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Nitrate removal from water by using *Cyperus alternifolius* plants in surface flow constructed wetlands

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ABSTRACT

In this research, nine systems were constructed and the flow of urban water was continuously maintained from May to November 2018. Three of these systems were considered as an emergent, three were set as floating canals and three other systems were unplanted and porous media. The nitrate inlet was 20 mg/L and hydraulic retention times (HRTs) were 1, 3, and 5 d. The experimental design consisted of a factorial split-plot design. The analysis of variance showed that the efficiency of nitrate removal was affected by the type of constructed wetland, HRT, temperature changes, and the reciprocal effects between these parameters ($P \leq 0.01$). In HRT of 1 d, the average efficiency of nitrate removal by the emergent system, floating canal system, and unplanted system were 14.34%, 12.09%, and 10.51%, respectively. With an HRT of 3 d, these average efficiencies became 17.62%, 15.76%, and 13.54%, respectively. With the HRT of 5 d, these percentages increased to 17.75%, 17.66%, and 16.08%, respectively. The comparison of mean values pertaining to the effect of temperature on nitrate removal showed significant differences between the efficiencies of nitrate removal in some months of the year ($P \leq 0.05$). The reciprocal showed that the highest nitrate removal efficiency was 17.75% by the HRT of 5 d in the emergent system and in the month of June.

Keywords: Constructed wetland; Nitrate; Retention time; *Cyperus alternifolius*

1. Introduction

Considerable amounts of contaminants emanate from urban sewage and wastewater [1]. Furthermore, the diverse types of culture-systems which include the extensive use of chemical fertilizers, pesticides, and the increase in livestock waste have caused changes in the quality of water resources [2,3]. A prominent environmental problem that is common nowadays is the prevalence of nitrate in underground waters and in waters that are in contact with domestic and industrial sewage [4]. Nitrate is one of the most hazardous sources of water contamination and is a great threat to aquatic ecosystems. Nitrate is produced by the decomposition of human and animal waste, by industrial productions, and the run-off that results from agricultural activities, all of which can enter surface waters and underground waters. Intensive use of nitrogen fertilizers, sewage disposal by drainage systems, and high atmospheric deposition can cause contamination of shallow groundwater with nitrates [5,6]. The occurrence of high amounts of nitrate in drinkable water can cause diseases such as methemoglobinemia in newborn babies and could cause an increase in the occurrence of cancer in adults by the formation of nitrosamines [2,7]. Physical, chemical, and biological methods exist for removing these contaminants from water resources, but their high costs and limitations in efficiency have encouraged researchers to turn their attention to natural processes for wastewater treatment – methods that

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are more cost-effective. A natural method for wastewater treatment that is increasingly gaining popularity is phytoremediation. The process can be carried out with the help of microbial populations, by absorbing the contaminants from the soil and water, and then accumulating them around the root zone or plant tissues [8].

Garsia-Avila [9] evaluated the performance of *Phragmites australis* and *Cyperus papyrus* in the treatment of municipal wastewater by vertical flow subsurface constructed wetlands. The results indicated that the *C. papyrus* presented a greater capacity of pollutants removal as biochemical oxygen demand (80.69%), chemical oxygen demand (69.87%), ammoniacal nitrogen (69.69%), total phosphorus (50%), total coliforms (98.08%), and fecal coliforms (95.61%). In the case of *P. australis* retains more solids. The species with greater efficiency in the treatment of municipal wastewater for this study was *C. papyrus*.

Researchers are trying to achieve cheaper and more compatible solutions that can coexist with the environment in an effort to remove contaminants from natural waters. Over the past two decades, research has shown that wetland structures are one of the most suitable methods for this purpose. Through the different physical, biological and chemical structures, wetlands can efficiently reduce the concentration of different chemical contaminants and pathogenic bacteria [10].

Wetlands can be divided into two major groups: natural or constructed wetlands are designed by humans and are used for wastewater treatment, ranging from urban, industrial, and agricultural sewage, surface run-off, or simply for the treatment of surface flows or lakes that may have been contaminated. Constructed wetlands can be used for treating contaminated wastewaters (which may contain nitrate) and for sewage purification with the help of aquatic plants during processes that would require lower amounts of cost in comparison with other methods. They can contribute to the efficient removal of contaminants such as nitrate [11].

In relevant research, nitrate removal experimented with sunflower and maize stems which were ground and then used as wastewater treatments. The biomass of sunflower and maize stems removed nitrate from wastewaters by 84% and 91%, respectively [12].

Maxwell et al. [13] enhanced nitrate reduction within a constructed wetland system, nitrate removal within groundwater flow was performed. The wetland waters had a mean nitrate as nitrogen (NO_3–N) concentration of 19.80 mg/L, which is a magnitude larger than the measured NO_3–N concentration in the upgradient groundwater of 1.53 mg/L. As water travels in the subsurface away from the wetland, the NO_3–N concentrations decrease to 10.99 mg/L and 44.5% reduction.

