

## Development of the Horizontal Flow Wetland Using Palm Waste Biochar for Greywater Reclamation

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### ABSTRACT

Wetlands technology is one of the main sustainable and successful treatment processes. Similarly, biochar is an organic, effective, and low-cost adsorbent material used for the treatment of diverse wastewaters. The combination between wetland system and biochar, as a media, can greatly enhance the treatment efficiency. The aim of this study is to assess the performance of two horizontal flow constructed wetlands planted with *Bacopa monnieri* L. for the treatment of household greywater. The objectives were to investigate the raw and treated greywater characteristics, compare the removal efficiency of pollutants by using gravel bed, and biochar-gravel bed, monitor the growth and survival of the plants. Findings indicated that the simulated treatment systems were able to improve all the greywater characteristics. The wetland with biochar enhanced the removal efficiency of biological oxygen demand (BOD<sub>5</sub>), ammonia (NH<sub>3</sub>), and other parameters compared with the wetland with gravels alone.

**Keywords:** greywater treatment, subsurface flow, *Bacopa monnieri* L., constructed wetland, biochar.

### INTRODUCTION

The current water shortage problems moved the attention of scientists towards water conserving approaches. This achieved by applying the sustainable wastewater treatment processes (Gizinska-Górna et al., 2016) and reuse the treated water as a non-conventional source of water (Abunaser and Abdelhay, 2020). In this context, reclamation of household greywater in rural areas, and recycling the treated water for irrigation or cleaning is a successful approach to reduce the pressure on the natural water resources (Juan et al., 2016; Abdelhay and Abunaser, 2021). Greywater is including the wastewater disposed from the bathroom, lavatories, kitchen sink and the dishwashers (Laaffat et al., 2016), and its components are varied based on the formation sources (Spychala et al., 2019). For example, wash basins and bathrooms discharged water consists of dead skin, hair, soap, toothpaste, shaving and skin care materials. Whereas, kitchen wastewater characterized by high level of detergents, pH, salts, food parts, nitrogen, organic

compounds, turbidity, suspended solids, fats and oils. On other hand, clothes washing machine discharged wastewater consists of viruses, dyes, heavy metals, fibers and bleaches and detergents (Couto et al., 2015). It is estimated that greywater covers around fifty to seventy percent of the household discharged water (Laaffat et al., 2016). Therefore, it is considered a good source to provide a valuable quantity of recycled water after treatment (Chripim and Nolasco, 2017).

Among all wastewater treatment types, constructed wetlands (CWs) technology is widely recommended due to its effectively, sustainability, thriftily, and environmentally friendly. Constructed wetlands are artificial engineered systems simulate the natural wetlands in terms of utilizing the combination between the plants, organisms, and soil for the treatment of diverse domestic sewage, industrial wastewater, storm, agricultural, and greywater (Yaseen and Scholz, 2016; 2018). All CWs configurations have proven as successful treatment systems. However, most of last studies mentioned that horizontal flow CW (HSSFCW)

is the preferable among other wetlands due to its effectiveness for the purification of diverse pollutants (Shukla et al., 2021). In addition to the direction of flow, the combination of the main CW components is the key role of wastewater treatment performance. Planted systems showed higher wastewater reclamation than unplanted ones (Shaikh and Ahammed, 2020). In terms of filter media, authors confirmed that sand is more efficient than gravel, and the mixture of sand and soil is the best case for removing different contaminants (Priya et al., 2013). Recently, other types of media were utilized in CWs to improve the treatment efficiency, such as zeolite, biochar, active carbon, biofilm carriers (Zaboon et al., 2022).

Biochar is one of the preferable and effective adsorbents for heavy metals, nutrients, dyes and other pollutants adsorption from water. This due to its features as a carbon rich substance, porous material, have diverse functional groups, and very cheap comparing with other adsorbents (Gupta et al., 2016). Biochar was examined as a mixture with other substrate materials to enhance the treatment of different loading rate of low C/N wastewaters (Zhou et al., 2018), synthetic wastewater (Abedi and Mojiri, 2019), and domestic wastewater (Xing et al., 2021) by vertical wetland. Also, it is utilized as a media in HFCW to examine the treatment of synthetic wastewater (Gupta et al., 2016).

Although, biochar was widely investigated to purify of diverse effluents by vertical and horizontal wetlands. However, the performance and efficiency of HFCW combined with biochar to treat household greywater has not been surveyed yet. Therefore, it is required more investigations with a significant extent to cover all the gaps in the field of greywater reclamation by modified CWs. This research is motivated by the challenges of wastewater discharges in low-income developing countries, involving the environmental pollution resulting from the discharge of the untreated or poorly treated wastewater linked with the water shortage problems, which require a crucial solution by treating these effluents using effective and low-cost technology for the protection of the eco-system, and consequently recycling of the treated wastewater for irrigation purposes or reuse for other processes.

The research aims to assess the performance of two horizontal flow constructed wetlands (HF-CWs) planted with *Bacopa monnieri* L. for the treatment of household greywater, as a secondary treatment stage. The objectives were to investigate

the raw and treated greywater characteristics, compare the removal efficiency of pollutants by using gravel bed, and biochar-gravel bed, monitor the growth and survival of the plants.

## EXPERIMENTAL DESIGN AND OPERATION

### Materials

Two types of the substrates were used in this research. The gravel material supplied by a local laboratory in Basra governorate. The pebbles, in different sizes of 5-10 mm, 10-20 mm, and 40-80 mm based on the design requirements, rinsed with deionized water to remove any impurities. The biochar material collected from the date palms (*Phoenix dactylifera* L.). Biochar was prepared by cutting and collecting the date palm fronds from a palm grove in the Abu Al-Khaseeb area. Then, the palm fronds washed with tap water to remove any dust and suspended matters, and placed below the sun for 15 days to dry very well. After that, the dried fronds were cut into small parts and placed in an oven at temperature of 500 to 600 °C for two hours, then cooled. Later, biochar was collected, grounded and sieved (Salem et al., 2021). Finally, the desired sizes of biochar placed in the wetland filters for drainage and treatment purposes.

The selected aquatic plant in this study was *Bacopa monnieri* L. A small pond located in Basra Province was the source of collection enough quantity of the selected plant. The pond was not attached with any sources of greywater or other wastewater types. The aquatic plant washed cautiously by distilled water to remove any dust. The greywater used in this research was taken from the washing machines, kitchen sinks, and lavatories from different houses at Abu Al-Khaseeb District. The household greywater water was not affected by any source of black water.

