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ORIGINAL PAPER

Feasibility removal of BOD_5, COD, and ammonium by using Gambusia fish and *Phragmites australis* in H-SSF wetland

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Abstract

Today, there is a wide range of wastewater treatment procedures, but most of them have major problems, such as high construction costs, high energy consumption, complex operation, and the need for sludge treatment and disposal, and requirement to use high technology for treatment. The main objective of this study was to evaluate the effectiveness of horizontal subsurface flow constructed wetlands (H-SSF CWs) by Gambusia fish and *Phragmites australis* (plant) for municipal wastewater treatment. This study focused on the removal of BOD_5, COD, and ammonium. The wastewater was used as the raw input effluent to the Shahid Mahallati Wastewater Treatment Plant in Tehran, Iran. The pilot used included five 120-liter tanks. In tank 1, coded 2-A, the plant of *P. australis* was planted and from tank 4 used as a control sample (without *P. australis*). In the remaining two tanks, tank 2-B and 3, Gambusia fish were placed. At the beginning of the study, the dilution of the input effluent was done for the adaptation. The removal efficiency of BOD_5 in the tanks of 1, 2, 3, and 4 was 91.83%, 91.97%, 4.12%, and 71.16%, respectively. The removal efficiency has increased in all tanks in the summer compared to spring. The results of the study showed that the maximum removal efficiency was observed in tank 2 due to the presence of *P. australis*–Gambusia fish. The mean concentration of ammonium in the spring and summer was 14.37 mg/l and 19.7 mg/l, respectively. The removal efficiency of NH_4^+ in the tanks of 1, 2, 3, and 4 was 36%, 11.38%, and 4.56%, respectively. So, the NH_4^+ removal efficiency has increased in all constructed wetlands in the summer compared to spring. Based on the results, H-SSF CWs can be considered as a good alternative for wastewater treatment in small communities.

Keywords Wastewater · Wetland · *Phragmites Australis* · Gambusia fish · H-SSF CWs

Introduction

Water is necessary for all living organisms on earth. But recently water has been considerably polluted by rapid urbanization and industrialization (Ali 2010; Burakova et al. 2018; Dehghani et al. 2013; Godini et al. 2010). One of the main sources of environmental pollution is the industries, and the textile industry is one of the most important ones (Ali et al. 2012a; Ali and Jain 2004). These industries have different pollutants, such as heavy metals (like Cr), colors, very high COD, low biodegradability, suspended solids, various nitrogen and phosphorus compounds, surfactants, thickeners, carboxylic acids (mainly acetic), aromatic amines, and oils (Ali et al. 2016c; Dehghani et al. 2016; Sharma and Imran 2011; Ali et al. 2016a). Today, there is a wide range of wastewater treatment systems, but most of them have major problems, such as high construction costs, high energy consumption, complex operation, and the need for sludge treatment and disposal, and they usually use high

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technology for treatment (Ali et al. 2016b, 2017a, b, 2018). Natural wastewater treatment systems are divided into three categories of aquatic, terrestrial, and wetland, which all of these categories are based on physical, chemical, and biological mechanisms (Ali et al. 2016a; Mahmood et al. 2013). The literature review shows that wetlands have been used for a long time for pollution control by human (Fan et al. 2009; Janbazi and Gorijiyanarabi 2012). Wetlands have been applied for many years to enhance the quality effluent and urban runoff (Fan et al. 2009). The wetland is a land where the water level is so large that it provides the soil with saturated water and conditions for plant growth. The wetlands are divided into two categories of natural and artificial. Constructed wetlands (CWs) are also engineered systems that have been designed as a suitable alternative to conventional wastewater treatment (CWT) technologies that have high energy consumption (Kadlec 2009; Mantovi et al. 2003). Compared to CWT systems, the CWs are low cost, easily operated and maintained, and have a strong perspective for application in developing countries. Also, the CWs have a higher rate of biological activity, which enables conversion of many of the contaminants that are contained in the effluent into non-toxic byproducts or essential nutrients that can be reused for additional biological activity (Ali et al. 2012b; Mustafa 2013). In CWs, the treatment processes are carried out under more controlled conditions than a natural wetland. Because of the simple operation, maintenance, and management, CWs are an economical and cost-effective method for wastewater treatment which can be used in developing countries (Odinga et al. 2013). Contaminants uptake in the CWs is a complex process and depends on a variety of removal mechanisms, such as filtration, sedimentation, plant uptake, precipitation, adsorption, volatilization, and various microbial processes (Basheer 2018; Wu et al. 2014, 2015). Generally, the processes are generally directly or indirectly influenced by the temperatures, operation strategies, the different loading rates, soil types, and redox conditions in the wetland bed (Wu et al. 2014; Yang et al. 2011). The CWs are divided into two categories of horizontal subsurface flow (H-SSF) and free water surface (F-WS). According to the design principles, there also are three groups of the CWs that include surface flow (SF), subsurface flow (SSF), and hybrid system (HS) (Odinga et al. 2013; Werker et al. 2002; Wu et al. 2015). Between F-WS-CWs and H-SSF-CWs, F-WS-CWs are more efficient in the removal of organic materials and suspended solids, compared with the removal of nitrogen and phosphorus compounds. As compared to F-WS-CWs, H-SSF-CWs are very effective in the removal of nitrogen and phosphorus compounds, microbial pollution, and heavy metals, and they are less cold sensitive and easier to insulate for winter operation (Babatunde et al. 2010; Wu et al. 2015). While in the H-SSF CWs, the treatment target is the removal of BOD and total suspended solids (TSS), H-SSF-CWs are an appropriate option for the treatment (Yousefi et al. 2013). So far, these systems have been used to remove various pollutants in the environment. The CWs have been used to remove urban wastewater and remove heavy metals and bacteria (Fan et al. 2009). Since the first full-scale CWs were built during the late 1960s, there are now more than 50,000 units of the CWs in Europe and more than 10,000 units of the CWs in North America (Wu et al. 2015). In 2005, Greenway used the CWs in secondary effluent treatment and water reuse in subtropical and arid Australia (Greenway 2005). Rozema et al. (2016) used the CWs to treat wastewater. The average reduction of BOD_5, TSS, TKN, and TP were 81%, 83%, 75%, and 64%, respectively. They also were managed to remove pathogenic bacteria (Rozema et al. 2016). In 2018, Sgroi et al. reported H-SSF-CWs had the highest efficiency for total nitrogen removal (60–69%). The highest removal of TOC, COD, BOD_5, and fluorescing organic matter of 72%, 67%, 81%, respectively, was observed in the unsaturated vertical subsurface flow (UVF) wetland (Sgroi et al. 2018). The CWs have been used successfully as a green technology to treat various effluents such as domestic sewage, agricultural and industrial effluent, mine drainage, landfill leachate, storm water, and urban runoff for decades (Wu et al. 2015). Innovations of this study were: (I) application of this system in different phases (five steps), which is observed in less previous studies, (II) simultaneous use of the *Phragmites Australis* plant and the Gambusia fish in a tank, (III) use of real effluent to do the study; on the other hand, the use of this hybrid system to treat a textile industry wastewater, and (IV) study in different seasons. So, the main goal of this study was to evaluate the effectiveness of H-SSF-CWs by Gambusia fish and *P. australis* plant in municipal wastewater treatment. This study focused on the removal of BOD_5, COD, and ammonium.

