Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2014.968631

56 (2015) 3357–3367
December

Taylor & Francis
Taylor & Francis Group

The effects of substrate type, HRT and reed on the lead removal in horizontal subsurface-flow constructed wetland

S. Taheri Ghannad[a,1,*], S. Boroomandnasab[b], H. Moazed[b], N. Jaafarzadeh[c,d]

[a]*Faculty of Water Sciences Engineering, Shahid Chamran University, Ahvaz, Iran, Tel. +98 611 3330635; Fax: +98 611 3331066; email: staheri2007@yahoo.com (S.T. Ghannad)*
[b]*Department of Irrigation and Drainage Engineering, Shahid Chamran University, Ahvaz, Iran, Tel. +98 611 3330635; emails: boroomandsaeed@yahoo.com (S. Boroomandnasab), hmoazed955@yahoo.com (H. Moazed)*
[c]*Environmental Technology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, Tel. +98 916 3184501; email: Jaafarzadeh_n@ajums.ac.ir (N. Jaafarzadeh)*
[d]*School of Public Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

Received 16 March 2014; Accepted 17 September 2014

ABSTRACT

In this research, between June 2013 and October 2013, the effects of three substrate types, hydraulic retention time (HRT), and Phragmites (common reed) on the removal of lead in horizontal subsurface-flow constructed wetlands (CWs) were investigated in the Islamic Republic of Iran. The results showed that the more HRT increased, the more removal efficiency (RE) increased, so that there was a significant difference between RE in sand substrate and retention times of 1, 3, and 5 d ($p < 0.05$), while no significant difference was observed between 5 and 10 d retention time at 5% level. Moreover, there was a significant difference between retention times of 1 and 3 d in two fine- and medium-gravel substrates ($p < 0.05$), but no significant difference was observed between retention times of 3, 5, and 10 d at 5% level. Therefore, the best HRT for sand, gravel, and medium-gravel substrate was recommended 5, 3, and 3 d, respectively, with the maximum efficiency of 88.51, 81.53, and 80.35%. The analysis results of substrate type also showed that sand substrate had higher efficiency than the other two substrates. Moreover, the results indicated the root of reed is highly capable of assimilating and accumulating influent lead and plays an important role on the lead removal in horizontal subsurface-flow CW.

Keywords: Synthetic wastewaters; Horizontal subsurface-flow constructed wetland; Lead removal efficiency; Substrate type

1. Introduction

Lead has gained considerable attention as a persistent toxic pollutant of concern partly because it has been prominent in the debate concerning the growing anthropogenic pressure on the environment. Indeed, lead has been used by people since the dawn of civilization, industrialization, urbanization, and mining, and many other anthropogenic activities have resulted in the redistribution of lead from the Earth's crust to

*Corresponding author.

[1]Department of Water Engineering, Dezful Branch, Islamic Azad University, Dezful, Iran, Tel. +98641 6260601- 9; Fax: +98641 6270019.

the soil and to the environment [1]. Wastewaters containing lead and other heavy metals are not only poisonous for aquatic and other beings, but also will make natural waters inappropriate for drinking and agricultural consumptions. Such elements usually cause the disorder of liver, lungs, bones, blood circulation, lips, and vital organs such as brain and kidney and also have negative effects on individuals' intelligence [2]. Therefore, wastewater treatment and the removal of heavy metals from water resources are considered as a basic factor to protect the environment and human health. The use of constructed wetlands (CWs) (reed beds) is a low-cost, low-technology method, often used for the removal of heavy metals from wastewater [3,4]. CWs are engineered systems that have been designed and constructed to utilize the natural processes involving wetland vegetation, soils, and the associated microbial assemblages to assist in treating wastewaters. They are designed to take advantage of many of the same processes that occur in natural wetlands, but do so within a more controlled environment. CWs for wastewater treatment may be classified according to the life form of the dominating macrophyte, into systems with free-floating, floating leaved, rooted emergent and submerged macrophytes [5,6]. Macrophytes play important roles in CW for the removal of pollutants. They not only assimilate nutrients, but are also able to concentrate and accumulate metals [7]. In the recent years, much attention has been paid to the phytoremediation and removal of heavy metals from water and soil by macrophytes in wetland systems [8,9]. Wetlands are planted with common reed (*Phragmites australis*), a rhizomatous plant of the Graminae which produces a good yield in green biomass and whose roots can reach a considerable depth [10] and plays a significant role in the wetland self-purification [4]. Treatment efficiency in these systems depends on the type and design of wetland, hydraulic retention time, hydraulic loading rate (HRT), and microorganisms' activities and climate. In order to have optimal efficiency, low hydraulic loading rate and long retention time are required [11]. The hydraulic retention time, including the length of time the water is in contact with the plant roots, affects the extent to which the plant plays a significant role in the removal or breakdown of pollutants. Whereas plants significantly affect the removal of pollutants in horizontal subsurface systems with long HRTs used to clean municipal wastewater, their role is minor in pollutant removal in periodically loaded vertical filters, which usually have short HRTs [12,13]. There are a number of physical, chemical, and (micro) biological processes in purification, such as sedimentation, filtration, adsorption, microbial decomposition, and chemical transformation [14]. Adsorption may play an important role in the removal process. Consequently, it is important to select those substrates of high ecological activity and adsorption capacity. There has been some recent work that has attempted to investigate the influence of different substrates [15,16]. But, those researches mainly focus on the treatment of wastewater containing P and N. There remains a lack of information on heavy metals purification effects in the CWs systems with different substrates. Therefore, the aim of the present study was to determine the effects of substrate type, HRT, and reed on the lead RE in horizontal subsurface-flow CW (HSSF) to reduce the negative impact generated by lead in the environment.

