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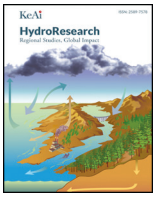

The removal of ciprofloxacin from synthetic wastewater in constructed wetland

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ABSTRACT

Considering the health effects of antibiotics in the environment, effective monitoring and treatment technologies are needed to mitigate social and environmental impacts. The present study was carried out to investigate the efficiency of the constructed wetland (CW) on the removal of Ciprofloxacin (CIP) from aqueous samples. Experiments were conducted in pilot scale CWs planted with single plants of *Cyperus alternifolius*, *Canna indica* and one planted with both plant species. Analysis of CIP concentrations in the influent and effluent samples was done using Cary 60 UV–Vis spectrophotometer, while physical-chemical parameters were monitored for the influent and effluent samples. The removal efficiency of physico-chemical parameters was ˃70% for Nitrate, ˃60% for Phosphate, ˃70% for BOD and ˃77% for COD. The maximum removal of CIP (77.1%) was observed in CW planted with *Cyperus alternifolius* during a 7 days hydraulic retention time (HRT). The results of this study show superior performance of *Cyperus alternifolius* than *Canna indica*. There was no significance difference ($p > 0.05$) produced by mixing the two plants in a CW. However, mixing of plants especially ornamental plants in CWs brings good visual impression of the systems while treating the wastewater. This study demonstrate that CW can remove antibiotics from wastewater. The best performance depends on best selection and best combination of the plants.

1. Introduction

Pharmaceuticals are either synthetic or natural chemical compounds that have pharmacological characteristics. They are used by humans to treat or prevent a variety of diseases (Fernandes et al., 2021; Jennifer et al., 2017; Tambosi et al., 2010; Viana et al., 2021) A significant number of pharmaceuticals are also used as veterinary medicine. They are used on farms to prevent and treat animal diseases, as well as to promote growth and productivity (Gworek et al., 2021; Ortúzar et al., 2022). Pharmaceuticals, their associated metabolites, and transformation products have been detected in a variety of aquatic environments, including tap water, ground water, surface water and wastewater (Shi et al., 2020; Słósarczyk et al., 2021). The primary issues with pharmaceuticals in aquatic environments are their toxicity, bioaccumulation, and persistence (Jennifer et al., 2017).

As a result of inappropriate treatment of pharmaceuticals-containing waste from companies, hospitals, or households, these compounds are dumped into the environment untreated (Gworek et al., 2021). Wastewater treatment systems have been identified as potential sources of pharmaceutical contamination in aquatic environment (Michael et al., 2013). These systems' inability to effectively remove pharmaceuticals from wastewater results in their continued detection in secondary effluents, which are then released into the aquatic environment (Huber et al., 2005). Another source is the improper disposal of unused or expired pharmaceuticals, which are finally discharged into sewage systems (Karungamye, 2022a; Miettinen and Khan, 2022). When pharmaceutical chemicals are present in aquatic environments, they can have detrimental side effects such as aquatic toxicity, bacterial resistance development, endocrine disruption, and genotoxicity (Deblonde and Hartemann, 2013; Tambosi et al., 2010). Because most pharmaceutical compounds are lipophilic and persist in aquatic environments for longer periods of time at low concentrations, the toxic effects are more likely to be chronic rather than acute (Khan et al., 2020b).

Antibiotics are one of the pharmaceutical classes that are most commonly utilized worldwide (Mahdi et al., 2021). Antibiotics are used to treat various bacterial infections and diseases in both humans and other animals (Amiri et al., 2020; Kaur et al., 2019; Kordestani et al., 2020). Because of their widespread use (Kordestani et al., 2019), they contribute a higher proportion in pharmaceutical wastewater (Zhao et al., 2018). It is challenging to treat waste water that contains these

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complex components (Puddoo et al., 2017; Thalji, 2021). Because of their high solubility in aquatic environments, longer half-life (AttariKhasraghi et al., 2021), and limited biodegradability, they accumulate over time (Mahmoud et al., 2020). Antibiotics in the environment can cause microbes to develop resistance, and their effects are transferred down the food chain to other organisms, disrupting the ecological balance. Antibiotic contamination in the aquatic environment has resulted in the emergence and spread of antibiotic-resistant bacteria and genes, posing a severe public health risk (Karungamye et al., 2022). According to the WHO, one of the three biggest risks to human health is antimicrobial resistance (Huang et al., 2021). There have been considerable adverse impacts observed even at low exposure concentrations (Khorsandi et al., 2019). Thus, in order to prevent negative effects, it is necessary to effectively treat wastewater and limit the discharge of these components into the environment.

