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Determination of Vetiver Plant's Efficiency in The Artificial Wetland for Removal Linear Alkyl Benzene Sulfonate (LAS) From Hospital Laundry Wastewater

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Abstract: *Background and objectives: Natural plant-assisted filtration is a convenient method in addition to having a low cost, easy manipulation, low technology and less energy consumption compared to other purification methods. One of specific plants for wastewater treatment is the vetiver plant. Nowadays, subsurface wetland is not only used for major wastewater and sewage pollutants but also for such special compounds as linear alkyl benzene sulfonates (LAS). This study aimed to determination of vetiver plants efficiency in the artificial wetland for removal leanr alkylbenzene sulfonate(LAS) from hospital laundry wastewater. Materials and methods: In this qualitative-analytical study, various parameters such as LAS, COD, BOD, phosphate and nitrate in the hospital wastewater and LAS content of laundry unit sewage (LAS) were measured before and after treatment with vetiver plant. All conditions for sampling and performing other tests were based on the Standard Method handbook guidelines. After different tests, the results were analyzed by SPSS and Excel software. Results: Results of BOD, COD, LAS, phosphate and nitrate concentrations in the clarifier effluent of hospital WWTP, effluent of vetiver pilot outflow, and control pilot showed that the highest post-treatment efficiencies of vetiver plant for the removal of LAS BOD, COD, phosphate and nitrate of the WWTP were 70, 73, 84, 33, and 44%, respectively. However, removal efficiencies of 40, 30, 22, 23 and 16%, respectively, were obtained for the same parameters in the control pilot without the plant. Discussion and conclusion: Overall, our study showed that vetiver plant's root in reducing the LAS and pollutants in effluent of the hospital WWTP was much more efficient than filtration and overland treatment. Also, the effluent of vetiver plant pilot had an appropriate filtration and certified for environmental wastewater discharge.*

Keywords: *Vetiver, Wetland, Surfactant, Laundry unit, Wastewater, Hospital*

INTRODUCTION

Plant-filtration is a purification method that involves absorption, transformation, accumulation and/or sublimation of pollutants using plants to remove contaminations from water, soil and air. Bacteria, fungi and plants are used in bio filtration to detoxify the environment for the health of both humans and the environment. In other words, bio filtration is a method for the elimination or safeguarding of various contaminants through the use of biological activities. Application of this method requires a low level of pollution and its instability. This method also involves a long time (Mulligan et al., 2001).

Vetiver plant is capable of floating and emergent growth forms in the wetland. The plant is a fast-growing, deep-rooted permanent grass with a high potential in dry matter production and a high photosynthesis efficiency. Vetiver is used in many cases, such as stabilization of wall slops and environmental protection owing to its unique morphological and ecological characteristics to withstand high levels of heavy metals and various environmental conditions. In addition, if the plant is harvested, it can be used in handicrafts, gable roofs, livestock feed, fertilizer production, and an organic source with appropriate degradability (Smeal et al., 2003). Vetiver is not an aquatic plant, though, it prefers saturated soil conditions and can naturally grow even when most of its stalk is submerged for a long time. Many researchers have confirmed the high ability of this plant in the removal of water nitrogen and phosphorus considering it as an appropriate plant for the purification of nutrient-rich waters (Zheng et al., 1997). Recent studies have further shown that vetiver plant is able to absorb and tolerate high levels of water nutrients (Wagner et al., 2003). In general, this environmental friendly approach is less costly compared to other conventional technologies. The plant filtration technology costs in the US is estimated to be 60 to 80 percent lower than those of other physical and chemical methods for controlling hazardous point and nonpoint pollution sources in the soil, surface and groundwater, and sediments (Morikawa and Ozgur, 2003).

As occurs in conventional trickling filters, wastewater in aquatic systems is primarily treated with microbial metabolism and physical sedimentation. In fact, aquatic plants *per se* play a trifling role in wastewater treatment; they function in these systems to provide proper conditions for improving wastewater treatment capacity and/or rendering the system more reliable for wastewater treatment (Siber and Eckenfelder, 2000).

