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RESEARCH ARTICLE

Chromium removal efficiency of plant, microbe and media in experimental VSSF constructed wetlands under monocropped and co-cropped conditions

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Abstract

Chromium (Cr), one of the most abundant and hazardous heavy metals, is generally observed to be widely distributed in environment, primarily due to the inter-mixing of the untreated domestic and industrial wastewaters. There has been an increased interest to replace conventional centralized treatment technologies with the low energy, low cost, and zero sludge producing decentralized constructed wetland technology. Therefore, a long-term investigation on the comparative metal removal efficiency of the experimental vertical sub-surface flow (VSSF) constructed wetland systems, irrigated with Cr-spiked ground waters, under both mono and mixed-culture conditions planted with five different macrophytes viz. Typha (T), Phragmites (P), Acorus (V), Arundo (A), and Vetiver (K), in as mono- and {viz. (TP), (PA), (KV), (AT), and (VT)} as co-cropped combinations along with unplanted (U) systems as controls was conducted at the ICAR-Indian Agricultural Research Institute, New Delhi, India. Long-term investigations revealed significant differences between metal removal efficiencies of the planted (61.6% to 78.5%) and the unplanted systems (32.8% to 47.9%). However, these long-term average metal removal efficiencies were found to be insignificantly different for the mono (78.5%) and the co-cropped systems (77.6%). On further compartmentalization of the experimental wetland system's Cr-removal efficiencies amongst the major components viz. plant, microbe, and substrate, it was observed that vegetation contributed the maximum (i.e., 33–48%) while the microbes and the substrate contributed only 4–20% and 8–28%, respectively. It was further observed that due to reduced microbial diversity under unplanted conditions, the planted systems were associated with 2–7% higher microbial and equivalently lower substrate removal efficiencies. Thus, microbial activity-mediated metal mobilization and plant uptake were observed to be the principal processes governing Cr removal in the test VSSF constructed wetland systems exposed to varying Cr concentrations. Amongst all test macrophytes and their combinations, Arundo (81.9%) and Acorus (84.5%) based monocropped systems and Arundo+Typha (89.3%) based co-cropped systems emerged to be the most superior Cr-removing systems.

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Introduction

Rapid urbanization and industrialization is adversely impacting the environment globally. Environmental degradation and pollution by inappropriate management of wastewater is one of the major environmental problems all around the world, including India. In India, the estimated sewage generation has reached 61,754 MLD (CPCB 2016) as against the total developed treatment capacity of 22,963 MLD (i.e., 38%) The remaining, 38,791 MLD of untreated sewage (i.e., 62%) just flows directly into the nearby water bodies. As a result, daily, about 14,353 tons of BOD load and the associated hazardous pollutants get discharged into the surface water bodies and its surrounding environments. Amongst all pollutants, heavy metals are the most important due to their toxicity, non-biodegradability, and bio-accumulating tendency and extreme persistence. Over last one decade, annual worldwide release of chromium has been estimated to have reached about 5,87,000 tons (Pacyna and Pacyna 2001). In fact chromium, primarily discharged by the steel, alloy, leather, tanning, paper, paint, dye, textile, photography, electroplating, and metal finishing industries, has been observed to be one of the most abundant hazardous heavy metals. In absence of proper treatment, such chromium-enriched industrial effluents generally get mix-up with the nearby municipal sewage streams, and thereby distribute into the environment.

In environment, chromium exists in several forms. However, the most stable and common forms are the trivalent, Cr (III) and the hexavalent, Cr (VI) forms. Cr(VI) compounds are mostly used for steel production and chrome plating while Cr(III) compounds are used as leather tanning agents. Cr(VI), as chromate (CrO_4^{2-}), dichromate ($Cr_2O_7^{2-}$), and chromium oxide (CrO_3), is considered to be the most toxic forms of chromium to the plants, animals, micro-organisms, and the humans due to its carcinogenic nature and high oxidizing potential, solubility, and mobility in the environment and across the membranes of the living organisms, under all pH conditions, while Cr(III), generally present as oxides, hydroxides, and sulphates, is less toxic due to its insolubility in water, particularly under neutral to basic pH conditions and has lower mobility due to its strong binding with the organic matter present in the soil and aquatic environments. However, under acidic conditions, Cr(III) also solubilizes and becomes toxic. Long-term exposure of chromium to humans leads to liver, kidney, and nerve tissue damage besides gastric problems, dermatitis, and lung cancer. As a result, maximum permitted discharge level of total Cr in the surface/ potable waters has been prescribed as below 0.05 mg/l while that for the wastewaters as 0.1 mg/l.

For removal of heavy metals from water and wastewaters, worldwide several methods such as adsorption, precipitation, reverse osmosis, coagulation, ion exchange, electro-dialysis, electro-winning, electro-coagulation, and photo-catalysis are in practice. However, these methods are extremely expensive especially for treating streams with low Cr-concentrations (ranging from 1 to 100 mg/l) and usually produce large quantities of toxic chemical sludge, of which disposal is a major problem. In view of this, the decentralized wastewater treatment methods are gaining popularity mainly due to their cost-effectiveness, lower operational and maintenance costs, and green and sustainable character with promoting business and job opportunities.

Amongst different decentralized treatment methods, constructed wetlands (CWs) has been recognized as an efficient technology for wastewater treatment. It is an artificially maintained bio-filtration system and a worldwide-proven wastewater treatment technology designed to utilize natural processes involving wetland vegetation, media, and the associated microbial assemblages (Mitsch and Gosseink 2007). Adsorption, microbial degradation/ transformation, and sequestration by plants are the major mechanisms that act to eliminate or transform much of the pollutant load in the wastewaters moving through the constructed wetlands (Toet et al. 2005). Compared to the conventional treatment systems, constructed wetlands need lesser energy, are easily operated, and have no sludge disposal problems (Morari and Giardini 2009). Further, these systems have low construction, maintenance, and operational costs.

Scanning of literature has revealed that depending on the constructed wetland type, such systems have the potential to remove metals and metalloids to varying degrees. Generally, there metal removal rates have been reported to be higher than 70%. However, few, if any, of such systems have been planted with particular species because of a deliberate choice regarding their efficacy. Further, till date, most of the research related to the constructed wetland technology is focused on the development and evaluation of a monoculture-based wetland system. Selected diverse plant communities may further enhance the treatment capacity and applicability of such systems. However, documented comparative nutrient and heavy metal removal efficiency of mono and mixed-culture constructed wetlands are limited, and with inconsistent results (Fraser et al. 2004; Picard et al. 2005; Zhang et al. 2007). Thus, in order to optimize designs for metal removal through constructed wetlands, there appears to be a need for the standardization of phyto-remediation protocols particularly with respect to the role of substrate, micro-organisms, macrophytes, and their interactions in the constructed wetlands.

Based on the aforementioned research gaps, a study was conducted at ICAR-Indian Agricultural Research Institute, New Delhi, India, where five different macrophytes viz. Typha (T), Phragmites (P), Acorus (V), Arundo (A), and Vetiver (K) were grown in the chromium spiked microcosms, under both monocropped and co-cropping combinations along with the unplanted-control (U) systems to (a) compare chromium removal efficiency of simulated wetland systems, (b) quantify percentage metal uptake by the individual plants and/or plant combinations, and (c) understand role of plant associated microbe(s) and substrate in metal bio-accumulation and precipitation.

Materials and methods

Experimental layout

The experiment was conducted near seed production site of ICAR-Indian Agricultural Research Institute, New Delhi, India, (28.08° N and 77.12° E), where an experimental vertical sub-surface flow (VSSF) constructed wetland facility (Fig. 1(a)) comprising of 54 microcosms, placed in completely randomized design, was set up. The test facility was assembled from commercially available plastic tanks of about 60 cm depth and 33 cm diameter (i.e., 50-L capacity). Each tank was placed on a 1 ft high earthen brick base, attached with a plastic tap at 2 cm height above its base for collecting temporal effluent water samples, and painted white from outside to avoid excessive heating of its outside surface. As illustrated in Fig. 1(b), these tanks were filled with a bottom (30 cm) layer of coarse gravel (of about 25 mm diameter) and an upper (20 cm) layer of fine gravel (of about 5 mm diameter). The balance 10 cm top space, in each tank, was kept empty for introducing 22 l of irrigation water. Each monocropped microcosm, planted (in triplicate) with five 30-day-old plantlets of each of the following emergent wetland plants viz. *Typha latifolia* (T), *Phragmites karka* (P), *Acorus calamus* (V), *Arundo donax* (A), and *Vetiver zizanioides* (K), along with an unplanted control (in triplicate),was spiked with 3 levels (i.e., 1.5, 3.0, and 5.0 ppm) of Cr-enriched ground waters (in triplicate) from Dec., 2013 to Jun., 2014; after its initial stabilization with (un-spiked) normal ground waters for 3 months (from Sep., 2013 to Nov., 2013). The Cr-spiked irrigation waters of 1.5, 3.0, and 5.0 ppm concentration were prepared from a 1000-ppm standard Cr solution. Simultaneously, fifteen (15) co-cropped microcosms planted (in triplicate) with 3 plants (each) of the following 5-wetland vegetation combinations viz. Typha+Phragmites (TP), Phragmites+Arundo (PA), Vetiver+Acorus (KV), Arundo+Typha (AT), and Acorus+Typha (VT) were established in Sep., 2014. After initial system stabilization for 3 months, these systems were irrigated with the highest (i.e., 5.0 ppm) chromium-enriched ground waters and monitored for their metal removal efficiency, along with the monocropped and the unplanted microcosms, for further 8 months from Nov., 2014 to Jun., 2015. The chemical characteristics of groundwater used for spiking at the test site are presented in Table 1.

