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Comparing the performance of *Cyperus papyrus* and *Typha domingensis* for the removal of heavy metals, roxithromycin, levofloxacin and pathogenic bacteria from wastewater

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Abstract

Contamination of heavy metals and antibiotics would threaten the water and soil resources. Phytoremediation can be potentially used to remediate metal and antibiotics contaminated sites. The current study was carried out over a period of 12 months to assess the efficiency of the macrophytes *Typha domingensis* and *Cyperus papyrus* with different substrate materials to remove heavy metals and two antibiotics, roxithromycin and levofloxacin, from wastewater for reuse in agriculture. The concentrations of seven heavy metals (copper, nickel, iron, cadmium, zinc, lead, and chromium) in water and plant tissues were determined. The results showed that *C. papyrus* had a greater capacity than *T. domingensis* to remove biochemical oxygen demand (BOD) (80.69%), chemical oxygen demand (COD) (69.87%), and ammonium (NH^{4+}) (69.69%). *Cyperus papyrus* was more effective in retaining solid pollutants. The bioaccumulation factors (BCF) roots of *C. papyrus* were higher levels of most metals than those of *T. domingensis*. The highest root–rhizome translocation factor (TF) values of *C. papyrus* were higher than *T. domingensis*. The bacterial indicators (total and fecal coliforms, as well as *Faecal streptococci*) and the potential pathogens (*Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*) showed removal efficiencies ranging between 96.9% and 99.8%. The results indicated that the two systems could significantly reduce the concentration of antibiotics in wastewater, with roxithromycin showing higher elimination rates than levofloxacin. The results showed maximum removal of the heavy metals in constructed wetlands CWs planted with *T. domingensis*. The presence of zeolite and *C. papyrus* in the effluent of CWs significantly improved treatment capacity and increased pollutant removal efficiency.

Keywords Constructed wetland, *Typha domingensis*, *Cyperus papyrus*, Wastewater treatment, Heavy metals, Pathogenic bacteria

Introduction

The scarcity of natural water resources is a growing problem globally, affecting many developing countries in the Middle East, such as Egypt, Libya and Jordan. It is crucial to use non-conventional water sources to close the gap between supply and demand for freshwater used for irrigation purposes. Most of the Middle Eastern countries already suffer from absolute water scarcity, i.e., their annual water supply from natural freshwater sources is below 500 m^3 per person to cover domestic, agricultural and industrial demand [1]. Wastewater is a feasible option for alleviating water supply shortages due to various factors, such as s growing population, climate change, higher standard of living, and industrialization [2]. In Egypt, around 17 billion cubic meters of

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agricultural drainage water are discharged into agricultural drains [3]. The Bahr El-Baqar drain is one of Egypt's most polluted drains. It drains heavily populated wastewater (about 3 million m^3 per year) from Governorates, Qalubeya, Sharkia, Ismailia, and Port Said and into Lake El-Manzala. The drainage water of the Bahr El-Baqar drain consists of 58% agricultural drainage, 40% domestic and commercial drainage, and 2% industrial drainage [4].

Prolonged use of wastewater in agriculture has led to the deterioration of soil characteristics and decreased productivity of crops grown in these soils due to accumulation of heavy metals, such as nickel, lead, mercury, and cadmium, to levels that exceed permissible thresholds [5]. Discharges of drainage water polluted with organic compounds, suspended solids, dyes, pathogens, heavy metals, colorants, pesticides, and nutrients into natural watercourses causes serious deterioration in the aquatic environment and poses a threat to flora and fauna. In addition, irrigation with this wastewater poses a serious hazard to public health, the lives of farmers, food safety, and environmental quality. Poor microbiological quality poses the greatest threat to municipal wastewater reuse, because most pathogenic organisms can survive in the wastewater, on the crops, or in the soil long enough to be transmitted to humans [6]. Survival periods of the eggs of resistant helminths vary from a few days up to 1 year [7]. Moreover, the pollution of our environment by heavy metals is a global issue that has both environmental and health implications.

Heavy metals such as cadmium, copper, chromium, and lead can affect various tissues, including the nerves, kidney, liver and bones, by inhibiting functional groups of vital enzymes, and they can be carcinogenic to humans [8]. Heavy metal accumulation in the soil and plants has been observed in fields irrigated with wastewater for an extended period of time [9]. The removal of toxic heavy metals from industrial wastewaters using conventional chemical approaches, such as adsorption, oxidation and reduction, and chemical precipitation, among others, proves to be costly. These processes produce substantial amounts of secondary pollutants and hazardous sludge, which raises concerns about their sustainability [10]. Conventional treatments of drainage water are very expensive, require a high initial investment, high power demands, highly trained operators for operation and maintenance, and are ineffective at removing organic and inorganic substances.

Constructed wetlands CWs have become a viable alternative to treatment of drainage water due to their numerous advantages, which include: low energy consumption, easy operation and maintenance, cost-effectiveness, and environmental friendliness [11]. In addition, it has been deemed suitable for use in small urban communities with no sewerage systems [2]. There are two types of constructed wetland systems: free water surface (FWS) and subsurface flow (SSF). Typically, the FWS consists of parallel beds with relatively impermeable bottom soil or subsurface barrier, emergent vegetation, and shallow water depth [12]. Macrophytes play a crucial role in the removal of pollutants from free water surface systems, because they provide an ideal environment for microbial growth around the root zone, which is responsible for filtering, entrapping suspended particles, and excreting substances that can be toxic to pathogenic microorganisms.

Numerous plant species, including *Cyperus papyrus*, *Typha domingensis*, *Phragmites_australis*, *Panicum_elephantipes*, *Scirpus spp*, and *Canna spp*, among others, have been used as essential components of CWs and soil-based wastewater treatment systems [13–15]. The plants and substrate, which provide support for plant growth, are essential components of the CWs system and contain a wide range of biochemical and microbial processes that degrade organic and inorganic waste present in the wastewater from various sources into nontoxic products [16, 17]. The CW systems should also be designed with the smallest possible area footprint that would comply to reduce water losses via evapotranspiration (ET) [1]. CWs have been successfully used as a green technology to treat domestic sewage under hot and arid climates, such as in the Middle East [1], agricultural wastes [18] industrial effluents [19], mine drainage, landfill leachate, storm water, and urban runoff for decades [20].

In constructed wetlands, contaminants are removed through filtration, sedimentation, plant uptake, precipitation, adsorption, volatilization, and various microbial processes [21]. Recent research has focused on the ability and effectiveness of wetlands to remove human pathogens, including total coliforms, fecal coliforms, *E. coli,* and *Salmonella* spp., from wastewater by aquatic plant species, namely, *C. alternifolius, C. papyrus, T. latifolia, P. mauritianus*, *I. pseudacorus*, and *S. lacustris* [22, 23]. Significant reductions in the number of total coliforms (99.2%) and fecal coliforms (99.6%) have been observed in *C. papyrus* ponds [13]. According to studies [24], aquatic plants can effectively remove waterborne pathogens from constructed wetlands. You et al. [25] reported that *Leersiahexandra* Swartz has the potential to remove 84–97% of Cr, Cu, and Ni ions from electroplating wastewater.

In recent decades, constructed wetlands technology has gained popularity worldwide, including in the United States, China, Argentina, the Czech Republic, Greece, the Netherlands, and Europe [8, 14]. Numerous studies have demonstrated that municipal and hospital wastewater treatment plants are not always sufficiently efficient and do not always remove all pharmaceuticals from the wastewater. Antibiotics and related compounds have consequently been detected in various bodies of water at concentrations ng$\times$L^{-1} [26]. Domestic and municipal wastewater is enriched with pharmaceutical compounds derived from human and animal feces [27]. The concentration of ciprofloxacin (CIP) in Pakistan in streams ranges from 42 to 332 µgmL^{-1}, with ofloxacin > ampicillin > levofloxacin > sulfamethoxazole coming in last [28]. Antibiotic use in humans and animals may also contribute to the emergence and spread of antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARGs) in the environment [29]. Therefore, antibiotics and ARGs are, therefore, regarded as emerging environmental contaminants and have been detected in numerous environmental compartments [30]. Ciprofloxacin can promote the generation of resistance genes (ARGs), and the spread and diffusion of resistance genes may accelerate the mass reproduction of resistant bacteria, posing a secondary threat to human health and ecological and environmental security [31].

Several studies demonstrate that conventional wastewater treatment plants (WWTPs) are not designed to remove pharmaceuticals, metabolites, or drugs [32]. In recent years, CWs have proven effective at reducing a broad range of micro-contaminant concentrations (including antibiotics residues) in municipal sewage; the antibiotic-resistant bacteria contents in CWs effluents were significantly lower than those of conventional activated sludge systems [33]. The effect of *C. papyrus*/*T. domingensis* combined with zeolite and limestone on its efficacy during the remediation of roxithromycin and levofloxacin contaminated water has not been documented. Both vertical flow (VF) and free-water surface (FWS) systems, in particular, have demonstrated their suitability for wastewater treatment and reclamation [20]. However, the VF and FWS systems are limited by their low removal efficiencies (antibiotic) when constructed using normal filter layers or simple designs. In addition, the lack of normal rapid infiltration plants and low filter media height in FWS systems are some additional disadvantages [34, 35]. Gorgoglione and Torretta [36] confirmed the findings of previous research indicating that certain media substrates, such as sand, granular activated carbon, gravel, and rock, may not be effective for long-term phosphorus storage, antibiotics and removal of pathogenic bacteria in constructed wetlands. To overcome the shortcomings of the FWS systems with regard to wastewater treatment, the materials, designs, and operations of these systems have been improved. Today, there is a growing interest in providing constructed wetland plant growth with substrate layers, which play a crucial role in removing pollutants from wastewater. In constructed wetlands, substrates including limestone, crushed stones, biochar, zeolite, and composite materials (e.g., BAZLSC) have been utilized [37, 38]. Due to their structural properties, such as ion exchange capacity, dehydration, and adsorption, natural zeolites, particularly clinoptilolite, have been extensively studied for the removal of pollutants from wastewater [39]. Their open-framework lattice comprises movable cations such as Mg, Ca, K, and Na that are easily exchangeable with cations found in the water medium, resulting in materials with antibacterial properties [40]. These improvements include the use of a novel FWS [41] with ecology filter-integrated rapid infiltration [42], multi-layer artificial wetlands [35, 43], zeolite-containing filter sands [44], and a combination of multi-gravel-layering systems and zeolite [45]. The combination of CW and zeolite has been reported to partly remediate the chemical oxygen demand (COD, contents of nutrients and organic compounds, turbidity and antibiotics [46–50]. These amendments have increased the level of wastewater purification via CWs. However, the integration of these improvements has still not received much attention, and there are many additional aspects that can be exploited. In recent years, several studies have demonstrated that natural zeolite tuffs are effective sorbents for the removal of Cu^{2+}, Zn^{2+}, and Ni^{2+} from wastewater [51]. Furthermore, they have a high capacity for attracting microorganisms and removing ammonia from wastewater treatment [52].

The novelty of the present study is the evaluation of the potential of free water surface constructed wetland treatment systems vegetated by *T. domingensis* and *C. papyrus* combined with zeolite and limestone to remove specific pollutants from water. These plants have not been used in this manner before and so this study will help to determine the efficacy of this combination in removing suspended solids, nutrients, drug-resistant microbial strains, heavy metals, and indicator pathogenic bacteria. This could potentially lead to a more cost effective and efficient way of treating wastewater. The main objectives of this study were to: 1. Evaluate the efficiency of a FWS at the experimental pilot scale. 2. Examine the effects of contact time on the ability of FWS to tolerate cadmium (Cd), copper (Cu), nickel (Ni), zinc (Zn), and lead (Pb) in root tissues. 3. Study the effects of the presence of aquatic macrophytes (*T. dominguensis* and *C. papyrus*) on the ability of FWS to tolerate heavy metals. 4. Investigate the capacity of *T. dominguensis* and *C. papyrus* to transport various heavy metals to the shoot systems. 5. Determine the antibiotic removal efficiency of FWS for wastewater treatment. 6. Evaluate the ability of FWS to remove pathogens (total coliforms, faecal coliforms, *Faecal streptococci*, and *E. coli*) from wastewater.

Materials and methods

Study site

The Bahr El-Baqardrain system is 170 km long and includes the main drain that begins near the City of Zagazig, where it collects discharges from the Bilbeis drain and the Qalubeya drain, and discharges the untreated wastewater (about 3 BCM/year) into Lake Manzala [53]. The Bahr El-Baqar is located between 31°50′ and 32°20′ longitude and 30°50′ to 30°10′ latitude (Fig. 1). The system operation treatment was launched in June 2021 to May 2022. The laboratory-scale experiment was carried out in the Central Laboratory for Environmental Quality Monitoring CLEQM at National Water Research Center, Cairo, Egypt.