Advantages of the wetland methodology in relation to other waste treatment approaches is simple operation, low cost of construction, no attraction of insects, and prevention of unpleasant odors. Although natural filtration systems (e.g. artificial wetlands) come with a low technology, they have a high efficiency (Kadlec and Wallace, 2009; Hammer, 1989). In addition to cost-effectiveness, application of low-technology wastewater treatment systems with no and/or low energy consumption (e.g. artificial wetlands) helps improve the environment. In artificial wetlands, physical, chemical and biological processes are used to treat various wastewater pollutants including organic matter, detergents, nitrogenous and phosphorous compounds, heavy metals, and suspended solids (Donald et al., 1994; Greenberg et al., 1998). Artificial wetlands are divided based on different parameters. Two major categories of these features are the flow regime (surface and subsurface) and the type of aquatic plant growing in the wetland (floating, submerged, and emergent plants). Accordingly, surface flow wetlands (SFWs) have the ability to cultivate a variety of aquatic plants including emergent, submerged, free floating, and floating leaved species (Vymazal, 2001). SFWs are filled with sand, gravel and soil of proper grain sizes, and such a substrate provides a good surface for vegetable and microbial growth (Thurston et al., 2001; Kadlec, 2009; Saeed and Sun, 2012).

Kadlec and Knight (1996) reported that nitrogen removal from sewage flowing into wetland systems depended on microbial activity and the presence of bacteria around the root, and that phosphorus removal was dependent upon temperature and sufficient oxygen content (Kadlec and Knight, 1996). Bavor (1995) found that over 90% of artificial wastewater systems had the potential to reduce concentrations of organic matter, suspended solids and typical bacteria while having low utilization requirements. Moreover, these systems have increasingly been considered for complementary treatment of treated wastewater along with conventional wastewater treatment technologies (Bavor, 1995).

In aquatic environments, detergents remain as a superficial layer on the water surface, thereby, they form an ill-scene environment, reduce gas exchange, and jeopardize the health of the aquatic animals by reducing dissolved oxygen. These compounds further result in water taste and odor changes, produce surface foams, disturb water treatment processes, increase water treatment costs, and lead to death of aquatic organisms. These substances produce stable foam on water surface at concentrations over 1 mg/L. The growth of aquatic plants and algae increase dissolved oxygen consumption in water leading to mortality of aquatic animals. Other consequences of these substances include degradation and destruction of ecosystems, disruption of coagulation, sedimentation and water filtration, occurrence of eutrophication due to increased phosphates, lack of proper degradability, and physiological reactions in contaminated water consumers. A large amount of foam formed on water surface prevents the light passing through the water and inhibits the vital phenomenon of photosynthesis. Detergents are able to change the state and quality of proteins and, as a result, deactivate viruses, disrupt bacterial metabolism and retard their functions. Detergents rupture the membranes of microorganisms giving rise to the destruction of enzymes, and also retard and disturb the activities of enzymes affecting bacterial respiratory functions (Hosseini et al., 2007; Chapman's, 1996; Nori and Shahriari, 2001; Roshany et al., 2003).

EPA (1989) recommended a maximum secondary concentration of of 0.5 mg/L for foaming agents. WHO (1984) announced that no foaming agent should exist in raw water. A maximum surfactant amount of 0.2 mg/L has been noted for drinking water. There are higher values for the standard cationic surfactants (Roshany et al., 2003). The Institute for Standardization and Industrial Research in Iran (1996) determined a maximum permissible level of 200 µg/L for detergents in drinking water (Nordin, 2006).

Laundry units at hospitals are one of wastewater production paths, where detergents, disinfectants, and bleaches are used to wash and disinfect the clothes of patients, bed sheets, blankets, and other items. Typically, 250 g of detergent powder, 200 g of bleach powder and half a liter of Javel water (for bed sheets, hood and white cloth) are used per 40 L of water (10 kg of washing machine capacity) (Jaqueline, 2017). The largest group is anionic surfactant decomposed by bacteria up to 90-97% amounting 3-21 mg/L in domestic wastewater, which decomposes slightly in anaerobic conditions (Sharvelle et al., 2007; Ch Fan et al., 2009). LAS is used in household detergents such as washing powder, dishwashing liquid, and other domestic detergents. The toxicity of detergents is mostly related to their alcoholic and aryl groups. By skin defatting and its stimulation, anionic surfactants cause redness, pain and general dermatitis, and even thickening, cracking, and blistering of the skin. Oral use also causes diarrhea, bowel swelling, and occasionally nausea. Upon oral intake, detergents are absorbed in the stomach and intestines, but there is no or very low cutaneous absorption. LAS brings about ocular, dermal, and mucosal sensitizations (Greenberg et al., 1998). Another study demonstrated that LAS detergents could decompose up to 98% in sewage treatment systems. Adverse effects of detergents on wastewater treatment include reduction of sewage treatment efficiency and increase of bacteria in the effluent (Nour et al., 2006). LaTaike and Charles et al. examined the removal of detergents along with the elimination of COD in wetlands and noticed that only 40-70% of detergents underwent normal decomposition. The low removal efficiency was attributed to the measurement of non-degradable detergents (Sharvelle et al., 2007).

