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Article

Comparative Study of Three Two-Stage Hybrid Ecological Wastewater Treatment Systems for Producing High Nutrient, Reclaimed Water for Irrigation Reuse in Developing Countries

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Abstract: In this study, three different two-stage hybrid ecological wastewater treatment systems (HEWTS) with combinations of horizontal flow (HF) constructed wetlands (CWs), vertical flow (VF) CWs and stabilization ponds (SP) were evaluated for the removal of Organic-N, NH_4^+, NO_3^-, Total N, Total P, Total Coliforms (TCol) and *Escherichia Coli*, BOD, COD and TSS. The overall goal of the study was novel in comparison to most other studies in that we sought to evaluate and compare the efficiency of the three HEWTSs for water quality improvements, while minimizing nutrient removal from the wastewater in order to generate high quality reclaimed water for reuse for irrigation of crops. The most effective systems were those systems containing a vertical flow component, either HF-VF or VF-HF. In these two HEWTS, NH_4^+ was reduced by 85.5% and 85.0% respectively, while NO_3^- was increased to 91.4 ± 17.6 mg/L and to 82.5 ± 17.2 mg/L, respectively, an artifact of nitrification. At the same time, *E. coli* was reduced by 99.93% and 99.99%, respectively. While the goal of most wastewater treatment is focused on reducing nutrients, the results here demonstrate that two-stage HEWTSs containing VF components can be used to produce a high quality effluent while retaining inorganic nutrients, thereby conserving this valuable resource for reuse as irrigation water for agriculture in subtropical developing countries where water and fertilizer resources are scarce or expensive.

Keywords: horizontal flow constructed wetlands; vertical flow constructed wetlands; stabilization aerobic ponds; nutrients; sustainability; developing countries; subtropical

1. Introduction

Irrigation of crops with raw, municipal wastewater has been a common practice for many decades in developing countries such as China, Mexico, Peru, Egypt, Lebanon, Morocco, India and Vietnam, mainly due to its nutrient value recognized by farmers [1]. Moreover, in some poor areas of developing countries like Mexico, wastewater reuse represents a critical opportunity of improving living standards by increasing income and ensuring food supplies [2]. Unfortunately, the use of untreated municipal wastewater in an agricultural setting poses risks to human health due to the potential presence of excreta-related pathogens (viruses, bacteria, protozoan and multicellular parasites), skin irritants and toxic chemicals including heavy metals; although it is uncommon to find unsafe levels of heavy metals in municipal wastewater [3]. Consequently, it is important to both treat the wastewater and select wastewater treatment processes that reduce pathogen while retaining nutrients if the water is to be applied for irrigation purposes [4]. Reuse of treated, high-quality reclaimed wastewater for agriculture not only protects human health but is also a good conservation strategy by reducing the consumption of limited drinking water for irrigation and reducing fertilizer costs to the agricultural sector in low-income countries.

Constructed wetlands and waste stabilization ponds are the most widely used ecological wastewater treatment systems in use in the world [5]; although they require significantly more land area than other treatment options. These technologies have proven to be effective treatment alternatives, using natural processes for treating wastewater in small and medium communities, mainly, worldwide. These systems are capable of reaching nearly 100% removal of parasitic eggs due to longer retention times in comparison to more expensive and energy-intensive conventional technologies [6]. In general, a one-stage system is usually not sufficient to effect pathogen reduction to safe target levels [7]. Nitrogen removal in constructed wetlands (CWs) and in particular, stabilization ponds (SPs) is often limited due to the lack of a sophisticated, controlled series of environmental conditions that promote settling, then oxidation followed by reduction which is required for organics N removal, and for promotion of coupled nitrification -denitrification [8]. The same is true for phosphorus removal.

Constructed wetlands have been extensively evaluated mostly in temperate climate prevalent in developed countries; in contrast, the experiences are less abundant under tropical and subtropical areas. In rich countries, the design criteria and guidelines have emphasized nutrient removal. However, the limited capacity of natural systems for nutrient removal is an advantage when the treatment goal is to produce a reclaimed wastewater for irrigation to promote plant growth. A low pathogen concentration, high nutrient content (in particular N) and low presence of heavy metals or other toxic pollutant in reuse water are very desirable in reclaimed wastewater for agricultural irrigation [9]. Hybrid ecological wastewater treatment systems take advantages of the strengths of each different type of CWs and SPs for improving water quality and combine them in order to produce high quality reclaimed water, such as a partially or fully nitrified effluent with low concentrations of indicator organisms and BOD [10,11].

Therefore, in this study three, relatively low cost hybrid ecological wastewater treatment systems (HEWTSs) were evaluated for the treatment of a high-ammonium concentration wastewater generated at a university over one year of operation. The goal was to compare the efficiency of the three HEWTSs arranged as two-stage systems for pollutant removal in order to produce high-quality reclaimed water appropriate for agricultural irrigation claiming the coupled benefits of water and nutrient recycling.