Mesocosm experimental design

The experimental system was composed of a septic tank, a sedimentation tank, and a free-surface flow wetland. The sedimentation tank, with a capacity of 40 L was filled with a bottom layer of coarse-grade gravel (7–10 mm diameter, 35 cm thickness), followed by a fine-gravel layer (3–6 mm diameter, 20 cm thickness) to reduce the pollution load and prevent clogging of the forward filters. Primary settled sewage was continuously fed into the beds through PVC distribution pipes of 76 mm in diameter, adjusted by a mechanical gate valve to ensure uniform inflow at a flow rate of 0.01 m^3day^{-1}. Three units were established, each having internal dimensions of 2 m length, 1 m width, and 0.45 m depth, and a slope of 0.5%. In the first 4 weeks before actual wastewater feeding, the plantation was allowed to develop and adapt. Then, 20 L of contaminated water containing roxithromycin, levofloxacin, and (100 mg L^{-1}) was added to the septic tank. The constructed beds were covered with a polyethylene of 1 mm thickness to avoid seepage of wastewater. The plants were cultivated in wetland units with rhizomes at a rate 6 plants per unit. The first unit (non-vegetated) served as the control bed, the second and third units consisted of four parts, the entire unit was vegetated with three stems *T. domingensis* or *C. papyrus* with an average

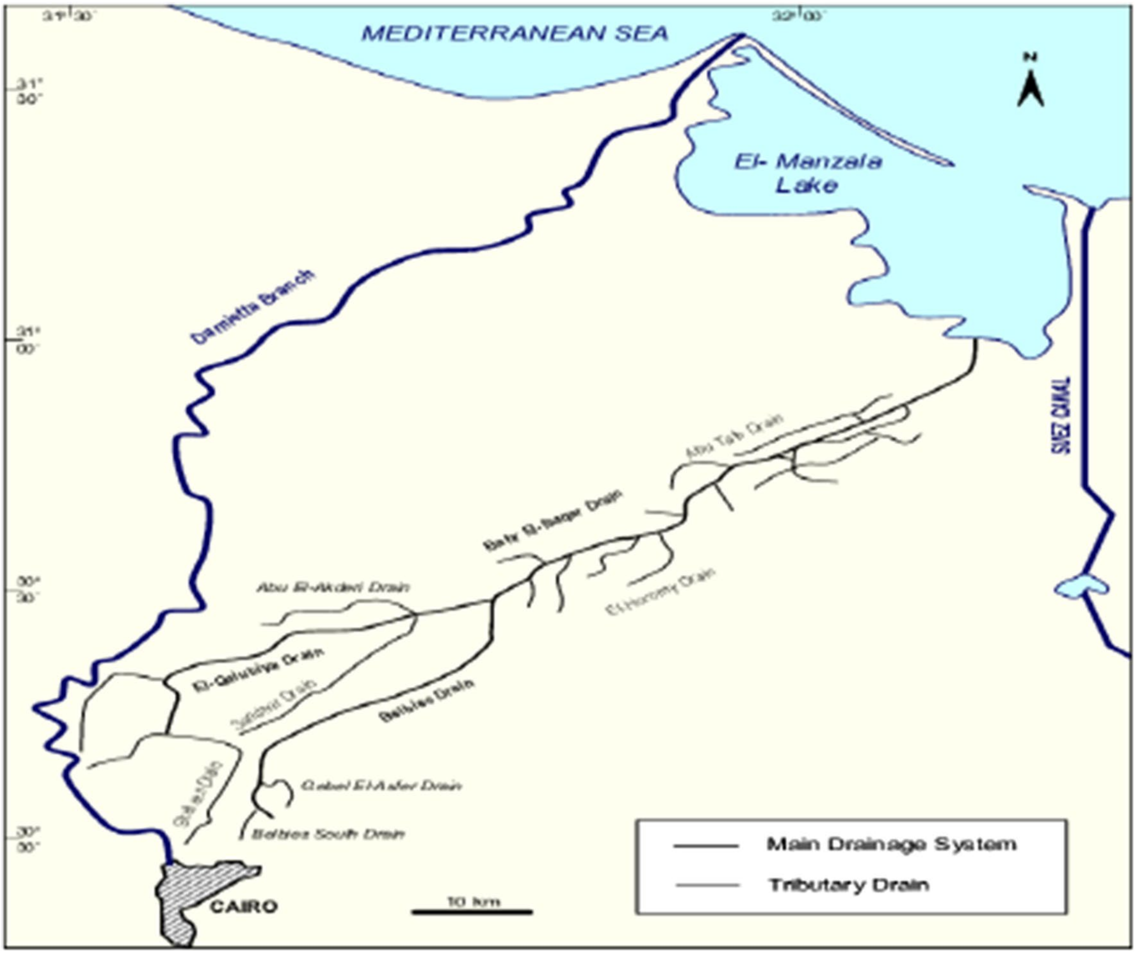

Fig. 1 Map of the main drains

size of 50 cm, a central baffle, part II was filled with 30 cm zeolite (4–8 mm), part III was vegetated with two stems *T. domingensis* and the last part was covered with a limestone bed with a width of 25 cm, as shown in Fig. 2. The study was performed two plant species *T. domingensis* and *C. papyrus*, which are known to be suitable for usage in constructed wetlands CWs. These plants have been used elsewhere to remove suspended solids, nutrients, heavy metals, toxic organic compounds, and indicator bacteria [54, 55]. The plants were collected from Lake Burullus (Kafr El-Shaikh) Governorate, Egypt. Before beginning the experiment, *T. domingensis* and *C. papyrus* were given 1 month to acclimatizes on the new growth environment. The plants were planted in the wetland units with rhizomes at a rate of 6 plants per unit. After cultivation, the wetland units were fed with wastewater for 1 month. ADT-161 data logger was used to monitor meteorological parameters (temperature and relative humidity) each month.

Physico-chemical analyses

Five liters of water samples were collected from the treatment beds at monthly intervals throughout the research period at the influent and effluent of the treatment wetlands, from June 2021 to May 2022. The samples were collected in sterile polyethylene plastic bottles, which and were placed in ice boxes before they were transported to the laboratory for analysis following the standard methods for examination of water and wastewater published by the APHA (2012). Physicochemical parameters including temperature, pH, and electrical conductivity (EC) were measured using the WTW–multi-probe analyzer Multi-3630-IDS. Total suspended solids (TSS) and turbidity was measured by the ThermoOrionAQ 4500. Ammonium (NH^{4+}) was measure by the photo Lab® 7100VISvon 320–1100 nm. BOD was measured using WTWOxiTop®-iIS6 Respirometric BOD system, and the seven heavy metals including chromium (Cr), copper (Cu), zinc (Zn), cadmium (Cd), iron (Fe), nickel (Ni), and lead (Pb) were measured by the (ICP-MS), Perkin Elmer Sciex, ELAN 9000.

Heavy metal analysis of plant

The collected plant samples were properly washed to eliminate debris. The washed samples were cut into small pieces and dried at 105 °C for 24 h. To facilitate heavy metal analysis, the substance was reduced to a fine powder using an agate mortar. The homogenized plant tissue (0.5 g) was then digested with a solution of hydrochloric acid:nitric acid (a ratio of 3:1) at 60 °C for 2 h on a hotplate. Before ICP–OES analysis, the samples were filtered through 0.6 mm Whatman filter paper and 0.45 µm cellulose nitrate membrane filter paper (APHA, 2012).

Microbiological analysis

Bacteriological samples, including total coliforms (TC) and fecal coliforms (FC) were cultured on (Difco) M-Endo agar LES and MFC agar at 35 °C and 44.5 °C for

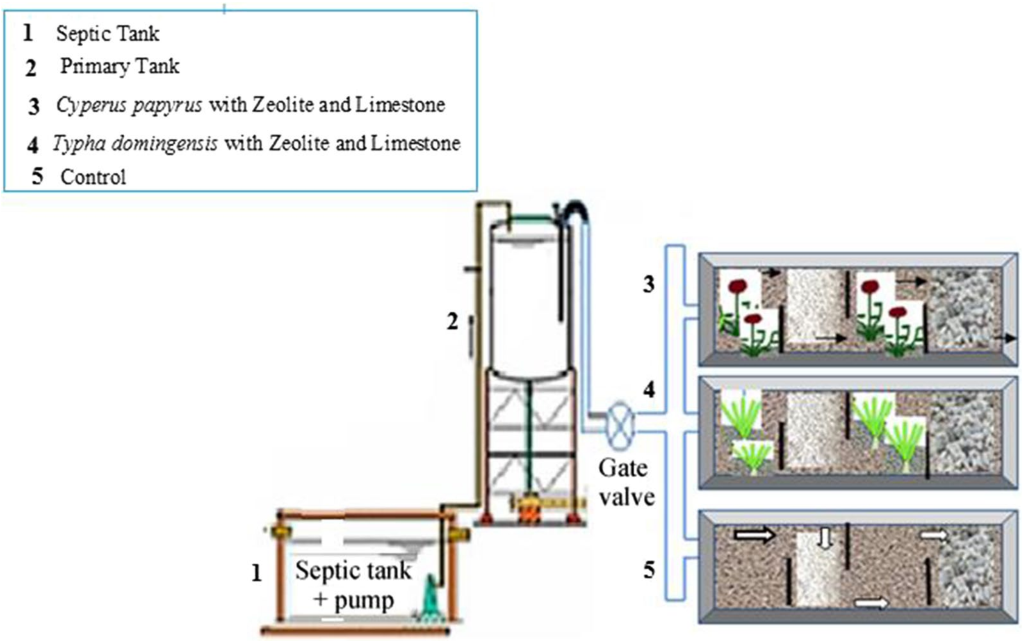

Fig. 2 Schematic diagram of the treatment units

24 h, respectively, before enumeration. Fecal streptococci (FS) was cultured on Difco TM m Enterococcus agar at 35 °C for 48 h, *E. coli* was cultured on M-TEC agar at 44.5 °C for 24 h, *Pseudomonas aeruginosa* was incubated on BBLTM-M-PA-C agar at 41.5 °C for 72 h and *Staphylococcus aureus* was cultured on Baired parker agar base at 35 °C for 48 h before enumeration were analyzed using the membrane filtration technique (APHA, 2012) following the standard methods. The results were expressed as colony forming units (cfu) per 100 mL using the following equation:

$$\frac{\mathrm{cfu/ml} = (\mathrm{no.\ of\ colonies} \times \mathrm{dilution\ factor})}{(\mathrm{volume\ of\ culture\ plate})} \tag{1}$$

Escherichia coli was confirmed by streaking on Eosin Methylene Blue agar plates, which would give apink growth with a golden metallic shine. *Staphylococcus aureus* was also confirmed by transferring to Mannitol salt agar plates to give golden yellow colonies. The *P. aeruginose* isolates were confirmed by streaking on DifcoTMCetrimide Agar Base plates to enhance fluorescein and pyocynin production (Blue green pigments). The BIOLOG GIN III system, Biolog Inc., California, USA was randomly selected for further species identification.

Bioinformatics' analysis

Sequences of DNA were identified using Basic Local Alignment Search Tool (blast) on the NCBI database. Using Krona software performed for many alignments of sequences (USA, version 5.2) [56]. Development of Molecular Genetic Analysis (MEGA) software (version 6.0) [57]. Identification for relationship of phylogenetic between microbial community. Evaluation using unweighting two method with arithmetic mean (UPGMA) through MEGA 6.0 software, and boot strap analysis (1000 replicates) was performed to assess the reliability of the constructed phylogenetic. Nucleotide sequences data determined by the National Center for Biotechnology Information (NCBI) GenBank database, USA.

Preparation of plant extracts

The antibacterial activity of root extracts from *C. papyrus* (L.) was tested against three potentially pathogenic bacteria present in wastewater influent: *E. coli, P. aeruginosa*, and *S. aureus*. The selected root parts were thoroughly washed with tap water and sterile distilled water, dried in an oven at 40 °C until completely dry, pulverized using an electric mixer, and stored in closed labeled containers for future use (Al-Samarrai et al. [58]). Approximately 11 g of dried roots were homogenized with 100 mL of sterile, boiled, distilled water for 2 h. The flasks were kept on a rotary shaker at 220 rpm for 30 min before being left at room temperature for 6 h. The extracts were filtered through muslin cloth, centrifuged at 4000 rpm for 10 min, and then filtered through Whatman no. 1 filter paper. The aqueous extract was allowed to evaporate. The dry residues were weighed and reconstituted to make the final water volume, resulting in 150, 300, and 500 mg.mL^{-1} concentrations.