Saeed et al. [14] evaluated the efficiency of constructed wetlands in the purification of river water by using the *common reed* and *edible canna* as Phyto-remedial plants for the removal of several contaminants, namely, ammonium nitrate, phosphorous, biochemical oxygen demand, and chemical oxygen demand, and the average removal efficiency of these contaminants by the plants were 0.66, 0.08, 0.59, and 2.49 g/m^2 each day, respectively. Chen et al. [15] used pre-constructed wetlands for the purification of contaminated flows, leading to promising results for the treatment of river water. Chen et al. [15] evaluated hydroponic root mats for wastewater treatment – a review. HRM$_s$ have been used for the treatment of various types of polluted water, including domestic wastewater, agricultural effluents, polluted river, lake, stormwater, groundwater, and even acid mine drainage. This article provides an overview of the concept of applying floating HRM and non-floating HRM filters for wastewater treatment.

The *Cyperus alternifolius* is a plant that has the capability of growing in wetlands in the form of a floating or an emergent plant. The *C. alternifolius* can have important roles in the process of phytoremediation in wetlands. Through its roots, stem, and leaves, this plant can absorb contaminants from water and wastewater. The purification efficiency of this plant depends on the type and design of wetland, retention time, concentration of contaminants, activity of microorganisms, and climatic conditions. Aquatic plants are more suitable than terrestrial plants for phytoremediation. Their ability to grow fast in wetlands is accompanied by their propensity for greater biomass production, and thus a stronger capacity for the absorption of contaminants.

So far, the *C. alternifolius* plant has not been used for nitrate removal in the constructed wetland with the surface flow. Therefore in the current study, the removal efficiency of nitrate was evaluated in constructed wetlands with a surface-flow. The constructed wetlands hosted *C. alternifolius* plants which were cultivated either as floating or as an emergent plant in the soil. The control group remained unplanted.

The variables included the hydraulic retention time (HRT) and temperature which were evaluated to affect the efficiency of nitrate removal. Here, it was endeavored to set up pilot constructed wetlands of the surface-flow type so as to use phytoremediation for improvement and purification of urban wastewater outlets in the future.

2. Materials and methods

This research was carried out in a greenhouse located in the research field of Gonbad Kavoos University. The duration of the experiment was from May to November 2018.

Nine rectangular ponds were constructed (to resemble the constructed wetlands). These comprised three types of systems, and each system had three replications. In order to establish the surface flow in the pilot-constructed wetlands, an outlet valve was placed at a height of 20 cm from the pond floor. A container (with a volume of 2,000 L) was situated beside each system so as to provide water for the ponds. The surface level of water inside the reservoir was controlled by a buoy, and the outlet flow was regulated by a stopcock valve. Each constructed wetland was structured as a rectangular cuboid (i.e., 2 m in length, 30 cm in width, and 20 cm in height) on the ground. These ponds were made of galvanized sheets, and superglue was used for insulating the systems and for protecting them from externally unwanted factors.

The experiment comprised three treatments, each of which had three replications, and each replication had seven plants. In three of the systems, the *C. alternifolius* were cultured in the soil, thereby being called the emergent system. In another three systems, the *C. alternifolius* were used as floating plants, while three remaining systems without porous media and unplanted were considered as control. The field soil texture used for porous media systems was silty-loam. The height of porous media was 15 cm above ground level.

The water loss in these systems happened in the form of leakage from the walls and bottom of constructed wetlands, evaporation from the water, and transpiration from plants. Since the walls were insulated with superglue, the water loss through leakage was reduced to negligible amounts. In reviewing the available literature on cases of similar research where galvanized sheets and superglue were used in pilot constructed wetlands, there were no indications of elemental removals from contaminated waters by the galvanized sheets. Accordingly, it was assumed here that the galvanized sheets had insubstantial effects on changing the amount of nitrate in the wastewater, and thus the evaluation of their ability in nitrate removal was left out. The most important sources of water loss in the systems of this research were evapotranspiration. Kadlec and Knight [16] reported that the amount of evapotranspiration in wetlands with surface flows can amount to 80% of evaporation from the evaporation pan. Accordingly, relevant data from the evaporation pan in the weather station, which was located 2 km away from the experimental site of this research, were used for calculating the amount of water loss in the systems. Then, the same amounts of water were compensated and added into the systems through the inlet flow.

Forty-two cuttings of *C. alternifolius* plants were collected from the greenhouse and the cuttings were placed upside down inside containers for a week to allow the formation of rooting under experimental conditions. When the cuttings had grown roots, they were placed under direct sunlight for 3 d. Fig. 1 shows the schematic plan of the plant establishments in the emergent, floating, and unplanted systems.