Materials and methods

Influent wastewater

The wastewater used in this study was the raw wastewater entering the Shahid Mahallati Wastewater Treatment Plant (SM-WTP) in Tehran, Iran. The area of the SM-WTP plant was 13,328 m^3. The plant became operational in 2000. The treatment plant treats a wastewater of 30,000 people. The secondary treatment method in this plant is an activated sludge process (extended aeration system). The average flow rate for the treatment plant was 200 m^3/h. Also, the average value of BOD_5, COD, and TSS was 240 mg/l, 352 mg/l, and 2.5 mg/l, respectively (Gholikandi et al. 2018).

Construction and operation of H-SSF-CWs

The studied H-SSF CWs were located in the northern part of the plant and near the exit channel of grit chamber. The pilot used included five 120-l tanks. In tank 1, 2-A, the plant of *P. australis* was planted and tank 4 used as a control sample (without *P. australis*). In the remaining two tanks, tank 2-B and 3, Gambusia fish were placed. Tank 4 was used as a controlled wetland without *P. australis* plant and Gambusia fish. A view of the pilot used is shown in Fig. 1. Tank 2 contained tanks A and B that were connected in series. In tank A and B, *P. australis* plant and Gambusia fish were placed, respectively. Tank 1, 2-A, was filled up to 35 cm from the grain (in height). The gravel bed used in each of the three tank units varied between 5 and 25 mm diameters. All tanks had an output at a height of 30 cm. In the first 45 days of the study, the influent wastewater to the treatment plant was diluted into 9-l tanks connected and diluted through a dosing pump, or metering pump, with a flow rate of 15 l/day for each tank. Sampling was performed in the outlet of the tanks. Before the start of the study as well as every 2 weeks, the pump calibration was carried out to ensure the correct operation of the pump in supplying the desired flow. The calibration of the pump was carried out by chronometry and volumetric measurements. At the beginning of the study, the dilution of the influent wastewater was done for the adaptation of *P. australis* and Gambusia fish. So this was done as follows: In the first week 80%, the second week 70%, the third week 60%, the fourth week 50%, the fifth week 40%, the sixth week 30%, the seventh week 20%, the eighth week 10%, and at the ninth week, dilution of wastewater with urban water was carried out. The amount of dilution of input effluent to the CWs in the first 2 months of the study is given in Table 1. Equation (1) is used to calculate the input flow into the tanks:

$$Q = \frac{V * n}{t}, \tag{1}$$

Table 1 Percentage of dilution of influent wastewater to the CWs in the first 2 months of the study

Time	Dilution rate
The first week	20% wastewater + 80% urban water
The second week	30% wastewater + 70% urban water
The third week	40% wastewater + 60% urban water
The fourth week	50% wastewater + 50% urban water
The fifth week	60% wastewater + 40% urban water
The sixth week	70% wastewater + 30% urban water
The seventh week	80% wastewater + 20% urban water
The eighth week	90% wastewater + 10% urban water

