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Improving Wastewater Reclamation Using Constructed Wetlands by Artificial Plastic Biofilm Carriers

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ABSTRACT

Increasing the demand for potable water, followed by the high quantity of discharged effluents linked with the water scarcity problems has necessitated giving more attention to improving wastewater treatment processes and operations. The constructed wetland has proven to be an excellent green sustainable technique for purification. This study aimed to examine the performance of four experimental free water surface constructed wetlands (FWSCWs) for the depuration of sewage effluents as a secondary treatment stage during winter season conditions. The objectives were to assess the raw and treated wastewater concentrations, evaluate the removal efficiency of chemical oxygen demand (COD), biological oxygen demand (BOD), nutrients, and total suspended solids (TSS) of each treatment line, and compare the impact of plastic rings (biofilm carriers) and *Lemna minor* L. with the presence of gravel bed on the treatment efficiency and bacterial growth, as well as assess the plant's adaption and growth. The results showed that all treatment systems improved the water characteristics, and adding biofilm carriers enhanced the efficiency of water purification, especially BOD reduction. The combination of the plants, biofilm carriers, and gravel in the wetland filter significantly enhanced ($\rho < 0.05$) the treatment efficiency in terms of TSS, COD, BOD, Ammonia (NH_3), Nitrates (NO_3), and Orthophosphate (PO_4) compared to the control treatment system (gravel bed). Plant growth was restricted in the presence of carriers in the system. Further study for examining the system performance under summer conditions, which may improve the nutrient reduction rates by biofilm carriers, is underway.

Keywords: wastewater treatment, free water surface, *Lemna minor*, constructed wetland.

INTRODUCTION

Background

The extreme demand for potable water for different human activities has resulted in an increase in the wastewater discharge to the receiving watercourses, which adversely affects the aquatic environment (Fitton et al., 2019; Yaseen et al., 2019; Jiang et al., 2020; Al-Nabhan and Al-Abbawy, 2021). This problem crucially affects the developing countries that suffer from both water shortage issues (Yousif et al., 2022) and deficit in wastewater purification (Oliveira et al., 2021), which consequently discharge the untreated or poorly treated effluents to the natural water resources. The discharged wastewater is loaded with different contaminants, such as dyes, solid particles, heavy metals, nutrients, bacteria, and others, which has necessitated the importance of applying treatment technologies that clean up the polluted water (Rahman et al., 2020; Yaseen et al., 2021).

The recent attention was tending towards the improved purification systems connected with treated effluents in high quality, with low operation and maintenance costs (Oliveira et al., 2021). In comparison with the conventional methods of treatment, constructed wetlands (CWs) is a practical, eco-friendly, and low-cost green and engineered system that is widely applied for the treatment of various sewage, such as domestic, municipal, and industrial wastewaters, as well as landfill leachates, stormwater and others (Wu et al., 2014; Yaseen and Scholz, 2018). The efficiency of pollutant reduction by CW is mainly functioned by the wetland configuration, appropriate media, suitable plants, microbes, and operation (Ilyas and Masih, 2017; Shiwei et al., 2019). Among the CWs types, many authors agreed that free water-surface constructed wetlands (FWSCW) offer an acceptable treatment efficiency, flooding problems management, and improvement the biodiversity (Borin and Malagoli, 2015; Semeraro et al., 2015; Dal Ferro et al., 2018). In addition, it is the cheapest system in operation and maintenance compared with other CWs systems (Vymazal, 2010; Dal Ferro et al., 2018; Yaseen, 2018). However, the limited available surface area for biofilms attachments in conventional FWSCWs that consist of popular natural substrates (gravel, soil, and/or sands) (Zhang et al., 2016; Yaseen, 2018) has encouraged the designers to use wetlands integrated with another natural, man-made, and industrial substrates (Vohla et al., 2011). These substrates are available in different shapes and sizes and offer various colonies for the biofilms due to their characteristics of providing an additional specific surface area and intensive hydrophilicity. Biofilm carriers enhanced the microorganism attachment (Corzo and Sanabria, 2019) and consequently improved the treatment efficiency by increasing nutrient assimilation and oxygen transfer (Al-Amshawee et al., 2020).

In this regard, some studies have examined the impact of diverse biofilm carriers coupled with floating bed wetlands (Li et al. 2010; Cao and Zhang 2014; Zhang et al. 2016), subsurface flow wetlands (Corzo and Sanabria, 2019; Shiwei et al., 2019; Zamora et al., 2019), and hybrid systems (Chyan et al., 2013). However, no research has yet examined the performance of free water surface wetland planted with free-floating plants integrated with biofilm carriers prepared from waste plastic materials as a support media. In addition, it is required to study the system performance under different climate conditions, especially the winter and summer seasons. Therefore, this area of study needs more interest for better interpretation of the best CWs design, mechanism of pollutants reduction, and plants–support media relationship (Rahman et al., 2020).

Lemna minor L.

Among various species of FWS wetland macrophytes, researchers have agreed that the plant species *Lemna minor* L. (Duck weed) has an extra accumulation capacity for diverse pollutants. It is a free-floating plant that acclimatizes to different climate conditions and wastewater types. The high removal efficiency is linked with their ability to grow rapidly and consequently doubling the number of fronds, which accumulate the pollutants in their tissue within a few weeks, as the life cycle of *Lemna minor* L. is four to six weeks (Azeez and Sabbar,2012). Moreover, this plant could be reused several times in the treatment process and as fodder due to the limited fiber content and increasing percentage of protein in their tissue (Yaseen and Scholz, 2016; 2017).