Antibacterial activity assay of *C. papyrus*

The antibacterial activity of the root extracts of *C. papyrus* (L.) was evaluated using agar well diffusion method as described by Mostafa et al. (2018). The microbial suspension was grown in 10 mL of nutrient broth (Oxoid, UK) at 37 °C for 24 h. One hundred microliters (10^6 CFU.mL^{-1}) fresh microbial culture was spread on a nutrient agar plate. Four wells of 6 mm diameter were punched off into the agar medium with sterile cork-borer (6 mm) and filled with 100 µL (100–500 mg.mL^{-1}) of plant extract using a micropipette in each well under aseptic conditions. Dimethylsulfoxide (DMSO) was used as a negative control. The plates were allowed to stand for 1 h at refrigerator to allow for pre-diffusion of the extract into the medium. The plates were incubated at 37 ± 2 °C for 24–48 h. The antibacterial screening was evaluated by measuring the zone of inhibition (mm) [59]. The test compound was evaluated at various concentrations, including 100, 150, 300, 400, and 500 µL, and compared to both a positive control (amoxicillin) and a negative control (sterile distilled water). The Petri dishes were incubated at 37 °C overnight for 24 h. The extracts showed antimicrobial activity were later tested to determine the Minimal Inhibitory Concentration (MIC) for each bacterial sample. Three bacterial samples (*P. aeruginosa, E. coli,* and *S. aureus*) were grown in nutrient broth for 6 h. After, 100 µL of 10^6 cells.mL^{-1} was inoculated in tubes with nutrient broth supplemented with different concentrations (100–500 µL) of the extracts, respectively. Afterwards, 24 h at 37 °C, the MIC of each sample was determined by measuring the optical density in the spectrophotometer (620 nm), comparing the sample readout with the was non inoculated nutrient broth.

Gas chromatography–mass spectrum (GC/MS) analysis

The quantification *C. papyrus* was analyzed by GC–MS using (Agilent 7890A gas Chromatography (USA) equipped with a 5975cInert Mass selective detector and HP-5MS capillary column (30 m, 320 mm, 0.25 mm). Approximately 1 µL of the extracted sample was injected into the GC–MS for 45 min. The oven was set to 60 °C for 2 min., ramped at 10 °C min^{-1} to 280 °C, and held for 8 min. Compounds were identified by comparing their spectra to a typical library of retention time and mass spectra supplied by the GC–MS system software [60].

Chemical and sampling and analytical method

All antibiotics were obtained from Sigma Aldrich (Germany) and were of HPLC grade (N 98%) purity. The influent and effluent of the CWs were sampled daily for the analysis of organic contaminants and every 2 days over a 10 day period for the analysis of physicochemical properties and nutrients. The antibiotic concentrations were determined using HPLC, as described previously. Analyses were performed using methods described by [61]. Prior to injection into the HPLC, all wastewater samples were passed through sterile polyethylene plastic bottles, acidified to a pH of 2.5 (± 0.2), and refrigerated at 4 °C.

Statistical analysis

Statistical analyzes were performed using the statistical package for the social sciences version 18.0. The analyzes included a one-way analysis of variance for normally distributed data at a significance level of $p = 0.05$ to test for differences in the performance of wastewater treatment by wetlands between *C. papyrus* and *T. domingensis*. The Tukey multiple comparison test was used to perform pair wise comparison of group means. The differences results of the study were presented in the form of mean ± standard deviation.

Experimental design by response surface methodology

The response surface methodology is a statistical and mathematical tool that uses a second-order equation to find the best conditions between the controllable input parameters and the response variable was determined using Box–Behnken design (BBD) statistical software design expert version (13). The effects of factors, such as area of wetland (X1), plant densities (X2), flow rate (X3) and contact time (X4), on the removal process, were studied using the Box–Behnken design, are depicted in Table 1. Twenty seven experimental runs were obtained according to a the three levels of each variable; low (− 1), middle (0) and high (1) were used to design and analyze the experiments, respectively. The second-order polynomial equation was developed to predict the optimum value between the dependent and independent variables. The correlation's general form can be stated according to the following equation:

$$Y = b_0 + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n} b_{ii} x_i^2 + \sum_{i=1}^{\mathrm{n}-1} \sum_{j=i+1}^{n} b_{ij} x_i x_j \qquad (2)$$

where Y is the predicted response factor (the removal of *E. coli*), x is input variable and β_0 (the intercept), β_j (the linear effect), β_{jj} (the square effect) and β_{ij} (the interaction effect) and N is the quantity of input controlling coded variable. The coefficient of determination, R^2, and Fisher's F test were used to describe the quality of the quadratic model equation. The statistical significance of the model was assessed using analysis of variance (ANOVA) using the Design-expert 13.

Table 1 Level of different process variables in coded and uncoded form for the removal of *E. coli* from wastewater using *Cyperus papyrus*

Variable	Factor	Range and level		
		Level (− 1)	Level (0)	Level (+ 1)
Area of wetland(m2)	X1	1	2	3
Initial *E. coli* concentration(cfu/100 ml)	X2	3	6	9
Flow rate (L)	X3	20	40	60
Contact time (day)	X4	1	2	4

Characterization and preparation of zeolite

The natural zeolite used in this study was purchased from the National Company Alex Trade in Egypt. The physical and chemical properties were determined by classical analytical methods; meanwhile, the crystalline phase was characterized using X-ray diffraction (XRD) according to the standard methods followed by the Metallurgical Development Research Center, Egypt. The zeolite material was divided in to fractions by mechanical sieves to obtain a particle size of 0.8–1 mm, suitable for supporting both agricultural [62] and treatment objectives [63]. Preliminary activation of the particles was carried out using 10% aqueous NaCl for 24 h. The treated zeolite was then washed several times with distilled water and left to dry at room temperature [64].

First-order removal rate constants

First-order coliform removal rate constants were calculated using a first-order volume-based kinetic model showing the relationship between inlet concentrations and HLR. Experimental K values were calculated by referring to at least two samples collected at different times [65]. As long as inflow bacterial populations are high, pathogenic bacteria removal follows a first-order relationship (Eq. 3):

$$\mathrm{K} = \mathrm{Q} \ln(\mathrm{Co} - \mathrm{Ci}) \qquad (3)$$

where Co and Ci are bacteria numbers (CFU/100 mL) in the outflow and inflow, respectively, K is areal first-order rate constant ($\mathrm{m.d}^{-1}$) and Q is HLR ($\mathrm{m.d}^{-1}$).

Results and discussion

Evaluation of water quality improvement

Next, the removal efficiency of bacterial contaminants and chemical pollutants was calculated using the following formula:

$$\text{Percent Removal}\,\% = \frac{A^{\circ} - A}{A^{\circ}} \times 100 \qquad (4)$$

where RE represents the removal percentage, A represents the influent concentration, and B represents the effluent concentration.

Physicochemical effluent

The parameters evaluated in the present study included DO, pH, and EC of the system influent Table 2. The difference between the pH and EC of the influent and effluent was not significant ($p > 0.09$, df = 3 and F value = 2.4) and varied between 6.04 and 7.25 mg.L^{-1} and 1.2 to 1.54 ds.m^{-1}, respectively. Whereas the mean DO of the influent (0.23 mg.L^{-1}) rapidly increased to 6.62 mg.L^{-1} for the effluents of the FWS. The temperature for the FWS influent ranged from 24.5 °C to 32.4 °C (28.4 ± 2.3), the *T. domingensis* and *C. papyrus* free water surface constructed wetlands (FWS–CWs) effluents ranged from 25 to 34.2 °C (27.8 ± 6.1). The effluents from the control FWS–CWs had a temperature ranging from 25.1 °C to 31.5 °C (28.3 ± 6.2).

Table 2 Physico-chemical characteristics of Bahr El-Baqar drain before treatment

Physcio-chemical parameters	Units	Mean ± SD
Temp	oC	28.4 ± 0.538
pH	–	7.20 ± 0.02
EC	dS/m	1.54 ± 0.16
TSS	mg/L	88.9 ± 1.5
CO3	mg/L	ND
HCO3	mg/L	146 ± 0.814
NH4+	mg/L	13.1 ± 1.1
BOD	mg/L	38 ± 1.3
Turbidity	NTU	40.8 ± 0.8
Major anions		
Cl –	mg/L	1100 ± 5.7
SO4 2 –	mg/L	346 ± 2.6
PO4 3 –	mg/L	1.3 ± 0.2
Major cations		
Ca 2+	mg/L	165 ± 29
Na+	mg/L	734 ± 11.3
K+	mg/L	30 ± 1.7
Mg 2+	mg/L	82 ± 12
Trace metals		
Cd	mg/L	0.045 ± 0.005
Cr	mg/L	0.61 ± 0.01
Cu	mg/L	0.72 ± 0.01
Fe	mg/L	3.2 ± 0.01
Pb	mg/L	0.29 ± 0.005
Mn	mg/L	0.28 ± 0.006
Zn	mg/L	0.57 ± 0.01

NH4^{+}–N reduction by *C. papyrus* and *T. domingensis*

In winter, the removal efficiencies of NH^{4+} in the units of *C. papyrus*, *T. domingensis* and the control were 81.3%, 91.9%, and 20.6%, respectively. In summer, the removal efficiency of NH^{4+} was 94.8%, 84.4%, and 40.1%, respectively, as shown in Fig. 3a, NH^{4+} may be decreased by several mechanisms, such as adsorption, plant and microbial activities, volatilization, and nitrification [18]. Mojiri et al. [38] stated that *T. domingensis*, as well as substrate zeolite could remove 86% of ammonia. Fu et al. [66] reported removal efficiency of NH^{4+} of 52% by CWs planted with *Acoruscalamus land* substrate with the zeolite. Plants enhanced ammonia reduction to 45% relative to the non-vegetated wetland most probably by enhancing nitrification via oxygen through the rhizosphere. The autotrophic bacteria in the biofilm adhering to the gravel may be responsible for ammonia elimination in the non-planted unit [67]. Hussien et al. [68] demonstrated that horizontally CWs of *C. papyrus* could remove up to 82% of ammonia. Ammonia was eliminated depending on plant uptake and water chemistry, such as a temperature and pH being within the range that promote microbial nitrification and denitrification processes [69]. A relatively high correlation was observed between initial NH^{4+}concentration and removal percentage of NH$^{4\,+}$ (p value > 0.01, df = 3 and F value = 2.1).

Turbidity reduction by *C. papyrus* and *T. domingensis*

The FWS influent had a turbidity of 19 to 50 NTU. As shown in Fig. 3b, the turbidity removal efficiency of the *C. papyrus*, *T. domingensis*, and the control wetlands were 94.1%, 89.2%, and 61.1%, respectively. The values are in harmony with the current FAO guidelines for wastewater reuse in irrigation, which stipulate that turbidity should not exceed, 5 NTU [70].

TSS reduction by *C. papyrus* and *T. domingensis*

TSS showed different patterns throughout the study. In summer, the TSS values of the influent ranged from 65 to 112 mg.L^{-1}, whereas in winter, a decrease was observed. The hydraulic retention time was designed for less than 4 days in each unit. The levels of effluent TSS for the FWS were in the range of 10–17 mg.L^{-1} and 22–38 mg.L^{-1}, respectively, with mean values of 13.5 and 30 mg.L^{-1}, respectively. The total removal efficiency for TSS

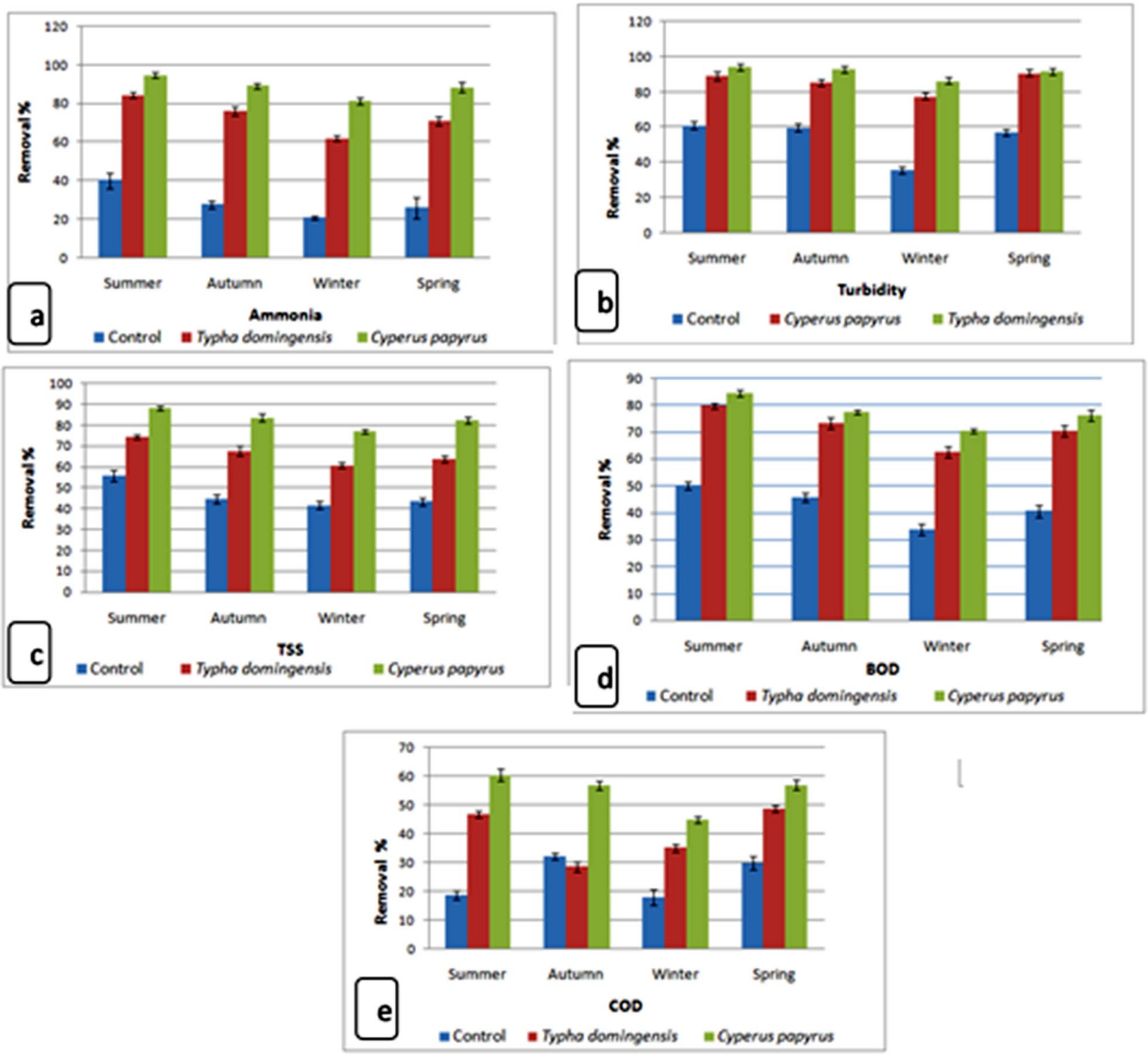

Fig. 3 Removal efficiencies of physicochemical (**a**) ammonia, (**b**) Turbidity, (**c**) TSS, (**d**) BOD and (**e**) COD pollutants versus seasonal variation using *Cyperus papyrus* and *Typha domingensis*