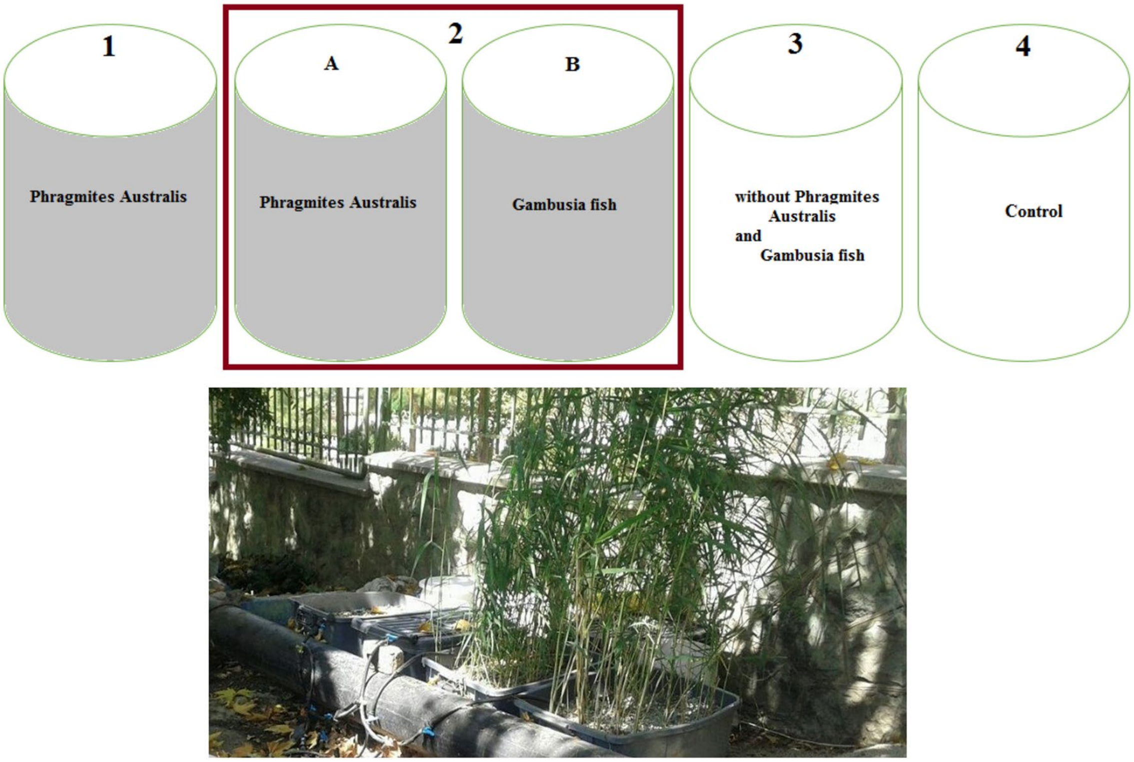


Fig. 1 A view of the pilot used in this study

where Q is the influent flow in l/day. V and n are tank volume in a liter and porosity (%), respectively. T is hydraulic retention time in the day (Ali et al. 2018).

Wetland of the plant and fish in series

In this study, tank 2 (A and B) was used separately as a series CW (Fig. 1). The two tanks were connected in serial mode. In the tanks of A and B, there were *P. australis* and Gambusia fish, respectively. At first, raw wastewater entered the CWs of the *P. australis* and after leaving the tank through into the Gambusia fish CWs by a pipe. From the outlet effluent of the tank B, the Gambusia fish CWs, sampling was done.

Phragmites Australis and Gambusia fish

Phragmites Australis is a plant which has been widely used in H-SSF-CWs and F-WS-CWs. This plant is a perennial plant and flood resistant. The roots of these plants penetrate up to a depth of 0.6–1 m in the soil. The stems of these plants are rigid and hollow with a height of 0.5–4.5 m. These plant species are commonly found in saline water, and it is found abundantly in Iran and Tehran, so these species were selected for the purpose of the current study (Vymazal 2013; Wu et al. 2015). In this study, the *P. australis* plant with a density of 12 was planted in each CW. Gambusia fish has a maximum size of 5.5 cm, which is a small and usually in grayish color (Wen et al. 2013). This is a native fish of the USA and is omnivorous. Gambusia fish is a category of freshwater fish and is known as mosquito fish. This fish is resistant to chemicals and organic waste from wastewater (Kengne et al. 2003; Willems et al. 2005). In this study, we investigated the role of this fish in the removal of organic waste from wastewater. The Gambusia fish in this study were transferred to a tank in which the water was previously chlorinated. To adapt the fish for the new environment, aeration was carried out into the tank. Then, 50 fish were transferred to any fish tanks. The fish's behavior in the tank was controlled by eye. Due to the beginning of the study by diluting the input wastewater, the mortality rate of the fish was negligible. Furthermore, as mentioned before, Gambusia fish is a resistant fish in adverse environmental conditions.

Sampling

After system stability, when the range of output fluctuations reached less than 10%, samples were taken for analysis. Samples were taken once in every 3–2 days. The sample volume was estimated to be 236. For sampling, a 1-l polyethylene was used. Samples were taken twice a week from the influent wastewater and effluent of the CWs. The first stage of the sampling was conducted from April 15 to June 31, 2017. This time was the first months of planting and the stems and leaves; this plant did not grow significantly (Ali et al. 2009; Basheer 2018). The second phase of the sampling was conducted from July 3 to September 29, 2017. At this time, the growth of the straw had increased. Samples were stored under standard conditions. The sampling of the algal mass was done in glass containers 8 times per season for constructed wetland containing Gambusia fish. Finally, the samples were analyzed for BOD_5 (5231B), COD (5221B), ammonium (4511), and analysis of algal mass (31211H) was made (Ali et al. 2009; Eaton et al. 2012). In this study, UV–visible spectrum spectrophotometer was used to measure chlorophyll-a (Ali and Aboul-Enein 2006; Gupta and Ali 2013; Peck and Walton 2008).

Results and discussion

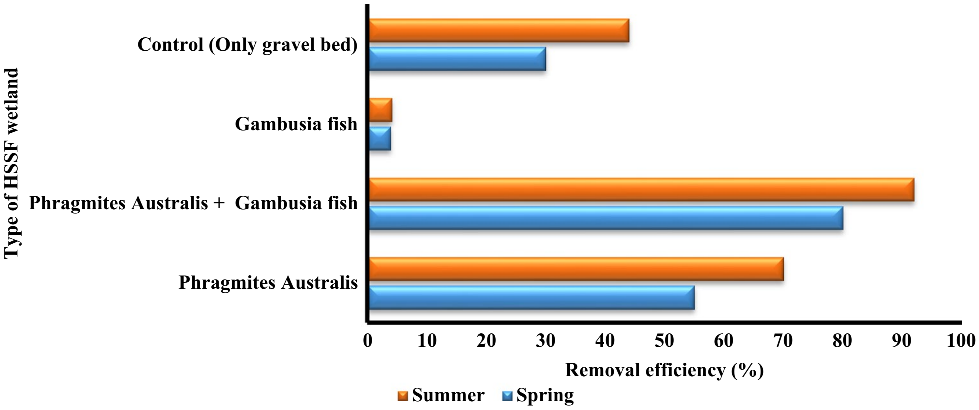
The removal efficiency of BOD_5, COD, and ammonium in the H-SSF-CWs