Sampling and analysis

Water sampling and analysis

In order to account for the total chromium removing efficiency of the experimental wetland systems, monthly effluent water samples were collected from each of the test microcosms. About 50 ml of the effluent water sample, from each of the three test-replicated microcosms, was collected and a composite effluent sample of 150 ml was prepared. These composite water samples were then immediately acidified with 1 N nitric acid (HNO_3) and stored at 4 °C. To determine metal concentrations in the influent and the effluent water samples, 50 ml water sample was digested using di-acid mixture (HNO_3: $HClO_4$:: 9:4) and its chromium concentration was determined using an atomic absorption spectrophotometer (AA8000, Lab India), as per the standard procedure.

Plant sampling and analysis

In order to screen efficient chromium removing macrophytes and their combinations, plant samples were collected at monthly intervals. Each month, just before the next irrigation cycle, one plant per each treatment from the replicated monocropped systems, and two plants (one per each plant combination), from each of the replicated co-cropped treatments was uprooted and air dried. Thereafter, their root and shoot portions were separated, oven dried, and ground. Each, 0.5 g of the ground plant sample was digested using di-acid mixture (9:4::HNO_3:$HClO_4$) and its chromium concentration was determined through AAS, as per the standard procedure (Willis 1962).

Gravel sampling and analysis

In order to assess long-term chromium deposition on the media of the test experimental wetland systems, under mono/ co-cropped and unplanted conditions, 50 g of representative gravel sample was collected from each of the test microcosms in June, 2015 (i.e., at the end of the experiment) and air/oven dried (at 55 °C) for 24 h. Thereafter, these samples were transferred to 150-ml pre-labeled acidified plastic bottles and subjected to an overnight treatment with 50 ml of 1 N- HNO_3. The following day, these samples were shaken for 2 h on a mechanical shaker (at 150 rpm, at room temperature) and the extracted surface-deposited metal containing sample was filtered through a Whatman filter paper no. 42. Thereafter, each

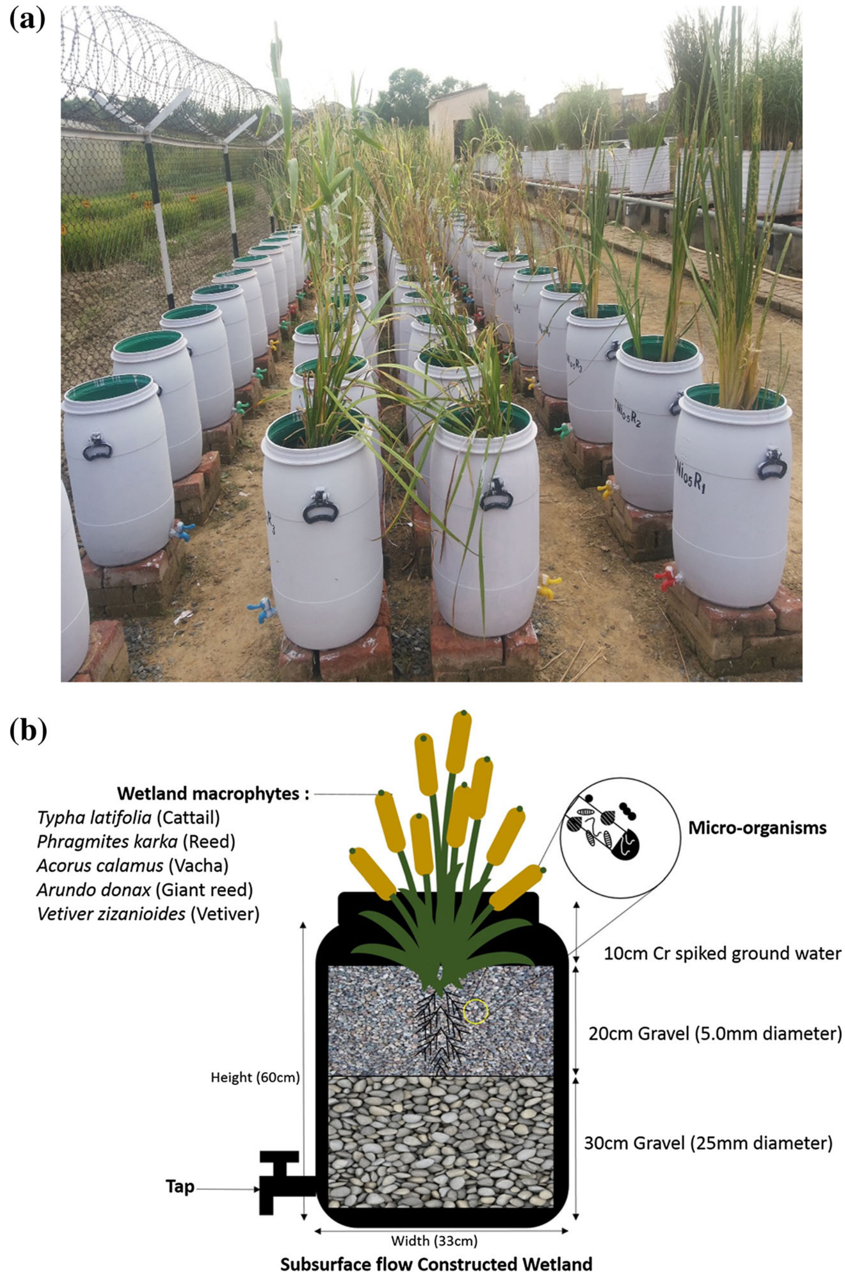

Fig. 1 (a) Experimental layout of treatments at the project site. (b) Verical profile of test microcosm

10 ml of filtrate sample was digested using di-acid mixture (9:4::HNO_3:$HClO_4$) and its chromium concentration was determined using AAS, as per the standard procedure (Willis 1962).

Rhizospheric microbial water suspension sampling and analysis

In order to account for long-term chromium removing efficiency of the micro-organisms in the test experimental wetland systems, a 10-ml microbial water suspension sample was collected from each of the test experimental wetland systems, at the end of the experiment (i.e., in June 2015). The microbial water suspension samples collected from respective replicated treatments were composited and immediately transferred to laboratory for culturing. For microbial culturing, a nutrient media (NM) broth comprising of sodium chloride (1.0%), peptone (1.0%), and beef extract (0.3%) was prepared and maintained at pH 6.8. The nutrient media (NM) broth was primed with chromium at 1.5, 3.0, and 5.0 ppm concentration levels. Thereafter, each 100 ml of NM broth was transferred to a 150-ml capacity conical flask, cotton plugged, autoclaved for 20 min at 121 PSI and inoculated with 1-ml microbial suspension, cotton re-plugged, labeled, and shaken on a mechanical shaker for 30 days at 30 °C. After inoculation, 50 ml of each microbial cultured NM broth was centrifuged, at 10,000 rpm for 8 min, and the dispensed supernatant was

Test parameter	Value	Permissible limit (s)	Reference
pH	8.08 ± 0.38	6.5-8.5	Acharya et al. (2008)
EC (dS/m)	2.32 ± 0.05	2.25	
Ca+Mg (me/l)	10.07 ± 2.19	6	
Bicarbonate (me/l)	7.90 ± 1.19	120	
Carbonate (me/l)	2.23 ± 2.58	-	
Potassium (me/l)	0.09 ± 0.09	0.5	
Sodium (me/l)	8.83 ± 4.74	50	
Fluoride (mg/l)	0.29 ± 0.08	1.0	
Phosphate (mg/l)	10.19 ± 4.87	50	BIS (1986)
Sulphate (mg/l)	68.35 ± 4.61	400	
Chloride (mg/l)	630.8 ± 10.09	355	FAO (1985)
Nitrate (mg/l)	8.42 ± 1.31	30	Ayres and Westcot (1985)
Iron (mg/l)	1.56 ± 1.03	5.0	
Chromium (mg/l)	0.17 ± 0.15	0.1	
Manganese (mg/l)	0.08 ± 0.11	0.2	
Lead (mg/l)	0.07 ± 0.07	5.0	
Zinc (mg/l)	0.02 ± 0.05	2.0	
Nickel (mg/l)	0.02 ± 0.06	0.2	

Table 1 Ground water quality at test site

acidified with 1 N HNO_3 and stored at 4 °C. Ten milliliters of supernatant was later digested in microwave digester (TITAN MPSTM, Perkin Elmer) using 5 ml nitric acid and its chromium concentration was determined through AAS, as per the standard procedure (Willis 1962).

Estimation of metal removal efficiencies

System metal removal efficiency

Based on the concentration of metals in the influent and the effluent water samples, total metal removal efficiency of each microcosm was computed as per the following formula:

$$Mt\ (\%) = (Miw - Mow)*100/Miw$$

Where Mt = total metal removal efficiency of the test planted/unplanted microcosm (%), Miw = metal concentration in the influent water (mg/l), and Mow = metal concentration in the effluent water (mg/l).

Plant metal removal efficiency

The plant metal removal efficiency of each of the test planted microcosms was estimated by subtracting the total metal removal efficiency of the unplanted microcosms (maintained at a particular metal concentration) from the respective planted microcosms:

$$Mp(\%) = Mv(\%) - Mu(\%)$$

Where Mp = plant-metal removal efficiency (%), Mv = metal removal of test planted microcosm (%), and Mu = metal removal efficiency of corresponding unplanted microcosm (%).