ranged from 77.04% to 88.7% and 60.6% to 74.1% after treatment by *C. papyrus* and the *T. domingensis* CWs system, respectively (Fig. 3c).The control FWS–CWs had a TSS ranging from 38 to 64 mg.L^{-1} and the total removal efficiency for TSS ranged from 41.6% to 55.8%. Physical methods such as sedimentation and filtration are used to remove TSS [71] accompanied by aerobic or anaerobic microbial degradation inside the substrate [61]. Carballeira et al. [72] reported that TSS removal was related to both physical settling, number of stem and absorption by plants [73]. Alayu and Leta [74] reported a removal efficiency of TSS of 90% for *C. alternifolius*. Mustapha [73] recorded removal efficiency of TSS of 85% for *T. latifolia*.

BOD reduction by *C. papyrus* and *T. domingensis*

Levels of BOD_5 varied from 34 to 40 mg.L^{-1} in the inlet wastewater. BOD in the effluent by the *T. domingensis* wetland was reduced by 79.9% to a range of 7–9 mg.L^{-1} in summer, whereas the lowest achieved in winter was in the range of 12–16 mg.L^{-1} with a removal efficiency of 62% (Fig. 3d). BOD was reduced by 84.4% in summer in the effluent by *C. Papyrus* and 70.2% in winter over 4 day detention time. The effluents in the high density *C. papyrus* treatment beds were under the recommended WHO discharge standards not more than 30 mg L^{-1} for BOD. The levels of BOD_5 at the inlet and outlet showed highly significant differences. Seasonal variations in performance of nutrient removal were observed. The removal efficiency of BOD_5within the effluent were relatively higher in summer and fall than in winter and spring [13, 75] reported that CWs planted with *C. papyrus* had an 85% BOD_5 removal efficiency. Most of the organic matter is removed in two ways, physical settling [76] and microbial activity, which involves organic material degradation by heterotrophic organisms that occur in the biofilm along the plant roots, stems and the surface of the substrate [24, 77]. The reduction of $BOD_5$was significantly different ($p < 0.05$, df = 3 and F value = 2.9) between the primary treatment and effluent treatment within wetlands. There is no significant difference between *T. domingensis* and *C. papyrus*, in their efficiency to reduce BOD ($p < 0.07$, df = 3 and F value = 2.3). The high removal efficiencies for BOD and nutrients in *papyrus*-based CWs indicates the system's ability to treat high oxygen demanding and nutrient rich wastewater effectively. This is in agreement with previous findings [69].

COD reduction by *C. papyrus* and *T. domingensis*

The municipal wastewater from Bahar El-Baqar drain (Egypt) was treated using a constructed wetland. The performances of *C. papyrus and T. domingensis* were assessed for COD removal. Following treatment by the *T. domingensis* unit, the COD content of the wastewater decreased from 129 mg.L^{-1} to 44 ± 0.05 mg.L^{-1} ($p < 0.01$ df = 3 and F value = 2.1) with a removal efficiency of 65.8% over 4 days, Fig. 3e. On the other hand, COD level in the *C. papyrus* unit were decreased from 129 mg.L^{-1} to 27 ± 0.01 mg.L^{-1} (79% efficiency, $p < 0.019$) over 4 days. A low rate of COD removal has been attributed to along retention time required for organic biodegradation by bacteria and the plant and animal detritus in the wetland [78]. The COD reduction obtained in this study was higher than that recorded by Li et al. [79] of a COD reduction of 40% by *T. angustifolia* Ebrahimi et al. [80] also reported that the CWs planted with *C. alternifolius* with sand and gravel can remove up to 72% of COD. Jegatheesan [81] showed that the CWs vegetated with *Phragmites australis* and a zeolite could remove 85% of COD. Liu et al. [82] concluded that the adsorption on substrates and biofilms and the metabolism of plants and microorganisms were essential factors for removal of COD in CWs.

Heavy metals removal from wastewater

The heavy metal concentrations in the influent and effluent samples collected from the wetlands are presented in Table 3. Cr, Cu, Zn, Cd, Pb, and Fe concentration variations in *C. papyrus* and *T. domingensis* effluent samples were significantly lower ($p < 0.05$) than in the control effluent. The total chromium concentration in the FWS–CWs influent was 0.61 (0.08) mg.L^{-1}, while the mean effluent Cr concentrations for the *C. papyrus, T. domingensis*, and unplanted control were 0.07 ± 0.057, 0.13 ± 0.01, and 0.42 ± 0.14, and 0.41 (0.384) mg.L^{-1}, respectively. The planted wetland units reduced Cr levels by 88.2% and 78.6% compared to the unplanted constructed wetland unit (31.1%). The total cadmium concentration in the influent was 0.046 ± 0.029 mg.L^{-1}, and the mean effluent Cd concentrations were 0.007 ± 0.01, 0.04 ± 0.03, and 0.033 ± 0.024 mg.L^{-1} for, *T. domingensis*, *C. papyrus* and unplanted control, respectively. The planted constructed wetland units reduced Cd levels by 91.3% and 84.7% compared to the unplanted constructed wetland unit (23.9%). The total zinc concentration in the influent was 0.58 ± 0.03 mg.L^{-1}, and the mean Zn concentrations in the *C. papyrus*, *T. domingensis*, and unplanted control effluents were 0.05 ± 0.01, 0.07 ± 0.006, and 0.32 ± 0.22 mg.L^{-1}, respectively. The planted wetland units reduced Zn levels by 91.3% and 87.9% compared to the unplanted constructed wetland unit (44.8%). The total copper concentration in the influent was 0.73 ± 0.11 mg.L^{-1}, whereas the mean effluent Cu concentrations for the *C. papyrus*, *T. domingensis*, and unplanted control were 0.086 ± 0.03, 0.11 ± 0.05, and 0.5 ± 0.14 mg.L^{-1}, respectively. The planted wetland units reduced Cu levels by 88.2% and 84.9% compared to the unplanted constructed wetland unit 45.2%. The total nickel concentration in the influent was 0.278 ± 0.6 mg.L^{-1}, and the mean effluent Ni concentrations were 0.015 ± 0.08, 0.47 ± 0.06, and 0.184 ± 0.3 mg.L^{-1} for *T. domingensis, C. papyrus* and unplanted control FWS–CWs, respectively. The planted wetland units reduced Ni levels by 94.6% and 83.3% compared to the unplanted constructed wetland unit (53.3%). The total

Table 3 Heavy metal concentrations in the constructed wetlands before and after treatments

Samples	Cr (mg/L)	Cu (mg/L)	Zn (mg/L)	Cd (mg/L)	Ni (mg/L)	Fe (mg/L)	Pb (mg/L)
Influent	0.61 ± 0.08	0.73 ± 0.11	0.58 ± 0.03	0.046 ± 0.02	0.278 ± 0.6	3.23 ± 0.6	0.3 ± 0.03
Typha domingensis	0.13 ± 0.01	0.11 ± 0.05	0.07 ± 0.01	0.004 ± 0.034	0.015 ± 0.08	1.04 ± 0.01	0.05 ± 0.03
Cyperus papyrus	0.07 ± 0.05	0.086 ± 0.03	0.05 ± 0.006	0.007 ± 0.001	0.047 ± 0.06	0.8 ± 0.06	0.06 ± 0.02
Control	0.42 ± 0.14	0.5 ± 0.14	0.32 ± 0.12	0.033 ± 0.023	0.184 ± 0.3	1.23 ± 0.014	0.18 ± 0.07
WHO guideline	0.05	1.00	3.00	0.003	0.20	0.10	0.01

Fe concentration in the influent was 3.23 ± 0.6 mg.L^{-1}, and the mean effluent Fe concentrations were 0.8 ± 0.06, 1.04 0.01, and 1.23 ± 0.014 mg.L^{-1} for the *C. papyrus*, *T. domingensis*, and unplanted control, respectively. The planted constructed wetland units were able to reduce Fe levels by 91.8% and 89.6% compared to the unplanted constructed wetland unit (46.1%). The total lead concentration in the influent was 0.3 ± 0.03 mg.L^{-1}, and the mean effluent pb concentrations were 0.05 ± 0.03, 0.06 ± 0.02, and 0.18 ± 0.07 mg.L^{-1} for the *C. papyrus*, *T. domingensis*, and unplanted control, respectively. The planted wetland units reduced Cr levels by 80.6% and 77.6% compared to the unplanted constructed wetland units (40%). *Typha domingensis* culture removed significantly more Cd and Ni than *C. papyrus* culture, whereas *C. papyrus culture* removed significantly more Cu, Pb, Cr, Fe, and Zn. A possible explanation would be that the selected plant species have varying heavy metal absorption efficiencies. These results were comparable to those reported by Tripathi [83] regarding the removal of heavy metals from wastewater using *P. australis* and *T. latifolia*. According to Tripathi [83] and Yadav et al. [10], the mechanisms of heavy metal removal in CWs involve multiple pathways: adsorption to the substrate, chemical precipitation, longer retention times, microbial interactions, and plant uptake. Compared to the control, the effluent from the free water surface (FWS)–CWs showed a significant reduction in heavy metal concentrations (non-vegetated CWs). However, the concentrations of heavy metals in the effluent varied significantly between the two FWS–CWs that were planted. Other studies have demonstrated the efficacy of constructed wetland systems for the removal of heavy metals [84], who reported a higher removal of Cr (90%) from wastewater by *C. papyrus.* According to Yadav et al. [10], *C. alternifolius* has achieved removal efficiencies of Cu (72.7%) and Cr (68.4%) in wetlands.

Heavy metal concentrations, translocation, and bioaccumulation in plant parts

According to Cheng et al. [85], bioaccumulation factors (BCF) of heavy metals in plants were calculated using the following formula:

$$\mathrm{BCF} = \frac{(\text{Metals content in plant})}{(\text{Metals concentration in influent})} \tag{5}$$

Here, the metal concentrations in the plant are in mg.kg^{-1} DW (dry weight), and the metal concentrations in the influent are calculated in mg.L^{-1}.

The translocation factor (TF) is the ratio of a heavy metal's concentration in the stem, leaves, or shoots of a plant to its concentration in the roots. Deng et al. [86] proposed the following equation to estimate the TF:

$$\mathrm{TF} = \frac{C_{\text{shoot}}}{C_{\text{Root}}} \tag{6}$$

where C_{shoots} and C_{Roots} are the metal concentrations in plant shoots and plant roots (mg.kg^{-1} DW), respectively.

The concentration of heavy metals in plant tissues, as well as translocation and bioaccumulation factors showed that a variation in the concentrations of heavy metals in the plant tissues was plant-specific (Table 4). For instance, the concentrations of heavy metals in *C. papyrus* and *T. domingensis* ranged from 2.2 to 5733 mg per kg and 0.2 to 2732 mg.kg^{-1}, respectively. The bioaccumulation factor (BCF) ranged from 0.7 to 1988 mg. kg^{-1} for *T. domingensis*, and from 0.3 to 955.5 mg. kg^{-1} for *C. papyrus.* The variation in concentration was greater in subterranean tissues than it was in aboveground tissues. For example, the bioaccumulation factor of the two plants' roots was greater than that of their stems and leaves. *T. domingensis* was able to translocate metals in the following order: Cd > Cr > Pb > Cu > Ni > Fe > Zn (ranging from 0.41 to 1.2). The translocation ability of *C. papyrus* was Cd > Pb > Zn > Cu > Cr > Fe (ranging from 0.2 to 1.73). The accumulation of heavy metals in *T. domingensis* and *C. papyrus* root tissues may indicate a metal tolerance strategy in the root cells. Similar results were reported for the bioaccumulation of heavy metals by other wetland plants [86].

