

The effect of plant density on the organic matter removal from municipal wastewater by vertical flow constructed wetlands (*Phragmites australis* case study)

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Abstract

Vertical flow constructed wetlands with varying plant densities of *Phragmites australis* (0, 3, 6, and 9 plants /m²) were evaluated for domestic wastewater treatment over a 12-month period. The experimental system consisted of four independent circular units (0.125 m²) filled with three substrate layers: coarse gravel (20–25 mm), medium gravel (10–15 mm), and fine sand (3 mm). Results demonstrated that increased plant density significantly enhanced treatment performance. The high-density configuration (9 plants/m²) achieved superior removal efficiencies for biochemical oxygen demand ($93.86 \pm 2.49\%$), chemical oxygen demand ($81.26 \pm 7.19\%$), and TSS ($85.40 \pm 5.66\%$) compared to medium-density, low-density, and unplanted controls. Statistical analysis confirmed significant differences between planted and unplanted systems ($p < 0.05$), with strong positive correlations between plant density and contaminant removal ($r = 0.99$ for biochemical oxygen demand, $r = 0.94$ for chemical oxygen demand). high-density also improved dissolved oxygen levels (from 0.46 ± 0.14 mg/L in influent to 4.75 ± 0.73 mg/L in high-density effluent) and stabilized effluent quality, with high-density treatments showing 19.12% and 57.71% lower standard deviations than low-density treatments for biochemical oxygen demand and chemical oxygen demand, respectively. Treatment mechanisms included enhanced root surface area for biofilm development, increased oxygen transfer to the rhizosphere, and greater production of exudates supporting microbial activity. All configurations achieved effluent quality compliant with Algerian regulatory standards, demonstrating Vertical flow constructed wetlands as effective, low-cost wastewater treatment solutions.

1. Introduction

Wastewater poses substantial environmental and public health challenges (Obilonu et al. 2013). Different determinants, such as population growth, agricultural intensification, and industrial activity, stimulate water pollution. Today represents a serious challenge (Nellyyat 2016; Negm 2020). In addition, problems of mobilization and poor reuse of water resources due to a lack of proper control of wastewater treatment plants have led to the lack of conservation of water resources (Boudjadja 2003). The wastewater contains chemicals from household residues and industrial facilities. Wastewater discharged directly into natural ecosystems without treatment poses significant environmental risks. They can lead to prevailing diseases in aquatic organisms and disturb the ecosystem. Such pollutants can then potentially pollute water sources used for human consumption and hygiene, leading to public health issues (Kumar and Joshiba 2018). Consequently, it becomes important for us to provide wastewater treatment before we allow it to flow into natural environments. Treatment systems such as wastewater treatment plants, using biological and physical chemical processes, can eliminate most of the pollutants (Zhou and Smith 2001). The effectiveness of these treatment systems depends on the design, maintenance, and the specific technologies employed to address varying types of contaminants (Kumar and Joshiba 2018).

There are different methods of treating the wastewater to make it clean again. It includes physical, chemical, and biological combined processes. Typically, a mix of these methods is employed to remove

various forms of contaminants from wastewater. The processes they choose must be dependent on the pollutants and the level of purification being sought (Berland et al. 2001).

The installation of conventional wastewater treatment plants in small, isolated communities faces several obstacles that can hinder their effectiveness and feasibility, including high costs, limited technical expertise, and challenges in maintaining infrastructure over time (Engin and Demir 2006). These challenges often lead communities to seek alternative solutions, such as decentralized treatment systems or innovative technologies that can provide more sustainable and cost-effective wastewater management options (Mann 1974). By embracing these alternatives, communities can not only reduce their environmental impact but also enhance local resilience and adaptability to changing conditions and demands (Corvea et al. 2012). This led us to consider wastewater treatment using unconventional, suitable, and inexpensive treatment plants. This system is based on the use of plants; it is an ecological, economical system that does not cause noise or odor and is simple in its implementation (Vymazal 2005).

Constructed wetlands (CWs) are artificial systems specially designed for wastewater treatment. These systems use natural processes that involve the vegetation, substrate, and microbial communities. These elements work simultaneously to remove pollutants from wastewater, making CWs an eco-friendly alternative to traditional treatment methods that often rely on chemical processes (Sudarsan et al. 2022). The use of CWs as a means of wastewater treatment is constantly gaining popularity. Numerous configurations and operational conditions have been thoroughly researched and implemented treating various types of wastewater, including domestic and industrial effluents. CWs offer an environmentally friendly approach to wastewater treatment and are relatively simple to maintain (Polprasert 2004). Wastewater treatment in CW encompasses a range of processes, such as filtration, adsorption, precipitation, ion exchange, absorption by plants, and aerobic and anaerobic microbial degradation. Compared to conventional treatment systems, CWs require lower investment and operating costs, making them more accessible and economically sustainable. Therefore, toilets have significant application potential in developing countries such as India, where environmental impact and accessibility considerations are paramount (Wu et al. 2015).

Plants play a key role in the functioning of artificial wetlands for wastewater treatment. The diversity of plant species can, in particular, improve the productivity and purification efficiency of the system. However, the influence of planting density has mainly been neglected so far (Han et al. 2020; Sandoval-Herazo et al. 2020) and understanding its impact could lead to optimized designs that enhance both the ecological and functional performance of these systems. Investigating various planting densities could reveal the optimal balance between plant competition and cooperation, ultimately maximizing nutrient uptake and pollutant removal rates in artificial wetlands (Webb et al. 2013).

To fill this gap, a recent experimental study planted a common plant species (*Phragmites australis*) in three different densities (three, six, and nine plants per square meter). Several parameters were then measured: Total suspended solids (TSS), turbidity, salinity, total dissolved solids (TDS), biological and

chemical oxygen demand (BOD₅ and COD) (Coleman et al. 2001; Barbagallo et al. 2013). This study highlights the importance of an adapted choice of the density of plants to optimize the treatment with artificial wetlands. This study aims to quantitatively evaluate the ability of *Phragmites australis* to remove organic matter from different levels of wastewater crossing a vertical flow constructed wetland (VFCWs).

2. Materials and Methods

2.1. Characteristics of the Experimental Facility

The study was conducted over 12 months, from January to December 2023, at the Mohamed El-Bachir Ibrahimi University in Bordj Bou Arreridj, Algeria (36° 02' 23" N, 4° 49' 06" E). The site has a Mediterranean climate with temperatures ranging from 10°C to 30°C and an average annual rainfall of 500 mm (Fig. 1).

The lab-scale VFCWs (Fig. 2) were dimensioned by considering a surface aeration coefficient $K_a = 30 \text{ gO}_2/\text{m}^2 \text{ d}^{-1}$ (Cooper 1999; Noorvee et al. 2005), and its height is taken equal to 0.4 m. The area obtained is increased by 25%; the vertical surface is then calculated using Eq. (1).

$$A = \frac{DO}{VA} \cdot 1.25$$

1

Where: $A \text{ (m}^2\text{)}$ is the area of the Vertical surface flow wetland. Dissolved oxygen (DO) is the oxygen demand of the wastewater entering the wetland system ($\text{gO}_2 \cdot \text{d}^{-1}$). VA represents the aeration potential of a VF wetland ($30 \text{ gO}_2 \text{ m}^{-2} \text{ d}^{-1}$). The Dissolved oxygen DO can be given as follow in Equations (2):

$$DO = C_{\text{DBO}_5} \cdot Q$$

2

Where: $Q \text{ (m}^3/\text{d}\text{)}$ is the average flow measured during the winter period and distributed over the proposed cells. C_{BOD_5} is the oxygen content eliminated by the vertical filter in Kg/m^3 .

The hydraulic retention time (HRT) corresponding to the average time that makes a drop of wastewater in the beds was determined using Eq. (3):

$$\text{HRT} = \frac{V}{Q}$$

3

Where: HRT is Hydraulic retention time (d), V is Media pore volume (m^3), and Q is Water flow rate (m^3/d). The pilot-scale VFCW consisted of four independent plastic beds, each with a circular surface (0.125 m^2) and a diameter of 40 cm, with a total height of 40 cm.

