Remoción de cromo y zinc de aguas residuales sintéticas en un humedal construido plantado con *Cyperus odoratus* L.

Chromium and Zinc removal from synthetic industrial wastewater in pilot-scale constructed wetlands planted with *Cyperus odoratus* L.

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Abstract

Introduction— Constructed Wetlands (CWs) are a recognized technology to treat industrial wastewater.

Objective— A pilot system of two horizontal subsurface flow CWs was used to remove Cr and Zn from industrial synthetic wastewater.

Methodology— The study was carried out at UA in Barranquilla, Colombia. Two containers of 0.375 m^2 were filled with a gravel bed (~10 mm and 40% of porosity), and a 0.3 m water column. One container was planted with *Cyperus odoratus* L. and another without plants was used as a control.

Results— The removal efficiency of Cr and Zn was 93% and 96% in the CW planted, respectively, and 67% and 98% removal were obtained in the unplanted system with statistical differences ($P< 0.05$). The observed difference in biomass production (0.1 kg m^2 and 0.6 kg/m^2) could be related to seasonal weather that could have favored the growth of the plant. *C. odoratus* reached a Translocation Factor greater than 1.5 for Cr and Zn, which is greater than that, reported by others for Cyperus species. However, a Bioconcentration Factor > 13.6 for Zn and < 7.7 for Cr indicated that *C. odoratus* is an accumulator species for Cr and Zn. Sorption metal processes in gravel can be occurring due to the high removal efficiency of Zn in unplanted systems.

Conclusions— These results show that *C. odoratus* could be recommended for use in constructed wetlands technology due to fast-growing and absorption and translocation heavy metals capacity.

Keywords— Subsurface flow constructed wetlands; heavy metals; phytoremediation; bio-concentration; translocation; *Cyperus odoratus*

Resumen

Introducción— Los Humedales Construidos (HC) son una tecnología reconocida para tratar aguas residuales industriales.

Objetivo— Remover Cr y Zn del agua residual sintética a través de un sistema piloto de humedales construidos de flujo subsuperficial horizontal.

Metodología— El estudio se realizó en la UA, Barranquilla, Colombia. Dos contenedores de 0.375 m^2 de altura fueron rellenados con grava (~10 mm y 40% de porosidad) y una columna de agua de 0.3 m. Uno de los humedales se plantó con *Cyperus odoratus* L. y otro sin plantas se usó como control.

Resultados— La eficiencia de remoción de Cr y Zn en el humedal plantado fue de 93% y 96%, respectivamente y se obtuvo 67% y 98% de remoción en el sistema sin plantar con diferencias estadísticas ($P < 0.05$). La diferencia observada en la producción de biomasa (0.1 kg m^2 y 0.6 kg m^2), estuvo relacionada con el climática estacional que pudo haber favorecido el crecimiento de la planta. *C. odoratus* alcanzó un Factor de Translocación mayor de 1.5 para Cr y Zn, lo cual fue mayor que el reportado para otras especies de Cyperus. Sin embargo, un factor de bioconcentración > 13.6 para Zn y < 7.7 para Cr indicó que *C. odoratus* es una especie acumuladora de Cr y Zn. Los procesos de sorción de metales en la grava pudieron ocurrir debido a la alta eficiencia de eliminación de Zn en los sistemas no plantados.

Conclusiones— *C. odoratus* podría recomendarse para su uso en tecnología de humedales construidos debido a su capacidad de rápido crecimiento, absorción y translocación de metales pesados.

Palabras clave— Humedales construidos de flujo subsuperficial; metales pesados; fitorremediación; bio-concentración; traslocación; *Cyperus odoratus*

I. Introduction

Heavy metals in industrial effluents can cause significant environmental impacts such as toxicity in aquatic and terrestrial ecosystems and harmful effects on human health. In Colombia, 6.2 million people, 13% of the population, inhabit the Cauca and Magdalena river basins [1]. Deficiencies in the treatment of domestic and non-domestic wastewater increase the contamination of these basins. Metallurgical foundry and galvanization industries discharge considerable heavy metal contaminants such as Pb, Cr, Hg, and Zn into the Magdalena River, the main river of Colombia [2]. Although Cr and Zn maximum permissible values to surface water discharges in Colombia are 0.5and 3.0 $mg \cdot L^{-1}$ respectively [L1], the wastewater industrial concentrations of these heavy metals can exceed 2 and more than 100 $mg \cdot L^{-1}$ respectively [3], [4].

