International Journal of Design & Nature and Ecodynamics
Vol. 21, No. 3, March, 2026, pp. 715-726
Journal homepage: http://iieta.org/journals/ijdne

Comparative Effectiveness of Common Reed *Phragmites australis* and *Juncus effusus* in Hybrid Constructed Wetlands Toward the Integrated Treatment of Pathogenic Microorganisms and Heavy Metal Contaminants in Wastewater

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https://doi.org/10.18280/ijdne.210310

Received: 11 January 2026
Revised: 4 March 2026
Accepted: 24 March 2026
Available online: 31 March 2026

Keywords:
surface-flow created wetland, Phragmites australis, Juncus effusus, heavy metals, wastewater treatment, pathogenic bacteria, phytoremediation

ABSTRACT

In addition to its use for agricultural purposes, the presence of heavy metals and other microbiological pollutants in wastewater is a significant problem and poses a serious threat to both the environment and human health. This study aimed to evaluate the relative effectiveness of a surface-flow wetland system, utilizing zeolite/limestone, common reed (*Phragmites australis*), and *Juncus effusus*, in removing trace contaminants, concentrating on lowering bacterial and heavy metal pollution in wastewater over a ten-month period. The inlet water concentrations of chromium, copper, zinc, and cadmium were 0.91 mg/L, 0.88 mg/L, 0.63 mg/L, and 0.034 mg/L, respectively. These contaminants were removed using the surface flow constructed wetlands (CWs). *Phragmites australis* showed a removal efficiency of 88.2% for Cr, 75.0% for Cu, 87.3% for Zn, and 58.8% for Cd, while *Juncus effusus* showed a removal efficiency of 78.6% for Cr, 91.3% for Cu, 90.5% for Zn, and 20.6% for Cd. An increased accumulation of many heavy metals in plant roots has been observed, and a greater bioaccumulation and translocation efficiency was observed for *Phragmites australis*. Removal of the pathogen was another significant factor. In summer season, *P. australis* wetlands removed 99.7% total coliforms (TC), 98.9% fecal coliforms (FC), and 97.9% fecal streptococci (FS), corresponding to a 2-3 log reduction. *J. effusus* wetlands removed slightly lower concentrations of 94-96%, whereas non-vegetated controls removed less than 80%. Both hydraulic retention time (HRT) and flow rate are important parameters for improving the quality of *E. coli* removal, according to optimization and modification studies using response surface methodology (RSM). Under ideal conditions, removal efficiency reached 99%. These results led to the conclusion that artificial wetlands containing common reed (*P. australis*) are highly efficient at removing pathogenic bacteria and heavy metals, and therefore have significant potential for use now and in the future as a sustainable and economical wastewater treatment technology in areas contaminated with heavy metals.

1. INTRODUCTION

Water reuse has become a widely recognized strategy for sustainable water management, aiming to mitigate water scarcity problems, particularly in arid and semi-arid regions [1, 2]. However, the presence of harmful microorganisms and traces of heavy metals makes the safe reuse of wastewater challenging. Furthermore, aquatic environments are contaminated with heavy metals such as chromium (Cr), copper (Cu), cadmium (Cd), zinc (Zn), and lead (Pb) due to the accumulation of industrial waste, agricultural runoff, and domestic sewage [3, 4]. These heavy metals are characterized by their ability to accumulate and bioaccumulate in soil and crops, and they are considered resistant and not biodegradable, thus creating long-term ecological and health hazards to humans [5, 6]. Conversely, wastewater is frequently replete with pathogenic bacteria like *Escherichia coli*, fecal coliforms (FC), and fecal streptococci (FS), which are major impediments to agricultural reuse in agricultural applications due to their potential linkage with waterborne diseases [7].

Many traditional, older heavy metal removal processing technologies consume large amounts of energy and require many highly qualified personnel to set them up and operate them [8, 9]. In addition, the problem of the formation of secondary sludge also exists. For the removal of pathogens, for example, the use of chemical disinfectants has the drawback of high operational costs and the formation of harmful by-products. This has resulted in the use of nature-based solutions, namely, constructed wetlands (CWs), which are associated with low operational requirements, simplicity, and sustainability [10, 11].

It should be noted that physical, chemical, and biological processes are used in engineered wetland systems to remove water pollutants. These processes include sedimentation, adsorption onto plant substrates, ion exchange, microbial transformation, and phytoaccumulation, all of which remove heavy metals from the wetlands [12, 13]. When the pathogens are eliminated, sedimentation, filtration, natural decay, exposure to UV, microbial predation, and rhizosphere-mediated antimicrobial activity are the mechanisms involved. The presence of aquatic macrophytes enhances the removal of water pollutants. The reason for this is the increased surface area of the roots for the growth of microorganisms, oxygenation of the rhizosphere, and enhancement of the removal and transformation of water pollutants [14].

Among the commonly employed wetland vegetation, *Phragmites australis* and *Juncus effusus* have been found to possess significant tolerance to polluted habitats and high treatment performance for wastewater treatment systems [15]. Various studies have already established the removal potential of these plant species for nutrients, organic matter, and some heavy metals [8, 11], while others have established significant removal of pathogenic bacteria in wetland systems [9, 13]. Despite this, much of the research has focused either on eliminating harmful microbes or germs or heavy metals. Systematic comparative studies on the simultaneous removal of pathogens and heavy metals in surface-flow systems remain scarce.

Moreover, there is a scarcity of information on the comparative bioaccumulation potential, translocation factors, and antibacterial properties of the plant species under identical conditions. The addition of natural materials such as zeolite, which is known for its cation-exchange capacity and enhancement of antibacterial properties [12, 15], also deserves thorough investigation for hybrid surface-flow wetland systems.

Therefore, this study aims to clarify the dimensions of a comprehensive comparison between common reed (*Phragmites australis*) and flowing Juncus (*Juncus effusus*) in surface-flow hybrid wetlands, reinforced with limestone and zeolite. The study aimed to: (i) evaluate and assess the effectiveness of simultaneous elimination of pathogenic bacteria and trace heavy metals; (ii) investigate metal bioaccumulation and translocation in plant tissues; (iii) investigate the antibacterial properties of plant root extracts; and (iv) optimize operational conditions for *E. coli* removal using Response Surface Methodology (RSM). By combining physicochemical, microbiological, phytochemical, and statistical approaches in a single experimental design, the current study offers original comparative information on the synergistic co-removal capabilities of two different macrophyte species in hybrid CWs.

