

# Removal of perchlorate by a lab-scale constructed wetland using achira (*Canna indica* L.)

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## Research Article

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# Abstract

Achira (*Canna indica* L.) has not yet been tested for its potential in removing perchlorate ( $\text{ClO}_4^-$ ) from wastewater. In this study, constructed wetlands with and without achira were used to investigate the removal efficiency and removal mechanism of perchlorate. The results showed that more  $\text{ClO}_4^-$  was removed by the wetlands with achira relative to those without. Perchlorate removal in the wetlands without achira decreased with time, whereas perchlorate in the wetlands with achira was stably removed. In terms of  $\text{ClO}_4^-$  content, the achira tissues were in the descending order of: leaf > aerial stem > flower or rhizome > root. Perchlorate was concentrated in leaves (more than 55.8%) rather than in root (less than 0.67%). Mass balance calculation showed that plant uptake accounted for 5.81–7.34% of initial  $\text{ClO}_4^-$  input, while microbial degradation accounted for 29.39–62.48%. The wetlands with achira were favorable for soil microorganism growth and proliferation and in turn  $\text{ClO}_4^-$  biodegradation. Furthermore, the effluent pH increased in achira wetland columns and in turn promoting  $\text{ClO}_4^-$  removal. The results indicating that the wetlands with achira promote  $\text{ClO}_4^-$  removal by improving the rhizosphere environment.

## Introduction

Used in such industrial productions as fireworks, rubber, and paint manufacturing, perchlorate has emerged as a contaminant in soil, water, and food. United State Environmental Protection Agency (U.S. EPA) reported that perchlorate concentrations up to 2000 mg/kg, 3700 mg/L, 120 mg/L, and 811  $\mu\text{g/L}$  have been documented in soil, groundwater, surface water, and drinking water, respectively (U.S. EPA, 2005), as well as airborne particulate matter (Wang et al. 2017). In addition, perchlorate has also been detected in food stuffs, breast milk, baby formulas, soft drinks, and human body fluids (Her et al. 2010; Abt et al. 2016; Zhang et al. 2010; Kirk et al. 2012; Shelor et al. 2012; Calderón et al. 2017). In 2009, the US EPA identified perchlorate as a drinking water contaminant (U.S. EPA. 2019), and the State of California controls the maximum contaminant level of perchlorate at 6  $\mu\text{g/L}$  in drinking water (Xie et al. 2018). This has caused a major concern due to the health effects of perchlorate, which is documented to inhibit iodine uptake by the thyroid gland, decrease thyroid production, and lead to thyroid disease (Abt et al. 2016; Kumarathilaka et al. 2016).

As perchlorate ion ( $\text{ClO}_4^-$ ) is of low reactivity and high mobility, it is very stable and persistent in the environment (Sevda et al. 2018). Physico-chemical and biological methods have been used for  $\text{ClO}_4^-$  remediation (Choe et al. 2013; Sevda et al. 2018; Nair et al. 2020). Bioremediation, *e.g.*, perchlorate reduction by perchlorate-reducing bacteria (PRB) or chlorate-reducing bacteria (CRB) to chloride (Cl), has been proven to be more environmentally friendly and economical (Bardiya and Bae 2011).

Phytoremediation is a bioremediation technology where perchlorate is reduced *via* plant uptake, phyto-degradation, and rhizo-degradation (Yifru and Nzungung 2008; Bhaskaran et al. 2013). Plant uptake and phyto-degradation are slow processes, whereas rhizo-degradation is rapid. Utilizing dissolved organic carbon as a carbon and energy source, PRB can rapidly reduce perchlorate to nontoxic chloride (Dahan et al. 2017; Yifru and Nzungung 2008).

Constructed wetlands are established mainly for wastewater treatment (Sehar and Nasser 2019; Abdel-Mohsein et al. 2020). As constructed wetlands are easy for operation and maintenance, environmentally friendly, and efficient, they have been widely used in treating domestic wastewater (Bilgin et al. 2014; Fang et al. 2018), industrial wastewater (Calheiros et al. 2012; de la Varga et al. 2020), and agricultural wastewater (Vymazal and Březinová 2015; Smith et al. 2019).

In a constructed wetland system, plants play an important role in pollutant removal. Plants can bind pollutants on their roots and remove pollutants from the water. In addition, plants can mediate redox transformation of pollutants in their oxygen-rich rhizosphere. Therefore, plant species is an important factor influencing a wetland's performance. Our previous research has demonstrated that achira (*Canna indica* L.), a wetland species with beautiful flowers, is tolerant to perchlorate stress (He et al. 2013). Therefore, perchlorate-contaminated waters might be effectively treated by a constructed wetland using achira.