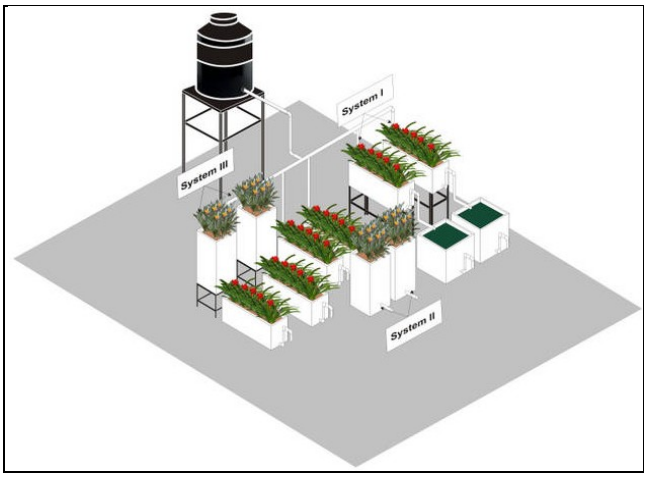
2. Materials and Methods

2.1. Description of the Wetland Systems

The study was carried out at the Centro Universitario de la Ciénega in Ocotlán, Jalisco, México from September 2009 to August 2010. The climate in the area is classified as warm and wet with rainfall in summer (ACw). The altitude is between 1530 and 1600 m above sea level. A 1100 L tank was used to store the wastewater which was pumped daily from the sewer line located on the campus. The tank was adapted to provide some opportunity for sedimentation of solids. The wastewater was a mixture of gray water (from a cafeteria), and sewage and wastewater from teaching and research laboratories. A total flow rate of ~200 L/d of wastewater was treated and distributed equally among the HEWTSs. The design hydraulic loading rate for the HF-CW, VF-CW and SP were 6.9 cm/d, 14.5 cm/d and 6.8 cm/d.

Three two-stage HEWTSs were evaluated in duplicate (Figure 1). System I consisted of a horizontal flow CW followed by a stabilization pond (HF-SP). The CWs were continuously fed with a theoretical hydraulic retention time of 3 days. The effluent from the CWs flowed by gravity to the stabilization ponds. System II was also configured with a horizontal flow CW as a first stage which was then followed by a vertical flow CW as a second stage (HF-VF). The horizontal flow CW operated in the same way as in the system I but the effluent was collected in a tank and pumped intermittently every 2 h on to the substrate of the vertical flow CW. System III was configured with a vertical flow CW followed by a horizontal flow CW (VF-HF). The vertical flow CW was intermittently fed by a pump programmed to discharge 2.8 L every 2 h on to the surface, specifically over the plant without a distribution system. The effluent flowed by gravity to the next stage.

Figure 1. Hybrid ecological wastewater treatment systems. System I: HF-SP, System II: HF-VF and System III: VF-HF.



The dimensions of the horizontal flow CWs, stabilization ponds and vertical flow CWs were 120 cm × 40 cm × 50 cm (L × W × H); 70 cm × 70 cm × 70 cm (L × W × H) and 48 cm × 48 cm × 110 cm (L × W × H), respectively. All the units were constructed of fiberglass and reinforced with external iron bars for supports. The horizontal flow CWs were planted with 6 (25–30 cm-height) individual of *Zantedeschia aethiopica* plants and the vertical flow CWs were planted with 1 individual, adult plant of *Strelitzia reginae*. These species were previously used in CWs for domestic wastewater treatment with successful results [12]. After six months of experimentation, the *Z. aethiopica* plants were replaced with *Canna indica* (a well-known wetland plant) due to the fact that the former plants desiccated during the dry season characterized by low air humidity and high ambient temperatures. Ground tezontle rock was used as the media in all the CWs after first being sieved through a 0.5-mm-opening sieve to remove finer particles which would typically clog subsurface-flow systems. The media sieve analysis revealed a d_{10} of 0.645 mm, d_{60} of 2.3 mm and a uniformity coefficient (UC) of 3.6.

2.2. Water Quality Parameters

The systems were fed with wastewater since the beginning but allowed to stabilize for four months and then monitored weekly for the following eight months. Organic-N, Ammonia, Nitrate, total N, BOD, COD, TSS, total P, TCol, *E. Coli*, pH, OD and Conductivity were measured at the influent and effluent of each system. Chemical and biological water quality parameters were determined as described in the Standard Methods for the examination of Water and Wastewater [13]. Total coliforms and *E. Coli* were quantified by the Colilert method. Samples were analyzed immediately after they were taken, in the Quality Environmental Laboratory at the university. When this was not possible, samples were preserved at 4 °C and analyzed within 24 h. A potentiometer (Thermo Scientific 3 Star) was used to measure pH and conductivity.

2.3. Data Analysis

A randomized block design was used to analyze the data in this study. Multifactor analysis of variance (ANOVA) was carried out using the Statgraphics Centurion XVI software package to check differences amongst treatments (influent and the three HEWTSs or influent and the two stages in each individual HEWTS). A significance level of $p = 0.05$ was used for all statistical tests, and values reported are the mean (average) ± standard error of the mean. When a significant difference was observed between treatments in the ANOVA procedure, multiple comparisons were made using the Least Significant Difference (LSD) test for differences between means.

3. Results and Discussion

The mean characteristics of both the influent water and the treated water for each of the three HEWTSs are presented in Tables 1–3. Overall, the influent was dominated by ammonium-N, comprising almost 92% of total N with a mean TP value of 12.4 mg/L. Other water quality parameters of the influent include 140 mg/L BOD, 273 mg/L COD and 61.8 mg/L TSS (Tables 1–3).