Performance of H-SSF-CWs in the removal of BOD_5, COD, and ammonium from the five tanks 1, 2, 3, and 4 was investigated on the basis of removal efficiency (%).Table 2 presents the mean value of influent and effluent of BOD_5, COD, and NH_4^+ of the CWs and the removal efficiency of pollutants in the various CWs. The mean concentration of value of BOD_5 in the spring and summer was 111.42 mg/l and 154.8 mg/l, respectively. In spring, the removal efficiency of BOD_5 in the tanks containing *P. australis*, *P. australis*–Gambusia fish, Gambusia fish, and control was 86.26%, 87.45%, 3.89%, and 64.64%, respectively. In summer, the removal efficiency of BOD_5 in the tanks of 1, 2, 3, and 4 was 91.83%, 91.97%, 4.12%, and 71.16%, respectively. So, the BOD_5 removal efficiency has increased in all CWs in the summer compared to spring. By passing wastewater from the bed, the same as a trickling filter, active microorganisms are formed on the surface of the bed. Thus, a biofilm layer was gradually formed on the surface of the substrate that can mechanically and biologically remove existing organic matter. So initially, the treatment is mechanical and continues to be biological. Figure 2 shows removal efficiency of BOD_5 in the H-SSF-CWs. The results of the study showed that the maximum removal efficiency was observed in tank 2 containing *P. australis*–Gambusia fish. Also, the minimum removal efficiency was observed in tank 3 containing Gambusia fish only. Kaseva (2004) reported that the presence of plants in the CWs leads to an increase in the removal efficiency of organic matter (Kaseva 2004). Mechanisms in the removal of biodegradable materials in the wetland system are the biological transformation of BOD_5 by bacteria (aerobic, optional, and anaerobic), adsorption, filtration, and flocculation and settling of suspended BOD (Ehrampoush et al. 2013). In fact, the microbial layer adhering to the roots (biofilm) provides good conditions for biological oxidation, which also causes organic matters, suspended solids, and other contaminants to decrease in the CWs (Yousefi et al. 2013). Of course, the role of this plant is also important due to the adsorption of these materials in the growing and

Table 2 Mean concentration of influent and effluent of COD, BOD_5, and NH_4^+ to the CWs and the removal efficiency of pollutants in the H-SSF-CWs

Season	Type of CWs		Average input concentration (mg/l)			Average outlet concentration (mg/l)			Removal efficiency (%)		
			COD	BOD5	NH4+	COD	BOD5	NH4+	COD	BOD5	NH4+
Spring	*Phragmites Australis*	1	156.43	111.42	14.37	35.54	14.7	10.53	74.43	86.26	33.16
	Phragmites Australis + Gambusia fish	2 (2–3)				32.92	13.73	12.91	76.82	87.45	21.07
	Gambusia fish	3				150.57	107.03	16.61	3.77	3.89	− 8.8
	Control (only sand)	4				65.57	39.48	13.66	54.7	64.64	3.83
Summer	*Phragmites Australis*	1	202.95	154.8	19.7	31.25	14.3	13.16	84.63	91.83	26
	Phragmites Australis + Gambusia fish					28.55	12.54	15.54	85.97	91.97	11.38
	Gambusia fish	3				193.13	148.32	21.42	4.83	4.12	− 14.35
	Control (only sand)	4				63.31	44.78	18.93	66.38	71.16	4.56