Microbial metal removal efficiency

Based on the concentration of metals in the blank (non-inoculated) and the sample (inoculated) broths, the microbial metal removal efficiency of each of the test microcosms was estimated as:

$$Mm\ (\%) = (Mb - Ms)*100/Mb$$

Where Mm = microbial metal removal efficiency (%), Mb = metal concentration in blank broth (mg/l), and Ms = metal concentration in sample broth (mg/l).

Media metal removal efficiency

The metal removal efficiency of media in each of the test microcosms was estimated as:

$$Md\ (\%) = (Mt - Mp - Mm)$$

Where Md = media-metal removal efficiency (%), Mt = total system metal removal efficiency (%), Mp = plant metal removal efficiency (%), and Mm = microbial metal removal efficiency (%).

Results and discussion

Plants, micro-organisms, and media play an important role in metal removal. In natural environments, microbes live in close association with the plant and media surfaces in the form of multi-cellular aggregates/consortia/clusters called bio-films (Branda et al. 2005) that assist immobilization/mobilization of heavy metals and stimulate their acquisition by plants (Aafi et al. 2012). Plants uptake and sequester these metallic pollutants in their large root biomass. Metallic pollutants are also removed through their sedimentation, binding to porous media and precipitation (i.e., through media filtration). Hence, total metal removal in any wetland system is generally the sum of its removal through plant uptake, microbial activity, and media filtration (i.e., precipitation, deposition, and adsorption). The contribution of each of these components to the total metal reduction efficiency of the test wetland systems (or microcosms) is as illustrated in the following sections:

System metal removal efficiency

Monthly chromium (Cr) removal efficiencies of the planted (mono/co-cropped) and the unplanted microcosms, subjected to Cr-spiked irrigation waters of 1.5, 3.0, and 5.0 mg/l concentrations, are presented in Fig. 2(a, b, c, d) and Tables 2 and 3. Figure 2(a, b, c) and Table 2 clearly illustrate that with increasing applied chromium concentrations, the chromium removal efficiency increased ($r = 0.80$) by about 12.20%, from 1.5 to 3.0 mg/l and by 5.02%, from 3.0 to 5.0 mg/l respectively. As anionic complexes (such as $Cr_2O_7^{2-}$) get adsorbed to the positively charged surfaces only therefore at increasing Cr concentrations, adsorption first increased and then ceased to increase as it reached its maximum adsorption capacity (AL-Hamdan and Reddy 2006).

It was also observed that the overall average chromium removal efficiency of the monocropped microcosms (Table 2) at 1.5, 3.0, and 5.0 mg/l Cr-concentrations was 61.61%, 74.74%, and 78.49%, respectively. Further, compared to the unplanted systems, the planted microcosms were observed to be associated with significantly (32.76 to 47.52%) higher chromium removal efficiencies. Amongst monocropped systems, the chromium removal efficiency of test macrophytes at 1.5 mg/l metal concentration was observed to be as Arundo ≥ Acorus ~ Typha ~ Vetiver ≥ Phragmites. Similarly, at 3.0 and 5.0 mg/l metal concentrations, the chromium removal efficiency of the monocropped systems was observed to be as Arundo ≥ Acorus ~ Typha ~ Phragmites ~ Vetiver and as Arundo ≥ Acorus ≥ Phragmites ≥ Typha ~ Vetiver, respectively. Thus, at lower (i.e., 1.5 mg/l) metal concentrations, the chromium removal efficiencies of the Arundo (69.3%), Acorus (66.9%), Typha (66.4%), and Vetiver (66.2%) based systems were observed to be the highest and comparable. While at higher chromium concentrations, Arundo (81.03%, at 3 mg/l to 84.5%, at 5 mg/l) and Acorus (81.9%, at 5 mg/l) appeared to be the superior macrophytes. These observations were found to be in close conformity with the earlier investigations (Gikas et al. 2013; Chen et al. 2014; Abdurahman et al. 2015).

Comparative temporal and overall chromium removal efficiencies of the co-cropped systems, maintained at 5.0 mg/l concentration, are also illustrated in Fig. 2(d) and Table 3, respectively. Investigations revealed that the co-cropped systems, maintained at 5 mg/l Cr-concentration, were associated with an average removal efficiency of 77.56%, i.e., ranging from 76.8% (for Typha+Phragmites: TP), 81.8% (for Phragmites+Arundo: PA), 59.3% (for Vetiver+Acorus: KV), 89.3% (for Arundo+Typha: AT), and 80.5% (for Acorus+Typha: VT), respectively. As evident, though these removal efficiencies were observed to be significantly (i.e., 46.59%) higher than those for the unplanted systems yet these were not significantly higher than those for the monocropped systems (maintained at the same Cr-concentrations). Arundo+Typha (AT) based systems seemed to be the most superior and closely followed by the Arundo ≥ Acorus ~ Phragmites+Arundo (PA) ~ Acorus+Typha (VT) ≥ Phragmites ~ Typha+Phragmites (TP) ~ Typha ≥ Vetiver > Vetiver+Acorus (KV; 59.29%) based systems. Thus, though the chromium removal efficiency of the co-cropped Arundo+Typha (AT) system was observed to be about 13.43% higher than the monocropped Typha-based system yet that for the Vetiver+Acorus (KV) based co-cropped system was 13.3% and 22.6% lower than the monocropped Vetiver- and Acorus-based systems. Similarly, no significant differences in the metal removal efficiencies of the mono and the co-cropped Typha+ Phragmites (TP), Phragmites+Arundo (PA), and Vetiver+ Typha (VT) based systems were observed. In fact, amongst different mono and co-cropped systems, chromium removal efficiencies were observed to be the highest and at par for the co-cropped Arundo+Typha (AT; 89.33%) and the monocropped Arundo (84.54%) based systems. Hence, it could be observed from Table 3 that the metal removal efficiency of the Arundo (84.54%), when co-cropped with Phragmites (PA, 81.83%) and Typha (AT, 89.33%) and of Acorus (V, 81.87%), when co-cropped with Typha (VT, 80.50%), was not significantly reduced while that for the Acorus (V, 81.87%) co-cropped with Vetiver (K: 72.59%; KV: 59.29%) was significantly reduced in comparison to their monocropped systems.

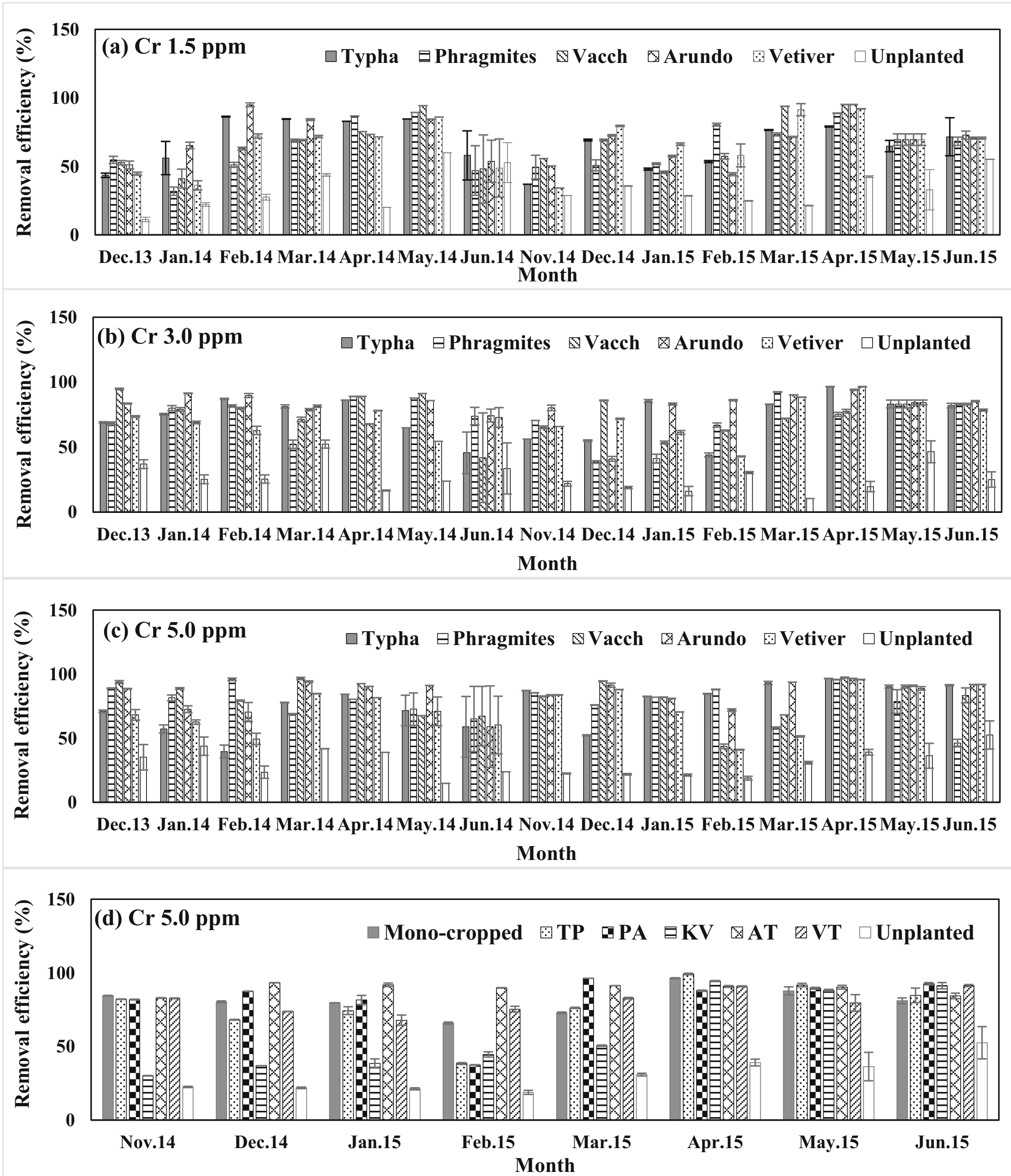

Fig. 2 Temporal chromium removal efficiency of monocropped systems at (a) 1.5 ppm, (b) 3.0 ppm, (c) 5.0 ppm, and of (d) co-cropped systems at 5.0 ppm. * TP, Typha+Phragmites; PA, Phragmites+Arundo; KV, Vetiver+Acorus; AT, Arundo+Typha; and VT, Acorus+Typha