Concentrations of Cr (1.82 $mg \cdot L^{-1}$) and Zn (3.800 $mg \cdot L^{-1}$) were reported by UMNG (CO) in galvanic wastewater [4]. UTP (CO) reported metallurgic levels in industrial effluent between 0.7 and 2.0 $mg \cdot L^{-1}$ for Cr and between 0.0 and 6.0 $mg \cdot L^{-1}$ for Zn [3]. Similarly, UCM (CO) reported Zn concentrations between 1 and 4 $mg \cdot L^{-1}$ in the effluent from the metal mechanics industry [5]. Therefore, due to these concentrations emitted by the metallurgical industry, Cr and Zn metals were selected to carry out the present study. Zn is an essential element for plants at low concentrations; however, it is toxic at elevated concentrations. Cr of the forms Cr VI and Cr III can be present in nature, while Cr VI is toxic to biota [6]. In the Colombian Caribbean region, only 75% of discharged wastewater is treated and the conventional treatment technologies used are insufficient to reduce heavy metals contamination of surface waters. Consequently, it is necessary to consider other options such as constructed wetlands [7]. Constructed wetlands can be classified in two types: Surface Flow (SF), and subsurface flow (SSF). In SF systems the wastewater flows across shallow ponds planted with aquatic macrophytes in direct contact with the atmosphere. In SSF CWs the wastewater is maintained at a constant depth and flows below the surface and through a granular media, such as gravel, which is planted with aquatic macrophytes. According to the direction of the flow the SSF can be classified in horizontal-flow or vertical flow and are mainly designed to treat primary settled wastewater [6].

In the Colombian Caribbean region, only 75% of discharged wastewater is treated and the conventional treatment technologies used are insufficient to reduce heavy metals contamination of surface waters. Consequently, it is necessary to consider other options such as constructed wetlands [9]. Therefore, the purpose of this study was to determine the removal efficiency of Cr and Zn in constructed wetlands planted with *C. odoratus*.

II. Literature Review

Constructed wetlands are used to treat industrial wastewater with high concentrations of heavy metals. Removal mechanisms include adhesion to granular material and sediments, chemical precipitation, and plant uptake [8]. Plants can remove concentrations of heavy metals through a complex internal system that involves uptake, accumulation in leaves and roots, trafficking, and detoxification of metals [9], [10]. Additionally, the heavy metal accumulation varies with plant species, biomass type, and time of harvest. The institutions UCAS and IHB (CN) [11], NUI (IE) and LJMU (UK) [12], reported that *Phragmites australis* L. accumulated 80% more Cr in belowground biomass than aboveground biomass in a few months.

More than 400 hyperaccumulator plants have been described to accumulate and detoxify high levels of metal ions, such as Ni, Co, Pb, Zn, Mn, Cd, etc. [9], [13]. The principal removal mechanisms for plants are uptake/absorption of the pollutants through roots and translocation to the upper part of the plant [14]. Although several studies reported that some Cyperaceae species are heavy metal hyperaccumulators of contaminants such as Zn and Cr [15]-[17], *C. odoratus* has not been studied for heavy metal accumulation. UB and ULM (ID) reported that *C. odoratus* is a candidate for mine wastewater treatment with low pH values and Fe concentrations lower than 70 $mg \cdot L^{-1}$ [18].

In Colombia, the Cyperaceae family has more than 80 reported plant species [19], however, only a few species have been evaluated for phytodepuration processes [20]. This study evaluated Cr and Zn removal efficiency of horizontal subsurface flow constructed wetlands (pilot scale) planted with *C. odoratus* (Cyperaceae) a common local species in tropical wetlands from Colombia. *C. odoratus* is a native grass adapted to live in flooded soils and widely distributed in all regions of Colombia, including rice cultivation systems [21]. UCV (VE) reported that *C. odoratus* is resistant to certain herbicides and common weed in agricultural areas [22]. Although there is sufficient information about heavy metals removal with experimental constructed wetlands, there is little information about *C. odoratus* removal of Cr and Zn from industrial wastewater and its resilience and potential to accumulate heavy metals.

Through phytoremediation processes the plants and their associated rhizospheric microbes, treatment environmental contaminants with heavy metals, organic substances, or industrial wastes. The contaminants can be uptake (absorbed) from soil or water and distribute into the plant tissues [24]. This relation can be quantified through Bioconcentration Factor (BCF). When the contaminants are transported from roots until shoots the translocation phenomena occur and can be calculated like Translocation Factor (TF) according to with relation between of the aerial parts concentrations and roots concentrations [15]. Values of BCFs and TFs show whether macrophytes are tolerant (BCF and TF of 0.1–1), accumulator (BCF > 1 and TF > 1), or hyperaccumulator. if additionally, the concentrations of metals in the plant exceed 0.1% by weight of dry plant [13].