The pilot-scale VFCWs were filled from bottom to top with three distinct layers: 0.1 m of coarse gravel ($\emptyset$: 20/25 mm) for the drainage layer, 0.2 m of medium gravel ($\emptyset$: 10/15 mm) for effective filtration, and 0.05 m of fine sand ($\emptyset$: 3 mm) for planting, with a porosity (n) coefficient of 36%. The height of 0.05 m is used to protect wastewater from flowing out of the beds. All gravel was thoroughly washed to eliminate clay, silt, and organic matter contaminants. Three beds were planted with *Phragmites australis* at increasing densities: low-density (LD) 3 plants/ m^2 , medium-density (MD) 6 plants/ m^2 and high-density (HD) 9 plants/ m^2 , respectively, and the fourth bed remained unplanted as a control. Wastewater was distributed individually and directly to each unit via a non-continuous distribution system (14 L/day/bed). The pilot-scale VFCW systems were designed in a batch feed mode. For effluent collection, sampling valves were installed at the outlet of each unit (5 cm above the bottom). The Fig. 3 provides a schematic representation of the VFCW system.

2.2. Wetland vegetation

The pilot-scale VFCWs were planted with the plant species *Phragmites australis* at varying densities. The plants of *Phragmites Australis* (Fig. 4) were harvested at the beginning of February. The healthy plants were thoroughly washed several times with distilled water to remove dead plant tissues as well as particles and microalgae that adhered to the plants. Subsequently, the plant species were planted in the basins of the wastewater treatment system on the same day.

During the period of acclimatization and growth, the system was supplied with fresh water; the wastewater was gradually added to reach 100% wastewater.

Phragmites australis is a native plant in North America. It is often used in CWs for wastewater treatment due to its ability to absorb nutrients such as nitrogen and phosphorus (Lee and Scholz 2007).

The plant is easily recognizable thanks to the length of the stubble (flowering stems), which can reach 6 meters. The root of *Phragmites australis* is a typical fascicular root whose high-density section is 50 cm below the surface. Further down, to a depth of 60 cm, the density decreases, and the roots are easily accessible (Milke et al. 2020).

2.3. Wetland Substrates

In CWs, granular materials such as sand, gravel, bark, perlite, and expanded clay are used as substrates. Due to its cost, its availability, and its large contact surface for the microorganisms involved in the degradation of pollutants, gravel is often preferred in CWs (Fig. 5). The chemical composition of the substrate also plays a role in plant growth and the effectiveness of nutrient removal. It is therefore advisable to select a substrate with a pH and conductivity that are adapted to the chosen plant species and local conditions (Castro et al. 2000; Ji et al. 2022).

2.4. Water sampling and analysis

To control the effect of plant density, water samples were taken from January to December 2023 in the influent and after three days in the effluent (Table 1). All parameters were analyzed in the laboratory of the faculty of SNV University Bordj Bou Arreridj, Algeria. The sampling of influent and effluent was carried out immediately for temperature (T, °C), (DO, mg O₂/l), potential hydrogen (pH), salinity (mg/l), (TDS, mg/L), and electric conductivity (EC, mS/cm) using a portable multimeter instrument model HI9829. The TSS, (mg/L) were measured according to the standard method for water and wastewater examination, NF T90-105 (AFNOR 1999). The BOD₅, (mg/L) was quantified by the 5-day BOD test with OxiTop head gas sensors (OxiTop® WTW box). The COD, (mg/L) was measured using the dichromate method following ISO guideline 6060 (ISO 1989).

2.5. Calculation and Statistical Analysis

Removal efficiency (RE%) for each variable was calculated by applying Eq. (4) by comparing influent (C_i) and effluent (C_o) concentrations in water (Kadlec and Wallace 2009)

$$RE(\%) = \left(\frac{C_e - C_s}{C_e} \right) \cdot 100 \quad (4)$$

Where C_e, and C_s are the inlet and outlet concentrations expressed in mg/L, respectively.

One-way analysis of variance (ANOVA) was used for all statistical analyses to determine significant statistical differences in the water treatment performance used by VFCWs. An ANOVA test was used, with a level of statistical significance set at $p \leq 0.05$, and species types and flow types were used as factors. The analysis of variance (ANOVA) was performed using the Origin software (2018).

3. Results and Discussion

3.1. MWW characterization and quality

The results of the measured parameters of raw wastewater (Table 1) are compared with the typical values established by Metcalf and Eddy (2003). The national standards JORA (2006) and the WHO (1973) reveal several remarkable characteristics of the influent.

The observed temperature averages sit squarely in the expected $10 < (19.25 \pm 5.71^\circ\text{C}) < 25^\circ\text{C}$ band for residential effluent, although the peaking upper limit (29°C) is marginally over the outlined thresholds. Warmer conditions can accelerate microbial kinetics and enhance treatment rates. However, they may simultaneously drive the demand for supplemental aeration, lest dissolved oxygen be constrained (Ahsan et al. 2005). Conversely, the recorded pH average (7.59 ± 0.20) is consistent with JORA (2006), which cites an acceptable range of 6.5–8.0 for residential effluent. Such moderate alkalinity is indeed favorable, for nitrogen conversion in biological filter systems is optimized within the specified pH

envelope (Vymazal 2007, Aruna et al. 2020). The observed levels, therefore, reflect the environmental conditions under which conventional microbial processes are deliberately calibrated (Ferrarezi et al. 2022, Weinberger and Yee 1984). Moreover, the pH range in this study promotes the growth of microorganisms, enhancing effective nitrogen removal and improving treatment efficiency (Kodukula et al. 2018). DO (0.46 ± 0.14 mg/L) is lower than the saturation values (> 5 mg/L) expected at the temperature range, which menaces aquatic life (Rao 2002). However, anoxic conditions are characteristic of raw wastewater influent (WHO 1973). The comparison of DO concentrations before and after treatment provides insight into the rate of biological activity (Kimwaga 2015; Lajçi et al. 2017). Higher DO after treatment indicates increased microbial activity and improved organic matter degradation. However, tracking oxygenation changes enhances wastewater treatment and ensures environmental standards.

Measured EC of 4.38 ± 0.29 mS/cm conveniently aligns with the standard bracket of 2.5–8.5 mS/cm, authenticated for municipal wastewater by Metcalf and Eddy (2003). Such a reading suggests a significant load of dissolved salts and minerals characteristic of urban effluent. The enhancement in EC levels reflects a typical response to elevated concentrations of mobile ionic species often retained in raw sewage (Le Bonté 2008; Lajçi et al. 2017; Wang et al. 2023). The temperature, pH, and EC parameters all align with typical domestic wastewater profiles, suggesting this influent would respond favorably to conventional treatment methodologies.

The TDS concentration varied between 1723 and 2414 mg/L in wastewater, with an average of 1991.58 ± 203.62 mg/L. All TDS measurements obtained in this study fell below the WHO (2006) standards. The elevation of TDS may be attributed to high concentrations of various dissolved substances, including chloride, sulfate, phosphate, carbonate, bicarbonate, sodium, calcium, magnesium, potassium, and other inorganic and organic compounds (Chapman and McPherson 2016). These compounds can originate from natural sources and anthropogenic activities, such as agricultural runoff and industrial discharges, resulting in significant environmental and health implications (Pushpalatha et al. 2022). Based on the comprehensive wastewater analysis data, it became clear that many parameters associated with water quality alarmingly exceed the regulatory limits established by Algerian environmental standards (JORA 2006).

The influent sample had an extraordinarily high level of turbidity, quantified at 109.25 ± 13.12 NTU, coupled with an increased concentration of suspended solids at 154.50 ± 49.91 mg/L, which significantly exceeds the provided limit of 35 mg/L (JORA 2006). This data suggests a critical need for immediate intervention to mitigate the pollution levels in the water system. Implementing in situ turbidity measurements can provide actual monitoring and help control the concentrations of technical support services effectively (Torres et al. 2013). Moreover, the BOD₅ and COD concentrations present an alarming scenario, as they are documented at 139.33 ± 32.72 mg/L and 233.00 ± 62.19 mg/L, respectively, both of which are markedly above their designated thresholds, specifically 35 mg/L for BOD₅ and 120 mg/L for COD (JORA 2006).