3.1. Organic Nitrogen

Organic-N in the output of the HEWTSs was significantly different in comparison to the input ($p < 0.05$). In the (HF-SP) an increase to a mean of 10.5 mg/L was observed due to the presence of algae in stage II which assimilated ammonia and nitrate producing Organic-N [14]. In contrast, Organic N was reduced in the other two hybrid HEWTS (HF-VF; VF-HF) reaching statistically similar values of 1.6 and 1.2 mg/L, respectively in the effluents (Table 1). The increase in Org-N in the first system and the reduction in the other two hybrid constructed wetlands demonstrate the superior performance of these two systems for the reduction of organic N.

Table 1. Performance summary for the three hybrid ecological wastewater treatment systems (HEWTSs) with respect to nutrient removal. Average ± standard error of the mean. Entire system removal percentages are in parentheses with bold letter.

Parameter	Influent	System I: HF-SP		System II: HF-VF		System III: VF-HF	
		1st stage HF-CW	2nd stage SP	1st stage HF-CW	2nd stage VF-CW	1st stage VF-CW	2nd stage HF-CW
Org-N (mg/L)	7.1 ± 1.2	3.2± 1.0	10.5± 1.0	2.8 ± 0.7	1.6 ± 0.7	1.4 ± 0.8	1.2 ± 0.8
Org-N Removal (%)		54.9	−228.1 (**−47.9**)	60.6	42.9 (**77.5**)	80.3	* (**83.1**)
NH_4^+-N (mg/L)	128.2 ± 11.4	103.1± 11.4	35.8 ± 12.2	103.4 ± 12.4	18.6 ± 5.6	25.1 ± 6.7	19.2 ± 6.2
NH_4^+-N Removal (%)		19.6	65.3 (**72.0**)	19.3	82.0 (**85.5**)	80.4	* (**85.0**)
NO_3^--N (mg/L)	4.2 ± 1.4	1.95± 0.3	14.1 ± 1.8	1.97 ± 0.6	91.4 ± 17.6	108 ± 16.3	82.5 ± 17.2
NO_3^--N Removal (%)		53.6	−623 (**−236**)	53.1	−4540 (**−2076**)	−2471	23.6 (**−1864**)
TN (mg/L)	139.5 ± 12.1	108.3±12.1	60.4 ± 9.9	108.2 ± 22.2	111.6 ± 13.2	134.5 ± 21.1	102.9 ± 13.1
TN Removal (%)		22.4	44.2 (**56.7**)	22.4	* (**20**)	*	23.5 (**26.2**)
TP (mg/L)	12.4 ± 1.1	11.8± 1.0	11.4 ± 1.0	12.1 ± 1.1	12.2 ± 1.0	11.3 ± 1.1	12.4 ± 1.1
TP removal (%)		*	*	*	*	*	*

Note: * No significant difference with regard to the concentration in the previous stage.

3.2. Ammonium

Ammonium concentration in the raw wastewater was unexpectedly high (Table 1), perhaps due to the use of liquid culture medium employed in the Biology cell and Microbiology labs and subsequent disposal down the sinks. The NH_4^+-N was reduced significantly in all three systems ($p < 0.05$) with a higher similar decrease in the HF-VF and VF-HF systems. These results support the findings by other authors with respect to the important role that vertical flow CWs plays for treating high-ammonia wastewater [15]. The reduction of ammonium concentrations was achieved mainly in the VF unit (>80%), regardless of position in the HEWTs. The presence of vertical flow CWs in the HEWTSs significantly increased the efficiency of the treatment by more than 13% with respect to the (HF-SP) system containing no VF component. The aerobic conditions present in VF systems likely contributed to this ammonium reduction due to the oxidation of NH_4^+ to NO_3^- (nitrification) under aerobic condition [16]. This result is in line with other studies using a similar VF system with gravel as a substrate, such as that in which a reduction of ammonium of 47.5% was achieved under similar influent concentrations [17] and another one under Mediterranean weather, where a reduction of 74%

was reached with an influent concentration of 43.1 mg/L [18]. In addition, the ammonium-reduction performance of these two HEWTS containing VF components were similar to 88% of ammonia-N removal found in other study [9] in a hybrid VF-HF system as the mean reduction in our systems averaged 85.3%.