III. Methodology

A. *Pilot system set up*

The pilot system consisted of Horizontal Subsurface Flow Constructed Wetlands (HSSF CW) in two containers made of polymer resin and fiberglass of 0.75 m large × 0.5 m width × 0.5 m height connected to a storage tank of 0.1 m^3 (Fig. 1). The experimental constructed wetlands were operated outdoors in the wastewater treatment facilities of the UA (Barranquilla, Colombia). This tropical, coastal city is located at 11°01'08" N and 74°52'19" W, and the most relevant environmental conditions include an altitude of 47 MASL, an average annual temperature of 32°C, average annual precipitation of 767 mm, a relative humidity of 82% and two dominant seasons, wet and dry. The principal wet season occurs between October and November and the dry season between January and March. The containers were backfilled to a depth of 0.5 m with a 10 mm granitic gravel layer (40% space porosity) and the water level was 0.3 m providing an effective volume of 45 L. The granitic gravel (calcite-dolomite content) was acquired in a commercial market in the city of Barranquilla. The HSSF CW operated with three days of Hydraulic Retention Time (HRT) as is recommended for bacteria growth [6], and other aquatic macrophytes in constructed wetlands of the Colombian Caribbean [20]. A flow of 15 $L.d^{-1}$ was applied in an intermittent regimen. The intermittent hydraulic regimen consisted of pouring 15 L of fresh wastewater influent into the inlet zone for approximately 20 min once per day. The intermittent feeding strategy was implemented to improve aerobic conditions in the HSSF CW. Intermittent feeding causes more internal turbulence and mixing, providing more oxygen to anaerobic zones [23]. The experiment was carried out in two study stages, to compare results in wet and dry seasons.

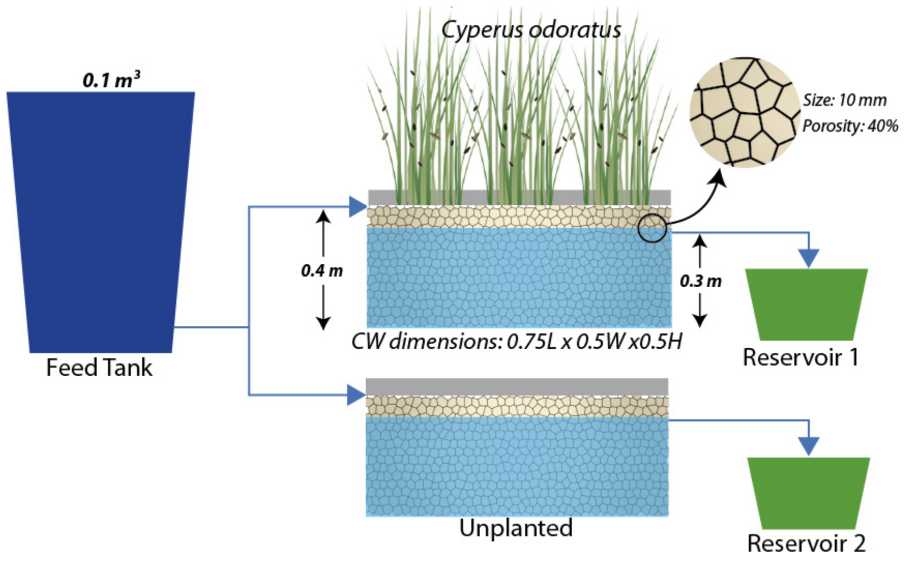

Fig. 1. Schematic representation of the pilot-scale industrial synthetic wastewater treatment plant using feed tank and SSF CWs (with C. odoratus and unplanted). Reservoirs were two small plastic containers to take the water samples.
Source: Authors.

B. *Collect acclimation, plantation, and monitoring of C. odoratus*

C. odoratus plants were collected in natural wetlands next to the university campus and acclimated in pots for three weeks applying approximately 1 $L.d^{-1}$ of synthetic industrial wastewater. Synthetic industrial wastewater was used due to the high costs of transporting industrial wastewater to the University. Cuttings of *C. odoratus* measuring 20 cm high were transplanted at a density of 12 $plants.m^{-2}$ in one of the containers while another remained without plants and was used as a control. The study was carried out in two phases due to the fast growth and senescence of *C. odoratus*. Phase I took place from September to October 2016 (wet season) and phase II, from February to March 2017 (dry season). Before the start of phase II, the containers were washed with drinking water and planted with seedlings of *C. odoratus,* which had previously been acclimated.

Measurements of plant growth were made by taking height measurements in all plants for each week of sampling in both study phases. The total biomass (aboveground and belowground) was obtained after each study phase (I and II) and was pruned, harvested, and quantified separately (roots and shoots). The biomass was placed in an oven at 105° C until the weight remained constant. To establish the levels of accumulated Cr and Zn in *C. odoratus* plant tissue from belowground and aboveground, samples of roots, shoots, and leaves were taken separately at the beginning and the end of both phases of the experiment. One gram of biomass was processed for the determination of heavy metals using an X-ray fluorescence Spectrometry protocol. Bioconcentration (BCF) and Translocation Factors (TF) were calculated according to similar studies with saturated water systems as in in each study phase [24], [13], as:

$$\mathrm{BCF} = \frac{\mathrm{Cp}}{\mathrm{Cw}} \tag{1}$$

Where:

BCF: Bioconcentration factor ($kg \cdot L^{-1}$).

Cp: Metal concentration in the whole tissue (mg/kg-dry).