2. Materials and methods

2.1. Physical characteristics of CWs

To conduct this research, artificial reed-bed system was prepared containing nine metal boxes made of galvanized iron in mesocosm scale (1 × 0.3 × 0.35 m) placed adjacent to the Faculty of Agriculture, Islamic Azad University, Dezful Branch, Iran (48˚25′ E, 32˚,16′ N) under ambient conditions. Then, they were filled by three different substrate types containing river sand (0.01–5 mm), fine gravel (5–10 mm), and medium gravel (10–20 mm) and a depth of 30 cm. In each system inlet and outlet, rubble was used with a diameter 50–100 mm so that the wastewater would immediately percolate into the bed and would be prevented from flowing over it. Moreover, a fine metal mesh was placed in front of outlet hole to prevent the washout of substrate from the reed-bed system into outlet samples of wastewater due to drainage. A large number of young and healthy *Phragmites australis* seedlings were gathered from local irrigation canals and were immediately cultivated a number of 20 plants in each wetland in 7 June 2013. While planting seedlings, at least a space 10 cm from each other and a depth 20 cm between the roots was considered which increased the buds and shoots during the reeds growth and prevented them from dying out. Fig. 1 shows schematic view of CW systems used in this research, and Fig. 2 also shows longitudinal cross section of a horizontal flow CW.

2.2. Hydraulic characteristics of CWs

The surface loading rate and HRT were selected with four different retention time including 1, 3, 5, and 10 d. The required flow rate and the surface loading rate of lead were calculated according to the wastewater concentration 10 mg/L lead (Table 1). Through the concentration of 10 mg/L lead, it was

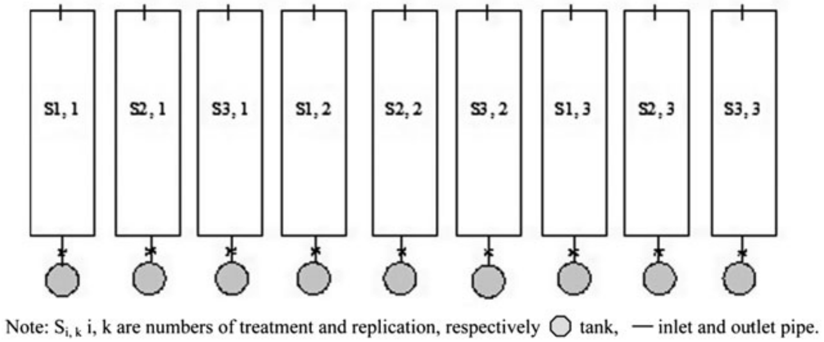

Fig. 1. Layout of CW systems.

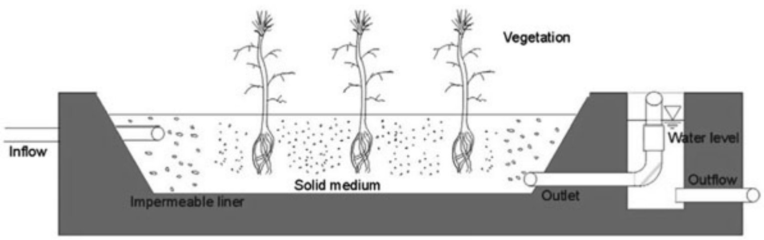

Fig. 2. Longitudinal cross section of a horizontal flow CW.

Table 1
Hydraulic conditions for operating the CWs system

HRT[e] (d)	V[d] (L)	Q[c] (L/d)	HLR[b] (mm/d)	SLR[a] (mg pb/d m^2)
1	59	59	198	1,955
3	69	23	77	756
5	79	16	53	526
10	104	10	35	331

[a]SLR = surface loading rate, which is the ratio of lead mass flow rate to surface area of wetland cells.
[b]HLR = hydraulic loading rate, which is the flow rate (Q) divided by total surface area of wetland cells.
[c]Q = flow rate of the recirculating water from the culture tank to the treatment unit.
[d]V = consumed volume of synthetic wastewater in liter.
[e]HRT = nominal hydraulic retention time, which can be computed as (surface area × water depth × porosity of wetland(s))/flow rate.

Table 2
Physical and chemical characteristics of wetland substrates

Physical	Unit	Sand	Fine gravel	Medium gravel	Chemical	Value	Unit
Size	mm	0.01–5	5–10	10–15	EC_e	0.03	ds/m
Hydraulic conduction (k)	cm/min	1.795	18.6	25	pH	7.2	–
Porosity (n)	%	47	36	30	Pb^{2+}	0.002	mg/kg
Bulk density (ρ_b)	g/cm^3	1.51	1.4	1.35	PO_4-P	1.3	mg/kg
Uniformity coefficient (d_{60}/d_{10})	–	3.7	2.19	1.93	NO_3-N	0.56	mg/kg

possible to achieve the synthetic wastewater higher than the standard of Iran's Environment Protection Organization and WHO in order to treat wastewater for irrigation consumptions. Moreover, a calibrated 200-L reservoir equipped with outlet valve was used in order to provide the required flow rate. The required flow rate for each system was calculated according to the wetland volume, the rate of daily evapotranspiration, and physical and chemical characteristics of substrate. The results of physical and chemical characteristics of substrate have been shown in Table 2. The source of lead was prepared from lead nitrate with high solubility by Merck Company.

2.3. Sampling and measuring

After planting, about 45 d was spent to provide appropriate conditions for the experiments; therefore, the experiments were delayed until 23 July 2013. In order to set different retention times in systems, different flow rate and hydraulic loading rate were replicated. At the end of each retention time and after discharging of used wastewater, ordinary water was added into the systems for 5 d to clean up the substrate and provide the necessary conditions for next experiment stages. Thus, the four selected retention times were completed for one month, and all the experiments were carried out with two replications for about 2 months until 3 October 2013. Meanwhile, during experiments period, the lead concentration of influent was measured again because the lead nitrate that was consumed containing impurities. At the end of each time stage, the plant samples were carefully uprooted, washed thoroughly with water and soap, and then were rinsed twice with distilled water in order to wash off any soil particles and were extracted after being air-dried based on relevant literature [17]. All samples root were introduced into special digestion tubes (Buchi 430 Digestor) with concentrated 25 mL from (97%) nitric acid. The digestion process included a 24-h period with the mixture at room temperature (left undisturbed), followed by a 4-h digestion at a range of high temperatures. For the first hour, the temperature was 100℃, for the second hour 150℃, and finally 200℃ for 2 h The remaining liquid, which most times was about one quarter of the original acid volume, was filtered using Whatman GF/C filters. Deionized water was added until the new solution reached the volume of the acid originally used. All influent and effluent samplings and plant samples were measured by Perklin Elmer A Analyst 700 atomic absorption whose relevant results were briefly shown in Table 3.