Table 1
Physicochemical characterization of the influent, and limit values of the discharge parameters. Number of samples (n = 12), (Average $\pm$ S.D) and (Min - Max). Experiments between January and December 2023.

Parameters	Wastewater	Limit values of the discharge parameters		
	Influent	WHO 1973	Metcalf & Eddy 2003	JORA 2006
Water T (°C)	19.25 $\pm$ 5.71 (13.00–29.00)	/	/	30
pH (/)	7.59 $\pm$ 0.20 (7.23–7.98)	6.5–8.5	/	6.5–8.5
DO (mg/L)	0.46 $\pm$ 0.14 (0.29–0.79)	> 5 mg/L	/	/
EC (mS/cm)	4.38 $\pm$ 0.29 (3.84–4.76)	2000	/	3.00
TDS (mg/L)	1991.58 $\pm$ 203.62 (1723.00–2414.00)	/	250–850	/
Salinity (mg/L)	1.91 $\pm$ 0.50 (1.30–2.80)	/	/	/
Turbidity (NTU)	109.25 $\pm$ 13.12 (89.00–132.00)	/	/	/
TSS (mg/L)	154.50 $\pm$ 49.91 (98.00–268.00)	> 70 mg/L	110–400	35
BOD ₅ (mg/L)	139.33 $\pm$ 32.72 (97.00–221.00)	> 25 mg/L	100–360	35
COD (mg/L)	233.00 $\pm$ 62.19 (165.00–373.00)	> 80 mg/L	250 – 1000	120

The BOD₅/COD ratio stands at 0.59, suggesting that the wastewater exhibits a moderate level of biodegradability; conversely, the COD/BOD₅ ratio is determined to be 1.67, indicating a more complex relationship between these two parameters. In alignment with the insights provided by Metcalf and Eddy (2003), BOD₅/COD ratios that fall within the range of 0.5 to 0.7 are indicative of wastewater that is only partially biodegradable, thus implying that such wastewater would substantially benefit from the

implementation of biological treatment processes designed to enhance its degradability and reduce pollutant loads.

High standard deviations, especially observed in the TDS measurement at 203.62 mg/L, TSS at 49.91 mg/L, and COD at 62.19 mg/L, are clear indicators of significant temporal fluctuations in the composition of wastewater throughout the designated sampling period, likely reflecting the erratic and inconsistent nature of the pollutant loads entering the treatment system. Such variability in the data underscores the critical need for a thorough understanding of the sources and dynamics of the inputs contributing to the wastewater characteristics, as these factors are essential for devising effective management and treatment strategies. Consequently, addressing these issues will not only be vital for compliance with regulatory standards. Still, it will also play a crucial role in protecting the environmental integrity of the aquatic ecosystems affected by the discharge of such wastewater.

3.2. Performance and removal efficiencies

3.2.1. Water temperature, pH, and DO

Table 2 shows the results of the control parameters monitored in the study period. The analysis of monthly temperature changes in wastewater treatment systems reveals significant differences between planted VFCW systems and non-planted (NP) systems.

Data collected over 12 months show that influent wastewater temperatures fluctuate between 13.0°C and 29.0°C, with an annual average temperature of $19.25 \pm 5.71^\circ\text{C}$. The planted systems demonstrate a higher temperature regulation capacity compared to the Np system. The mean temperature of low density (LD) is considered the most stable ($16.88 \pm 3.24^\circ\text{C}$), whereas the average temperature dropped in medium density (MD) to $16.85 \pm 3.07^\circ\text{C}$ and $17.18 \pm 3.79^\circ\text{C}$ in high density (HD) (Fig. 6a). The Np system showed more temperature variability, while the average under this condition was $17.78 \pm 4.07^\circ\text{C}$. This is consistent with the study by Gaballah and Lammers (2025), which emphasizes the importance of plants for temperature management in CWs.

This vegetation-based thermal buffering effect is particularly evident during summer when peak temperatures are substantially reduced in CWs. The optimal planting density appears to be 6 plants/m², as higher densities yield minimal additional benefits. These stabilized thermal conditions likely enhance biological treatment processes and reduce energy requirements, making planted systems particularly valuable in regions with significant seasonal temperature variations. It is noted that plant mulching directly affects the thermal characteristics of processing systems.

Table 2

Mean concentrations with standard deviations (Average $\pm$ S.D), (Min - Max) of effluent water quality parameters (n = 12) between January and December 2023.

Parameters	Control (NP)	Low Density (LD)	Medium Density (MD)	High Density (HD)
Density	0 plants/m ²	3 plants/m ²	6 plants/m ²	9 plants/m ²
Water T (°C)	17.78 $\pm$ 4.07	16.88 $\pm$ 3.24	16.85 $\pm$ 3.07	17.18 $\pm$ 3.79
Min – Max	(12.60–24.00)	(10.80–20.80)	(11.20–21.00)	(10.90–22.00)
pH	7.19 $\pm$ 0.28 ^a	6.95 $\pm$ 0.21 ^a	6.70 $\pm$ 0.22 ^{a,b}	6.77 $\pm$ 0.20 ^{a,b}
Min – Max	(6.65–7.56)	(6.71–7.35)	(6.23–6.99)	(6.32–6.99)
DO (mg/L)	2.97 $\pm$ 1.30 ^a	3.95 $\pm$ 0.68 ^{a,b}	4.08 $\pm$ 0.93 ^{a,b}	4.75 $\pm$ 0.73 ^{a,b}
Min – Max	(0.63–4.96)	(2.44–4.98)	(2.37–5.23)	(2.75–5.50)
EC (µS/cm)	3.54 $\pm$ 0.61 ^a	2.48 $\pm$ 0.42 ^{a,b,c}	2.11 $\pm$ 0.52 ^{a,b}	1.73 $\pm$ 0.36 ^{a,b}
Min – Max	(2.08–4.22)	(1.83–3.22)	(1.33–2.98)	(1.14–2.33)
TDS	1623.4 $\pm$ 154.0 ^a	1463.5 $\pm$ 360.5 ^a	1255.25 $\pm$ 277.3 ^a	1240.3 $\pm$ 158.28 ^a
Min – Max	(1452–1925)	(489–1854)	(567–1567)	(986– 1532)
R (%)	17.79%	26.63%	36.78%	36.78%
Salinity	1.61 $\pm$ 0.44	1.19 $\pm$ 0.32 ^a	1.08 $\pm$ 0.29 ^{a,b}	1.03 $\pm$ 0.25 ^{a,b}
Min – Max	(1.20–2.50)	(0.70–1.70)	(0.80–1.60)	(0.70–1.50)
R (%)	14.66	34.96	41.68	43.67
Turbidité	12.83 $\pm$ 3.11 ^a	10.16 $\pm$ 4.91 ^a	10.69 $\pm$ 4.48 ^a	9.23 $\pm$ 4.53 ^a
Min – Max	(8.10–18.90)	(4.00–22.60)	(3.00–19.00)	(3.00–16.00)
R (%)	88.06	90.32	89.86	91.19
TSS (mg/L)	32.50 $\pm$ 12.10 ^a	25.75 $\pm$ 5.33 ^a	24.50 $\pm$ 5.76 ^a	21.17 $\pm$ 6.74 ^a
Min – Max	(14.00– 65.00)	(12.00– 34.00)	(13.00– 33.00)	(11.00– 36.00)
R (%)	78.10	82.24	82.89	85.40
BOD ₅ (mg/L)	13.92 $\pm$ 4.56 ^a	11.67 $\pm$ 4.60 ^a	10.17 $\pm$ 4.90 ^a	8.42 $\pm$ 4.19 ^a
Min – Max	(10.00–24.00)	(4.00–21.00)	(3.00–21.00)	(5.00–20.00)
R (%)	89.92	91.33	92.66	93.86

Parameters	Control (NP)	Low Density (LD)	Medium Density (MD)	High Density (HD)
Density	0 plants/m ²	3 plants/m ²	6 plants/m ²	9 plants/m ²
COD (mg/L)	71.68 ± 17.47 ^a	59.99 ± 22.12 ^a	43.57 ± 12.57 ^a	40.84 ± 10.91 ^a
Min – Max	(54.20–119.00)	(41.50–122.40)	(32.10–76.00)	(26.90–69.00)
R (%)	67.41	72.73	79.90	81.26

^a High significant difference (HSD of Tukey test: $p < 0.05$) between WW and the rest of the beds, ^b High significant difference (HSD of Tukey test: $p < 0.05$) between unplanted and planted beds and ^c High significant difference (HSD of Tukey test: $p < 0.05$) between the planted beds.