Ciprofloxacin (CIP), a fluoroquinolone-family broad-spectrum antibiotic, is utilized in both human and veterinary medicine around the world (Diniz et al., 2021). The scientific name for CIP (Fig. 1) is (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid) (Akhtar et al., 2016). This is the most commonly given fluoroquinolone antibiotic, and it is effective against a wide range of Gram-negative and Gram-positive bacteria (Girardi et al., 2011). CIP works by inhibiting some topoisomerases, DNA gyrase, and subsequent cell division (Weber et al., 2011). Because to CIP's long-term persistence, it is repeatedly detected in many environmental compartments. These synthetic pharmaceuticals are partially metabolized in animal physiological systems, and the majority of these compounds are eliminated as the parent compounds (Nas et al., 2021). As a result of their existence in the aquatic environment, bacterial drug resistance may spread, posing major risks to the ecosystem and to human health (Sayed et al., 2016). This necessitates investigating at several technologies that can remove CIP from environmental components.

The effective approach to deal with pharmaceutical residues is to prevent them from getting into water sources and streams by improving wastewater treatment (Behera et al., 2011; Epold et al., 2012). Conventional wastewater treatment methods incorporate physical, chemical, and biological processes. These techniques are designed to remove typical wastewater parameters such as organic matter, soluble contaminants, nutrients, and colloids (Crini and Lichtfouse, 2019; Semreen et al., 2019). The removal of emerging contaminants, such as pharmaceuticals, was not their intended purpose (Ghezali et al., 2022; Ramírez-Durán et al., 2019). These techniques therefore fail to effectively remove pharmaceutical components from wastewater (Simon et al., 2021; Valipour and Ahn, 2017; Yi Yang et al., 2017). As a result, it is important to establish efficient and environmentally friendly techniques for breaking down these components in the aquatic environment (Amiri et al., 2020; Kaur et al., 2019; Mahdavi and Bagherifar, 2018; Zhou et al., 2021).

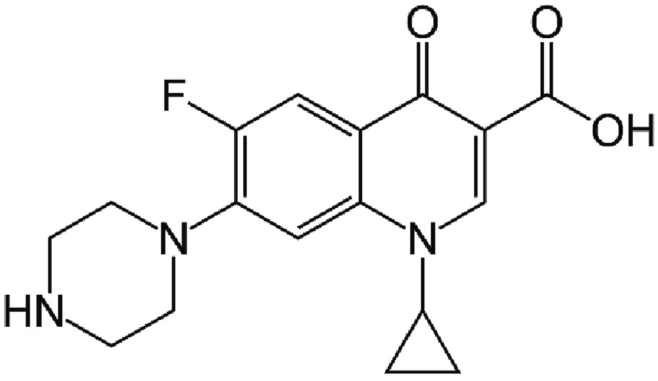

Fig. 1. Chemical structure of ciprofloxacin.

A constructed wetland (CW) is a man-made system that uses the combined effects of microbes, plants and substrate to treat water and wastewater. The mechanisms used to treat the water or wastewater include filtration, precipitation, adsorption, ion exchange, and microbial decomposition (Huang et al., 2021). CWs is an environmentally friendly treatment method that mimics natural wetland processes. The technology has been widely utilized to treat a variety of wastewater types, including industrial effluent, contaminated river water, urban runoff, agricultural wastewater, storm water, residential sewage, landfill leachate and mine drainage (Karungamye, 2022b). In comparison to a number of pollution management techniques, CWs are seen to be a simple and affordable alternative (Carvalho et al., 2014; Shelef et al., 2013). CWs are simple and have lower operating and maintenance expenses than a number of wastewater treatment techniques (Thalla et al., 2019). They have a great capacity to tolerate variations in flow and influent quality (Abdel-Shafy and El-Khateeb, 2013). They can decompose a wide range of organic and inorganic pollutants, including pharmaceuticals (Hijosa-Valsero et al., 2010). Despite their effectiveness, few researches have studied the mechanisms involved in pharmaceuticals degradation in CWs (Jones et al., 2004).

