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Removal efficiency of nitrate, phosphate, fecal and total coliforms by horizontal subsurface flow-constructed wetland from domestic wastewater

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Abstract
Background: Constructed wetlands are systems designed based on the utilization of natural processes, including vegetation, soil, and their associated microbial assemblage to assist in treating different types of wastewater.
Methods: Two local Appalachian plants (*Louis latifolia* and *Phragmites australis*) were planted into small-scale constructed wetlands to treat domestic wastewater in the North of Iran. The influent wastewater and the effluent from each wetland were sampled daily for 120 days. Experiments were conducted based on the mean ± standard deviation (SD) by analysis of variance (ANOVA).
Results: It was found that nitrate, phosphate, fecal and total coliforms were reduced by 84.4%, 94.4%, 96.3%, 93.9% for *P. australis* and 73.3%, 64.0%, 94.4%, 92.1% for *L. latifolia*, respectively.
Conclusion: According to the results, by using the HF-CW technology with *L. latifolia* and *P.* australis plants, the treated wastewater fully meets the wastewater discharge parameters of WHO standards.
Keywords: Nitrate, Phosphate, Wetland, Wastewater treatment, Iran
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Introduction

In the last decade, one of the most important challenges in the world has been the sustainable use and reuse of water and wastewater. Water scarcity is a serious problem, which is accentuated in developing countries around the world. Tourist population growth and agricultural or industrial development have exacerbated the water scarcity issue. Changes in climate and ecosystem will affect water supply, quality, and demand. Therefore, finding sustainable solutions is necessary (1). One of the possible solutions for the sustainable management and conservation of water resources is the use of eco-friendly methods such as wetland method to treat wastewater containing high concentrations of phosphate, nitrate, and coliforms (2,3). Currently, different conventional wastewater treatments (screening, grit removal, primary sedimentation, and biological treatment) have been applied to treat wastewater to meet the regulatory discharge limits (4). However, they are not useful to treat wastewater full of organic and inorganic products, because of power demand, component cost, technology intensity, etc (5). As a part of outgoing research, constructed wetlands are promising green technologies to treat domestic wastewater due to the following advantages: energy and cost-effective, easily operated and maintained, and environmentally friendly (6). Constructed wetlands are systems in which the plants grown in the system naturally, contribute to wastewater treatment, directly or indirectly, by physicochemical mechanisms (7-11). Recent studies have shown that vegetation provide significant wastewater treatment efficiency for decreasing chemical oxygen demand (COD), total suspended solids (TSS), nitrogen, phosphorus, and coliforms (12). Some studies reported that removal of pollutants including biochemical oxygen demand (BOD), TSS, nitrogen, phosphate, and coliforms in wetlands is due to physical and biological processes (sedimentation and microbial degradation) principally by aerobic bacteria attached to plant roots (13-15). Moreover, Hammer and Knight reported that the poor removal of nitrogen is attributed to nitrification being limited by low oxygen and high carbon concentrations derived from the influent sewage (16).

Most marsh plants have been successfully used in wastewater treatment. The effect of some factors such as the climate condition and the temperature on wetland plant growth was investigated. *Louis latifolia* and *Phragmites australis* are local plants from the North of Iran. Currently, few studies have explored the use of *L. latifolia* and *P. australis* as wetland plants for wastewater treatment in Iran. The aim of this study was to evaluate the efficiency of a horizontal subsurface flow constructed wetland (HF-CW) for the removal of nitrate, phosphate, total and fecal coliforms from the municipal wastewater of Babol city.

Materials and Methods

Study area

Babol city is located in the North of Iran close to the Caspian Sea (36°40N 52°50E), where the wastewater after conventional treatment is discharged into the Caspian Sea via Babol river. Babol city experienced maximum and minimum temperatures of 21.13°C and 20°C, respectively, as well as the average annual rainfall of 889 mm (17). The raw wastewater in the treatment plant comes from various sources such as hostels, laundry service, and the bathroom washing wastewater coming from the city. It is estimated that a total domestic wastewater of 24.5 million liters per day (MLD) is generated in Babol city urban with a population of 495 472 people (18). Thus, there is a big gap in the treatment of domestic wastewater in Babol city, and it is necessary to decrease nitrate, phosphate, and fecal and total coliforms in the effluent of wastewater treatment plant.