2. METHODOLOGY

2.1 System design and setup

The system of treatment was made up of an artificial wetland with surface runoff, a sedimentation basin, and a tank. The 40-liter sedimentation tank, lined with a layer of fine gravel (3–6 mm in diameter and 20 cm thick), was constructed on top, adding a coarse gravel foundation layer (8–12 mm in diameter and 39 cm thick). The design aimed to minimize the pollutant load and reduce the likelihood of blockage. Using 76 mm diameter PVC pipes, the initially treated wastewater was continuously conveyed to the constructed wetland. The flow was controlled by a mechanical valve to maintain a constant rate of 0.01 m³/day.

Furthermore, each of the three test units, measuring two meters long, one meter wide, and 0.45 meters deep, was constructed with a slope of 0.5%. Before adding, the septic system was filled with 20 liters of effluent that contained heavy elements such as lead (Pb), zinc (Zn), nickel (Ni), copper (Cu), and cadmium (Cd). The plants were given four weeks to get used to the marsh setting. A 1 mm-thick polyethylene film was placed over the substrate to stop wastewater leaks. After that, six stems of *Juncus effusus* and *Phragmites australis* were planted in each wetland unit.

Ten months were spent on the investigation (June 2024 to April 2025). The experimental timeline consisted of four phases: (1) a four-week acclimatization period during which plants were irrigated with primary treated wastewater without heavy metal supplementation; (2) a two-week heavy metal spiking phase; (3) an eight-month routine operational phase under controlled hydraulic conditions; seasonal monitoring was conducted during both summer and winter within the operational phase. Influent and effluent samples were collected monthly throughout the operational phase.

The second and third units each had four compartments, while the first unit functioned as a non-vegetated control. *Phragmites australis* was planted in one compartment, *Juncus effusus* in another, and zeolite particles (4–8 mm) were added to the remaining compartments, along with limestone beds (Figure 1). All plants used in this study were collected from local wetland environments. A 30-day acclimatization period (Phase 1 of the experimental timeline) was conducted, during which wastewater was fed to the wetland units to allow plant adaptation. During this period, meteorological parameters, including temperature and relative humidity, were continuously monitored using ADT-161 data loggers.

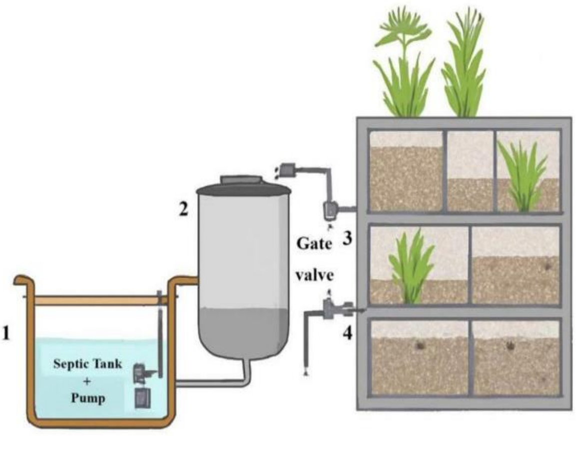


Figure 1. Diagrammatic representation of the treatment units

2.2 Physical-chemical evaluation

Between June 2024 and April 2025, five liters of water were taken each month from each wetland treatment system's influent and effluent. In accordance with the established protocols described by the American Public Health Association (APHA), the collected samples were put into sterile plastic bottles and brought to the laboratory in a chilled container for examination [16]. Total suspended solids, turbidity, ammonium, temperature, pH, electrical conductivity (EC), biochemical oxygen demand (BOD), and heavy metals such as chromium, copper, zinc, cadmium, iron, nickel, and lead were then measured using inductively coupled plasma mass spectrometry (ICP-MS) (Perkin Elmer Six, Elan 9000).

2.3 Assessment of plant tissue heavy metal levels

The plant samples were sliced into little pieces and dried at 100 ℃ for an entire night after being carefully cleaned to remove contaminants and unnecessary material. In an agate mortar, the dried plant material was ground into a fine powder. 0.5 g of the tissue homogenate was then digested for two hours at 60 ℃ using a 2:2 acid combination. Whitman filter paper (APHA, 2017) [16] was used to filter the samples for quantification.

2.4 Microbiological evaluation

FC and TC, which were incubated at 44.5 ℃ and 35 ℃, respectively, were part of the microbiological analysis. To make bacterial counting easier, *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli,* and fecal streptococci (FS) were cultivated at 35 ℃ for 48 hours, 44.5 ℃ for 24 hours, and 41.5 ℃ for 72 hours, respectively. The membrane filtration technique was used to measure bacterial colonies in accordance with standard protocols (APHA, 2017) [16]. The following formula was used to express the results as colony-forming units (CFU) per 100 milliliters:

$$\text{CFU/ml} = \frac{(\text{number of colonies} \times \text{dilution factor})}{(\text{culture dish volume})} \tag{1}$$

2.5 Plant material preparation for extraction

To evaluate the antibacterial activity of *Phragmites australis* root extracts, three frequently prevalent pathogenic bacteria in wastewater—*Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*—were chosen. After a thorough cleaning, the roots were ground into a fine powder, dried at 40 ℃, and placed in containers with labels. After homogenizing 15 g of the dried root powder with 150 mL of sterile, boiling distilled water for two hours, the mixture was shaken for thirty minutes at 220 rpm. The extracts were centrifuged for ten minutes at 2000 rpm and filtered through cloth after being allowed to stabilize at 30 ℃ for six hours. After the aqueous extract had evaporated, the residues were reconstituted to final concentrations of 100, 300, and 600 mg/mL.

2.6 Evaluation of antimicrobial action

The antimicrobial properties of the common reed plant were evaluated using the well diffusion method on agar. Following an overnight incubation period in 50 mL of nutrient-rich broth at 35 ℃, 100 µL of freshly generated bacterial cultures were transferred onto nutrient agar plates. One hundred microliters of plant extract (at a concentration of 100–500 mg/mL) were then added to each of four wells (6 mm in diameter) drilled in the agar. Dimethyl sulfoxide (DMSO) was used as a negative control. Areas of inhibition were measured to assess antibacterial activity after overnight incubation of the plates at 37 ℃. The extracts were assessed at several concentrations (100–500 µL) and contrasted with sterile distilled water, which served as a negative control.

2.7 Analysis with gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC–MS) was used to examine the chemical components of the plant extracts. An HP-7MS column (20 m × 290 mm × 0.30 mm) and an Agilent 8821A gas chromatograph were used for the analysis. Initially, the oven was preheated at 55 ℃ for two minutes; a 1 µL sample was introduced into a GC-MS, and then increased at a rate of 15 ℃ per minute until 280 ℃, where it was held for eight minutes. To identify the chemical compounds, the mass spectra obtained by the GC-MS software were compared with their own spectra.