Fig. 2 Removal efficiency of BOD_5 in the H-SSF-CWs



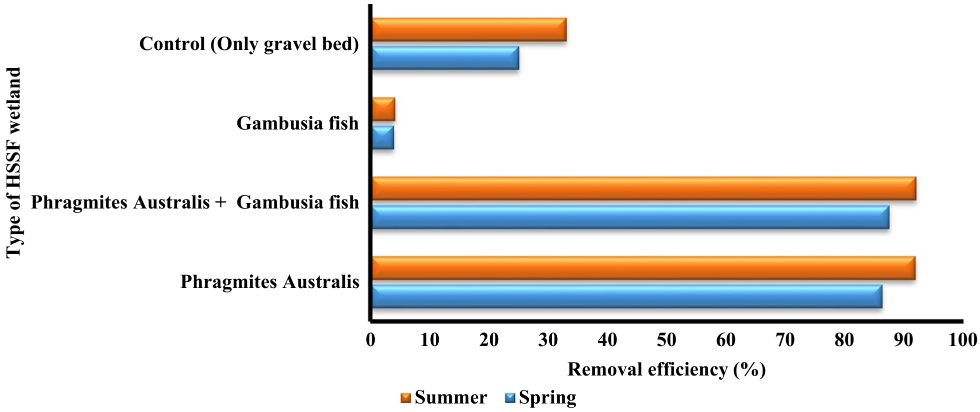


Fig. 3 Removal efficiency of COD in the H-SSF-CWs

surviving seasons that are suitable for the plant. According to the previous experiments, gravel size in the range of 5 mm to 25 mm was suitable for the CWs. The previous studies showed that there was no significant difference between gravel sizes in this range (Fig. 3). The removal of organic matters in wetlands occurs through anaerobic degradation and as an anaerobic degradation. Oxygen required for aerobic decomposition is supplied through atmospheric oxygen penetration, air transfer through the wind or oxygen in the root of the plants. An anaerobic degradation is also performed on parts of a bed that does not have oxygen. According to discharge effluent standards, the effluent BOD_5 for agricultural and irrigation purposes and for discharge to surface water is 100 mg/l and 30 mg/l, respectively (Salari et al. 2012). Based on the results of Table 2, the effluent BOD_5 from the tanks of *P. australis* and *P. australis*–Gambusia fish was less than 30 mg/l, so it can be discharged to surface waters. It is also possible in both spring and summer. Trang et al. (2010) reported that BOD_5 removal by using a tropical H-SSF-CWs was in the range of 65–83% (Trang et al. 2010). Upadhyay et al. (2016) used two plants of Potamogeton Crispus and Hydrilla Verticillata for treatment of urban wastewater. The results of this study showed that the average removal of conductivity, TDS, TSS, BOD, NO_3^-, and PO_4^- was 60.42%, 67.27%, 86.1%, 87.81%, 81.28%, and 83.54%, respectively, at 72 h of retention time (Upadhyay et al. 2016). The mean concentration of COD in spring and summer was 156.43 mg/l and 202.95 mg/l, respectively. In the spring, the removal efficiency of COD in the tanks containing *P. australis* (1), *P. australis*–Gambusia fish (2), Gambusia fish (3), and control (4) was 74.84%, 82.87%, 77.3%, and 54.7% respectively. In summer, the removal efficiency of COD removal in the tanks of 1, 2, 3, and 4 was 84.63%, 85.97%, 77.3%, and 54.7%, respectively. So, the COD removal efficiency has increased in all CWs in the summer compared to spring, like BOD_5. Most effective removal of COD was observed in the *P. australis*–Gambusia fish CW. In *P. australis* CW, the removal efficiency of COD in the summer was more than that of spring. This increase in the efficiency was due to rising temperatures during the spring and an increase in the rate of chemical reactions. The minimum removal efficiency of COD was related to Gambusia fish CWs. Because Gambusia fish is omnivorous and feeds on plankton and organic particles, it is expected that organic matter removal by Gambusia fish will be higher. However, the waste of fish that enters the CWs increased the amount of organic matter, which finally reduced the removal efficiency of the CWs in removing COD and BOD_5 (Kaseva 2004). According to the discharge effluent standards, the effluent BOD_5 for agricultural and irrigation purposes and for discharge to surface water is 200 mg/l and 60 mg/l, respectively (Salari et al. 2012). Based on the results of Table 2, the effluent COD from the tanks of *P. australis* and *P. australis*–Gambusia fish was less than 60 mg/l, so it can be discharged to surface waters. It is also possible in both spring and summer, like BOD_5. These results were consistent with other studies (Salari et al. 2012). Trang et al. (2010) reported that COD removal by using a tropical H-SSF-CWs was in the range of 57–84% (Trang et al. 2010). The mean concentration of ammonium in the spring and summer was 14.37 mg/l and 19.7 mg/l, respectively. In the spring, the removal efficiency of NH_4^+ in the tanks of *P. australis*, *P. australis*–Gambusia fish, and control was 33.16%, 21.07%, and 3.83%, respectively. In summer, the removal efficiency of NH_4^+ in the tanks of 1, 2, 3, and 4 was 36%, 11.38%, and 4.56%, respectively. So, the NH_4^+ removal efficiency, like BOD_5 and COD, has increased in all CWs in the summer than in spring. Figure 4 shows removal efficiency of ammonium in the H-SSF-CWs. The CWs are known to act as biological filters through a combination of physical, chemical, and biological ways which may participate to decrease pollutants in wastewater. Hiley (1995) reported which roots, shoots, and litter from the plants, like *P. australis*, can support the biofilm (aerobic bacteria and associated animals and plants), which treats wastewater in the H-SSF-CWs (Hiley 1995). By comparing the results, it can be observed that

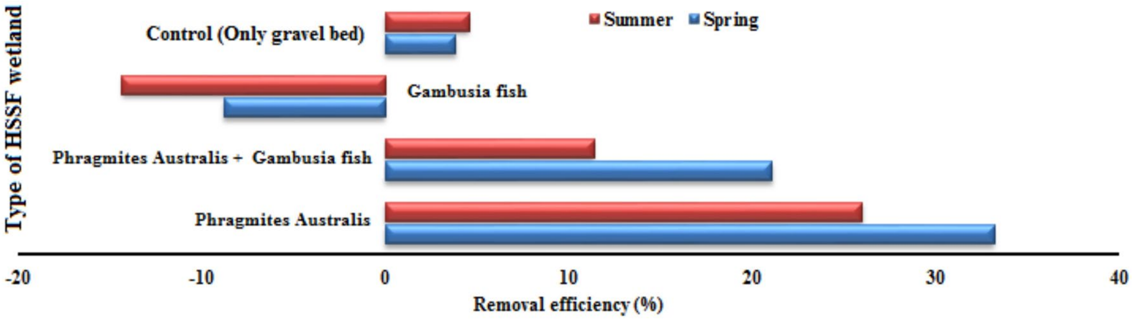


Fig. 4 Removal efficiency of ammonium in the H-SSF-CWs

Table 3 Concentration of chlorophyll-a (mg/l) in *Phragmites Australis* + Gambusia fish and Gambusia fish