Cw: Metal concentration in water ($mg \cdot L^{-1}$).

$$\mathrm{TF} = \frac{\mathrm{Ci}}{\mathrm{Cr}} \tag{2}$$

Where:

TF: Translocation factor.

Ci: Metal concentration in the aboveground tissues (mg/kg-dry).

Cr: Metal concentrations in the roots (mg/kg-dry).

C. *Industrial wastewater analysis*

The variables and measurement methods of the physicochemical quality of the synthetic industrial wastewater are shown in Table 1. Influent and effluent water samples were collected approximately twice per week and analyzed for Chemical Oxygen Demand (COD), and sulphates according to SMEWW [25]. Cr VI and Zn total concentrations in water and tissues were measured by X-Ray Fluorescence Spectrometry (Bruker S2 PICOFOX). *In situ* variables such as temperature, pH, redox potential, and DO were measured using a WTW Multi 3420 probe. Synthetic industrial wastewater was prepared in Universidad del Atlántico laboratory. Because Cr and Zn concentrations reported in effluents from the metallurgical industry in Colombia vary widely, we used influent concentrations of 25 $mg \cdot L^{-1}$

for Cr and Zn in the experiments. Consequently, the synthetic industrial wastewater was prepared using $K_2Cr_2O_7$ and $ZnCl_2$ salts, which were acquired from JT Baker, a chemical producer. Glucose, ammonium, phosphate, and sulphate concentrations were 100, 16, 1, and 60 $mg \cdot L^{-1}$ respectively and were added to water to simulate nutritional requirements of bacterial growth in CWs [26].

Table 1.
Variables and measurement methods of physicochemical quality of the synthetic industrial wastewater.

Variable	Units	Method	Quantification limit lower	Equipment
Temperature	°C		10	WTW Multi 3420 probe
pH	Units	Potentiometric	2	
Redox Potential	mV	Electrometric	–2000	
DO	$mg \cdot L^{-1}$	Electrometric	0.1	
Elect. Cond.	$\mu S.cm^{-1}$	Electrometric	10	
COD	$mg \cdot L^{-1}$	Colorimetric	3	Spectrometer DR 3900HACH
SO4	$mg \cdot L^{-1}$	Colorimetric	0.03	
Cr total	$mg \cdot L^{-1}$	Fluorescence-Spectrometry	0.1	Bruker S2 PICOFOX
Zn total	$mg \cdot L^{-1}$	Fluorescence Spectrometry		

Source: Authors.

C. *Experimental design and Data analysis*

The experimental design was based on the fixed effect of the input variables such as Cr and Zn concentrations in constructed wetlands with *C. odoratus* and another without plants. Cr and Zn removal efficiency in constructed wetlands, planted and unplanted, were analyzed following mixed linear models, which consist of the extension of the classic model to situations where the assumptions of independence between the measurements of the subjects and the homogeneity of covariance matrices are not required. Normality tests (Shapiro Wilks) were carried out and the differences between planted and unplanted systems were examined using repeated ANOVA test measurements in PAST software in phases I and II separately.

IV. Results

Measurements of temperature, Dissolved Oxygen (DO), redox potential, and electrical conductivity varied in the different treatment cells and study phases (Table 2), however, they were not statistically significant differences ($P > 0.05$). Wastewater temperature was higher than 29 °C in influent and effluent, which are considered good conditions for biogeochemical processes in tropical zones, as the rate of reactions increases at temperatures higher than 20°C [26]. Effluent pH values, although near neutral, increased in planted and unplanted effluents, in agreement with the buffer capacity reported in many HSSF CW [6]. pH values between planted and unplanted cells were significantly different ($P< 0.05$). The effluent DO values greater than 2 $mg \cdot L^{-1}$ were measured in all cells indicating optimum oxidation and interaction of heavy metals. These DO values are related to redox potential showing aerobic conditions in planted and unplanted wetlands in both study phases [26]. Electric conductivity demonstrated similar values in both HSSF CW and in both study phases with higher values in effluents, probably by salt concentrations [20]. COD concentrations in influent were around 90 $mg \cdot L^{-1}$ while the effluents were lower than 27$mg \cdot L^{-1}$ for planted and unplanted systems in both study phases. The COD removal efficiency was 85 and 70% for *C. odoratus* and unplanted systems respectively in both study phases. Similarly, sulfate values in effluents did not surpass 47 $mg \cdot L^{-1}$, and efficiency removal was around 30 and 23% in both phases for *C. odoratus* and unplanted systems, respectively.

TABLE 2.
VALUES OF INFLUENT AND EFFLUENT INDUSTRIAL SYNTHETIC WASTEWATER CHARACTERISTICS IN SCALE-LAB CONSTRUCTED WETLANDS DURING A TWO-PHASE STUDY. STANDARD DEVIATION IN BRACKETS. N = 12-13.