Plants in CWs play an important role in the removal of contaminants from wastewater due to complex interactions between plants, wastewater, substrate, and microorganisms (Türker et al., 2016b). Their treatment-related properties make them an important element of the design (Vymazal, 2011). They promote microbial degradation by giving a favorable environment for the development of biofilms (Kurzbaum, 2022). They are responsible for evapotranspiration and nutrient uptake, as well as providing a sustainable carbon source for denitrification (Caselles-Osorio and Garcia, 2007). Plants are the main source of oxygen in CWs by a process called radial oxygen loss that occurs in the root zone. This process increases pollutants removal by providing aerobic conditions as compared to anaerobic condition which is predominant in wastewater (Sandoval et al., 2019). Exudates secreted by plants contain nutrients, organic matter, and acids that support microbial-mediated degradations in the rhizosphere (Hdidou et al., 2022; Rachman, 2018). The performance of CW systems is said to be enhanced by greater plant diversity (Liang et al., 2011). More research is needed to decide whether maintaining monoculture or creating mixed wetlands based on the nature of plant species, plant density and mode of mixing.

According to studies, the most common plants used in CWs worldwide are *Typha latifolia*, *Phragmites australis*, *Cyperus papyrus* and *Cyperus alternifolius*. *Canna indica* has been the subject of some research that demonstrates its potential for treating wastewater in CWs (Karungamye, 2022a). *Canna indica* performs well in terms of removing conventional waste-treatment parameters, but it also has a high biomass yield, a quick growth rate, and is generally available throughout Tanzania. *Canna indica* has received little research on its capacity to remove emerging contaminants like pharmaceuticals, despite having the qualities needed for a plant to be used in CWs (Ali et al., 2020). CIP is one of the pharmaceuticals that Tanzanian healthcare facilities dispense the most (Mbwasi et al., 2020; Sangeda et al., 2021; Seni et al., 2020). Therefore, the goal of this study was to investigate the potential use of *Cyperus alternifolius* and *Canna indica* for removing CIP from synthetic wastewater samples in CWs.

2. Materials and methods

2.1. Description of the study area

The Constructed Wetlands Cells were created in a greenhouse (120 m^2) on the Nelson Mandela African Institution of Science and Technology (NM-AIST) campus in Arusha, Tanzania. The location of NM-AIST is at 03^0 24′ S and 036^0 47′ E, and it is 1205 m above sea level. The region is defined by distinct rainy and dry seasons, as well as frigid, dry air, during much of the year. The temperature ranges from 13 to 30 °C, with an average of roughly 25 °C.

2.2. Designing of the constructed wetland

Each of the four rectangular concrete CW cells measured 1.5 m (length), 1.0 m (depth), and 0.5 m (width). For the purpose of preventing leaks, the sides and bottom of these cells were lined with polyethylene plastic sheets (S. I. Abou-Elela and El-Khateeb, 2015; Heike et al., 2011; Thathong et al., 2019). Then, pre-washed gravels with a size range of 12 to 20 mm were arranged within. In order to have a better root growth and a better rhizosphere, the effective depth of the gravel column was 0.8 m in all cells (García-Ávila et al., 2019; Tuttolomondo et al., 2020). Based on experimental data and the relationship in Eq. (i) (Kaseva, 2004), the average media porosity (p) was 0.35.

$$p\ (\%) = (pd - bd) \times 100$$

Where bd is the bulk density of gravel determined as the ratio of the dry weight of the gravel sample to its volume, and pd. is particle density calculated as the ratio of the dry weight of the gravel sample to the difference between the volume of gravel and the volume of waste required to replace the pores (Raphael et al., 2019). The first cell left unplanted to serve as control (A), the second was planted with *Cyperus alternifolius* (B), the third with *Canna indica* (C) and the fourth with both plant species (D) as shown in Fig. 2. These plants were transplanted from natural wetland areas near the NM-AIST in March 2022 and were planted on the same day. The plants were planted in low density such as 8 plants per $0.5m^2$ (Chen et al., 2020; Rahmadyanti et al., 2020; Sandoval et al., 2019) In a cell with mixed plants, half number of each specie was used to give similar planting density. All of the cells were irrigated with tap water for 4 months prior to the commencement of the experimental period to allow the plants to stabilize (Mustapha et al., 2018; Yan Yang et al., 2018).

2.3. Synthetic wastewater

In this study, synthetic wastewater that had been CIP-spiked was used as the experimental influent. The features of domestic wastewater were best simulated by synthetic wastewater. Even though synthetic wastewater cannot completely replicate real domestic wastewater, it does have several advantages, such as better experimental condition control, production of desirable redox conditions (van Loosdrecht et al., 2016), elimination of batch-to-batch variability, health risks, and storage issues (Prieto et al., 2019). The synthetic wastewater should have an organic source, a source of nitrogen, phosphate, and other elements (Lima et al., 2018). The synthetic wastewater used in this study was composed with 400 mg/L glucose (source of carbon) (Haritash et al., 2015; Tang et al., 2019), 50 mg/L acetic acid (source of organic acids), 10 mg/L K_2HPO_4 (source of phosphorus) and 60 mg/L Urea (source of nitrogen) (Stefanakis and Tsihrintzis, 2009). For the trace elements, 100 mg/L $MgCl_26H_2O$, 0.5 mg/L $MnCl_24H_2O$, 0.5 mg/L $FeCl_26H_2O$ and 7.5 mg/L $CaCl_22H_2O$ were used (Biplob et al., 2011). All these were dissolved in tap water. 0.5 mol/L $NaHCO_3$ was used to control pH variations (Nasseri et al., 2014). In this study Aarciflox tablets 500 mg brand of CIP was used in experiments.