This study aimed to determine vetiver plant's root with sub-surface cultivated wetland for the removal of LSA anionic surfactant and effluent treatment of clarifier in the WWTP at Sari Imam Khomeini Hospital.

Materials and Methods

This descriptive-analytical research studied the laundry unit sewage (LUS) and WWTP at Imam Khomeini Hospital in Sari. A part of the LUS outflow was collected and stored after washing the clothes. Also, wastewater (120 L) from the secondary clarifier of WWTP at Sari Imam Khomeini Hospital was stored in a polyethylene container and transferred to vetiver planting site. Then, a faucet was installed on the bottom of grit chambers to continuously flow the laundry unit sewage and WWTP wastewater through drip-gravity manner into the artificial wetland system containing vetiver plant.

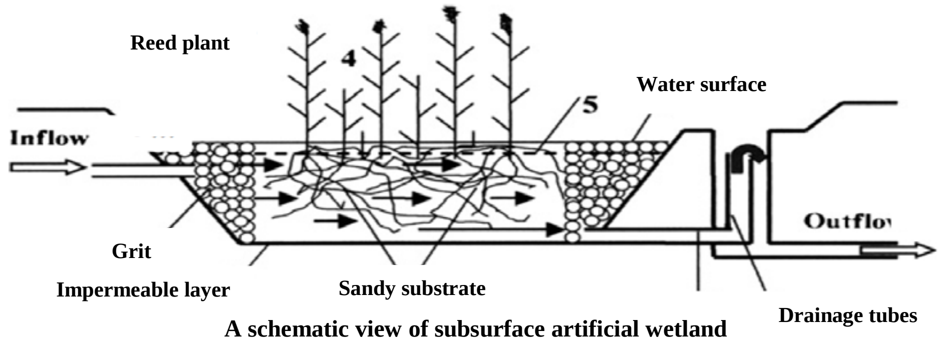

Figure 1: vetiver grass pilot for LAS removal

This experimental pilot study was carried out at Sari Faculty of Public Health during summer and autumn 2017. Four polyethylene pilot tanks (40 × 60 × 60 cm) were prepared and filled with soil mixed with sand, gravel and clay. Irrigation was performed with subsurface and continuous system methods in this research. The WWTP effluent and LUS at the hospital were first completely homogenated with a mixer. The mixture was stored in two 120 L tanks for initial settling and then introduced into the four pilot tanks through the faucet with a discharge rate of 0.7 L/h. In order to evaluate the efficiency of this plant in the treatment of wastewater and LUS of the hospital, concentrations of TP, TN, BOD, COD and LAS parameters in the inflow and outflow were measured according to the standard method handbook instructions. Finally, the efficiency of vetiver plant in pollutant removal was determined on the graph. Wastewater samples were analyzed for total nitrogen, phosphorus and LAS by spectrophotometry, COD using recycle distillation method, and BOD5 by titration and sodium azide method. No plant was used in the first pilot as a control, but 10 vetiver plants per pilot were used in the second pilot. Wastewater was sampled (120 L) from the WWTP effluent and LUS at Sari Imam Khomeini Hospital. Pilots were located in the WWTP of Sari Faculty of Public Health. Vetiver was planted in the pilots under sunlight from April to September 2017. The plant showed a very good growth performance. The inflow and outflow concentrations of pollutants were tested in these pilots, which were sampled once every two days. All methods for the preparation of reagents, solutions, and concentration measurements of samples followed the handbook of standard methods for water and wastewater tests (Apha, 1998).

Preparation of half-strength Hoagland solution

The experimental plantlets were transferred to the Faculty of Public Health, Mazandaran University of Medical Sciences. In order to adapt to the new milieu and prepare for planting, the plantlets were maintained aquiculturally in the half-strength Hoagland solution (the following formula) under artificial light for a period of 2-5 weeks. The half-strength Hoagland solution (1 L) was formulated as below:

- KH_2PO_4 0.067^g
- KNO_3 0.253^g
- $\mathrm{Ca}(No_3)_2 .4H_2O$ 0.590^g
- $MgCl_2 .6H_2O$ 0.200^g

After incubation in the half-strength Hoagland solution for 2-5 weeks, the plants were weighed and transferred to the conditions provided in both artificial and natural light settings.