Variable	Influent phase 1 (Sep-Oct/16)	Effluents phase II		Influent phase II (Feb-Mar/17)	Effluents phase II	
		Planted	Unplanted		Planted	Unplanted
Temperature (°C)	33.6 (±2.8)	29.9 (±2.2)	30.5 (±2.0)	34.1 (±1.7)	29.9 (±1.8)	30.2 (±1.9)
pH (Units)	6.7 (±0.1)	7.3 (±0.1)	7.6 (±0.1)	6.9 (±0.1)	7.4 (±0.2)	7.5 (±0.2)
EH (mV)	318 (±48.3)	197.3 (±79.6)	193.1 (±83.7)	359.5 (±12.3)	249.9 (±89.5)	255.2 (±40.7)
DO ($mg \cdot L^{-1}$)	7.2 (±0.2)	5.1 (±1.0)	5.5 (±1.0)	7.4 (±0.5)	3.6 (±1.1)	3.7 (±1.2)
Elect. Cond. ($\mu S.cm^{-1}$)	575 (±9.0)	647.8 (±53.2)	648.4 (±75.2)	606.8 (±8.0)	635.2(±65.8)	632.0 (±87.1)
COD ($mg \cdot L^{-1}$)	86.7 (±7.8)	12.5 (±12.1)	26.4 (±12.9)	90.3 (±3.7)	13.3(±10.9)	25.9 (±10.8)
SO4 ($mg \cdot L^{-1}$)	58.6 (2.9)	40.3 (±7.8)	44.5 (±6.6)	60.3 (±1.4)	41.8(±8.5)	46.3 (±10)
Cr ($mg \cdot L^{-1}$)	25.3 (±0.5)	1.3 (±126)	9.2(±5.9)	25.5 (±0.5)	1.7 (±1.6)	8.2 (±3.4)
Zn ($mg \cdot L^{-1}$)	26.1 (±0.9)	1.1 (±1.2)	0.5 (±0.4)	25.3 (±0.4)	0.8 (±0.5)	0.6 (±0.4)

Source: authors

The planted HSSF CW reduced Cr concentrations from 25.5 to 1.3 $mg \cdot L^{-1}$ and 1.7 in phases I and II, respectively. The unplanted HSSF CW reduced Cr to 9.2 $mg \cdot L^{-1}$ and 8.2 $mg \cdot L^{-1}$ in phases I and II, respectively. Zn was reduced in the planted wetland from 26.1 to 1.1 and 0.8 $mg \cdot L^{-1}$ in phases I and II, respectively. The unplanted HSSF CW, Zn was reduced to 0.5 and 0.6 $mg \cdot L^{-1}$, in phases I and II, respectively (Table 2).

Cr and Zn removal efficiency was higher than 92% in *C. odoratus* in both study phases (Fig. 2). However, the unplanted wetlands reached Cr and Zn removal efficiency higher than 62 and 96%, respectively. Removal efficiency between planted and unplanted systems in each Phase was statistically different. Cr was significantly reduced in the planted system ($P < 0.05$), while Zn was significantly reduced in both planted and unplanted systems in the I and II study phases.

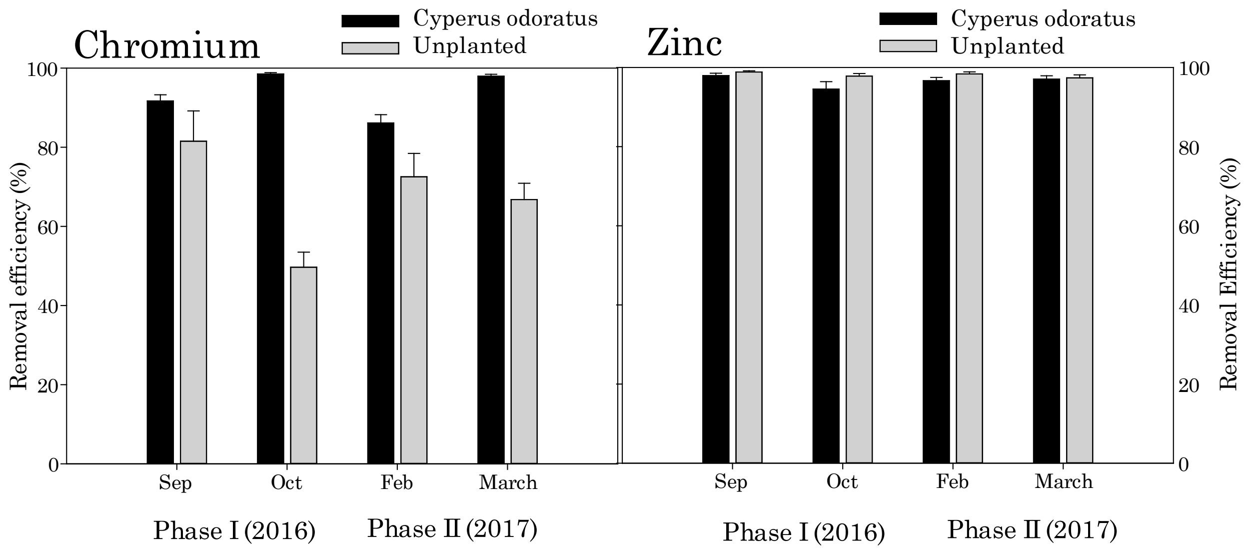

Fig. 2. Chromium and Zinc removal efficiency in constructed wetlands planted with C. odoratus and unplanted.
Source: Authors.