3.3. Nitrate

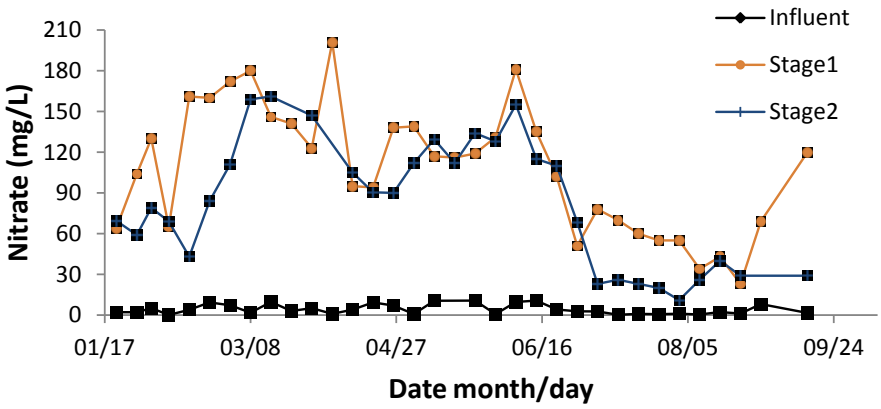
As expected, nitrate increased significantly in the three HEWTSs ($p < 0.05$). In concert with the high reduction rates of ammonium, there were higher and analogous increases in nitrate concentration in both systems containing VF treatment components (Table 1, Figure 2a–c). This result was driven by the greater nitrification capacity in the VF components in comparison to the stabilization ponds which anchored the other system. In the three different systems, there was a significant difference between the two stages in the nitrate concentration ($p < 0.05$). The system composed of VF followed by HF had a 23.6% nitrate reduction from the first stage to the second stage. The HF wetland systems typically have greater reduction rates for nitrate [19,20]. However, our system may have lacked sufficient organic carbon to stimulate denitrification [21]. The BOD was almost entirely removed in the first stage leaving little bioavailable carbon for denitrification in the second stage, having as a consequence a nitrate accumulation [8]. According to several researchers, a source of dissolved organic matter should be provided to improve nitrate removal by recirculation or by adding an external organic source such as methanol to provide the needed electron donors for denitrification [22,23]. However, in the case where the goal of treatment is to provide a nitrified effluent, these results indicate that the two systems containing VF systems are preferred and are equally effective in maintaining bioavailable N which can be used for crop production. Other authors have arrived at the same conclusions when the treated wastewater is to be reused in irrigation [24].

3.4. Total N

Total N was significantly reduced in the three HEWTSs ($p < 0.05$) with greater removal in the HF-SP system in comparison to systems containing VF components (Table 1, Figure 3). It is likely that ammonia volatilization, common in algae dominated, aerobic stabilization ponds was responsible for this higher removal of TN. Photosynthetic activity in surface waters can drive up pH during the day leading to ammonium volatilization [25]. The pH (measured from 8–10 am each day) of the HF-SP system, averaged above 8 during all stages of treatment. Since TN was dominated by ammonium in the influent, a greater removal of TN by the system suggests removal of N from the system by either ammonia volatilization or coupled nitrification-denitrification versus simply converting ammonium to nitrate (denitrification) as was the case in the two VF systems. Although aerobic conditions generally predominate in aerobic stabilization ponds during the day driven by photosynthetic activity of algae, dissolved oxygen can drop as a result of diurnal variations to a very low level during night time permitting denitrification to take place [26]. Recall in the two systems containing VF components, it is noticeable (Figure 3) that nitrogen in the effluent is composed primarily of nitrate while in contrast, in the system containing the stabilization pond, nitrogen is primarily in the ammonium form. As previously stated, this nitrate accumulation was due to the depletion of biodegradable organic compounds in the previous stage that inhibited denitrification, as has been reported by others [8].

Moreover, the average total N removal in the HF-VF and VF-HF systems were similar to the 29% removal, achieved in HF-VF systems under warm climate in Mediterranean region. An increase to 66% total N removal was only possible through 100% wastewater recirculation through the system [15].

Figure 2. Nitrate concentration in the three HEWTSs along the monitoring period. (**a**) HF-SP; (**b**) HF-VF; (**c**) VF-HF.



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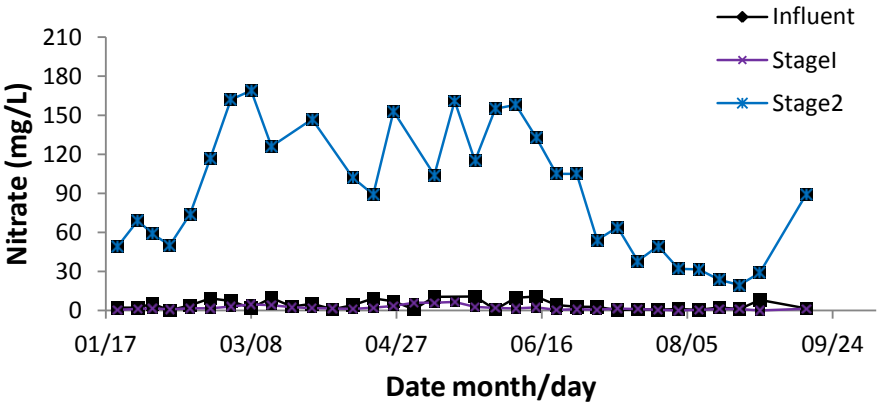

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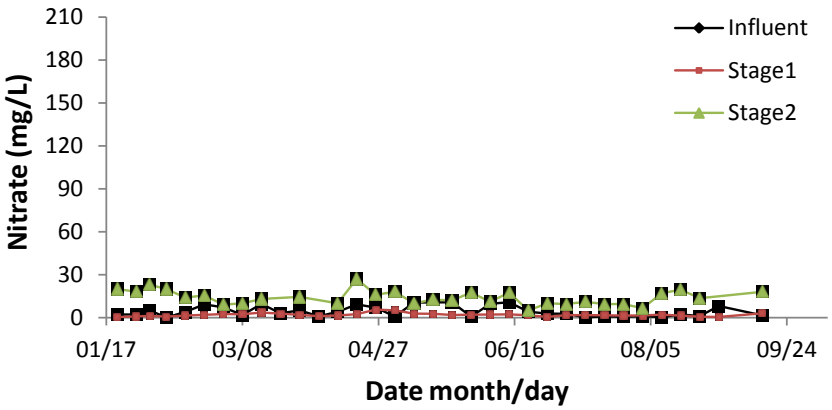