In this study, the main objectives of this work were to explore perchlorate removal potential of achira in vertical flow constructed wetlands. The selected plant species has not yet been tested for use in treatment of wastewaters containing  $\text{ClO}_4^-$ . Furthermore, achira is a beautiful plant with ornamental value, easily reproductive and has the ability to absorb nutrient contaminants. The present work studied the accumulation of  $\text{ClO}_4^-$  in soil layer, roots, stem and leaves of achira in order to know the removal mechanism and mobility of  $\text{ClO}_4^-$  in constructed wetlands. According to previous reports (Tan et al., 2004b; Seyfferth and Parker, 2008; Yifru and Nzungung, 2008), microbial degradation may play a more important role than plant uptake in  $\text{ClO}_4^-$  degradation in natural wetland system. Thus, microbial populations,  $\text{NO}_3^-$  in water which proved as a potential competitive electron acceptor with  $\text{ClO}_4^-$  were also discussed. The findings of this study are expected to provide support for high efficient wastewater treatment using constructed wetlands of high ornamental value.

## Materials And Methods

### Preparation of constructed wetland columns

Wetland columns were prepared using cylindrical ceramic pots (30 cm height and 26 cm inner diameter) (Fig. 1). For each pot, there was a side opening at the bottom connected to a tube for effluent collecting. Each pot was first filled with a 5-cm layer of cobble (4 kg/pot) and then a 20-cm layer of soil (8 kg/pot). The cobbles, white 1-3 cm diameter, purchased from Goldstone Powder Materials Co., LTD (China), which had been washed with tap water before use. The soil was collected from the surface layer of a paddy field in the Experimental Farm of the South China Agricultural University, air dry and through 2 mm sieve before use. The main properties of the soil are shown in Table 1.

### Plant species

The wetland plant species, achira, was selected for its large biomass and high tolerance to perchlorate (He et al. 2013). Plants of the same size at their three-leaf stage were collected from a local nursery. The seedlings height were about 10-15 cm.

### Experimental details

Constructed wetland columns with or without plants (achira) were prepared to treat waters containing 0 ( $C_0$ ), 40 ( $C_{40}$ ), or 100 mg/L ( $C_{100}$ ) perchlorate. For the three treatments with achira, two achira seedlings at three-leaf stage were transplanted to each wetland column.

Before the experiment started, the columns were first flooded with 6 L tap water for 7 days, drained, and let stand for one day. Then, they were fed with 6 L tap water from the top containing 0, 40, or 100 mg/L perchlorate. Water was added every day for seven days to compensate for evaporation loss. Then, effluents were collected on the 8th day, and the wetland columns were let Plant and bulk soil samples were collected at the end of the experiment. During the whole experiment (53 days), the 18 wetland columns were sheltered from rain. The properties of the tap water used in the experiment are shown in Table 2.

### Sample preparation

The collected effluent samples were stored in plastic bottles and kept in a refrigerator until analysis. The plants including roots were washed with tap water and blotted dry. After plant height, root length, and fresh weights of shoot and root were measured, shoots and roots were dried, ground into powder, and sieved through a 35-mesh (0.5 mm) sieve. Soil samples were freeze-dried, ground, and homogenized with a 35-mesh (0.5 mm) stainless steel sieve.

For  $\text{ClO}_4^-$  concentration determination, the soil and plant samples were processed as described by He et al. (2013). Briefly, 2.0 g of plant powder were added into a 50-mL conical flask with 30 mL Milli-Q water (Milli-Q Water System, Millipore Corporation, Bedford, MA), shaken at 200 rpm and room temperature for 3 h, and centrifuged at 6 000 rpm for 25 min. The supernatant was vacuum filtered through a 0.22  $\mu\text{m}$  polyethersulfone filter (Millipore Corp., Bedford, MA) and passed through a pre-conditioned ENVI-18 solid-phase extraction (SPE) cartridge (Supelco, Bellefonte, PA) to remove organic materials. The first 1.0 mL filtrate is discarded and the remaining aliquots are used for analysis. Soil sample (5.0 g) was weighed into a 50-mL conical flask with 20 mL deionized water, shaken at 200 rpm and room temperature for 3 h, and centrifuged at 6 000 rpm for 25 min. The supernatant was filtered through a 0.22  $\mu\text{m}$  polyethersulfone membrane filter, an OnGuard H cartridge, and an OnGuard RP successively to remove solid substances, metallic ions, and hydrophobic compounds, respectively.