The variance analysis reveals significant differences between the groups ($F > 2.83$, $p < 0.05$), confirming the influence of planting density on thermoregulation. Correlations ($R^2 = 0.75$) with raw wastewater vary according to density (Np, LD, MD, and HD systems). Despite this, it was found to be in a suitable range (between 16.5 and 32°C) to favor the removal of contaminants in the CWs (Zhu et al. 2018). On the side of increased bacterial activity, which is initiated at a temperature of $> 20^\circ\text{C}$ (Morvannou et al. 2013). The effect size ($\eta^2 = 0.025$) is small, suggesting that within this dataset, the various VFCW and wastewater measurements yield statistically similar results.

Seasonal fluctuation suggests that all VFCW systems experience peak temperatures during summer (July-August) and lowest temperatures during winter (February-March). Nevertheless, these fluctuations are to some extent moderated by the buffering effect of vegetation and evaporation. This thermoregulation also enhances biological treatment performance and maintains the culture for microbial action in CWs (Kadlec and Knight 1996).

From the one-way ANOVA results on pH data across the four treatment conditions (Np, LD, MD, and HD system), it can be concluded that there is a significant difference between the pH values of wastewater influent and the remaining groups ($p < 0.05$). Furthermore, the effect size ($\eta^2 = 0.41$) was large, indicating that nearly 41.71% of the variance in pH was associated with the treatment conditions. Descriptive statistics tell us there was an apparent gap among groups. The wastewater group had the highest mean pH value of 7.59 ± 0.20 , followed by Np 7.19 ± 0.28 , then LD 6.95 ± 0.21 , HD 6.77 ± 0.20 , and MD 6.70 ± 0.22 (Fig. 6b). The outcomes of this research contradict those published by Ouattara et al. (2011). The reduction in pH observed over the treatment stages can be attributed to the metabolic activity of microorganisms. The breakdown of phosphate and nitrogen compounds by microorganisms results in the formation of acids. Concurrently, acid-forming bacteria during the breakdown of organic material release volatile fatty acids. The cumulative result of these biological processes explains the shift of water from slightly alkaline to more neutral over the course of the treatment system (Kumar et al. 2020; Sandri and Reis 2021). Post-hoc Tukey's HSD tests confirm significant differences, especially between

wastewater and other treatment groups (especially MD and HD). This suggests that processing techniques have varying effects on the acidity and alkalinity of water. She also emphasizes the complex interactions between plant density, seasonal variations, and biogeochemical processes in VFCWs.

Statistical analysis of DO concentrations across the four VFCWs reveals highly significant differences between the WW influent and the other groups ($p < 0.05$), with treatment type accounting for approximately 24.61% of the variation in DO levels ($\eta^2 = 0.2461$). The results reveal a transparent oxygenation gradient across the treatment process, with raw wastewater (WW) showing severely oxygen-depleted conditions (0.46 ± 0.14 mg/L). In comparison, each subsequent treatment stage demonstrates progressively higher oxygenation: VFCWNpl (2.97 ± 1.30 mg/L), planted LD system (3.95 ± 0.68 mg/L), planted MD system (4.08 ± 0.93 mg/L), and planted HD system (4.75 ± 0.73 mg/L) (Fig. 6c). The results reveal a robust positive correlation ($R^2 = 0.8296$) between the number of plant species and treatment performance.

Post-hoc Tukey's HSD comparisons confirm significant differences between the wastewater and all treatment stages ($p < 0.05$), as well as between the first VFCW Np and the planted (LD, MD, and HD systems). The elevation in dissolved oxygen levels can be attributed to oxygen transport mechanisms through plant roots in the CW systems. This oxygen delivery establishes optimal conditions for several treatment processes, including enhanced microbial growth, accelerated organic matter degradation, improved nitrification efficiency, and effective bacterial inactivation (Wang et al. 2012).

These findings demonstrate the VFCW's effectiveness in re-oxygenating wastewater, with the final stage (planted HD system) providing optimal oxygen conditions to support aerobic biological treatment processes essential for effective wastewater purification.

3.2.2. Electrical Conductivity, TDS, and Salinity

A comprehensive multivariate investigation of VFCWs revealed statistically significant spatio-temporal variations in electrochemical parameters, demonstrating the role of plant density in modulating wastewater treatment processes. The analyses performed by a one-way ANOVA showed significant differences in means across study groups ($p < 0.001$). This supports the hypothesis that vegetation density affects the electrochemical processing of the waste constituents in wastewater.

The EC (Fig. 7a) displays a clear stepwise progression with the following average values: for wastewater influent, 4.38 ± 0.29 mS/cm; for the Np system, 3.54 ± 0.61 mS/cm; for the LD system, 2.48 ± 0.42 mS/cm; for the MD system, 2.11 ± 0.52 mS/cm; and for the HD system, 1.73 ± 0.36 mS/cm. The TDS exhibited a dynamic change in concentration (Fig. 7b), with average values ranging from 1991.58 ± 203.62 mg/L in the wastewater influent to 1623.4 ± 154.0 mg/L in the Np system, 1463.5 ± 360.5 mg/L in LD system, 1255.25 ± 277.3 mg/L in MD system, and 1240.3 ± 158.28 mg/L in HD system. There were significant inter-configuration differences ($P = 0.001$), greater with the Npl system and the other planted systems, LD and HD. Also, for TDS, the LD-planted system was significantly different ($P = 0.001$) from the HD-planted system.

Salinity levels (Fig. 7c) revealed complex biogeochemical relationships with mean values ranging from 1.30 to 1.91 and statistically significant variations across plant density gradients ($F = 4.836$, $p = 0.019$). Post-hoc Tukey's HSD test revealed significant contrasts between the NP system and HD system vegetated treatments, suggesting a strong plant-mediated electro geochemical process.

Variability tests substantiated the intrinsic complexity of the system, where EC exhibited moderate heterogeneity ($SD = 0.29$ to 0.61), TDS exhibited extensive fluctuations ($SD = 158.28$ to 360.52), and salinity exhibited uniform variations ($SD = 0.25$ to 0.50). These findings reveal the sophisticated, multidimensional interactions between plant density, seasonal processes, and electro geochemical processes in VFCWs as sophisticated, adaptive bioengineered systems with immense worth to future wastewater remediation practices.

3.2.3. Removal of TSS and turbidity

Total TSS is defined as the solid components that can be removed from water by filtration. These components include sediments, algae, plankton, and any other insoluble particles. TSS is a parameter essential to water quality (Rossi et al. 2006). In addition to TSS, water has other quality indicators such as turbidity. The latter is a measure of the level of cloudiness of liquid as a result of the presence of suspended particles that scatter light. It quantifies the degree to which material in suspension reduces the passage of light through water. Higher values of turbidity indicate greater amounts of suspended particles in water (Adjovu et al. 2023).

Mean TSS concentration decreased from 154.50 ± 49.91 mg/L in the wastewater influent down to 32.50 ± 12.10 mg/L in the Np and down further to 25.75 ± 5.33 mg/L in LD, 24.50 ± 5.76 mg/L in MD, and 21.17 ± 6.74 mg/L in HD systems (Fig. 8a). Similarly, turbidity was lowered from 109.25 ± 13.12 NTU in the wastewater influent down to 12.83 ± 3.11 NTU in the Np, 10.16 ± 4.91 NTU in LD, 10.69 ± 4.84 NTU in MD, and 9.23 ± 4.53 NTU in HD (Fig. 8b). After treatment, both TSS and turbidity measurements were compliant with Algerian water quality regulatory standards for discharge, with values that did not exceed the 35 mg/L for TSS and 45 NTU for turbidity thresholds (JORA 2006). TSS removal followed similar patterns to organic matter removal, with mean annual removal efficiencies of 78.10%, 82.24%, 82.89%, and 85.40% for Np, LD, MD, and HD density treatments, respectively (Fig. 8a).