Microbiological analysis

Removal of bacterial indicators of pollution

The mean concentrations of TC, fecal coliforms, and *FS* showed varied patterns during the period of the experiment. TC, FC, and FS values of the influent ranged from 4 to 4.7, 5.3–5.6, and 4–4.5 log units throughout the study, respectively. In summer, the highest removal efficiency of TC of 99.8% (2.07–2.2 log units), 99.5% of FC (2.3–2.6 log units) and 98.1% for FS (1.9–3.2 log units) were achieved in the effluent of *C. papyrus* unit, whereas *T. domingensis* had a lower removal efficiencies of TC, FC, and FS of 95.6%, 96.3% and 94% at retention time of 4 days, respectively. Non-vegetated control wetlands, the removal efficiencies of the non-vegetated control wetland of 78.5%, 79.5%, and 80.5% of TC, FC, and *FS*, respectively, were lower than those of wetlands with *C. papyrus* and *Typha domingensis.* The values showed a higher efficiencies of TC, FC, and FS removal in *C. papyrus* wetland ($p < 0.03$ df = 3 and F value = 3.3) than in the *T. domingensis* wetland ($p < 0.16$ df = 3 and F value = 2.2) CWs compared to unplanted control ($p < 0.36$ df = 2 and F value = 3.3). A plot of fitted regression line for all treatments showed a very good linear correlation with R^2

Table 4 Bioaccumulation and translocation factor of heavy metals in aquatic plants

Plant species	Tissues	Heavy metals concentrations (mg/kg)						
		Cd (II)	**Cu (II)**	**Zn (II)**	**Fe (II)**	**Pb (II)**	**Cr (VI)**	**Ni (II)**
Typha domingensis	Stem	2.1 ± 0.12	4.9 ± 0.005	220.5 ± 0.5	49 ± 0.1	4.3 ± 0.01	11.4 ± 0.005	10.366 ± 0.1
	Shoot	12.8 ± 0.14	10.8 ± 0.005	399.5 ± 0.09	496 ± 1.7	7.8 ± 0.3	21.1 ± 0.1	19.206 ± 0.2
	Root	30.6 ± 0.2	16.9 ± 0.005	329.7 ± 0.01	2832 ± 1.1	10.1 ± 0.006	45.1 ± 0.1	27.26 ± 0.01
TF		0.4183	0.63905	1.2117	0.8751	0.593	0.4678	0.7045
BCF	Stem	45.652	5.45831	380.172	17.232	4.567	17.1908	61.837
	Shoot	278.260	45.8948	688.79	174.437	9.233	31.818	114.451
	Root	665.217	6.46085	568.448	995.9807	5.4518	68.007	244.638
Cyperus papyrus	Stem	0.1 ± 0.005	1.7 ± 0.1	221.4 ± 0.5	223 ± 1.1	16.7 ± 0.2	6.5 ± 0.02	8.25 ± 0.001
	Shoot	15.8 ± 0.1	9.2 ± 0.1	393.4 ± 0.09	1553 ± 1.5	35.9 ± 0.3	8.6 ± 0.005	14.653 ± 0.007
	Root	9.1 ± 0.09	5.8 ± 0.1	419.2 ± 0.01	5653 ± 1.7	30 ± 0.2	17.3 ± 0.01	24.933 ± 0.01
TF		1.736	1.58	0.9384	0.274	1.1966	0.4971	1.6508
BCF	Stem	2.173	7.4633	381.72	78.42	8.2026	9.8018	29.68
	Shoot	286.457	12.03	678.27	546.17	17.632	14.098	52.68
	Root	328.26	18.87	722.75	1988.092	14.734	26.0871	88.71

Table 5 Bacteriological characteristics of Bahr El-Baqar drain before treatment

Bacteriological parameters	Units	Mean ± SD
Total coliforms	cfu/100 mL	$49\times10^3\pm0.5$
Fecal coliforms	cfu/100 mL	$38\times10^3\pm0.2$
Fecal streptococci	cfu/100 mL	$27\times10^3\pm0.4$
Escherichia coli	cfu/100 mL	$36\times10^3\pm1.5$
Pseudomonas aeruginosa	cfu/100 mL	146 ± 0.5
Staphylococcus aureus	cfu/100 mL	$21\times10^3\pm1.01$

above 0.84 (Table 5). The percentage removals of *E. coli*, *S. aureus*, and *P. aeruginosa* in the wetlands during the study are shown in Fig. 4a–e. Removal efficiencies ranging from 96.9% to 98.8% in the *C. papyrus* vegetated wetland and from 91% to 96.7% in the *T. domingensis* vegetated wetland and 40.9–71.6% in the non-vegetated control were recorded in the summer season. In the winter season, removal efficiencies ranging from 89.3% to 95.9%, 72.4–90.3% and 24.3–63.04% were recorded in the *C. papyrus, T. domingensis* and non-vegetated control wetlands, respectively. Statistical analysis revealed significant differences between *C. papyrus* and *Typha* wetlands ($p<0.04$, df = 3 and F value = 2.4). The lower removal of FC during the winter in the vegetated systems can be attributed to the lower metabolic activity and significant reductions in the populations of predator microbes [87]. The higher efficiency of *C. papyrus* in pathogen reduction might be due to some antibacterial properties of the rhizomes of this plant [22]. The removal of indicator organisms in wetlands is accomplished by a combination of mechanisms including physical processes (e.g., temperature, filtration and sedimentation), chemical processes (e.g., oxidation, UV solar radiation by sunlight, aggregate on substrates, and antimicrobial compounds excreted by macrophyte roots), as well as biological processes (e.g., die-off, competition for nutrients, predation by (protozoa and copepods) and attachment within biofilm [88]. Furthermore, there are hydraulic loading rates and long HRT [89]. The vegetated systems showed higher DO levels than the non-vegetated control due to the aeration and oxygen level around plant roots, which assist in pathogen elimination [90]. The electrostatic forces between bacteria cells and filter media depend on electrolytes of the surface of materials, such as metal ions [91]. Natural zeolites contain Cu^{2+}, Ag+, and Zn^{2+}, which have antibacterial activity against *P. aeruginose*, *S. aureus* and *E. coli* (Hrenovic et al. [40]), [50]. Effective removal of total coliform, fecal coliform, FS and *E. coli* (95.61–99.8%) from wastewater has been observed previously in wetland wastewater treatment systems elsewhere [75, 92]. About 99.9% removal of TC, 99.6% FC, 99.8% *P. aeruginosa*, and 99.9% *Streptococci* have been reported from a wetland planted with *C. papyrus* after a retention time of 3 days (Hussien et al. 2021). Removal efficiency of FC in surface flow wetlands with the plant *T. domingensis* greater than 90% have been recorded [93].

Screening of phytochemical compounds in the plant extracts

The GC–MS analysis of aqueous root extracts obtained from *C. papyrus* (L.) showed the presence of six major compounds identified according to their mass spectra

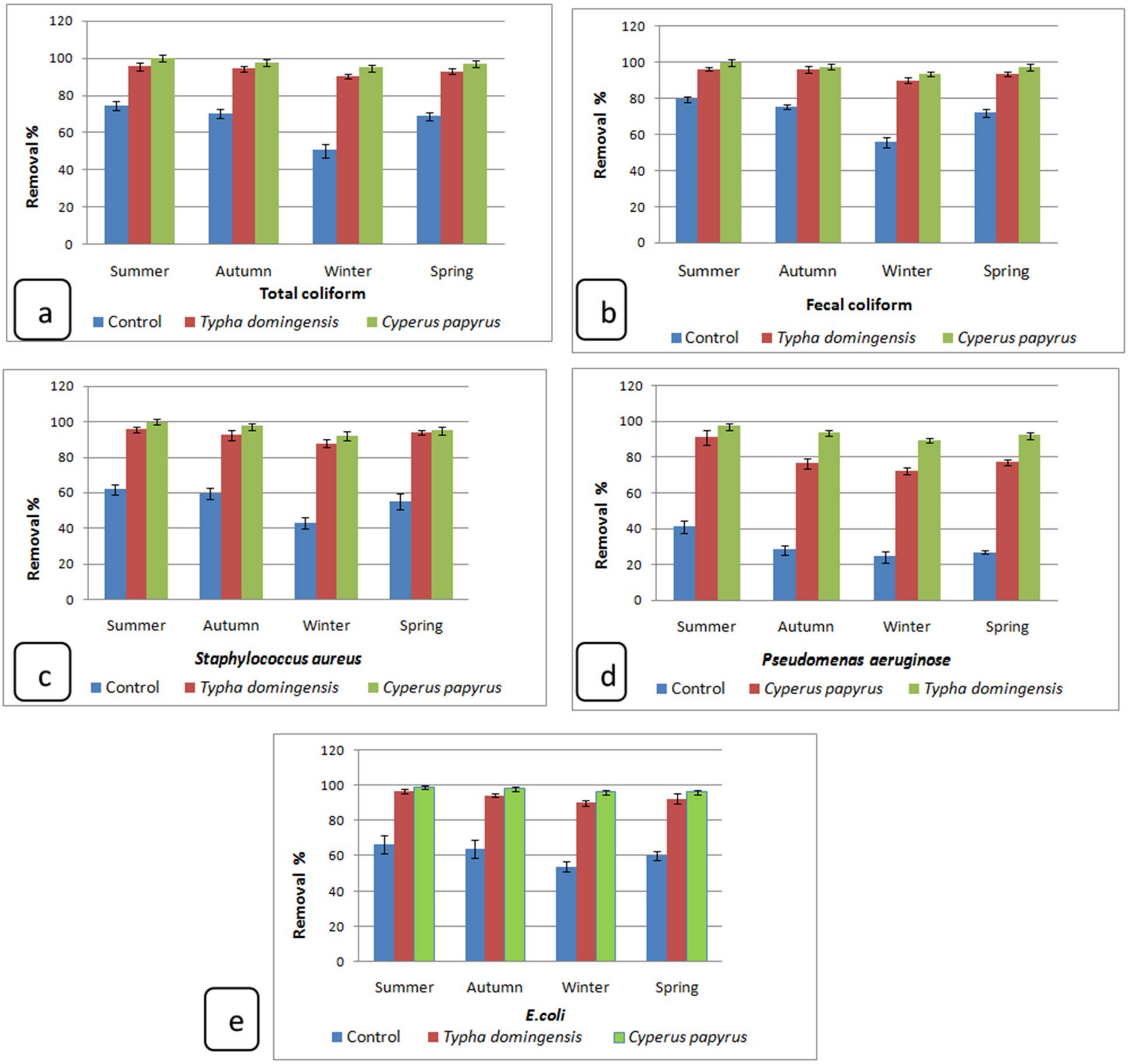

Fig. 4 Removal efficiencies of physicochemical (**a**) ammonia, (**b**) Turbidity, (**c**) TSS, (**d**) BOD and (**c**) COD pollutants versus seasonal variation using *Cyperus Papyrus* and *Typha domingensis*

(Additional file 1: Fig. S1 and Additional file 2: Table S1), which were found to be responsible for the antibacterial activity detected in the rhizosphere zone. The antibacterial activity was directly proportional to the decrease in bacterial pollution during the treatment process. Our findings are consistent with those of previous studies on the antibacterial activity of compounds against both Gram-positive and Gram-negative bacteria, including *E. coli*, *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, and *Listeria* [94]. In addition, the antioxidant activity of these compounds has been reported in other studies [95]. These findings indicate that plant extracts have a great potential as antimicrobial compounds against microorganisms, which can increase the efficiency of constructed wetland for the removal of pathogenic bacteria.