3. Results and discussion

3.1. Models and removal efficiencies

According to measurements, removal efficiency of lead (RE) and the ratio of concentration of inflow to outflow (C/C_0) in synthetic wastewater were calculated. In addition, removal time ratio (RTR) was calculated at different retention times demonstrated in Table 3. (According to RTR, it was possible to calculate the contribution of each retention time on the lead removal.) Accordingly, the relationship between the RE of influent and the retention time was examined using CurveExpert1.4 software. The equation obtained was as the exponential equation $\mathrm{RE} = a \cdot (b - e^{c \cdot t})$, so that among the fitted equations that had the highest

Table 3
Pilot performance results during the study period (mean ± standard deviation, $n = 22$)

HRT (d)	Substrate	C_0[e] (mg/L)	C[d] (mg/L)	C/C_0[c]	RE[b]	RTR[a]
1	Sand	9.88 ± 0.11	1.57 ± 0.03	0.159 ± 0.005	84.1 ± 0.47	95.4
	Fine gravel	9.88 ± 0.09	2.35 ± 0.02	0.237 ± 0.003	76.3 ± 0.4	94
	Medium gravel	9.88 ± 0.06	2.41 ± 0.03	0.244 ± 0.004	75.6 ± 0.447	94.9
3	Sand	9.81 ± 0.08	1.25 ± 0.04	0.128 ± 0.006	87.2 ± 0.45	98.9
	Fine gravel	9.81 ± 0.13	1.88 ± 0.03	0.191 ± 0.005	80.9 ± 0.63	99.7
	Medium gravel	9.81 ± 0.07	2.00 ± 0.06	0.204 ± 0.007	79.6 ± 0.75	99.9
5	Sand	9.95 ± 0.12	1.18 ± 0.03	0.119 ± 0.005	88.1 ± 0.41	100
	Fine gravel	9.95 ± 0.12	1.89 ± 0.04	0.190 ± 0.006	81.0 ± 0.62	99.9
	Medium gravel	9.95 ± 0.02	2.02 ± 0.05	0.203 ± 0.005	79.7 ± 0.55	100
10	Sand	9.52 ± 0.06	1.13 ± 0.02	0.118 ± 0.002	88.2 ± 0.28	100
	Fine gravel	9.52 ± 0.04	1.80 ± 0.04	0.189 ± 0.005	81.1 ± 0.5	100
	Medium gravel	9.52 ± 0.05	1.93 ± 0.04	0.203 ± 0.005	79.7 ± 0.6	100

Note: six replicates and per every group, (±) standard deviation containing three spatial replicates and two time replicates.
[a]RTR = removal time ratio was determined by calculating temporal RE divided by 10-d RE.
[b]RE = removal efficiency was determined by calculating the percentage of concentration decrease from influent to effluent.
[c]C/C_0 = relative concentration of wastewater which is defined as the ratio of lead concentration influent to effluent.
[d]C = lead concentration of effluent.
[e]C_0 = lead concentration of influent.

correlation coefficient that the relevant results were presented in Table 4. This equation can be used to simulate the removal of lead at the subsurface wetland (SSF). Measurements show the more HRT increases, the more RE also increases, but the increasing rate of RE decreased with increasing HRT and was in the range of 75.15–88.48% at four HRT. In addition, the results showed that the effluent concentration in all cases was below standard permissible limit of Iranian Environment Protection Organization and World Health Organization (5 mg/L) for irrigation consumptions. Therefore, in this study, SSF wetland had acceptable performance in treating lead-contaminated wastewater.

The different design models were used to calculate the required surface area of a horizontal subsurface constructed treatment wetland, able to produce an effluent in compliance with the legal standards. Considering the fact that the majority of the investigations on treatment wetlands have mainly been focused on output–input (O/I) data rather than on internal processes data, regression equations seem to be a useful tool in interpreting and applying these O/I data to calculate the required surface area; therefore, at this paper, relationship between hydraulic loading rate and relative concentration of wastewater was evaluated in three different substrates, and the relationship between independent and dependent variables was fitted to a linear regression which indicated high correlation between them (Fig. 3). Table 5 also shows the results of this fitting. According to these relationships, with the reduction of HLR from 19.8 to 3.5 cm/d, C_0/C_i decreased significantly and in the form of a linear relationship. Therefore, through the increase in retention time and the decrease in hydraulic loading rate, more opportunity has been provided for physical, chemical, and biological processes to remove lead in wetland system. The results showed that the intensity of the effective processes in sand substrate was more than the other two substrates and fine-gravel substrate was also more than medium-gravel substrate. Crites and Tchobanoglous [18] suggested relationships for the removal of some pollutants in surface and subsurface wetlands upon which a linear relationship with high correlation coefficient was defined between relative concentration of wastewater (C/C_0) and hydraulic loading rate [18]. Weerakoon et al. [19] evaluated the potential of the removal of synthetic wastewater pollutants under different hydraulic loading in horizontal subsurface-flow CW by planting Typha in which concluded with the reduction of HLR, the RE increased [19] which with the results, this research was similar.

Table 4
Regression coefficients of exponential equation of RE

Substrate	*a*	*b*	*c*	*r*	SD error
Sand	0.085	10.39	0.718	0.975	0.004
Fine gravel	0.21	3.85	1.464	0.982	0.0045
Medium gravel	0.154	5.16	1.32	0.965	0.0055

Table 5
Results of linear regression between HLR and C/C_0

Substrate	R^2	*N*	Eq.
Sand	0.991	12	$\frac{C_0}{C_i} = 0.1073 + 0.0003$ HLR
Fine gravel	0.964	12	$\frac{C_0}{C_i} = 0.1729 + 0.0003$ HLR
Medium gravel	0.954	12	$\frac{C_0}{C_i} = 0.1891 + 0.0003$ HLR

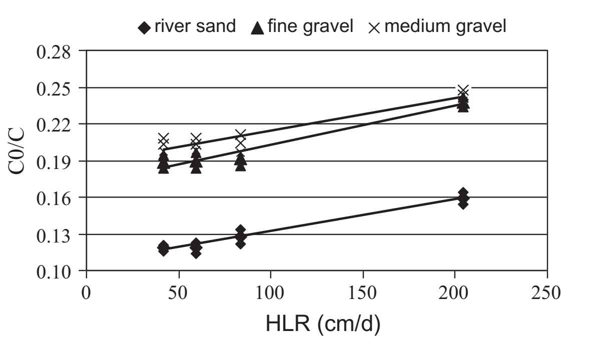

Fig. 3. Correlation between HLR and C/C_0 with linear regression in different substrates.