The current study has shown that the plant density is a key factor for TSS and turbidity removal in VFCWs. Results reveal a robust positive (TSS: $R^2 = 0.92$, $p < 0.05$; turbidity: $R^2 = 0.76$, $p < 0.05$) between the number of species and the performance of the treatments (Fig. 8a and 8b), with the mean efficiencies of TSS rising from 78.10% (Np) to 85.40% (HD) and turbidity from 88.06% for Np to 91.19% for HD density. This result is comparable to TSS removal recorded by Zorai et al. (2025) in the same study region. ANOVA shows the differences among treatments are significantly different ($p < 0.001$). The progressive improvement in performance is explained by several synergistic mechanisms: increased microbial biofilm surface area on roots, improved substrate oxygenation through root oxygen transfer, and optimization of physical filtration processes (Vymazal 2019). The observed seasonal variations, particularly pronounced during winter periods (minimum efficiencies), highlight the importance of plant

diversity for maintaining stable treatment throughout the year. These results confirm the interest in favoring multi-species systems to optimize total suspended solids removal in CWs.

Enhanced filtration effects from dense root networks and reduced flow velocities resulting from greater hydraulic resistance explain the superior performance of HD plantings in TSS removal. The results of this study remain higher compared to other studies (Table 3): Rahmadyanti and Wiyono (2017), with a density of 10 plants/m² and HRT from 20 days (>35.4%); García-Ávila. (2020), with a density of 4 plants/m² and HRT from 1.25 days (>41.28%); and Khouloud et al. (2024), with an intensity of 3 plants/m² and HRT for 3 days (>22.55%).

Examination of effluent solids (TSS and turbidity) revealed differences in particle size distribution between treatments. The HD density showed more effective removal compared to other treatments, likely due to the finer root structure and associated biofilm development, which provides more efficient filtration of small colloidal particles.

3.2.4. Organic matter removal (COD and BOD₅)

The BOD₅ is a laboratory procedure used to assess the level of dissolved oxygen utilized by microorganisms to decompose organic materials in a given water sample over 5 days at 20°C. It is a measure of organic pollution of water bodies like rivers and lakes. In other words, it shows the level of organic contaminants in a given water sample (Galinha et al. 2018). In addition, the oxygen required for the complete oxidation of organic carbon-to-dioxide carbon, water, and ammonium is termed COD. This measurement is not accurate since it cannot tell the difference between materials that can undergo biological oxidation and inanimate substances (Méndez-Mendoza et al. 2015). It can be said that the higher value of BOD₅ in a wastewater sample, the more contaminated that sample is.

In this study, the average BOD₅ concentrations were reduced from 139.33 ± 32.72 mg/L in the wastewater inflow to 13.92 ± 4.56 mg/L with Np systems to 11.67 ± 4.60 mg/L with LD, 10.17 ± 4.90 mg/L with MD, and 8.42 ± 4.19 mg/L with HD systems, respectively (Fig. 9a). Although the COD values have declined from 233.00 ± 62.19 mg/L in influent to 71.68 ± 17.47 mg/L in the Np, 59.99 ± 22.12 mg/L in the LD, 43.57 ± 12.57 mg/L in the MD, and 40.84 ± 10.91 mg/L in the HD systems, respectively (Fig. 9b). After treatment, the BOD₅ and COD measurements met the Algerian regulatory standards for water quality discharge, with BOD₅ and COD values below the required 35 and 125 mg/L, respectively (JORA 2006).

The Fig. 9a and 9b illustrates the removal efficiencies for BOD₅ and COD across different plant density treatments. The HD system consistently outperformed the other configurations, achieving mean annual removal rates of $93.86 \pm 2.49\%$ for BOD₅ and $81.26 \pm 7.19\%$ for COD. The MD system demonstrated intermediate removal rates with $92.66 \pm 3.29\%$ for BOD₅ and $79.90 \pm 8.17\%$ for COD, while the LD treatment showed the lowest removal efficiencies among planted systems with $91.33 \pm 3.64\%$ for BOD₅; $72.73 \pm 12.46\%$ for COD. The Np control units exhibited significantly lower removal rates with $89.92 \pm$

2.56% for BOD₅ and $67.41 \pm 11.63\%$ for COD. This result is lower than the results recorded by Zorai et al. (2025) in the same study area, using the baffled horizontal flow CW system, and is higher than those reported by Rahmadyanti and Wiyono, (2017), with a density of 10 plants/m² and an HRT of 20 days; García-Ávila, (2020), with a density of 4 plants/m² and an HRT of 1.25 days; and Khoulood et al. (2024), with a density of 3 plants/m² and an HRT of 3 days.

The ANOVA results ($P = 0.018$) suggested statistically significant differences between Np and HD planted configurations in BOD₅ removal efficiency, and ($P = 0.02$, $P = 0.0085$) between Np and MD and LD planted configurations, respectively, in COD removal efficiency. The findings of this study show similar results when compared to the data presented by Tee et al. (2012) and Ouattara et al. (2011). Statistical analysis revealed data showing robust positive relationships between the density of plants and organic matter elimination, with correlation coefficients of 0.99 for BOD₅ and 0.94 for COD removal rates. Effluent quality showed greater stability in the HD treatment, with standard deviations in effluent concentrations approximately 19.12% lower than in the LD treatment in BOD₅, and 57.71% in COD. This improved stability is particularly valuable for applications requiring consistent compliance with discharge standards. Several complementary mechanisms explain the enhanced performance of higher-density HD plantings in organic matter removal. These include an increased root surface area that provides greater attachment sites for biofilm development compared to lower-density LD plantings, and creates more opportunities for microbial degradation of organic compounds (Kurzbaum 2022). In addition, enhanced oxygen transfer to the rhizosphere stimulates aerobic degradation processes. Oxygen measurements indicated rates of 3.95, 4.08, and 4.75 mg O₂/L from roots in the LD, MD, and HD treatments, respectively. This increased oxygen availability supports more efficient aerobic degradation pathways with higher energy yields (Tao et al. 2010), and greater production of exudates that support microbial activity.

3.3. Current Study vs. Previous Studies

The current study, with a plant density of 9 plants/m² and a 3-day time HRT, demonstrated superior contaminant removal efficiencies compared to previous CW investigations (Table 3).

For TSS, the system achieved 85.40% removal, significantly outperforming García-Ávila. (2020) by 22.55%, Khoulood et al. (2024) with 3 plants/m² by 41.28%, and Rahmadyanti and Wiyono (2017) by 35.4%. However, the results of this study remain close to the results of Ouattara et al. (2018). In the same way, BOD₅ removal efficiency was as high as 93.86%, which is 18.47% more than that of García-Ávila. (2020), 39.08% more with a 3-day HRT as compared to the study of Khoulood et al. (2024), and 24.53% more than that of Rahmadyanti & Wiyono (2017). Besides, it should be pointed out that the present structure got the similar success as that of the longer HRTs (6–20 days) and different plant densities, while only the work of Oladejo et al. (2015), with a 9-day HRT, and shows a higher rate of BOD₅ removal (100%). This study's findings demonstrate that the integration of moderate plant density (9 plants/m²) and comparatively short HRT (3 days) provides an optimized balance between treatment

efficacy and operating parameters for VFCWs, which is a great step for wastewater treatment in less resourceful areas.

Table 3
Current Study vs. Previous Studies removal comparison

Reference	Density	HRT (days)	Parameter	Previous Studies	Current study	$\Delta \backslash \text{varvec{R}} \backslash \text{varvec{E}}$ (%)
García-Ávila, 2020	4	1.12	TSS	62.85	85.40	22.55
			BOD ₅	75.39	93.86	18.47
			COD	64.78	81.26	16.48
Khouloud et al. 2024	3	3	TSS	44.12	85.40	41.28
			BOD ₅	54.78	93.86	39.08
			COD	58.66	81.26	22.6
	3	7	TSS	83.30	85.40	2.1
			BOD ₅	84.38	93.86	9.48
			COD	77.45	81.26	3.81
Rahmadyanti and Wiyono, 2017	10	20	TSS	50.00	85.40	35.4
			BOD ₅	69.33	93.86	24.53
			COD	78.52	81.26	2.74
Oladejo at al. 2015	6	3	TSS	63.34	85.40	22.06
			BOD ₅	58.45	93.86	35.41
	6	6	TSS	67.41	85.40	17.99
			BOD ₅	85.52	93.86	8.34
	6	9	TSS	64.75	85.40	20.65
			BOD ₅	100	93.86	-6.14
Ouattara et al. (2018).	20	/	TSS	86.8	85.40	-1.4
			COD	86.3	81.26	-5.04

3.4. Potential limitations and future work

The study has key limitations, including the small 0.125 m² scale, which may not accurately reflect full-scale system dynamics; the 12-month duration, which may overlook long-term issues such as clogging and biofilm maturation; and the constant influent loading, which doesn't represent real municipal variability. Future research should address these by using larger pilot systems exceeding 5 m², conducting longer studies of more than 3 years to examine root structure and microbial evolution, testing different plant species across various climates, investigating pathogen removal mechanisms, and conducting life cycle and economic assessments of dense planting configurations.