### Perchlorate quantification

Chromatographic column IonPac AG20/AS20 was used at 30°C, the mobile phase was 30 mM KOH at 1.0 mL/min, and the injection volume was 10  $\mu\text{L}$ . After filtration through a 0.22- $\mu\text{m}$  filter, the sample solution was injected into the system. The retention time of  $\text{ClO}_4^-$  was 15.5 min. The concentrations of  $\text{ClO}_4^-$  in the samples were quantified by external calibration. Standard curves were calculated from injection of 50 to 50,000  $\mu\text{g/L}$  calibration standards. A calibration standard was run along with every 10 samples, while a laboratory reagent blank, a laboratory fortified blank, and a laboratory fortified duplicate were run long with every 20 samples. Based on injection of 10  $\mu\text{L}$  injections, the instrument detection limit was 20  $\mu\text{g/L}$ . Quality control samples were prepared as the effluent, plant, or soil samples. The mean recovery of  $\text{ClO}_4^-$  was  $100 \pm 10\%$ .

### Enumeration of soil microbial populations

Populations of culturable bacteria, fungi, and actinomycetes in the rhizosphere samples were estimated using the serial dilution technique. Ten gram soil were mixed with 100 mL sterile water, shaken for 20 min, let stand for 3–5 min, serially diluted, and plated onto 1/10-strength **beef-protein agar** (BPA), potato dextrose agar (PDA), and starch nitrate agar (SNA) for enumeration of bacteria, fungi, and actinomycetes, respectively.

### Statistics analysis

The experiment adopted a completely randomized design in triplicates. Data were analyzed using the analysis of variance (ANOVA) and Duncan's multiple range tests with SPSS-13 statistical package (SPSS Inc., Chicago, IL, USA) at a significant level of  $P < 0.05$ . Results are presented as mean  $\pm$  standard error. Perchlorate removal rate was computed as follows:

$$\text{Removal rate} = \frac{C_0 * V_0 - C_i * V_i}{C_0 * V_0} * 100\%$$

where  $C_0$  is the initial perchlorate concentration (mg/L);  $V_0$  is the initial volume (L);  $C_i$  is perchlorate concentration at day  $i$  (mg/L); and  $V_i$  is the volume at day  $i$  (L).

## Results And Discussion

### Perchlorate removal by the constructed wetland columns

Differences in perchlorate removal were observed in the different treatments (Fig. 2). In terms of average  $\text{ClO}_4^-$  removal rate, the treatments were in the order of: planted  $C_{40}$  (68.29%) > planted  $C_{100}$  (52.22%) > unplanted  $C_{40}$  (48.16%) > unplanted  $C_{100}$  (35.71%). For a same perchlorate level in influent, significantly more perchlorate was removed by the planted columns than by the unplanted columns. Furthermore, perchlorate removal in the unplanted columns diminished noticeably with running time. By contrast, perchlorate removal in the planted columns did not show a clear reduction, but increased significantly at the sixth period, being 78.46% and 55.25% in the planted columns of  $C_{40}$  and  $C_{100}$ , respectively. In comparison, only 41.52% and 20.20% of perchlorate were removed from the influent at day 53 in the unplanted  $C_{40}$  and  $C_{100}$  columns, respectively.

Tan et al. (2004) also demonstrated that perchlorate removal by wetland columns planted with Bulrush (*Scirpus* sp.) was higher as compared with columns without plants. Plants are a key component of constructed wetlands and their presence is known to improve pollutant removal (Ballesteros et al. 2016). Plants absorb nutrients, heavy metals, and toxic substances from waters. In addition, they introduce an aerobic root zone and increase aerobic processes as compared with unplanted "wetlands" (Faulwetter et al. 2009). Furthermore, wetland plant roots secrete such substances as enzymes, organic acids, and organic carbon, which are favorable for microbial metabolism, growth, and reproduction (Wu et al. 2017) and subsequently promote perchlorate biodegradation.

### Plant uptake

Plant height, and plant biomass in columns  $C_{40}$  and  $C_{100}$  were significantly lower as compared with column  $C_0$  (Table 3), indicating that achira growth was inhibited by  $\text{ClO}_4^-$ . However, the results also demonstrated that achira could tolerate high  $\text{ClO}_4^-$  (100 mg/L). Perchlorate content in the achira tissues increased with increasing perchlorate level in the wetland system (Table 4). These findings are in line with previous findings for other wetland plants (Seyfferth and Parker 2008).

Perchlorate content in the above-water tissues (aerial stem, leaf, and flower) of achira was much higher than that in the underwater tissues (root and rhizome). The order of perchlorate content in different tissues of achira was: leaf > aerial stem > flower or rhizome > root. Perchlorate content in achira leaf was 2 868.51 and 10 441.06 mg /kg DW in column  $C_{40}$  and  $C_{100}$ , respectively. In contrast, lower perchlorate content in root was observed, 141.03 and 703.51 mg/kg DW in column  $C_{40}$  and  $C_{100}$ , respectively. It is evident that perchlorate mainly accumulated in leaf (more than 55.8%) rather than in root (less than 0.67%). Similar results have been reported in other plants (Tan et al. 2004b).