In general, the average amount of time that takes for contaminated water to go through a wetland system is called the HRT. In a relevant study by Persson and Wittgren [17], the use of tracer substances led to the conclusion that a stream-flow type of water can be the best type of flow in terms of the nominal retention time (volume of inlet flow ratio) which has to correlate with the actual retention time red. Based on the constant volume of each constructed wetland, this research was carried out by having different inlet volumes of flow, and therefore the HRTs were 1, 3, and 5 d.

A continuous flow of urban water was maintained into each system for specific HRTs. In the beginning of each retention time, the inlet flow of water had specific concentrations of nitrate in each system. Samples were taken from the inlet and the outlet flows in the beginning and end of each retention time, respectively, so as to measure the concentration of nitrate. The samples were immediately taken to the laboratory by putting them in iced containers. The concentration of nitrate in each sample was measured by a spectrophotometer device at a wavelength of 410 nm, according to the method in the book of standard experiments on water and sewage [18]. The effects of temperature and different months on nitrate removal by plants and systems were assessed by considering the temperature data which had been collected from the mentioned weather station. In the beginning and end of each experiment, other parameters were also measured besides nitrate. These included the pH, electrical conductivity (EC), total dissolved solids (TDS), and dissolved oxygen (DO) which were measured by the HQ40D portable multi-meter. The mentioned parameters were measured in samples taken from the inlet and the outlet flows of the systems. By completing and collecting the data, the efficiency of nitrate removal was calculated according to the following formula:

$$R = \frac{\left(C_e - C_i\right)}{C_e} \times 100 \quad (1)$$

where R is the output of nitrate removal efficiency, while C_e and C_i are concentrations of nitrate in the outlet and inlet of flows (mg/L). For the analysis of variance (ANOVA), the SAS software was used and the figures were illustrated by Microsoft Excel.

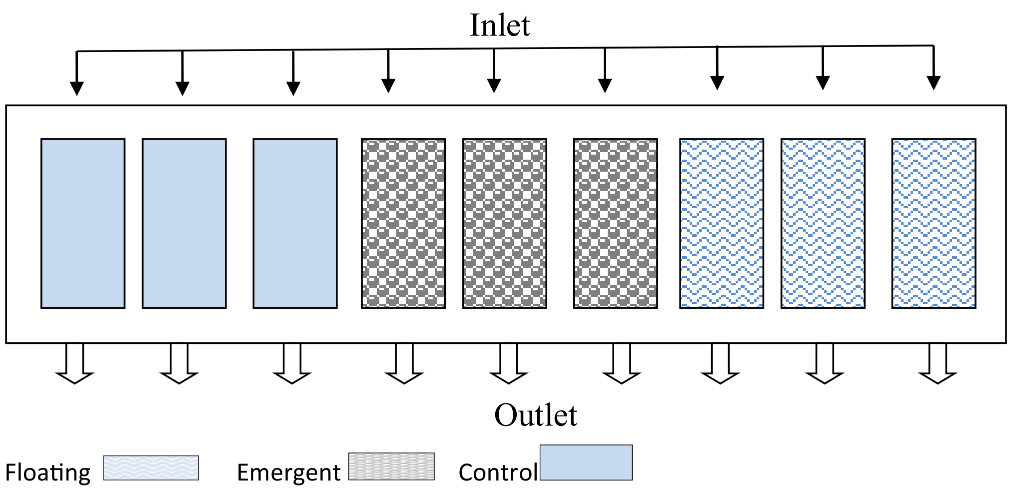

Fig. 1. Schematic plan of the plant establishments in the emergent, floating, and unplanted systems.

The following first-order decay model was used to determine the first-order decay rates in this constructed wetland system [15,19]:

$$C_e = C_0 \exp(-kt) \tag{2}$$

$$\text{HRT} = \frac{V}{Q} \tag{3}$$

$$\text{HLR} = q = \frac{Q}{A} \tag{4}$$

$$\text{PLR} = q \times C_0 \tag{5}$$

where C_e is the mean effluent concentration, C_0 is the mean influent concentration, k is the temperature-dependent decay rate constant (1/d), and t is the HRT (d); Q is the average flow rate (m^3/d), A is the wetland surface area (m^2), and V is the available wetland volume (m^3).

The treatment efficiency was determined using the averaged influent and effluent concentrations of the above major water quality parameters.

2.1. Statistical analysis

Split plot designs were used for comparing the parameters in different locations and times. The Kolmogorov–Smirnov test was used for checking the normality of the variables. On the occasion that the data series were not normal, the data were normalized using the appropriate conversion of BAX-COX and the logarithmic. Finally, the effects of each system (emergent, floating, and control) at different HRTs (i.e., 1, 3, and 5 d) and in different months were evaluated in the SAS environment, using a factorial test with split-plot design. The comparison of mean values was performed by the least significant difference (LSD) test.