### Treatment system description

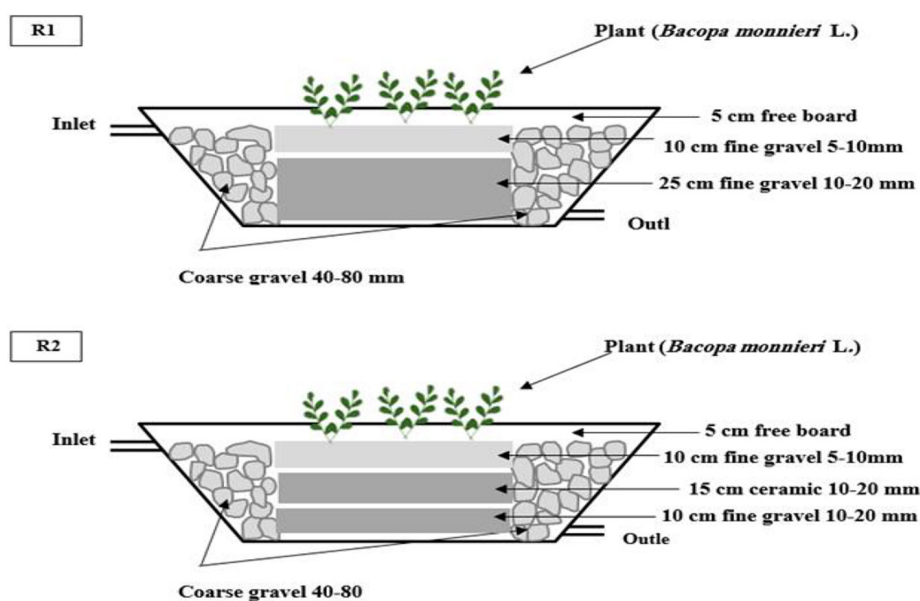
Two experimental scale HFCWs were operated using rectangular plastic basins for the period from 4/10/2022 to 26/12/2022 (with extra two weeks as a setup period). The basins, with dimensions of 26 cm length, 85 cm width, and 45 cm depth, placed in the yard of a house at Abu Al-Khaseeb District under semi-natural conditions (30.46662°, 47.865902°, Basrah, Iraq).

Both basins received the same quantity of 25 liters of greywater, which was equivalent to 35 cm depth. The first basin (R1) consisted of gravel as media whereas the second basin (R2) consisted of gravel and biochar. The EPA (2000) declared that the recommended media sizes in treatment zone of HFCW ranged between 5 and 20 mm. Tanner et al. (2011) mentioned that the media basic size in treatment zone is from 10 to 20 mm. Therefore, in this study the size of media (gravel and biochar) was selected between 10–20 mm in the bottom layer as a treatment zone, and between 5–10 mm in the top layer as a treatment zone and to support the plants' roots. The sides of the treatment system filled with gravel of 40 to 80 mm size to distribute the water evenly and minimize clogging, as recommended for the inlet and outlet zones (Tanner et al., 2011). The schematic diagram of the wetland basins is shown in Figure 1. The greywater was collected and placed in a plastic storage tank of 250 L capacity. The inlet tap position was at depth of 25 cm from bed of the storage basin. This was to assure that the greywater enters to the systems by gravity and without any sediments. The GW was discharged to the wetland basins after the time of filling by around 2 hours, which was suitable time for settling the particles, as a pre-treatment stage. Both basins were filled and drained each five days, regularly. This because the contact time of five days was recommended by (Tanner et al., 2011) for best TSS, TDS, TU, BOD<sub>5</sub>, COD, NH<sub>3</sub>, NO<sub>3</sub>, and PO<sub>4</sub>,

reduction. Table 1. Summarized the experimental wetlands description and properties.

### Analysis of samples

The outlet water from each filter and the raw greywater were collected regularly for analysis. The volume of each collected sample was three liters. Most of the analyses were done based on the standard methods (APHA, 2012). The temperature, total dissolved solids (TDS), electrical conductivity (EC), and the pH were tested using pH meter (Hanna/ Romania). Turbidity (TU) was measured using Turbidity meter (TB 300IR/ Lovibond/Germany). Total suspended solids (TSS) was tested by passing the samples through filter paper of 0.45 µm. Biological oxygen demand (BOD<sub>5</sub>), chemical oxygen demand (COD), and dissolved oxygen (DO) were measured using Winkler's method (APHA, 2005), spectrophotometer DR 5000 Hach Lange (Germany), and oxi Top/WTW, respectively. The spectrophotometer (V-1100D /Germany) was used to test reactive nitrate (NO<sub>3</sub>) at wavelengths of 220 and 270 (APHA, 1999), and orthophosphate (PO<sub>4</sub>) at a wavelength of 650 (EPA1978). Total hardness (TH), magnesium and calcium were determined using Titrimetric Method (APHA 2005). The ammonia (NH<sub>3</sub>) samples were sent to the College of Agriculture and tested by the technicians at the central laboratory. The efficiency of pollutants reduction (R) and the porosity (P) were calculated by Equation 1 and 2, respectively (Zaboon et al., 2022).



**Figure 1.** Schematic diagram of the experimental setup: R1 – plant + gravel filter; R2 – plants + biochar + gravel filter

**Table 1.** Treatment systems details

Details	R1	R2
Length (cm)	26	26
Width (cm)	85	85
Depth (cm)	45	45
Influent volume (liter)	25	25
HRT (days)	5	5
Water depth (cm)	35	35
Porosity (%)	29	29
Vegetation	<i>B. monnieri</i> L.	<i>B. monnieri</i> L.
Biochar weight (kg)	-	40
Gravel weight (kg)	85	65
Size of gravel /sides	40–80 mm	40–80 mm
Size of gravel/ bottom layer	10–20 mm of 25 cm depth	10–20 mm of 10 cm depth
Size of biochar/ middle layer	-	10–20 mm of 15 cm depth
Size of gravel/ top layer	5–10 mm of 10 cm depth	5–10 mm of 10 cm depth

**Note:** R1 – plant + gravel filter; R2 – plants + biochar + gravel filter.

$$R = ((Ic - Oc)/Ic) \times 100\% \quad (1)$$

$$Porosity = ((Vt - Vs)/Vt) \times 100\% \quad (2)$$

where:  $Ic$  – inlet concentration,  $Oc$  – outlet concentration;  $Vt$  – total volume;  $Vs$  – volume of the solids.

### Fecal and total coliforms

Fecal coliform (FC) was calculated according to APHA (2005), as described by Zaboon et al. (2022). The total coliform (TC) test according to APHA (2005), which was similar to FC test, except the following steps: the dissolved weight of Endo agar medium was 20.75 g, the boiled solution was then sterilized for 15 minutes by autoclaving at 15 lbs pressure at 121°C and then transferred to the dishes, and finally set the incubator temperature at 37.5°C. The bacteria colonies were counted by Eq. 3.

$$Colonies \text{ forming unit } \frac{(CFU)}{100} \text{ mL} = Rd \times 100 \times \frac{n}{FS} \quad (3)$$

where: CFU – colonies forming unit,  $Rd$  – reciprocal dilution,  $n$  – the number of colonies in the plate;  $FS$  – filter sample size.

### Data analysis

Microsoft Excel (www.microsoft.com) was used to analyze all the study records. The statistical analyses were computed using the IBM SPSS

Statistics (www.ibm.com) version 22. Shapiro-Wilk test, t-test, and Mann-Whitney U test were used to check the data normality, observed the significant differences between the filters for the parametric and non-parametric data, respectively.

## RESULTS AND DISCUSSION

### Raw greywater characteristics

The untreated greywater characteristics for the period between 4/10/2022 and 26/12/2022 are presented in Table 2.

### Characteristics of treated water

The physiochemical parameters of water are reflecting the biological activities that occur in wetlands systems to improve the treatment performance. The main biological parameters are the temperature, pH and dissolved oxygen (Kadlec and Wallace, 2008).