3.2. The effect of HRT in different substrates

Changes trend of the RE in relation to HRT showed that the more retention time increased, the more RE increased. It seemed that when the retention time increased, the hydraulic loading rate and lead surface loading rate decreased in every three bed (Figs. 4–6); thus, enough opportunity was provided for physical, biological, and chemical processes in systems substrate for lead removal operation. As shown in Figs. 4–6, with increasing HRT, the RE was increasing in every three substrates, but their increasing rate was decreasing, so it seems that the initial times had greater effect on effective processes of the lead removal. Therefore, the RTR was calculated (Table 3) according to which the effect of each retention time on the lead removal was assessed. Accordingly, the highest effect of time share occurred for 1-d retention time which was equal to 95.4, 94.9, and 94% from maximum RE (10-d RE) which based on the mean RE were 84.1, 76.3, and 75.6% in the sand, fine-gravel, and medium-gravel substrates, respectively. Hence, it was

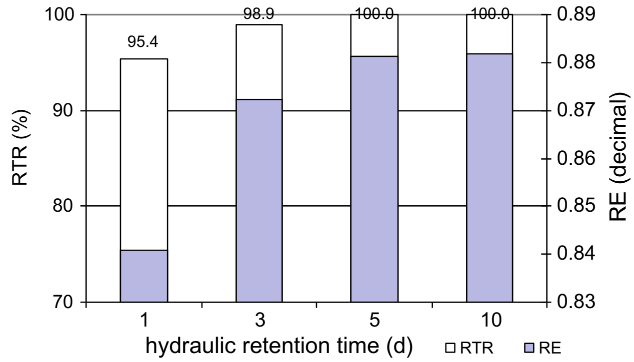

Fig. 4. RE and RTR in effluent in relation to HRT in sand substrate.

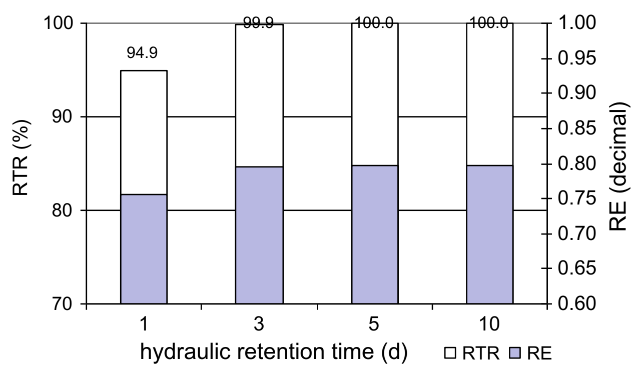

Fig. 5. RE and RTR in effluent in relation to HRT in fine-gravel substrate.

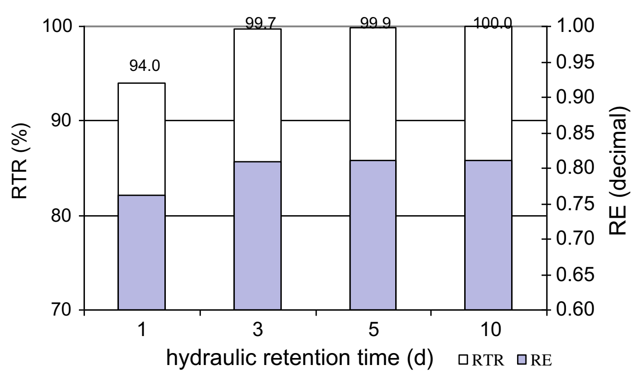

Fig. 6. RE and RTR in effluent in relation to HRT in medium-gravel substrate.

concluded that chemical and biological processes affecting on the lead RE at 1-d retention time was in maximum intensity, but while the retention time increased, the intensity of affective processes decreased and according to these results, the increasing rate of RE decreased in relation to the HRT (Figs. 4–6). Examining other retention times in this research showed that it would be possible to approach the potential RE to an acceptable level by choosing 3-d HRT. Moreover, in order to investigate the significance testing of the retention time on RE, the average RE at different retention times was compared together by SPSS 18 software and Duncan's test that the relevant results have been shown in Table 6. According to ANOVA, there was a significant difference between removal efficiencies at retention time 1, 3, and 5 d ($p < 0.05$) in sand substrate, but no significant difference was observed between efficiencies at retention time 5 and, 10 d ($p < 0.05$). Therefore, 5-d retention time is suggested in optimum conditions with RE of 88.1%. In addition, there was a significant difference between RE of lead at HRT 1 and 3 d in fine- and medium-gravel substrate, but no significant difference was observed between RE at retention time 3, 5, and 10 d ($p < 0.05$). Therefore, 3-d retention time is suggested in optimum conditions in fine- and medium-gravel substrates with the RE of 80.9 and 79.6%, respectively. Similar studies have been done on lead RE in CWs by different researchers, and similar results have been obtained, so that the lead RE was mainly concluded in the range of 76–95%. Moreover, the formation of insoluble sulfides and filtration of solids and colloids and combining with iron and manganese oxide have been introduced as major mechanisms of lead removal in wetland systems [11,20–22]. Lead can form insoluble sulfide (PbS) under anaerobic conditions in SSF wetlands, but carbonate precipitation may be effective for the removal of lead as well [23]. Obarska-Pempkowiak [24] reported a comparable removal (53.5%) of lead in HF CW in Przywidz, Poland [25]. Lesage [26] reported removal of Pb higher than 90% in two HF CWs in Belgium [26]. Also Vymazal [27] reported high Pb removal (98%) in Nučice, Czech Republic [27]. In a research conducted in subsurface-flow CW as a laboratory scale with plant species Typha and Phragmites, similar results were obtained. In that research, the lead concentration of influent was in the range of 1–20 mg/L, and the RE

Table 6
The effect of retention time on the mean lead RE

HRT (d)	Sand	Fine gravel	Medium gravel
1	84.1 ± 0.47^a	76.3 ± 0.4^a	75.6 ± 0.447^a
3	87.2 ± 0.45^b	80.9 ± 0.63^b	79.6 ± 0.75^b
5	88.1 ± 0.41^c	81.0 ± 0.62^b	79.7 ± 0.55^b
10	88.2 ± 0.28^c	81.1 ± 0.5^b	79.7 ± 0.6^b

Note: Different letters indicate significant difference between HRT at the level of $p < 0.05$.

was achieved in the range of 75–96% [28]. In another research conducted on lead and cadmium was observed that with increasing retention time from 2 to 6 d, the percentage of heavy metals removal increased and the maximum removal of 75% was reported [2]. Manshouri and Vosoughi used pilot units with subsurface flow to study the efficiency of constructed reed-bed systems. The results showed that the highest increase of RE belonged to 1-d retention time and as the concentration of copper and chromium increased, the RE decreased from 100 to 98% [3]. In addition, the increase in retention time led to the increase in RE and they pointed out that reed had a significant role on reduction of pollutants in wastewater containing heavy metals so that copper and chromium were accumulated in roots and rhizomes of reed and larger amounts of them were mainly stored in sand substrate reported to be due to biological processes, precipitation, and adsorption [3].