3. Results and discussion

The values of pH, EC, and NO_3 concentration were examined in the soil and plants, before and after the experiment (Table 1). Higher concentrations of nitrate can serve as the main source for plant nutrition which could lead to enhanced rates of stem growth, more optimum levels of chlorophyll in the leaves, and greater root mass. The fresh and dry weights of the *C. alternifolius* were different throughout the duration of the experiment (Table 1). As can be seen, the fresh and dry weights of the plant increased in response to the presence of higher concentrations of nutritional elements in the emergent system, thereby increasing plant biomass. The emergent system hosted the greatest growth of shoots, wherein the stems reached heights of up to 130 cm. Meanwhile, the maximum height of plants in the floating system reached 60 cm. Table 1 shows changing in pH, EC, and concentration of nitrate in the soil and plants, before and after the experiment.

After the experiments, the EC had increased in the roots, stems, and leaves by 2.9, 2.4, and 1.9 times, respectively, compared to the control (Table 1). The amount of nitrate in the roots increased by 1.4 and 1.1 times in the emergent and floating systems, respectively. The increase in nitrate in the stems and leaves of the two systems (emergent and floating) was 1.12, 1.02, 0, and 1.13, respectively. The amount of nitrate in the soil had decreased by the end of the experiments. Therefore, the adsorption of nitrate had occurred more significantly by the roots, as compared to the amounts adsorbed by the shoots. Since perennial plants have the habit of storing nutritional elements in their roots for their future use, the levels of EC and nitrate in the roots of plants in this research appeared to be greater in comparison to other organs (Table 1). Furthermore, the plants adsorb nitrate and salts from the soil which explains why the levels of nitrate and EC were reduced by the end of the experiment. According to Table 1, the dry weight of the roots and shoots of plants in the emergent system had increased more in comparison to those of the floating system. The amount of nitrate removal correlates positively with the root weight of plants (Table 1). High-weight roots and rhizomes indicate more nitrate removal in the system.

3.1. Weather temperature

The effects of temperature on the efficiency of nitrate removal from water in the constructed wetland systems

Table 1
pH, EC, and concentration of nitrate in the soil and plants, before and after the experiment

Environment			pH	EC (μmhos/cm)	NO_3^- (mg/L)	Wet weight (g)	Dry weight (g)
Root	Before experiment		7.01	40.37	10.46	0.548	0.14
	After experiment	Emergent	3.11	115.57	14.8	3.28	0.39
		Floating	2.55	115.73	11.52	1.5	0.21
Shoot (stem)	Before experiment		7.5	50.04	7.89	0.687	0.074
	After experiment	Emergent	3.08	120.5	8.82	1.48	0.314
		Floating	4.85	118.87	8.11	0.832	0.18
Shoot (leaf)	Before experiment		6.86	60.21	8.1	0.467	0.012
	After experiment	Emergent	3.57	118.33	9.16	0.715	0.259
		Floating	3.47	113.77	7.54	0.44	0.034
Soil	Before experiment		6.11	175	8.52	–	–
	After experiment	Emergent	5.66	42.13	6.15	–	–
		Floating	–	–	–	–	–

were evaluated by the daily values of environmental temperature. The average change of weather temperature in 2018 (during the summer and autumn) were recorded (Fig. 1). It is observed that the temperature fluctuated between 4°C in October to 46°C in July during the experiments. Therefore, the trend of temperature changes from May to October was in a manner that the repetition of the experiments in the consecutive months could provide the opportunity to evaluate the effect of temperature increase on the efficiency of nitrate removal.

Nitrate concentrations of the inlet and outlet flows, in addition to the efficiency of nitrate removal, are presented in Table 2. The efficiency of nitrate removal had an average, maximum, minimum, and standard deviation which were 18.49, 20.81, 17.81, and 1.08 mg/L, respectively (Table 2). Among the HRTs, the retention time of 5 d caused a greater decrease in the nitrate concentration of the outlet flow. The emergent system, in which plants were cultured in the soil, led to greater nitrate removal efficiency. In all HRTs, the concentration of nitrate in water from July to October exceeds the allowed level of standard concentration of nitrate (15 mg/L) in urban waters that eventually sink into groundwater. Accordingly, there is an urgent need to employ purification systems for the treatment of urban waters in the region where this experiment was carried out. The ANOVA and the comparison of mean values were carried out by the LSD test, and the relevant figures were illustrated.

Table 3 shows the ANOVA pertaining to the effects of treatments on the percentage of nitrate removal. The ANOVA (Table 3) showed that the effects of temperature are embedded in the months during which the experiment was performed (i.e., minor factor), and the type of plant cultivation in the wetland or the type of constructed wetland system (major factor), HRT (major factor), and the reciprocal effects of these factors on nitrate removal were statistically significant ($P \leq 0.01$). Values with similar letters indicate non-significant difference ($P \leq 0.05$). Values with non-similar letters are statistically significant.