Description and parameters affecting reduction of *E. coli* using Box–Behnken design

According to Box–Behnken design, experimental runs of 27 were performed with four different independent parameters and their combined interaction effect was studied for *E. coli* removal (Additional file 2: Table S2) presents the results calculated from the experiments and predicted data found from the model. The empirical relation between the coded units of independent parameter and percentage of *E. coli* removal was expressed by a second-order polynomial equation (see in Additional file 2). Table 6 represents the analysis of variance (ANOVA) results of the fitted quadratic polynomial model for the removal of *E. coli* by *C. papyrus*. The model's significance is calculated by the regression coefficient (R^2), and adjusted R^2 values [96]. The model *F* value of removal percentage of *E. coli* was 370.36, which was significant. The model *p* values for *E. coli* removal showed statistical significance and there was only a 0.01% chance that an *F* value this large could occur due to noise. The *p* values of less than 0.0500 indicate that the model terms were significant. In this case, A^2, B^2, D^2, C^2, AB, AD, BC, BD and CD were significant model terms for *C. papyrus*. Values greater than 0.1 indicate the model terms are not significant. On the other hand the *F* value for *E. coli's* lack of fit was 3.13, indicating that the lack of fit is not significant in comparison with the pure error. There is a 14.15%, chance that a lack of fit *F* value this large could occur due to noise. The non-significant lack of fit indicated that the quadratic model was valid for the present study. Based on these results, a relationship between the removal percentage of *E. coli* and selected variables was expressed by the second-order polynomial equation. The regression equation obtained after the ANOVA showed that the correlation coefficient (R^2) was 0.997 for *E. coli* removal by *C. papyrus*, revealing that only 0.2% of the total variance could not be explained by the model. A high R^2 value close to 1 demonstrates good agreement between the calculated and observed results within the range of the experiment, and shows that a desirable and reasonable agreement with adjusted R^2 is necessary. The predicted R^2 of *C. papyrus* of 0.985 is in reasonable agreement with the adjusted R^2 of 0.994. These values showed that the developed model is good and the values of the independent variables are accurate. An adequate precision is a measure of the range in predicted response relative to its associated error. A ratio greater than four is desirable. The ratio of 92.7 of *C. papyrus* was high, indicating the reliability of the experimental data. Hence, the quadratic model can be used to navigate the design. The coefficient of variation (CV) % is the ratio of the standard error of the estimate to the mean value of the observed response (as a percentage) and it indicates reproducibility of the model. A model can typically be considered reproducible if its CV% is not more than 10% [97]. The CV% values of *C. papyrus* obtained in this study are relatively small with 0.8%, and indicated that the deviations between experimental and predicted values were low.

Table 6 ANOVA for the treatment of *E. coli* wastewater using *Cyperus papyrus*

Source	Sum of squares	df	Mean square	*F* value	*p* value	
Model	6071.29	14	433.66	370.36	< 0.0001	Significant
A—Are	24.41	1	24.41	20.85	0.0004	
B—*E. coli*	1623.65	1	1623.65	1386.66	< 0.0001	
C—rate	40.05	1	40.05	34.21	< 0.0001	
D—day	238.32	1	238.32	203.53	< 0.0001	
AB	148.18	1	148.18	126.55	< 0.0001	
AC	3.09	1	3.09	2.64	0.1265	
AD	273.14	1	273.14	233.27	< 0.0001	
BC	343.95	1	343.95	293.74	< 0.0001	
BD	480.64	1	480.64	410.48	< 0.0001	
CD	16.98	1	16.98	14.50	0.0019	
A^2	359.02	1	359.02	306.62	< 0.0001	
B^2	2231.74	1	2231.74	1905.99	< 0.0001	
C^2	25.67	1	25.67	21.93	0.0004	
D^2	604.31	1	604.31	516.10	< 0.0001	
Residual	16.39	14	1.17			
Lack of fit	14.53	10	1.45	3.13	0.1415	Not significant
Pure error	1.86	4	0.4647			
Cor total	6087.69	28				
Std. Dev.	1.08				R^2	0.9973
Mean	84.23				Adjusted R^2	0.9946
C.V. %	0.8				Predicted R^2	0.9858
					Adeq precision	92.294

The average differences between the predicted and experimental values of *E. coli* removal were less than 0.1, which indicated that most of the data variation was explained by the regression model (Fig. 5a–c).

Interpretation of variable interaction on MG removal

Three-dimensional response surfaces (3D) plots were produced using Design Expert version (13) program. The plots highlight the relationship between independent variables (X1, X2, X3 and X4) and the response (*E. coli* removal), as presented in Fig. 7. The concentration of *E. coli* in the wastewater entering the beds was about log 5.1 cfu.100 mL^{-1}. The concentration of *E. coli* along the beds was the lowest at the end of the bed, with about 99% removal efficiency. Improved *E. coli* removal rates were achieved by decreasing the contact time and consequently increasing contact time (Fig. 6). The average reduction of *E. coli* concentration was 65.6% at bed

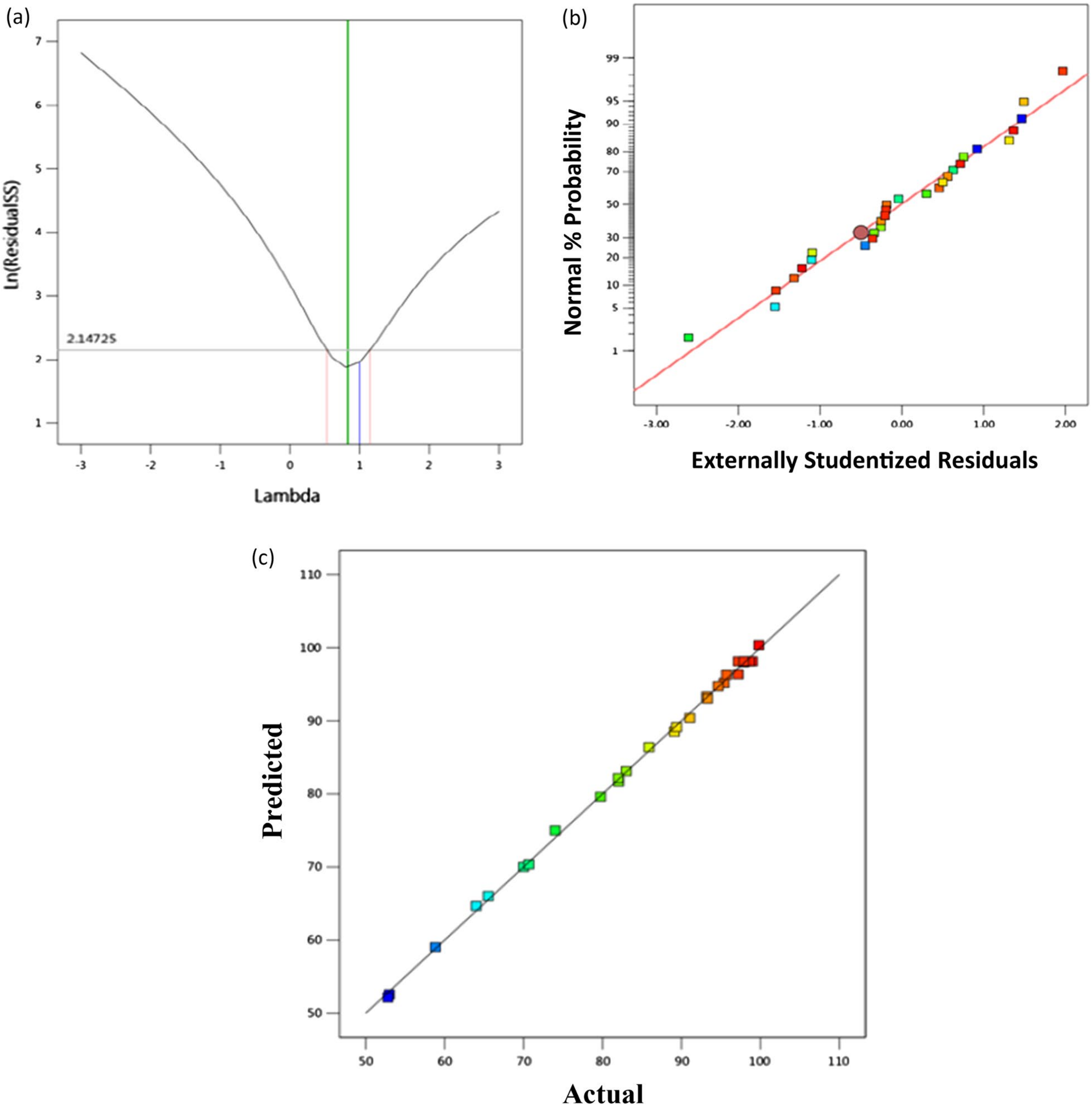

Fig. 5 Box–Cox plot of model transformation (**a**), the studentized residual and normal % probability plot (**b**), and (**c**) the actual and predicted values for the reduction of *E. coli* by *Cyperus papyrus*

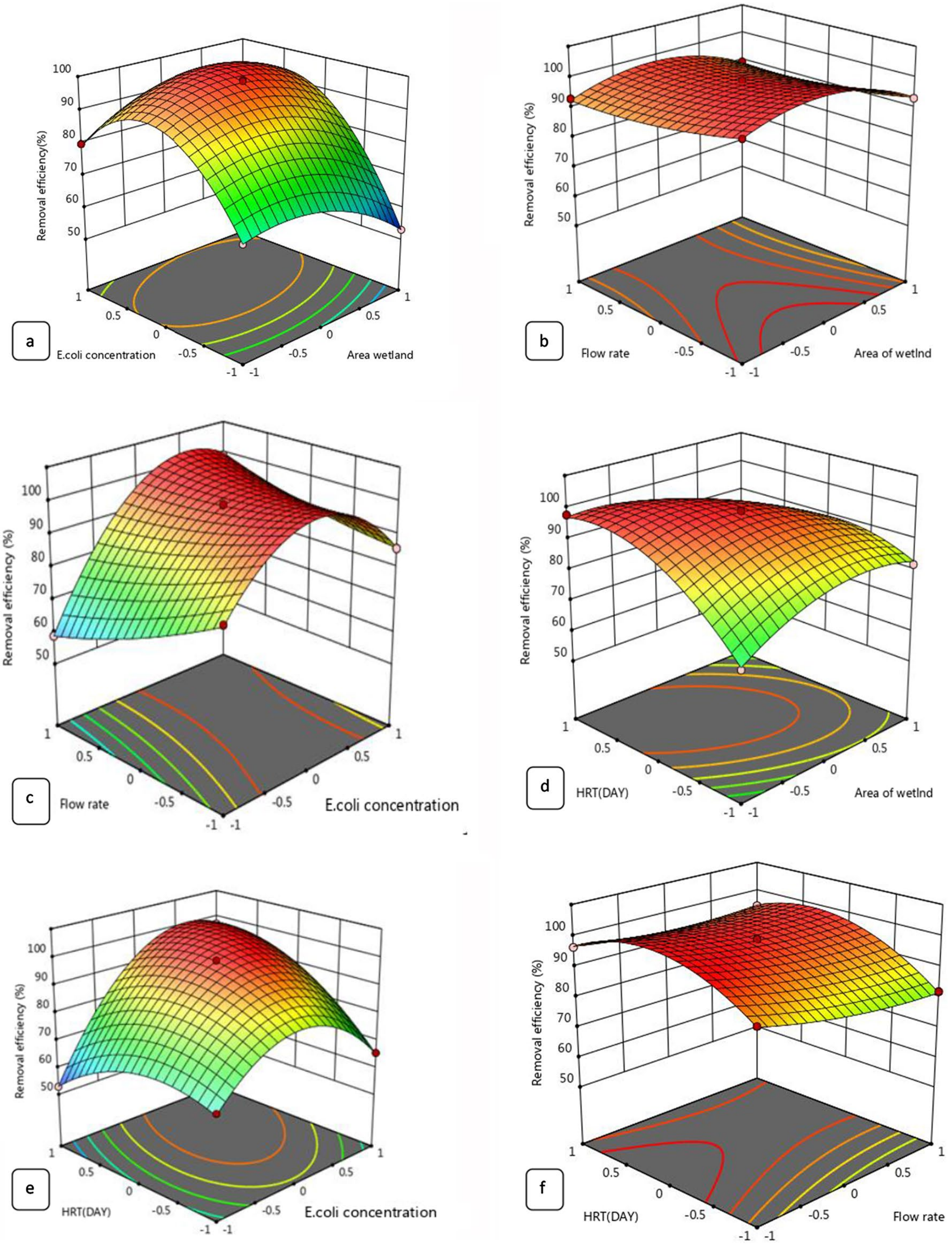

Fig. 6 **a** 3D response surface plot of interactions of area of wetland and initial *E. coli* concentration for E. coli reduction. **b** 3D response surface plot of interactions of flow rate and area of wetland for *E. coli* reduction. **c** 3D response surface plot of interactions of flow rate and initial *E. coli* concentration for *E. coli* reduction. **d** 3D response surface plot of interactions of HRT (day) and area of wetland for *E. coli* reduction. **e** 3D response surface plot of interactions of HRT (day) and initial *E. coli* concentration for *E. coli* reduction. **f** 3D response surface plot of interactions of HRT (day) and flow rate for *E. coli* reduction