Aim and objectives

This study aimed to analyze the performance of four experimental FWSCWs for the depuration of sewage effluents as a secondary treatment stage. The objectives were to: assess the raw and treated wastewater concentrations, evaluate the removal efficiency of COD, BOD, nutrients, and SS of each treatment line, and compare the impact of plastic rings (biofilm carriers) and plant with the presence of gravel bed on the treatment efficiency and bacterial growth, as well as assess the plant's adaption and growth.

MATERIALS AND METHODS

Materials and wastewater

The wastewater used in this study was collected from the main point of a wastewater treatment unit located at the University of Basrah, the campus of Garmmat-Ali (30^o 33' 20.6676" N, 47^o 44' 55.5612" E). This unit received the wastewater from all the buildings on campus.

The used biofilm carriers were plastic rings made of polyurethane, polyethylene, and polyvinyl chloride material, prepared by cutting the unwanted plastic hoses to the same size (47 pieces, each has; 3.5 cm diameter, 4 cm height, and 3.035 gm weight). The selected macrophytes, *Lemna minor* L., were collected from a pond in Basra Province, which has no contact with any wastewater source. The plants were carefully rinsed with deionized water to be clean from any dirt. The gravel bed, which was supplied from a local shop (Basra, Iraq), was washed with deionized water and then used in wetlands filters for drainage purposes and treatment.

Experiment setup and operation

A small-scale treatment system of FWSCWs was placed at the University of Basrah, Garmmat-Ali campus (Figure 1), in the roofed yard closed to the College of Science (30.5670° N, 47.7499° E, Basrah, Iraq) using plastic basins under semi-natural conditions. The system consists of four treatment lines and has been operated for three months from 1/12/2021 to 1/3/2022 (in addition to the prior two weeks for plant acclimatizing and growth) to examine the treatment performance based on the presence and absence of the plants and/or carriers in gravel bed wetland filters.

The experimental setup was constructed using rectangular plastic containers (as filters) with a height of 45 cm. The length and width of each filter were 93 cm and 35 cm, respectively. Each treatment line received the same wastewater quantity of 40 liters (equivalent to a depth of 35 cm) from the inlet taps connected with an elevated plastic storage tank of 500-liter capacity. The storage tank was filled with the wastewater weekly and used as a pre-treatment stage for particle sedimentation. All inlet taps were placed at the height of 30 cm from the tank bed to avoid the discharge of settled particles and to ensure that the wastewater distribution occurred by gravity. The wastewater was distributed from each tap to the wetlands filters after a specific period from the filling time, enough for particles to settle.

The first treatment line (filter 1; F1) contained plant, plastic biofilm carriers, and gravel. The second line (F2) consisted of plants and gravel to assess the impact of the carriers. The third line (F3) comprised plastic biofilm carriers and gravel to assess the impact of the plants. The fourth line (F4) contained gravel only as a control to assess the effect of gravel. The hydraulic retention time (HRT) was seven days, which was decided based on a preliminary test. Many authors applied this contact time within the recommendation for a typical FWS treatment system (Yaseen and Scholz, 2016; 2017). Each gravel layer was placed up to a depth of 8 cm in each filter using the size of 5–10 mm followed by 10–20 mm (top to bottom, see Figure 1). The carriers were placed in the first and third filter, using a filling ratio of 50% (47 units per m^3). Many researchers suggested this ratio within the allowable recommended range of 50–70% to achieve optimum biofilm growth, bacterial activity, and oxygen diffusion (Al-Amshawee et al., 2020).

Then, fresh weight of 600 g (equivalent to 80% covered surface area) was placed in the first and second treatment lines, as recommended by (Oliveira et al. 2021). This biomass density was chosen to avoid overcrowding problems and maintain enough coverage area to minimize algae

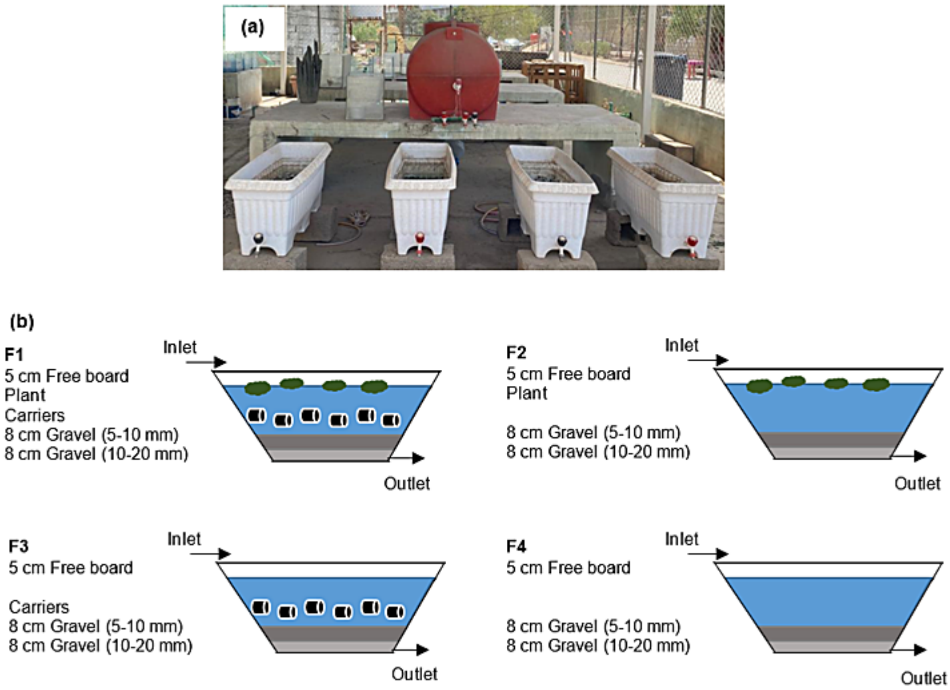

Figure 1. The experimental setup: (a) photograph, (b) diagram of each treatment system; F1 – contained plant, plastic carriers, and gravel; F2 – consisted of plants and gravel; F3 – comprised plastic biofilm carriers and gravel; F4 – contained gravel only

growth in the system (Zimmo, 2003). The system details are declared in Table 1. After the acclimatizing period of two weeks, the system was fed weekly based on fill and drain mode with an average flow rate of 40 l/week and hydraulic loading rate of 0.0175 $m^3/m^2 \cdot day$.