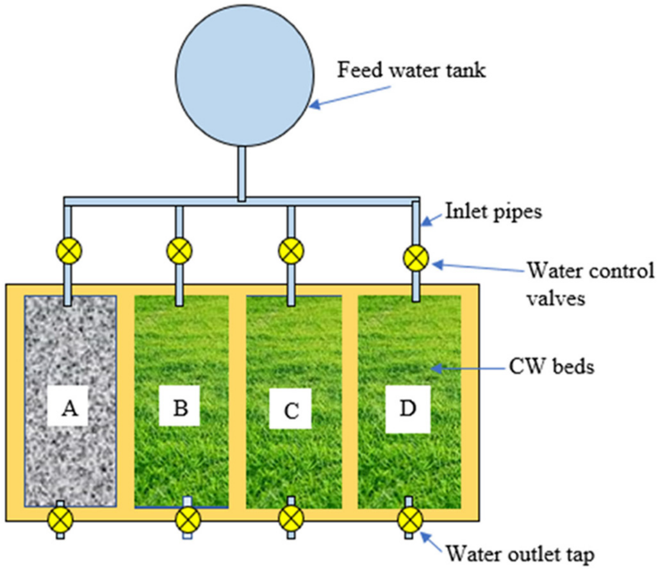

Fig. 2. Layout of the experimental constructed wetlands.

2.4. CW operation and wastewater sampling

All four CW cells were run in batch mode, which involves holding the wastewater in the media bed for a predetermined retention period before draining it out (Kiiza et al., 2020; Nguyen et al., 2021). The experiments were conducted to examine the impact of plant species and hydraulic retention time on the removal of CIP. The hydraulic retention times was set up to 7 days which means sampling was done at the outlets every morning in 7 days. The samples were collected using pre washed and rinsed 500 mL polypropylene bottles. The samples were stored at 4 °C in a refrigerator after being acidified with 2% HNO_3 until analysis. The experiment was carried out twice to ensure the validity of the results (Rana and Maiti, 2018; Yadav et al., 2010). Fig. 3 depicts the view of the CW cells during the experiments.

2.5. Wastewater analysis

2.5.1. Physico-chemical parameters

Unless otherwise stated, all of the analyses were conducted using Standard Methods for the Examination of Water and Wastewater (APHA, 2017). The methods are summarized in the Table 1 below.

2.5.2. Quantification of CIP

Previously optimized method was used for analysis of CIP (Naveed and Waheed, 2014) in the inlet and outlet samples. This was done with 278 nm using Cary 60 UV–Vis spectrophotometer (Agilent technologies) and data collected using Cary win-UV software. The calibration graphs were produced using the standard solutions made by dissolving accurate weight of the standards CIP powder to make 10, 50, 100, 250 and 500 mg/L. This gave a linear equation, Abs $= 0.00560 \times$ Conc $+0.03881$ and $R^2 = 0.99912$.

2.6. CIP removal efficiency

The removal efficiency of CW denoted as the removal percentage (r %) was calculated by the using following eq. 2 below.

$$Removal\% = \frac{C_{in} - C_{out}}{C_{in}} \times 100$$

where, C_{in} = Concentration of CIP in influent and.

C_{out} = Concentration of CIP in effluent.

3. Results and discussions

3.1. Reduction of physico-chemical parameters

The reduction of some physico-chemical parameters from synthetic wastewater in CWs planted with different plants was measured after 3, 5 and 7 days hydraulic retention time (HRT) and the results are summarized in the Table 2. According to these results, there is small change in electrical conductivity in all cells. The CW with mixed plants had highest removal of EC (10.2%) and phosphate (66.3%). The highest reduction rate of NO_3-N (76.6%) and COD (79.5%) was observed in CW planted

Fig. 3. The view of the CW cells during experiments.

with *Cyperus alternifolius*. The CW planted with both *Canna indica* and *Cyperus alternifolius* was the best for reduction of BOD (72.7%).