4. Conclusions

A vertical flow CW planted with *Phragmites australis* was applied for one year as an effective, sustainable municipal wastewater treatment process where plant density was found to be a key operational factor. HD configuration with 9 plants/m² always yielded better results than MD, LD, and the Np configuration for all measured parameters with higher removal efficiencies for BOD₅ at 93.86 ± 2.49%, COD at 81.26 ± 7.19%, and TSS at 85.40 ± 5.66%.

Statistical analysis affirmed that significant differences do exist between planted and Np system, with high positive correlations between densities of planting and contamination removal. More specifically, a HD of plantings significantly improved DO from 0.46 ± 0.14 mg/L in the influent to 4.75 ± 0.73 mg/L in the HD effluent, thus creating favorable conditions for aerobic microbial processes. This indicates a great potential application insight when VFCWs are well designed as nature-based interventions among decentralized wastewater management practices within resource-constrained environments toward surpassing considerable environmental and economic benefits against commonplace treatment technologies.

As a practical recommendation, we propose that the investigated type of technological system, which consists of VFCW systems, *Phragmites australis* plant and 3 days, should be used in locations where legal provisions require a high efficiency in the organic matter treatment.

Declarations

Author Contributions

All authors contributed to the study conception and design (material preparation, data collection, and analysis). The first draft of the manuscript was written by Yahi Takai-Eddine, and all authors commented on the manuscript. All authors read and approved the submitted manuscript.

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Data Availability Statement

For anyone interested, the data can be obtained by emailing the corresponding author

Conflict of Interest

The authors declare no competing interests.

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Figures



Figure 1

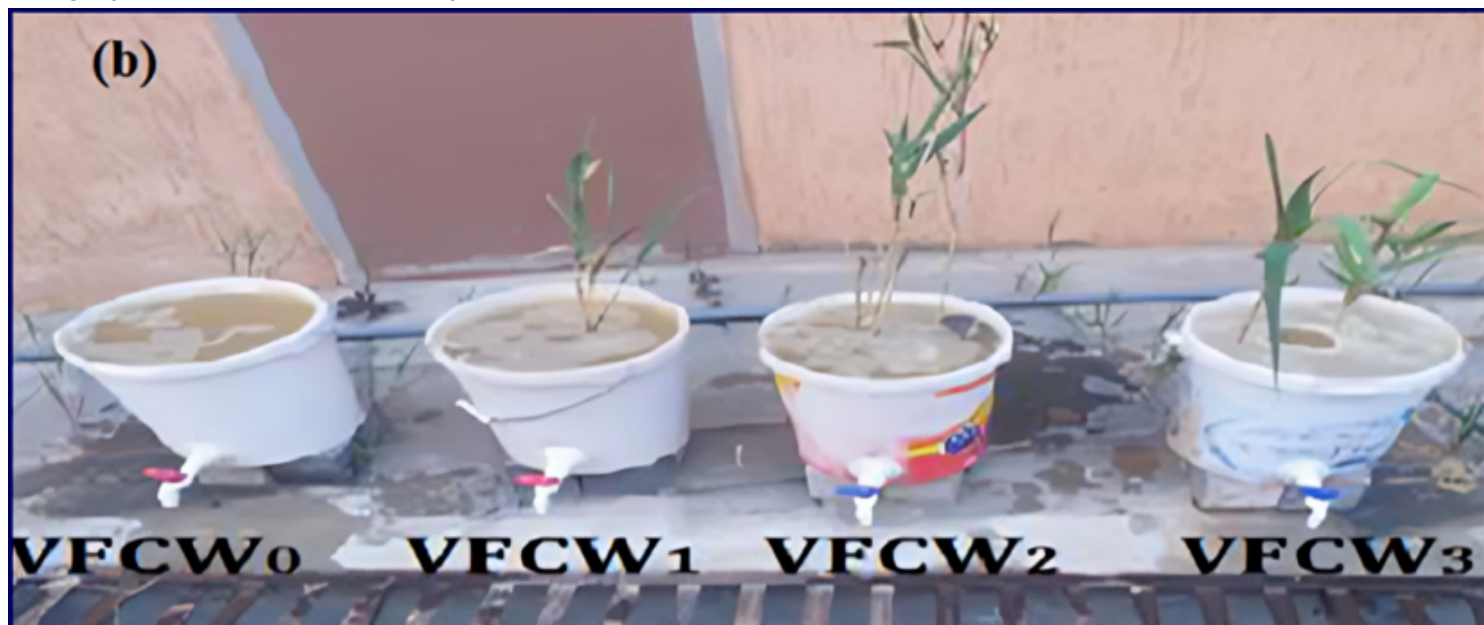


Figure 2

Profile photo of the four beds: VFCW₀ (Control), VFCW₁ (low-density: *P. australis*), VFCW₂ (medium-density: *P. australis*), and VFCW₃ (high-density *P. australis*).

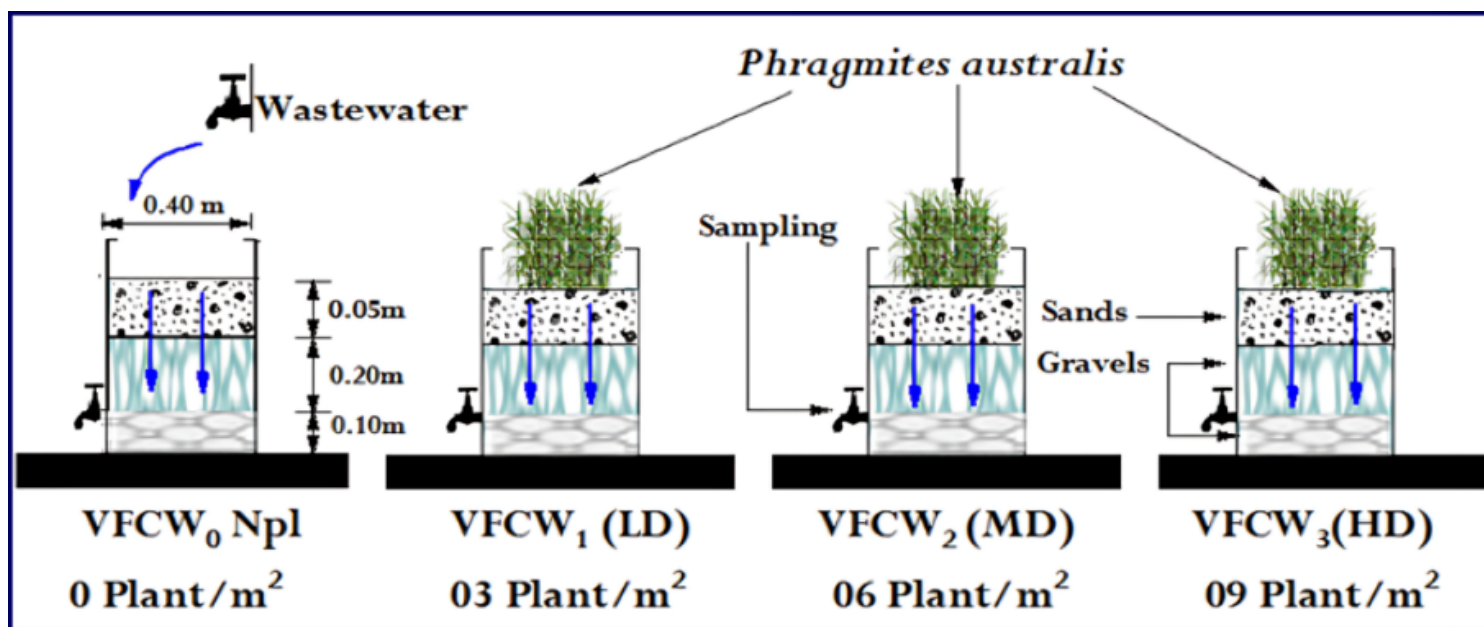


Figure 3

Schematic illustration of the VFCWs systems: (a) Nonplanted, (b) low-density, (c) medium-density, and (d) high-density