3.3. Effect of substrate type on the lead RE

Relevant measurements showed that the change of average RE was from 83.63–88.48% in sand substrate, 75.15–81.63% in fine-gravel substrate, and in the range of 75.15–80.5% in medium-gravel substrate. The effect of substrate type on the lead RE of influent at different retention times has been statistically analyzed by SPSS 18 software that the results of ANOVA have been demonstrated in Table 7. According to which the difference between the RE at fine- and medium-gravel substrates was not significant at 1-d retention time at 5% level, but the difference between the RE in sand substrate was significant at 5% level compared to other two substrates, so that the highest RE belonged to sand substrate by 84.57%, while in fine- and medium-gravel substrates were 76.7 and 76%, respectively. The results of statistical analysis at 3-, 5-, and 10-d retention times showed that there was a significant difference among all three types of substrate at 5% level. Therefore, substrate type had a significant effect on lead RE. RE changes for the three substrates in relation to retention time were shown in Fig. 7. As shown in Fig. 7, the maximum efficiencies of 88.51, 81.53, and 80.35% in sand, fine-, and medium-gravel substrates were respectively obtained at retention times of 5, 3, and 3 d. Statistical analyses showed that the efficiency of sand substrate was more than other two substrates. Therefore, it was concluded that sand substrate was more effective in enhancing physical, chemical, and biological processes which influence the lead removal. It seemed when porous media of substrate particles increased, effective surface of substrate increased which in turn led to the increase of contact surface of substrate with wastewater and more activities of microorganisms. Akratos and Tsihrintzis [29] investigated the effects of substrate type and HRT on efficiency of subsurface CW at pilot scale. The results showed that fine-gravel substrate had higher RE than medium-gravel substrate in Typha cultivation conditions [29] that these results are similar to the finding of this research. Arroyo et al. [30] studied the effects of substrate type and plant species on the colony of bacteria in the removal of Zn and As and concluded that have remarkable effect on abundance and diversity of bacteria which are effective in the removal of contaminants [30]. Cortes-Esquivel et al. [7] evaluated the RE of Cu and Zn from swine wastewater as effected by three variables: the HRT (HRT) (24, 48, 72, and 96 h), two different plant species (*Typha domingensis* Pers. and *Eleocharis cellulosa*), and two different sizes of filter media (5 and 15 mm) using a horizontal subsurface-flow CW. They reported higher RE was achieved in the HRT 96 h for Zn, and in the case of Cu, the highest efficiency was recorded in the HRT of 72 h. Therefore, HRT was a factor to take into account in order to achieve the desired removal, moreover concluded the particle size of filter media had no statistically significant difference with respect to the percentage of metals removal though greater averages were recorded with the fine gravel of 5 mm. The results of this research indicated that HSSF-wetlands could be a very useful tool for the removal of heavy metals (HMs) like Zn and Cu in swine wastewater [7]. Kröpfelová et al.

Table 7
The effect of substrate type on the lead RE

	1-d HRT		3-d HRT		5-d HRT		10-d HRT	
Substrate	RE	Duncan Test	RE	Duncan Test	RE	Duncan Test	RE	Duncan Test
Sand	84.1	A	87.2	A	88.1	A	88.2	A
Fine gravel	76.3	B	80.9	B	81	B	81.1	B
Medium gravel	75.6	B	79.6	C	79.7	C	79.7	C

Note: Different letters indicate significant difference between HRT at 5% level ($p < 0.05$).

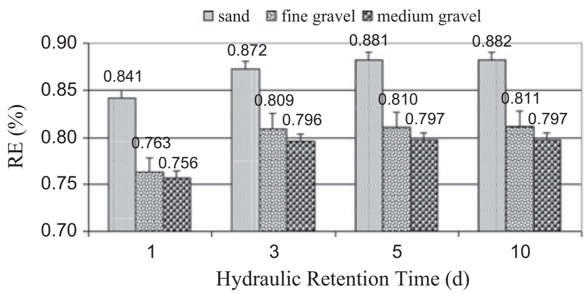

Fig. 7. Comparison of RE in different media.

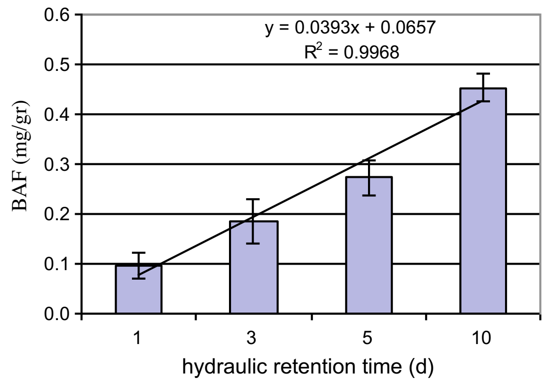

Fig. 8. BAF in relation to HRT. (HRT had significant effect on the BAF at the level of $p < 0.05$.)