Samples analysis

The samples of 2 liters was collected from the inlet wastewater and the outlet water from each filter weekly to assess the treatment performance based on the water quality tests (APAH, 2012). A DR 5000 Hach Lange (Ger.) spectrophotometer was used to measure the chemical oxygen demand (COD). Total suspended solids (TSS) were measured by filtering the sample using sterile Millipor 0.64 mm filter paper. Turbidity was obtained by a turbidity meter (TB 300IR/ Lovibond/Germany). The pH, electric conductivity (EC), temperature (°C), and total dissolved solids (TDS) were determined with a pH meter (Hanna/ Romania). Dissolved oxygen (DO) and biological oxygen demand (BOD_5) were measured with Winkler's method (APHA, 2005). The spectrophotometer (V-1100D /Germany) was used to measure the reactive nitrate (NO_3) at wavelengths of 220 and 270 (APHA, 1999) and orthophosphate (PO_4) at a wavelength of (880) (APHA, 1999). Ammonia (NH_3) was tested by sending the samples to the central laboratory at the College of Agriculture. The removal efficiency R was determined using Eq. (1) (Mahdi and Al-Abbawy, 2019). Porosity P was calculated by Eq. (2):

$$R = ((I_c - O_c)/I_c) \times 100\% \quad (1)$$

where: I_c – inlet concentration, O_c – outlet concentration.

$$P = ((V_T - V_S)/V_T) \times 100\% \quad (2)$$

where: V_T – total volume, V_S – volume of the solid.

Plant growth monitoring

To assess the effect of plastic rings on the plant's growth, the growth rate of *Lemna minor* L. was measured. The plants were harvested after covering the system's surface area to avoid the case of overcrowding. The fresh weight of the plant was recorded during the setup period and after each time plants are harvested. The fresh biomass weights were taken after placing the harvested plants on absorbent paper for five minutes. The growth rate was calculated using equation 3 (Zhang et al., 2016).

$$G_r = \frac{\ln(W_f) - \ln(W_i)}{T_f - T_i} \quad (3)$$

where: G_r – relative growth rate, W_f – final dry weight of the plant, W_i – initial dry weight of the plant, T_f – final experimental time, T_i – initial experimental time.

Fecal coliform

Calculating the fecal coliform (FC) according to APHA (2005) was done by dissolving 4.96 M-FC medium in distilled water to achieve a 100 mL volume. The solution was then boiled and poured into the dishes. For a dilution ratio of 1/10, a sterilized mug and funnel (as filter devices) was used, and then 9 mL of the distilled water was taken by sterile syringes (10 mL volume) and added to the sterile tube of 10 mL size. The second dilution of 1/100 was also filtered using a sterile Millipore 0.64 µm filter paper, then the walls of the mug were washed

Table 1. Studies treatment systems details

Details	Filter 1	Filter 2	Filter 3	Filter 4
Dimension (m)	0.93*0.35*0.45			
Flow rate l/week	40			
Influent volume (liter)	40			
HRT (days)	7			
HLR(m^3/m^2.day)	0.0175			
Bottom layer	10–20 mm Gravel 8 cm height			
Top layer	5–10 mm Gravel 8 cm height			
Total volume (liter)	60			
Water depth (m)	0.35			
Porosity (%)	66	66	66	66
Vegetation	*Lemna minor*	*Lemna minor*	-	-
Plastic rings units	47	-	47	-

with sterile distilled water using a sterile syringe during filtration. After that, the first dilution was filtered, another plate was and planted on it; the sample number and the dilution 1/10 or 1/100 were written on it. As for the third dish, 10 mL of the concentrated sample (wastewater sample) was taken and filtered without dilution on a sterile filter paper. The paper was planted on the third dish, and only the sample number was written on it without dilution. The dishes were placed upside down in a water bath at a temperature of 44.5°C for 24 hours, after which the number of developing colonies was counted (in blue color only and ignoring lead or brown). The number of bacteria was calculated from the Eq. 4:

$$\frac{CFU}{100}\,(mL) = R_d \frac{n}{F_s} \times 100\% \tag{4}$$

where: *CFU* – colonies forming unit, R_d – Reciprocal dilution, n – the number of colonies in the plate, F_S – filter sample size.

Method of data analysis

All data were analyzed using the standard software Microsoft Excel (www.microsoft.com) and the IBM SPSS Statistics Version 22 (www.ibm.com). The normality test was applied using the Shapiro-Wilk test. The personality test was applied to determine the correlation coefficients of the parametric parameters. One-way ANOVA was performed to examine the differences among the operation variables within the treatment design.

RESULTS AND DISCUSSION

Raw water quality

Table 2 shows the characteristics of the raw wastewater collected from the collection point in the treatment unit (before the treatment application). The standard deviation, maximum and minimum values of each parameter demonstrated the variability of the raw wastewater characteristics from 1/12/2021 to 2/3/2022.

Treated water characteristics

The biological activities modify the wetland's environment in terms of the physical and chemical parameters. Usually, the temperature, pH, and dissolved oxygen are the main biotic parameters that affect the treatment performance in wetland systems (Kadlec and Wallace, 2008; Chyan, 2013).