Fig. 3 shows the comparison of mean values pertaining to the months of the experiment and their effects on the percentage of nitrate removal which is statistically significant in some of the months, including the period between the first and the fourth months and between the

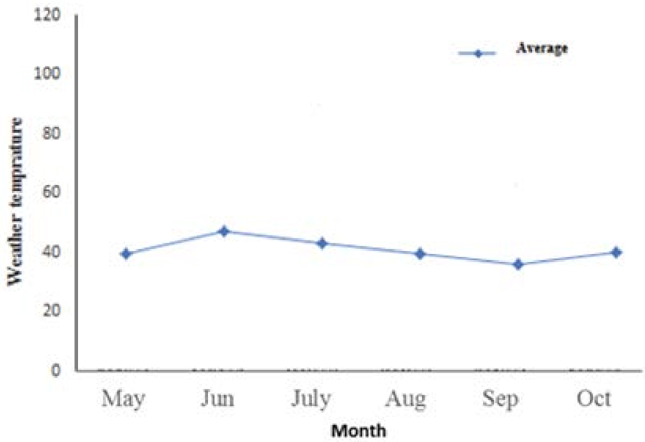

Fig. 2. Variation of average temperature of the environment from the start to the end of the experiment.

Table 2
Concentrations of nitrate in the inlet and outlet flow of urban water and the percentage of nitrate removal per HRTs

Types of constructed wetland system									
Emergent		Floating			Control				
Removal rate	NO_3^- out (mg/L)	Removal rate	NO_3^- out (mg/L)	Removal rate	NO_3^- out (mg/L)	NO_3^- in (mg/L)	Trial period		Retention time (d)
7.11	19.33	7.35	19.28	21.67	16.30	20.81	2018/06/28	2018/06/27	1 d
0.73	17.79	17.52	14.78	35.27	11.60	17.92	2018/07/23	2018/07/22	
2.77	17.53	23.57	13.78	30.23	12.58	18.03	2018/08/31	2018/08/30	
1.92	17.84	9.57	16.45	11.49	16.10	18.19	2018/09/28	2018/09/27	
2.30	17.40	5.11	16.90	6.23	16.70	17.81	2018/10/26	2018/10/25	
3.30	17.60	4.01	17.47	5.71	17.16	18.20	2018/11/29	2018/11/28	
8.75	18.99	30.75	14.41	31.43	14.27	20.81	2018/12/29	2018/12/28	3 d
0.89	17.76	34.21	11.79	45.42	9.78	17.92	2018/07/1	2018/06/29	
3.05	17.48	37.33	11.30	46.20	9.70	18.03	2018/08/1	2018/07/29	
2.42	17.75	13.52	15.73	13.85	15.67	18.19	2018/09/1	2018/08/29	
4.21	17.06	5.56	16.82	7.47	16.48	17.81	2018/10/1	2018/09/29	
5.82	17.14	10.44	16.30	19.01	14.74	18.20	2018/11/1	2018/10/29	
7.21	19.31	52.72	9.84	58.87	8.56	20.81	2018/12/1	2018/11/29	5 d
1.28	17.69	39.40	10.86	44.81	9.89	17.92	2018/12/27	2018/12/25	
3.16	17.46	50.47	8.93	66.11	6.11	18.03	2018/09/24	2018/09/20	
4.51	17.37	29.85	12.76	36.17	11.61	18.19	2018/10/28	2018/10/24	
5.73	16.79	11.90	15.69	24.14	13.51	17.81	2018/11/29	2018/11/25	
5.99	17.11	20.49	14.47	26.37	13.40	18.20	2018/12/29	2018/12/25	

Table 3
Analysis of variance pertaining to the effects of treatments on the percentage of nitrate removal

Possibility	Value F	Average of squares	Sum of squares	Degree of freedom	Sources of changes
0.0001	307.69	292.93	585.86	2	Month (A)
0.0001	138.75	132.10	264.19	2	System type (B)
0.0001	33.87	32.24	128.97	2	Month × System type ($A \times B$)
0.0001	64.67	61.57	307.84	2	Retention time (C)
0.0001	25.14	23.93	239.34	10	Retention time × Month ($A \times C$)
0.0001	6.48	6.17	61.72	10	System type × Retention time ($B \times C$)
0.0003	2.88	2.75	55.04	20	Month × System type × Retention time ($A \times B \times C$)
		0.95	85.68	90	(Error)
	579.48	552.65	1,728.65	143	Sum

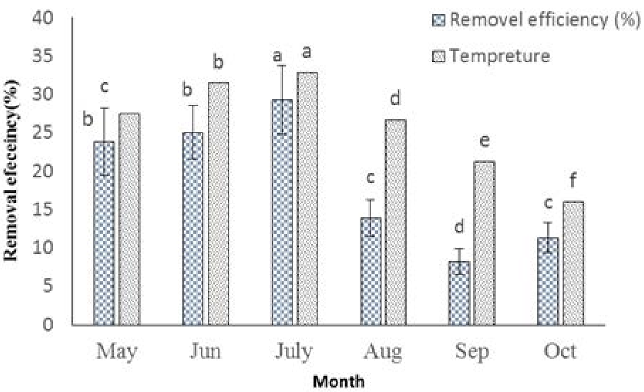

Fig. 3. Effect of month of the experiment on the percentage of nitrate removal. Different letters indicate significant differences. *Note*: Means denoted by a different letter indicate significant different at the 5% level according to an LSD test.