Preparation of methylene blue solution

To test the amount of LAS (mg/L), methylene blue (100 mg or 0.1 g) was dissolved in 100 ml of water, 30 ml of which was transferred to a 1000 ml flask, followed by addition of water (500 ml). Afterwards, 6 N sulfuric acid (41 ml) and monohydrate sodium hydrogen phosphate (50 g) were added, dissolved well by stirring, and the solution was diluted to 1000 ml with distilled water.

Preparation of washing solution

First, 41 ml of 6 N sulfuric acid was poured into 500 ml of distilled water in a 1000 cc flask. Then, 50 g of sodium monosodium phosphate (NaH_2PO4H_2O) was added, dissolved well by stirring, and diluted to 1000 ml with distilled water.

Spectrophotometric determination of LAS concentration using methylene blue active substance (MBAS)

To carry out the test, 1 N sulfuric acid, phenolphthalein, and methyl orange were first prepared followed by formulation of methylene blue, chloroform, and propanol solutions. The surfactants were extracted and measured by methylene blue and spectrophotometric methods according to the APHA (1989). The extraction was carried out with a decanter funnel with a mixture of methylene blue solution (12.5 cc) and chloroform (5 cc), which was stirred vigorously for 30 seconds to separate liquid and organic phases. The extraction process was repeated 2 times each with 5 ml of chloroform addition. The chloroform layer was poured into a second funnel containing the wash solution (25 ml) and stirred vigorously for 30 seconds. Afterwards, two more separation process were performed with 5 ml of chloroform per separation. The organic phase was transferred to a 50 ml volumetric flask by means of a Pyrex funnel and silk filter, and the final solution volume was adjusted to 50 ml with chloroform. The sample absorption was then read by spectrophotometry at a wavelength of 652 nm. In this study, detergents were measured by MBAS method according to the instructions given in the standard method handbook (Apha, 1998).

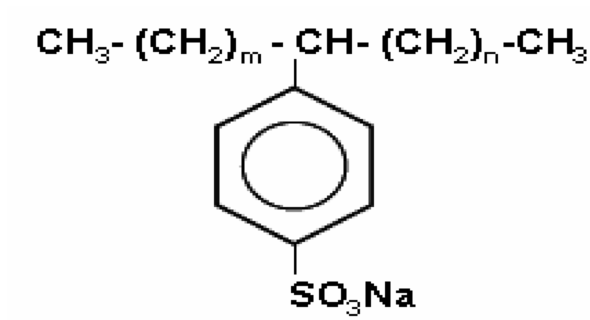

Figure 2: leaner alkyl benzene sulfonate(LAS) structure formula

Results

Table 1. Concentrations of inflow and outflow effluents of the hospital before and after plant-filtration with vetiver plant

Sampling site / Parameter	WWTP inflow	WWTP outflow	WWTP effluent before vetiver filtration	WWTP effluent after vetiver filtration
BOD	362	42	42	7
COD	700	75	75	20
LAS	0.25	0.1	0.1	0.03
Phosphate	15	9	9	6
Nitrate	140	52	52	29

According to the results, LAS concentrations in the hospital WWTP averaged 0.1 and 0.03 mg/L in vetiver pilot inflow and outflow, respectively. Vetiver plant showed a LAS removal efficiency of 70%. Also, clean LUS was continuously irrigated into vetiver pilots through gravity-drip method after dilution and determination of its concentration using a calibration curve, followed by measuring and recording the results of inflow and outflow concentrations as well as the efficiency of each concentration.

1. LAS outflow concentration was 0.13 mg/L with an initial concentration of 1.5 mg/L, and a vetiver efficiency of 87.9% for LAS removal.
2. LAS outflow concentration was 0.2 mg/L with an initial concentration of 2 mg/L, and a vetiver efficiency of 90% for LAS removal.
3. LAS outflow concentration was 0.43 mg/L with an initial concentration of 5 mg/L, and a vetiver efficiency of 91.4% for LAS removal.