The mean temperature values (Tables 2 and 3) of the treated water during the study duration were lower (25 °C) compared with the corresponding values of the untreated water (27 °C). In addition, all mean records were slightly higher (25.7 °C) in gravel filter (R1) than the coal filter (25 °C) (R2). Figure 2a clearly showed that there was a fluctuation in the treated water temperature depending on the weather temperature fluctuation during the study period, as the treated water temperature increased in some weeks compared to the raw water

**Table 2.** Characteristics of the raw greywater

Parameter (unit)	Average	Standard deviation	Minimum	Maximum
Temp. (°C)	27.5	2.0	24.3	30.9
pH	9.6	0.8	8.4	11.3
EC (mS/cm)	2.9	1.7	1.1	6.2
TU (NTU)	284.1	138.5	105.0	648.0
TDS (mg/L)	2070.7	828.4	766.0	3212.0
TSS (mg/L)	558.5	434.2	140.0	1420.0
DO (mg/L)	5.0	0.8	3.1	6.0
BOD <sub>5</sub> (mg/L)	288.0	48.9	200.0	400.0
COD (mg/L)	1313.4	270.0	876.0	1646.0
NO <sub>3</sub> (mg/L)	46.2	7.6	36.6	63.1
NH <sub>3</sub> (mg/L)	1238.0	202.3	866.0	1652.0
PO <sub>4</sub> (mg/L)	12.5	3.0	8.1	20.0
TH (mg/L)	1011.26	310.81	600.0	1700.00
Ca <sup>2+</sup> (mg/L)	250.6	73.7	160.3	416.8
Mg <sup>2+</sup> (mg/L)	184.8	60.8	87.4	329.3

**Note:** Number of readings –15..

temperature due to the high weather temperature in those weeks. The low temperature of treated water could explain by system configuration as the water level was below the top layer, which reduce the penetrated sunlight and consequently decreases the water temperature (Borne et al., 2014). Maximum and minimum temperature values of the treated water were more than the range that could slowing down the nitrification process. Also, all the values were out the limits that could impact adversely on the removal mechanism of COD and BOD<sub>5</sub> (Vymazal, 2007).

The pH level affects the growth of bacteria, which is highly enhance the biological activities in wetland. Bacterial growth and activities achieved when the level of pH in water within the range from 4 to 9.5. When pH value is higher than 7.2, nitrification process occurs very well. However, nitrogen removal was increased at pH value between 6.5 and 9 (Qian et al., 2019; Zaboon et al., 2022). The minimum and maximum values of pH of the treated water were less (8.1–9.9) than the raw water (8.4–11.3) (Tables 2 and 3). This was due to the impact of *B. monnieri* L. roots on the filters R1 and R2, as many authors confirmed the ability of *B. monnieri* L. in reducing the pH level of water (Lastiri-Hernández et al., 2023). All outflow pH records were within the allowable limits for nitrification occurrence, and more than the required limits for increasing the denitrification. The mean pH values of the R1 and R2 were not significantly different ( $p > 0.05$ ), ( $p = 0.131$ ,

Mann-Whitney U test). This result indicated that the impact of coal on pH level was not valuable. This was clearly noticed in the longitudinal profile of pH levels within the treatment duration (Fig. 2b). The figure 2b also showed that the pH values of the treated water increased in some weeks during December because of the change in temperature that affects the plant activity, which in turn affects the pH values as mentioned above.

The outflow electrical conductivity values (0.1–4.6 mS/cm) were less than the inflow (1.1–6.2 mS/cm) values (Tables 2 and 3). This reduction in salinity level was due to the presence of plant in both treatment systems. Same results were concluded and explained by Lastiri-Hernández et al. (2023) confirming the great ability of *B. monnieri* L. to reduce the water salinity while absorbing Na<sup>+</sup> cations by their roots. This because Na<sup>+</sup> absorbing makes the process of Ca<sup>2+</sup>/Na<sup>+</sup> exchange reaction to be slow and the low flow rate enhanced the efficiency of desodification through sodium translocation to the harvestable parts of the plants (Lastiri-Hernández et al., 2023). Yaseen and Scholz (2017) also concluded a reduction in water salinity by aquatic plants in wetland due to the passing of some salts from wastewater within the semipermeable membrane. Most of the outflow EC records were acceptable (less than 4 mS/cm) for plant survival and organism growth (Zaboon et al., 2022). No dissimilarity was founded between R1 and R2 in terms of EC records ( $p = 0.901$ , Mann-Whitney U test), indicating that

**Table 3.** Treated greywater characteristics

Parameter	Filter	Average	Standard deviation	Minimum	Maximum
Temp. (°C)	R1	25.7	2.6	22.6	30.9
	R2	25.0	3.2	20.8	30.8
pH	R1	9.1	0.4	8.3	9.9
	R2	9.0	0.3	8.1	9.3
EC (mS/cm)	R1	1.7	1.2	0.40	4.1
	R2	1.7	1.4	0.1	4.6
TDS (mg/L)	R1	1105.3	426.8	423.0	1642.0
	R2	930.9	384.3	341.0	1456.0
TSS (mg/L)	R1	71.8	14.2	33.0	93.0
	R2	69.2	9.1	53.0	86.0
TU (NTU)	R1	53.1	13.6	21.2	77.1
	R2	50.6	15.7	20.1	88.5
DO(mg/L)	R1	6.9	0.9	5.3	8.4
	R2	7.9	0.9	6.1	9.2
BOD <sub>5</sub> (mg/L)	R1	124.0	51.7	20.0	260.0
	R2	97.3	44.3	20.0	220.0
COD (mg/L)	R1	204.5	54.1	99.0	268.0
	R2	183.8	67.0	68.0	310.0
NO <sub>3</sub> (mg/L)	R1	34.6	8.0	24.5	51.4
	R2	34.9	7.9	24.4	51.0
NH <sub>3</sub> (mg/L)	R1	8.1	0.9	6.8	10.1
	R2	6.1	1.3	4.1	8.4
PO <sub>4</sub> (mg/L)	R1	5.5	0.8	4.4	6.9
	R2	5.5	1.7	2.7	8.1
TH (mg/L)	R1	699.6	286.9	380.0	1320.0
	R2	615.3	276.2	340.0	1220.0
Ca <sup>2+</sup> (mg/L)	R1	166.9	54.6	104.2	272.5
	R2	146.1	58.9	88.2	256.5
Mg <sup>2+</sup> (mg/L)	R1	128.1	54.2	67.0	235.1
	R2	112.7	50.5	61.2	216.6

**Note:** R1 – treated water from plant + gravel filter; R2 – treated water from plants + biochar + gravel filter; number of readings –15.

there is no a valuable impact for coal on the EC level. This was clearly noticed in the longitudinal profile of EC levels within the treatment duration as shown in Figure 2c, which also showed a noticeable fluctuation in the electrical conductivity values, as the treated greywater values increased in some weeks and decreased in others. This is because of plants in the treatment basins on the conductivity values, as explained above.

All the values of TU, TDS and TSS for both treatments filters were highly less than those for the raw water (Table 2 and 3). These results clearly shown in the longitudinal profile of the raw and treated water (Figs. 2d, e, and f). Also, it is noticed from these figures that the values of

turbidity, dissolved and suspended solids fluctuated depending on their fluctuation in the raw greywater. No increase was recorded in the values of the treated water during the study period, both basins were highly efficient in reducing the turbidity values, dissolved and suspended solids, with some differences, as the lowest values were recorded in the biochar filter. The reduction of TSS values explained by the TSS trapping and the high porosity of media used in both treatment filters (Zidan et al., 2015; Hdidou et al., 2021).