fifth and the sixth months. From the comparison of mean values pertaining to the effect of temperature on nitrate removal, it can be suggested that the increase in temperature can enhance the efficiency of nitrate removal (Fig. 3). However, the trend of increase in the efficiency of nitrate removal is slower than the rate of increase exhibited by the trend of temperature increase. Furthermore, the highest percentage of nitrate removal was observed from July to August (29.21%). Similar to this experiment and according to reports by it is hypothesized that the rate of nitrogen removal is decreased parallel to the decrease in the accessibility to enough oxygen in months with lower temperatures. Therefore, the most successful condition and the highest rate of wastewater treatment are achieved in warm temperatures due to the increase in microbial activity. Nonetheless, Liu et al. [20] reported that the rate of nitrate removal is not significantly less during the colder months of the year, and that the difference between cold and warm months in this respect is less than 10%. Another report by Kadlec and Knight [16], Khosh Navazaz et al. [21] showed that the increase in temperature can enhance the efficiency of nutritional adsorption by plants as a result of the effect of temperature on the rate of physiological processes such as the growth and development of plants. Furthermore, the processes of biological treatment are dependent on temperature. Similar to the reproduction and distribution of aquatic organisms which affect the rate of chemical activities and the metabolism of organisms, biological treatments, and their processes have important roles in the transport of environmental oxygen to wetlands, besides increasing the amount of soluble oxygen in the water and ultimately assisting in the oxidation of organic materials. In relevant research, it was reported that the temperature conditions for the process of nitrification in the Golestan Province are suitable during most months of the year and that the wetlands of the province have acceptable levels of efficiency for wastewater treatment. One of the most suitable mechanisms for nitrate removal in wetland systems is denitrification.

Fig. 4 shows the comparison of mean values pertaining to the effect of the type of constructed wetland system on the percentage of nitrate removal. Significant differences ($P \leq 0.05$) were observed between the efficiencies of nitrate removal by the three different types of constructed wetland. Using the *C. papyrus* for wastewater treatment led to the observation that the nitrate removal efficiency is 17.68% in the emergent system where plants are cultivated in the soil, compared to the efficiency of the floating system (14.17%) and the control system (13.27%) without plants. According to Fig. 4, the emergent system in which plants were cultivated in the soil managed to show a higher efficiency of nitrate removal due to the dominance of denitrification, as compared to nitrate removal in the floating system. In wetland systems, nitrogen removal is partly dependent on microbial activity and the population of bacteria surrounding the root zone. This is why nitrate removal is mostly dependent on factors such as temperature and the presence of enough oxygen [16]. In a similar context, Falahi et al. [22] examined hydroponic cultivations of three different plant species (i.e., the common reed, bamboo, and *C. papyrus*) for nitrate removal from urban waters under experimental conditions. In the mentioned experiment, the concentrations of nitrate were 15, 20, and 25 mg/L, and the 6 months of that experiment showed that the highest amount of nitrate removal is achieved by the roots and rhizomes of plants. By comparing the wetland systems in the mentioned research, among all the HRTs, it was observed that canals with

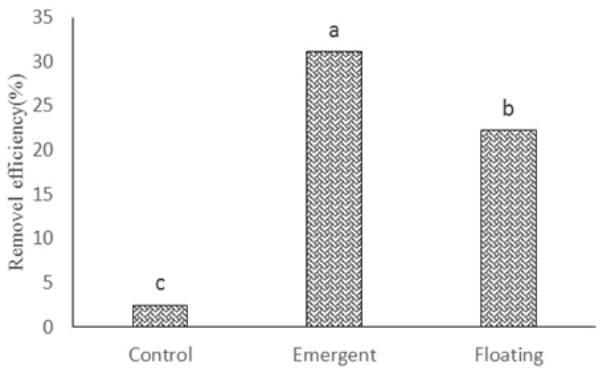

Fig. 4. Effect of type of constructed wetland system on the percentage of nitrate removal. Different letters indicate significant differences.

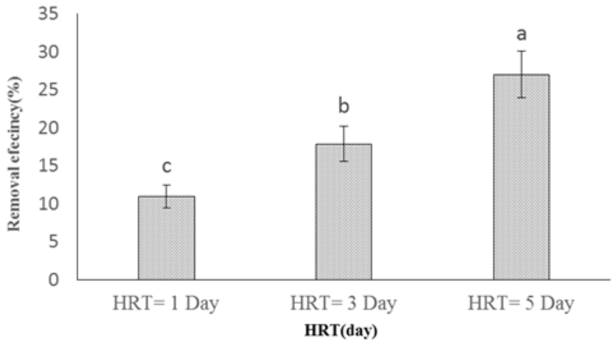

Fig. 5. Effect of HRT on nitrate removal. Different letters indicate significant differences.