Description of the constructed wetlands

The pilot unit was designed based on the Environmental Protection Agency (EPA) and Central Pollution Control Board (CPCB) guidelines. Three HS-CWs pilot-scale wetlands were set up and seeded with *L. latifolia* and *P. australis*, and the third one remained unplanted. The HF-CWs consisted of concrete basins (0.40 m height × 3.00 m length × 1.00 m width). The wetland media consisted of 0.20 m gravel (mean diameter of 10-20 mm) at the bottom followed by 50 mm sand (mean diameter of 8-15 mm) and finally, with 0.50 mm granulated Blast Furnace slag (1-5 mm particle size) in the upper part (Figure 1) (Table 1).

Plants

Louis latifolia and *P. australis* were collected from nearby natural wetlands located in Babol city. The collected plants were maintained in laboratory conditions, including optimum temperature (30-20°C, day-night), nutrients, carbon dioxide, water, and suitable soil moisture to grow the plants for 10 days. Subsequently, the plants were transported to the wastewater treatment plant and cultivated in the pilot-scale wetlands. Wastewater flowing in the wastewater treatment site was injected on the wetland pilot by pump. Then, nutrients, phosphate, and bacteria removal efficiency was investigated continuously (Table 2).

Wastewater sampling and analysis

This study was conducted from May 2016 to August 2016. Nitrate, phosphate, and total and fecal coliforms in the treated wastewater effluent were examined. The efficiency of the wetland system on the reduction of each parameter for 10 days was investigated continuously. Each parameter was measured three times, and the average values were recorded. All systems were operated continuously at hydraulic retention times (HRT) of 1, 3, and 5 days. Wastewater effluent samples were collected daily for 10 days, stored in polyethylene plastic bottles, transported to the laboratory on the same day, and stored in a dark

Table 1. Characteristics of influent wastewater

Parameters	Influent	Unit
TDS	593±73.57	mg/L
TSS	21.12±3.55	mg/L
BOD	21.87±7.86	mg/L
COD	36.67± 1.55	mg/L
Phosphate	6.63±1.09	mg/L
Nitrate	25.50±10.51	mg/L
Total coliform	200.00±47.67	MPN/1000 mL
Fecal coliform	91.00±19.61	MPN/1000 mL

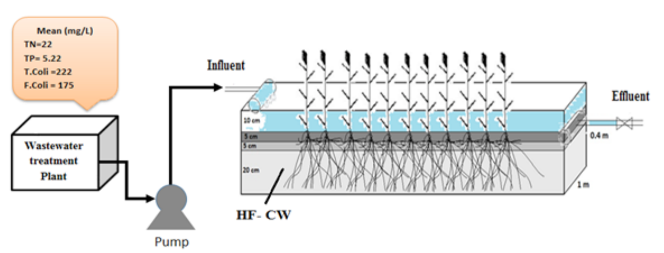

Figure 1. Structure of the constructed wetland system.

Table 2. Comparison of the wetlands output with standards

Parameters	*Phragmites australis*			*Louis latifolia*			Wastewater Discharge Limitation			Drinking Water Standard
	HRT						Agricultural and Irrigation	Discharge in Well	Discharge in Surface Water	MCL
	1	3	5	1	3	5				
Nitrate	7.77	4.65	2.85	10.15	6.08	4.20	50	10	-	50
Phosphate	2.59	1.17	1.16	3.00	1.38	1.41	6	6	-	3
Total coliform	17.80	12.43	9.49	21.80	15.93	14.56	10^3	10^3	10^3	0
Fecal coliform	12.43	15.10	14.50	17.70	16.50	14.96	400	400	400	0

Abbreviations: MCL, maximum contaminant level; HRT, hydraulic retention times.

place at 4°C until use. All the analyses including nitrates, phosphate, total and fecal coliforms were performed according to the Standard Methods for the Examination of Water and Wastewater (19).

Statistical analysis

Experiments were conducted based on the mean ± standard deviation (SD) by ANOVA. Efficiency of wetland process on the removal of nitrate, phosphate, and fecal and total coliforms was analyzed using analysis of variance (ANOVA) test by SPSS version 17.0.