Table 2 System chromium removal efficiency at varying metal concentrations for monocropped systems

Chromium Conc.	Typha	Phragmites	Acorus	Arundo	Vetiver	Unplanted	Mean
Cr 1.5 ppm	66.41^{ab}	64.19^{b}	66.92^{ab}	69.29^{a}	66.22^{ab}	33.84^{c}	61.15
Cr 3.0 ppm	72.94^{b}	72.26^{b}	75.35^{b}	81.03^{a}	72.11^{b}	26.83^{c}	66.75
Cr 5.0 ppm	75.90^{c}	77.54^{bc}	81.87^{ab}	84.53^{a}	72.59^{c}	30.96^{d}	70.57
LSD at 0.05% for Cr 1.5 ppm (4.57), Cr 3.0 ppm (5.22), Cr 5.0 ppm (5.34)							

Different letters (a, b, c, d) in the superscript represent different treatment groups on the basis of least significant difference (LSD) value

Table 3 System chromium removal efficiency at 5 ppm metal concentration for mono and co-cropped microcosms

Treatment	System chromium removal efficiency (%)
Typha (T)	75.90[cd]
Phragmites (P)	77.55[cd]
Acorus (V)	81.87[bc]
Arundo (A)	84.54[ab]
Vetiver (K)	72.59[d]
Unplanted (U)	30.96[f]
Typha + Phragmites (TP)	76.83[cd]
Phragmites + Arundo (PA)	81.83[bc]
Vetiver + Acorus (KV)	59.29[e]
Arundo + Typha (AT)	89.33[a]
Acorus + Typha (VT)	80.50[bc]
MEAN	72.72
LSD at 0.05%	6.31

Different letters (a, b, c, d, e, f) in the superscript represent different treatment groups on the basis of least significant difference (LSD) value

Plant metal removal efficiency and translocation

In wetland system, macrophytes play important role as production of biomass serves as food for a variety of organisms, perpetuates different biogeochemical processes, and removes pollutants from wastewater by uptake. Besides, it helps in transporting oxygen from the atmosphere to the rhizosphere and in the release of the carbon compounds (as rhizo-deposits such as exudates, mucigels, dead cells etc.) which are rich in sugars, amino acids, organic acids, siderophores, proteins, and vitamins (Stottmeister et al. 2003) and are used for the maintenance of habitats for micro-organisms (Marchand et al. 2010). It has been observed that the wetland systems planted with monocots are often more efficient in metal removal than the dicots. This is probably because in monocots root systems are adventitious or fibrous in nature and have higher density than dicot plants, with tap root system. This provides more biomass for metal storage as well as higher surface area for microbial growth and produces higher phyto-siderophores like mugineic acids, which efficiently chelate metals due to the presence of amine and carboxyl groups in such systems (Kidd et al. 2009).

The overall chromium removal efficiency of the test monocot plant-based (mono/co-cropped) microcosms and the unplanted systems, subjected to long-term (Dec. 2013 to Jun. 2015) Cr-spiked irrigation waters of 1.5, 3.0, and 5.0 mg/l concentrations, is presented in Fig. 3. As evident from Fig. 3, with increasing applied chromium concentrations, the plant chromium removal efficiency increased (rroot =0.71; rshoot = 0.530) by 46.23% from 1.5 to 3.0 mg/l and thereafter decreased by 0.81% from 3.0 to 5.0 mg/l respectively. Thus, overall average plant chromium removal efficiency of the monocropped microcosms (Fig. 3) was observed to be 32.76%, 47.91%, and 47.52% of the total system chromium removal efficiency (Table 2) of 61.61%, 74.74%, and 78.49%, at 1.5, 3.0, and 5.0 mg/l, respectively. This was observed to be in close conformity with the observations of Sheoran and Sheoran (2006) who have reported that overall, only one-third (i.e., 33%) of the metal, in general and the chromium, in particular (Guo et al. 2010) is absorbed by the plants. Thus, amongst test monocropped systems, the plant chromium removal efficiency (Fig. 3) at the lowest (i.e. ,1.5 mg/l) metal concentration was observed to be as Arundo > Acorus > Typha > Vetiver > Phragmites (30.35%). While, at the higher (i.e., 3.0 and 5.0 mg/l metal concentrations), it was observed to be as Arundo > Acorus > Typha > Phragmites > Vetiver (45.28%), and Arundo > Acorus > Phragmites > Typha > Vetiver (41.63%), respectively. Thus, as also reported by Zhao-hui and Xu-feng (2010), the Arundo-based systems, associated with higher growth rate, biomass, and deeper roots (Yeh 2008), were observed to be associated with the highest plant chromium removal efficiencies (ranging from 35.44 to 54.21%) at varying metal concentrations.

Comparative studies on the metal reduction efficiency of the mono and the co-cropped systems have been very limited, with most studies focussing on only nutrient removal. Hence, comparative plant chromium removal efficiencies of the mono and the co-cropped systems, maintained at 5.0 mg/l concentration, were also investigated and are illustrated in Fig. 3. Investigations revealed that the co-cropped systems (viz. Typha+Phragmites: TP, Phragmites+Arundo: PA, Vetiver+Acorus: KV, Arundo+Typha: AT and Acorus+Typha: VT) were associated with average plant-metal removal efficiency of 47.19%. As evident, though the average plant Cr-removal efficiency of the co-cropped systems was in general at par with that for the monocropped systems (i.e., 47.52%, maintained at the same Cr-concentrations). Yet, the Arundo+Typha (AT; 58.96%) based systems were observed to be more superior to the Arundo (53.57%) and the Typha (T: 44.94%) based monoculture system. Similarly, the plant chromium removal efficiency of the Phragmites+Arundo (PA; 51.46%) and Acorus+Typha (VT; 50.13%) based system, though not superior to the Arundo (53.57%) and the Acorus (V; 50.91%) based monoculture systems, were more superior to the monoculture Phragmites (P: 46.58%) and the Typha (T: 44.94%) based systems, respectively. This could be accounted to the fact that compared to the monocropped, in poly-cultures, or mixed cultures, the distribution of root system is more effective thereby allowing more growth of diverse microbial population. This enhances the release of root exudates that might stimulate higher metal removal through either rhizodeposition or uptake (Karathanasis et al. 2003; Wu et al. 2012; Calheiros et al. 2015). However, this synergistic effect could not be observed in the case of the Vetiver (K; 41.63%)

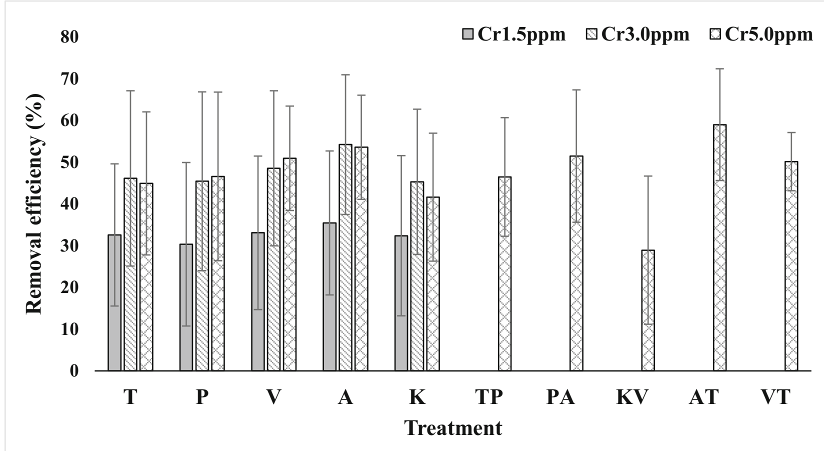

Fig. 3 Plant chromium removal efficiency at varying metal concentrations in mono- and co-cropped systems. * TP, Typha+Phragmites; PA, Phragmites+Arundo; KV, Vetiver+Acorus; AT, Arundo+Typha; & VT, Acorus+Typha

based monoculture and the co-cropped Vetiver+Vacha (KV; 28.93%) based systems.