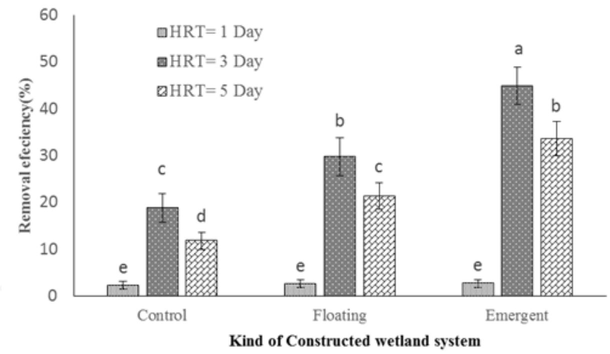

Fig. 6. Effect of HRT and the type of constructed wetland system on nitrate removal.

plants are significantly capable of staging higher efficiencies in terms of nitrate removal. The results of the current study also confirmed this fact by showing that constructed wetlands of the emergent type are more capable of nitrate removal because of hosting plants that have roots in the soil.

Fig. 5 shows the comparison of mean values pertaining to the effects of HRTs on nitrate removal. Here, the treatments significantly affected the amount of nitrate removal from urban wastewater ($P \leq 0.05$). The efficiency of nitrate removal by the HRT of 5 d was 16.53%, compared to the HRT of 3 d which had a removal efficiency of 15.18%, and the HRT of 1 d which showed an efficiency of 13.41%. In this regard, Pickard et al. [23] stated that one of the most important parameters in designing constructed wetlands for the purpose of achieving high efficiencies is the HRT. The reason is that systems of constructed wetlands are intensively dependent on natural sources of energy (such as sunlight which relate to photosynthesis and oxygenation) and wind (which facilitates the transport of oxygen into the water). Accordingly, by having enough value of HRT, the system can have sufficient time to make adequate use of these energies. Furthermore, the microbial population inside a constructed wetland increases through time and as the wetland ages, which leads to enhanced levels of nitrate removal and greater adsorptions of organic and nutritional substances from the water.

The evaluation of reciprocal effects between the major factors in this experiment (Fig. 6) were considered to evaluate the efficiency of HRT at 3 and 5 d, thereby showing that the type of wetland system determined the degree of nitrate removal efficiency per the HRTs of 3 and 5 d. In the emergent systems where plants were cultivated in the soil, the highest efficiency was achieved by the mentioned HRTs. On the other hand, the HRT of 1 d did not depend on the type of constructed wetland, and all three systems did not show significant differences in nitrate removal when treated with the HRT of 1 d.

According to Fig. 6, among all HRTs, the efficiency of nitrate removal from urban water is greater when using the emergent system as compared to the control system. The average efficiency of nitrate removal in the emergent, floating, and control systems were 14.34%, 12.09%, and 10.51%, respectively, based on the HRT of 1 d. When exposing the same systems to the HRT of 3 d, the efficiency of nitrate removal became 17.62%, 15.76%, and 13.54%, respectively, while the HRT of 5 d caused the efficiency of nitrate removal by the three systems to become 17.75%, 17.66%, and 16.08%, respectively. Accordingly, the maximum efficiency of nitrate removal was achieved in the emergent system with the HRT of 5 d. The results of the present study showed that the emergent system is more efficient in removing nitrate from wastewater due to the dominance of denitrification which is observed less in the floating and unplanted systems.

Vymazal [24] stated that constructed wetland systems containing aquatic plants are capable of reducing organic and nutritional materials from contaminated wastewater, and that the efficiency of removing organic matter is more than that of removing nutritional substances. Nonetheless, the overall result of the research showed that constructed wetlands with plants can have a greater role in removing nutritional substances from wastewater in comparison with constructed wetlands without plants, which was similar to the findings of other cases of research [23,25]. In addition to the role of plants in adsorbing nutritional substances, there is the role of microorganisms in putting to reason that the roots of plants in wetlands are a good site for microbial activity which, in turn, cause an increase in the microbial population of constructed, planted wetlands, as compared to unplanted ones. Furthermore, the removal of nutritional substances from any wetland can be facilitated by microbial processes which have major roles in this realm [26]. Aquatic plants contribute to the diffusion of oxygen by the process of photosynthesis in aquatic environments and, therefore, they provide the necessary amount of oxygen for the oxidization of ammonium to nitrate via bacteria. On the other hand, plant respiration can reduce the level of oxygen in wetlands and thus activate the process of denitrification, thereby converting nitrate to nitrogen gas [27].