The averages TU, TDS, and TSS records of the outflow water for R2 (gravel-biochar bed) were slightly lower than the corresponding values of R1 (gravel bed). Same results were concluded

and explained by Dalahmeh et al. (2016) and Mwenge and Seodigeng (2019). No dissimilarity ( $p > 0.05$ ) was founded between the two filters in terms of TU, TDS, and TSS ( $p = 0.654$ , Mann-Whitney U test), ( $p = 0.137$ , T-test), ( $p = 0.575$ , Mann-Whitney U test), respectively. These results indicated that bio-char doesn't affect these parameters significantly. All TSS outflow values ranged between sound to acceptable (Zamora et al., 2019). Whereas, the TDS records extended from poor to unacceptable water quality.

Dissolved oxygen level in water is a sign to the aerobic or anaerobic environment in treatment system. Aerobic conditions are essential for plant growth, ammonia and  $BOD_5$  removal. Anaerobic conditions are suitable for nitrate removal (Wang et al., 2016). The minimum and maximum values of DO varied between 5.3 and 9.2 mg/L, indicating the occurrence of high nitrification and limited denitrification. The DO records of the outflow water were significantly higher in comparison with the inflow water (Tables 2 and 3). This because the depth of the designed wetland was within the shallow depth range, which is letting the oxygen in the system to be affected by the atmospheric diffusion and consequently enhances the concentration of DO in the treated water (Wang et al., 2016). Throughout the study period, higher oxygen values were recorded in the bio-char filter, this was clearly noticed from the longitudinal profile of DO values presented in Figure 2g. The presence of aquatic plants also increased the concentration of DO in the system. The DO records were significantly higher ( $p \leq 0.05$ ) in R2 compared with R1 ( $p = 0.005$ , T-test). This reflecting the impact of bio-char that reduces the bio-requirement for oxygen and ammonia. As a result of the activity of microorganisms and their ability to remove pollutants, they consume dissolved oxygen, but are compensated through the process of photosynthesis by plants, which were highly active in the R2 (Paul and Hall, 2021).

The outflow COD and  $BOD_5$  records were less than the values of raw water (Tables 2 and 3), which attributed to the organic matters breakdown in both filters. The mean COD and  $BOD_5$  records of the treated water were less in coal filter than the control filter, although the dissimilarity was not significant ( $p > 0.05$ ) in terms of COD ( $p = 0.141$ , Mann-Whitney U test) and  $BOD_5$  ( $p = 0.154$ , T-test). This means that the COD and  $BOD_5$  reduction rate in wetland is slightly more by biochar than the gravel only. This small impact

of biochar attributed to the bacterial growth and biological activities on the coarse coal surfaces (Paul and Hall, 2021), in addition to the presence of more reactive sites with a strong presence of  $\pi$  bones, which consequently simply absorbed the organic pollutants by the electrostatic attraction and intermolecular hydrogen bonding onto the biochar due to the  $\pi$  bones (Gupta et al., 2016). Figures 2h and i describe the organic pollutants variation of treated and untreated water during the study period. The fluctuation of the COD and BOD values was dependent on the fluctuation of their values in the raw greywater, and throughout the period, the biochar filter recorded the lowest values compared to the control filter.

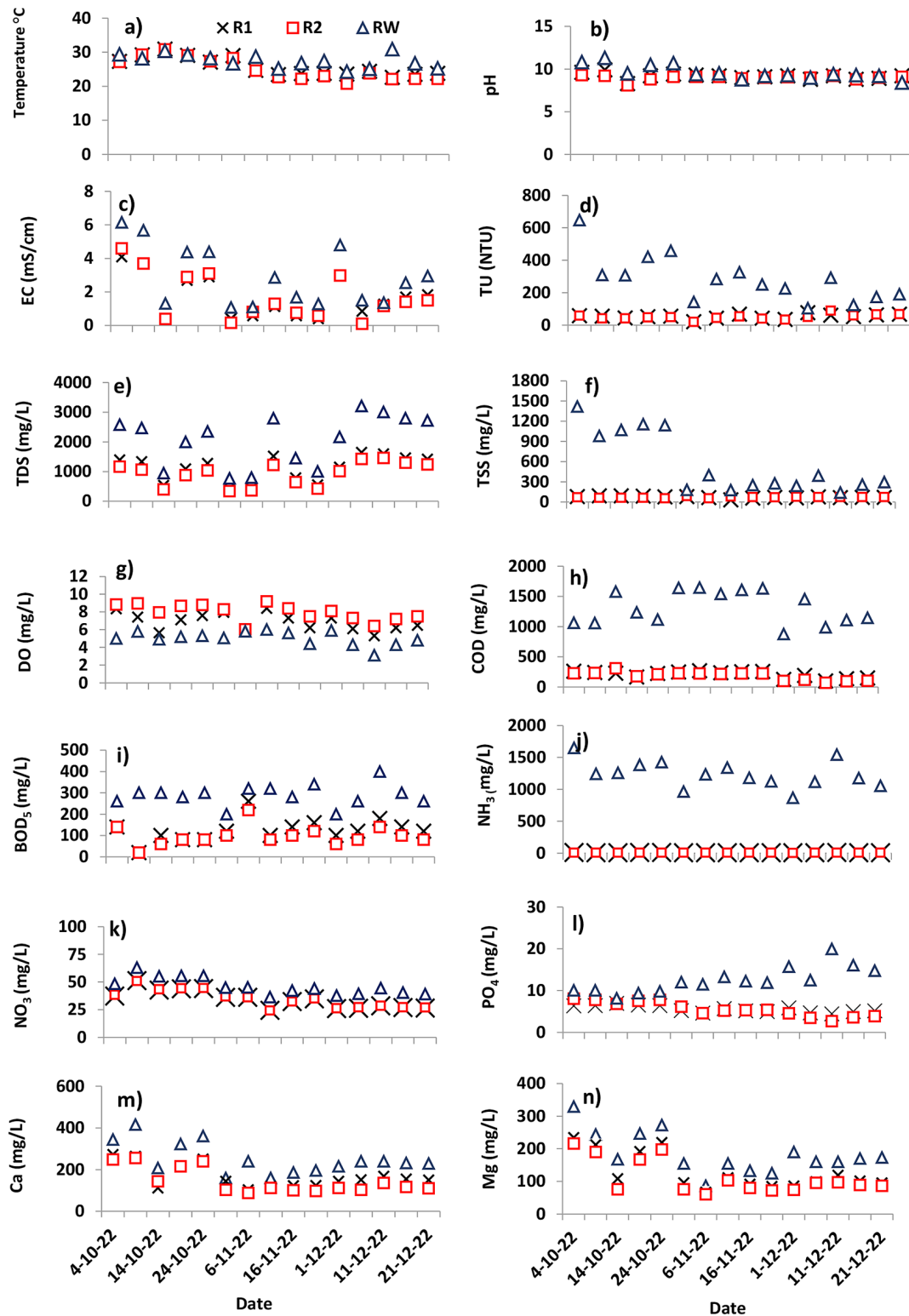
Nitrogen reduction in wetland is mainly depends on the ammonia concentration because it is the key form of nitrogen in the system. The outflow ammonia values were noticeably less than the inflow values. This attributed to the presence of aerobic environment in both filters (high DO level). This high oxygen level occurred due to the depth of the studied wetlands, as mentioned above. The mean ammonia outflow values were significantly ( $p = 0.00$ , T-test) less in R2 than R1. This because the pores on biochar in R2 provide suitable conditions for microbial growth (for example ammonia-oxidizing bacteria), which consequently enhance nitrification process (Verhamme et al., 2011). Same outcomes were demonstrated by Zhou et al. (2018) using vertical wetland with biochar for wastewater treating. Same results were concluded and explained by Gold et al. (2017), Mwenge and Seodigeng (2019) and Xing et al. (2021).