Constructed wetlands	Season			
	Spring		Summer	
	Chlorophyll-a	Algae biomass	Chlorophyll-a	Algae biomass
Phragmites Australis + Gambusia fish				
Sample-1	0.06	3.12	0.1	6.9
Sample-2	0.04	2.7	0.11	7.44
Sample-3	0.03	1.6	0.17	11.06
Sample-4	0.02	1.37	0.2	13.1
Gambusia fish				
Sample 1	0.12	8.24	0.22	14.3
Sample-2	0.12	8.26	0.23	15.12
Sample-3	0.13	8.92	0.26	17.22
Sample-4	0.14	9.67	0.3	18.08

ammonium removal was less than BOD_5 and COD. The findings of this study are consistent with the study of Kaseva (2004). Kaseva (2004) reported nutrients resulted in the least removal efficiency in all units compared to coliforms and COD (Kaseva 2004). Caselles-Osorio et al. (2017) reported ammonium removal was significantly improved in a planted wetland, averaging 85% vs. 40% for unplanted wetlands (Caselles-Osorio et al. 2017). As shown in Fig. 4, the concentration of ammonium in the fish tank has increased. In the tank of Gambusia fish, the ammonium concentration increased about 8.8 mg/l and 14.35 mg/l in spring and summer, respectively. Therefore, this fish is not suitable for the removal of effluents containing ammonium. Unlike BOD_5 and COD removal, the most efficient process was observed in the tank containing *P. australis*, because the Gambusia fish have a negative effect on the removal of ammonium and increased its concentration in the effluent. Afrous et al. (2010) reported which plant of *P. australis* and Typha latifolia have a high efficiency in removing the nutrients from sewage in aerobic and anaerobic conditions (Afrous et al. 2010). Nitrogenous compounds in the H-SSF-CWs are decreased by nitrification and denitrification. However, nitrification is limited due to lack of dissolved oxygen. Also, anoxic/anaerobic conditions are suitable for denitrification. Therefore, H-SSF-CWs are not suitable for ammonia removal (Reinhardt et al. 2006; Zhang et al. 2014). Tan et al. (2017) reported the removal efficiencies of NH_4^+ and dissolved inorganic nitrogen were both highest in summer, and then decreased significantly in autumn and winter (Tan et al. 2017). The previous studies reported that H-SSF-CWs play an active role in reducing BOD, COD, and TSS from wastewater, and this issue was confirmed by *P. australis* for the weather conditions in Tehran in this study (Yousefi et al. 2013). Sewage temperature has a great impact on the wetland performance. Temperature variations also affect the metabolism of the plant, and changes in the population of organisms affect the amount of dissolved oxygen, pH, and electrical conductivity (Shahi et al. 2012). In all samples, the removal efficiency of COD was temperature dependent and was directly related to it.

The role of algae in the H-SSF-CWs

After fungi and bacteria, alga is the third most dominant microorganism in CWs. Algae are often the first consumers in a wetland and may be multicellular or single-cell photosynthetic organisms (Klinepeter 2017). Table 3 lists the concentration of chlorophyll-a and algae biomass in *P. australis*–Gambusia fish and Gambusia fish in different seasons. Chlorophyll-a is a major component of algae and is a good indicator of algae biomass (Jalilzadeh et al. 2014). There are five chlorophylls a, b, c, d, and e in algae. Chlorophyll-a is present in all algae, so its concentration was measured (Lally et al. 2012). In this study, only the mass of algae in CWs simultaneous *P. australis*–Gambusia fish and Gambusia fish was analyzed. The results showed that the algal mass in simultaneous *P. australis*–Gambusia fish CW in spring and summer was 0.84 mg/l and 2.96 mg/l, respectively. Also, the algal mass in Gambusia fish CW in spring and summer was 0.67 mg/l and 1.76 mg/l, respectively. As it is clear from the results, the growth rate of algae was higher in *P. australis*–Gambusia fish constructed wetland. This increase in algal mass may be due to the growth of algae around the roots of plants in the CWs, which is likely to be that part of the algae in the plant's wetland through the outlet from the wetland, which immediately enters the Gambusia fish CW. Reports indicate that plant tissues have physical effects such as filtering, increasing sedimentation, and reducing the risk of rebounding. The plants also increase the time of contact between the sewage and the bed (Leto et al. 2013). Many studies have shown that macrophytes in the upper and lower parts of the bed provide a large surface for the development of biofilms that are responsible for most microbial processes in wetlands (Bosak et al. 2016). Kant and Conservation (2016) have argued that plants help in the removal of nitrogenous compounds, especially in low loads, but plant adsorption is less important compared to nitrogen removal by microbial processes. Peck and Walton (2008) reported phytoplankton biomass was significantly different depending on location and sampling time.

Conclusion

In general, the proper performance of a constructed wetland depends on the good interaction between plants, the type of bed, and microorganisms in relation to the type of wetland and the type of wastewater treatment. The findings of this study showed that the simultaneous use of *P. australis* and Gambusia fish has the most effective role in removing COD and BOD_5. Also, the highest removal of ammonium has occurred in the wetland containing *P. australis*. The highest removal of studied pollutants was observed in summer. The studied results showed that removal efficiency of organic matter was very low by Gambusia fish, but this fish has high resistance to high ammonium concentration. It is found in the present study that plants can play an important role in removing organic matter and ammonium. The concentration of COD and BOD_5 in the output effluent from wetland containing *P. australis* and combined wetland, *P. australis* and Gambusia fish, has reached to less than standards. So H-SSF wetlands can be considered as a good alternative for wastewater treatment of small communities. In addition, the CWs are a promising alternative for wastewater treatment in developing countries, and especially in Iran.

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References

Afrous A, Hedayat N, Liaghat A, Mohammadpour M, Manshouri M (2010) Accumulation and uptake of nitrogen and phosphorus by four species of aquatic plants under arid and semi-arid conditions of Dezful, Iran. World Appl Sci J 10:886–891

Ali I (2010) The quest for active carbon adsorbent substitutes: inexpensive adsorbents for toxic metal ions removal from wastewater. Sep Purif Rev 39:95–171

Ali I, Aboul-Enein HY (2006) Instrumental methods in metal ion speciation. CRC Press, Boca Raton

Ali I, Jain CK (2004) Advances in arsenic speciation techniques. Int J Environ Anal Chem 84:947–964

Ali I, Aboul-Enein HY, Gupta VK (2009) Nanochromatography and nanocapillary electrophoresis: pharmaceutical and environmental analyses. Wiley, Hoboken