Over the entire period of the monitoring and operation, the records of water temperature varied between 14 and 22.7°C, compared with the raw water temperature of 15 and 27.5°C. The average records (Table 3, Figure 2a) showed that the values were similar (17.2°C) for F1, F2 and significantly lower if compared with F3 and F4 (22°C). The difference in temperature values between the planted and unplanted filters was evident through the entire treatment period, as shown in Figure 2a. This is interpreted by the impact of the plants, which prevented the penetration of sunlight and consequently reduced the temperature in the system (Borne et al., 2014; Zhang et al., 2016). Note that temperature records in this study were within the range that does not affect the COD and BOD

Table 2. Characteristics of the raw wastewater

Parameter	Average	Standard deviation	Maximum	Minimum	Reading No.
Temp. °C	18.6	3.2	27.5	15.0	14.0
pH	8.4	0.7	9.2	6.9	14.0
EC (mS/cm)	6.0	0.8	7.3	5.0	14.0
TU (NTU)	114.2	10.7	135.0	99.0	14.0
TDS (mg/L)	1996.5	223.3	2470.0	1713.0	14.0
TSS (mg/L)	136.7	6.4	150.0	130.0	14.0
DO (mg/L)	3.7	0.8	5.1	2.2	14.0
BOD_5 (mg/L)	40.1	5.2	51.0	33.0	14.0
COD (mg/L)	134.4	29.5	191.0	98.0	14.0
NO_3 (mg/L)	26.6	2.3	29.9	22.4	14.0
NH_3 (mg/L)	46.5	3.6	51.3	39.4	14.0
PO_4 (mg/L)	2.9	0.4	3.9	2.2	14.0

reduction in a wetland system. This is because temperature between 2–26°C does not affect the COD and BOD reduction in wetlands; moreover, the nitrification process is significantly affected by temperatures between 30–40°C and occurs very slowly in the case of temperatures below 20 to 10°C (Vymazal, 2007; Chyan et al., 2013). This study's temperature records showed that most values were lower than the range for nitrification occurrence.

The pH values could alter the chemistry and biology of wetland water. This is because bacteria are only available within a pH environment between 4 and 9.5. In addition, the nitrification and denitrification bacteria activities are dominated at pH values of more than 7.2 and from 6.5 to 7.5, respectively (Chyan et al., 2013). In this study, the minimum and maximum pH values were ranged from 7.5 to 9.5, corresponding to raw values of 6.9 to 9.2 (Tables 2 and 3). These outcomes were within the allowable range for nitrification and denitrification occurrence. The mean values of pH of treated water for all filters were slightly increased compared with the raw water. There is no significant variation of mean pH values among the filters, which is shown clearly in the longitudinal profile (Figure 2b). The low respiration may be due to *Lemna minor* roots, which consequently reduces the amount of carbon dioxide released in F1 and F2. Moreover, the photosynthesis process in F1 and F2 is affected by increasing carbon dioxide consumption linked with higher pH and DO levels (Borne et al., 2014). The recommended pH range for plant growth is between 4.5–8.3 (Yaseen and Scholz, 2016).

The EC of wetland water affects the plant's growth and microbial activity, consequently affecting the degradation and uptake of the contaminants. Jurries (2003) suggested that the best EC values for plant and biota growth are equal to or less than 4 mS/cm. The minimum and maximum EC values of treated water were 4.1 and 7.7 mS/cm, corresponding to 5 and 7.3 mS/cm for the raw water. These results imply that EC could be a limiting factor for plant and bacteria growth. The mean values of EC were slightly increased in treatment filters compared with the raw water (Tables 2, 3). In addition, the EC values in treated water were higher in F3 and F4 than in the planted filters (no significant differences, $\rho > 0.05$), indicating no impact for the carriers in terms of EC, and only the plants played their role in reducing the EC. The same outcomes were proposed by Yaseen and Scholz (2017), confirming the plants' ability to pass a few salts within their semi-permeable membrane. Through the study period, the EC values increased weekly, reflecting the increase in raw water salinity (Figure 2c).

The values of TU, TDS, and TSS were decreased clearly in all treatment filters compared with corresponding parameters in the raw water (Tables 2 and 3). The wastewater turbidity after treatment was reduced from 114 NTU to 40, 45, 55, and 63 NTU in F1, F2, F3, and F4, respectively. For TDS, the values range as follows: F2>F3>F1>F4 without any significant differences. The mean inlet TSS concentration was 136.7 ± 6.4 mg/L (Figure 3), which was reduced when passing the water through the wetland treatment filters, which reflected, in turn, the removals (Table 3). The TSS values of treated water were significantly higher in F4 ($\rho < 0.05$), than F3 followed by F2 and F1. This indicates that the high performance is linked with planted wetlands and carriers. However, the prominent role was due to the plant's activities, as the mean values in F1 were significantly lower ($\rho = 0.029$) than in F3, and no differences were found between F1 and F2. Usually, TSS decreasing results from the high void space and porosity of substrates, in addition to the TSS trapping (Zidan et al., 2015). The authors mentioned that the excellent water quality achieved at TSS was less than 25 mg/l, the sound quality was achieved at TSS values ranging between 26 and 75, and acceptable quality was achieved at values between 76 and 150 mg/L (Zamora et al., 2019). In this study, all TSS values ranged between acceptable and good water quality, except the control values (F4, gravel bed) were within the allowable water quality only. The profile of TU, TSS, and TDS is shown in Figures 2d to f.