The root and shoot metal translocation pattern of the test plants grown under mono and co-cropped conditions is also illustrated in Fig. 4(a, b) and Tables 4 and 5. As evident from Fig. 4(a) and Table 4, average chromium accumulation in the monocropped macrophytes grown under three different Cr-concentrations was found to be varying between 184.08 mg/kg, 343.79 mg/kg, and 429.79 mg/kg with 75.41%, 73.92%, and 79.24% retained in the roots and 24.59%, 26.08%, and 20.76% retained in the shoots, respectively. Similarly, of the total 479.60 mg/kg of the metal in the co-cropped systems, maintained at 5.0 mg/l, 80.44% got retained in the roots and only 19.56% got retained in the shoots of the co-cropped plants. Thus, only small amount of absorbed metals were observed to be translocated to the shoots

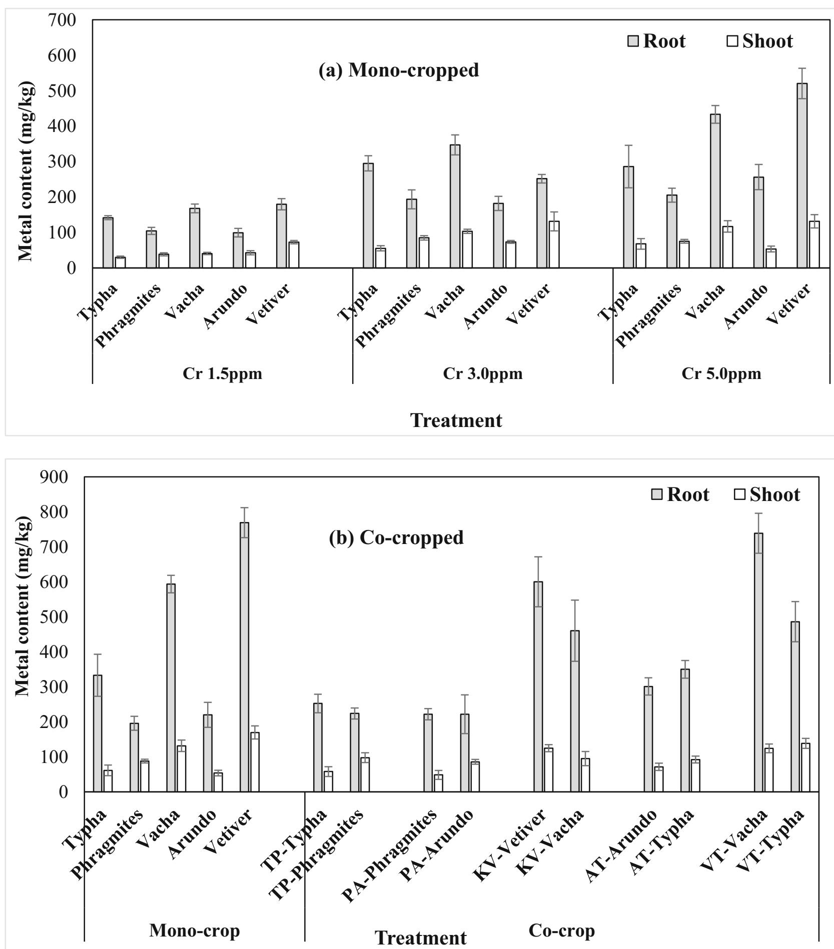

Fig. 4 Chromium translocation to root and shoot of test macrophytes in (a) monocropped systems at 1.5, 3.0, and 5.0 ppm concentrations and (b) mono and co-cropped systems at 5.0 ppm concentration. * TP, Typha+Phragmites; PA, Phragmites+Arundo; KV, Vetiver+Acorus; AT, Arundo+Typha; and VT, Acorus+Typha

Table 4 Chromium translocation to root and shoot of monocropped macrophytes at varying metal concentrations

Treatment	Cr 1.5 ppm		Cr 3.0 ppm		Cr 5.0 ppm	
	Root	Shoot	Root	Shoot	Root	Shoot
Typha	142.01[bc]	30.30	295.26[ab]	55.53	286.39	68.13
Phragmites	104.71[cd]	38.84[bc]	193.77	85.23[bc]	205.52	75.30
Acorus	168.05[ab]	41.02[bc]	347.27[a]	103.40[ab]	433.40[a]	117.15[a]
Arundo	99.60	43.12[b]	182.40	72.61[bc]	256.52	53.73
Vetiver	179.74[a]	73.01[a]	251.94[bc]	131.53[a]	520.67[a]	131.73[a]
MEAN	138.82	45.26	254.13	89.66	340.50	89.21
LSD at 0.05%	37.33	12.48	71.39	36.29	93.84	24.04

Different letters (a, b, c, d) in the superscript represent different treatment groups on the basis of least significant difference (LSD) value

(as phyto-extraction) basically to avoid metal toxicity to the plant. Similar low Cr-translocations from the root to the shoot have also been reported by Abdurahman et al. (2015) in *Arundo donax*, and by Bareen and Khilji (2008) and Bose et al. (2008) in case of *Typha angustifolia*.

At the lowest Cr concentration (i.e., 1.5 ppm), total metal accumulation was observed to be the highest in Vetiver (K; 252.75 mg/kg) followed by Acorus (V; 209.07 mg/kg), Typha (T, 172.31 mg/kg), Phargmites (P; 143.55 mg/kg), and Arundo (142.72 mg/kg). While at 3.0 ppm Cr level, total metal accumulation was observed to be the highest in Acorus (V; 450.67 mg/kg) followed by Vetiver (K; 383.47 mg/kg), Typha (T, 350.79 mg/kg), Phargmites (P; 279.0 mg/kg), and Arundo (255.01 mg/kg). Similarly, at 5 ppm Cr- concentration, total metal accumulation was observed to be the highest in Vetiver (K; 652.40 mg/kg) followed by Acorus (V; 550.55 mg/kg), Typha (T, 354.52 mg/kg), Arundo (310.25 mg/kg), and Phargmites (P; 280.82 mg/kg). While in the co-cropped systems (Fig. 4(b) and Table 5), these ranged from 1488.68 mg/kg in Acorus+Typha (VT) followed by Vetiver + Acorus (KV: 1280.9 mg/kg); Arundo+Typha (AT: 815.59 mg/kg); Typha+Phragmites (TP: 632.74 mg/kg); and Phragmites+Arundo (PA: 578.06 mg/kg). Hence, as evident from Table 5, Arundo (A) based mono and co-cropped (viz. PA and AT) systems were observed to be associated with non-significantly different root and shoot metal translocations while the Acorus- and Typha-based co-cropped systems (VT) were observed to be associated with significantly higher root/ shoot-metal translocations than those observed in their respective monocropped systems (i.e., V and T, respectively). Thus, the only macrophyte combination which resulted in significantly reduced root/ shoot metal translocations than their respective monocropped systems was again observed to be the Acrous co-cropped with Vetiver (i.e., KV) thereby clearly showing some sort of antagonistic functional relationship, and hence competition between these two co-cropped macrophytes probably due to significant differences in their root architecture and hence bio-chemistry (Lai et al. 2011; Leiva et al. 2018).

Table 5 Plant root and shoot chromium translocation in mono and co-cropped systems at 5 ppm metal concentration

Treatment		Root (mg/kg)	Shoot (mg/kg)
Typha (T)		332.98[e]	61.45[ef]
Phragmites (P)		195.87[f]	87.86[cdef]
Acorus (V)		593.67[bc]	131.75[abc]
Arundo (A)		220.12[f]	54.03[ef]
Vetiver (K)		769.25[a]	169.83[a]
Typha + Phragmites (TP)	Typha	252.74[ef]	58.18[ef]
	Phragmites	224.13[f]	97.69[bcde]
Phragmites + Arundo (PA)	Phragmites	221.85[f]	48.55[f]
	Arundo	221.70[f]	85.96[def]
Vetiver + Acorus (KV)	Vetiver	600.28[b]	124.87[bcd]
	Acorus	460.38[d]	95.37[bcde]
Arundo + Typha (AT)	Arundo	301.33[ef]	71.67[ef]
	Typha	350.13[e]	92.46[cdef]
Acorus + Typha (VT)	Acorus	739.07[a]	124.50[bcd]
	Typha	486.33[cd]	138.78[ab]
MEAN (mg/kg)		397.99	96.20
LSD at 0.05%		108.16	44.19

Different letters (a, b, c, d, e, f) in the superscript represent different treatment groups on the basis of least significant difference (LSD) value

According to Lai et al. (2011), wetland plants can be divided into two groups: fibrous root plants and thick-roots plants. A previous study of Leiva et al. (2018) showed that fibrous-root plants have significantly higher growth rates (16.07 g/m^2) than thick-root plants (7.56 g/m^2). In our study also, we observed that in contrast to the Acorus-based monocropped system which were associated with shallow thick/ knotty fibrous roots, the Vetiver-based monocropped system was observed to be associated with deep fine fibrous roots along with fibrous secondary roots. As reported by Kidd et al. (2009), this provides more biomass for nutrient absorption and metal storage, as well as higher surface area for microbial growth and hence higher production of phyto-siderophores like mugineic acids, which efficiently chelate metals and other nutrients thereby resulting into significantly increased Vetiver biomass as compared to the Acorus, and hence competition to Acorus in the KV-based system.

As evident from Table 5, the Vetiver-based systems were also observed to be associated with 1.29 to 3.92 times higher root and 1.28 to 1.93 times higher shoot metal translocations than the other systems (based on Acorus, Typha, Phragmites, and Arundo) with relatively thicker and shallow to moderately deep fibrous root systems. However, though total metal accumulation, at higher metal concentrations in particular, was observed to be the highest for the Vetiver-based microcosms and the lowest for the Arundo-based microcosms, yet total metal removal efficiencies, as observed from Fig. 2(a, b, c, d) and Tables 2 and 3 were observed to be the highest for the Arundo and the Arundo+Typha (AT) based systems, associated with the higher growth rate and plant biomass (Tawde and Bhalerao 2012; Paz-Alberto and Sigua 2013; Singh et al. 2015). Generally, plants with higher growth rate, higher biomass, and higher root depth sequester more metals (Yeh 2008; Abdurahman et al. 2015). Thus, a suitable selection of plant species is therefore desirable for avoiding unstable plant communities and decreasing competition amongst such co-cropped systems (Katharina et al. 2002).