[25] employed three HSSF-CWs with the plant species Phragmites to evaluate the influence of filter media size in the removal of Zn and Cu from municipal wastewater for a period of 2 years. The authors employed three different CWs. The first wetland, called Břehov, contained gravel of 4–8-mm filter media, and the percent of removal was 86% for Zn and 84% for Cu. The second wetland, called Mořina, contained crushed rock of 4–8 mm, and the percent of removal obtained was 90.5% for Zn and 73.8% for Cu. Finally, the third wetland, called Slavošovice, contained gravel of 3–20 mm and reached 58.3 and 41.7% removal for Zn and Cu, respectively, and obtained higher removal efficiencies in the CWs with finer gravel [25]. Therefore, CWs with the finer filter media allowed for retention of metals more effectively because the empty spaces between stacked gravel are smaller. According to Gambrell, fine textured soils containing appreciable organic matter content tend to accumulate contaminants; nevertheless, they create a clogging effect in less time because retained solids are higher in comparison with the retained solids in coarse gravel [31]. In the present study, particle size of filter media had statistically significant effect on the lead RE so that the greater averages were recorded with the river sand substrate, and similar result was also obtained by Kröpfelová et al.

3.4. Lead uptake and bioaccumulation factor

The linear relationship between HRT and bioaccumulation factor (BAF) in the root tissue of reed was examined. Accordingly, a linear relationship with high correlation coefficient ($R^2 = 0.99$) was obtained based on the equation of $\text{BAF} = 0.0393\,\text{HRT} + 0.0657$ in which HRT was HRT in day and BAF was lead BAF in the root tissue of reed in mg/g (Fig. 8). The results showed that there was a significant relationship between BAF at different HRT so that with the increase HRT, cumulative uptake of reed increased up to 0.435 mg/g dw of below-ground organs biomass. In addition, statistical analysis (ANOVA) showed HRT had a significant effect on the accumulation of lead in the root tissue of reed ($p < 0.05$). Thus, the root of reed is highly capable of assimilating and accumulating lead and plays an important role in the lead removal in subsurface wetland system. Aquatic plants are among the main biological processes in wetlands because they not only directly absorb oxygen but also make it enter around the root zone which leads to nutrition absorption, oxidation, and direct spoilage of contamination and microorganisms activities. Consequently, different plant species play important roles in removing heavy metals [7,32]. Heavy metals usually accumulate in the roots of aquatic plants particularly reed and can be used as a bio-indicator for monitoring water pollution or sewage sludge [33]. Biological removal is an important pathway for heavy metal removal in the CWs, it includes plant and microbial uptake. The rate of metal removal by plants varies widely, depending on plant growth rate, plant species, and concentration of the heavy metals in the wastewater [34]. Maximum concentration of metals in plants was observed in roots. Barley et al. [35] also reported the highest metal concentrations in the roots of wetland plants [35]. Once lead has penetrated into the root system, it may accumulate there or may be translocated to aerial plant parts. For most plant species, the majority of absorbed lead (approximately 95% or more) is accumulated in the roots, and only a small fraction is translocated to aerial plant parts. There are several reasons why the transport of lead from roots to aerial plant parts is limited. These reasons include immobilization by negatively charged pectins within the cell wall, precipitation of insoluble lead salts in intercellular spaces, and sequestration in the vacuoles of rhizodermal and cortical cells. The endoderm, which acts as a physical barrier, plays an important role in this phenomenon. Indeed, following apoplastic transport, lead is blocked in the endodermis by the Casparian strip and must follow symplastic transport. In endodermis cells, the major part of lead is sequestered or excreted by plant detoxification systems [1].

Lead forms various complexes with soil components, and only a small fraction of the lead present as these complexes in the soil solution are phyto-available. Despite its lack of essential function in plants, lead is absorbed by them mainly through the roots from soil solution and thereby may enter the food chain [1]. The absorption of lead by roots occurs via the apoplastic pathway or via Ca^{2+}-permeable channels. The behavior of lead in soil and uptake by plants is controlled by its speciation and by the soil pH, soil particle size, cation-exchange capacity, root surface area, root exudation, and degree of mycorrhizal transpiration. Excessive lead accumulation in plant tissue impairs various morphological, physiological, and biochemical functions in plants, either directly or indirectly, and induces a range of deleterious effects. It causes phytotoxicity by changing cell membrane permeability, by reacting with active groups of different enzymes involved in plant metabolism and by reacting with the phosphate groups of ADP or ATP, and by replacing essential ions. Lead toxicity causes inhibition of ATP production, lipid peroxidation, and DNA damage by over production of reactive oxygen species (ROS). In addition, lead strongly inhibits seed germination, root elongation, seedling development, plant growth, transpiration, chlorophyll production, and water and protein content. The negative effects that lead has on plant vegetative growth mainly result from the following factors: distortion of chloroplast ultrastructure, obstructed electron transport, inhibition of Calvin cycle enzymes, impaired uptake of essential elements, such as Mg and Fe, and induced deficiency of CO_2 resulting from stomatal closure. Under lead stress, plants possess several defense strategies to cope with lead toxicity. Such strategies include reduced uptake into the cell; sequestration of lead into vacuoles by the formation of complexes; binding of lead by phytochelatins, glutathione, and amino acids; and synthesis of osmolytes. In addition, activation of various antioxidants to combat increased production of lead-induced ROS constitutes a secondary defense system [1]. Several hyperaccumulator plant species, such as *Brassica pekinensis* and *Pelargonium*, are capable of translocating higher concentrations of lead to aerial plant parts, without incurring damage to their basic metabolic functions. A specific hyperaccumulator species can accumulate more than 1,000 ppm lead [36]. Therefore, in the present study, reed was not a hyperaccumulator species; however, the results indicated that this aquatic plant was resistant to high concentrations of lead.

3.5. Contributions of reed and substrate in the lead removal

According to the mass balance of the lead in wetland, the percentage contribution of substrate and plant in the lead removal was calculated separately. As shown in Fig. 9, the contribution of reed in lead removal during four retention times was in the range of 7.66–22.01% of influent lead in three substrate so that the contribution of plant increased, while BAF increased in relation to retention time (Fig. 9), but in contrast, the contribution of substrate decreased with the increasing retention time in the range of 77.3–90.6%, 75.6–85.6%, and 74.4–83% of influent lead in sand, fine-gravel, and medium-gravel substrates, respectively (Fig. 10). As a result, it seemed that the contribution of substrate at lower HRT was more effective due to higher HLR and lead surface loading rate in the other word when the HRT increased, the storage capacity of substrate decreased. Furthermore, the results showed that the adsorption capacity of sand substrate was higher than the other two substrates. Therefore, it was seemed that sand substrate with more porous media was more effective than the two other substrates to intensify physical, chemical,

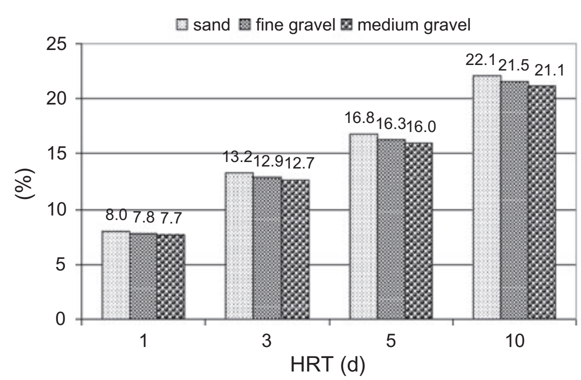

Fig. 9. The contribution of reed in the lead removal.

