VFCW cells varied among four metal species. *Canna indica* achieved the highest Cu and lowest Fe removal. Whereas, the potential of *A. calamus*, was highest for Cu and lowest for Cr. Unplanted cell showed negligible potential for heavy metals removal through the natural process. The results indicated that two macrophytes *C. indica* and *A. calamus* were very good performers for the HM removal and have significant elimination capacity for HMs removal through VFCW. The high performance of the two wetland plants may be attributed to their high metal uptake potential and accumulation capacity.

The likely mechanism for the removal of heavy metals in CW is very complex, which involves physicochemical and biological processes such as binding to the substrate, plant uptake and microbial metabolism.[32, 33] Uptake potential of HMs is governed by accumulation, mobilization, and translocation factors in the macrophytes.[34, 35] The retention and transportation of HMs from root to shoot differs with macrophytes species used in wetlands.[36] In the constructed wetland, HMs may undergo precipitation and co-precipitation as insoluble salts, adsorption, and breakdown of metals by microbes.[37, 15]

Table 2: The initial and final concentration of heavy metals

Heavy Metals	Initial concentration (mg L^{-1})	Final concentration (mean ± standard deviation in mg L^{-1})		
		Unplanted	*Canna indica*	*Acorus calamus*
Zn	5	4.14±0.01	0.21±0.01	0.52±0.03
Fe	5	4.20±0.03	0.42±0.01	0.97±0.02
Cu	5	4.30±0.02	0.19±0.01	0.42±0.01
Cr	5	4.64±0.01	0.33±0.02	2.65±0.01

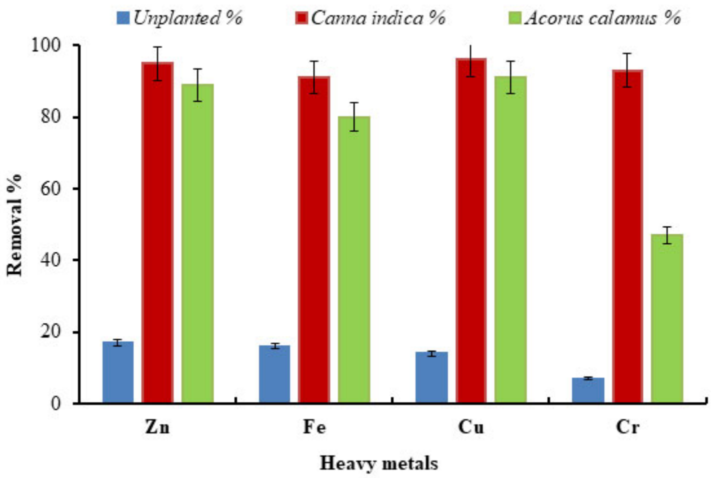

Fig. 2: Percent removal of heavy metals through unplanted and two macrophytes

Role of Macrophytes

The order of HMs removal performance in the present study was Cu>Zn>Cr>Fe in case of VFCW cell planted with *C. indica* and Cu>Zn>Fe>Cr in case of VFCW cell planted with *A. calamus* and Zn>Fe>Cu>Cr in case of unplanted wetland setup (Fig 3). *Canna indica* have fleshy and high stem biomass when compared with *A. calamus*. Hence, the translocation of HMs and its uptake from the wastewater to stem biomass, have probably resulted in low HM concentration after 144 hr of retention time in VFCW.[2] High removal of Cu (96.0%), Zn (95.0%), Cr (93.0%), and Fe (80.0%) in the present study was recognized when compared to the macrophytes reported by.[38] High HRT favours high removal rate of HMs from wastewater by *C. indica*, when the current study was compared with the similar model of CW designed by[2] having 72 hr of HRT, the removal efficiency of Zn, Cu, and Cr was found to be more at 144 hr HRT (present study). High removal of Cu, Zn and Cr in this study may be due to the high retention time, substrate, and uptake of HMs by plant species. These findings suggest that the duration of operation for the removal of heavy metals in wastewater is significantly affected.