Results

Characterization of the pilot-scale wetland

The results of wetland method revealed that vegetative structure of plants and ecosystem function play an important role in the dry-out period. Dry-out rarely occurs in CWs. Vegetation that endures continuous flooding, can survive. The effluent of the wastewater discharged from wastewater treatment plant was pumped to the HS-CWs by PVC pipe at an average daily influent flow rate of 0.9, 0.3, and 0.18 m^3 and a calculated HRT of 1, 3, and 5 days and a hydraulic loading rate (HLR) of 6, 10, and 30 cm/day, respectively. After providing HRT of 1, 3, and 5 days, output effluent was analyzed for nitrate, phosphate, and fecal and total coliforms (Figures 2-5). The results showed that nitrate (84%), phosphate (94.4%), fecal coliform (93.8%), and total coliform (96.3%) were reduced in vegetated concrete basins by *L. latifolia* and *P. australis* in effluent wastewater treatment plant. Also, as clearly shown in Figures 6-9, the plants show completely various results on their overall final effluent parameters. *P. australis* generally facilitated treatment and led to better results compared to *L. latifolia* species. Also, the performance of wetland system was different at different HRTs. Thus, *L. latifolia* had the highest removal efficiency for fecal and total coliforms (94%) at HRT of 3 days, while *P. australis* had a removal efficiency of 91% and 95% at HRT of 5 and 3 days, respectively. For reducing nitrate and phosphate, both plants showed similar results (97%) at HRT of 1 day. Removal of nitrate and phosphate increased with increasing the HRT.

Figures 6-9 illustrate further details of the study during 10

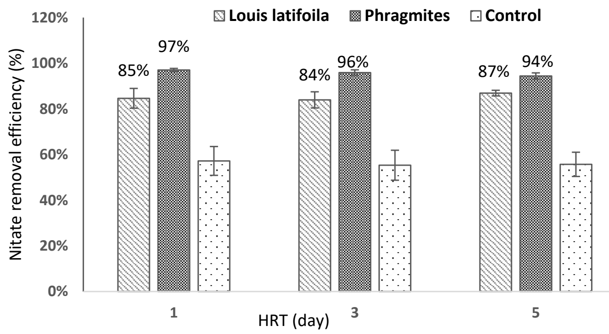

Figure 2. Removal of nitrate from influent wastewater by the studied plants at HRT of 5 days.

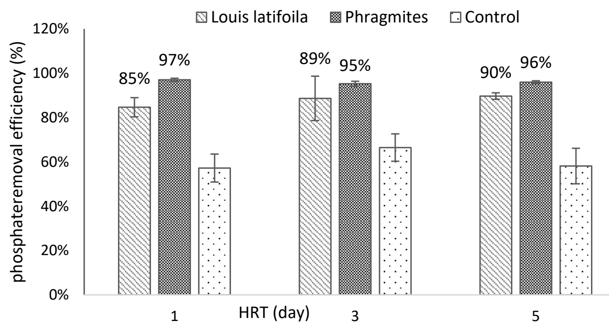

Figure 3. Removal of phosphate from influent wastewater by the studied plants at HRT of 5 days.

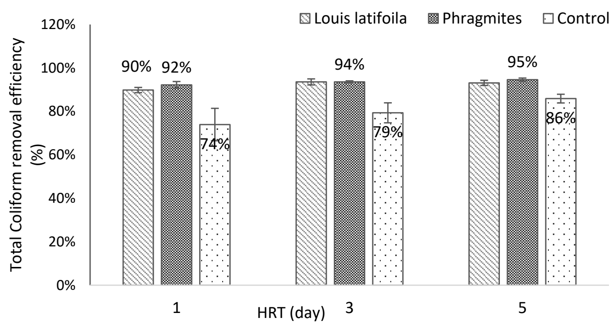

Figure 4. Removal of total coliform from influent wastewater by the studied plants at HRT of 5 days.

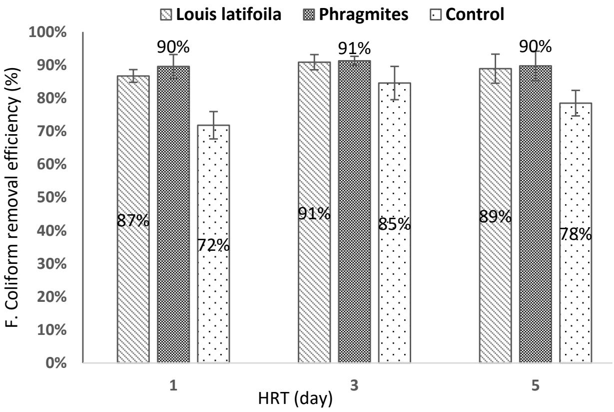

Figure 5. Removal of fecal coliform from influent wastewater by the studied plants at HRT of 5 days.

