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ORIGINAL PAPER

Removal of pollutants (COD, TSS, and NO_3^-) from textile effluent using Gambusia fish and *Phragmites australis* in constructed wetlands

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Abstract In developing countries, the discharge of polluted effluents into the environment has caused environmental problems. For this purpose, constructed wetlands are attracting great concern owing to their low cost and less operation and maintenance requirements. The main aim of this work was to study the effectiveness of constructed wetlands utilizing *Phragmites australis* plants and Gambusia fish in the treatment of textile effluent. The constructed wetlands are located in the eastern part of a wastewater treatment plant near a grit chamber unit. This research was carried out in four polyethene rectangular tanks with a capacity of 80 L. The tanks were filled to about 20% with sand with a porosity of 48% and the diameter of the gravel bed used in the horizontal subsurface flow unit varied between 5 and 25 mm. The results of different tanks showed the highest and lowest removal efficiencies of chemical oxygen demand (COD) were in the tanks containing *Phragmites australis*/Gambusia fish and *Phragmites australis*, respectively. The best tank for the removal of total suspended solids (TSS) was the tank containing the *Phragmites australis* and the Gambusia fish. In the tank containing the *Phragmites australis* plants, the removal efficiency of NO_3^-, COD, and TSS was in the range of 40–70, 68–72, and 49–71%, respectively. The maximum increase of nitrate, approximately 78%, was observed in tank 2, which contained only fish. In the control tank, the removal efficiency of NO_3^-, COD, and TSS was in the range of 0–10, 10–18, and 15–25%, respectively. The results of this study showed that if these systems were properly designed and operated, they could be used to treat various wastewaters, especially in developing countries.

Keywords Constructed wetlands · Gambusia fish · Natural treatment · Nutrients

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Introduction

Contaminated effluents, in addition to contaminated creatures, in surface and underground waters causes the disease and mortality of thousands of people in the short or long term. In developing countries, due to many problems, the discharge of polluted effluents into the environment has caused irreparable pollution (Kengne et al. 2002; Massoudinejad et al. 2018a, b, c). Water pollution is one of the main threats in the countries of the world, especially in developing countries (Jafari et al. 2018; Massoudinejad et al. 2018a, b, c). Therefore, it is important to protect existing water resources by treating polluted effluents from human activities (Ghaderpoori and Dehghani 2016; Mustafa 2013; Massoudinejad et al. 2018a, b, c). So far, various methods have been developed for the treatment of wastewater, but most of these systems are unusable in developing countries such as activated sludge process, trickling filter, and membrane bioreactors (Ghasemi et al. 2017; Massoudinejad et al. 2015). Therefore, in these countries, more focus is on the use of low-cost methods of treatment. Thus, selecting low-cost and efficient treatment systems for effluent treatment is very important. The results of various studies have indicated which natural treatment systems can be very useful in controlling environmental pollutants, especially in developing countries (Li et al. 2014; Wu et al. 2015). For this purpose, constructed wetlands (CWs), as a rational choice for treating polluted effluents, are attracting great concern owing to lower cost and less operation and maintenance requirements (Li et al. 2014; Sgroi et al. 2018). Compared to conventional wastewater treatment systems, CWs or black boxes (Sgroi et al. 2018) have a higher rate of biological activity, which can ultimately lead to the conversion of organic and inorganic pollutants into non-hazardous or inert materials. Wetlands have been used for secondary treatment and also in some cases for tertiary treatment and final reuse (Mustafa 2013). In recent decades, the use of this kind of treatment system has also been increasing rapidly, due to its advantages over the conventional wastewater treatment systems (Sgroi et al. 2018). Wetlands are one of the most important natural systems that have been used for many years. Also, CWs today have been used as a green technology to treat various effluents for several decades (Wu et al. 2015). In CWs, wastewater treatment processes are carried out under more controlled conditions than natural wetlands. CWs are a safe way to manage municipal and industrial wastewaters and to meet environmental standards. So their disposal in the most environmentally acceptable way can be acceptable in most cases. The use of advanced treatment systems in remote areas has some problems and the costs associated with the use of specialists in the field of operation and maintenance are high. Nutrient removal is another application of CWs (Salari et al. 2012; Farzadkia et al. 2013; Tan et al. 2017). Unlike conventional treatment systems to remove nitrogen and phosphorus, the wetlands are low-cost, functional, and effective (Tan et al. 2017). According to a literature review, CWs were first used by Seidel, Happle, and Kickuth in Germany in 1960. This system has been used to treat various wastewaters like domestic and agricultural wastewater and industrial landfill leachate (Harrington and Scholz 2010; Saeed and Sun 2012; Badhe et al. 2014). Bavor in 1995 reported that CWs have a very high potential for the removal of more than 90% of organic compounds, suspended solids and significant microorganisms. Generally, the cost of maintaining and operating these units is also low (Bavor et al. 1995). The CW system has three main forms: (1) surface flow, SF, (2) horizontal subsurface flow, HSSF, and (3) vertical subsurface flow, VSSF (Rozema et al. 2016). Each CW system has advantages and disadvantages and their use can vary depending on the region and weather conditions (Werker et al. 2002; Odinga et al. 2013; Wu et al. 2015). According to studies, the use of HSSF is more suitable for the reduction of biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) and the removal of pollutants such as total suspended solids (TSS) and nutrients (Babatunde et al. 2010; Yousefi et al. 2013; Wu et al. 2015). The novelty of this study is compared to other studies: (1) the use of this system in several separate steps, which has not been reported in other studies until now; (2) use of both *Phragmites australis* plants and Gambusia fish separately and in combination to treat the pollutants; and (3) use of real effluent from textile industries to perform all experiments. The main aim of this work was to study the effectiveness of the HSSF by the *Phragmites australis* plant and Gambusia fish in the treatment of textile effluent. This research focused on the removal of COD, TSS, and nitrate (NO_3^-).