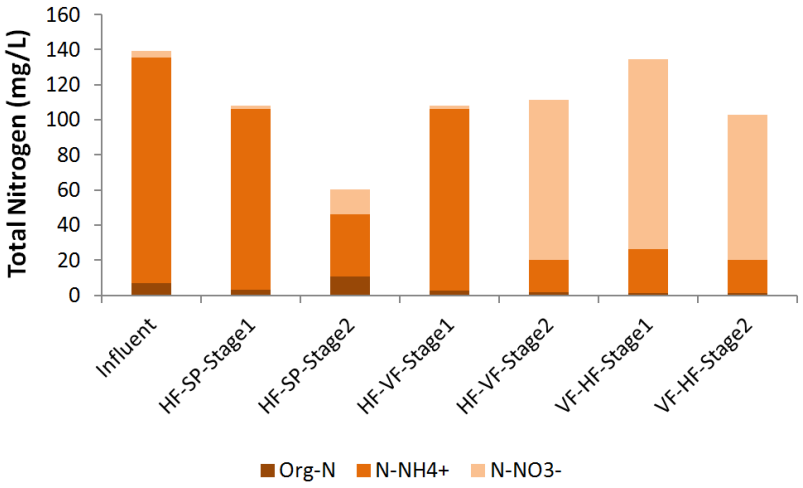
(**c**)

3.5. Total P

It is well known that TP removal in constructed wetlands can be complicated due to the coupling of both organic and inorganic removal mechanisms [27] available for P in wetland systems as well as

regeneration of P from organic matter back into the water column [28,29]. As a result many single treatment systems and hybrid systems have reported low TP removal rates [30,31]; in a VF-HF system with gravel, a removal of only 21% was achieved [11]. In our case, the decrease in TP concentration at the effluent of the three HEWTSs was not significant ($p > 0.05$). The same finding was obtained between the two stages in each system ($p > 0.05$). In previous studies, with the same substrate, ~45% and ~50% of phosphorus removal was achieved in the horizontal flow CWs and vertical flow CWs, respectively [12]. The lack of TP removal in the horizontal flow (HF) system in this work was likely due to the fact that six months after beginning the experiment (two months after beginning the monitoring period), the ornamental emergent plants (*Z. aethiopica*) began to desiccate in the horizontal flow CWs in all the three systems. As the plants senesced and decayed, they are essentially releasing P to the systems. In the vertical flow components, there was non-uniform distribution of the influent as there was no distribution system that evenly distributed the wastewater over the surface of the bed, but instead the wastewater was fed over the plant. This uneven distribution pattern may have contributed to the low P removal. In vertical flow CWs, adsorption and precipitation of phosphorus is effective in systems where wastewater continuously comes into contact with the filtration substrate [32]. While the substrate provides sites for P removal by sorption initially, the sorption sites can become saturated, over time [33]. It is at that point that the expansion of the root systems from the developing plants (plant uptake) would replace sorption to the substrate as the major P removal mechanism over time. However, if the goal of the water reuse is for agricultural purposes, the low removal rate of P in this case is preferable, much as it was for N, as the nutrients will be available for whatever crop receives the reclaimed water [11].

Figure 3. Average TN concentrations in the influent and effluents of the HEWTSs.



3.6. Disinfection Performance

Total coliform removal was significantly lower in the HF-SP system in comparison to the other two systems which contained VF components ($p < 0.05$) and whose removals were similar. An increase in TCol was observed in the stabilization ponds (Table 2) perhaps related to feces of birds frequently observed in the ponds. In addition, it is well known that TCol can reproduce in surface water when stimulated by high nutrient availability [34].

With regard to *E. coli* reductions, findings were similar to the results of TCol. The HF-VF and VF-HF configured systems were more effective (3.2 log unit reduction and 3.9 log unit reduction, respectively) ($p < 0.05$) than the HF-SP system (2.6 log unit reduction) (Table 2, Figure 4a–c). The predominant aerobic conditions in VF systems represent the main factor responsible for their higher efficiency in indicator organism removal; the aerobic conditions are not optimal for their success and allow higher predator abundance [8,12]. In this way, the HF-VF and VF-HF systems evaluated in this work in subtropical climate were highly efficient and achieved a reduction of *E. coli* that fulfills the levels established in the Mexico's current national guideline for reclaimed water reuse for agricultural irrigation purposes [35]. Similar findings were obtained in VF-HF systems in a study performed in a tropical climate [7] and in a HF-VF system which treated wastewater produced by a hotel under hot Mediterranean climate, where the authors report a 99.93%–99.99% removal of indicator organisms [24]. In addition, these results satisfy the 2006 WHO guidelines that require a 3–4 log unit pathogen reduction by wastewater treatment in order to protect the health of those working in wastewater-irrigated field and those consuming wastewater-irrigated food crops [36].