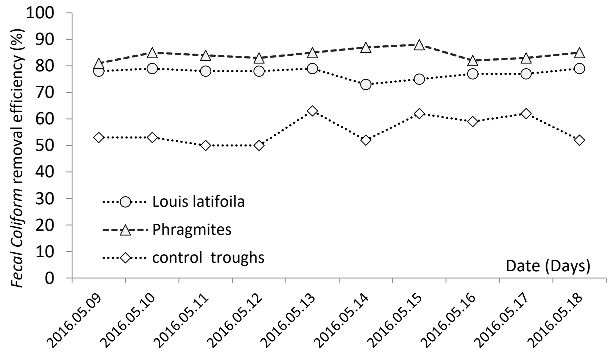

Figure 8. Trend of fecal coliform reduction during 10 days

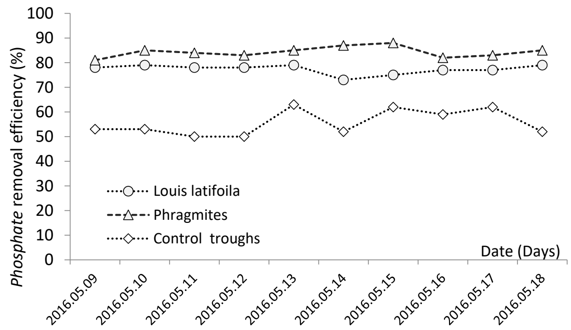

Figure 6. Trend of phosphate reduction during 10 days.

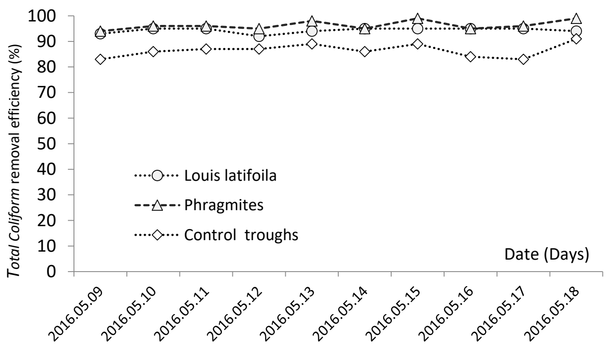

Figure 9. Trend of total coliform reduction during 10 days

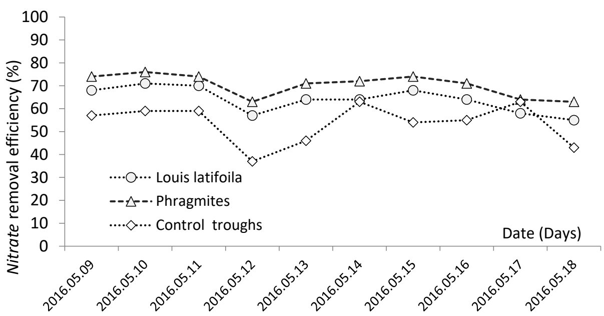

Figure 7. Trend of nitrate reduction during 10 days.

days. Nitrate in wastewaters is a critical issue because of its role in eutrophication and toxicity in aquatic ecosystems.