Although Cr is toxic for plant growth [27], *C. odoratus* showed high tolerance in phase I, principally where plant stems reached 527 mm in height. Stem growth reached 227 mm in phase II. During phase I, the biomass production was higher than phase II (Table 3) and the shoots were longer and healthy. The high reduction efficiency was related to high Cr concentration in leaf and root tissues, principally comparative with another *Cyperus* species (Table 4). However, in phase II the biomass production was lower and the shoots were weak with signs of chlorosis and necrosis. Several authors stated that Cr removal is favored by plants since they modify metals mobility and toxicity through root exudate release, and interactions with the rhizosphere microbial community [28], [29].

Table 3.
Cyperus odoratus biomass production in phases I and II.

Description	Phase I (Sep-Oct/16)	Phase II (Feb-March/17)
Aboveground biomass (kg/m^2)	0.052	0.036
Belowground biomass (kg/m^2)	0.073	0.033
Total	0.123	0.069

Source: Authors

Table 4.
Cr and Zn biomass accumulation and Bioconcentration and Translocation Factors reported for different Cyperus species.

Cyperus species	Metal accumulation (mg.kg^{-1})				Bioconcentration Factor		Translocation Factor		Authors
	Aboveground Biomass		Roots biomass		Cr	Zn	Cr	Zn	
	Cr	Zn	Cr	Zn					
C. alternifolius L.	84.7	108	407	693	20.3	34.6	0.129	0.121	[32]
	-	-	80a	130a	8.0*103	1.5*103	0.028-13	0.19-0.34	[24]
	0.028	0.097	0.026	0.074	-	-	1.0	1.3	[33]
C. articulatus L.	8.8-12.9	344-691	11.6-16.5	656-1100	0.69	16.89	0.5	0.58	[17]
C. esculentus L.	36.8	53.2	17.03	58.56	0.59	1.15	0.94	0.5	[15]
C. vaginatus R.Br	-	-	10.2*10-4 -1.2*10-3	0.017-0.022	-	-	-	-	[34]
C. odoratus	29.7 and 56.9	31.6 and 19.5	7.1 and 13.3	16.8 and 9.2	4.2 and 7.7	24.8 and 13.6	4.1 and 4.3	1.9 and 2.1	This studyb

b The metal accumulation and BCF and TF values are corresponding each phase of the study.
Source: Authors [32], [24], [33], [17], [15], [34].

Even though the constructed wetlands media were washed and made ready for the growth of *C. odoratus* seedlings before the start of phase II, it is probable that residual metal concentrations from phase I caused a greater negative effect on plant growth. The adsorption-desorption-re-adsorption cycle in a granular medium can modify contaminants removal processes and this variability could inhibit growth rate as reported in some studies [30], [31]. The Cr concentrations in aboveground and roots biomass were higher in phase II than phase I with values higher than 50 mg.kg^{-1} (Table 4) and according to Uni-Mysore and IISC (IND) [28], Cr values higher than 30 mg.kg^{-1} are toxic for plants.

Contrary, Zn concentrations in aboveground and roots biomass were lower in phase II, but these concentrations could also decrease *C. odoratus* growth and development [27]. Although Zn is an essential micronutrient for plants; it is well known that concentrations greater than 1.5g.kg^{-1} in water and soils cause phytotoxicity inhibiting many plant metabolic functions, resulting in retarded growth and causing senescence [12], [27], [35]. Moreover, the observed difference in biomass production between phases I and II could be related to seasonal weather as reported by IITKGP (IN) [36]. During Sep-Oct the rain could have favored the growth of *C. odoratus* and caused variation in dissolved oxygen and redox potential parameters (Table 2).

Adsorption of Zn on gravel is strongly influenced by changes in pH [37]. Table 2 shows a small but statically significant difference in the pH between the planted and unplanted wetlands. A greater reduction of Zn in the unplanted HSSF CW could be related to increased precipitation due to increases in pH values. Additionally, greater Zn removal in the unplanted HSSF CW could be related to the greater available surface area than in the planted HSSF CW in which the plant roots likely reduce available surface area. Uni-Mysore and IISC affirm that iron and manganese oxides are present as partial coatings on the silicate minerals in soils and freshwater sediments and their large surface area results in a high potential for Zn adsorption [28]. The vegetation-free gravel likely facilitated greater adsorption of Zn.