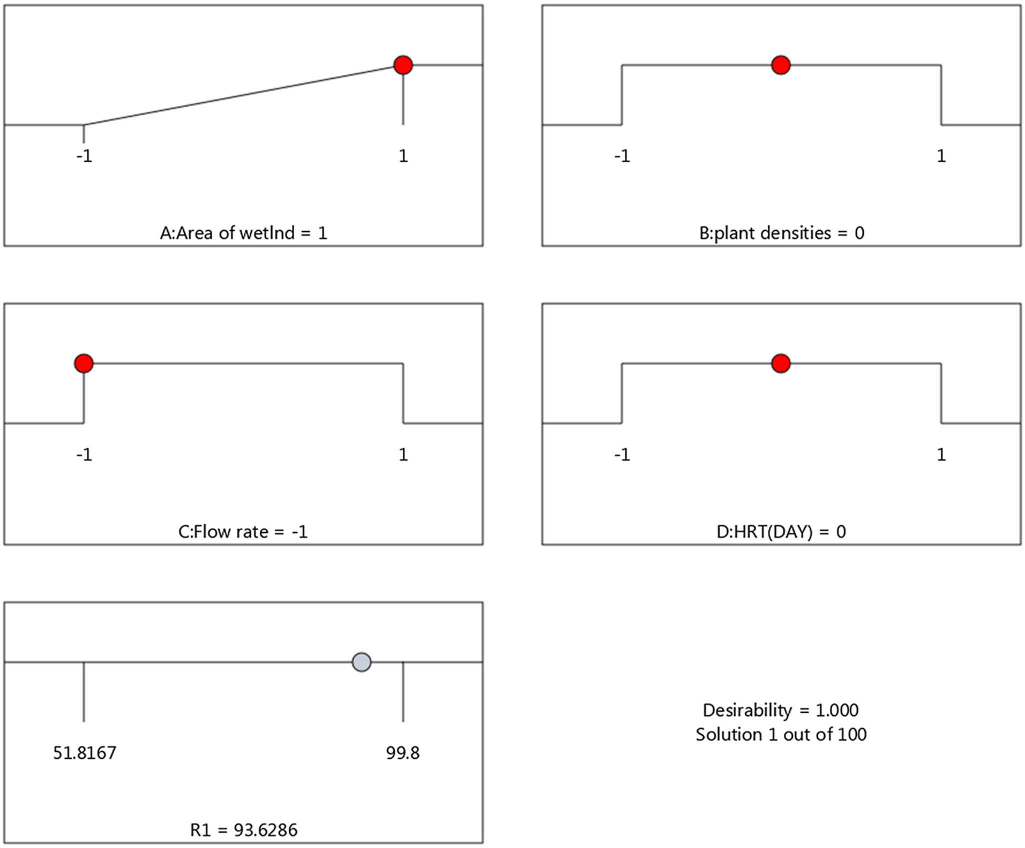

Fig. 7 Desirability ramps for numerical optimization of *E. coli* reduction by plant *Cyperus papyrus*

length of 2 m, flow rate of 40 L, initial *E. coli* concentration of 100 cfu.100 mL^{-1} and contact time of 1.8 days with a residual value of 40 cfu.100 mL^{-1}.The reduction reached 85.1% at bed length (2 m), flow rate of 40 L, initial *E. coli* concentration of 100 cfu.100 mL^{-1} and contact time of 2.2 days with a residual value of 15 cfu.100 mL^{-1}. The reduction finally increased to 95.1% at bed length of 2 m^2, flow rate of 40 L, initial *E. coli* concentration of 100 cfu.100 mL^{-1}, and contact time of 3.7 days. Shingare et al. [98] demonstrated a decrease in *E. coli* from 60.9% to 80.3% and 95.3% to 98.7%, respectively, when contact time was increased from 1 to 4 days, indicating that wetland treatment for these factors may require a long residence time to be effective. Figure 5 illustrates the effect of bed length and different types of flow rates on *E. coli* reduction. The high bacterial pollution load of the medium sewage had more adverse effect on the ability of the wetland to reduce the *E. coli* level. The average reduction of *E. coli* reached 58.82% at bed length of 2 m^2, flow rate of 60 L, initial *E. coli* concentration of 250 cfu.100 mL^{-1} and contact time of 2 days and also, the average reduction of *E. coli* reached 53.82% at bed length of 3 m^2, flow rate of 40 L, initial *E. coli* concentration of 250 cfu.100 mL^{-1} and contact time of 2 days. In contrast, the average reduction of *E. coli* reached 98.8% at bed length of 2 m^2, flow rate of 40 L, initial *E. coli* concentration of 100 cfu.100 mL^{-1}, and contact time of 2 days. Travaini-Lima and Sipaúba-Tavares [99] demonstrated that a high *E. coli* level in raw sewage contributed to the poor performance of a wetland to reduce *E. coli* level. This might be because domestic wastewater contains a lot of nutrients, which boosts the survival of microorganisms. In addition, the bacterial removal mechanisms involve biological processes, such as ingestion by protozoa, release of antibiotics by plant roots, and natural die-offs, as well as physical processes including filtration, sedimentation, adsorption, and die-off caused by toxins. It is also thought that plant coverage and hydraulic retention time are important factors in the effectiveness of the *E. coli* reduction process [67]. Since the majority of enteric bacteria, such as *E. coli*, is facultative or obligate anaerobes, the presence of oxygen inhibits their ability to develop. Moreover, the presence of oxygen makes it easier for bacterial predators including viruses, lytic bacteria, and protozoans to survive [61]. According to Stevik et al. [100], electrostatic charges between cells and particle surfaces lead to the adherence of bacterial cells to the surface of porous media, and natural zeolite has a high cation exchange capacity and cation selectivity [101].

Optimization of parameters affecting reduction of *E. coli* using desirability function

Factors affecting the percentage reduction of *E. coli* were optimized using the desirability function. The desirability function allows determination of the best variables influencing a response. The values vary from 0 (outside the range) to 1 (on target). Under the "Numerical optimization" option in the Design-Expert 13 program, the objective fields for response contain five possible values: none, maximum, minimum, target, and within range. In the current work, a "numerical optimization" of the software was chosen to find a specific point that maximizes the desirability function (% reduction). Figure 7 illustrates the results of optimization and the ramp desirability of solution 1 that was produced through numerical optimization (length bed = 3 m, initial *E. coli* concentration 150 cfu.100 mL^{-1}, flow rate (20 L) and retention time 2 (day).The removal efficiency of *E. coli* under these optimized operating conditions was 93%. Finally, it was shown that the Box–Behnken design and desirability functions could be employed to improve the elimination parameters for the removal of *E. coli* by *C. papyrus*.

Antibacterial activity of *C. papyrus* (L.) extracts

The study aimed to assess the ability of aqueous extracts obtained from the roots of *C. papyrus* (L.) to inhibit the growth of three potentially pathogenic bacteria isolated from wastewater influent. The results, presented in Table 7, show the reduction in bacterial growth measured in terms of inhibition zones. The aqueous root extract was found to be more effective in inhibiting the growth of *S. aureus* compared to *E. coli* and *P. aeruginosa*. The diameter of the inhibition zones ranged from 12.0 ± 0.8 mm to 21.3 ± 0.9 mm for *S. aureus*, and the minimum inhibitory concentration (MIC) was determined to be 100 mg.mL^{-1}. For *E. coli* and *P. aeruginosa*, the inhibition zones ranged between 12.4 ± 0.9 mm to 20.5 ± 0.7 mm and 17.0 ± 0.9 mm to 18.0.0 ± 0.7 mm, respectively, with MIC values of 150 mg.mL^{-1} and 300 mg.mL^{-1} for the same bacterial species in the same order. These findings suggest that the aqueous root extract of *C. papyrus* (L.) may have potential as an antibacterial agent against pathogenic bacteria commonly found in wastewater influent. In this investigation, it was found that aqueous root extract had a significant antibacterial effect, comparable to a synthetic standard antibiotic, amoxycillin. Other studies, such as Hassanein et al. [102] and Taha et al. [103], have reported similar findings. The mechanism behind this activity is believed to be the release of chemical compounds from the roots, which are toxic to pathogens. In addition, the release of these root exudates is specific to the pathogen and plant species, as indicated by el Zahar Haichar et al. [104].

Properties of natural zeolite

According to Rhodes [105], natural zeolite possesses several physical properties that make it a desirable material. It does not produce dust or cloud liquids, which can be attributed to the absence of clay. It is also non-toxic and non-flammable, as well as hard and resistant to wear. Physical zeolite analysis is presented in Table 8. SiO_2 made up the majority of zeolite's chemical composition, followed by Al_2O_3 (11.09%), Fe_2O_3 (4.03%), CaO (3.58%), K_2O (3.2%), and Na_2O (0.78%). This study revealed that natural zeolite contained sodium, potassium, and calcium ions that could be exchanged. Moreover, Si/Al ratios of 5.6 are typical for clinoptilolite [106], and the corresponding (Na + K)/Ca ratio is 1.05. As shown in Fig. 8, the XRD analysis confirmed that the natural zeolite contains approximately 70% clinoptilolite. Zeolite offers a formidable advantage compared with other

Table 7 Antibacterial activity of *Cyperus papyrus*

Concentrations (mg/mL)	Zones of inhibition (mm)								
	Escherichia coli			*Pseudomonas aeruginosa*			*Staphylococcus aureus*		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
150	0.0	0.0	0.0	0.0	0.0	0.0	11.0	13.0	12.0 ± 0.8
300	12.0	13.0	12.3 ± 0.9	0.0	0.0	0.0	13.0	14.0	13.3 ± 0.7
400	17.0	19.0	18.3 ± 0.8	16.0	18.0	17.0 ± 0.9	17.0	19.0	18.3 ± 0.6
500	19.0	21.0	20.3 ± 0.7	17.0	19.0	18.0 ± 0.9	21.0	22.0	21.3 ± 0.9
Negative control[a]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Positive control[b]	22.0	23.0	22.3	18.0	20.0	18.6	23.0	24.0	23.3

Values are mean zones of inhibition (mm) SD of 3 replicate tests; [a]sterile distilled water; [b]standard antibiotic (Amoxycillin/1 mg/mL)

Table 8 Chemical composition and physical properties of natural zeolite

Chemical composition	Percentage (%)	Physical properties
SiO_2	66.96	Appearance porosity (%), 31.5
Al_2O_3	12.04667	Appearance density (g/cm^3), 2.3
Na_2O	0.8	Average pore diameter (μm) (0.01–0.2)
MgO	0.7	Humidity (%) 6.7
CaO	3.4	Color-(Grayish-white)
Fe_2O_3	3.22	Surface Area—m^2/gm 89.9
K_2O	2.83	Particle size—6 mm
TiO_2	0.4	Moisture—%(6.78)
LOI	10	Cation Exchange Capacity (CEC)—MEq/gm (1.6–2.0)
		pH 7.5

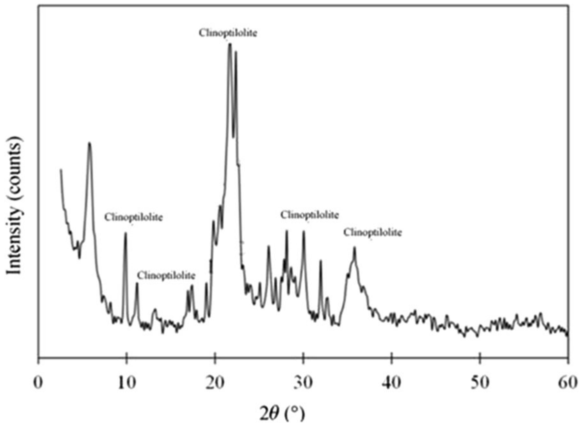

Fig. 8 X-ray diffraction pattern of natural zeolite

adsorbents because of its tunable physicochemical properties and the possibility of being regenerated without significant loss of performance at relatively low temperatures [107]. Zeolite contains negatively charged pores that are balanced by positively charged ions (cations), such as Na^+, K^+, Ca^{2+}, Mg^{2+} on the pore surfaces. These cations are weakly bonded to the aluminosilicate structure, allowing for exchange with certain cations in solutions. Because of this unique structure, Zeolites have a high cation-exchange capacity, which can be beneficial in removing NH^{4+} from wastewaters [108]. Treatment and modification greatly increase the average pore diameter, total pore volume and surface area of the original zeolite due to increasing Si/Al ratio [109].

First-order removal rate constants

Table 9 displays the average *k* values for various indicators and types of constructed wetland macrophytes. Total coliform, fecal coliforms, *fecal streptococci*, and *E. coli* values are in good agreement with those reported by Kadlec and Knight [71], who reported average *k* values for removal of FC in FWS of 0.205 m.d^{-1} and 0.260 m. d^{-1}, respectively.