2.8 Analysis of statistics

SPSS version 18.0 was used to perform the statistical analysis. Subsequently, one-way analysis of variance (ANOVA) with mean ± standard deviation was used to evaluate the normally distributed data at a significance level of 0.05.

2.9 Response surface methodology in experimental design

A statistical method known as RSM, this method uses a second-degree polynomial to model the interactions between two or more variables in order to determine ideal operating conditions [17]. A Box–Behnken design (BBD) was used in this study to assess how different parameters affected the removal of heavy metals. As shown in Table 1, the experimental design included 27 runs with each factor kept at three levels. The best values for the variables and the response were found using Eq. (2), and F-tests and ANOVA were used to verify the model's validity.

$$Y = \beta_0 + \Sigma\beta_{\mathrm{i}}X_{\mathrm{i}} + \Sigma\beta_{\mathrm{ii}}X_{\mathrm{i}}^2 + \Sigma\beta_{\mathrm{ij}}X_{\mathrm{i}}X_{\mathrm{j}} \tag{2}$$

Y is the expected response factor (elimination of *E. coli*), X is the input variable, β_0 is the intersection, β_j is the linear effect, β_{ii} is the quadratic effect, the interaction effect is represented by β_{ij}, and the quantity of input utilized to define the encoded variable is indicated by N.

Table 1. Using the common reed plant (*Phragmites australis*) to remove *E. coli* bacteria from wastewater by changing process parameters

Flexible	The Factors	Level		
		−1	0	+1
The Wetland area (m^2)	X1	1	1	3
The initial *E. coli* concentration (CFU/100ml)	X2	3	6	9
The rate of flow (L)	X3	20	40	60
The time of contact (day)	X4	1	2	4

2.10 Characterization and preparation of zeolite

The material's physicochemical characteristics were then determined using traditional classical analytical techniques, and its crystalline phase was examined using X-ray diffraction (XRD) according to approved protocols [18]. Sieving produced zeolite particles in a size range of 0.8 to 1 mm for use in agriculture and wastewater treatment processes. After a

24-hour treatment with a 10% aqueous NaCl solution, the zeolite was thoroughly cleaned with distilled water. As part of the initial activation procedure, the material was then allowed to air dry at ambient temperature.

2.11 Kinetic rate constants of first order

A volume-based kinetic model that relates input concentrations to hydraulic loading rates (HLR) was used to calculate the first-order removal rate constants for coliform bacteria [19]. Data from at least two samples taken at different times were used to calculate the K values of the experimentally obtained removal rate constants. First-order kinetics, which is represented by the following equation, was thought to govern the elimination of harmful bacteria: First-order kinetic rate constants.

$$K = HLR \cdot ln(C_i/C_0)$$

$$HLR = Q/A$$

In this case, C_0 and C_i stand for the bacterial concentrations (CFU/100 mL) in the influent and effluent, respectively, Q is the influent flow rate (m^3/day), A is the effective surface area of the wetland (m^2), and K is the areal removal rate constant (m/day). The K values were calculated using data obtained from at least two sampling events.

3. RESULTS AND DISCUSSION

3.1 Evaluating improvements and modifications in water quality

The elimination efficiency of chemical and microbiological pollutants was determined using the following formula:

$$RE\% = \frac{(A - B) \times 100}{A}$$

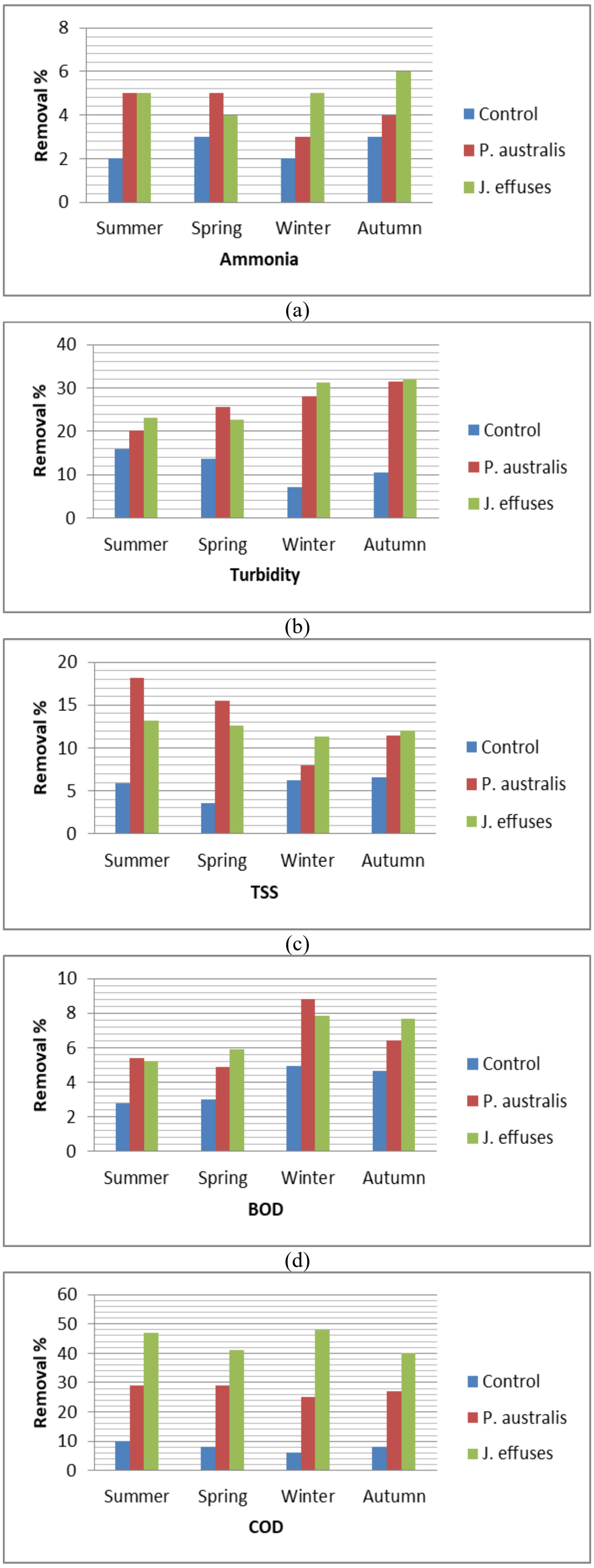
where, A is the influent concentration, B is the effluent concentration, and RE is the removal ratio.