Materials and methods

Input wastewater

The input effluent used in the research was the raw effluent entering the wastewater treatment plant of Hamrangkimia Company. The company is a textile company founded in 1968 in the province of Alborz in Iran. In Table 1, the characteristics of the input effluent to the wastewater treatment plant are presented. If the concentration of effluent COD is greater than 750, it will be in the category of high wastewater (Muserere et al. 2014). Therefore, the average COD is 856 mg/L, so the effluent classification of Hamrangkimia Company is high. The nominal effluent capacity of this plant is 200 m^3/day. At present, according to the plan of expansion, the amount of effluent has been increased to 400 m^3/day. The treatment system is a combination of a chemical treatment system and biological treatment, an extended aeration/activated sludge system. Materials used in the chemical unit includes polyaluminium chloride, sodium hydroxide, and poly-electrolyte.

Construction and operation of the HSSF

The CW is located in the eastern part of wastewater treatment plant of Hamrangkimia Company near a grit chamber unit. This research was carried out in four polyethene (PE) rectangular tanks with a capacity of 80 litres. The dimensions of the tanks were as follows: length 0.5 m, width 0.35 m, and depth of 0.5 m. Figure 1 shows the view of the used pilot study. The tank arrangement was as follows: tank 1 contained the *Phragmites australis* plant, tank 2 contained the Gambusia fish, tank 3 contained the *Phragmites australis* plant and the Gambusia fish, and tank 4 contained the control (without *Phragmites australis* plants or Gambusia fish). About 20% of the depth of the tanks was filled with sand with a porosity of 48%. According to previous studies, the gravel bed used in the HSSF unit varied between 5 and 25 mm (Yousefi et al. 2013). The number of the *Phragmites australis* plants in tanks 1 and 3 was 10. Gambusia fish from a fish farm in Rasht, Iran, in the Gilan province were added to tanks 2 and 3, for a density of 40. In order to stabilize and adapt the plants and the fish, all four tanks were fed with regular water for 1 week. All experiments were carried out in two steps. In the first step, due to the high COD of the input effluent (about 856 mg/L), it was diluted to 100 mg/L, a concentration the plants and fish were able to tolerate. Daily, the effluent (70 L/day) would enter the four tanks. Before entering the effluent into tanks, parameters COD, pH, and TSS were measured and were measured again after 24 h. Input flow, based on the volume of the reservoir, the contact time, and the porosity coefficient, was calculated using Eq. 1:

$$Q = \frac{V * n}{t} \Rightarrow \frac{70}{1} = 70\,\text{L/day} \tag{1}$$

where Q is the influent flow in L/day; V, n, and t are the tank volume (L), porosity (%), and hydraulic retention time (day), respectively. The second step was performed for more fish and plant compatibility. After exploring the results of the first step, dilutions of 20, 40, 60, 80, and 100% were selected to continue the experiments. At the beginning and end of this step, the parameters of COD, pH, TSS, and NO_3^- were measured.

Table 1 Characteristics of the input effluent to the Hamrangkimia Company treatment plant

Parameters	Unit	Mean concentration (mg/L)
pH	–	8.3
Turbidity	NTU	156
COD	mg/L	856
TSS	mg/L	150
Hardness	mg/L as $CaCO_3$	285
Nitrate	mg/L as NO_3^-	30
Sulphate	mg/L as SO_4^{-2}	541
Sodium	mg/L as Na	490
Calcium	mg/L as Ca	100
Magnesium	mg/L as Mg	8.4

NTU Nephelometric turbidity units, *COD* chemical oxygen demand, *TSS* total suspended solids

Results and discussion

This study was conducted in two steps. The main difference between these two steps was the contact time. The contact time in the first and second steps (with dilution) was 1 and 6 days, respectively. The histogram of the obtained data is shown in Fig. 2. As is