The average outflow records of  $NO_3$  were somewhat less than the inflow water (Tables 2 and 3). In addition, the mean  $NO_3$  records of the treated water for R1 and R2 doesn't showed any significant dissimilarity ( $p = 0.924$ , T-test). This results attributed to the absence of anaerobic condition in both treatment filters. The average  $PO_4$  concentrations were lower in treated water compared with the inflow water (Tables 2 and 3). The  $PO_4$  reduction in wetland is assimilated by plants or occurred by precipitation, sedimentation and adsorption (Kozak et al., 2014; Shukla et al., 2021). The expected mechanisms for  $PO_4$  removal in this research are assimilation, sedimentation, and adsorption. Statistical analysis showed that there is no significant dissimilarity ( $p = 0.917$ , Mann-Whitney U test) between R1 and R2. The variation of  $NH_3$ ,  $NO_3$ , and  $PO_4$  concentrations in the raw and treated water during the operation

period was presented in Figures. 2j, k, and l, respectively. Also, at the beginning of the study, it was noticed that the orthophosphate values of the treated greywater decreased by a small amount, but after a period from the start of the experiment,

the values began to decrease significantly. Treatment at the same level with little fluctuation depending on fluctuation values in raw greywater.

Maximum and minimum calcium concentrations were decreased in treated water and ranged



**Figure 2.** Outflow water characteristics along the study period: RW – raw wastewater; R1 – treated water from plant + gravel filter; R2 – treated water from plants + biochar + gravel filter



between 272.5 mg/L and 104.2 mg/L for R1, and between 256.5 mg/L and 88.2 mg/L for R2 compared with the raw water values, which ranged from 416.8 mg/L to 160.3 mg/L. Also, the maximum and minimum magnesium values were decreased in treated water and ranged from 235.1 mg/L to 67.0 mg/L for R1, and from 216.6 mg/L to 61.2 mg/L for R2 compared with the raw water (87.4–329.3 mg/L) (Tables 2 and 3). No significant difference ( $p > 0.00$ ) were founded between R1 and R2 in terms of the mean Ca and Mg concentrations. This because these two elements are absorbed by plant in the form  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions, as essential elements for plants survival (Kozak et al., 2014). Figures. 2m and n showed the longitudinal variation of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  during the experimental period. The biochar filter had lower calcium and magnesium values than the control filter.

The values of TH for both treatments filters were highly lower than the corresponding values of the untreated water (Tables 2 and 3). In addition, the TH values of the treated water of R2 were significantly lower than those of R1 ( $p = 0.018$ , Mann-Whitney U test). This confirm the impact of biochar in TH reduction rate. Same results were concluded and explained by Chaukura et al. (2020).

### Performance of processing systems based on removal efficiency

#### Removal rates of TSS, TDS, TU and EC

The removal rate of TSS is presented in Figure 3a. The mean reduction efficiency was slightly higher in R1 (79.40%) comparing with R2 (77.11%) without any differences between the two filters ( $p = 0.82$ , Mann-Whitney U test). This means that the TSS reduction in both R1 and R2 was attributed to the mechanical filtration, and the growth of the plant roots (Tsang, 2015; Gupta et al. 2016). The removal of TDS, TU, and EC was lower in R1 comparing with R2 (Fig.3a). Statistical analysis showed a significant difference between the two filters in terms of the mean removal of TDS only ( $p = 0.00$ , Mann-Whitney U test).

#### Removal rates of COD and $\text{BOD}_5$

The COD reduction was due to the organic matter decomposition in wetland systems. Organic pollutants degradation occurred in HFCWs under aerobic or anaerobic conditions due to the bacterial accumulation on the plant roots and the media layer represented by biochar (Oliveira et

al., 2021). The ratio of  $\text{BOD}_5$  to COD is considered as a sign for the biodegradability of organic matter in wetlands. In this study, the ratio was (0.219) within the range of lowest biodegradability (Zhang et al., 2020).

The treatment system was highly efficient in removing COD. The average reduction efficiency of COD was slightly more in R2 (86.05%) than R1(84.33%) (Fig. 3b). However, no dissimilarity founded between the gravel bed and biochar-gravel beds ( $p = 0.288$ , T-test) in terms of the COD removal rates. These results confirmed that the COD reduction was due to the presence of *B. monnieri* L. in the system. Organic matter degradation by plants in wetlands attributed to the roots perfusion and the oxygen presented from the vegetarian parenchymal system, which consequently promote the microbial activities for organic matters consumption (Zammora et al., 2019). Same results were discussed by Gupta et al. (2016), Mwenge and Seodigeng (2019), and Xing et al. (2021).

The mean biological oxygen demand ( $\text{BOD}_5$ ) removal (Fig.3b) in filter R2 (65.97%) was higher than filter R1 (56.49%). This confirms the effect of biochar in increasing microbes and organic substances that promote bio-cracking process (Paul and Hall, 2021). Significantly, no dissimilarity between the R1 and R2 regarding  $\text{BOD}_5$  reduction efficiency ( $p = 0.13$ , T-test). These data confirmed that there is a very small effect for the biochar in the filter R2 for  $\text{BOD}_5$  reduction, and the main factor was the plants. These plants are the predominate source of oxygen transfer in wetland, and convey the oxygen from the leaves to the roots. These plants do not remove the  $\text{BOD}_5$  directly, they work as a host for a variety of attached growth organisms, which are primarily responsible for the organics decomposition.

#### Removal rates of nitrate and orthophosphate

In wetlands, the predominate mechanism for nitrogen removal occurs are absorption by plants and microbes, and nitrification/denitrification processes (Vymazal, 2007; Chyan et al., 2013). Within the aerobic environment, the oxidation of ammonia ( $\text{NH}_3$ ) to nitrite ( $\text{NO}_2$ ) is occurred, which is then converted to nitrate ( $\text{NO}_3$ ) by nitrification. Later,  $\text{NO}_3$  under anaerobic conditions is converted to  $\text{N}_2$  by denitrification process.