There was a generally low decrease in EC where the trend in different CW is Mixed > *Cyperus alternifolius* > *Canna indica* > Without plants. The trend for NO_3-N is *Cyperus alternifolius* > Mixed > *Canna indica* > Without plants and for PO_4-P is Mixed > *Canna indica* > *Cyperus alternifolius* > Without plants. The trend for BOD is Mixed > *Cyperus alternifolius*> *Canna indica* > Without plants and for COD is *Cyperus alternifolius* > Mixed> *Canna indica* > Without plants. This is well presented in Fig. 4.

The reduction of Nitrates and Phosphates in all cells increase with increase in HRT. Nitrogen is removed from CW through both aerobic and anaerobic conditions in nitrification and enitrification processes (Khan et al., 2020a; Zhang et al., 2022). Nitrification is the microbial oxidation of ammonium (NH_4) to nitrite (NO_2) and finally nitrate (NO_3) (Vymazal, 2008). Because this process requires oxygen, it happens in aerobic wetland regions. The nitrate then dissipates into the wetland's anaerobic regions, where it may be denitrified (Jones et al., 2016; Shelef et al., 2013). This process is the rate-limiting process in the nitrogen removal. Nitrate (NO_3) is transformed into gaseous nitrous oxide (N_2O) and nitrogen gas (N_2) during the denitrification process, and these gases are then discharged into the atmosphere (Turpie et al., 2010). The reduction of nitrate in CW planted with *Canna indica* (71.5%), *Cyperus alternifolius* (76.6%) and mixed plants (74.7%) are in agreement with efficiency reported by other researchers such as 62.1% (Sudarsan et al., 2015). The larger root surface area of *Cyperus alternifolius*, which is greater than *Canna indica*, is believed to be responsible for the higher removal rate of nitrate in CW planted with *Cyperus alternifolius* than *Canna indica*.

Table 1
Methods used for physico-chemical analysis of synthetic wastewater.

Parameter	Measurement unit	Method/Instrument
pH	Unitless	Hanna HI98129 Combo meter
NO_3-N	mg/L	HACH test kits (NitraVer 5 Nitrate Reagent Powder Pillows)
PO_4-P	mg/L	HATCH test kit using the ascorbic acid method and PhosVer® (ascorbic acid) reagent pillows
Electrical conductivity (EC)	mg/L	Hanna HI98129 Combo meter
Chemical oxygen demand (COD)	mg/L	Reflux Titrimetric Method (Part 5220 method C)
Biochemical oxygen demand (BOD)	mg/L	WTW OxiTop® measurement unit in accordance with the manufacturer's instructions.

The presence of plants in CW facilitates for phosphorus elimination by plant uptake. The organic form of phosphate, which cannot be removed by plants can be transformed to the inorganic form through the action of enzymes. It has long been recognized that one key route to remove phosphorus in CWs is through plant growth metabolism (Sohair I. Abou-Elela et al., 2013). Phosphorus can also be eliminated by sorption and deposition on the substrate and microbial metabolism (Ravichandran and Philip, 2022). The results obtained in this study fall in the range of phosphates removal (50% - 80%) reported by other researchers (Pinninti et al., 2021). In comparison to an unplanted CW (36.7%) or a monoculture of *Canna indica* (65.4%) or *Cyperus alternifolius*(60.7%) there was high significant phosphate removal in CW with mixed plants (69.5%).

BOD and COD express the organic carbon content in wastewater. In this study, high rates of BOD and COD removal were achieved. The performance in COD and BOD removal increase with increase in HRT. In all cells, the highest reduction percentage is observed at 7 days HRT. The obtained results in this study for COD and BOD removal agree with results reported by other researchers which is >70% (Pinninti et al., 2021; Sudarsan et al., 2015). The reduction of COD and BOD can be explained by action of both aerobic and anaerobic microorganisms attached to the roots and rhizome, as well as the porous substrate (Papaevangelou et al., 2016). Under aerobic conditions COD acts as an electron donor utilized in denitrification process under anaerobic condition (Ding et al., 2014). COD reduction can also be accomplished using suspended particles filtration or sedimentation (Rahmadyanti et al., 2020). All these mechanisms require time for the organic matter to interact with the CW's components. There is significant difference in performance between CW with plants and without plants. This shows a role of plants in performance of CWs. For reduction of COD, the CW planted with *Cyperus alternifolius* (79.5%) had the highest performance and for removal of BOD the CW planted with both *Canna indica* and *Cyperus alternifolius* performed better (72.7%) than CWs planted with only *Canna indica* (70.1%) or *Cyperus alternifolius* (71.0%). This can be

Table 2
The Physico-chemical parameters measured in synthetic wastewater during the experiment.