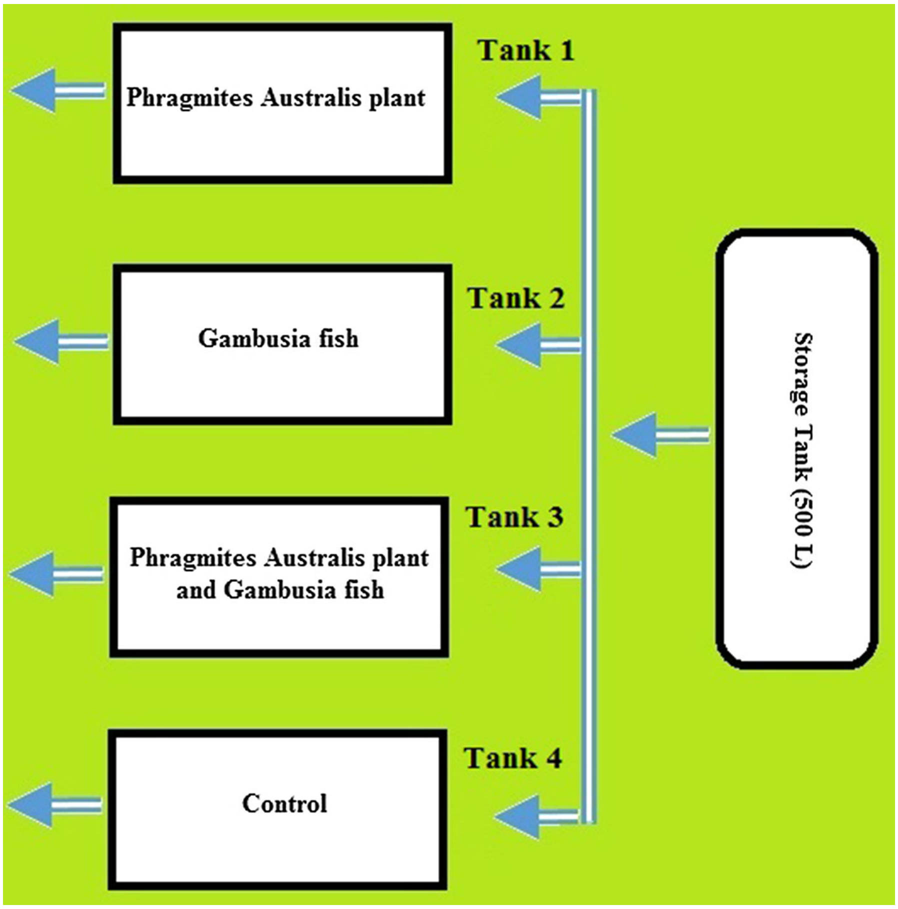

Fig. 1 View of the used pilot study

clear, the graphs are almost symmetric bells that indicate the normalization of the data. The results of the first step are presented in Table 2. Tank 1 contained the *Phragmites australis* plants, tank 2 contained the Gambusia fish, tank 3 contained the *Phragmites australis* plants and Gambusia fish, and tank 4 functioned as the control (without *Phragmites australis* plants or Gambusia fish). In the tank containing the *Phragmites australis* plants (tank 1), the removal efficiency of COD and TSS was in the range of 66–71 and 47–57%, respectively. As shown, there is no significant relationship ($P_{value} > 0.05$) between the amount of dilution and the removal efficiency. In the tank containing the Gambusia fish (tank 2), the removal efficiency of COD and TSS was in the range of 46–70 and 29–52%, respectively. As indicated, there is a significant relationship ($P_{value} < 0.05$) between the amount of dilution and the removal efficiency. As the dilution was increased, the removal efficiency increased. In the tank containing the *Phragmites australis* plants and Gambusia fish (tank 3), the removal efficiency of COD and TSS was in the range of 8–74 and 36–51%, respectively. As is clear, there is no significant relationship ($P_{value} > 0.05$) between the amount of dilution and the removal efficiency of COD. As the dilution was increased, the removal efficiency of TSS increased. In the control tank (tank 4), the removal efficiency of COD and TSS was in the range of 29–31 and 30–52%, respectively. Comparison of the COD removal efficiency in different tanks is shown in Fig. 3. The results of different tanks showed that the highest and lowest removal efficiency of COD was in tank 3 (*Phragmites australis* plant/Gambusia fish) and tank 1 (*Phragmites australis* plants), respectively. Comparison of TSS removal efficiency in different tanks is shown in Fig. 4. The findings of this study were similar to Trang's findings. The amount of COD removal was 57–84% (Trang et al. 2010). For TSS, the highest and lowest removal efficiency was in the *Phragmites*