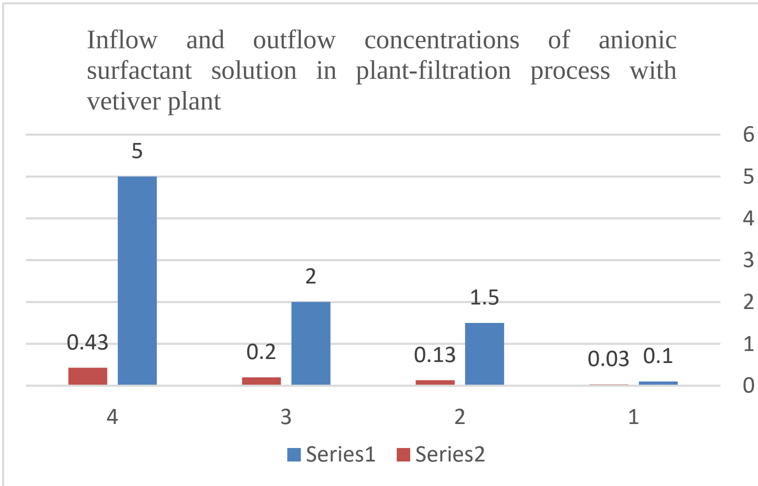

Figure 3: Inflow and outflow concentrations of LAS in the vetiver grass pilot

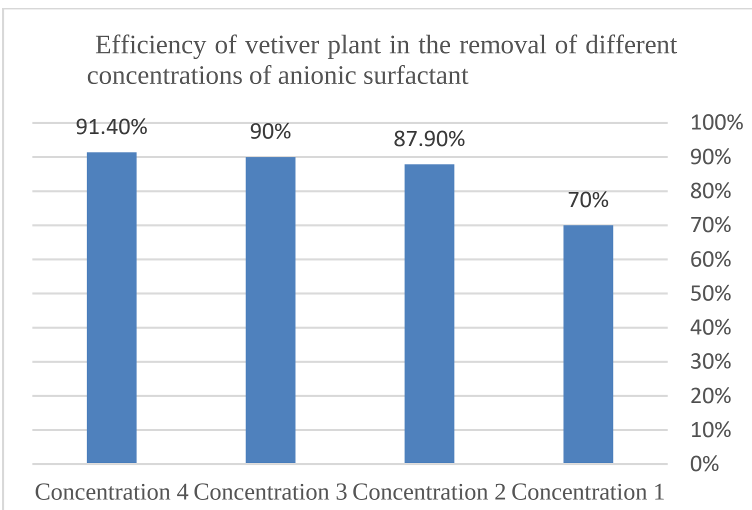

Figure 4: Efficiency of vetiver plant in the removal of different concentrations of anionic surfactant(LAS)

According to the experiments, LAS concentration was 10.7 mg/L in the LUS, which was diluted with wastewater from other hospital units and reached a concentration of 0.3 mg/L in the WWTP inflow with a dilution efficiency of 97.2%. After aeration, this amount reduced to 0.1 mg/L in the WWTP clarifier. After irrigation of the control and vetiver pilots, LAS concentrations in the clarifier effluent with retention times of 2 and 7 days reduced from 0.1 mg/L to 0.06 and 0.03 mg/L, respectively. LAS concentration in the LUS with a retention time of 7 days decreased from 10 mg/L to less than 1.0 mg/L by the root of vetiver plant. As a result of our analysis, the aeration pool unit, control pilot, and vetiver-containing pilot yielded efficiencies of 66%, 40%, and 70% with retention times of 2 h, 7 days, and 7 days, respectively, in LAS removal from WWTP at

Sari Imam Khomeini Hospital. For 10 mg/L of the hospital LUS, vetiver plant also yielded LAS removal efficiencies of 50, 70, 84 and 92% with retention times of 1, 2, 4, and 7 days, respectively.
Mahvi et al. investigated the sewage of Ghods Town by lagoon method through six-step sampling and measured detergent levels in the inflow and outflow of activated sludge treatment plant. The results revealed a removal efficiency of 95-90%, outflow values less than a standard limit of 1.5 mg/L, and a BOD removal rate of 91.9%. They further reported an appropriate efficiency of the treatment plant (Mahvi et al., 2004).