The mean removal efficiency of  $\text{NH}_3$  (Fig. 3c) was very high for both treatment filters (R1, 99.33%; R2, 99.43%) without any significant

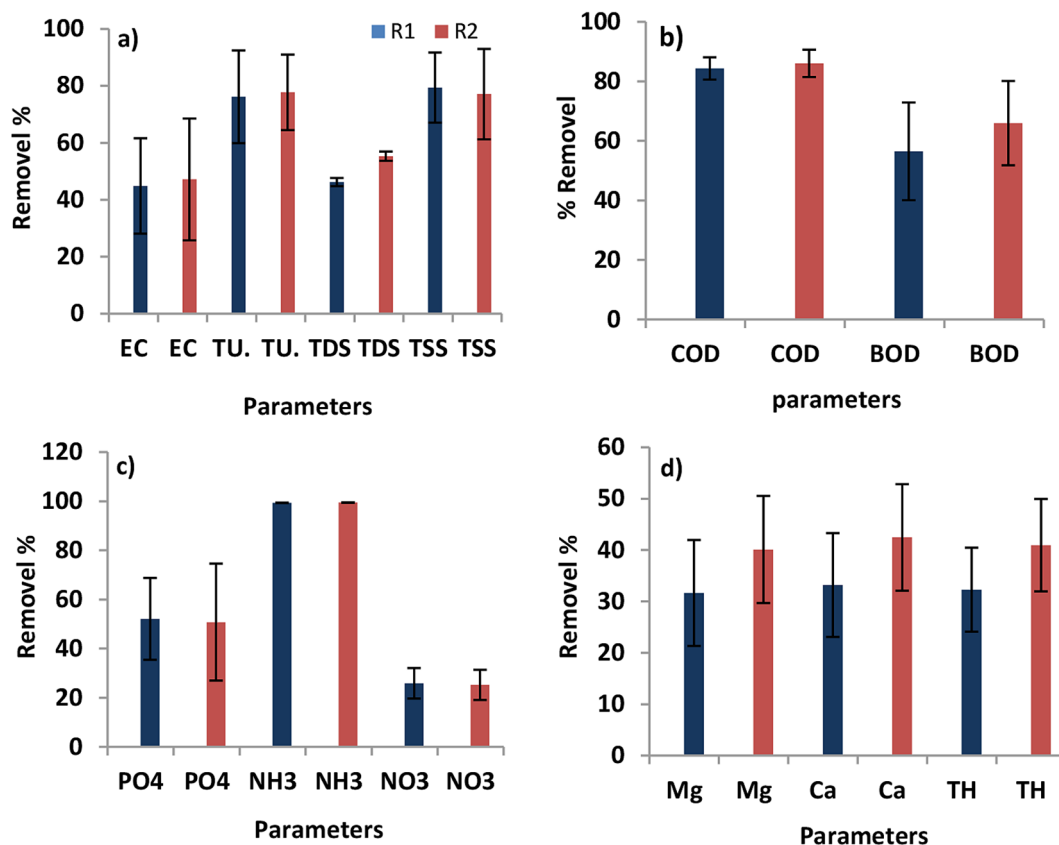
differences between them ( $p > 0.05$ ). this was due to the nitrification process, and plants and microbial absorption. The mean  $\text{NO}_3$  removal efficiency was very low due to the low denitrification level in both treatment systems (Fig. 3c). Also, no significant differences ( $p = 0.543$ , Mann-Whitney U test) were noticed between both of R1 (25.85%) and R2 (25.22%) indicating that both filters were similar in terms of microbes grown on the roots of plants and the media. This confirmed by Zhang et al. (2016). Low  $\text{NO}_3$  reduction in both filters attributed to the limited hypoxic conditions in the treatment systems that is also confirmed by the DO level in both treatment systems.

The reduction of  $\text{PO}_4$  was slightly higher (Fig. 3c) in R1 (52.10%), followed by R2 (50.58%). Statistically, both filters do not show any significant differences between the filters ( $p = 0.95$ , Mann-Whitney U test). Generally,  $\text{PO}_4$  reduction in wetlands achieved by the media (adsorption), plants and microbes (biotic uptake), and sedimentations. Also, it is mentioned that the level of water temperature improved the  $\text{PO}_4$  assimilation by the plants and microbes (Kadlec and Wallace, 2008). Therefore, it seems

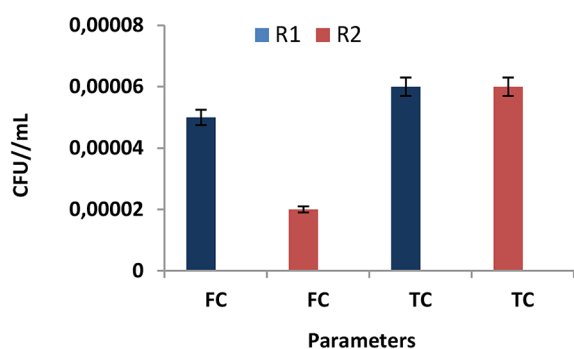
that all the above treatment mechanisms were responsible for  $\text{PO}_4$  reduction. Several authors confirmed that the anaerobic environment is the predominated conditions in HFCW, and therefore it is not efficient for organic matters and nutrients reduction (Hdidou et al., 2021). However, this study proved that the batch mode of wastewater filling and draining, the system and water depths, as well as the type and size of media could alert wetland performance by enhancing the DO level, and consequently achieving high level of  $\text{NH}_3$  reduction comparing with low  $\text{NO}_3$  removal. Andreo-Martínez et al. (2017) also proved a high improvement in HFCW efficiency by increasing the concentration of dissolved oxygen in the filter.

#### Removal rates of calcium and magnesium

The average calcium removal rates of treated water in filter R2 (42.45%) were higher than filter R1 (33.18%). Also, the average magnesium removal rates of treated water in the filter R2 (40.11%) were higher than the filter R1 (31.64%). Significant differences were founded



**Figure 3.** Mean removal efficiency of treatment filters: R1 – treated water by plant + gravel filter; R2 – treated water by plants + biochar + gravel filter



**Figure 4.** Fecal (FC) and total (TC) coliform bacteria concentrations. (R1, treated water by plant + gravel filter; R2, treated water by plants + biochar + gravel filter)

in terms of calcium ( $p = 0.039$ , T-test) and magnesium removal rates ( $p = 0.024$ , T-test) between both treatment filters confirming the impact of biochar as a beneficial adsorbent material (Fig. 3d). Chaukura et al. (2020) confirmed the same results; they mentioned that biochar was very efficient for enhancing the adsorption of calcium and magnesium.

#### Bacterial and plant surveillance

The outcomes displayed that the average fecal and total coliform concentrations for the raw water were  $20 \times 10^{-3}$  CFU/mL, and  $6 \times 10^{-6}$  CFU/mL, in that order. After three months of treatment period (during Winter season), the concentrations of fecal coliform per 10 mL of the sample were reduced to  $5 \times 10^{-5}$  CFU/mL and  $2 \times 10^{-5}$  CFU/mL in R1 and R2, respectively. The values were lower in R2 compared with R1 (Fig. 4). This confirming the impact of biochar in the second filter, which enhanced the fecal coliform removal from the greywater. In terms of total coliform, the concentrations remained  $6 \times 10^{-6}$  CFU/mL for both filters (Fig. 4). In wetlands, aquatic plants are the main factor that crucially affect fecal bacteria by enhancing the level of DO (preferable conditions for living microbes). Furthermore, the aquatic plants have antimicrobial properties by certain secretions (Vymazal, 2005). Bacterial concentrations in treatment systems are affected by the media through the mechanical filtration process (Wand et al., 2007). The regular monitoring of plants survival showed that the plants grew very well during the experiment period in both treatment systems. The green color of the plant was continued within the three months of operation.