On the basis of dry weight, perchlorate bio-concentration factor (BCF) of achira leaf was calculated to be 71.71 and 104.41 in column  $C_{40}$  and  $C_{100}$ , respectively (Table 4). The BCF of achira in the present study is close to that of other aquatic plants, such as smart-weed (*Polygonum* sp.), watercress (*Nasturtium* sp.), and bulrush (*Scirpus* sp.) (Tan et al., 2004a, b).

#### Mass balance analysis

The contribution of each component to the overall  $\text{ClO}_4^-$  removal by the wetland system was evaluated by mass balance analysis. The non-volatile  $\text{ClO}_4^-$  can be degraded to innocuous  $\text{Cl}^-$ . Assuming steady-state conditions, the following mass balance equation can be derived for perchlorate in the planted wetland columns:

$$M_{\text{in}} = M_{\text{out}} + M_p + M_m + M_s$$

where  $M_{\text{in}}$  is total  $\text{ClO}_4^-$  input to the wetland system (g),  $M_{\text{out}}$  is  $\text{ClO}_4^-$  output with the effluent from the wetland system (g),  $M_p$  is the part absorbed by the plants (g),  $M_m$  is the part degraded by microbes (g), and  $M_s$  is the part adsorbed by the soil and gravels.

As shown in Table 5, less than 6.32% of  $\text{ClO}_4^-$  input was adsorbed by the soil or gravels in any of the treatments, about 5.81–7.34% was absorbed by achira in the planted columns, and more than 29.39% was degraded by microbes in any of the treatments. Tan et al. (2004b) reported that 0–14.3% of initial  $\text{ClO}_4^-$  input (32 mg/L) was absorbed by plants, whereas 48.4–99.9% was biodegraded in planted columns. Nzengung et al. (1999) concluded that phytodegradation contributed to approximately 11% perchlorate removal in their 26-day experiment. Previous findings in the literature and our mass data (Table 5) indicated that microbial perchlorate reduction instead of plant uptake plays a major role in perchlorate removal by planted wetland systems.

A significantly higher percentage of the initial  $\text{ClO}_4^-$  input was biodegraded in the planted columns than in the unplanted columns. Of the initial  $\text{ClO}_4^-$  input, 62.48% and 43.27% was biodegraded in the planted  $C_{40}$  and  $C_{100}$  treatments, respectively. In comparison, 45.90% and 29.39% was biodegraded in the unplanted  $C_{40}$  and  $C_{100}$  columns, respectively. Interestingly, achira greatly increased the proportion of perchlorate being biodegraded,

indicating that plants play an important role by creating a more favorable environment for microbial growth and activity. In this study, no external carbon was supplied to the wetland columns. Carbon required by the perchlorate-reducing microbes must be self-supplied in the wetland systems. Therefore, it is expected that plant would play a more important role with time in such wetland systems as it provides both habitat and food for the indigenous perchlorate-reducing microorganisms.

#### Microbial populations in the constructed wetlands

As shown in Table 6, the CFUs of culturable bacteria, fungi, and actinomycetes in rhizosphere decreased with running time of the wetland systems, indicating that microbial growth and reproduction was inhibited by perchlorate and the inhibiting effect became more prominent with time. Sha et al. (2020) also showed that microbial community changed when exposed to perchlorate. For a same perchlorate input level, bacterial, fungal, and actinomycete counts were greater in the planted columns than in the unplanted ones, suggesting that achira helped to improve microbial growth in the wetland systems. Correlation analysis showed that there was a significant positive correlation between perchlorate removal and the microbial counts (Table 9). Additionally, bacteria were prevalent over fungi and actinomycetes in the wetland columns no matter with plants or not. Taken together, contaminants are removed in constructed wetlands under the synergistic action of plant-soil-microorganism, and the symbiotic interaction between microorganisms and animals and plants plays a central role (Lee et al. 2009). Microbial species and population in a constructed wetland directly affect the performance of the system.

#### Effluent pH value in the constructed wetlands

As shown in Table 7, the differences in effluent pH between treatments became bigger with running time, especially after the 44th day, when effluent pH in the planted columns was significantly higher than that in the unplanted columns, suggesting that achira helped to raise pH value in the wetland systems. Furthermore, in the later periods, the effluent pH values of the columns, planted or not, were in the order of:  $C_0 > C_{40} > C_{100}$ . Correlation analysis showed that there was a significant positive correlation between perchlorate removal and effluent pH (Table 9). Seyfferth et al. (2008a) demonstrated that increasing pH significantly reduced the bioavailability of perchlorate. The pH variation in the constructed wetland directly affected the perchlorate removal efficiency.