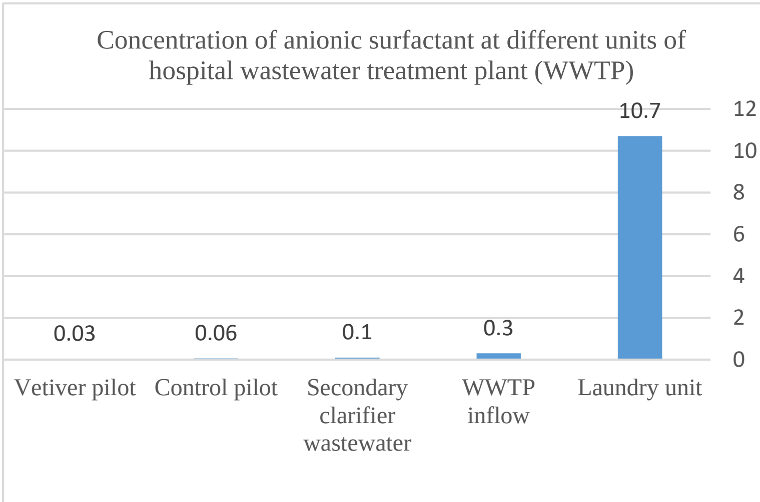

Figure 5: concentration of anionic surfactant at different units

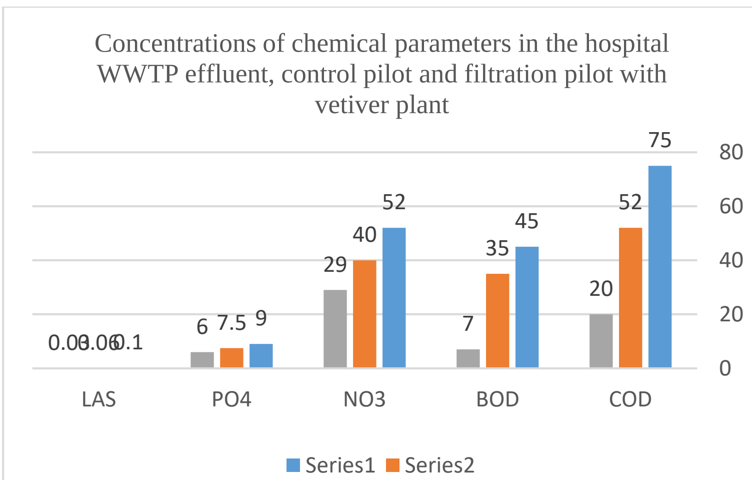

Figure 6: concentration of pollutant parameters in the hospital effluent, control pilot and vetiver pilot

COD discharge rates (a standard level of 60 mg/L for discharge into Iran's surface waters and of 200 mg/L for agricultural uses) from the WWTP in all months of the study were lower than environmental standards for agricultural uses.
This study recorded an average effluent COD of 75 mg/L in the activated sludge system of the hospital WWTP, while the outflow values for the control and vetiver pilots averaged 52 and 20 mg/L, respectively. Mean COD removal rates in the control and vetiver pilots were 30% and 73%, respectively.
Herrera Melián et al. investigated pilot-scale municipal wastewater treatment and recovery through wetland and revealed removal efficiencies of 80%, 86% and 96% for COD, BOD, and suspended solids, respectively (Herrera et al., 2010), which are consistent with our results in terms of COD levels.

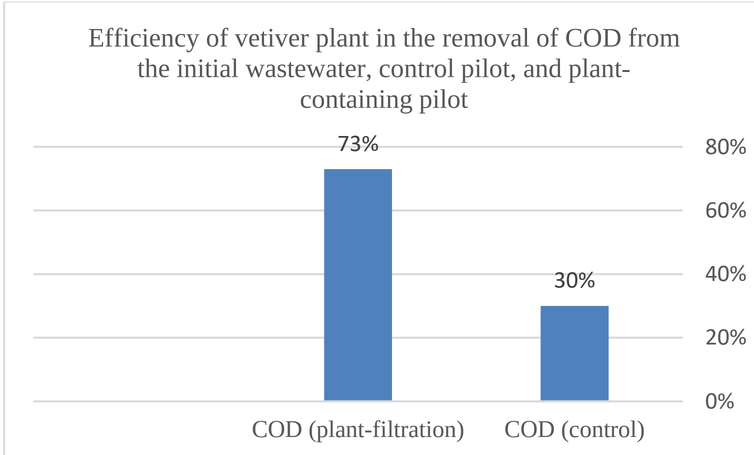

Figure 7: Efficiency of vetiver plant in the removal of COD from control pilot and vetiver pilot

In a study by Chihhao Fan on the effects of various types of reed plant in the removal of pollutants, a mean value of 89.9 ± 6.7% was obtained for BOD removal. A total phosphorus of 76.9 ± 12.2% was also eliminated by different reed species. It was further found that artificial wetlands had a great ability to remove suspended solids, BOD, nitrogen, and phosphorus, with a high removal efficiency (Ch Fan et al., 2009).