## CONCLUSIONS

The designed HFCW was successfully operated in Basra city. The conclusions are summarized as follow. Greywater characteristics enhanced for both treatment systems. High COD removal was achieved by gravel bed (86.05%) more than the gravel-biochar bed (84.33%). The COD reduction was mainly due to the presence of *B. monnieri* L. in the system. The mean BOD<sub>5</sub> removal in biochar-gravel filter 65.97% was higher than gravel bed 56.49% High NH<sub>3</sub> reduction rate in biochar-gravel filter 99.4% and gravel bed 99.3%. The NO<sub>3</sub> removal was very low in both treatment systems. The reduction of PO<sub>4</sub> was slightly higher in gravel bed (52.10%) followed by biochar-gravel bed (50.58%). The removal of Ca<sup>2+</sup>, Mg<sup>2+</sup>, TDS, TU, and EC was higher in biochar-gravel bed comparing with gravel bed only. The concentrations of fecal coliform were reduced to  $5 \times 10^{-5}$  CFU/mL in gravel bed and  $2 \times 10^{-5}$  in biochar-gravel filter. The plants monitoring showed that the plant grew very well and remaining green during the experiment period.

## Acknowledgments

The authors would like to express appreciation to the Department of Ecology in collaboration with the Department of Civil Engineering, at the University of Basrah for supporting laboratories and the facilities to carry out this research.

## REFERENCES

1. Abdelhay, A., Abunaser, S.G. 2021. Modeling and economic analysis of greywater treatment in rural areas in Jordan using a novel vertical-flow constructed wetland. *Environmental Management*, 67(3), 477–488. <https://doi.org/10.1007/s00267-020-01349-7>
2. Abedi, T., Mojiri, A. 2019. Constructed wetland modified by biochar/zeolite addition for enhanced wastewater treatment. *Environmental Technology & Innovation*, 16, 100472. <https://doi.org/10.1016/j.eti.2019.100472>
3. Abunaser, S.G., Abdelhay, A. 2020. Performance of a novel vertical flow constructed wetland for greywater treatment in rural areas in Jordan. *Environmental Technology*, 1–11. <https://doi.org/10.1080/09593330.2020.1841832>
4. Andreo-Martínez, P., García-Martínez, N., Quesada-Medina, J., Almela, L. 2017. Domestic wastewaters reuse reclaimed by an improved

- horizontal subsurface-flow constructed wetland: A case study in the southeast of Spain. *Bioresour. Technol.*, 233, 236–246. <https://doi.org/10.1016/j.biortech.2017.02.123>
5. APHA. 1999. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association (APHA), American Water Works Association, and Water Environment Federation, Washington DC.
  6. APHA. 2005. Standard methods for the examination of water and waste-water, 21st ed. American Public Health Association (APHA), American Water Works Association, and Water and Environment Federation, Washington DC.
  7. APHA. 2012. Standard methods for the examination of water and wastewater, 22nd ed. American Public Health Association (APHA), American Water Works Association, and Water Environment Federation, Washington DC.
  8. Borne, K.E. 2014. Floating treatment wetland influences on the fate and removal performance of orthophosphate in stormwater retention ponds. *Ecological Engineering*, 69, 76–82. <https://doi.org/10.1016/j.ecoleng.2014.03.062>
  9. Chaukura, N., Chiworeso, R., Gwenzi, W., Motsa, M.M., Munzeiwa, W., Moyo, W., Nkambule, T.T. 2020. A new generation low-cost biochar-clay composite ‘biscuit’ ceramic filter for point-of-use water treatment. *Applied Clay Science*, 185, 105409. <https://doi.org/10.1016/j.clay.2019.105409>
  10. Chrispim, M.C., Nolasco, M.A. 2017. Greywater treatment using a moving bed biofilm reactor at a university campus in Brazil, *Journal of Cleaner Production*. Elsevier Ltd, 142, 290–296. <https://doi.org/10.1016/j.jclepro.2016.07.162>
  11. Chyan, J.M., Senoro, D.B., Lin, C.J., Chen, P.J., Chen, I.M. 2013. A novel biofilm carrier for pollutant removal in a constructed wetland based on waste rubber tire chips. *International Biodeterioration & Biodegradation*, 85, 638–645. <https://doi.org/10.1016/j.ibiod.2013.04.010>
  12. Couto, E.A.D., Calijuri, M.L., Assemany, P.P., Santiago, A.F., Lopes, L.S. 2015. Greywater treatment in airports using anaerobic filter followed by UV disinfection: an efficient and low cost alternative. *J. Clean. Prod.*, 106, 372–379. <https://doi.org/10.1016/j.jclepro.2014.07.065>
  13. Dalahmeh, S.S., Lalander, C., Pell, M., Vinnerås, B., Jönsson, H. 2016. Quality of greywater treated in biochar filter and risk assessment of gastroenteritis due to household exposure during maintenance and irrigation. *Journal of applied microbiology*, 121(5), 1427–1443. <https://doi.org/10.1111/jam.13273>
  14. EPA, U.S. 1978. Method 365.3: Phosphorus, all forms (colorimetric, ascorbic acid, two reagent). US EPA. United States Environmental Protection Agency Washington, DC.
  15. EPA, U.S. 2000. Constructed wetlands treatment of municipal wastewater treatment. EPA 625/R-99/010, U.S. EPA Office of Research and Development: Washington, D.C., United States.
  16. Gizińska-Górna, M., Czekala, W., Józwiakowski, K., Lewicki, A., Dach, J., Marzec, M., Listosz, A. 2016. The possibility of using plants from hybrid constructed wetland wastewater treatment plant for energy purposes. *Ecological Engineering*, 95, 534–541. <https://doi.org/10.1016/j.ecoleng.2016.06.055>
  17. Gold, J., Afrooz, N., Boehm, A. 2017. Analysis of modified sand filtration for the capture and storage of grey water nutrients. *J Earth Environ Science*. <https://doi.org/10.29011/JEES-102.100002>.
  18. Gupta, P., Ann, T.W., Lee, S.M. 2016. Use of biochar to enhance constructed wetland performance in wastewater reclamation. *Environmental Engineering Research*, 21(1), 36–44. <http://dx.doi.org/10.4491/eeer.2015.067>
  19. Hdidou, M., Necibi, M.C., Labille, J., El Hajjaji, S., Dhiba, D., Chehbouni, A., Roche, N. 2021. Potential use of constructed wetland systems for rural sanitation and wastewater reuse in agriculture in the Moroccan context. *Energies*, 15(1), 156. <https://doi.org/10.3390/en15010156>
  20. Juan, Y.K., Chen, Y., Lin, J.M. 2016. Greywater reuse system design and economic analysis for residential buildings in Taiwan. *Water*, 8(11), 546. <https://doi.org/10.3390/w8110546>
  21. Kadlec, R.H., Wallace, S.D. 2008. *Treatment wetlands*. Boca Raton, Florida: CRC Press. <https://doi.org/10.1201/9781420012514>
  22. Kozak, C., Schirmer, W.N., Gomes, S., da Fonseca, A.F. 2014. Verifying the efficacy in removing nutrients using wastewater treatment stations by constructed wetlands. *recursos hídricos*, 35(1).
  23. Laaffat, J., Aziz, F., Ouazzani, N., Mandi, L. 2016. Biotechnological approach of greywater treatment and reuse for landscape irrigation in small communities. *Saudi journal of biological sciences*, 26(1), 83–90. <https://doi.org/10.1016/j.sjbs.2017.01.006>
  24. Lastiri-Hernández, M.A., Álvarez-Bernal, D., Cruz-Cárdenas, G., Silva-García, J.T., Conde-Barajas, E., Oregel-Zamudio, E. 2023. Potential of *Epipremnum aureum* and *Bacopa monnieri* (L.) Wettst for Saline Phytoremediation in Artificial Wetlands. *Water*, 15(1), 194. <https://doi.org/10.3390/w15010194>
  25. Mwenge, P., Seodigeng, T. 2019. Greywater Treatment Using Activated Biochar Produced from Agricultural Waste. *International Journal of Chemical and Molecular Engineering*, 13(3), 140–145. <http://doi.org/10.5281/zenodo.2643647>
  26. Oliveira, G.A., Colares, G.S., Lutterbeck, C.A., Dell’Osbel, N., Machado, Ê.L., Rodrigues, L.R.