#### Variation of $\text{NO}_3^-$ in the constructed wetlands

Perchlorate-contaminated water often contains high nitrate ( $\text{NO}_3^-$ ). Studies have demonstrated that simultaneous removal of perchlorate and nitrate is feasible at low nitrate level (Ziv-El and Rittmann 2009; Bardiya and Bae 2011). High nitrate level (above 5 mg/L) was found to inhibit  $\text{ClO}_4^-$  biodegradation (Tan et al. 2004b). In the present study,  $\text{NO}_3^-$  concentration in the effluent decreased gradually with the operation continuing, but increased with increasing  $\text{ClO}_4^-$  concentration in both the unplanted and planted columns (Table 8). Effluent  $\text{NO}_3^-$  concentration in the planted columns was significantly lower than that in the unplanted columns after 17 days of operation (Table 8). And as shown in Fig.2, for a same perchlorate level in influent, significantly more perchlorate was removed by the planted columns than by the unplanted columns. Thus, the results demonstrated that simultaneous  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  removal was achieved in the constructed wetlands, and

achira played an important role in it. Correlation analysis showed that there was a negative, though not significant, correlation ( $r = -0.398$ ,  $P = 0.054$ ) between  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  concentrations in effluent. This might be because  $\text{ClO}_4^-$  concentration was much higher than  $\text{NO}_3^-$  concentration in our wetland systems.

Nitrate is one of the important nutrients for the growth of plants and microorganisms. In higher plants,  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  may share transporters or transport pathways (Seyfferth et al. 2008b). Some findings have also shown that perchlorate-respiring organisms can grow with nitrate as terminal electron acceptor, and *vice versa* (Ziv-El and Rittmann 2009). Although interference between  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  was observed, simultaneous removal was achieved in our wetland system. Therefore, natural achira wetlands can be used to treat perchlorate-contaminated nitrate-eutrophic water. However, the design of a constructed wetland system with achira for co-treatment of perchlorate and nitrate should take nitrate concentration into account.

## Conclusions

In summation, we demonstrated the constructed achira wetlands have the potential to treated  $\text{ClO}_4^-$  contaminated wastewaters. Microbial degradation and pH raise played a major role in overall removal of perchlorate, plant uptake and transformation only contributed to a relatively small portion, but planting achira could greatly improve  $\text{ClO}_4^-$  biodegradation in this wetland system. As plants can provide a continuous carbon source and support medium for microbe growth, plants will play a more important role in long term wetland treatment systems.

## Declarations

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### Compliance with ethical standards

Conflict of interest All authors claim that they do not have any actual or potential conflict of interest including any financial, personal, or other relationships with other people or organizations.

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**Consent to participate:** Consent.

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**Authors' contributions:** Dongqin Li: Conduct an experiment, Writing- Original draft preparation; Haishuo Gao: Conduct an experiment; Xinyu Du: Visualization, Software; Junhao Qin: Supervision, Methodology; Huashou Li: Data curation; Hongzhi He: Supervision, Conceptualization; Guikui Chen: Writing-Reviewing and Editing, Validation.

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## Tables

**Table 1** Physical and chemical properties of the soil used in this study

pH	Eh (mV)	Organic matter (g/kg)	Total nitrogen (g/kg)	Total phosphorus (g/kg)	Total potassium (g/kg)	Available nitrogen (mg/kg)	Available phosphorus (mg/kg)	Available potassium (mg/kg)	Cl <sup>-</sup> (mg/kg)	ClO <sub>3</sub> <sup>-</sup> (mg/kg)	ClO <sub>4</sub> <sup>-</sup> (mg/kg)
5.94	66.4	20.92	0.932	0.954	18.67	56.41	71.91	229.96	60.82	n.a.	n.a.

Note: n.a. indicates not detected.

**Table 2** The characteristics of the tap water used in this study

pH	Electrical conductivity ( $\mu$ S/cm)	Total dissolved solids (mg/L)	Dissolved oxygen (mg/L)	Biochemical oxygen demand (mg/L)	Chemical oxygen demand (mg/L)	Total phosphorus (mg/L)	Total nitrogen (mg/L)	Cl <sup>-</sup> (mg/L)	ClO <sub>3</sub> <sup>-</sup> (mg/L)	ClO <sub>4</sub> <sup>-</sup> (mg/L)
6.66	146.90	75.40	5.00	0.61	12.13	0.02	0.20	12.66	n.a.	n.a.