The amount of organic matter in the WWTP outflow was below the standard level during the whole study period. According to the Iranian environmental standards, the standard levels of BOD5 outflow to discharge into surface water and for agricultural uses are 30 and 100 mg/L, respectively (Department of Environmental standard and critic a Tehran, 2001). However, the findings of this study imply that the year-round amount of outflow effluent is below the standard level for agricultural uses. BOD5 concentration in the outflow of activated sludge system averaged 45 mg/L. Mean outflow BOD5 in the control and vetiver plants were 35 and 7 mg/L, respectively. The control and vetiver plant pilots presented average BOD5 removal rates of 22% and 84%, respectively.

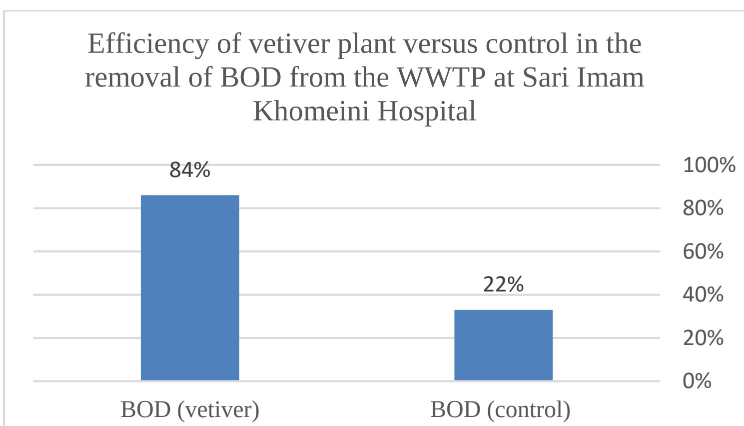

Figure 8: Efficiency of vetiver plant in the removal of BOD from control pilot and vetiver pilot

The US Environmental Protection Agency (EPA) reported that percentages of removal efficiency for BOD5, nitrogen, and phosphorus ranged 50-90%, 30-98%, and 20-90%, respectively (Rows and Isam, 1995). Sarano et al. (2011) and Cato et al. (2010) could eliminate 52 and 39% of TN using vertical and horizontal wetlands, respectively. Shutz et al. (2005) reported a 65.5% removal rate of nitrate. Using a horizontal artificial wetland, Saeed et al. (2012) could remove 25% of nitrate.

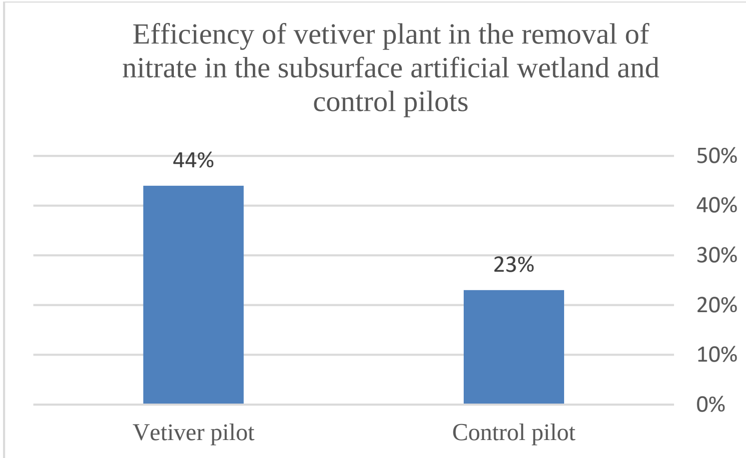

Figure 9: Efficiency of vetiver plant in the removal of nitrate from control pilot and vetiver pilot

The permissible amounts of nitrate (NO_3) discharge for WWTPs are reported in the standards to be 50 mg/L for discharge into surface water and 10 mg/L for release into absorbing wells, but no agricultural standard is available (Department of Environmental standard and critic a Tehran, 2001).

The amount of NO3 in the outflow of activated sludge system of the hospital WWTP averaged 52 mg/L, which reduced to 40 mg/L and 29 mg/L in the control and vetiver plant pilots, respectively. This means that nearly a 23% elimination by the control pilot and a 44% removal by vetiver plant pilot occurred within a retention time of over 7 days. Therefore, the effluent of the hospital WWTP is not allowable and has limitations in comparison with the standard levels for discharge into surface water, as well as for discharge into absorbing wells and agricultural uses. On the other hand, the results and analysis of this study demonstrated that the effluent of the hospital WWTP after vetiver filtration in the artificial wetland is suitable for discharge into surface water; however, it has limitations for discharge into absorbing wells, which is in line with the US EPA's survey results.