It is probable that adsorption Zn behavior is also related to Ca concentrations (> 50 mg · L^{-1} data not shown) in the interstitial water of the HSSF CW. The Zn adsorption in calcareous soils was studied by Iranian and Australian researchers, who concluded that high $CaCO_3$ [38], clay content, and specific surface area were closely related to the kinetics of Zn adsorption. Similarly, French studies reported that Zn sorption was also found to be highly correlated with the Ca contents of constructed wetland soil and its bonds with carbonates [39].

According to UF and LSU (USA) [26], sulfur cycling governs many microbial communities that regulate oxidation and reduction reactions and play a critical role in the biogeochemistry of wetlands. Although this study did not measure reduced sulfur species, sulfates reductions in both systems under high temperature (>28 °C) and adequate organic matter, (93 mg · L^{-1}) probably favored metals removal as demonstrated by studies at UGA (USA) [40]. The bacterial community and plant growth in wetlands can also modify the heavy metal dynamic. Several authors report that the emergent aquatic plants in HSSF CW tend to accumulate metals, mainly in the roots, because that is where physicochemical and biological processes occur [41], [24].

Additionally, the use of local native plants is highly recommended, since they show better survival percentage and adaptation properties [42]. However, in *C. odoratus*, the concentrations of Cr and Zn in aboveground biomass were superior to those registered in belowground biomass (Fig. 3), in both study phases. These results differ from those reported by the UNI (IE), LJMU (UK) [12], UAEM (Mx) [13], OCU and DOWA (JPN) [24] to other aquatic plants used in constructed wetlands or species of the *Cyperus* genera where the major metal accumulation occurred in belowground biomass from at least aquatic macrophyte species evaluated. Species like *C. sculentus* y *C. articulatus* accumulated more heavy metal in aboveground respected belowground tissues (Table 4) Higher Cr and Zn accumulation in aboveground biomass of *C. odoratus* could indicate evidence for the faster movement of these metals and that *C. Odoratus* is an aboveground biomass accumulator. Similar results were reported about *Typha angustifolia* L. where shoots accumulated more Zn than roots [15].

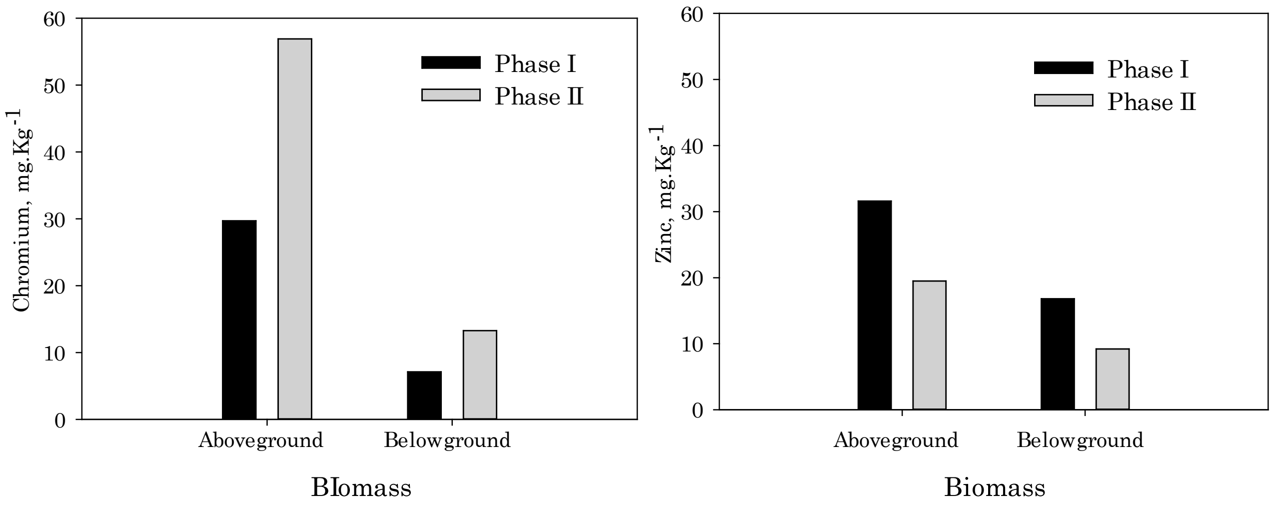

Fig. 3. Chromium and Zinc contents in aboveground and belowground biomass of C. odoratus in two study phases.
Source: Authors.

Frequently emerging aquatic plants tend to retain metals in root tissues and exclude them from aerial tissues as a metal tolerance strategy and protection against metals-induced injuries [43], [10]. Contrary, the higher concentrations of Zn in aboveground biomass of *C. odoratus* could be explained by the essential function of this metal in the synthesis of the hormones in stems of plants [44]. However, the direct role of plants is usually restricted to plant uptake of nutrients and heavy metals. The purpose of this study was to evaluate the amount of heavy metals sequestered in the aboveground biomass of Phragmites australis and thus, available for harvest and removal. The survey revealed that the amount of heavy metals accumulated in the aboveground plant biomass (aboveground standing stock. While for Cr, *C. odoratus* shows a high affinity for translocating to aboveground as has been reported for other Cyperaceae species [24], [45]. *C. alternifolius* could reach TF values upper 10 [32]. The Cyperacea family includes species declared hyperaccumulators such as *Cyperus alternifolious* L. [24], [46], and *Cyperus articulatus* L. [17].