**Table 3** Some growth parameters of achira (*Canna indica* L.) in the different wetland columns

Treatment	Root length (cm)	Plant height (cm)	Fresh weight (g /plant)		Plant biomass (kg DW /m <sup>2</sup> )	
			Root	Above-water	Root	Above-water
C <sub>0</sub>	45.87±3.82 a	88.27±0.69 a	51.80±2.36 a	206.59±4.56 a	1.87±0.14 a	7.28±0.36 a
C <sub>40</sub>	30.87±2.78 b	75.80±1.31 b	39.42±2.89 b	181.63±6.85 b	1.36±0.11 b	6.27±0.21 b
C <sub>100</sub>	26.67±1.32 b	64.23±1.86 c	38.85±2.85 b	178.11±7.07 b	1.26±0.08 b	6.09±0.23 b

The data are presented as mean  $\pm$  standard error ( $n = 3$ ). Different lowercase letters in a same column indicate significant differences between treatments (Duncan's multiple range tests,  $P < 0.05$ )

**Table 4** Distribution of perchlorate in different tissues of achira (*Canna indica* L.) at the end of the experiment

	ClO <sub>4</sub> <sup>-</sup> content		Bio-concentration factor (BCF)		ClO <sub>4</sub> <sup>-</sup> accumulation		Fraction	
	(mg/kg DW)				(mg/pot DW)		(%)	
	C <sub>40</sub>	C <sub>100</sub>	C <sub>40</sub>	C <sub>100</sub>	C <sub>40</sub>	C <sub>100</sub>	C <sub>40</sub>	C <sub>100</sub>
Root	141.03±4.98 d	703.51±12.06 d	3.53	7.04	0.34	1.78	0.41	0.41
Rhizome	1456.39±39.00 <sup>c</sup>	3541.41±38.58 c	36.41	35.41	6.96	14.65	8.31	5.15
Aerial stem	1767.26±77.40 <sup>b</sup>	5296.25±181.24 <sup>b</sup>	44.18	52.96	27.32	81.46	32.63	30.63
Leaf	2868.51±114.45 <sup>a</sup>	10441.06±631.59 <sup>a</sup>	71.71	104.41	46.72	162.32	55.8	61.59
Flower	1517.06±44.07 <sup>c</sup>	3371.75±87.61 c	37.93	33.72	2.39	4.82	2.85	1.75

The data are presented as mean ± standard error ( $n = 3$ ). In a same column, different lowercase letters indicate significant differences between tissues (Duncan's multiple range tests,  $P < 0.05$ ).

Bioconcentration factor (BCF) was calculated according to the following equation:  $BCF = C_{d_{plant}}/C_{d_{soil}}$

**Table 5** Mass balance estimation of perchlorate for different wetland columns

Treatment	ClO <sub>4</sub> <sup>-</sup> mass (mg/pot)					Percent of initial input (%)			
	Total influent	Column effluent	Plant uptake	Sorption by substrate	Degradation <sup>a</sup>	Column effluent	Plant uptake	Sorption by substrate	Degradation
Unplanted C <sub>40</sub>	1440	746.58	—	32.48	660.94	51.85	—	2.26	45.90
Unplanted C <sub>100</sub>	3600	2314.36	—	227.68	1057.96	64.29	—	6.32	29.39
Planted C <sub>40</sub>	1440	456.62	83.73	0	899.65	31.71	5.81	0	62.48
Planted C <sub>100</sub>	3600	1720.22	265.03	57.20	1557.54	47.78	7.36	1.59	43.27

<sup>a</sup> Degradation = Mass (Influent) - Mass (Effluent) - Plant uptake - Sorption by substrate

—: not applicable.