Fig. 7 shows the comparison of mean values pertaining to the reciprocal effects between the month and the type of wetland system on the efficiency of nitrate removal. According to Fig. 7, it is observed that the amount of nitrate removal differed in each month, depending on the type of constructed wetland system (i.e., floating or emergent). According to these differences, higher levels of nitrate removal were observed in the months of May, June, and August in comparison with the months of September, October, and November. The mean values of nitrate removal by the unplanted wetland system remained insignificantly different throughout all months. The maximum and minimum nitrate removal efficiency in the emergent and floating systems happened in July (48% and 38%) and in September (15% and 10%), respectively. The control system showed the lowest efficiency of nitrate removal in September and had a constant degree of nitrate removal efficiency in all other months. Therefore, the warmest months of the year had the greatest efficiency of nitrate removal by the soil and the plants.

The average values of EC, DO, and TDS of samples were taken from the outlet flows of the emergent, floating, and unplanted systems during each month of the experiment (Table 4). The highest amount of EC and TDS, along with the lowest value of DO, in the constructed wetlands was observed in May (Table 4). In November, the quality of outlet flow from the systems became better and the value of DO increased. Based on Tables 1 and 4, the decrease in the EC of water resulted from the accumulation of salts in the underground organs of the plants (i.e., in roots and rhizomes) which are consistent with previous reports by Almedia [28]. The amount of EC in samples taken from the wastewater in May, June, July, and August was significantly different compared to the EC values in other months, except in October and November (Table 4). The amounts of DO in November and August were significantly different compared to the DO values in other months, except in May, June, September, and October. Based on the results, it was observed that the species of *C. alternifolius* has good potential for nitrate removal when using the emergent system. This is to the extent that the increase in HRT led to the increase in the percentage of nitrate removal, and the highest efficiency was observed in the emergent system with an HRT of 5 d in June. This is an indication that other nutritional elements and HRTs can affect nitrate removal in the emergent system.

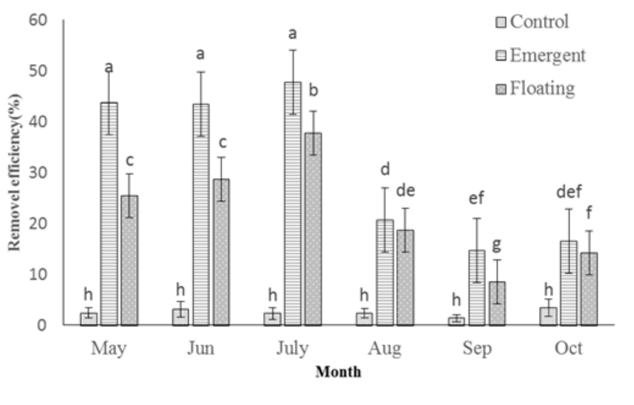

Fig. 7. Effects of month and the type of constructed wetlands on nitrate removal.

4. Conclusion

In the present study, the efficiency of wastewater treatment by surface-flow constructed wetlands was evaluated in the case of urban wastewater. The maximum percentage of nitrate removal was 17.75% in the month of June. Furthermore, the average percentage of nitrate removal by the emergent, floating, and unplanted systems was 16.53%, 15.18%, and 13.27%, respectively. The maximum percentage of nitrate removal was 17.68% by the HRT of 5 d. By considering the duration in which constructed wetland systems were established and used, as the entire period was in the growing stage of the plants, it was observed that the constructed wetland systems which included *C. alternifolius* plants in floating canals have a lower efficiency of nitrate removal compared to emergent system.

During a total period of 180 d, assuming that the concentration of nitrate remained 20 mg/L, almost 48% of the nitrate was removed by the *C. alternifolius* in the emergent system, almost 42% of it was removed by the same plants in the floating system, while 38% was removed by the unplanted system.

The highest amount of EC and TDS, along with the lowest value of DO, in the constructed wetlands was observed in the month of May. In November, the quality of outlet flow from the systems became better and the value of DO increased. Based on Tables 1 and 4, the decrease in the EC of water resulted from the accumulation of salts in the underground organs of the plants.

Plants in the emergent system grew more aerial and underground organs because the roots could adsorb the necessary nutrients from the soil. It shows the important role of other nutritional elements in the growth of the *C. alternifolius*, as the limitations of nutrients can reduce the growth of these plants. This is comparable to the weaker growth of roots and shoots in the floating system. In each system, it was observed that the efficiency of nitrate removal correlates directly with the weight of roots.

Table 4
Average of EC, DO values in the systems

Month	EC	DO
May	763.24^a	7.5659^d
June	319.70^e	7.7815^d
July	655.78^d	8.2352^c
August	690.18^c	9.113^b
September	733.93^b	9.1948^b
October	726.00^b	10.7226^a

Heavier roots and rhizomes can adsorb nitrate more efficiently. Due to the diversity of plant species in Iran (which comprises about 7,500 plant species) and the prevalence of an appropriate climate for vegetative growth, the use of *C. alternifolius* plants for constructed wetlands can be a cost-effective initiative for purposes such as nitrate removal from urban wastewaters. By carrying out future studies on this subject, more steps can be taken towards the provision of pragmatic solutions to the common problem of wastewater contamination and its treatment.

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