2021. Floating treatment wetlands in domestic wastewater treatment as a decentralized sanitation alternative. *Science of the Total Environment*, 773, 145609. <https://doi.org/10.1007/s11356-021-17395-5>
27. Paul, D., Hall, S.G. 2021. Biochar and Zeolite as Alternative Biofilter Media for Denitrification of Aquaculture Effluents. *Water*, 13(19), 2703. <https://doi.org/10.3390/w13192703>
28. Priya, S.G., Brighu, U. 2013. Comparison of different types of media for nutrient removal efficiency in vertical upflow constructed wetlands. *International Journal of Environmental Engineering and Management*, 4(5). <https://doi.org/10.1186/s42834-021-00087-7>
29. Qian, W., Ma, B., Li, X., Zhang, Q., Peng, Y. 2019. Long-term effect of pH on denitrification: High pH benefits achieving partial-denitrification. *Bioresource Technology*, 278, 444–449. <https://doi.org/10.1016/j.biortech.2019.01.105>
30. Salem, I.B., El Gamal, M., Sharma, M., Hameedi, S., Howari, F.M. 2021. Utilization of the UAE date palm leaf biochar in carbon dioxide capture and sequestration processes. *Journal of Environmental Management*, 299, 113644. <https://doi.org/10.1016/j.jenvman.2021.113644>
31. Shaikh, I.N., Ahammed, M.M. 2020. Quantity and quality characteristics of greywater: a review. *Journal of environmental management*, 261, 110266. <https://doi.org/10.1016/j.jenvman.2020.110266>
32. Shukla, R., Gupta, D., Singh, G., Mishra, V.K. 2021. Performance of horizontal flow constructed wetland for secondary treatment of domestic wastewater in a remote tribal area of Central India. *Sustainable Environment Research*, 31(1), 1–10. <https://doi.org/10.1186/s42834-021-00087-7>
33. Sychala, M., Niec, J., Zawadzki, P., Matz, R., Nguyen, T.H. 2019. Removal of volatile solids from greywater using sand filters. *Appl. Sci.*, 770, 2–13. <https://doi.org/10.3390/app9040770>
34. Tanner, C.C., Headley, T., Dakers, A. 2011. Guidelines for the Use of Horizontal Subsurface-Flow Constructed Wetlands in On-Site Treatment of Household Wastewaters. National Institute of Water & Atmospheric Research: Hamilton, New Zealand
35. Tsang, E. 2015. Effectiveness of Wastewater Treatment for Selected Contaminants Using Constructed Wetlands in Mediterranean Climates.
36. Verhamme, D.T., Prosser, J.I., Nicol, G.W. 2011. Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. *ISME J.*, 5, 1067. <https://doi.org/10.1038/ismej.2010.191>
37. Vymazal, J. 2005 Removal of enteric bacteria in constructed treatment wetlands with emergent macrophytes: A review. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering*, 40(6–7), 1355–1367. <https://doi.org/10.1081/ESE-200055851>
38. Vymazal, J. 2007. Removal of nutrients in various types of constructed wetlands. *Science of the total environment*, 380(1–3), 48–65. <https://doi.org/10.1016/j.scitotenv.2006.09.014>
39. Wand, H., Vacca, G., Kuschik, P., Kruger, M., Kastner, M. 2007. Removal of bacteria by filtration in planted and non-planted sand columns. *Water Research*, 41(1), 159–167. <https://doi.org/10.1016/j.watres.2006.08.024>
40. Wang, W., Ding, Y., Ullman, J.L., Ambrose, R.F., Wang, Y., Song, X., Zhao, Z. 2016. Nitrogen removal performance in planted and unplanted horizontal subsurface flow constructed wetlands treating different influent COD/N ratios. *Environmental Science and Pollution Research*, 23(9), 9012–9018. <https://doi.org/10.1007/s11356-016-6115-5>
41. Xing, C., Xu, X., Xu, Z., Wang, R., Xu, L. 2021. Study on the decontamination effect of biochar-constructed wetland under different hydraulic conditions. *Water*, 13(7), 893. <https://doi.org/10.3390/w13070893>
42. Yaseen, D.A., Scholz, M. 2016. Shallow pond systems planted with *Lemna minor* treating azo dyes. *Ecological engineering*, 94, 295–305. <http://dx.doi.org/10.1016/j.ecoleng.2016.05.081>
43. Yaseen, D.A., Scholz, M. 2018. Treatment of synthetic textile wastewater containing dye mixtures with microcosms. *Environmental Science and Pollution Research*, 25, 1980–1997. <https://doi.org/10.1007/s11356-017-0633-7>
44. Yaseen, D.A., Scholz, M. 2017. Comparison of experimental ponds for the treatment of dye wastewater under controlled and semi-natural conditions. *Environmental Science and Pollution Research*, 24(19), 16031–16040. <https://doi.org/10.1007/s11356-017-9245-5>
45. Zaboon, B.H., Al-Abbawy, D.A., Yaseen, D.A. 2022. Improving Wastewater Reclamation Using Constructed Wetlands by Artificial Plastic Biofilm Carriers. *Journal of Ecological Engineering*, 23(11), 241–253. <https://doi.org/10.12911/22998993/153459>
46. Zamora, S., Marín-Muñiz, J.L., Nakase-Rodríguez, C., Fernández-Lambert, G., Sandoval, L. 2019. Wastewater treatment by constructed wetland ecotechnology: Influence of mineral and plastic materials as filter media and tropical ornamental plants. *Water*, 11(11), 2344. <https://doi.org/10.3390/w11112344>
47. Zhang, L., Zhao, J., Cui, N., Dai, Y., Kong, L., Wu, J., Cheng, S. 2016. Enhancing the water purification efficiency of a floating treatment wetland using a biofilm carrier. *Environmental Science and Pollution Research*, 23(8), 7437–7443. <https://doi.org/10.1007/s11356-015-5873-9>

48. Zhang, B., Ning, D., Yang, Y., Van Nostrand, J.D., Zhou, J., Wen, X. 2020. Biodegradability of wastewater determines microbial assembly mechanisms in full-scale wastewater treatment plants. *Water Research*, 169, 115276. <https://doi.org/10.1016/j.watres.2019.115276>
49. Zhou, X., Liang, C., Jia, L., Feng, L., Wang, R., Wu, H. 2018. An innovative biochar-amended substrate vertical flow constructed wetland for low C/N wastewater treatment: impact of influent strengths. *Bioresource Technology*, 247, 844–850. <http://dx.doi.org/10.1016/j.biortech.2017.09.044>
50. Zidan, A.R.A., El-Gamal, M.M., Rashed, A.A., Eid, M.A.A.E.H. 2015. Wastewater treatment in horizontal subsurface flow constructed wetlands using different media (setup stage). *Water Science*, 29(1), 26–35. <https://doi.org/10.1016/j.wsj.2015.02.003>