The level of DO is used as an indicator of the aerobic and anaerobic conditions in wetlands. During the aerobic environment, the dissolved oxygen that is available via the transportation of plants and the photosynthesis process is consumed by the decomposition of the plants and the reduction of NH_3-N and BOD_5. During the anoxic and slightly anaerobic environments, nitrate reduction has occurred. It is suggested that the level of DO of more than 2 mg/L does not cease nitrification, and more than 0.09 mg/L is restricted the denitrification (Chyan et al., 2013). The outcomes of the DO values varied between 6 to 12 mg/L, indicating that they limited the denitrification processes. The levels of DO in all filters were increased sharply compared with the raw water

Table 3. Characteristics of the treated water (F1 – contained plant, plastic carriers, and gravel; F2 – comprised of plants and gravel; F3 – comprised plastic biofilm carriers and gravel; F4 – contained gravel only)

Parameter	Filter	Average	Standard deviation	Maximum	Minimum	Reading No.
Temp °C	F1	17.2	2.00	22.1	14.0	14
	F2	17.2	2.02	22.3	14.2	14
	F3	22.7	0.00	22.7	22.7	14
	F4	22.1	0.00	22.1	22.1	14
pH	F1	8.6	0.55	9.3	7.9	14
	F2	8.5	0.66	9.5	7.5	14
	F3	8.5	0.69	9.4	7.4	14
	F4	8.5	0.64	9.5	7.7	14
EC (mS/cm)	F1	6.1	0.90	7.2	4.1	14
	F2	6.2	0.91	7.2	4.5	14
	F3	6.3	0.82	7.3	5.1	14
	F4	6.4	0.97	7.7	5.0	14
TU (NTU)	F1	40.2	10.23	52.0	23.8	14
	F2	45.5	12.94	65.0	22.3	14
	F3	55.8	15.02	78.5	26.9	14
	F4	63.1	14.76	83.0	32.2	14
TDS (mg/L)	F1	1240.1	155.77	1523.0	1032.0	14
	F2	1294.9	223.07	1804.0	1021.0	14
	F3	1243.8	210.01	1754.0	1024.0	14
	F4	1219.5	123.29	1481.0	1019.0	14
TSS (mg/L)	F1	63.9	6.60	80.0	55.0	14
	F2	65.4	6.67	80.0	55.0	14
	F3	74.6	10.43	100.0	60.0	14
	F4	100.0	9.26	110.0	80.0	14
DO (mg/L)	F1	8.3	0.80	9.5	7.0	14
	F2	9.6	1.33	12.0	7.8	14
	F3	7.4	0.70	8.8	6.2	14
	F4	7.0	0.63	8.2	6.0	14
BOD_5 (mg/L)	F1	16.6	8.16	32.0	4.0	14
	F2	23.0	6.65	37.0	12.0	14
	F3	18.4	5.58	28.0	9.0	14
	F4	33.1	6.31	47.0	23.0	14
COD (mg/L)	F1	62.4	22.94	108.0	33.0	14
	F2	66.5	21.64	104.0	35.0	14
	F3	78.1	10.31	103.0	61.0	14
	F4	91.4	13.86	126.0	73.0	14
NO_3 (mg/L)	F1	17.9	3.35	24.5	13.6	14
	F2	18.5	3.22	24.9	14.6	14
	F3	19.5	3.16	25.1	14.8	14
	F4	22.0	2.94	27.6	17.2	14
NH_3 (mg/L)	F1	27.2	3.16	33.7	22.3	14
	F2	28.6	2.66	32.3	23.3	14
	F3	28.7	2.62	35.8	24.1	14
	F4	31.7	4.54	39.5	24.1	14
PO_4 (mg/L)	F1	1.8	0.42	3.0	1.3	14
	F2	1.9	0.42	3.1	1.4	14
	F3	2.1	0.49	3.4	1.1	14
	F4	2.4	0.43	3.5	1.9	14

(Tables 2 and 3). This is because the DO level in the shallow wetlands is generally affected by the atmospheric diffusion that leads to enhancing DO in all treatment filters (Yaseen and Scholz, 2016). However, the values were higher in F1 and F2, compared with F3 and F4. This confirmed the impact of plants during respiration and photosynthesis. The values were significantly ($\rho < 0.05$, 0.016) higher in F2 than F1 and other filters during all the treatment time (Figure 2g). This is likely due to the presence of plants only without carriers that enhance their growth and activities.

All outflow COD values were lower than the inflow ones (Tables 2 and 3), indicating the degradation of some organic matter in all treatment systems. The mean outflow values were lower in F1, F2, followed by F3 and F4. These results indicated that the plants and carriers together