				% Reduction			
Parameter	**Unit of measure**	**Influent**	**HRT (Days)**	**Without plants**	***Canna indica***	***Cyperus alternifolius***	**Mixed plants**
EC	mg/L	1690 ± 16.77	3	1.2	4.1	4.2	7.1
			5	1.1	4.4	4.3	8.5
			7	2.4	4.4	4.7	10.2
NO_3-N	mg/L	34.7 ± 3.48	3	13.5	57.4	62.2	64.2
			5	28.6	62.6	67.9	66.1
			7	34.6	71.5	76.6	74.7
PO_4-P	mg/L	3.84 ± 0.71	3	22.7	55.2	54.9	59.6
			5	27.6	62.2	58.1	66.4
			7	36.7	65.4	60.7	69.5
BOD	mg/L	86.67 ± 12.47	3	34.6	63.1	59.6	62.9
			5	41.9	66.6	65.5	67.7
			7	46.2	70.1	71.0	72.7
COD	mg/L	156.29 ± 19.56	3	37.2	63.6	62.9	61.7
			5	45.6	69.2	67.6	68.1
			7	49.2	77.7	79.5	77.9

the outcome of the rooting zone's effective partitioning in a system with diverse species of plants (Karathanasis et al., 2003).

3.2. Removal of CIP

The performance of CWs without plants, with *Canna indica*, *Cyperus alternifolius* and mixed plants on CIP removal from synthetic wastewater is presented in Table 3.

The results show variations in removal efficiencies between planted and unplanted cells, between single and mixed plants, between plant species and among HRTs. The maximum removal (77.1%) was observed in CW planted with *Cyperus alternifolius* followed by 74.3% (*Canna indica*), 72.0% (Mixed plants). These results are in line with earlier research' findings that CIP removal was >77% (Nas et al., 2021). The variations in CIP removal are presented in Fig. 5.

3.2.1. Comparing planted and unplanted CWs in CIP removal

In all experiments, the CWs with plants gave higher performance than CWs without plants. For instance, for unplanted CW, maximum removal of CIP was 43.6% in 7 days HRT whereas for planted CWs the maximum removal of CIP was 77.1% observed in CW planted with *Cyperus alternifolius* in 7 days HRT. This shows the role played by plants in CWs in removing contaminants from water and wastewater. Plants provide the majority of the oxygen in CWs via a mechanism known as radial oxygen loss (ROL), which occurs in the root zone (Jiang et al., 2020). Aerobic conditions in CWs support aerobic respiration pathways, which are more effective than anaerobic pathways at removing contaminants (Zhang et al., 2012). In the rhizosphere, where there is extensive microbial activity, plants produce root exudates that enhance the ability of wetlands to remove the contaminants (Punyapwar and Mutnuri, 2020). Antibiotics can also be removed from wastewater in CWs by plant uptake (Decezaro et al., 2018).

3.2.2. Comparing single and mixed plants CWs in CIP removal

The plant diversity in many CWs around the world is low, or they just use one main species (Arliyani et al., 2021). The literature indicates that mixed vegetation is more effective in removing pollutants than single-species vegetation (Shelef et al., 2013). In this study CWs planted with single plant species (*Cyperus alternifolius*) performed better than the one with mixed plant species. However, in many experiments the system with mixed plants performed better than the system planted with only *Canna indica*. Because of this, it is difficult to say if mixed plants are preferable than single plants in CWs. The lack of a distinct pattern in CW performance in single and mixed plants makes plant