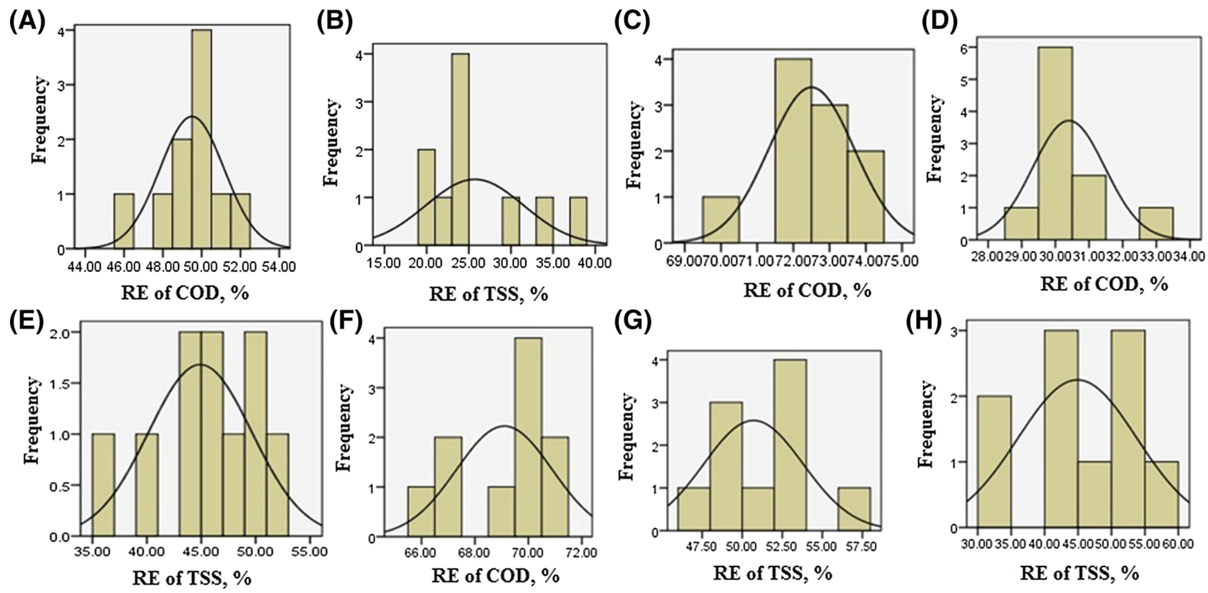

Fig. 2 Histogram of the obtained data. **a** Gambusia fish tank, **b** Gambusia fish tank, **c** *Phragmites australis* plant and Gambusia fish tank, **d** control tank, **e** *Phragmites australis* plant and Gambusia fish tank, **f** *Phragmites australis* plant tank, **g** *Phragmites australis* plant tank, **h** control tank

australis plant tank (tank 1) and the Gambusia fish tank (tank 2), respectively. Based on the discharge effluent standard of Iran, the maximum allowable effluent COD and TSS concentrations for agricultural and irrigation purposes are 200 mg/L and 100 mg/L, respectively (Salari et al. 2012). Also, for this purpose, pH should be 6–8.5 (Ehrampoush et al. 2013). Based on Table 2, the maximum of input COD to the tanks was 850 mg/L. Depending on the type of tank used, the maximum residual COD is 25 mg/L. So, its effluent can be used for agricultural and irrigation purposes. As previously mentioned, the best tank for the removal of COD was the tank containing the *Phragmites australis* plants. Findings from other researchers indicate that the presence of the plant, such as *Phragmites australis*, in CWs can significantly enhance the removal of organic and refractory compounds (Kaseva 2004). It also has been found that the main method of removing organic materials in these systems is by combining various processes such as adsorption, biological processes, flocculation, filtration, and sedimentation (Ehrampoush et al. 2013). The maximum of input TSS, based on Table 2, to the tanks was 150 mg/L. Depending on the type of tank used, the maximum residual TSS is 7 mg/L. So, its effluent can be used for agricultural and irrigation purposes. The best tank for the removal of TSS was the tank containing the *Phragmites australis* and the Gambusia fish. The study results of Melián et al. (2010) showed that the removal efficiency of the CWs in removing suspended solids (SS) was about 96%. In a study by Evanson, the efficiency of CW removal for TSS0 was reported to be 25–89.1% (Evanson and Ambrose 2006). The findings of Nordin (2006) showed that sedimentation and filtration mechanisms have a greater effect on the removal of total solids than biological processes associated with plants and bacterial populations (Nordin 2006). TSS actually is removed primarily through the mechanism of interception and settling (Mustafa 2013). Therefore, the primary method of TSS removal is the filtration process of the bed. The results of various studies indicate that plant roots act as a strong filtration agent (Nordin 2006; Salari et al. 2012). Reports have shown that pollutant removal from effluent may change as a function of the wetland hydrology, the type of soil or substrate, the type of used vegetation, and the concentration of pollutants (Yousefi et al. 2013). The study results of Yousefi et al. showed the removal efficiencies of the *Phragmites australis* plant for BOD_5, COD, TSS, TKN, and TP were 47.11, 70.26, 50.83, 19.69, and 91.69%, respectively (Yousefi et al. 2013). In this study, different dilution ratios, 0–90%, were used, which was the main reason for system