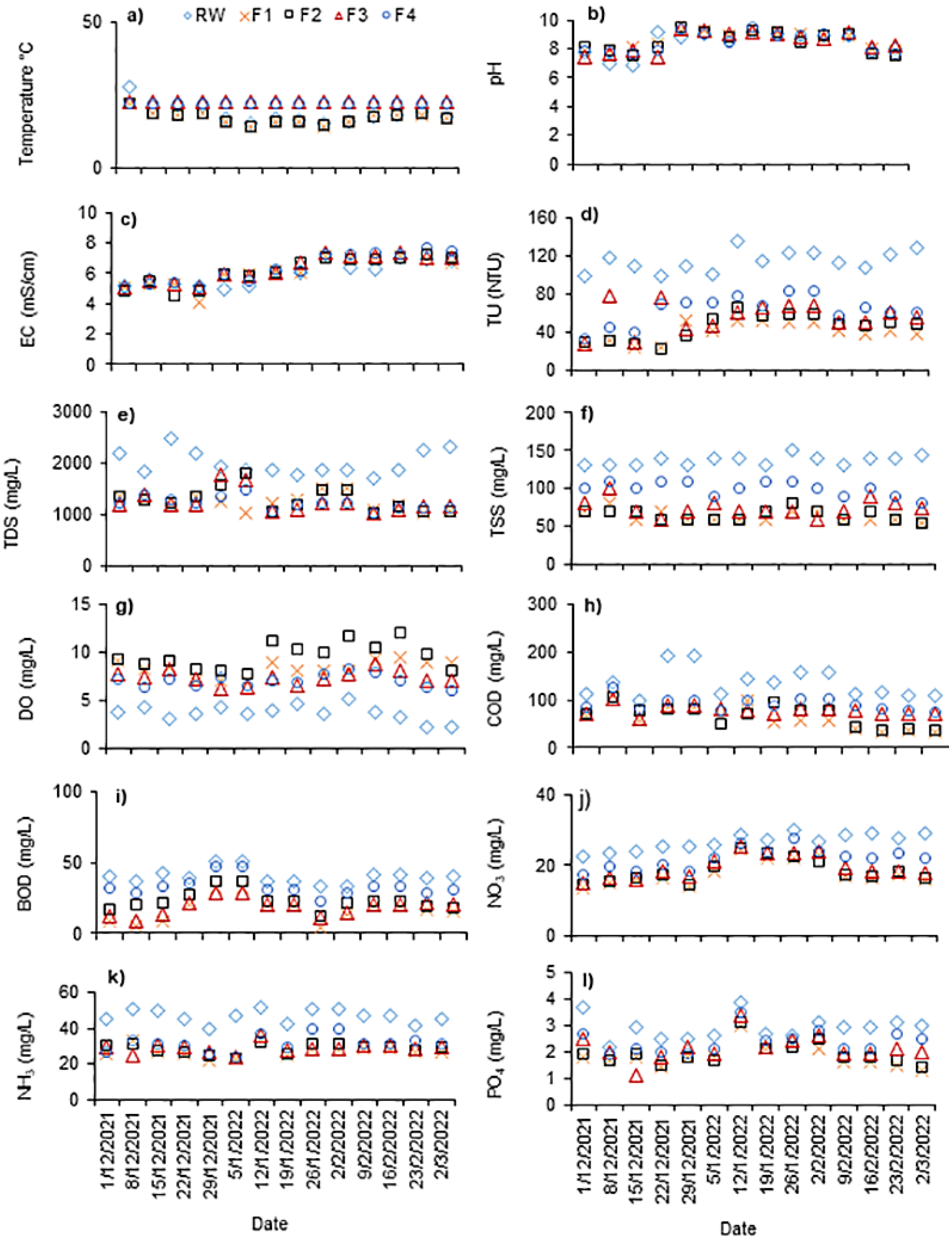

Figure 2. Longitudinal profile of treated water characteristics: RW – raw wastewater; F1 – contained plant, plastic carriers, and gravel; F2 – plants and gravel; F3 – comprised plastic biofilm carriers and gravel; F4 – contained gravel only

enhanced the COD reduction. Note that the significant differences ($\rho < 0.05$) were between F1 and F3 and F4, as well as between F2 and F4. This indicates that the presence of the plants with the carriers or one of them in the system bed decreased the COD concentrations to a greater extent than in the gravel bed. The longitudinal profile (Figure 2h) clearly showed the reduction of COD values between the raw and treated values during three months of the system operation. However, the values were nearly similar during the last month for F1 and F2, reflecting the dominant role of the plants in the system. The variation of outflow values may have been interpreted by the variation of COD in the raw water. Moreover, there was a clear fluctuation for the outflow values during January in F2, possibly due to plant harvesting from the system.

Regarding the BOD_5 values, the BOD_5 concentrations of treated water were lower than those for the raw water, especially in F1 and F3 (Tables 2 and 3). The values were significantly lower ($\rho = 0.02$) in F1 compared with F2. In addition, no difference was found between F1 and F3. These results reflected the beneficial impact of carriers in the system and consequently the bacterial growth for biological activities due to the effect of biofilm carriers. All BOD values were significantly higher in Filter 4, compared with other filters. During the study period (Figure 2i), all values ranged as follows F3<F1<F2<F4. The variation of outlet values followed the raw water variability.

The mean NO_3, NH_3, and PO_4 concentrations of the treated water were slightly lower than the raw water (Tables 2 and 3). The outflow values of nutrients were as follows F1<F2<F3<F4, although the significant differences were found only for F4 compared with other filters. This indicates that during the cold season, wetland performance in terms of nutrients was not highly affected by the plants and/or carriers. However, the gravel variation of the concentrations along the study period (Figures 2j, k and l) reflected the raw water variation and low nutrients removal in the systems.

Treatment systems performance based on removal efficiency

The degradation of organic pollutants in free water surface constructed wetlands occurred under aerobic and anaerobic conditions due to the combination of the bacteria that stick on the roots and the substrates, in addition to the rhizomes in the case of using plants with rhizome area (Oliveira et al., 2021). The removal of TSS (Figure 3a) was higher in F4 followed by F1 (53%), F2 (52%), and F3 (45%). The difference was significant in the case of F4 compared with other filters. The removal of TSS in wetland occurs by filtration, sedimentation, and root of a plant (Tsang, 2015).

The reduction rate of COD reflects the organic matter degradation in wetland systems. Some organic matters are not biodegradable; therefore, many authors suggested that the BOD_5 to COD ratio is the best indicator to examine the biodegradation of organic matter. It was found that high organic pollutants breakdown occurs at $0.4 < BOD_5/COD < 0.6$, optimum biodegradation occurs at $0.48 < BOD_5/COD < 0.53$, and low biodegradability at $0.3 > BOD_5/COD > 0.6$ (Zhang et al., 2020). In this study, the inlet BOD_5 to COD ratio ranged between 0.5 and 0.2, indicating that the biodegradability of the studied wastewater was mostly low. The values of COD removal were higher in F1 (53.4%), followed by F2 (50%), and then F3 (40.4%) and F4 (30.5%), the control one (Figure 3b). No significant difference ($\rho=0.504$) was found in the removal rate between F1 and F2, indicating that the COD removal was only performed by the gravel and plants. This may be because the efficiency of the biofilm carriers was restricted during the operation period due to the limited microbial growth in the cold season. The differences in COD elimination were significant in the case of F1 compared with F3 and F4, F2 with F3 and F4, and F3 with F4. These results confirmed that the combination of the plants and gravel only was enough to achieve a removal efficiency of 50%. The vegetation role in wetlands for organic matter reduction is explained by the exudation of their roots and the supplied oxygen via the parenchymal system of plants leading to enhance the bacterial growth for organic matter removal, which was predominated over the carriers' role (Zammora et al., 2019). The efficiency of a gravel bed in COD reduction (Figure 3b) was the highest at 30.5%, followed by the plants at 19.5%, and then the carriers at 9.9%. (Zammora et al. 2019) mentioned that the low water velocity enhanced the organic matter sedimentation. In this study, a contact time of 7 days could support the idea of low water velocity and interoperation the COD reduction in treatment systems. Some studies achieved a higher COD reduction, reaching 57.9% (Zhang et al., 2016), due to the impact of plants and biofilm carriers. In contrast, others attained lesser COD degradation rate of 27% (Mietto et al., 2013).