**Table 6** Microbial number in rhizosphere of different treatments at different times

Treatment	Fungi ( 10 <sup>3</sup> cfu/g DW)			Bacteria ( 10 <sup>4</sup> cfu/g DW)			Actinomycetes ( 10 <sup>3</sup> cfu/g DW)		
	17 <sup>th</sup> day	35 <sup>th</sup> day	53 <sup>th</sup> day	17 <sup>th</sup> day	35 <sup>th</sup> day	53 <sup>th</sup> day	17 <sup>th</sup> day	35 <sup>th</sup> day	53 <sup>th</sup> day
Unplanted C <sub>0</sub>	2.96±0.03b	2.55±0.05b	1.94±0.05b	11.51±0.76bc	9.55±0.79abc	9.15±0.83a	4.04±0.27b	4.62±0.16c	2.63±0.05c
Unplanted C <sub>40</sub>	2.51±0.01c	2.45±0.12b	1.16±0.05e	9.04±0.55cd	8.52±0.36bc	5.83±0.65b	4.88±0.47b	4.01±0.19d	1.63±0.03d
Unplanted C <sub>100</sub>	1.76±0.05e	1.78±0.17c	0.82±0.04f	7.16±0.71d	5.36±0.53d	3.83±0.16c	5.23±0.11b	2.47±0.26e	0.97±0.02e
Planted C <sub>0</sub>	4.11±0.09a	3.21±0.09a	2.60±0.03a	26.87±2.40a	11.45±1.50a	9.85±0.57a	7.14±0.22a	7.52±0.22a	3.38±0.07a
Planted C <sub>40</sub>	2.55±0.08c	2.47±0.13b	1.97±0.03b	13.12±1.57bc	10.59±0.74ab	7.02±0.79b	6.86±0.38a	5.74±0.07b	2.96±0.04b
Planted C <sub>100</sub>	2.26±0.12d	1.97±0.08c	1.46±0.08c	13.69±0.58b	7.40±0.91bcd	5.74±0.41b	4.12±0.73b	3.59±0.20d	2.24±0.03c

**Table 7** Effluent pH of every period

Treatment	8 <sup>th</sup> day	17 <sup>th</sup> day	26 <sup>th</sup> day	35 <sup>th</sup> day	44 <sup>th</sup> day	53 <sup>th</sup> day
Unplanted $C_0$	7.43±0.02a	7.46±0.06a	7.33±0.04ab	7.34±0.03c	7.35±0.04bc	7.38±0.01c
Unplanted $C_{40}$	7.47±0.15a	7.29±0.05b	7.24±0.13b	7.30±0.03c	7.29±0.04c	7.37±0.07c
Unplanted $C_{100}$	7.58±0.09a	7.04±0.01c	7.01±0.01c	7.08±0.02d	7.10±0.01d	7.02±0.06d
Planted $C_0$	7.51±0.21a	7.55±0.02a	7.55±0.03a	7.72±0.06a	7.73±0.03a	7.83±0.04a
Planted $C_{40}$	7.63±0.10a	7.46±0.01a	7.56±0.02a	7.58±0.02b	7.66±0.01a	7.73±0.09ab
Planted $C_{100}$	7.61±0.02a	7.24±0.08b	7.23±0.09b	7.35±0.01c	7.42±0.02b	7.56±0.03b

**Table 8** The concentration of  $\text{NO}_3^-$  in effluent of every period (mg/L)

Treatment	8 <sup>th</sup> day	17 <sup>th</sup> day	26 <sup>th</sup> day	35 <sup>th</sup> day	44 <sup>th</sup> day	53 <sup>th</sup> day	average
Unplanted $C_0$	3.58±0.75c	10.48±0.34b	5.47±1.52a	4.19±0.23c	2.35±0.04c	1.15±0.05b	4.54±0.76
Unplanted $C_{40}$	1.81±0.03cd	3.07±0.06e	3.09±0.10ab	5.35±0.39b	4.95±0.38b	1.72±0.01b	3.34±0.35
Unplanted $C_{100}$	3.26±0.48c	16.81±0.04a	6.04±1.66a	11.89±0.19a	8.51±0.03a	4.19±0.66a	8.45±1.17
Planted $C_0$	7.87±1.02b	4.84±0.06d	0.99±0.44b	0.60±0.14d	0.48±0.01d	0.80±0.01b	2.60±0.70
Planted $C_{40}$	1.20±0.64d	7.16±0.70c	1.34±0.62b	0.96±0.08d	0.44±0.02d	0.83±0.19b	1.99±0.59
Planted $C_{100}$	15.68±0.15a	8.02±0.06c	3.24±0.12ab	1.14±0.11d	0.47±0.02d	1.20±0.19b	4.96±1.31

**Table 9** Correlation coefficients between  $\text{ClO}_4^-$  removal and some wetland properties

	$\text{ClO}_4^-$ removal	pH	$\text{NO}_3^-$	Fungus	Bacteria	Actinomycetes
$\text{ClO}_4^-$ removal	1.000					
pH	0.803**	1.000				
$\text{NO}_3^-$	-0.398	-0.465*	1.000			
Fungus	0.833**	0.508	-0.204	1.000		
Bacteria	0.683*	0.666*	0.195	0.801**	1.000	
Actinomycetes	0.817**	0.648*	-0.265	0.847**	0.797**	1.000

\* =  $P < 0.05$ ; \*\* =  $P < 0.01$  (2-tailed)