Table 2 Qualitative characteristics of input and output effluent in different dilutions in the first step

Tank	Dilution	Input effluent			Output effluent			RE, % (COD)	RE, % (TSS)
	%	pH	COD	TSS	pH	COD	TSS		
Phragmites australis	90	8.2	83	13	7.5	25	7	70	47
	80	8.3	168	25	7.6	60	13	67	48
	70	8.5	270	35	7.2	83	17	70	52
	60	8.5	335	50	7.8	117	26	66	48
	50	8.4	423	63	7.5	160	30	67	53
	40	8.6	502	75	7.8	151	36	70	52
	30	8.2	585	90	7.9	186	47	69	48
	20	8.4	660	103	7.8	193	45	71	57
	10	8	750	120	7.7	220	61	71	50
Gambusia fish	90	8.2	83	13	7.3	41	10	51	24
	80	8.3	168	25	7,6	81	19	52	24
	70	8.5	270	35	7.5	142	27	48	23
	60	8.5	335	50	7.8	172	39	49	22
	50	8.4	423	63	7.9	218	48	49	24
	40	8.6	502	75	7.3	253	61	50	20
	30	8.2	585	90	7.5	298	72	50	20
	20	8.4	660	103	7.8	330	65	50	37
	10	8	750	120	7.3	380	86	50	29
	0	8.3	850	150	7.7	460	100	46	34
Phragmites australis plant and the Gambusia fish	90	8.2	83	13	7.4	23	8	73	39
	80	8.3	168	25	7.6	45	16	74	36
	70	8.5	270	35	7.2	75	20	73	43
	60	8.5	335	50	7.8	90	28	74	44
	50	8.4	423	63	7.5	120	35	72	45
	40	8.6	502	75	7.8	145	38	72	50
	30	8.2	585	90	7.9	163	48	73	47
	20	8.4	660	103	7.8	190	50	72	45
	10	8	750	120	7.7	225	62	70	49
	0	8.3	850	150	7.7	245	74	72	51
Control	90	8.2	83	13	7.3	59	7	29	47
	80	8.3	168	25	7.6	116	14	31	44
	70	8.5	270	35	7.5	189	15	30	58
	60	8.5	335	50	7.8	225	25	33	50
	50	8.4	423	63	7.9	295	37	31	42
	40	8.6	502	75	7.3	352	51	30	32
	30	8.2	585	90	7.5	410	63	30	30
	20	8.4	660	103	7.8	462	50	30	52
	10	8	750	120	7.3	525	70	30	42
	0	8.3	850	150	7.7	595	72	30	52

COD chemical oxygen demand. *TSS* total suspended solids. *RE* removal efficiency. COD and TSS concentration based on mg/L