Ali I, Gupta VK, Khan TA, Asim M (2012a) Removal of arsenate from aqueous solution by electro-coagulation method using Al-Fe electrodes. Int J Electrochem Sci 7:1898–1907

Ali I, Khan TA, Asim M (2012b) Removal of arsenate from groundwater by electrocoagulation method. Environ Sci Pollut Res 19:1668–1676

Ali I, Al-Othman ZA, Al-Warthan A (2016a) Removal of secbumeton herbicide from water on composite nanoadsorbent. Desalin Water Treat 57:10409–10421

Ali I, AL-Othman ZA, Alwarthan A (2016b) Molecular uptake of congo red dye from water on iron composite nano particles. J Mol Liq 224:171–176

Ali I, Al-Othman ZA, Alharbi OML (2016c) Uptake of pantoprazole drug residue from water using novel synthesized composite iron nano adsorbent. J Mol Liq 218:465–472

Ali I, Alothman ZA, Alwarthan A (2017a) Supra molecular mechanism of the removal of 17-β-estradiol endocrine disturbing pollutant from water on functionalized iron nano particles. J Mol Liq 241:123–129

Ali I, Alothman ZA, Alwarthan A (2017b) Uptake of propranolol on ionic liquid iron nanocomposite adsorbent: kinetic, thermodynamics and mechanism of adsorption. J Mol Liq 236:205–213

Ali I, Alharbi OML, Alothman ZA, Badjah AY, Alwarthan A, Basheer AA (2018) Artificial neural network modelling of amido black dye sorption on iron composite nano material: kinetics and thermodynamics studies. J Mol Liq 250:1–8

Babatunde A, Zhao Y, Zhao X (2010) Alum sludge-based constructed wetland system for enhanced removal of P and OM from wastewater: concept, design and performance analysis. Bioresour Technol 101:6576–6579

Basheer AA (2018) Chemical chiral pollution: impact on the society and science and need of the regulations in the 21st century. Chirality 30:402–406

Bosak V, VanderZaag A, Crolla A, Kinsley C, Gordon R (2016) Performance of a constructed wetland and pretreatment system receiving potato farm wash water. Water 8:183

Burakova EA, Dyachkova TP, Rukhov AV, Tugolukov EN, Galunin EV, Tkachev AG, Basheer AA, Ali I (2018) Novel and economic method of carbon nanotubes synthesis on a nickel magnesium oxide catalyst using microwave radiation. J Mol Liq 253:340–346

Caselles-Osorio A, Vega H, Lancheros JC, Casierra-Martínez HA, Mosquera JE (2017) Horizontal subsurface-flow constructed wetland removal efficiency using *Cyperus articulatus* L. Ecol Eng 99:479–485

Dehghani MH, Jahed G-R, Zarei A (2013) Investigation of low-pressure ultraviolet radiation on inactivation of Rhabitidae Nematode from water. Iran J Public Health 42:314

Dehghani MH, Sanaei D, Ali I, Bhatnagar A (2016) Removal of chromium(VI) from aqueous solution using treated waste newspaper as a low-cost adsorbent: kinetic modeling and isotherm studies. J Mol Liq 215:671–679

Eaton AD, Clesceri LS, Rice EW (2012) Standard methods for the examination of water and wastewater. American Water Works Association (AWWA), Washington

Ehrampoush M, Hossein SD, Ebrahimi A, Ghaneian M, Lotfi M, Ghelmani V, Salehi VA, Ayatollahi S, Talebi P (2013) Evaluation of the efficiency of sub-surface constructed wetland methods in wastewater treatment in Yazd City in 2011. Tolooebehdasht 2(1):22–43

Fan C, Chang F-C, Ko C-H, Sheu Y-S, Teng C-J, Chang T-C (2009) Urban pollutant removal by a constructed riparian wetland before typhoon damage and after reconstruction. Ecol Eng 35:424–435

Gholikandi GB, Ardakani MN, Moradi F (2018) Fered–Fenton technology for efficient waste-activated sludge stabilization: determination of the main specifications and optimization of the energy consumption. J Environ Chem Eng 6:1546–1557

Godini H, Rezaee A, Jafari A, Mirhousaini S (2010) Denitrification of wastewater containing high nitrate using a bioreactor system packed by microbial cellulose. World Acad Sci Eng Technol 62:283–287

Greenway M (2005) The role of constructed wetlands in secondary effluent treatment and water reuse in subtropical and arid Australia. Ecol Eng 25:501–509

Gupta VK, Ali I (2013) Environmental water: advances in treatment, remediation and recycling. Newnes, Oxford

Hiley PD (1995) The reality of sewage treatment using wetlands. Water Sci Technol 32:329–338

Jalilzadeh E, Salimi M, Roozbehnia P (2014) Detection and determination of chlorophylls a and b in Karaj, Taleghan and Latian reservoirs by high performance liquid chromatography (HPLC/UV-VIS). Water Wastewater 25(4):21–26

Janbazi A, Gorijiyanarabi M (2012) Evaluation of water quality based SavadkoohKslyan parameters of physical, chemical and hydrological. J Wetl Ecobiol 5:63–74

Kadlec R (2009) Comparison of free water and horizontal subsurface treatment wetlands. Ecol Eng 35:159–174

Kant S, Conservation CV (2016) Environmental benefit index for wetland restoration on private lands in the greenbelt region of ontario, Canada. Credit Valley Conservation

Kaseva M (2004) Performance of a sub-surface flow constructed wetland in polishing pre-treated wastewater—a tropical case study. Water Res 38:681–687

Kengne IM, Brissaud F, Akoa A, Eteme RA, Nya J, Ndikefor A, Fonkou T (2003) Mosquito development in a macrophyte-based wastewater treatment plant in Cameroon (Central Africa). Ecol Eng 21:53–61