3.2 Analysis of physical and chemical properties of outlet water

EC, pH, and other physical and chemical characteristics of the influent and effluent water were assessed (Table 2). The mean EC and pH values were 2.33 ± 0.43 dS/m and 7.69 ± 0.03, respectively. The average DO in the constructed wetlands (CWs) planted with *Juncus effusus* and *Phragmites australis* increased significantly from 0.46 mg/L in the influent to 9.01 mg/L in the effluent. Influent water temperature averaged 37.2 ± 0.65 °C.

3.3 Removal of ammonium nitrogen (NH_4^+–N) using *Phragmites australis* and *Juncus effusus*

The removal efficiency of ammonium ions (NH_4^+) varied across seasons and treatment systems (Figure 2(a)). The highest removal was observed in wetlands planted with *Juncus effusus*, followed by *Phragmites australis*, while the control units showed the lowest performance. Seasonal variation was evident, with relatively improved removal during the colder period. The reduction of ammonium ions can be attributed to multiple processes, including plant uptake, adsorption, and enhanced microbial activity. Previous studies [19, 20] have demonstrated that plant species such as *Phragmites australis* and *Juncus effusus* promote NH_4^+ removal through stimulation of microbial nitrification processes.



(a)

(b)

(c)

(d)

(e)

Figure 2. Seasonal differences in removal efficiency of (a) NH_4^+, (b) turbidity, (c) Total suspended solids (TSS), (d) biochemical oxygen demand (BOD), and (e) chemical oxygen demand (COD) by *P. australis* and *J. effusus*

Table 2. Sample physicochemical characteristics before treatment

The Physicochemical Parameters	Mean ± SD
The Temperature, °C	37.2 ± 0.654
pH	7.69 ± 0.03
The Electrical Conductivity (EC), dS/m	2.33 ± 0.43
The Total Suspended Solids (TSS), mg/L	99.7 ± 2.03
The Bicarbonate (HCO_3), mg/L	200 ± 0.099
The Ammonium (NH_4^+), mg/L	22.6 ± 2.3
The Biochemical oxygen demand (BOD), mg/L	42 ± 3.4
Chemical oxygen demand (COD)	129 ± 2.2
The Turbidity (NTU)	62.8 ± 0.9
The Major Anions	
The Chloride (Cl^-), mg/L	900 ± 6.09
The Sulfate (SO_4^{2-}), mg/L	222 ± 1.6
The Phosphate (PO_4^{3-}), mg/L	2.9 ± 0.5
The Major Cations	
The Calcium (Ca^{2+}), mg/L	180 ± 40
The Sodium (Na^+), mg/L	800 ± 10.9
The Potassium (K^+), mg/L	22 ± 2.1
The Trace Metals	
The Cadmium (Cd), mg/L	0.037 ± 0.109
The Chromium (Cr), mg/L	1.09 ± 0.07
The Copper (Cu), mg/L	0.83 ± 0.06
The Iron (Fe), mg/L	4.3 ± 0.04
The Lead (Pb), mg/L	1.29 ± 0.037
The Manganese (Mn), mg/L	0.54 ± 0.036
The Zinc (Zn), mg/L	0.79 ± 0.31

3.4 Reducing turbidity using *Phragmites australis* and *Juncus effusus*

In the free-water surface (FWS) system, influent turbidity ranged from 30 to 70 NTU. The seasonal turbidity removal efficiencies observed in the constructed wetlands are presented in Figure 2(b). The wetlands planted with *Phragmites australis* showed removal efficiencies ranging from approximately 25% in spring to 32% in autumn, while *Juncus effusus* achieved removal efficiencies ranging from 22% in spring to 32% in autumn. In contrast, the unplanted control system exhibited lower removal efficiencies, ranging from 7% in winter to 15% in summer.

Overall, both planted systems demonstrated improved turbidity removal compared to the control, with the highest efficiencies recorded during autumn. These variations can be attributed to seasonal differences in plant growth, hydraulic conditions, and enhanced sedimentation and filtration processes within the vegetated systems [21].

3.5 Total suspended solids removal by *Juncus effusus* and *Phragmites australis*

The TSS concentrations in the influent wastewater ranged from 77 to 150 mg/L, with higher values typically observed during summer and lower values during winter. The seasonal removal efficiencies of TSS are illustrated in Figure 2(c). The wetlands planted with *Phragmites australis* achieved removal efficiencies ranging from approximately 8% in winter to 18% in summer, while *Juncus effusus* showed removal efficiencies between 11% in winter and 13% in summer and autumn. The control system exhibited lower TSS removal efficiencies, ranging from 4% in spring to 7% in autumn. These results indicate that vegetated wetlands enhance TSS removal compared to unplanted systems, likely due to improved filtration, sedimentation, and microbial activity associated with plant root systems [22].

3.6 *Phragmites australis* and *Juncus effusus* for biochemical oxygen demand reduction

The amounts of BOD_5 in the influent varied from 41 to 50 mg/L. *Juncus effusus*-planted wetlands had BOD removal efficiency of 79.9% in the summer, lowering concentrations to 8–10 mg/L, and 67% in the winter, with effluent levels ranging from 18–20 mg/L (Figure 2(d)). In a similar vein, *Phragmites australis* wetlands showed removal efficiency of 68.7% in the winter and 79.5% in the summer. Overall, the results met the WHO-established treated effluent requirements [23].

3.7 Using *Juncus effusus* and *Phragmites australis* to reduce chemical oxygen demand

The influent's initial COD content was 129 mg/L. Treatment in *Juncus effusus*-planted wetlands reduced COD levels to 55 ± 0.05 mg/L by 57.36%, while *Phragmites australis*-planted wetlands reduced COD levels to 32 ± 0.01 mg/L by 75.19% are shown in Figure 2(e). These findings are in line with earlier research showing that *Phragmites australis*-planted artificial wetlands effectively remove COD [24].