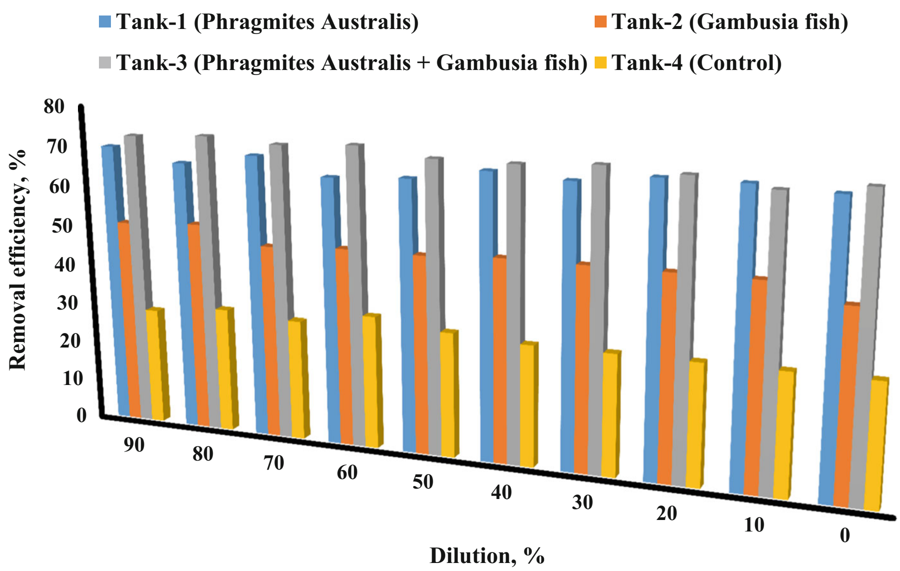

Fig. 3 Comparison of removal efficiency of COD in different tanks

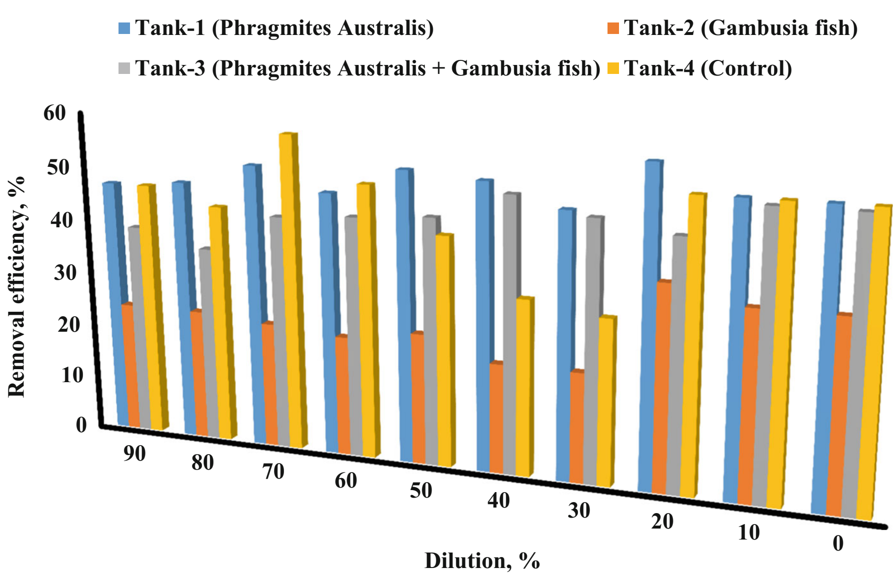

Fig. 4 Comparison of removal efficiency of TSS in different tanks

compatibility. In other words, for system compatibility, a low organic load was initially used. The reason for this was the inadequacy of the volume of biofilm formed on the surface of the bed, which plays a major role in the treatment of input effluent (Tan et al. 2017). At the beginning of the work, the roots and stems of the *Phragmites australis* were growing fast and required a lot of oxygen to breathe, which consumes more oxygen in competition with microorganisms. In later steps, due to the fact that the photosynthesis action is better done, the oxygen level is sufficient for microorganisms. Due to the rapid growth in the heat season, organic matter and suspended solids are eliminated better (Yousefi et al. 2013). The results of the two steps are presented in Table 3. Decreasing and controlling nitrogenous compounds is a main factors in the design of CWs because NO_3^- is one of the main forms of nitrogen in the effluents and can cause many health problems for the environment and human health (Mustafa 2013; Mohsenibandpei et al. 2016). In the

Table 3 Qualitative characteristics of input and output effluent in different dilutions in the second step

Tank	Day	Input effluent				Output effluent				RE, % (COD)	RE, % (TSS)	RE, % (NO_3^-)
	%	pH	COD	TSS	NO_3^-	pH	COD	TSS	NO_3^-			
Phragmites australis	1	8.6	848	152	12	7.8	278	78	5	68	49	58
	2	8.3	852	150	10	7.5	270	76	6	69	50	40
	3	8.3	850	154	13	7.4	265	77	6	69	50	54
	4	8.5	847	148	12	7.4	258	71	5	70	53	58
	5	8.4	853	145	9	7.3	250	63	4	71	57	56
	6	8.6	851	153	10	7.2	243	60	3	72	61	70
Gambusia fish	1	8.6	848	152	12	7.5	464	100	19	43	35	−58
	2	8.3	852	150	10	7.6	460	95	17	47	37	−70
	3	8.3	850	154	13	7.3	453	94	21	47	39	−62
	4	8.5	847	148	12	7.4	450	90	20	47	40	−67
	5	8.4	853	145	9	7.5	443	88	16	49	40	−78
	6	8.6	851	153	10	7.6	439	85	17	49	55	−70
Phragmites australis plant and the Gambusia fish	1	8.6	848	152	12	7.8	253	72	11	71	53	8
	2	8.3	852	150	10	7.5	241	70	12	72	54	−20
	3	8.3	850	154	13	7.4	233	69	12	73	56	8
	4	8.5	847	148	12	7.4	229	65	10	73	57	17
	5	8.4	853	145	9	7.3	220	68	10	75	54	−11
	6	8.6	851	153	10	7.2	215	69	11	75	55	−10
Control	1	8.6	848	152	12	7.3	590	75	12	63	51	0
	2	8.3	852	150	10	7.6	582	73	9	65	52	10
	3	8.3	850	154	13	7.5	576	74	12	64	52	8
	4	8.5	847	148	12	7.7	570	72	11	65	52	8
	5	8.4	853	145	9	7.8	564	70	9	63	52	0
	6	8.6	851	153	10	7.5	561	71	10	62	54	0