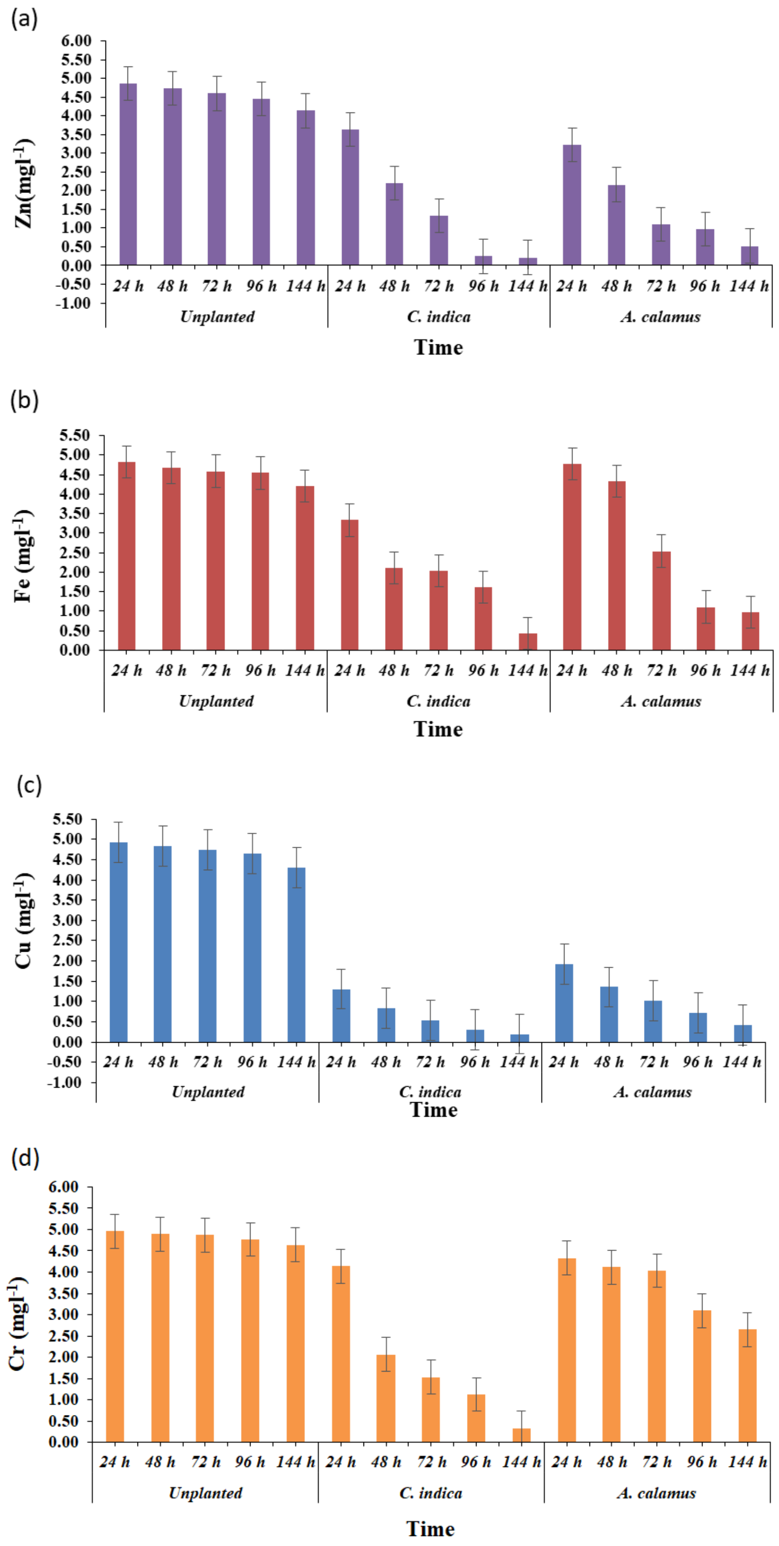

Fig. 3: Removal efficiency of experimental VFCW cell with respect to HRT (A) Zinc (B) Iron (C) Cupper and (D) Chromium

Role of Wetland Media in Heavy Metal Removal through Constructed Wetlands

The composition of the substrate is very important and carry out the major functions related to filtration, adsorption, sedimentation, flocculation, precipitation and ion exchange in the constructed wetland.[15] It is the substrate that withstands or holds the macrophytes and allows the microbial film to grow on it.[37] Commonly used substrates in constructed wetlands are gravel and sand. Washed gravels increase the filtration of wetlands and minimize clogging.[39, 40] Hydraulic retention time and adsorption capacity are the main characteristics of substrates in treating the wastewater as shown in Table 3.

Table 3: The removal efficiency of heavy metals in various constructed wetlands

Locality	Constructed wetlands	Retention Time	Substrate	Wastewater	Zn	Fe	Cu	Cr	References
Pakistan	VFCW	48 h	Sand, Gravel	Industrial	-	89	-	97	41
Japan	VFCW	72 h	Loamy soil	Synthetic landfill leachate	92	65	-	80	42
China	SSFCW	12.68 h	Coke, Gravel	Trace element solution	91	-	90	-	43
India	VFCW	36 h	Gravel	Sewage	>55	-	>95	>35	38
India	VFCW	144 h	Sand, Gravel	Sewage	>95	>90	>95	>90	Present study

The average performance of the highest removal Zn and lowest removal of Cr has been observed, through the media (sand and gravel) in the unplanted VFCW cells. The present study suggests that both sand and gravel were effective substrates for the removal of Zn, Cr, Cu, and Fe from primary treated sewage simulation discharges from rural areas. The sewage wastewater flowed through sand and gravel and resulted in the reduction of pore-clogging.

Conclusion

The present study had shown the effective treatment of sewage mixed HMs through experimental VFCW cells planted with two macrophytic species *C. indica* and *A. calamus*. The application of VFCW has been found a successful strategy for the treatment of heavy metals and various sewage quality parameters like BOD, nutrients (Nitrate and Phosphate). This study was quite consistent in removing the four HMs i.e. Cr, Cu, Fe Zn up to a removal efficiency of >90%. This study suggests that the use of vertical flow constructed wetlands can be adopted in rural and remote areas of India where the treatment of sewage and HMs is a challenge. The two macrophytes used in this study were selected from the local area and are available abundantly in the region. The wetland media (sand and Gravel) are also available locally at a low cost. A combination of various factors such as very low energy requirement, abundant availability space, media and plants can make this technology highly applicable for this region. However, more researches are needed on the removal capacity, type of substrate, diversity of plants for study and batch mode operation of constructed wetlands. The present study suggests that both sand and gravel were effective substrates for the removal of Zn, Fe, Cu, and Cr by through experimental VFCW cells.

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Conflict of Interest

The authors do not have any conflict of interest.

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