Bioconcentration (BCF) and translocation (TF) factors of *C. odoratus* metals were higher than 1.8 (Table 4). BCF for Zn was 24.8 and 13.6 in the first and second phases respectively, compared with Cr of 4.2 and 7.7. However, the TF was higher for Cr metal indicating high translocation capacity for this metal. These results indicate a tendency for metal accumulation in *C. odoratus* in the order Cr > Zn and aboveground > belowground.

Cr phytoremediation depends on several processes that occur simultaneously, such as phytoextraction, phytovolatilization, phytostabilization, rhizofiltration and phytosecuestration [29]. Considering that TF was higher for Cr, it is possible to suggest that *C. odoratus* was able to uptake significant amounts of Cr and translocate in aerial parts. However, these results showed that there was a greater Zn bioaccumulation compared to Cr in *C. odoratus* (Table 4). The average of the Zn concentrations in the tissues was lower, indicating, as is well known, that the BCF depends on the variations of the concentration of the metal in the medium, time of exposure, and physiology of the plant species [14], [29]. Therefore, the high removal efficiency values of Zn (96.6%) compared with Cr removal efficiency (94.3%) show that wastewater treatment systems do not only depend on the phytoremediation capacity of the species, but also of the different mechanisms of elimination in these systems. Recently, joint Chinese and Canadian studies reported that the microbial community in constructed wetlands may be adsorbing Cd and Zn as an effective strategy for heavy metal wastewater treatment [47]. The greater values of TF for Cr than for Zn in this study show an affinity of the plant for accumulating the Cr in an order of aboveground > belowground as well as the results obtained for Cr by Univalle (CO) [46].

Cr and Zn accumulations also vary in *Cyperus* spp. (Table 3). While *C. odoratus* can accumulate Cr and Zn in aboveground and roots biomass similarly to *C. alternifolius, C. esculentus* L., or *C. articulatus*, the TF is higher than other *Cyperus* species. It is important to highlight that the heavy metals such as Cr and Zn from industrial wastewater accumulated in aboveground biomass are available to harvest and therefore reach the remotion.

Several authors affirmed that to consider a macrophyte as a hyperaccumulator, the BCF and TF values must be ≥ 1 and the percentage of the metal concentration in the dry biomass greater than 0.1% [48], [13]. Although in this study both BCF and TF were > 1 for Cr and Zn in both phases (Table 4), the percentage of metal in dry biomass, estimated as the average concentration of the metal contained in Kg of plant biomass, was ≤ 0.003% for both metals in phases I and II. These results indicate that *C. odoratus* is an accumulator species for Cr and Zn. Large values of TF (>> 1) imply a high translocation capacity [46]. *C. odoratus* reached TF greater than 1.5 in both phases for Cr and Zn, indicating that *C. odoratus* is an accumulator species for Cr and Zn as described by Japanese and Colombian studies, for other species [45], [46].

Although the total biomass was greatest in phase I (Table 3), an increase in the concentration of Cr in biomass was recorded during phase II. These results could indicate that other factors associated with plant physiology, such as uptake capacity and intracellular transportation in the plants could explain this increment [44].

Irish and UK research suggest that the accumulation of metal in plants can be associated with the harvesting process and maximum content of the metal in the plant [12]. However, during the second experimental period (phase II) visible symptoms of toxicity were found in plants, the removal efficiency was high, and less belowground biomass was produced (Table 3). This could indicate the metal is affecting plant [49].

The unplanted system showed high removal efficiency of Zn, probably as a result of sorption-desorption on gravel surfaces [39]. These results also show how the gravel media can reduce Cr and Zn concentrations (Fig. 2). BASU (IRN) conclude that the sorption of metals by soil is related to increased pH and substrate type [50]. Zn removal in the unplanted system was higher than 90% indicating that sorption-desorption processes are responsible for higher efficiency [39].

V. Conclusions

C. Odoratus is an accumulator species for Cr and Zn due to large values of TF (>> 1) imply a high translocation removal of Cr and Zn were 93% and 96% respectively in the HSSF CW planted with *C. odoratus* and 67% Cr and 98% Zn removal were obtained in the unplanted system. The Cr removal was high in the planted system ($P < 0.05$), while Zn removal was high in both planted and unplanted systems in study phases I and II. The tendency for metal accumulation in *C. odoratus* was greater for Zn than Cr and greater in aboveground than belowground biomass. Bioaccumulation and Translocation phenomena in Cr and Zn in *C. odoratus* in this study is probably associated with plant physiology. *C. odoratus* is an accumulator species for Cr and Zn. Consequently, it could be an emergent aquatic macrophyte recommended for constructed wetlands treatment.

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