Average BOD removal rates (Figure 3c) in F4 were significantly lower than in other filters. However, a higher reduction was found in both F1 and F3, confirming the impact of the plastic rings as biofilm carriers for enhancing microbial growth and, as a result, microbial activities. However, the BOD_5 reduction fluctuated in all filters due to the variation of the inlet BOD_5 concentrations.

Organic nitrogen reduction occurs in wetlands by various mechanisms (Chyan et al., 2013). However, the primary and predominant processes are plant and microbial uptake and nitrification/denitrification (Vymazal, 2007). Under aerobic conditions, ammonia (NH_3) oxidizes into nitrite ($NO_{2)}$ and then into nitrate (NO_3) by nitrification. After that, the denitrification process occurs for transforming NO_3 into N_2 that occurs under anoxic conditions.

In this research, the NH_4 reduction rate (Figure 3d) was higher in F1 (41.4%), followed by F2 (38.4%) and F3 (37.9%), and then in F4 (31.9%). No significant differences were noticed between the first three filters. However, the NH_3 reduction in F4 was significantly lower ($p < 0.05$) than in other treatment filters indicating that the combination between the plant, gravel, and/ or carriers was better. Note that the impact of each factor alone in the removal rate of NH_4 was 31.9%, the highest, by gravel, followed by the plant, 6.5%, and then carriers at 6%. These results are attributed to the impact of nitrification due to the presence of aerobic conditions in all treatment systems linked with the high DO level, microbial uptake, and plant uptake.

The removal rates of NO_3 (Figure 3e) in F1 (32.8%), F2 (30.7%), and F3 (27%) were significantly ($p < 0.05$) greater than F4 (17.7%), which indicates that the plants and/or carriers play a vital role in denitrification process in treatment systems. This efficiency was due to the microbial activities on the plant's root and the biofilm. No significant differences were found in the NO_3 removal among F1, F2, and F3. These results may have been discussed by the impact of carriers' specific surface area, which matched the impact of *Lemna minor* roots. The same outcomes were discussed by Zhang et al. (2016). The low removal rate of NO_3 in all filters reflects the limited denitrification process due to the limited anoxic conditions in treatment systems that are also confirmed by DO level.

The reduction of PO_4 in wetlands occurred simply by the impact of the substrate due to the chemical reactions and physical adsorption, biotic uptake due to the plants and microbial uptakes, or accumulation in accreting sediments. Higher

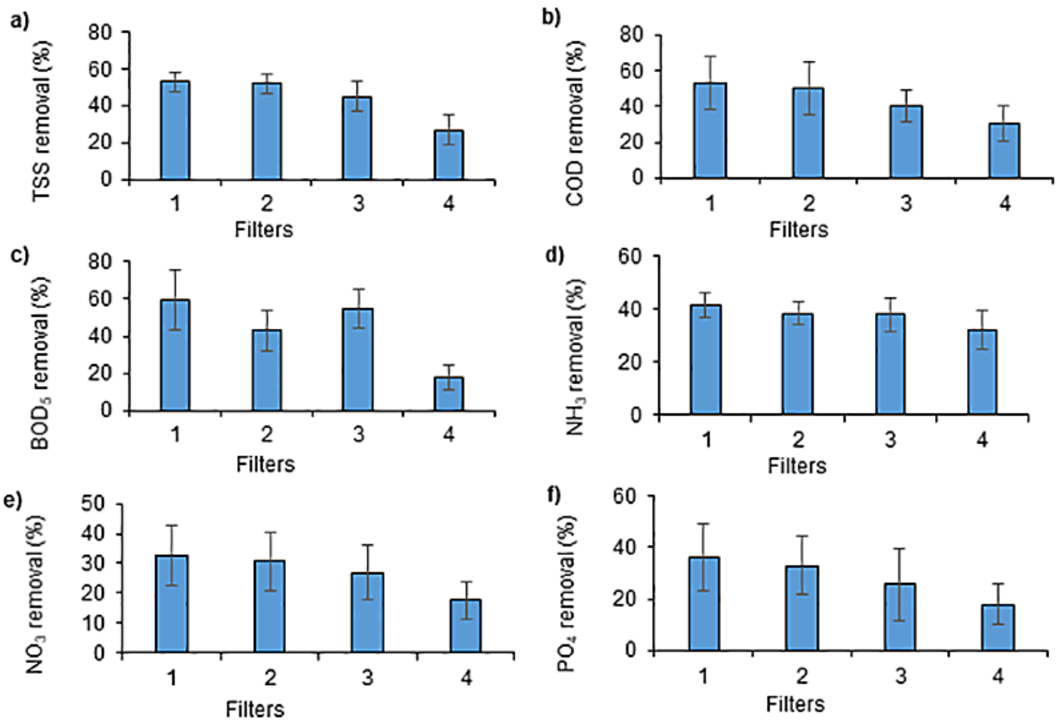

Figure 3. The removal rate of the treatment system: 1 – contained plant, plastic carriers, and gravel; 2 – consisted of plants and gravel; 3 – comprised plastic biofilm carriers and gravel; 4 – contained gravel only