Discussion

It is demonstrated that the phenomenon of treatment is related to sedimentation, adsorption, biotic processing, and nutrient retention (20). However, an increase in HRT implies that the increase of the surface area of the gravel and, as a result, a larger total root surface area is available to support aerobic bacteria. Some researchers reported that the use of wetland system followed by wastewater treatment plant, directly or indirectly, leads to the absorbance of phosphate and nitrate by plants root and reduces this contaminant in the wastewater effluent (21). As shown in Figures 2-5, in most cases, the effluent quality was further improved by the presence of vegetation. Some studies reported that the role of aerobic bacteria such as Rhizosphere in the uptake of nutrients (phosphate and nitrate) and oxygen transport is effective (22-25). Several biological and physicochemical reactions are involved in the transformation of nitrogen into different biologically useful forms. Since plants require nitrogen to grow, so, they can remove it from the wastewater (26). However, other researchers have proven that nitrification and denitrification are the major mechanisms for nitrogen removal in the wetland system (27,28). To remove nitrogen, wetland ecosystems need a proper functioning of the system and suitable conditions such as temperature and, etc. Though, it should be taken into account that temperature monitoring is necessary for nitrogen removal in an HF-CW system. Phosphate is another important parameter investigated in this study. It has various characteristics at different retention times. Therefore, a group of processes (physical, chemical, and biological) is involved in the removal of phosphate in wetland systems (29). In a wastewater treatment process, phosphate is decreasing by chemical reaction including absorption and desorption during the process time. On the other hand, microalgae have a major role in reducing phosphate microorganisms such as phosphate solubilizing bacteria. In spite of rapid uptake of phosphate by these microorganisms, they do not storage completely. Also, the uptake of phosphate from free-floating aquatic plants is questionable. So, using a wetland system for removal of phosphate by the harvested plants can be a useful and suitable alternative to treat wastewater containing high levels of phosphate (27). In removing pathogenic microorganisms in constructed wetlands, various chemical (oxidation, UV radiation by sunlight, exposure to biocides, adsorption of organic matter and biofilm), physical (filtration and sedimentation), and biological (predation, biolytic processes, antibiosis, and natural die-off) mechanisms play an important role (30,31). Sedimentation is one of the most important physical wastewater treatment processes (32). The removal of total and fecal coliforms from wastewater was remarkable (>90%), although they had not been removed by previous treatments. Several studies reported that bacteria can bind to sand particles and roots during sedimentation in wastewater treatment processes and form settable solid compounds. In addition, larger particles have a higher sedimentation rate (33). It means that the bottom layer of CW, settles coliforms particularly total and fecal coliforms such as E. coli, Streptococci, and Enterococcus, by precipitation mechanism (34). Reduction of the total and fecal coliforms during 10 days is shown in Figures 9 and 10. According to these figures, it seems that redox is one of the most important reactions in coliform removal. The wetland soil shifts from an aerobic to anaerobic or reduced condition due to soil saturation with water and in this condition, oxygen available for consuming microbial respiration and biological and chemical reactions. Wetland plants transfer oxygen from the atmosphere to their roots and exude it to the rhizosphere, so they create aerobic microsites. Since coliforms are generally obligate anaerobes, oxygen availability plays an important role in the survival and growth of these bacteria. The use of dissolved oxygen by plant roots has a positive effect on the coliform removal (35). Another important mechanism in coliform removal is solar radiation which inactivates the coliforms, especially at low temperatures in the wetland ecosystem (36). Finally, according to Table 1, by the use of wetland technology in the wastewater treatment process of Babol city, the concentration of nitrate, phosphate, and total and fecal coliforms in the final effluent was kept less than the maximum contaminant level according WHO standards (30 mg/L, 30 mg/L, 400.0 MPN/100 ml, and <2.2) (37). According to Table 2, HF-CW produced a high quality effluent amenable not only for wastewater reuse in agriculture, but also for discharge to well and surface water (WHO suggested standards) (32,38-42). The HF-CW used for the treatment of Babol city wastewater, shows a good capacity when compared to the other studies (Table 3).

Conclusion

The implementation of an HF-CW at the wastewater treatment plant in Babol city, can help clean up the Caspian Sea from pollutants which are continually discharged from the wastewater treatment plant. According to the results, by using a wetland system in wastewater treatment process, nitrate, phosphate, total and fecal coliforms met the current Central Pollution Control Board (CPCB) regulations for domestic wastewater discharge. However, both local plants (Louis latifolia and Phragmites australis) used in the HF-CW system showed high pollutants removal efficiency. So, implementation of the HF-CW technology using *L. latifolia* and *P. australis*, local plants of the north of Iran, seems to be a viable alternative for reducing the complex pollution in domestic wastewater treatment. The advantages of this technology over other conventional treatment processes include low energy consumption, biologically self-design strategies, social and economic adherence, and production of high-quality treated wastewater suitable for any type of reuse. Therefore, it is concluded that HF-CW system not only is suitable for municipal wastewater treatment and reclamation, but also saves a large amount of water that could be used for other purposes such as irrigation. However, it should be taken into account that wetlands are only one part of a multi-part system, as a unit of wastewater treatment process. Finally, a series of replicated long-term screening experiments is suggested to provide a stable and effective

Table 3. Comparative evaluation of the use of wetland in the various studies

Type of Wastewater	Removal Performance (%)				Wetland Design and Operation				Ref
	Total Nitrate	Total Phosphate	Fecal Coliform	Total Coliform	Dimension (m × m × m) (L × W × D)	HLR (m^3/day)	HRT (day)	State	
Municipal wastewater	5–28	31–76	-	-	-	52–58 mm/day		Tartu, Estonia	39
Municipal wastewater	7.7	26.8		-	-	6.3 cm/day	3.5	Korea	40
Domestic wastewater	-	76-89	-	-	2.4 × 0.4 × 0.2	520	4	Waterloo, Canada	41
Dairy wastewater	78.5	77.8	-		336 m^2	7.3–7.9 mm/day		Hokkaido, Japan	42
Dairy water after anaerobic lagoons and aerobic ponds	-	-	-	96	5000 m^2	-	5	USA	43
Dairy + domestic	-	-	42.38	-	75	-	10	Italy	44

wastewater treatment.

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Ethical issues
The author hereby certify that all data collected during the research are as expressed in the manuscript, and no data from the study has been or will be published elsewhere separately.

Competing interests
The authors have declared that they have no conflict of interests.

Authors' contributions
All authors contributed in data collection, analysis, and interpretation. All authors reviewed, refined, and approved the manuscript.

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