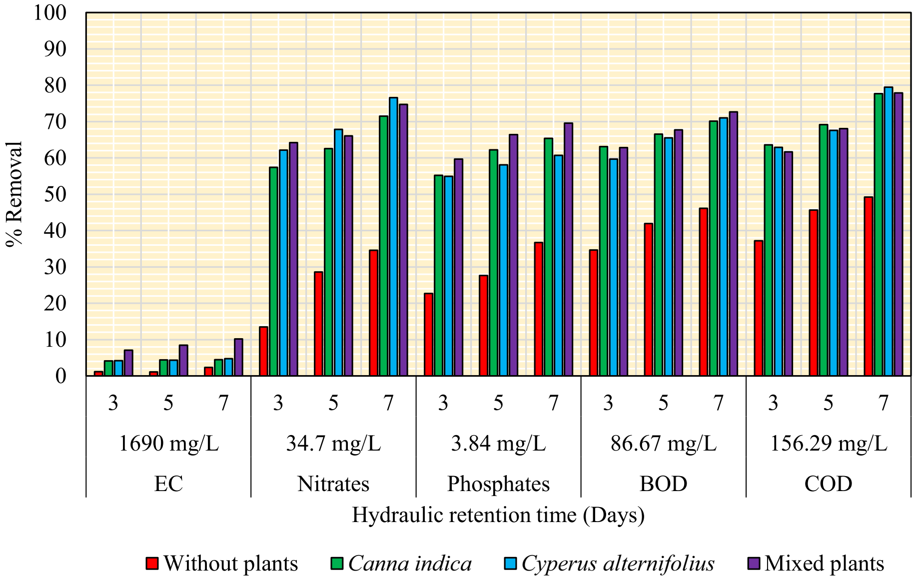

Fig. 4. Reduction of physicochemical parameters from synthetic wastewater.

Table 3
The removal of CIP from synthetic wastewater in CWs.

	Without plants		*Canna indica*		*Cyperus alternifolius*		Mixed plants	
HRT (Days)	Average (mg/L)	% Removal	Average (mg/L)	% Removal	Average (mg/L)	% Removal	Average (mg/L)	% Removal
Influent	65.97		65.03		66.17		65.93	
1	50.66	23.2	37.33	42.6	34.67	47.6	36.78	44.2
2	47.23	28.4	33.22	58.1	30.98	53.2	29.81	54.8
3	45.37	31.2	24.93	61.7	22.87	65.4	25.49	61.3
4	43.17	34.6	22.99	63.1	20.09	69.6	22.84	65.4
5	40.33	38.9	22.1	66.0	18.11	72.6	20.71	68.6
6	39.01	40.9	21.07	69.1	15.98	75.9	18.99	71.2
7	37.23	43.6	18.71	74.3	15.17	77.1	18.46	72.0

selection an important factor in CW design. Single plant systems are thought to be more vulnerable to plant death due to predation or disease than mixed and native plant systems, which are thought to be more resilient (Zhang et al., 2007). The reduced effectiveness in the system with mixed plants could be attributed to significant plant competition and other interactions (Leiva et al., 2018). Because the plants planted in CWs have the same or comparable growth patterns, individual size, and light demand competition in CWs may be more intense than in other plant communities (Liang et al., 2011). The capability of plants to absorb nutrients is constrained by competition. It also has a negative impact on the ongoing purification process of the stable plant community in CWs (Türker et al., 2016a). More study is required to determine the nature of dominance in CWs and how it affects growth and performance due to the likelihood that plant dominance can change with the seasons. This is because changes in the composition of plant species takes some years to become apparent due to the slow process of competition between plant species (Vymazal, 2011). Further research is necessary to identify optimized plant species matching, which will result in a good appearance of the CWs, less interspecific competition, and incredible performance in removal of pollutants.

3.2.3. Comparing Cyperus altenifolius and Canna indica planted CWs in CIP removal

Despite of the good performance of both plants in removal of CIP, the results show superior performance of *Cyperus alternifolius* than *Canna indica*. This variation could be attributed to microbial abundances in the rhizosphere. Previous research has found that *Cyperus altenifolius* has higher rhizosphere microbial abundance than *Canna indica*. This results from differences in root exudate and oxygen release levels (Wu et al., 2017; Zhang et al., 2014). Plant root exudates and compounds attract specific microbial communities (Shahid et al., 2020). Exudates from various species and even subspecies have varying chemical compositions (Kumar et al., 2020; Stottmeister et al., 2003). In comparison to *Canna indica*, *Cyperus altenifolius* has denser roots and a larger underground biomass, which promote reproduction of aerobes (Wu et al., 2017; Zhang et al., 2014). While *Cyperus alternifolius* has a robust, prolonged rhizome type, small size, and adventitious roots, *Canna indica* has a larger root type but is more delicate. *Cyperus alternifolius* roots have a greater ability to penetrate the media than *Canna indica* roots do. This makes *Cyperus alternifolius* enhanced oxygen supply because to its ability to successfully reach a greater region of media (Trifando et al., 2022).