3.8 Heavy metal removal from wastewater

Table 3 shows the heavy metal concentrations in the samples from this experiment. When comparing treated wastewater from wetlands planted with common reed (*Phragmites australis*) and juniper (*Juncus effusus*) with control wastewater samples, the amounts of all studied elements (chromium, copper, zinc, cadmium) were lower ($p < 0.05$). For example, the average removal concentration of chromium in treated wastewater from reed was 0.06 mg/L, while it was 0.15 mg/L, compared to 0.91 mg/L in the incoming wastewater. The removal percentage of chromium was 88.2% in reed and 78.6% in juniper, while it was 31.1% in the control wetlands. It should be noted that the planted wetlands showed significantly higher removal efficiency than unplanted wetlands for other heavy metals, such as copper, cadmium, and zinc. In line with earlier research on artificial wetlands, these results validate the efficiency of *Phragmites australis* and *Juncus effusus* in the removal and uptake of heavy metals [25]. Although the constructed wetland system achieved a considerable relative removal efficiency for cadmium, the final effluent concentrations did not fully comply with the WHO guideline value. The majority of Cd was retained within the plant root tissues and the substrate matrix (zeolite and limestone), indicating that immobilization and phyto-stabilization were the dominant removal mechanisms rather than complete elimination from the system.

Table 3. Heavy metal concentrations before and after processing treatment

Treatment Condition	Chromium (mg/L)	Copper (mg/L)	Zinc (mg/L)	Cadmium (mg/L)
Influent (Untreated)	0.91 ± 0.08	0.88 ± 0.14	0.63 ± 0.02	0.034 ± 0.02
P. australis System	0.06 ± 0.01	0.22 ± 0.05	0.08 ± 0.02	0.014 ± 0.067
J. effusus System	0.15 ± 0.05	0.077 ± 0.03	0.06 ± 0.023	0.027 ± 0.012
Unvegetated Control	0.51 ± 0.14	0.62 ± 0.14	0.41 ± 0.51	0.045 ± 0.066
WHO Limits	0.05	1.00	3.00	0.003

Table 4. Bioaccumulation of heavy metals and their transfer through aquatic plants

Plant Species	The Tissue	Cd	Cu	Zn	Fe	Pb	Cr	Ni
Phragmites australis	The Stem	3.2	5.000	230.598	59.098	14.398	21.498	20.464
	The Shoot	11.9	20.898	409.598	506.098	17.898	31.198	29.304
	The Root	29.5	26.998	339.798	2842.098	20.198	55.198	37.358
	TF	0.3456	10.7371	11.3097	10.9731	10.691	10.5658	10.8025
	The BCF Stem	56.232	15.5563	390.270	27.330	14.665	27.2888	71.935
	The BCF Shoot	300.00	55.9928	698.888	184.535	19.331	41.916	124.549
	The BCF Root	599.67	16.5589	578.546	1006.079	15.5498	78.105	254.736
Juncus effusus	The Stem	0.2	11.798	231.498	233.098	26.798	16.598	18.348
	The Shoot	13.5	19.298	403.498	1563.098	45.998	18.698	24.751
	The Root	8.4	15.898	429.298	5663.098	40.098	27.398	35.031
	TF	1.444	11.678	11.0364	10.372	11.2956	10.5951	11.7488
	The BCF Stem	3.342	17.5613	391.818	88.518	18.301	19.8998	39.778
	The BCF Shoot	209.56	22.128	688.368	556.268	27.730	24.196	62.778
	The BCF Root	300.34	28.968	732.848	1998.190	24.832	36.1851	98.808

TF: Transfer Factor; BCF: Bioaccumulation Factor

3.9 Accumulation, translocation, and bioaccumulation of heavy metals in plant parts

The factors for the presence of heavy metals in two factories were determined using the following mathematical formula [25]:

$$BCF = \frac{(Metals\ content\ in\ plant)}{(Metals\ concentration\ in\ influent)} \tag{3}$$

While influent values are displayed in mg/L, this method takes into account the metal concentrations in plant tissues represented in mg·kg^{-1} dry weight (DW). By comparing the concentrations in shoots (such as leaves and stems) to those in roots, the translocation factor (TF), which measures the transfer of metals from roots to aerial plant parts, may be computed. The following formula is used to determine the TF:

$$TF = \frac{C_{shoot}}{C_{Root}} \tag{4}$$

where, C_{shoot} and C_{root} are the metal concentrations in shoots and roots, respectively (mg·kg^{-1} DW). *Phragmites australis* and *Juncus effusus* exhibited species-specific differences in heavy metal concentrations, bioaccumulation, and translocation variables (Table 4). For example, bioaccumulation factors for *Phragmites australis* ranged from 0.5 to 875.4 mg·kg^{-1}, in contrast, *Juncus effusus* had values between 0.9 and 2008 mg·kg^{-1} DW. Compared to aboveground tissues, mineral values in the roots were significantly higher. *Juncus effusus* showed the highest mineral translocation as follows: cadmium > copper > nickel > iron > chromium > lead > zinc. While *Phragmites australis* followed a different order: Cd > Fe > Pb > Zn > Cu > Cr. This suggests that both plants accumulate metals in their roots as a tolerance mechanism, reducing the buildup in the aerial sections. Similar patterns in bioaccumulation have been reported for other species [26].

3.10 Removal of bacterial indicators of pollution

The concentrations of FC, FS, and TC varied significantly over the course of the trial. The range of TC, FC, and FS influent values was 21 × 10^3 to 39 × 10^3 CFU/100 mL. *Phragmites australis*-planted wetlands had the greatest removal efficiency throughout the summer, reaching 99.7% for TC, 98.9% for FC, and 97.9% for FS. Wetlands planted with Juncus *effusus*, on the other hand, showed somewhat lower removal efficiency; during a 4-day retention period, TC, FC, and FS decreased by 95.6%, 96.3%, and 94.0%, respectively. Lower removal efficiencies of 78.5%, 79.5%, and 80.5% for TC, FC, and FS were found in non-vegetated control wetlands. Pathogen clearance efficiency varied from 38.2% to 67.9% in the control wetlands, 92.0% to 97.5% in *Juncus effusus* wetlands, and 96.9% to 98.8% in *Phragmites australis* wetlands over the summer. *Phragmites australis* clearance efficiency was from 90.2% to 96.8% in the winter, *Juncus effusus* from 75.6% to 91.5%, and the control wetlands from 29.1% to 59.9%. These results highlight the strong pathogen removal capability of both *Phragmites australis* and *Juncus effusus*, with *Phragmites australis* demonstrating superior performance across both seasons. The enhanced efficiency of *Phragmites australis.* Its rhizomes' antimicrobial qualities may be responsible for this impact [27].

Its enhanced pathogen elimination effectiveness also occurs in the form of antimicrobial chemicals produced by its roots. There are several ways that pathogens are eliminated in artificial wetlands [28]. By raising the oxygen content of the surrounding substrate, root-mediated aeration facilitates pathogen inactivation [29]. Additionally, through electrostatic interactions, the presence of metal ions like Cu^{2+}, Ag^{+}, and Zn^{2+} in filter materials such as natural zeolites might enhance the removal of bacteria [30]. Previous research involving different plant species has revealed similar high pathogen clearance efficiency [31].