Antibiotics removal

As shown in Fig. 9, the antibiotic removal efficiency of the wetlands differs between roxithromycin and levofloxacin. Furthermore, the rate of levofloxacin removal in the vegetated units was 4–120 ng.L^{-1} with a removal efficiency of 91.5–80.3% and 89.3–76.25% during the summer and winter seasons for *C. papyrus* and *T. domingensis,* respectively, although significant antibiotic removal was observed in the absence of plants (20%). A similar variation in removal efficiency for roxithromycin was observed compared to levofloxacin, the influent concentration of roxithromycin was reduced from 4700 ng.L^{-1} to 788 ng.L^{-1} and 210 ng.L^{-1}, along with a 93.8–83.2%, and 91.4–78.7% reduction in removal efficiency of during, the winter and summer seasons for *C. papyrus* and *T. domingensis,* respectively. Our results are consistent with those of [61] reported that the ciprofloxacin was only eliminated by a free-water surface (FWS) planted with *Juncusacutus* (93.9%), and Ibuprofen was removed in FW systems planted with *Phragmites australis* (80%). In this study, nutrients (NH^{4+} and COD) positively correlated with the occurrence of the antibiotic groups. Similarly, Sabri et al. [110] discovered the effect between antibiotics and ARG concentrations with physicochemical parameters and nutrients. Furthermore, various mechanisms could be incorporated into the depuration of pharmaceuticals in a CWs, including chemical (breakdown of the contaminants) and biological (plant-assisted rhizoremediation, oxygen, and exudates not the rhizosphere) [111]. Moreover, plant uptake significantly affects Ciprofloxacin and sulfamethoxazole removal from wastewater. Ciprofloxacin conversion to ofloxacin and enrofloxacin for plant uptake has been observed in a study [112]. Hijosa-Valsero et al. [113] noted that the planted subsurface system with *T. angustifolia* and *P. australis* was more efficient in the removal of ampicillin and Erythromycin than its un-planted system. Chen et al. [30] demonstrated that the seven antibiotics and all 18 target genes could be reduced by the mesocosm-scale CWs planted *Cyperus alternifolius* and four substrates (oyster shell, medical stone, the zeolite, and ceramic had bigger specific surface areas) and found the aqueous removal rates of the total antibiotics ranged from 17.9% to 98.5%. Temperature greatly influences the rate of biological and chemical processes in CWs, including nitrification, denitrification, and BOD_5 decomposition. High temperatures promote the ET rate, which is directly associated with the removal of organic contaminants [114]. The removal of

Table 9 Average *k* values for removal of pathogenic bacteria in FWS

Pathogenic bacteria in wetland	Inflow (CFU/100 mL)	Outflow (CFU/100 mL)	k (m d − 1)
Total coliforms			
Cyperus papyrus	49×10^3	1290	0.21535
Typha domingensis		3000	0.214652
Fecal coliforms			
Cyperus papyrus	38×10^3	790	0.202977
Typha domingensis		1770	0.20716
Fecal streptococci			
Cyperus papyrus	27×10^3	1200	0.202977
Typha domingensis		3100	0.201391
Pseudomonas aeruginosa			
Cyperus papyrus	146	9	0.097776
Typha domingensis		27	0.09476
Staphylococcus aureus			
Cyperus papyrus	20×10^3	630	0.198143
Typha domingensis		1560	0.19725
E. coli			
Cyperus papyrus	36×10^3	1111	0.212452
Typha domingensis		2480	0.211661

antibiotics in CWs is affected by the type of plant, substrate, and microorganisms and involves biodegradation and substrate adsorption [115], and is directly proportional to plant growth [116]. Furthermore, Alsubih et al. [117] investigated constructed wetland treatment efficiency in the removal of antibiotics, and they found that CIP increased significantly during the summer season. Likewise, Chen et al. [30] have reported 75–99% ibuprofen and 95–100% removal efficiency in the horizontal sub-surface flow of CWs. Ma et al. [115] also reported a removal efficiency of 57–80% for sulfamethoxazole in treating Monsalves et al. [118] studied antibiotic-resistant gene (ARG) reduction in CWs that used zeolite as a support medium, they determined values of 95.3% for the sul and tet genes. Moreover, these results can be explained by the porous morphology and larger surface area of zeolite.

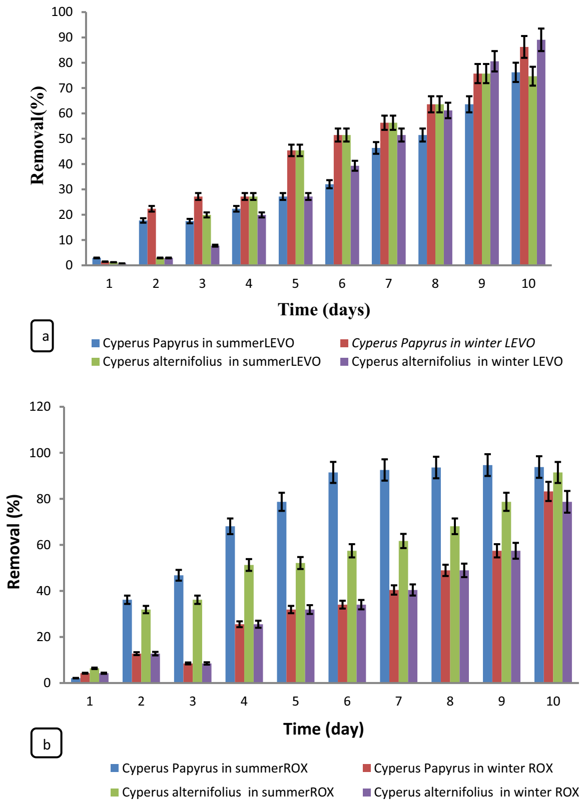

Fig. 9 Comparison of effluent concentration of antibiotics **a** LEVO and **b** ROX versus time

Bacterial composition analysis

Krona was used to examine the taxonomic composition based on the Ribosomal Database Project. Similar patterns of bacterial population composition at the phylum level were observed between influent and effluent, with Firmicutes and Proteobacteria accounting for over 42% and 35%, respectively (Fig. 10). At the genus level, however, the microbial communities of the two samples were clearly distinct. Streptococcus comprised nearly half of the influent sample, followed by *Staphylococcus* and Bacteroides. In contrast, *Pseudomonas aeruginosa* accounted for nearly half of the bacteria in the effluent, Fig. 10,

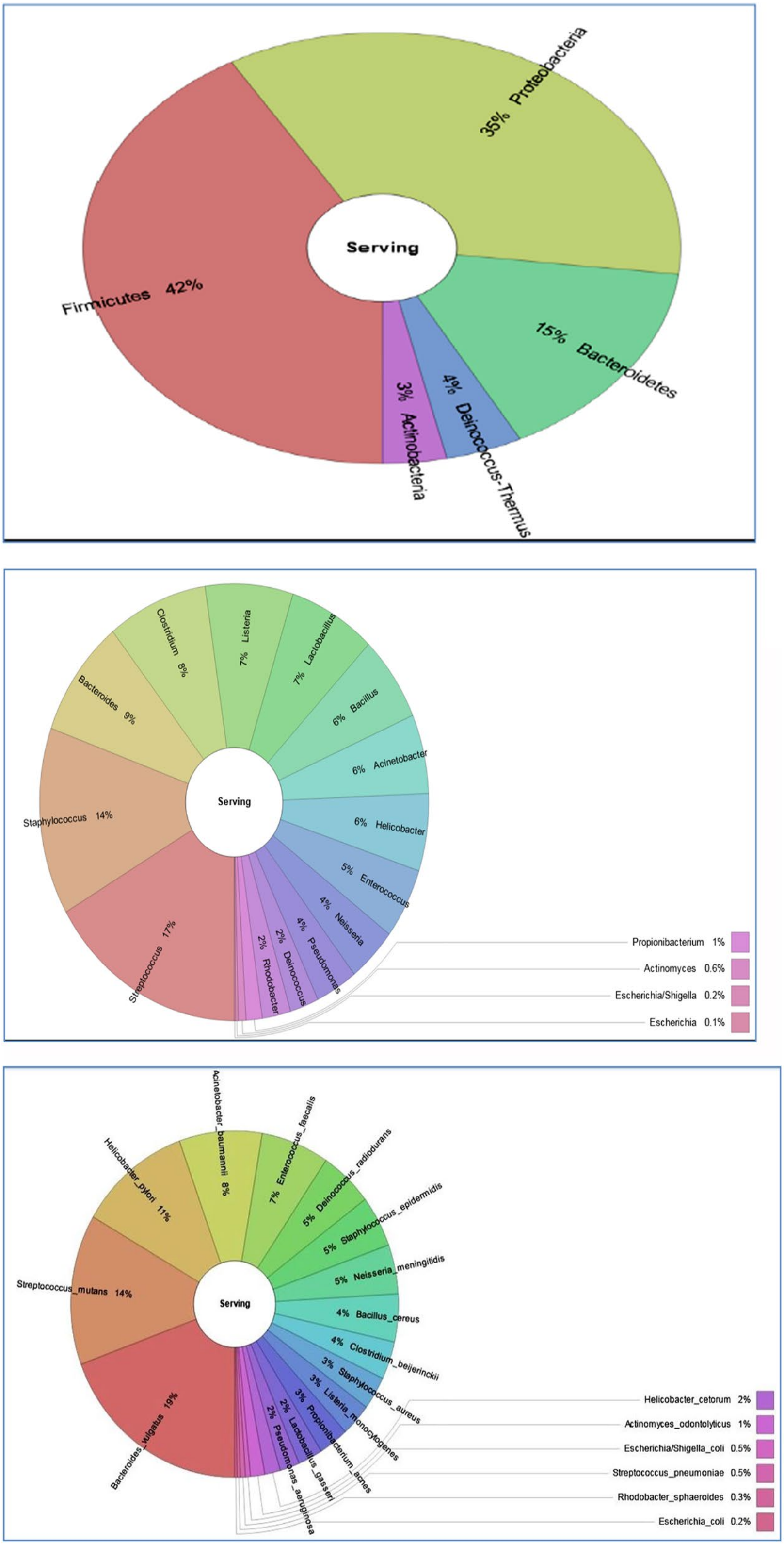

Fig. 10 Hierarchical tree representing the dominant bacterial phyla detected in influent

followed by *Escherichia coli* and *Clostridium sp*. Furthermore, at the species level, *Bacteroidesvulgatus* (19%), *Streptococcusmutans* (14%), *Helicobacterpylori* (11%), and *Acinetobacter* (8%) were found to be the most abundant species in influent, whereas *Pseudomonas aeruginosa* (32% and 23%), *Acinetobacter baumannii* (26% and 13%) and Helicobacterpylori (22% and 18%) dominated in the effluent *C. papyrus* and *T. domingensis,* respectively, microbial community. In contrast, the abundance of bacterial genera, including *Rhodobacter sphaeroides, Acinetobacter baumannii, Propioni bacteriumacnes*, and *Deinococcus radiodurans,* increased following treatment. In accordance with the findings. According to Yao et al. [50], reported that hospital wastewater had comparatively high abundances of opportunistic bacteria such *Acinetobacter* (3.59%), *Klebsiella* (2.07%), *Aeromonas* (8.84%), and *Pseudomonas* (7.60%).

Conclusion

The constructed wetland vegetated with *C. papyrus* and *T. domingensis* were continuously more efficient in the removal of fecal indicator bacteria, BOD, and TSS from wastewater than the non-vegetated control. Results showed that limestone and zeolite substrates in combination with *C. papyrus* effectively reduce the concentration of all bacterial pathogens and physicochemical parameters, pH, EC turbidity, TSS, BOD, COD and ammonia in the wastewater. *C. papyrus* showed markedly higher removal efficiencies for heavy metals than *T. domingensis.* Yearly average removal efficiencies for BOD_5, TSS, COD, total coliforms, FC, and ammonia were at 80.69%, 69.87%, 98.08%, 95.61%, 69.69% and 50.0% for *C. papyrus* and 75.39%, 64.78%, 96.02%, 93.74%, 70.70% and 49.38% for *T. domingensis,* respectively. The *C. papyrus*-vegetated free water surface (FWS) constructed wetland was continuously effective at removing heavy metals and bacteria indicators from wastewater for a period of 1 year. The average trace metal removal efficiency of the systems was approximately 73% for iron, 75% for copper, 64% for zinc, and 51% for lead. The removal rate constants of this study indicated that *C. papyrus* and zeolite media had different absorption efficiencies for NH^{4+} and BOD, indicating that the removal efficiencies of NH^{4+} and BOD were significantly influenced by the selection of substrates. The first-order removal kinetics obtained for BOD, COD, TSS and NH^{4+}, revealed a consistent relationship with the positive effect of vegetation on the removal of all pollutants, as demonstrated by previous research. Therefore, vegetation is an essential element for enhancing the FWS performance. The high contribution of *C. papyrus* to the removal of total coliform, fecal coliform, and physicochemical parameters suggests that this macrophyte should be utilized in wetland technology for the treatment of domestic wastewater. *Cyperus papyrus* extracts can be used as effective antibacterial agents, Furthermore, the treated wastewater can be reused for agricultural purposes without posing any health risks to farmers, who are usually associated with irrigation of polluted wastewater. Constructed Wetlands incorporate *C. papyrus* have the essential qualifications credentials, to represent a feasible solution for wastewater treatment in hot and arid climates.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-023-00748-x.

Additional file 1: Figure S1. Box–Cox plot of model transformation (a), the studentized residual and normal % probability plot (b), the actual and predicted values for the reduction of *E.coli* by *Cyperus papyrus*.

Additional file 2: Table S1. Phytochemical compounds detected by GC–MS analysis in root extracts from *Cyperus papyrus* (L.). **Table S2**. Box Behnken design-based experimental conditions for the treatment of *E.coli* wastewater using *Cyperus paperus*.

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Author contributions

MTM: provided conception and design of research; acquisition, analysis, and interpretation of data; drafted the manuscript, substantively revised it processed creation of new software used in the research, and revised the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are of our own work and it is our pleasure to be available publically.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The author consent to publishing the manuscript in environmental sciences Europe.

Competing interests

The author declares that they have no competing interests.

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