3.2.4. Effect of hydraulic retention time (HRT)

HRT is one of important hydraulic parameters influencing the removal efficiencies of pharmaceuticals in constructed wetlands (Li et al., 2014). This is a measure of the average length of time that wastewater remains in a constructed wetland (Cui et al., 2010). HRT has a distinct effect on different CWs with different plant species and communities. From the results for all cells the removal efficiency of CIP increases with increase in HRT. This is expected due to the fact that at smaller HRT the compounds have less contact time with the microbials and thus, lower removal efficiency (Haiming Wu et al., 2015). At 7 days HRT (maximum HRT in this study), the maximum removal of CIP was 43.6% (Without plants), 74.3% (*Canna indica*), 77.1% (*Cyperus alternifolius*) and 72.0% (Mixed plants). It is likely that adding more HRT than what was tested in this study would result in a higher removal efficiency. This is owing to the fact that antibiotics removal in CWs involves a number of mechanisms, including adsorption, photodegradation, aerobic and anaerobic biodegradation and plant uptake, all of which are slow processes that necessitate a longer HRT (Ilyas and van Hullebusch, 2019). However, if wastewater is left in CW for a prolonged period of time, the removal rate will decline since there won't be as much nutrients for the microbial growth (Rani et al., 2011). Additionally, a considerable amount of land will be needed,

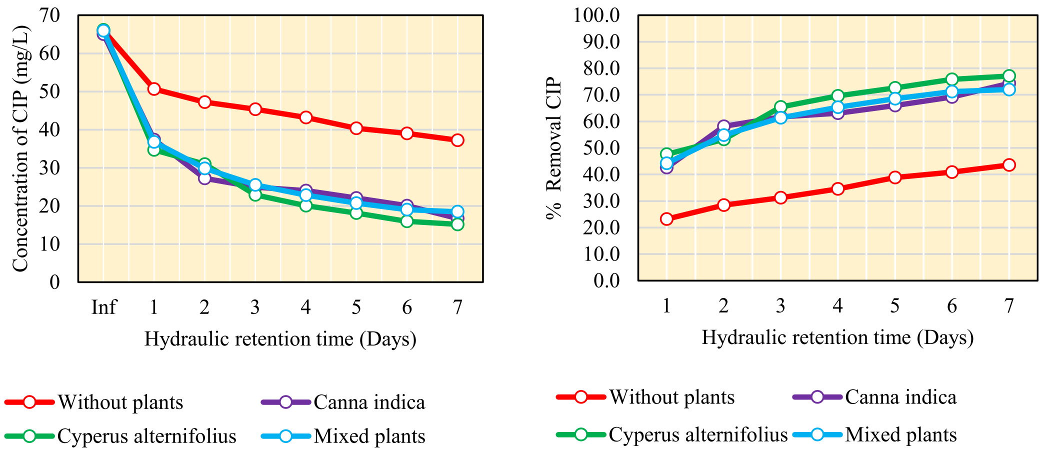

Fig. 5. Removal of CIP in CWs.

increasing the capital and operating costs (Kümmerer, 2009). Efficiency and cost must therefore be balanced when designing of CWs.

4. Conclusion and recommendations

In the present study removal of CIP was investigated in CWs planted with *Cyperus alternifolius*, *Canna indica* and mixture of the two plat species. The results show that, CWs are capable of reducing different physico-chemical parameters from wastewater. In this study the removal of these parameters was >70% of Nitrate, >60% of Phosphate, >70% of BOD and >77% of COD. CWs with plants gave higher performance than CWs without plants. Despite the excellent results both plants achieved in removing CIP, the results indicate that *Cyperus alternifolius* performed better than *Canna indica*. Combining the two plants in a CW didn't result in any significant difference ($p > 0.05$). However, mixing plants, particularly ornamental plants, in CWs improves the visual appearance of the systems while treating wastewater. More research is required to determine the ideal plant combination for improved system performance and stability. Seven days of HRT provided the best removal of CIP in all experiments. Metabolism and degradation of CIP in wastewater results into different metabolites and degradation and transformation products, which are not covered in this study. We recommend further study to investigate removal of these components in CW. It should be noted, however, that these experiments were conducted in a greenhouse using synthetic wastewater. This means that when used in real settings and with real wastewater, the same plants and antibiotic may yield different outcomes. This research is significant because it provides important details on the design of CW for pharmaceutical removal from wastewater. These variables include plant selection and whether plants should be monoculture or mixed culture.

Author contribution statement

The authors confirm contribution to the paper as follows:

a) Study conception and design: Petro Karungamye, Anita Rugaika, Kelvin Mtei, Revocatus Machunda
b) Samples collection and analysis: Petro Karungamye,
c) Data analysis and interpretation of results: Petro Karungamye, Anita Rugaika, Kelvin Mtei, Revocatus Machunda
d) Draft manuscript preparation: Petro Karungamye.
e) Review and manuscript finalization: Anita Rugaika, Kelvin Mtei, Revocatus Machunda
f) Supervision: Anita Rugaika, Kelvin Mtei, Revocatus Machunda

All authors reviewed the results and approved the final version of the manuscript.

Declaration of Competing Interest

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

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