3.11 Overview and factors impacting Box–Behnken design reduction

To assess the impact of four independent parameters and their interactions on the effectiveness of *E. coli* eradication, a total of 27 BBD experiments were carried out. The ANOVA findings for *Phragmites australis*-planted wetlands are shown in Table 5. The modified coefficient of determination (R^2) was used to evaluate the model's relevance, and the regression coefficients were then computed [32]. The probability of this F value occurring by chance was 0.0021%, and given that the typical F value for *E. coli* removal was 225.01, it was statistically significant. The model boundary was statistically significant when the p-value was less than 0.05. The adjusted R^2 value of 0.994 was very close to the expected R^2 value of 0.985; this demonstrates the model's resilience. The number of 92.7 for *Phragmites australis* shows that the experimental results were quite dependable, and an experimental precision ratio larger than four is typically regarded as ideal.

Table 5. Analysis of variance (ANOVA) results for *Phragmites australis*-planted wetland *E. coli* removal

Source	SS	df	MS	F	p-value
Model	5971.09	14	426.51	302.14	< 0.0001
A	29.31	1	29.31	20.76	0.0005
B	1723.15	1	1723.15	1220.36	< 0.0001
C	39.15	1	39.15	27.73	0.0001
D	250.66	1	250.66	177.61	< 0.0001
AB	150.22	1	150.22	106.39	< 0.0001
AC	4.02	1	4.02	2.85	0.113
AD	255.66	1	255.66	181.06	< 0.0001
BC	300.05	1	300.05	212.55	< 0.0001
BD	433.22	1	433.22	306.80	< 0.0001
CD	15.08	1	15.08	10.68	0.006
A^2	300.77	1	300.77	213.07	< 0.0001
B^2	2441.92	1	2441.92	1729.63	< 0.0001
C^2	29.07	1	29.07	20.59	0.0005
D^2	594.33	1	594.33	421.02	< 0.0001

3.12 Analysis of the interaction of variables for the elimination of *Escherichia coli*

As shown in Figure 3, to investigate the correlations between the independent variables, three-dimensional response surface plots were created. As the wastewater moved through the marsh, the *E. coli* concentration gradually dropped, eventually reaching approximately 99% of the elimination efficiency at the end of the basin. The efficiency of the elimination increased with increasing basin length and contact times. For example, a 70.2% reduction was achieved with a basin length of 2.5 m, a flow velocity of 45 L, and an initial *E. coli* concentration of 200 CFU/100 mL after two days of contact. The reduction increased to 90.1% when the contact period was extended to three days and to 97.2% when it was extended to four days.

E. coli reduction varied from 59.8% to 79.2% when the contact period was prolonged to four days, according to Shingare et al. [33], underscoring the significance of sufficient residence time for successful wetland treatment. Figure 4 shows how filter bed length and flow rate affect the effectiveness of removing *E. coli*. Wetlands showed weaker performance in reducing *E. coli* at high bacterial loads (i.e., moderately concentrated wastewater). For example, with a filter bed length of 2.5 m, a flow rate of 80 L/min, and an initial *E. coli*. After 2.5 days of contact time, a 61.02% reduction was seen at an initial concentration of 300 CFU/100 mL. On the other hand, after three days of contact time, a wetland with a 2.5 m bed length, a flow rate of 60 L, and an initial *E. coli* density of 200 CFU/100 mL achieved 99% removal efficiency.

A variety of biological processes are among the strategies used in wetlands to eliminate or get rid of microorganisms. Biological processes such as protozoan predation, the release of antibiotic chemicals by plant roots, and spontaneous cell death can reduce pathogens. Pathogen clearance is also facilitated by physical processes like adsorption, sedimentation, and filtering. Furthermore, the protective environment that plant cover provides improves these elimination processes; also, there are two crucial and important factors for enhancing the efficiency of the eradication or elimination of *Escherichia coli* bacteria, namely the HRT [34]. Most gut bacteria, including facultative or obligatory anaerobes like *E. coli,* cannot thrive when exposed to oxygen. Furthermore, oxygen increases the activity of bacterial predators, such as viruses and protozoa [35].

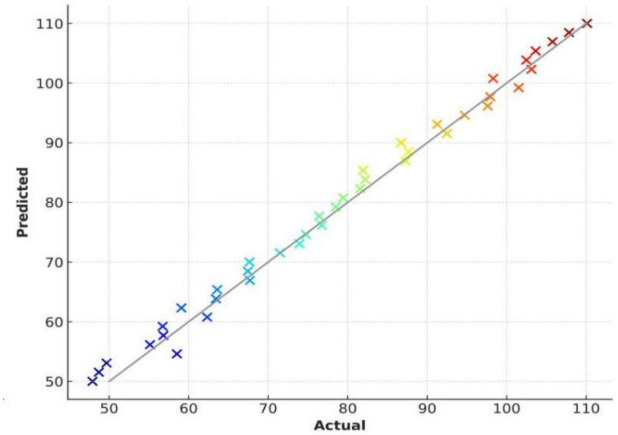


Figure 3. Evaluation of the *E. coli* elimination model using studentized residuals and a normal probability plot