Microbial metal removal efficiency

Contribution of microbes in removing chromium from the test planted (mono/ co-cropped) and the unplanted microcosms, subjected to long-term (Dec., 2013 to June, 2015) Cr-spiked irrigation waters of 1.5, 3.0, and 5.0 mg/l concentrations, is presented in Fig. 6 and Tables 6 and 7. As evident from Table 6, with increasing applied chromium concentrations, the microbial chromium removal efficiency increased ($r = 0.93$) at a much faster rate (i.e., by 89.93%, from 1.5 to 3.0 mg/l and by 65.40% from 3.0 to 5.0 mg/l respectively) than the total system chromium removal efficiency (Tables 2 and 3). However, the overall average microbial chromium removal efficiency of the monocropped microcosms (Table 8) was observed to be just 6.38%, 12.11%, and 20.03% of the total system chromium removal efficiency (Table 2) of 61.61%, 74.74%, and 78.49% at 1.5, 3.0, and 5.0 mg/l, respectively.

Table 7 Comparative microbial chromium removal efficiency of mono and co-cropped systems, at 5 ppm metal concentration

Treatment	Microbial chromium removal efficiency (%)
Typha (T)	21.56[ab]
Phragmites (P)	18.32[cd]
Acorus (V)	18.64[bcd]
Arundo (A)	20.36[abc]
Vetiver (K)	21.28[abc]
Unplanted (U)	15.56[d]
Typha + Phragmites (TP)	22.94[a]
Phragmites + Arundo (PA)	22.38[a]
Vetiver + Acorus (KV)	21.80[ab]
Arundo + Typha (AT)	22.09[a]
Acorus + Typha (VT)	22.09[a]
Mean	20.64
LSD at 0.05%	3.23

Different letters (a, b, c, d) in the superscript represent different treatment groups on the basis of least significant difference (LSD) value

Amongst monocropped systems, the microbial chromium removal efficiency of test macrophytes at 1.5 mg/l metal concentration was observed to be as Typha (7.8%) ~ Vetiver (8.7%) > Acorus ~ Arundo ~ Phragmites. Similarly, at 3.0 and 5.0 mg/l metal concentrations, the microbial chromium removal efficiency of the monocropped systems was observed to be as Typha (18.2%) > Vetiver ~ Acorus ~ Arundo ~ Phragmites, and Typha (21.6%) ~ Vetiver (21.3%) ≥ Arundo ≥ Acorus ~ Phragmites respectively. Thus, at all metal concentrations, the microbial chromium removal efficiencies of the Typha- and the Vetiver-based systems were observed to be the most superior. Further, compared to the unplanted systems (with 4.7, 8.4, and 15.6% microbial Cr - removal efficiencies at 1.5, 3.0, and 5.0 mg/l conc., respectively), the planted microcosms were observed to be associated with significantly (28.75% to 36.04%) higher microbial chromium removal efficiencies. This could probably be attributed to the higher microbial diversity and/or the chromium bio-remediating potential of the plant root-associated micro-organisms in the

Table 6 Microbial chromium removal efficiency percentage of monocropped systems, at varying metal concentrations

Chromium Conc.	Typha	Phragmites	Acorus	Arundo	Vetiver	Unplanted	Mean
Cr 1.5 ppm	8.66[a]	4.69[b]	5.35[b]	5.35[b]	7.84[a]	4.69[b]	6.10
Cr 3.0 ppm	18.20[a]	9.37[b]	11.32[b]	9.38[b]	12.30[b]	8.43[b]	11.50
Cr 5.0 ppm	21.56[a]	18.32[b]	18.64[b]	20.36[ab]	21.28[a]	15.56[c]	19.29
LSD at 0.05% for Cr 1.5 ppm (2.14), Cr 3.0 ppm (4.74) and Cr 5.0 ppm (2.29)							

Different letters (a, b, c) in the superscript represent different treatment groups on the basis of least significant difference (LSD) value

Table 8 Chromium deposition on gravel in monocropped systems at varying metal concentrations

Chromium Conc.	Typha (mg/kg)	Phragmites (mg/kg)	Acorus (mg/kg)	Arundo (mg/kg)	Vetiver (mg/kg)	Unplanted (mg/kg)	Mean (mg/kg)
Cr 1.5 ppm	5.86[d]	8.29[b]	6.78[c]	7.15[c]	6.31[cd]	11.74[a]	7.69
Cr 3.0 ppm	15.13[e]	26.99[b]	17.34[d]	18.16[c]	15.34[e]	34.95[a]	21.32
Cr 5.0 ppm	26.79[d]	57.31[b]	41.13[c]	40.03[c]	26.80[d]	68.34[a]	43.40
LSD at 0.05% for Cr 1.5 ppm (0.88), Cr 3.0 ppm (0.78), Cr 5.0 ppm (1.49)							

Different letters (a, b, c, d) in the superscript represent different treatment groups on the basis of least significant difference (LSD) value

Typha- and the Vetiver-based planted (aerobic-anaerobic) systems, in addition to the anaerobes and facultative species, normally found in the unplanted systems. However, contrary to the unplanted systems associated with 231.91% increased microbial Cr-removal efficiency at increasing metal concentrations, the microbial chromium removal efficiency of the planted systems was observed to rather decline (by about 20.2%) at higher metal concentrations.This could primarily be due to the reduced precipitation of chromium, spiked through the $(NH_4)_2Cr_2O_7$ salt in the high pH (8.1 ± 0.38) ground waters of the experimental site, and the consequent anionic nature of the chromium (as $Cr_2O_7^{2-}$) in the planted systems, as compared to the unplanted (anaerobic) systems (with near neutral pH and thus more immobilized metal forms; Gambrell 1994) that facilitated higher proportions of chromium in the mobile state, and thus its easy availability to the plants in the vegetated systems as compared to the non-vegetated systems associated with increased media deposition, and thus higher media-based microbial removal efficiencies (Garcia et al. 2010), as also illustrated in the following section on the media metal removal efficiency.

Comparative overall microbial chromium removal efficiencies of the co-cropped systems, maintained at 5.0 mg/l concentration, are also illustrated in Fig. 5 and Table 7, respectively. Investigations revealed that the co-cropped systems (viz. Typha+Phragmites: TP, Phragmites+Arundo: PA, Vetiver +Acorus: KV, Arundo+Typha: AT and Acorus+Typha: VT), maintained at 5.00 mg/l Cr-concentration, were associated with microbial-metal removal efficiencies of 22.94%, 22.38%, 21.80%, and 22.09% respectively; with an average removal efficiency of 22.26%. As evident, though these removal efficiencies were observed to be significantly (i.e., 43.03%) higher than those for the unplanted systems but were not significantly (i.e., only 11.09%) higher than those for the monocropped systems (maintained at the same Cr-concentrations). This could primarily be attributed to no significant increase in the metal sequestration potential (Berta et al. 2002) of the increased microbial biomass (Oliveira et al. 2001) and diversity under co-cropped conditions, as compared to the monocropped conditions.

Media metal removal efficiency

Contribution of media in removing chromium from the test planted (mono/co-cropped) and the unplanted microcosms, subjected to long-term (Dec., 2013 to June, 2015) Cr-spiked irrigation waters of 1.5, 3.0, and 5.0 mg/l

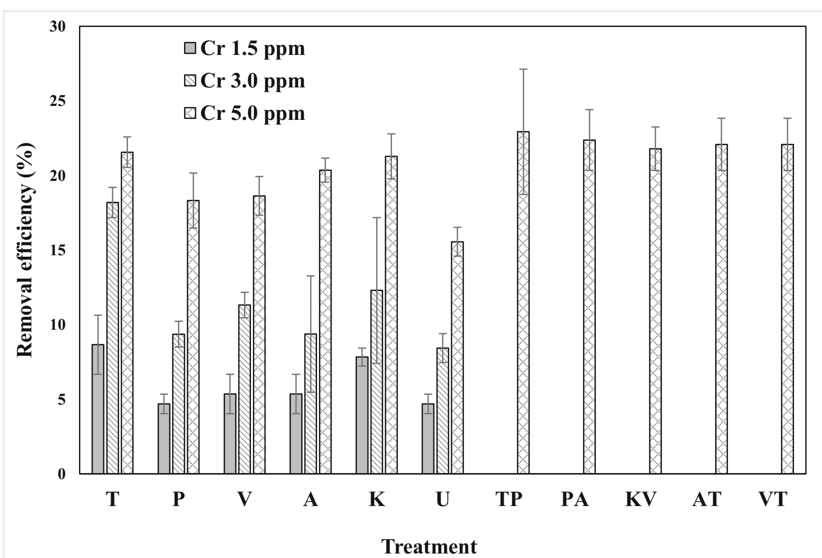

Fig. 5 Microbial chromium removal efficiency at varying chromium concentrations in mono- and co-cropped systems. * TP, Typha+Phragmites; PA, Phragmites+Arundo; KV, Vetiver+Acorus; AT, Arundo+Typha; and VT, Acorus+Typha

concentrations, is presented in Fig. 6 and Tables 8 and 9. The overall average media-based chromium removal efficiency of the monocropped microcosms (Table 8) was observed to be 27.47%, 14.71%, and 10.93% of the total system chromium removal efficiency (Table 2) of 61.61%, 74.74%, and 78.49% at 1.5, 3.0, and 5.0 mg/l, respectively. Further, amongst monocropped systems, the media-metal removal efficiency of the test macrophytes at 1.5 mg/l and 5.0 mg/l metal concentrations was observed to be the highest in the Phragmites followed by the Acorus, Arundo, Vetiver, and Typha-based systems. At higher (i.e., 3.0 and 5.0 mg/l) metal concentrations also, the media chromium removal efficiency of the monocropped systems was observed to be the highest in the Phragmites, followed by the Arundo, Acorus, Vetiver, and Typha-based systems. Thus, at all metal concentrations, the media-based chromium removal efficiencies under Phragmites (12.64% to 29.15%) and the Acorus (12.33% to 28.49%) based systems were observed to be the highest and comparable. Higher sedimentation of metals in case of *Phragmites australis*, possibly due to higher rhizo-deposition, has also been reported by (Lee and Scholz 2007). The observed results were thus found to be in close conformity with the existing literature that reports up to 25% of chromium removal through media precipitation/ adsorption in wetlands (Schiffer 1989). However, as evident from Fig. 6, with increasing applied chromium concentrations, the metal removal efficiency of media through deposition (particularly caused by metal precipitation and adsorption on the media surface; Kadlec and Wallace 2009) in the planted systems decreased ($r = 0.879$) by about 12.75%, from 1.5 to 3.0 mg/l and by 25.71% from 3.0 to 5.0 mg/l. Similarly, in the unplanted systems also, the media metal removal efficiency decreased by 10.76%, from 1.5 to 3.0 mg/l and