## Figures

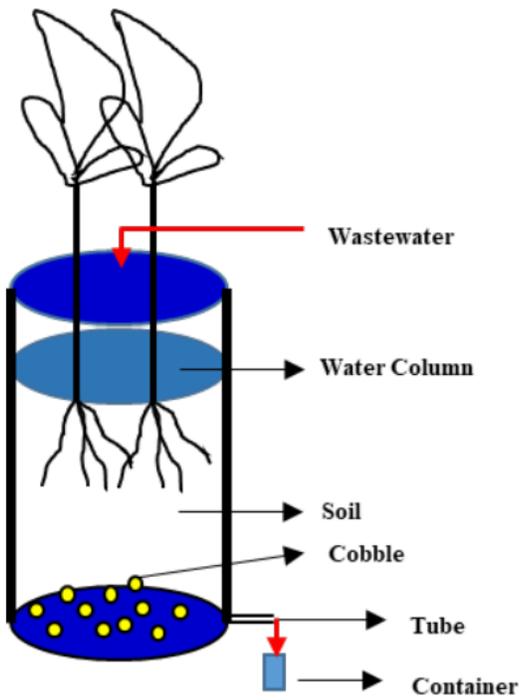
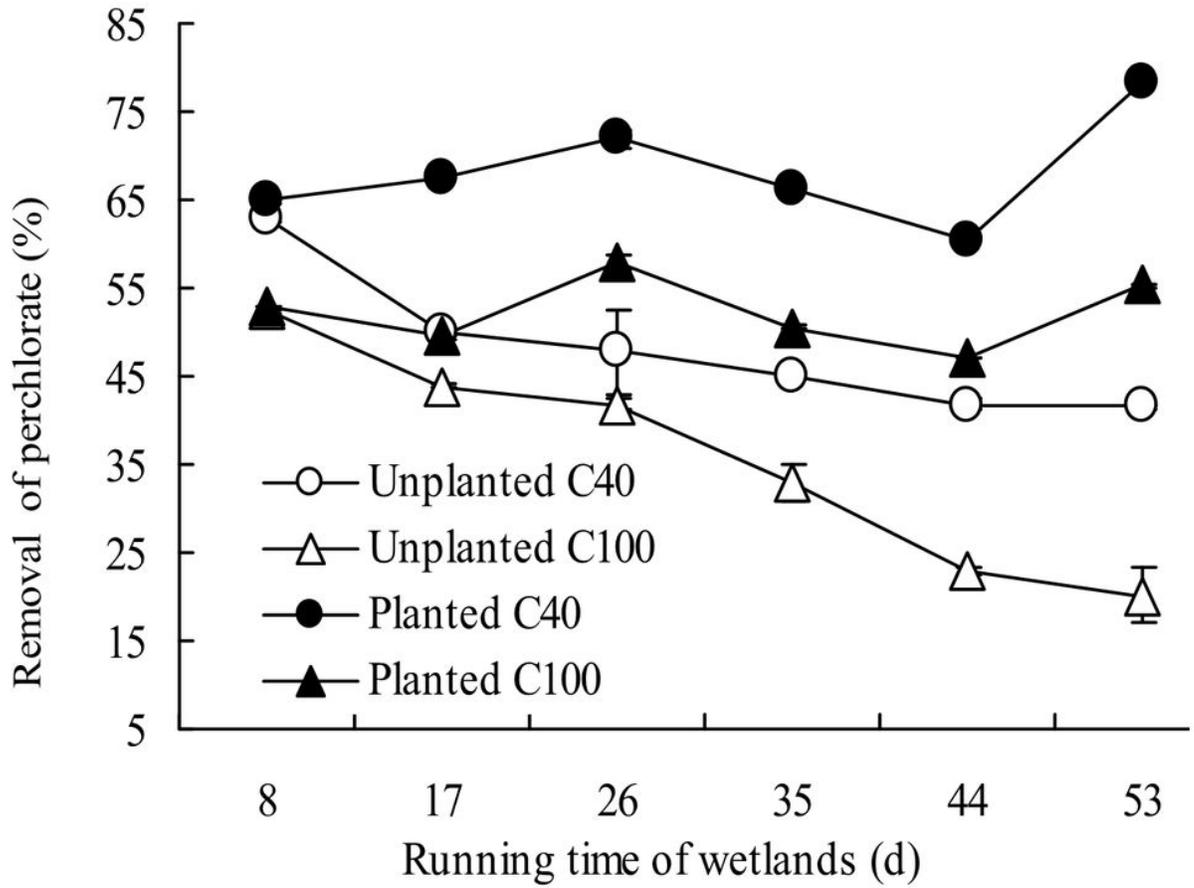


Figure 1

Schematic diagram showing a simulated wetland vegetated with achira (*Canna indica* L.)

**Fig.2**



**Figure 2**

Percent removal of perchlorate in different constructed wetland columns.