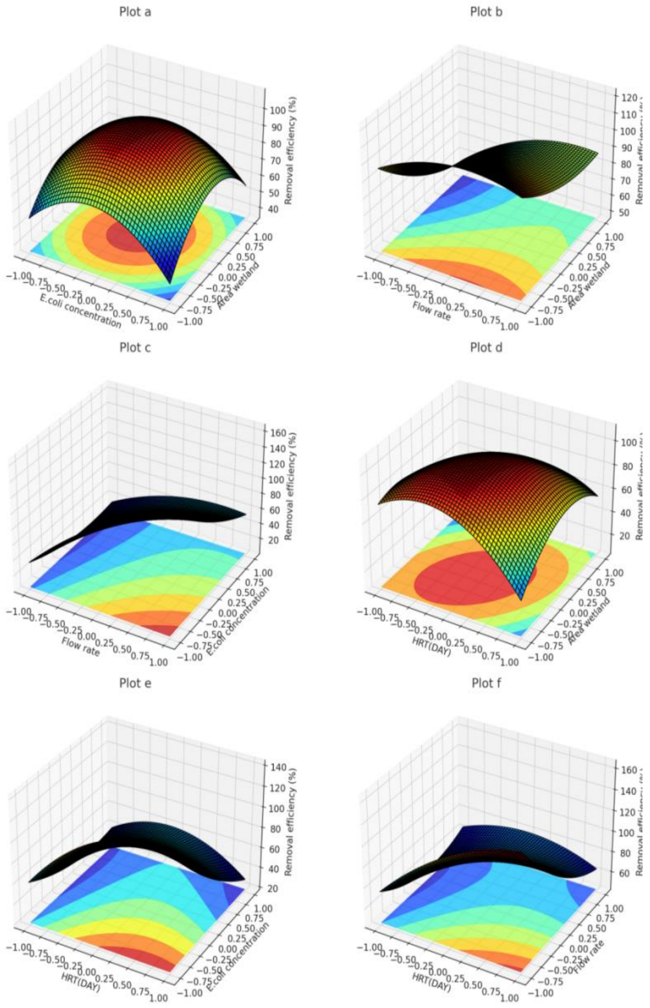


Figure 4. Combined effects of key operational parameters on *E. coli* removal in constructed wetlands (CWs) using 3D response surface plots: (a) initial *E. coli* concentration and wetland area; (b) flow rate and wetland area; (c) flow rate and initial *E. coli* concentration; (d) hydraulic retention time (HRT, days) and wetland area; (e) HRT and initial *E. coli* concentration; and (f) flow rate and HRT (days) for *E. coli* reduction

Bacterial adhesion is facilitated by electrostatic interactions between the surfaces of porous medium and bacterial cells, as shown by Hou et al. [36]. Furthermore, by strengthening the interaction between bacteria and the filter media, natural zeolites with a high cation exchange capacity further enhance bacterial clearance [37].

3.13 Optimizing the parameters for bacterial removal, the desirability function in use

The desire function, a technique used to identify the optimal variables that significantly influence the response, was employed to maximize *E. coli* colony reduction. The desire function is defined as a set of values from 0 (beyond the permissible range) to 1 (within the intended range), where the desirability levels vary. The goal was to use numerical optimization to identify a set of parameters that optimizes the desirability function for the eradication of *E. coli*. The optimization procedure found an ideal situation with a bed length of 3 m, an initial *E. coli* concentration of 150 CFU/100 mL, a flow velocity of 20 L, and a retention period of 2 days. The clearance effectiveness of *E. coli* was 93% under these circumstances. These results show that removal parameters in wetlands planted with *Phragmites australis* can be efficiently optimized using both the Box-Behnken Design and the desired function.

3.14 Antibacterial properties of *Phragmites australis* root aqueous extracts

The common reed (*Phragmites australis)* may possess antimicrobial properties. The ability of aqueous extracts of common reed roots, the root extract's ability to inhibit the growth of dangerous bacteria frequently seen in influent wastewater, was assessed. The three bacterial species' inhibitory zones, which varied from 15.0 mm to 26 mm, are shown in Table 6. When it came to *Staphylococcus aureus*, the extract showed the strongest antibacterial action.

Table 6. *Phragmites australis* root extracts' antibacterial efficacy against specific bacterial strains

Concentration (mg/mL)	*Escherichia coli* (mm)	*Pseudomonas aeruginosa* (mm)	*Staphylococcus aureus* (mm)
150	0.0	0.0	15
300	15.0	15.1	15
400	19.0	20.2	20.0
500	22.0	23.3	26
Negative Control (a)	0.0	0.0	0.0 – 0.0

3.15 Natural zeolite properties and characterization

The natural zeolite material is considered a good material for wastewater treatment due to its many good and useful properties. Zeolite that occurs naturally is a good material for long-term use in treatment systems since it is nontoxic, non-inflammatory, and resistant to wear. Table 7 shows that SiO_2 predominates in its chemical composition. XRD examination reveals that the zeolite is composed of around 70% clinoptilolite, which has a high cation-exchange capacity (Figure 5). This characteristic makes it easier to remove compounds like NH_4^+ by allowing zeolite to exchange ions like sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}) with wastewater contents. Zeolite's physicochemical characteristics can also be adjusted, and it can be renewed for further usage; it adds high value to its applications in wastewater treatment without suffering a significant loss of performance at relatively low temperatures [38].

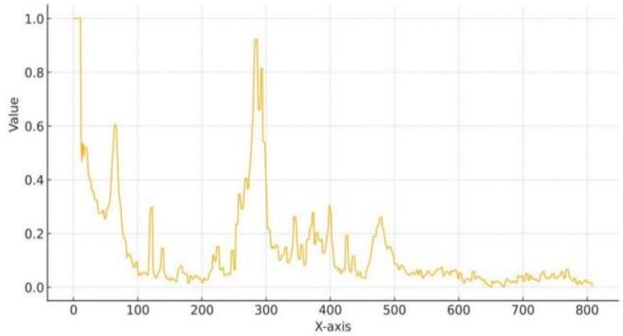


Figure 5. X-ray diffraction (XRD) pattern of natural zeolite

3.16 Removal rate constants, which are of the first order

Table 8 displays first-order removal rate constants (k values) for a range of indicators and wetland macrophytes. Langergraber and Dotro [39] found similar k values for FC and other harmful bacteria in FWS artificial wetlands, and these results are in line with their findings.

Table 7. Properties of natural zeolite

Property	Value
SiO_2	66.96%
Appearance (Porosity)	31.5%
Al_2O_3	12.05%
Appearance (Density)	2.3 g/cm³
Na_2O	0.8%
Average Pore Diameter	0.01–0.2 µm
MgO	0.7%
Humidity	6.7%
CaO	3.4%
Color	Grayish-white
Fe_2O_3	3.22%
Surface Area	89.9 m²/g
K_2O	2.83%
Particle Size	6 mm
TiO_2	0.4%
Moisture	6.78%
Loss on Ignition (LOI)	10%
Cation Exchange Capacity (CEC)	1.6–2.0 meq/g
pH	7.5

3.17 Wetlands' microbial community structure and removal efficiency planted alongside *Juncus effusus* and *Phragmites australis*

Analysis using Krona software was conducted to evaluate the taxonomic composition of microbial communities. Similar microbial patterns appeared in both the proportion of bacilli and proteobacteria, which constituted more than 40% and 30% of the incoming and outgoing water, respectively (Figure 6).