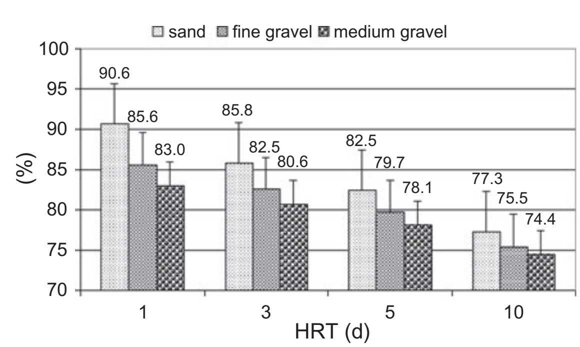

Fig. 10. The contribution of substrate in the lead removal.

and biological processes, which are effective in the lead removal of synthetic wastewater. According to these results and similar studies, physical and chemical characteristics of substrate can mainly play a remarkable role in the removal of contaminants in the subsurface CWs [10,11].

Liu et al. [37] studied the rate of lead accumulation by 19 plant species in CWs. The results showed the removal efficiencies were greater than 90% and plants had a significant effect on the lead absorption and accumulation so that lead was absorbed an average of about 22% of influent lead at the first harvest [37]. Cheng et al. [32] investigated a twin-shaped CW comprising a vertical flow (inflow) chamber with *Cyperus alternifolius* followed by a reverse-vertical flow (outflow) chamber with *Villarsia exaltata that* was assessed for decontamination of artificial wastewater polluted by lead. Based on it concluded that lateral roots of C. *alternifolius* removed 13% of the applied lead and a vertical flow CW with *C. alternifolius* is an effective tool in phytoremediation of water polluted with heavy metals [32].

4. Conclusions

(1) The results showed that the effluent concentration in all cases was below standard permissible limit of Iranian Environment Protection Organization and World Health Organization (5 mg/L) for irrigation. Therefore, in this study, SSF wetland has acceptable performance in treatment of lead-contaminated wastewater.

(2) The ratio of concentration of effluent to influent (C/C_0) decreased significantly with the increasing of HRT and the decreasing of hydraulic loading rate from 19.8 to 3.5 cm/d and as a linear relationship with high correlation coefficient. Therefore, when the retention time increased and the surface loading decreased, more opportunity was provided for chemical and biological processes affecting lead removal in wetland system.

(3) Statistical analyses showed that there was a significant difference between RE in sand substrate and retention times of 1, 3, and 5 d at 5% level, while there was no significant difference between 5 and 10 d retention time ($p < 0.05$). Moreover, there was a significant difference between retention times of 1 and 3 d ($p < 0.05$) for fine- and medium-gravel substrates, but no significant difference was observed between retention times of 3, 5, and 10 ($p < 0.05$). Therefore, the best HRT in sand, gravel, and medium-gravel substrate was 5, 3, and 3 d, respectively, with the maximum efficiency of 88.51, 81.53, and 80.35%.

(4) The analysis results of substrate type also showed that no significant different was observed between fine- and medium-gravel substrate at 1-d HRT, whereas there was a significant difference between the sand substrate with two other substrates; in addition, there was a significant difference between the three different substrate types at HRT 3, 5, and 10 d so that sand substrate had higher efficiency than the other two substrates. Therefore, it was concluded that sand substrate has been more effective in relation to intensify physical, chemical, and biological processes which are effective in the lead removal of synthetic wastewater. The results of this research indicated that CWs could be a very useful tool for the removal of lead in synthetic wastewater and that physical characteristics of substrate and reed play an important role on the lead removal in the subsurface CWs.

Acknowledgment

This paper was derived from a Ph.D thesis whose authors would like to thank those who have contributed in this work and the Faculty of Water Sciences Engineering, Shahid Chamran University, Ahvaz, Iran, due to financial support and provision of the research executive facilities.

References

[1] B. Pourrut, M. Shahid, C. Dumat, P. Winterton, E. Pinelli, Lead uptake, toxicity, and detoxification in plants, Rev. Environ. Contam. Toxicol. 213 (2011) 113–136.
[2] Z. Usefi, A. Mashayekhi, A. Mohammadpour, Performance evaluation a hybrid constructed wetland in removal of lead and cadmium from Mazandaran university of Medical Sciences campus complex wastewater, J. Mazandaran Univ. Med. Sci. 97 (2012) 258–269 (in Persian).
[3] M. Manshouri, M. Vosoughi, Removal of heavy metals by constructed wetland, in: 2th National Conference on Environmental Health, Tehran, 2000, 12 pp. (in Persian).
[4] S. Lavrova, B. Koumanova, The role of *Phragmites Australis* in wetlands self-purification, J. Environ. Prot. Ecol. 9 (2008) 531–539.