Table 2. Performance summary for the three HEWTSs with respect to indicator organisms. Average ± standard error of the mean. Entire system removal percentages are in parentheses with bold letter.

Parameter	Influent	System I: HF-SP		System II: HF-VF		System III: VF-HF	
		1st stage HF CW	2nd stage SP	1st stage HF-CW	2nd stage VF-CW	1st stage VF-CW	2nd stage HF-CW
Tot.Coliform (MPN/100mL)	$2.5 \times 10^6 \pm 9.9 \times 10^5$	$2.0 \times 10^5 \pm 6.3 \times 10^4$	$3.8 \times 10^5 \pm 1.4 \times 10^5$	$2.1 \times 10^5 \pm 6.6 \times 10^4$	$1.6 \times 10^4 \pm 5.7 \times 10^3$	$1.1 \times 10^5 \pm 4.1 \times 10^4$	$7.7 \times 10^4 \pm 2.5 \times 10^4$
Tot.Coliform Removal (%)		92.0	−90.0 (84.8)	91.6	92.38 (99.36)	95.6	30.00 (96.92)
E. coli (*MPN/100 mL*)	$1.6 \times 10^6 \pm 6.8 \times 10^5$	$3.1 \times 10^4 \pm 9.6 \times 10^3$	4210 ± 1457	$3.1 \times 10^4 \pm 1.3 \times 10^4$	1060 ± 326	$3.8 \times 10^4 \pm 6.2 \times 10^3$	213.1 ± 59.0
E. coli Removal (%)		98.06	86.42 (99.74)	98.06	96.58 (99.93)	97.63	99.44 (99.99)

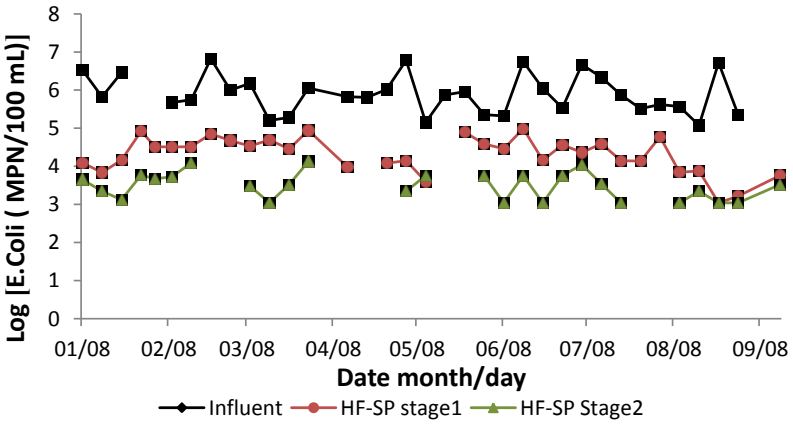
With respect to the individual components that made up these hybrid in-series treatment systems, the removal rate of TCol in each of the components were similar to those obtained in previous studies with either one-stage horizontal flow CWs and vertical flow CWs treatment systems [12]. Therefore, these results demonstrate that at least two stages of treatment in HEWTs are required to achieve the disinfection level in order to qualify the reuse of reclaimed water in a safe approach for irrigation to agricultural fields.

3.7. BOD and COD

The HF-VF and VF-HF systems were more effective for BOD removal as well as for COD removal with significant greater reductions compared to the HF-SP system ($p < 0.05$) (Table 3). In the latter system, both BOD and COD increased in the stabilization ponds after a decrease in the first-stage due to presence of algae, which essentially converted sewage-BOD to algal-BOD [37]. In the VF-HF system, BOD was almost completely reduced (>95%) in the VF first stage, so that no significant

reduction was observed in the second stage ($p > 0.05$); analogous results were reported in VF-HF systems where the authors found a removal of 89% and 90% of BOD by using gravel and lapilli as substrate, respectively, and no additional BOD removal in the HF stage [11]. A similar pattern was observed with respect to the COD (Table 3).

Figure 4. *Escherichia coli* concentration in the three EWTSs along the monitoring period. (**a**) HF-SP; (**b**) HF-VF; (**c**) VF-HF.



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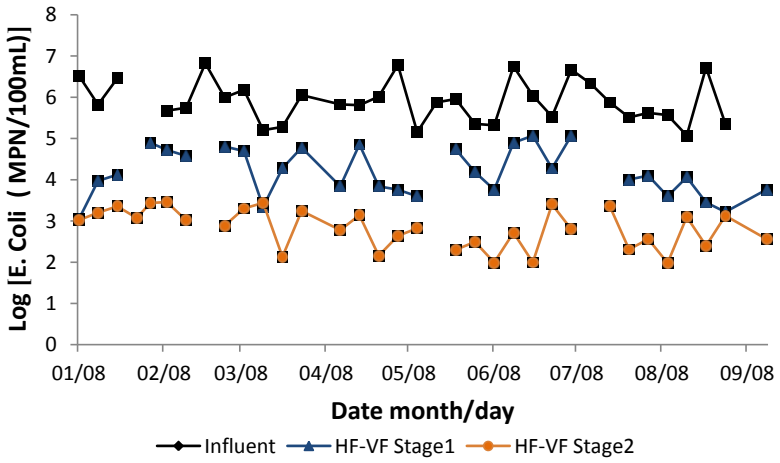