Table 8. Average k values for the elimination and removal of harmful bacterial colonies in free-water surface (FWS) systems

Pathogenic Bacteria	Plant Species	Inflow (CFU/100 mL)	Outflow (CFU/100 mL)	k ($m \cdot d^{-1}$)
Total Coliforms	*Phragmites australis*	50 × 10^3	1300	0.2354
	Juncus effusus		2897	0.2756
Fecal Coliforms	*Phragmites australis*	44 × 10^3	681	0.2948
	Juncus effusus		1230	0.2435
Fecal Streptococci	*Phragmites australis*	3227 × 10^3	1000	0.2837
	Juncus effusus		2900	0.2847
Pseudomonas aeruginosa	*Phragmites australis*	133	11	0.0533
	Juncus effusus		32	0.0565
Staphylococcus aureus	*Phragmites australis*	20 × 10^3	550	0.1365
	Juncus effusus		1230	0.1465
Escherichia coli	*Phragmites australis*	36 × 10^3	1221	0.2643
	Juncus effusus		2020	0.2755

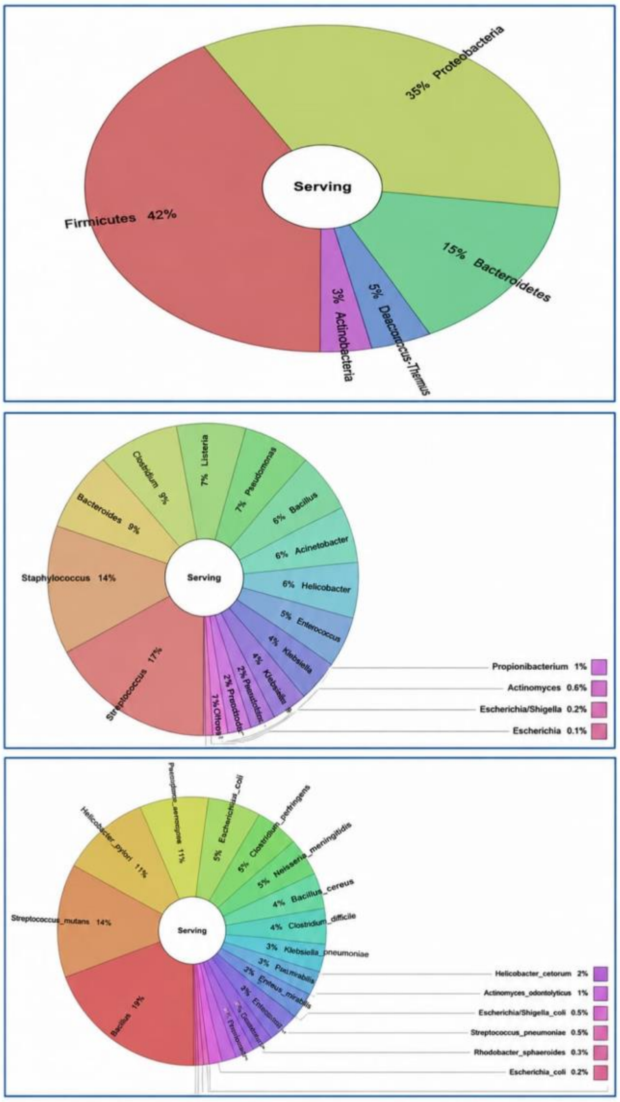


Figure 6. Hierarchical clustering dendrogram illustrating the predominant bacterial phyla identified in the influent samples, displaying patterns of relative abundance and taxonomic relationships

Furthermore, microbial diversity differed considerably between incoming and outgoing waters at the genus level for bacteria. Streptococcus was the most prevalent genus in incoming waters, followed by *Staphylococcus* and then *Bacillus*. On the other hand, almost half of the bacteria in the effluent were *Pseudomonas aeruginosa*, followed by *Escherichia coli* and *Clostridium* species. *Bacillus* (19%), *Streptococcus mutans* (14%), *Helicobacter pylori* (11%), and *Acinetobacter* (8%) were the most common species in influent waters. In contrast, distinct species compositions were found in the effluent from wetlands planted with *Phragmites australis* and *Juncus effusus*, such as Pseudomonas aeruginosa (32% and 23%), *Acinetobacter baumannii* (26% and 13%), and *Helicobacter pylori* (22% and 18%). Additionally, after treatment, the abundance of taxa, including *Arthrobacter globiformis*, *Streptomyces griseus, Flavobacterium johnsoniae,* and *Nitrosomonas europaea,* increased. Previous investigations, such as Cui et al. [40], have shown similar results, emphasizing high quantities of opportunistic bacteria in hospital wastewater. This is consistent with Al-Ibady [41], who found that Pb(II) and Cr(VI) ions were effectively removed from contaminated water by a manganese oxide nanocomposite that was green-synthesized using star anise extract. The results also agree with the results of Nassrullah et al. [42], who demonstrated its potential as an efficient and environmentally friendly adsorbent for use in water treatment applications. The findings highlight the urgent need to improve wastewater management, impose more stringent environmental regulations, and to establish regular and continuous water monitoring to protect the environmental and public health functions of the Tigris River.

4. CONCLUSIONS

Plant-based systems demonstrated high effectiveness and efficiency in removing and eliminating heavy metals and pathogenic microorganisms, outperforming non-plant-based systems. Among the tested species, the common reed (*Phragmites australis*) showed outstanding performance in this experiment, particularly in inhibiting microbial activity and removing chromium and zinc, achieving a removal efficiency of approximately 99% under optimal experimental conditions. The results of this study indicate that the treatment efficiency depends on synergistic interactions between substrate uptake (zeolite and limestone), microbial processes, and plant-mediated mechanisms, especially root zone oxygenation and plant fixation. The concentration of metals in root tissues suggests limited translocation, thus reducing the potential environmental risks associated with biomass disposal. One of the most important contributions of this study is the identification of the antibacterial activity of extracts from the roots of the common reed (*Phragmites australis*), indicating that plant-derived bioactive compounds play a direct role in suppressing and eliminating pathogens within engineered wetlands. Furthermore, optimization analysis confirmed that HRT and flow rate are critical factors in controlling the efficiency of Escherichia coli removal. However, the cadmium concentrations in the treated water consistently did not meet the limits recommended by the World Health Organization, highlighting the need for further system optimization or integration with other complementary treatment processes.

Finally, the results demonstrate that hybrid wetlands based on common reed (*Phragmites australis*) offer a reliable, low-cost, environmentally sustainable, and eco-friendly approach or protocol for wastewater treatment, particularly in arid regions. Future research should also focus on long-term operational stability, large-scale application, and safe management of heavy metals accumulated in plant biomass.

ACKNOWLEDGMENT

The facilities and technical assistance required to finish this study were provided by the linked institution, for which the authors are grateful. Additionally, they value the lab staff's support throughout the trial.

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