Table 9 Chromium deposition on gravel in mono and co-cropped systems at 5 ppm metal concentration

Treatment	Chromium deposition (mg/kg)
Typha (T)	26.79[g]
Phragmites (P)	57.31[b]
Acorus (V)	41.13[c]
Arundo (A)	40.03[c]
Vetiver (K)	26.80[g]
Unplanted (U)	68.34[a]
Typha + Phragmites (TP)	32.68[e]
Phragmites + Arundo (PA)	24.96[h]
Vetiver + Acorus (KV)	9.88[i]
Arundo + Typha (AT)	35.62[d]
Acorus + Typha (VT)	29.37[f]
Mean	35.72
LSD at 0.05%	1.32

Different letters (a, b, c, d, e, f, g, h) in the superscript represent different treatment groups on the basis of least significant difference (LSD) value

by 2.99%, between 3.0 and 5.0 mg/l, respectively. Generally, adsorption of chromium on media surface, particulate matter, and organic matter is greater and so it is generally less labile/ bio-available than say, nickel (Marchand et al. 2010; Huang et al. 2016). However, in this study, as the chromium metal salt used during spiking was $(NH_4)_2Cr_2O_7$ (i.e., 1000 mg/l standard solution of Cr dissolved in 2% nitric acid) and as at > 6 pH range, chromium mostly exists as CrO_4^{2-} and Cr_2O7^{2-} forms (Lin 1995). Therefore, due to the type of the chromium salt used for spiking and the consequent anionic nature of the chromium (as $Cr_2O_7^{2-}$) in the system and the high pH (8.1 ± 0.38) of the ground waters used for the present spiking studies, most of the chromium in the wetland

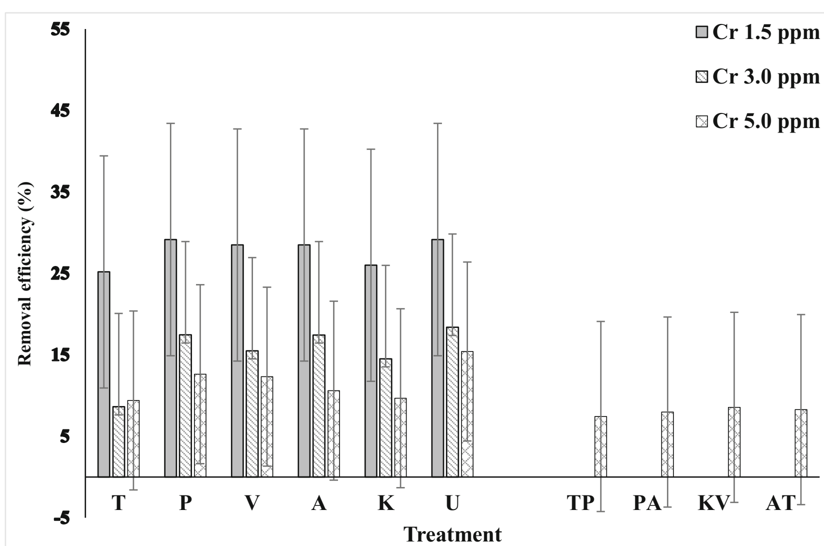

Fig. 6 Media chromium removal efficiency at varying chromium concentrations in mono and co-cropped systems. * TP, Typha+ Phragmites; PA, Phragmites+ Arundo; KV, Vetiver+Acorus; AT, Arundo+Typha; and VT, Acorus+Typha

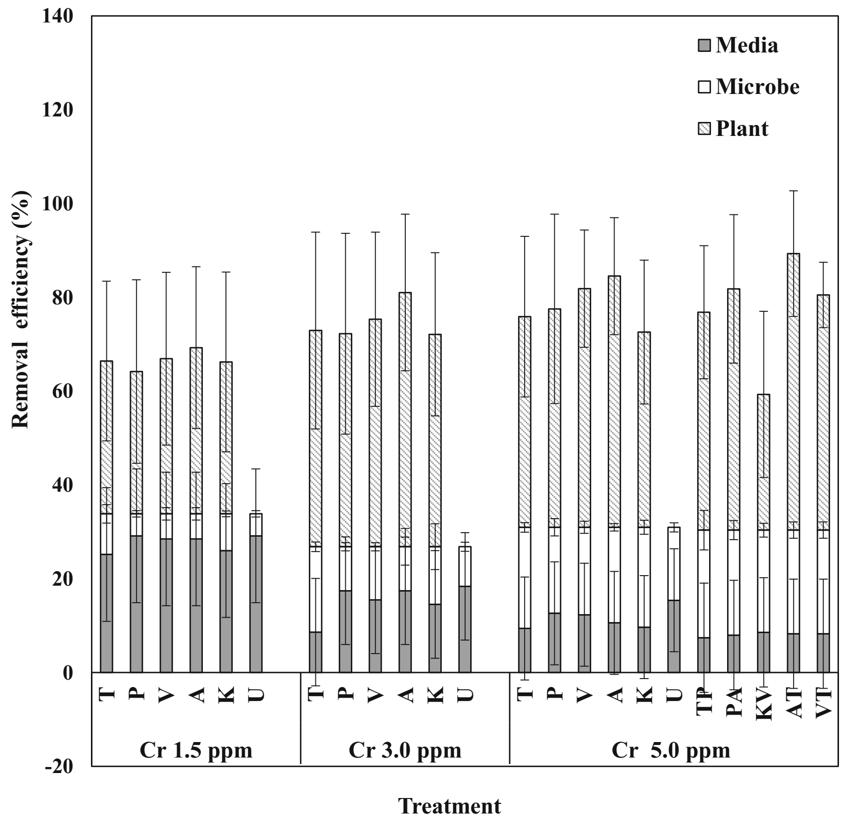

Fig. 7 Long-term average plant, microbe, media chromium removal efficiencies in mono and co-cropped systems at varying chromium concentrations. * TP, Typha+Phragmites; PA, Phragmites+Arundo; KV, Vetiver+Acorus; AT, Arundo+Typha; and VT, Acorus+Typha

systems, particularly at higher metal concentrations, could have remained mobile, and thus easily available to the plants, and hence not retained appreciably on the negatively charged substrate media.

Comparative overall media-based chromium removal efficiencies of the co-cropped systems, maintained at 5.0 mg/l concentration, are also illustrated in Fig. 6 and Table 9, respectively. Investigations revealed that the co-cropped systems (viz. Typha+Phragmites: TP, Phragmites+Arundo: PA, Vetiver +Acorus: KV, Arundo+Typha: AT and Acorus+Typha: VT), maintained at 5.00 mg/l Cr-concentration, were associated with comparable media-metal removal efficiencies of 7.43%, 7.99%, 8.57%, 8.28%, and 8.28%, respectively, with an average removal efficiency of 8.11%, which was observed to be about 2.82% lower than that for the corresponding monocropped systems. Further, like monocropped systems associated with 1.69 to 4.47% lower media metal removal efficiencies than the unplanted sytems, the media metal removal efficiencies of the co-cropped planted systems were also observed to be about 7.29% lower than the corresponding unplanted systems.This could probably be due to the absence of plants and the associated micro-organisms in the unplanted systems, which play an active role in the mobilization and uptake of a significant fraction of the deposited metals on the media surface. Accumulation of metals on the particulates in the sediments and litter and their uptake by microbial community has also been reported by Garcia et al. (2010).

Conclusions

Thus, long-term investigations clearly revealed significantly (32.76 to 47.52%) higher chromium removal efficiency of the planted systems over the unplanted systems. However, there appeared to no significant improvement in the overall average chromium removal efficiency of the co-cropped sytems (77.56%) over that of the monocropped systems (78.49%), at same chromium concentration level. Of all plants and plant-combinations tested, Arundo (81.9%) and Acorus (84.5%) based monocropped systems and Arundo+Typha (AT; 89.33%) based co-cropped systems appeared to be associated with the the highest system Cr-removal efficiencies. Quantification of the contribution of the plants, microbes, and media (Fig. 7) in the overall percent chromium removal efficiency of the test planted-experimental wetland systems (or mesocosms) revealed increased plant and microbial metal removal efficiencies at increased chromium concentrations, ranging from 32.76% (at 1.5 mg/l), 47.91% (at 3.0 mg/l), to 47.91% (at 5.0 mg/l), and from 6.38% (at 1.5 mg/l), 12.11% (at 3.0 mg/l), to 20.03% (at 5.0 mg/l) respectively. However, the contribution of media (deposition/ adsorption/ precipitation) in the total chromium removal efficiency decreased from 27.47% (at 1.5 mg/l) to 14.71% (at 3.0 mg/l), and 8.11% (at 5.0 mg/l), thereby clearly indicating increased microbial activity-mediated metal mobilization and plant uptake as the principal processes governing chromium removal in such VSSF constructed wetland systems exposed to varying chromium levels.