COD chemical oxygen demand. *TSS* total suspended solids. *RE* removal efficiency. COD, TSS, and NO_3^- concentration based on mg/L

tank containing the *Phragmites australis* plant, the removal efficiency of NO_3^-, COD, and TSS was in the range of 40–70, 68–72, and 49–71%, respectively. With increasing contact time from 1 to 6 days, the removal efficiency of NO_3^-, COD, and TSS was increased. In the tank containing the Gambusia fish, the removal efficiency of NO_3^-, COD, and TSS was in the range of −58 to −78, 43–49 and 35–55%, respectively. As is clear, the concentration of nitrate increased after treatment. In the tank containing the *Phragmites australis* plant and the Gambusia fish, the removal efficiency of NO_3^-, COD, and TSS was in the range of −20–17, 71–75 and 53–57%, respectively. As is clear, there is no significant relationship between contact time and the removal efficiency of COD and TSS ($P_{value} > 0.05$). In this study, the contact time of the treatment was 1 to 6 days. In Table 3, the role of the contact time is well illustrated. The results showed with increasing contact time, the removal efficiency of pollutants increased, although it was not statistically significant ($P_{value} > 0.05$). These results are consistent with Yousefi et al. (2013) and Spieles and Mitsch (1999). Like the tank containing the Gambusia fish, nitrate concentration increased. In the control tank, the removal efficiency of NO_3^-, COD, and TSS was in the range of 0–10, 10–18, and 15–25%, respectively. The main strategy for removing biodegradable materials in the wetlands systems is thebiological transformation of soluble COD by bacterial degradation (aerobic, optional, and anaerobic), adsorption, filtration, flocculation, and sedimentation of particles contributing to COD (Ehrampoush et al. 2013). The present study, like some of its other studies, has suggested that combined systems have more efficiency in removing organic waste from effluents (Yeh and Wu 2009; Yousefi et al. 2013). According to the discharge effluent standard of Iran, the nitrate concentration for agricultural and irrigation purposes has not been determined (Salari et al. 2012; Ehrampoush et al. 2013). Changes in nitrate concentration in different tanks are presented in Fig. 5. As mentioned earlier and shown clearly in Fig. 3, the concentration of nitrate in tanks containing Gambusia fish, tanks 2 and 3, increased significantly. The maximum increase of nitrate, approximately 78%, was observed in tank 2, which contained only fish. Findings of Upadhyay showed that the use of plants such as and Hydrilla verticillata and Potamogeton crispus can play a significant role in eliminating NO_3^- (Upadhyay et al. 2016). As shown in Fig. 3, the amount of nitrate added in the tank containing the plant and fish is lower in comparison to the fish. The most important reason for increasing the concentration of nitrate in the tank containing the Gambusia fish is that it is a source of organic matter (Kaseva 2004). The results of various studies indicate that the amount of BOD_5 and COD removal in a tank containing fish can be less than that of a tank containing plants and plant + fish. There are several reports that confirm the proper removal of nitrogen compounds with plants. The Ehrampoush et al. (2013) results showed that the removal efficiency of nutrients such as NO_3^-, NH_4^+, and total phosphor (TP) by sub-surface CW methods was 40, 36, and 33%, respectively. The findings of Fenxia showed that towery hybrid CWs cab be effectively used to treat domestic wastewater for small rural communities (Fenxia and Ying 2009). The removal of TSS, COD, NH_4^+, total nitrogen (TN), and TP by this system was 89, 85, 83, 83, and 64%, respectively (Ye and Li 2009). The *Phragmites australis* plant can effectively absorb nutrients. This plant has a large biomass mass in two upper regions (leaves) and in a lower region (stems and roots) which are considered as substrate levels. Subsurface tissues of plants grow horizontally and vertically and can create a massive matrix which binds the soil particles and provides a wide surface for absorbing nitrides and ions (Vymazal 2005; Yousefi et al. 2013). The *Phragmites australis* plant can cause the development of biofilms, the oxidation of organic compounds, and the decomposition of environmental pollutants in wastewater. Afrous et al. (2010) used four plant species including *Phragmites australis*, Typha latifolia, Alisma plantago, and Scirpus (Bulrush) to remove nitrogen and phosphorus. The findings of this study showed that *Phragmites australis* and Typha latifolia had the highest removal efficiency (Afrous et al. 2010). In 2001, Park et al. (2001) used Phragmites japonica to