Klinepeter M (2017) An assessment of constructed wetland treatment system cells: removal of excess nutrients and pollutants from municipal wastewater in Lakeland, Florida. University of South Florida

Lally H, Gormally M, Higgins T, Gammell M, Colleran E (2012) Phytoplankton assemblages in four wetlands created on cutaway peatlands in Ireland. In: Biology and environment: proceedings of the Royal Irish Academy. JSTOR 207-216

Leto C, Tuttolomondo T, La Bella S, Leone R, Licata M (2013) Effects of plant species in a horizontal subsurface flow constructed wetland–phytoremediation of treated urban wastewater with *Cyperus alternifolius* L. and *Typha latifolia* L. in the West of Sicily (Italy). Ecol Eng 61:282–291

Mahmood Q, Pervez A, Zeb BS, Zaffar H, Yaqoob H, Waseem M, Afsheen S (2013) Natural treatment systems as sustainable ecotechnologies for the developing countries. Biomed Res Int 2013:1–19

Mantovi P, Marmiroli M, Maestri E, Tagliavini S, Piccinini S, Marmiroli N (2003) Application of a horizontal subsurface flow constructed wetland on treatment of dairy parlor wastewater. Bioresour Technol 88:85–94

Mustafa A (2013) Constructed wetland for wastewater treatment and reuse: a case study of developing country. Int J Environ Sci Dev 4:20

Odinga C, Swalaha F, Otieno F, Ranjith KR, Bux F (2013) Investigating the efficiency of constructed wetlands in the removal of heavy metals and enteric pathogens from wastewater. Environ Technol Rev 2:1–16

Peck GW, Walton WE (2008) Effect of mosquitofish (*Gambusia affinis*) and sestonic food abundance on the invertebrate community within a constructed treatment wetland. Freshw Biol 53:2220–2233

Reinhardt M, Müller B, Gächter R, Wehrli B (2006) Nitrogen removal in a small constructed wetland: an isotope mass balance approach. Environ Sci Technol 40:3313–3319

Rozema ER, VanderZaag AC, Wood JD, Drizo A, Zheng Y, Madani A, Gordon RJ (2016) Constructed wetlands for agricultural wastewater treatment in Northeastern North America: a review. Water 8:173

Salari H, Hasani A, Borghei M, Yazdanbakhsh A, Rezaei H (2012) Investigation of performance wetland in removal N and P in wastewater treatment (case study: Morad Tapeh). J Water Wastew 83:40–47

Sgroi M, Pelissari C, Roccaro P, Sezerino PH, García J, Vagliasindi FG, Ávila C (2018) Removal of organic carbon, nitrogen, emerging contaminants and fluorescing organic matter in different constructed wetland configurations. Chem Eng J 332:619–627

Shahi DH, Ebrahimi A, Esalmi H, Ayatollahi S, Dashty N (2012) Efficiency of straw plants in removal of indicator pathogens from sub surface flow constructed wetlands of municipal wastewater in Yazd, Iran. J Health Dev 1:147–155

Sharma S, Imran A (2011) Adsorption of Rhodamine B dye from aqueous solution onto acid activated mango (Magnifera indica) leaf powder: equilibrium, kinetic and thermodynamic studies. J Toxicol Environ Health Sci 3:286–297

Tan E, Hsu T-C, Huang X, Lin H-J, Kao S-J (2017) Nitrogen transformations and removal efficiency enhancement of a constructed wetland in subtropical Taiwan. Sci Total Environ 601:1378–1388

Trang NTD, Konnerup D, Schierup H-H, Chiem NH, Brix H (2010) Kinetics of pollutant removal from domestic wastewater in a tropical horizontal subsurface flow constructed wetland system: effects of hydraulic loading rate. Ecol Eng 36:527–535

Upadhyay A, Bankoti N, Rai U (2016) Studies on sustainability of simulated constructed wetland system for treatment of urban waste: design and operation. J Environ Manag 169:285–292

Vymazal J (2013) Emergent plants used in free water surface constructed wetlands: a review. Ecol Eng 61:582–592

Wen R, Xie Y, Wan C, Fang Z (2013) Estrogenic and androgenic effects in mosquitofish (*Gambusia affinis*) from streams contaminated by municipal effluent in Guangzhou, China. Aquat Toxicol 132:165–172

Werker A, Dougherty J, McHenry J, Van Loon W (2002) Treatment variability for wetland wastewater treatment design in cold climates. Ecol Eng 19:1–11

Willems KJ, Webb CE, Russell RC (2005) A comparison of mosquito predation by the fish *Pseudomugil signifer* Kner and *Gambusia holbrooki* (Girard) in laboratory trials. J Vector Ecol 30:87

Wu S, Kuschk P, Brix H, Vymazal J, Dong R (2014) Development of constructed wetlands in performance intensifications for wastewater treatment: a nitrogen and organic matter targeted review. Water Res 57:40–55

Wu H, Zhang J, Ngo HH, Guo W, Hu Z, Liang S, Fan J, Liu H (2015) A review on the sustainability of constructed wetlands for wastewater treatment: design and operation. Bioresource Technol 175:594–601

Yang Y, Zhao Y, Wang S, Guo X, Ren Y, Wang L, Wang X (2011) A promising approach of reject water treatment using a tidal flow constructed wetland system employing alum sludge as main substrate. Water Sci Technol 63:2367–2373

Yousefi Z, Hoseini SM, Tahamtan M, Alia R, Zazouli MA (2013) Performance evaluation of artificial wetland subsurface with horizontal flow in wastewater treatment. J Mazandaran Univ Med Sci 23:12–25

Zhang DQ, Jinadasa K, Gersberg RM, Liu Y, Ng WJ, Tan SK (2014) Application of constructed wetlands for wastewater treatment in developing countries—a review of recent developments (2000–2013). J Environ Manag 141:116–131