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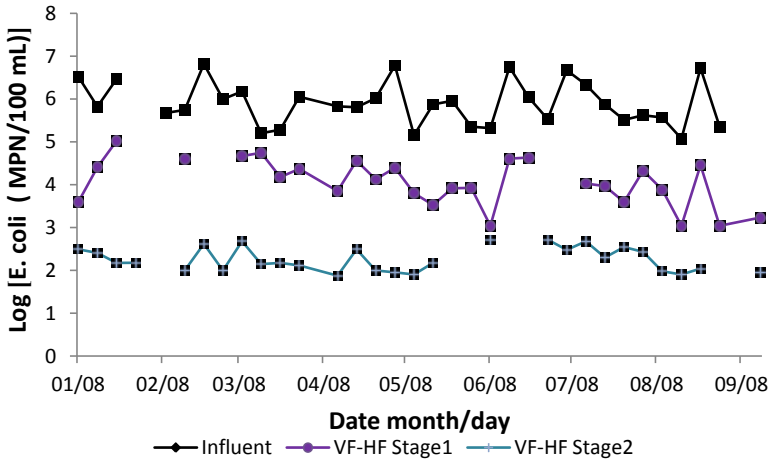


(**c**)

Table 3. Performance summary for the three HEWTSs with respect to organic load and control parameters. Average ± standard error of the mean. Entire system removal percentages are in parentheses with bold letter.

Parameter	Influent	System I: HF-SP		System II: HF-VF		System: VF-HF	
		1st stage HF-CW	2nd stage SP	1st stage HF-CW	2nd stage VF-CW	1st stage VF-CW	2nd stage HF-CW
BOD (mg/L)	140.6 ± 28.2	27.3 ± 5.1	55.5 ± 9.6	24.3 ± 3.8	6.1 ± 0.9	6.7 ± 1.5	4.8 ± 0.72
BOD Removal (%)		80.6	−103.3 (**60.5**)	82.7	74.9 (**95.7**)	95.2	* (**96.6**)
COD (mg/L)	273.5 ± +50.0	96.9 ± 11.8	277.8 ± 43.5	95.4 ± 17.5	56.9 ± 7.4	66.7 ± 8.9	55.8 ± 8.2
COD Removal (%)		64.6	−186.7 (**0.0**)	65.1	40.4 (**79.2**)	75.6	* (**79.6**)
TSS (mg/L)	61.8 ± 11.7	12.3 ± 2.4	138.3 ± 31.0	8.3 ± 2.0	4.6 ± 1.0	10.6 ± 3.6	4.5 ± 1.0
TSS Removal (%)		80.1	−1024.4 (−**123.8**)	84.9	49.5 (**92.4**)	82.8	57.4(**92.7**)
Conductivity (µS/cm)	1797 ± 359	1774 ± 119	1387 ± 119	1693 ± 375.5	1369 ± 291	1381 ± 285	1457 ± 409
pH	8.2 ± 0.08	8.0 ± 0.08	8.1 ± 0.08	8.2 ± 0.14	6.4 ± 0.13	6.7 ± 0.12	6.8 ± 0.12
DO (mg/L)	1.5 ± 0.5	5.5 ± 0.6	8.8 ± 1.7	4.7 ± 0.7	4.3 ± 0.6	6.9 ± 0.5	5.2 ± 0.5

Note: * No significant difference with regard to the concentration in the previous stage.

3.8. Total Suspended Solids

The concentration of TSS in the influent was low due to the partial sedimentation process which took place in the holding tank. The TSS increased for the HF-SP system due to the presence of algae in the stabilization pond while TSS was significantly reduced in the other two systems ($p < 0.05$), averaging 92% for both the HF-VF and VF-HF systems.