Figure 4

Plants used in this study (*Phragmites australis*)

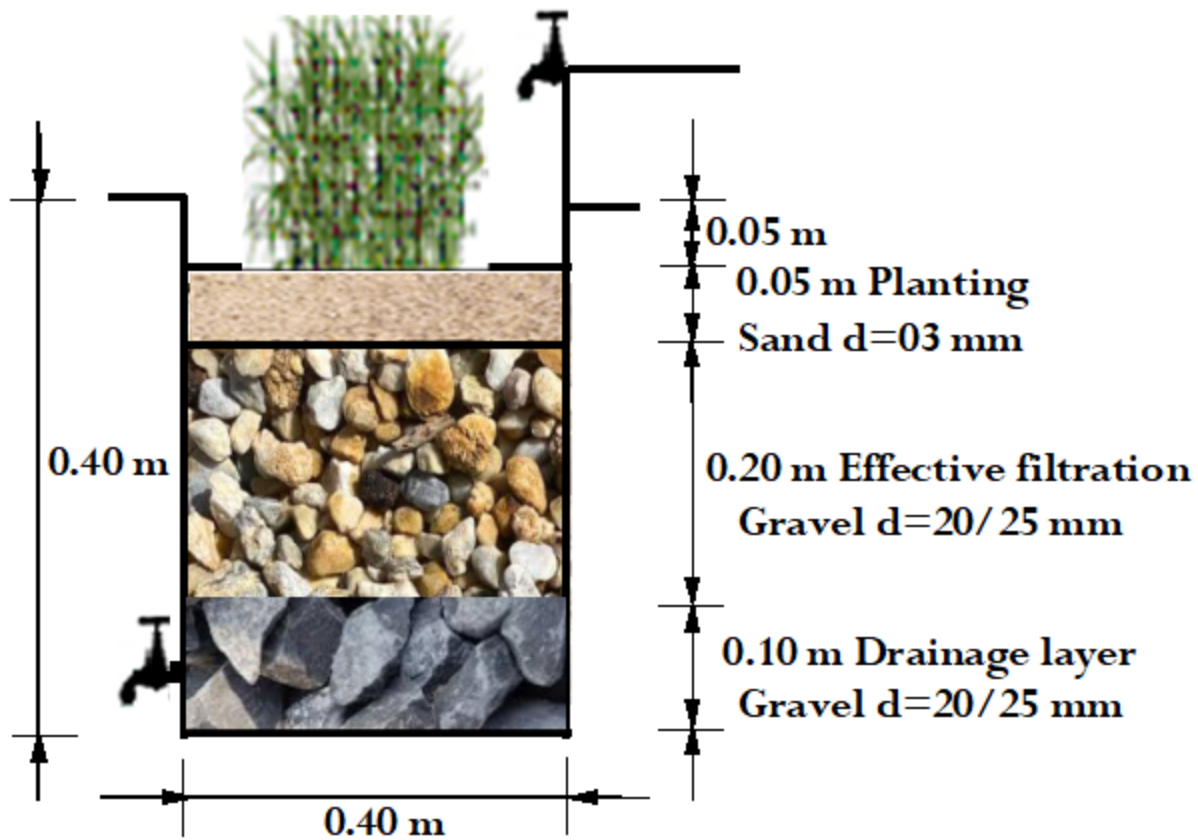


Figure 5

The substrates used in this study

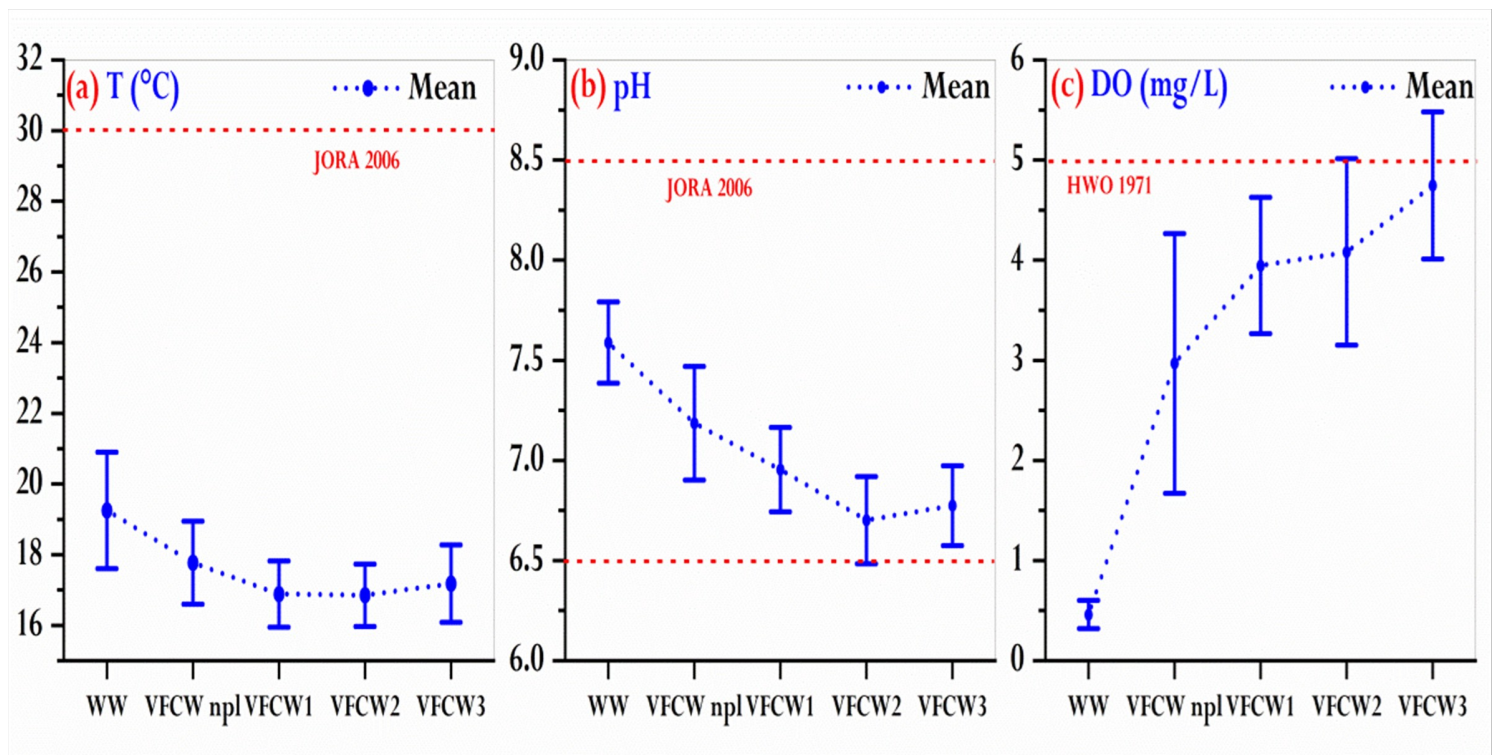


Figure 6

(a) Water temperature, (b) pH, and (c) dissolved oxygen in the inlet and in the different VFCWs outlet (unplanted control and the VFCWs planted with *Phragmites australis*).