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References

Aafi NE, Brhada F, Dary M, Maltouf AF, Pajuelo E (2012) Rhizostabilization of metals in soils using *Lupinus luteus* inoculated with the metal resistant rhizobacterium *Serratia* sp. MSMC 541. Int J Phytoremediation 14:261–274

Abdurahman S, Stockle C, Harsh J, Beutel M, Zaher U (2015) Arundo Donax (Giant Reed) phytoremediation function of chromium (Cr) removal. World Academy of Science, Engineering and Technology. Int J Agric Biosyst Eng 2(3):200

Acharya GD, Hathi MV, Asha D, Patel, Parmar KC (2008) Chemical properties of groundwater in Bhiloda Taluka Region, North Gujarat, India. E-J Chem 5(4):792–796

AL-Hamdan AZ, Reddy RK (2006) Adsorption of heavy metals in glacial till soil. Geotech Geol Eng 24:1679–1693

Ayres RS, Westcot DW (1985) Water quality for agriculture. Irrigation and Drainage paper no. 29, Food and Agricultural Organization of the United Nations, Rome, pp 1–117

Bareen F, Khilji S (2008) Bioaccumulation of metals from tannery sludge by *Typha angustifolia* L. Afr J Biotechnol 7(18):3314–3320 https://www.ajol.info/index.php/ajb/article/view/59295/47593

Berta G, Fusconi A, Hooker JE (2002) Arbuscular mycorrhizal modifications to plant root systems: scale, mechanisms, and consequences. In: Gianinazzi S, Schüepp H, Barea JM, Haselwandter K (eds) Mycorrhizal Technology in Agriculture. Birkhäuser Verlag, Basel, Boston, Berlin, pp 71–85

BIS (1986) Guidelines for the quality of irrigation water. Indian Standard Institute, New Delhi

Bose S, Vedamati J, Rai V, Ramanathan AL (2008) Metal uptake and transport by *Tyaha angustata* L. grown on metal contaminated waste amended soil: an implication of phytoremediation. Geoderma 145: 136–142

Branda SS, Vik S, Friedman L et al (2005) Biofilms: the matrix revisited. Trends Microbiol 13:20–26

Calheiros CSC, Bessa VS, Mesquita RBR, Brix H, Rangel AOSS, Castro PML (2015) Constructed wetland with a polyculture of ornamental plants for wastewater treatment at a rural tourism facility. Ecol Eng 79:1–7

Chen Y, Hong X, He H, Luo H, Qian T, Li R, Jiang H, Yu H (2014) Biosorption of Cr (VI) by Typha angustifolia: mechanism and responses to heavy metal stress. Bioresour Technol 160:89–92

CPCB (2016) Central pollution control board, bulletin vol-I, July 2016, 24 pages

FAO (1985) Water quality for agriculture. Irrigation and Drainage Paper, 29 Rev. 1. FAO, Rome, 174 pages

Fraser LH, Carty SM, Steer D (2004) A test of four plant species to reduce total nitrogen and total phosphorus from soil leachate in subsurface wetland microcosms. Bioresour Technol 94:185–192

Gambrell RP (1994) Trace and toxic metals in wetlands—a review. J Environ Qual 23:883–889

Garcia J, Rousseau DPL, Morato J, Lesage E, Matamoros V, Bayona JM (2010) Contaminant removal processes in subsurface-flow constructed wetlands: a review. Crit Rev Environ Sci Technol 40:561–661

Gikas P, Ranieri E, Tchobanoglous G (2013) Removal of iron, chromium and lead from waste water by horizontal subsurface flow constructed wetlands. J Chem Technol Biotechnol 88(10):1906–1912

Guo H, Luo S, Chen L, Xiao X, Xi Q, Wei W, Zeng G, Liu C, Wan Y, Chen J, He Y (2010) Bioremediation of heavy metals by growing hyperaccumulaor endophytic bacterium *Bacillus* sp. L14. Bioresour Technol 101(22):8599–8605

Huang FC, Han YL, Lee CK, Chao HP (2016) Removal of cationic and oxyanionic heavy metals from water using hexadecyltrimethylammonium-bromide-modified zeolite. Desalin Water Treat 57(38):17870–17879

Kadlec RH, Wallace SD (2009) Treatment wetlands, 2nd edn. CRC Press, Boca Raton

Karathanasis AD, Potter CL, Coyne MS (2003) Vegetation effects on fecal bacteria, BOD, and suspended solid removal in constructed wetlands treating domestic wastewater. Ecol Eng 20:157–169

Katharina A, Engelhardt M, Ritchie ME (2002) The effect of aquatic plant species richness on wetland ecosystem processes. Ecology 83:2911–2924

Kidd P, Barcelo J, Bernal MP, Navari-Izzo F, Poschenrieder C, Shilev S (2009) Trace element behaviour at the root–soil interface: implications in phytoremediation. Environ Exp Bot 67:243–259

Lai WL, Wang SQ, Peng CL, Chen ZH (2011) Root features related to plant growth and nutrient removal of 35 wetland plants. Water Res 45(13):3941–3950

Lee BH, Scholz M (2007) What is the role of Phragmites australis in experimental constructed wetland filters treating urban runoff? Ecol Eng 29:87–95

Leiva AM, Núñez R, Gómez G, López D, Vidal G (2018) Performance of ornamental plants in monoculture and polyculture horizontal subsurface flow constructed wetlands for treating wastewater. Ecol Eng 120:116–125

Lin LY (1995) Wastewater treatment for inorganics. Encyclopedia of Environmental Biology, vol 3. Academic Press, pp 479–484

Marchand L, Mench M, Jacob DL, Otte ML (2010) Metal and metalloid removal in constructed wetlands, with emphasis on the importance of plants and standardized measurements: a review. Environ Pollut 158:3447–3461

Mitsch WJ, Gosseink JG (2007) Wetlands, 4th edn. John Wiley, New York, p 722

Morari F, Giardini L (2009) Municipal wastewater treatment with vertical flow constructed wetlands for irrigation reuse. Ecol Eng 35:643–653

Oliveira RS, Dodd JC, Castro PML (2001) The mycorrhizal status of Phragmites australis in several polluted soils and sediments of an industrialised region of Northern Portugal. Mycorrhiza 10:241–247

Pacyna JM, Pacyna EG (2001) An assessment of global and regional emissions of trace metals to the atmosphere from anthropogenic sources worldwide. Environ Rev 9:269–298

Paz-Alberto AM, Sigua GC (2013) Phytoremediation: a green technology to remove environmental pollutants. Am J Clim Chang 2:71–86

Picard CR, Fraser LH, Steer D (2005) The interacting effects of temperature and plant community type on nutrient removal in wetland microcosms. Bioresour Technol 96:1039–1047

Schiffer DM (1989) Water quality variability in a Central Florida wetland receiving highway runoff. In: Davis FE (ed) Water: Laws and Management. American Water Research Association, Bethesda, pp 7A-1–7A-11

Sheoran AS, Sheoran V (2006) Heavy metal removal mechanism of acid mine drainage in wetlands: a critical review. Miner Eng 19:105–116

Singh V, Thakur L, Mondal P (2015) Removal of lead and chromium from synthetic wastewater using *Vetiveria zizanioides*. CLEAN – Soil, Air, Water 43(4):538–543

Stottmeister U, Wiebner A, Kuschk P, Kappelmeyer U, Kastner M, Bederski O, Muller RA, Moormann H (2003) Effects of plants and microorganisms in constructed wetlands for wastewater treatment. Biotechnol Adv 22:93–117

Tawde SP, Bhalerao SA (2012) Uptake of heavy metals by Vetiveria zizanioides, l. Nash from ETP sludge. ISSN 0974-0678. Bionano Frontier 5(2-II):1–4
Toet S, Bouwman M, Cevaal A, Verhoeven, Jos TA (2005) Nutrient removal through autumn harvest of Phragmites australis and Typha latifolia shoots in relation to nutrient loading in a wetland system used for polishing sewage treatment plant effluent. J Environ Sci Health A 40:1133–1156
Willis JB (1962) Determination of lead and other heavy metals in urine by atomic absorption spectrophotometry. Anal Chem 34:614–617
Wu FY, Chung AKC, Tam NFY, Wong MH (2012) Root exudates of wetland plants influenced by nutrient status and types of plant cultivation. Int J Phytoremediation 14(6):543–553
Yeh TY (2008) Removal of metals in constructed wetlands: review. Pract Period Hazard Toxic Radioact Waste Manag 12(2):96–101
Zhang Z, Rengel Z, Meney K (2007) Nutrient removal from simulated wastewater using Canna indica and Schoenoplectus validus in mono- and mixed-culture in wetland microcosms. Water Air Soil Pollut 183:95–105
Zhao-hui G, Xu-feng M (2010) Growth changes and tissues anatomical characteristics of giant reed (*Arundo donax* L.) in soil contaminated with arsenic, cadmium and lead. J Cent S Univ Technol 17:770–777