3.9. PH

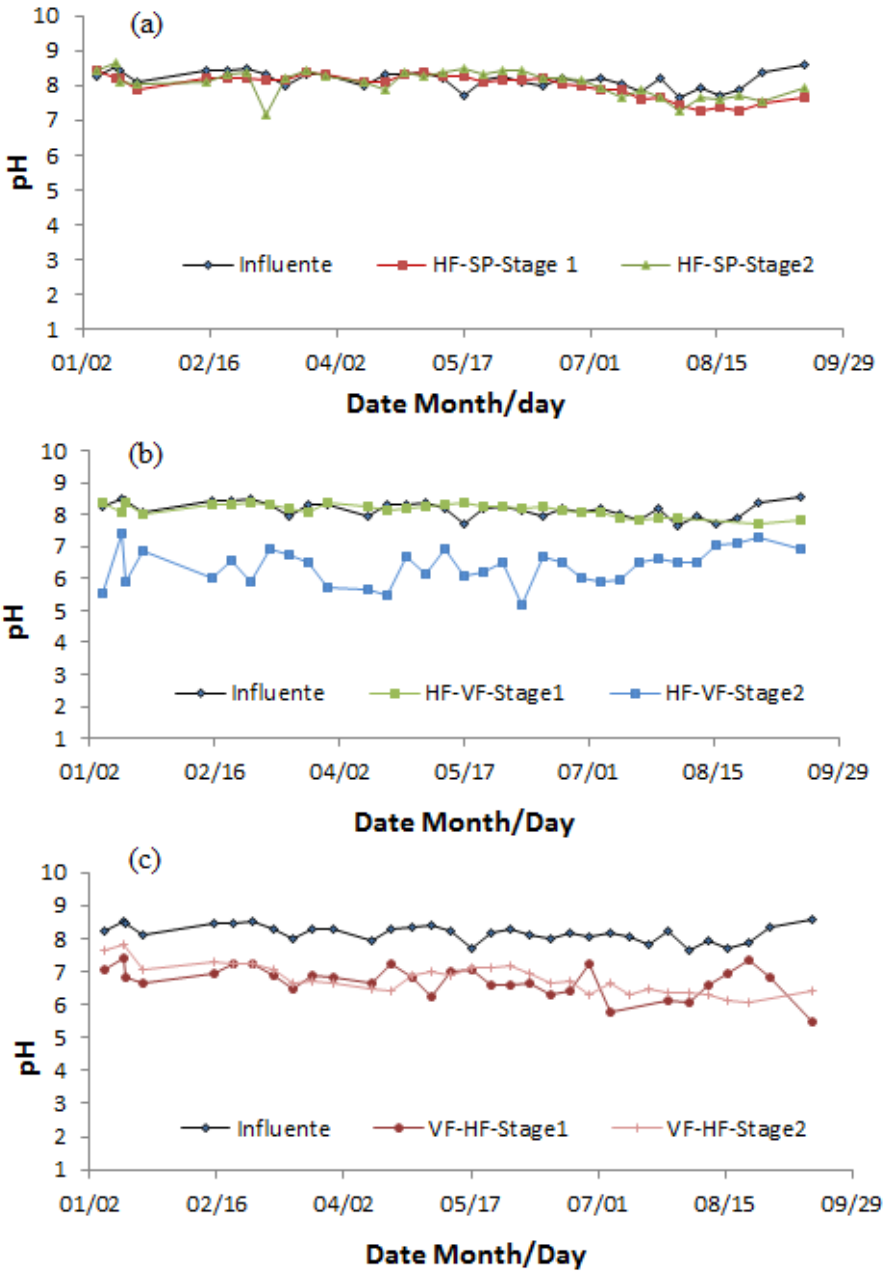
The pH is an important characteristic of water when considering reuse of reclaimed water for irrigation [38]. It is well documented that irrigation water should have a pH ranging between 5 and 6.5 in order to maximize nutrient uptake by plants [39]. The values of pH averaged above 8 for the HF-SP system likely driven by photosynthetic activity in the stabilization pond (Figure 5a). In contrast, the HF-VF and VF-HF systems effectively modified the pH to an average value of 6.6 (Table 3, Figure 5b,c) due mainly to the nitrification process in the vertical flow CWs.

3.10. Electrical Conductivity (EC)

Although an increase in EC might be expected in the effluent of CWs, some studies have found that this does not frequently happen with a HRT < 3 days but for a HRT > 10 days [11,40]. In this study, EC was reduced significantly in the three systems ($p < 0.05$) reaching similar values. According to some authors [41], a decrease in EC despite significant water losses is explained by uptake of micro and macroelements and ions by plants and their removal through adsorption to plant roots, litter and settled suspended particles. Moreover, the ground tezontle rock used as the media in the CWs has demonstrated a high capacity for total dissolved solid removal in previous studies [42]. The final values

in the three effluents averaged less than 1.5 dS/m, which is considered as the threshold value from which a reduction in crop yield potential due to salinity, can be expected in salt-sensitive species [43,44].

Figure 5. PH in the three EWTSs along the monitoring period. (**a**) HF-SP; (**b**) HF-VF; (**c**) VF-HF.



4. Conclusions

Two-stage hybrid ecological wastewater treatment systems comprised of constructed wetlands and stabilization ponds can be configured and combined in order to optimize the inorganic nutrient retention and the removal of pathogens and other detrimental water quality parameters. In this study, the most effective systems capable of providing high quality, nutrient-rich water for agriculture, for the water quality parameters we evaluated, were those systems containing a vertical flow CW component, either HF-VF or VF-HF. A high-nitrified effluent, desirable in reclaimed water reuse for crop

irrigation, was produced in the two systems due to efficient nitrification in the vertical flow components. There was no significant difference amongst the three systems with regard to TP removal. Regarding the indicator organisms, the two most effective systems achieved a >3 log unit *E. coli* reduction, fulfilling the WHO guidelines for wastewater treatment systems and complying the <1000 MPN/100 mL Mexican standard for treated wastewater reuse in agriculture. In this way, we have demonstrated that it is possible to remove harmful pathogenic organisms by using at least two-stage systems, essentially disinfecting the reclaimed water using natural processes and thereby negating the need to use expensive conventional disinfectants. The production and reuse of high-quality, reclaimed water is a recommended practice in order to protect human health, maximize recycling of nutrients and reduce demand on high quality drinking water sources that are used for irrigation in many parts of the world, conserving this vital resource for human consumption. Moreover, in the developing world, where fertilizers costs are relatively high, the coupling of low-cost wastewater treatment while maximizing reuse of nutrients makes both economic sense and improves long-term sustainability of the coupled human-agricultural system.

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Conflicts of Interest

The authors declare no conflict of interest.

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