temperature significantly enhanced the PO_4 assimilation by macrophytes and bacteria (Kadlec and Wallace, 2008). In this study, the rate of PO_4 abatement (Figure 3f) was higher in F1 (35.8%), followed by F2 (32.9%), and then F3 (25.7%) and F4 (17.9%). No significant differences (ρ= 0.521) were found between the removal rate of PO_4 in F1 and F2. However, the gravel bed showed significantly lower adsorption capacity than other filters, which means the presence of plants and carriers increases the uptake and adsorption capacity, respectively. Note that the results confirmed that orthophosphate adsorption by gravel was the dominant mechanism (17.9%) in the studied wetland filters, followed by the uptake process by the plants (15%). It is worth noting that harvesting plants positively affects the removal of nutrients in wetlands.

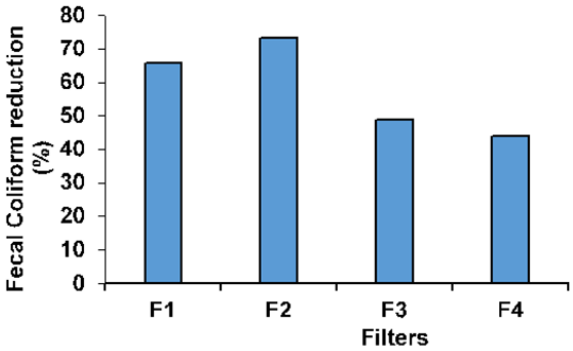

Figure 4. Removal rate of bacteria in each treatment system: F1 – contained plant, plastic carriers, and gravel; F2 – comprised plants and gravel; F3 – comprised plastic biofilm carriers and gravel; F4 – contained gravel only

Plants growth monitoring

During the setup period of the experiment, the plants' fresh weight was 0.08 kg, nearly covering 80% of the surface water. On the basis of the regular monitoring, the plants were growing in the systems (F1 and F2) and covered all the water surfaces. Therefore, to avoid overcrowding, plants were harvested when it was required. The growth rate of *Lemna minor* L. was significantly higher in F2 (0.036 kg/day) compared with F1 (0.007 kg/day). This is because the presence of plastic rings restricted the growth of the plant. Table 4 shows each system's fresh biomass weight harvested over the study period.

Bacterial monitoring

The main mechanisms for bacterial removal in wetlands are sedimentation, natural die-off, death by low temperature, oxidation, biofilm interaction, filtration by plant roots and media, and exposure to UV radiation. The results indicated that the total mean value of Fecal coliform bacteria for the pre-treatment wastewater sample was 0.041/10 ml. After a treatment period of three months during the winter season, the lowest proportion of bacteria per 10 ml of the filtered sample was in F2 (0.011), followed by F1 (0.014), and then F3 (0.021) and F4 (0.023). Figure 4 shows the removal rate of bacteria in each treatment system. These outcomes confirmed the significant impact of plants in the treatment system for increasing bacteria removal. This is because plants play a crucial role in bacterial removal by enhancing the DO level in the system, consequently providing a favorable environment for organisms. In addition, the plant has antimicrobial characteristics due to some exudates (Vymazal, 2005). The lowest removal in F1 compared with F2 is due to the impact of biofilm carriers, which enhanced the microbial activities and communities in the treatment system (Stott and Tanner, 2005). The reduction rate achieved by gravel in all filters was due to mechanical filtration, which refers to the attachment to the filter bed (Wand et al., 2007).

Table 4. Fresh weight of the plant biomass harvested (F1 – contained plant, plastic carriers, and gravel; F2 – consisted of plants and gravel)

Date	Harvested fresh weight (kg)	
	F1	F2
29/12/2021	0.064	0.068
19/1/2022	0.0181	0.079
2/2/2022	0.0136	0.869
9/2/2022	0.0156	0.0114
16/2/2022	0.0146	0.087
2/3/2022	0.0298	0.905

CONCLUSIONS

The treatment system in this study was successfully operated for three months during the winter season conditions in Basra city. The main conclusions are following. All treatment systems enhanced the characteristics of the raw wastewater. The mean pH and EC values of treated water were slightly increased compared with the raw water without any significant variation among the filters. The levels of DO in all filters confirmed the presence of aerobic conditions. The mean outflow concentrations of TDS, turbidity, TSS, COD and BOD_5 of the treated water were lower than those of the raw water. However, the mean NO_3, NH_3, and PO_4 concentrations of the treated water were slightly lower than those of the raw water. Higher TSS and COD removal was in F1, followed by F2 and F3, confirming the impact of plants and/or carriers. The mechanisms of TSS reduction were filtration and sedimentation. However, COD degradations occurred by microbial activities. Higher BOD removal was founded in F1 and F3, confirming the impact of the plastic rings as biofilm carriers for enhancing microbial growth and, as a result, microbial activities. The reduction rates of NH_3, NO_3, and PO_4 were significantly higher in F1, F2, and F3 compared with F4, indicating that the combination between the plant, gravel, and carriers is better. Nitrification, denitrification, microbial uptake, and plant uptake were the main mechanisms for the NH_4 and NO_3 removal. However, the orthophosphate reduction mechanisms were adsorption by gravel and the uptake process by the plants. The growth rate of *Lemna minor* was significantly higher in F2 compared with F1, as the plastic rings restricted the plant growth. The bacterial removal was higher in F2 compared with F1 due to the impact of biofilm carriers, which enhanced the microbial activities and communities in the treatment system.

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