[5] J. Vymazal, Constructed wetlands for wastewater treatment, Water 2 (2010) 530–549.
[6] J. Vymazal, Removal of nutrients in various types of constructed wetlands, Sci. Total. Environ. 380 (2007) 48–65.
[7] J. Cortes-Esquivel, G. Giacoman-Vallejos, I.D. Barcelo, R. Mendez, M.C. Ponce, Heavy metals removal from Swine wastewater using constructed wetland with horizontal subsurface flow, J. Environ. Prot. 3 (2012) 871–877.
[8] G.H.P. Art, J.D.M. Belgers, C.H. Hoekzema, J.T.N.M. Thissen, Sensitivity of submersed freshwater macrophytes and endpoints in laboratory toxicity tests, Environ. Pollut. 153 (2008) 199–206.
[9] A. Hazrat, E. Khan, M. Anwar Sajad, Phytoremediation of heavy metals—Concepts and applications, Chemosphere 91 (2013) 869–881.
[10] P. Mantovi, M. Marmiroli, E. Maestri, S. Tagliavini, S. Piccinini, N. Marmiroli, Application of a horizontal subsurface flow constructed wetland on treatment of dairy parlor wastewater, Bioresour. Technol. 88 (2003) 85–94.
[11] A. Kumar Choudhary, S. Kumar, C. Sharma, Constructed wetlands: An approach for wastewater treatment, Elixir. Pollut. 37 (2011) 3666–3672.
[12] U. Stottmeister, A.Wießner, P. Kuschk, U. Kappelmeyer, M. Kästner, O. Bederski, R.M. Müller, H. Moormann, Effects of plants and microorganisms in constructed wetlands for wastewater treatment, Biotechnol. Adv. 22 (2003) 93–117.
[13] F. Wissing, Wasserreinigung mit Pflanzen, Verlag Eugen Ulmer, Stuttgart, J.B. Yavitt, A.K. Knapp, Methane emission to the atmosphere through emergent cattail (*Typha latifolia* L.) plants, Tellus 47B (1995) 521–534.
[14] S. Sirianuntapiboon, M. Kongchum, W. Jitmaikasem, Effects of hydraulic retention time and media of constructed for treatment of domestic wastewater, Afr. J. Agric. Res. 1 (2006) 027–037.
[15] R.H. Kadlec, R.L. Knight, Treatment Wetlands, CRC Press, Bankok, 1996.
[16] M. Chen, Y. Tang, X. Li, Z. Yu, Study on the heavy metals removal efficiencies of constructed wetlands with different substrates, J. Water Res. Prot. 1 (2009) 1–57.
[17] G. Sposito, C.S. LeVesque, J.P. LeClaire, A.C. Chang, Trace metal chemistry in arid-zone field soils amended with sewage sludge: III. Effect of time on the extraction of trace metals, Soil Sci. Soc. Am. J. 47 (1983) 898–902.
[18] R. Crites, G. Tchobanoglous, Small and Decentralized Wastewater Management Systems, WCB McGraw-Hill, Boston, MA, 1998.
[19] G. Weerakoon, K. Jinadasa, G. Herath, M. Mowjood, J. Van Bruggen, Impact of the hydraulic loading rate on pollutants removal in tropical horizontal subsurface flow constructed wetlands, Ecol. Eng. 61 (2013) 154–160.
[20] B.N. Noller, P.H. Woods, B.J. Ross, Case studies of wetland filtration of mine waste water in constructed and naturally occurring systems in northern Australia, Water Sci. Technol. 29 (1994) 257–266.
[21] T.L. Sinicrope, R. Langis, R.M. Gersberg, M.J. Busnardo, J.B. Zedler, Metal removal by wetland mesocosms subjected to different hydroperiods, Ecol. Eng. 1 (1992) 309–322.
[22] J. Vymazal, Removal of heavy metals in a horizontal sub-surface flow constructed wetland, J. Environ. Sci. Health A 40(6–7) (2010) 1369–1379.
[23] L.Y. Lin, Wastewater treatment for inorganics, in: Encyclopedia of Environmental Biology, vol. 3, Academic Press, San Diego, CA, 1995, pp. 479–484.
[24] H. Obarska-Pempkowiak, Removal and retention of selected heavy metals in components of a hybrid wetland system, in: U. Mander, P. Jenssen (Eds.), Constructed Wetlands for Wastewater Treatment in Cold Climates, WIT Press, Southampton, 2003, pp. 299–309.
[25] L. Kröpfelová, J. Vymazal, J. Švehla, J. Štíchová, Removal of trace elements in three horizontal sub-surface flow constructed wetlands in the Czech Republic, Environ. Pollut. 157 (2009) 1186–1194.
[26] E. Lesage, Behavior of Heavy Metals in Constructed Treatment Wetlands, PhD thesis, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium, 2006.
[27] J. Vymazal, Distribution of iron, cadmium, nickel and lead in a constructed wetland receiving municipal sewage, in: J. Vymazal (Ed.), Wetlands-Nutrients, Metals and Mass Cycling, Backhuys Publishers, Leiden, 2003, pp. 341–363.
[28] A.S. Mungur, R.B.E. Shutes, D.M. Revitt, M.A. House, An assessment of metal removal by a laboratory scale wetland, Water Sci. Technol. 35 (1997) 125–133.
[29] C. Akratos, V. Tsihrintzis, Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands, Ecol. Eng. 29 (2007) 173–191.
[30] P. Arroyo, G. Ansola, L. Miera, Effects of substrate, vegetation and flow on arsenic and zinc removal efficiency and microbial diversity in constructed wetlands, Ecol. Eng. 51 (2013) 95–103.
[31] R.P. Gambrell, Trace and toxic metals in wetlands—A review, J. Environ. Qual. 23 (1994) 883–881.
[32] S. Cheng, W. Grosse, F. Karrenbrock, M. Thoennessen, Efficiency of constructed wetlands in decontamination of water polluted by heavy metals, Ecol. Eng. 18 (2002) 317–325.
[33] G. Bonanno, R. Lo Giudice, Heavy metal bioaccumulation by the organs of *Phragmites australis* (common reed) and their potential use as contamination indicators, Ecol. Indic. 10 (2010) 639–645.
[34] A.S. Sheoran, V. Sheoran, Heavy metal removal mechanism of acid mine drainage in wetlands: A critical review, Min. Eng. 19 (2006) 105–116.
[35] R.W. Barley, C. Hutton, M.M.E. Brown, J.E. Cusworth, T.J. Hamilton, Trends in biomass and metal sequestration associated with reeds and algae at Wheal Jane Biorem pilot passive treatment plant, Sci. Total. Environ. 338 (2005) 107–114.
[36] E. Maestri, M. Marmiroli, G. Visioli, N. Marmiroli, Metal tolerance and hyperaccumulation: Costs and trade-offs between traits and environment, Environ. Exp. Bot. 68 (2010) 1–13.
[37] J. Liu, Y. Dong, H. Xu, D. Wang, J. Xu, Accumulation of Cd, Pb and Zn by 19 wetland plant species in constructed wetland, J. Hazard. Mater. 147 (2007) 947–953.