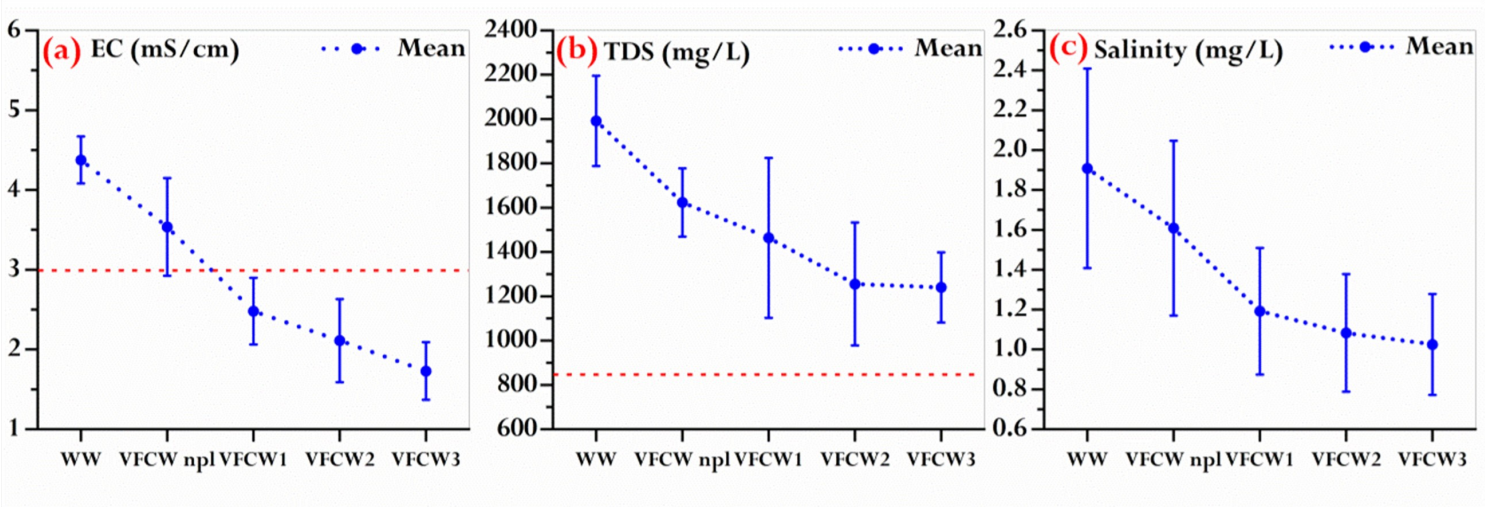


Figure 7

(a) Electrical conductivity, (b) TDS, and (c) salinity, in the inlet and in the different VFCWs outlet (unplanted control, and the VFCWs planted with *Phragmites australis*).

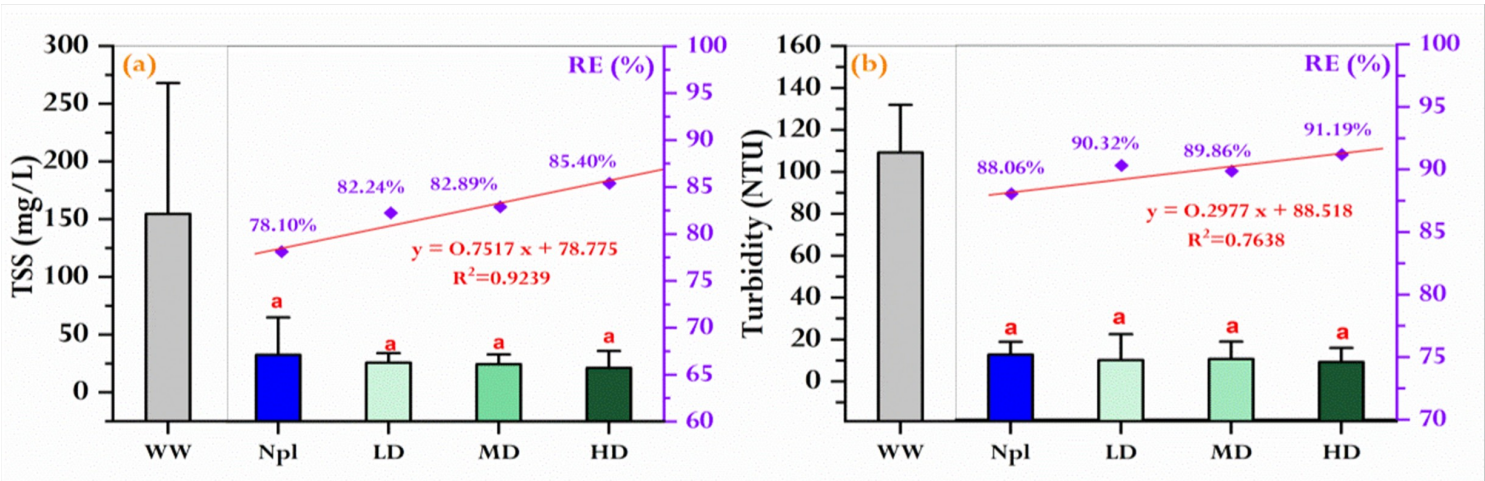


Figure 8

Comparison of the concentrations (mg/L) in the inlet and in the different VFCWs outlet, removal efficiency (%) and coefficient of correlation (R^2) for (a) TSS and (b) turbidity. Letter a, indicates significant difference between WW and the rest of the beds

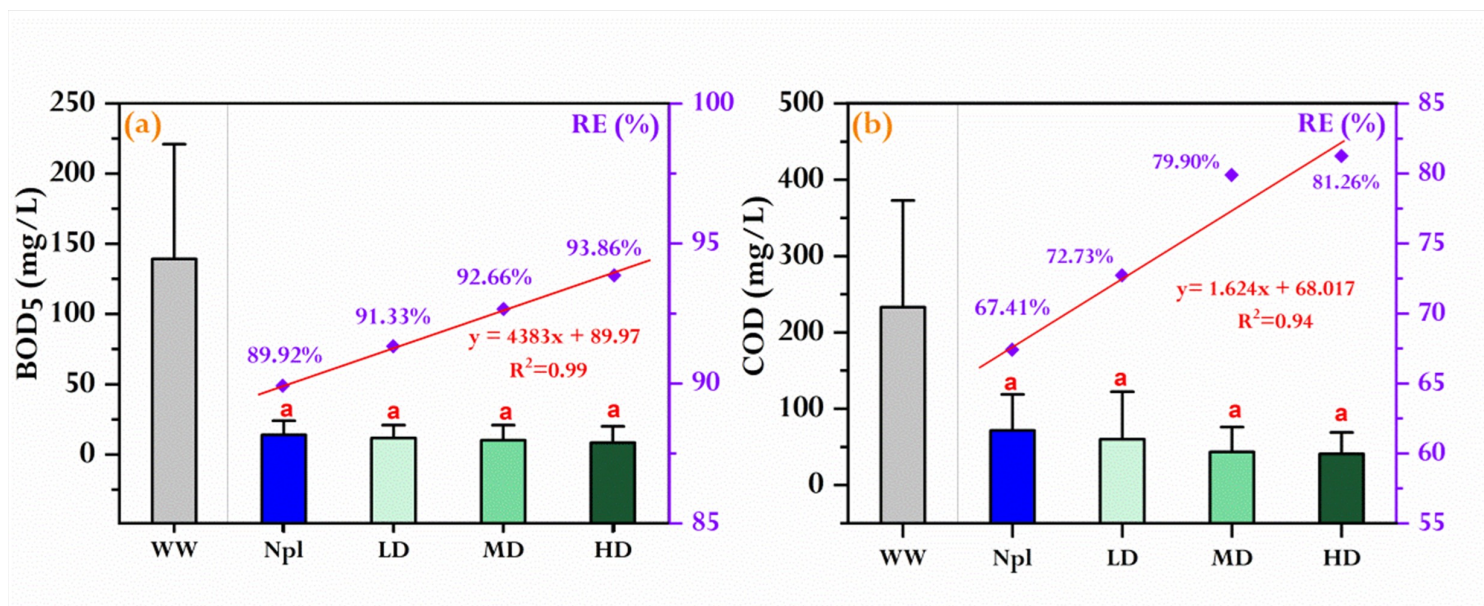


Figure 9

Comparison of the concentrations (mg/L) in the inlet and in the different VFCWs outlet, removal efficiency (%) and coefficient of correlation (R^2) for (a) BOD_5 and (b) COD. Letter a, indicates significant difference between WW and the rest of the beds