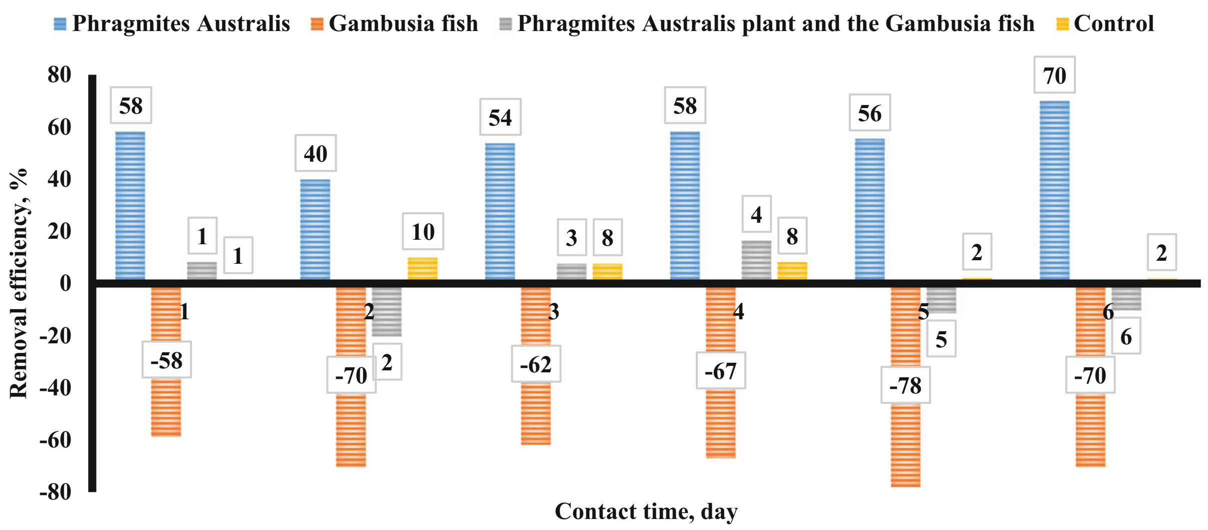

Fig. 5 Changes in nitrate concentration in different tanks

remove pollutants in wastewater. The results showed that the efficiency of removing TP and TN was 80 and 84%, respectively (Park et al. 2001). In general, the removal of nitrogenous compounds in wetlands is aerobic and anaerobic. Aerobic removal is carried out by the oxygen around the roots. Also, anaerobic removal in the distant regions of the roots and bottom of the bed is carried out by optional nitrogen microorganisms and by nitrification and denitrification processes (Salari et al. 2012). In this study, the nitrate concentration in the output effluent was lower than the input. The reason is that in this kind of system, e.g. CWs, nitrification and denitrification processes are carried out simultaneously. Due to denitrification, nitrate is converted to nitrogen and released into the atmosphere (Yousefi et al. 2013). In general, various nitrogen compounds are converted via nitrification and denitrification processes (Salari et al. 2012; Tan et al. 2017). Gersberg et al. (1984) used artificial wetlands to remove nitrogen from wastewater. The results showed that removal efficiency for mineral nitrogen and TN was 97 and 94%, respectively (Gersberg et al. 1984). Rozema et al. (2016) used CWs to remove NO_3^-–N from wastewater. The results showed that the removal efficiency of nitrate was lower than other nitrogen [total Kjeldahl nitrogen (TKN) and NH_4^+–N] forms (Rozema et al. 2016). Previous studies have reported that wetland systems play an active role in reducing pollutants such as BOD_5, COD, and TSS from wastewater. This topic has been verified in the present study with the native *Phragmites australis* in Iranian climatic conditions. The present study also has suggested that CWs can have good efficiencies in the removal of NO_3^- from industrial effluents.

Conclusion

The input effluent used in the research was the raw effluent entering the wastewater treatment plant of Hamrangkimia Company. Nominal effluent capacity of this plant is 200 m^3/day. At present, according to the plan of expansion, the amount of effluent has been increased to 400 m^3/day. The arrangement of tanks was as follows: tank 1 contained the *Phragmites australis* plant, tank 2 contained the Gambusia fish, tank 3 contained the *Phragmites australis* plant and the Gambusia fish, and tank 4 functioned as the control (without *Phragmites australis* plants or Gambusia fish). The number of the *Phragmites australis* plants in each tank was 10. The fish density in each tank was 40. In the first step, there was no significant relationship between the amount of dilution and the removal efficiency of COD. In the second step, with increasing contact time from 1 to 6 days, the removal efficiency of NO_3^-, COD, and TSS was increased. The concentration of nitrate in tanks containing Gambusia fish, tanks 2 and 3, increased significantly. The amount of nitrate added in the tank containing plants and fish was lower in comparison to the fish-only tank. The maximum increasing of nitrate, approximately 78%, was observed in tank 2, which contained only fish. CWs, as an efficient treatment option, can reduce resistant wastewater pollutants to the standards of secondary wastewater treatment. Furthermore, CWs can remove nutrients from effluent discharges more effectively than conventional systems.

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