The standard level of phosphate (mg/L phosphorus) is 6 mg/L in the effluent of WWTPs for discharge into surface waters and absorbing wells, but no limits are available for agricultural and irrigation purposes.

According to the results of this study, mean phosphorus concentration in the effluent of the hospital WWTP was 9 mg/L in the inflow of vetiver plant pilot. Therefore, the amount of phosphate in the effluent of active sludge system in the hospital WWTP has no restrictions for agricultural consumption, but it has limitations for discharge into absorbing wells and surface waters. However, phosphate concentration in the outflow of vetiver plant wetland was 6 mg/L with a 7-day retention time. This suggests that the concentration of phosphate reached a reliable limit of 6 mg/L through wastewater treatment by vetiver plant system with a very good efficiency making it allowable and without limitation for discharge into surface water and absorbing wells.

Concentrations of vetiver plant pilot amounted 8, 7, and 6 mg/L in three replicates with retention times of 2, 5 and 7 days, respectively. Phosphorus removal efficiency rates averaged 33% and 17% by the plant and the control wetland, respectively, which is consistent with the US EPA's report.

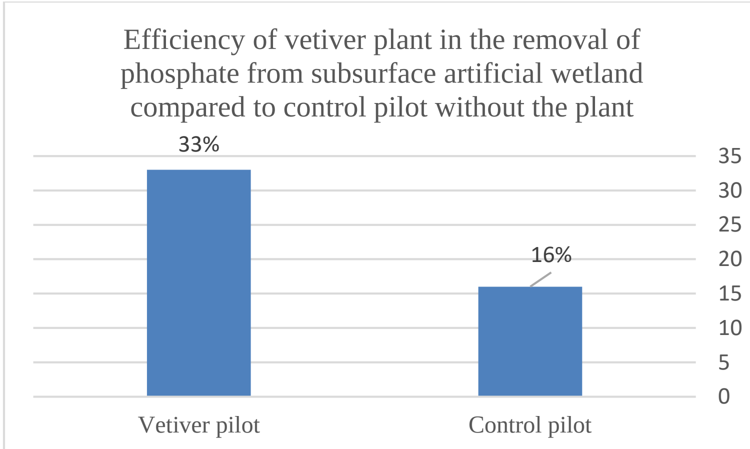

Figure 10: Efficiency of vetiver plant in the removal of phosphate from control pilot and vetiver pilot

Statistical analysis

Data were analyzed using the SPSS software with a significance level of $P \geq 0.05$ in all tests. ANOVA test was used to evaluate vetiver plant in the main and control wetland systems. Inflow and outflow concentrations of individual pollutants in the wetlands were compared by paired T-test.

Discussion and Conclusion

1. In the present study, the removal percentage of detergents is 70-92%, which is a relatively good efficiency. The efficiency rates for COD and BOD are 70-75% and 85% -80%, respectively. Nitrate and phosphate removal efficiency rates are in the ranges of 30-44% and 20-33% respectively.
2. Results of ANOVA test indicate that BOD5 concentration in the outflow of vetiver plant pilot is significantly lower than its outflow concentration in the control ($P < 0.05$). It is also lesser than the inflow concentration of vetiver pilot.
3. Our overall findings indicate that the hospital WWTP has generally created a situation that significantly reduced plenty of main pollution indices due to the appropriate conditions. Nonetheless, the removal of pollutants in this system has not been acceptable compared to environmental discharge standards. Accordingly, the effluent of hospital WWTP has undergone the process of plant-filtration with vetiver plant. Eventually, this wastewater received environmental discharge standards.
4. The limited water resources and the need for optimal use of these resources signify the importance of using treated wastewater in cases of demand for a better quality water. Additionally, untreated sewage can cause numerous environmental problems. The use of low-technology wastewater treatment systems with no and/or low energy consumption reduce environmental costs and also help improve the environment.
5. Considering the unique characteristics of vetiver plant, including its high resistance to adverse environmental conditions and a proper efficiency, this plant is very suitable for final filtration of the WWTP and surfactant-containing LUS. Also, vetiver planted in the artificial wetland within 5 months (mid-spring until late summer) with a suitable irrigation reached the highest growth rate (1.5 - 1.8 m in height). It is, therefore, very useful as livestock fodder after harvesting and chopping. The plant is also widely used in the pharmaceutical and sanitary industry.

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