



Evaluating the Efficiency of Salicornia, Typha, and Juncus Aquatic Plants in Extra Phosphate Phytoremediation from the Aqueous Solutions

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Abstract

Background: The phosphorous as one of the agricultural, industrial, and urban pollutants has an important role in the eutrophication phenomenon of the surface waters.

Objectives: In this research, the effect of three species of aquatic plants, including Salicornia (Persica), Typha, and Juncus, were investigated and compared with the potential of phosphate uptake.

Methods: After preparing the condition and cultivation of plants, they were irrigated by synthetic wastewaters with various phosphate concentrations (7, 18, and 28 mg. L⁻¹) for 28 days. In this test, the adsorbed phosphate was calculated based on mg. g⁻¹ of the plant dry tissues weight using two methods: mass balance and wet digestion methods.

Results: The results showed that increase in the pollutants concentration and passing time had a significant effect on the accumulative increase in phosphate uptake in all three plants at 5% level. Thus, the maximum adsorption capacity was obtained on the 28th day in high concentration of pollutant which was measured for Salicornia, Juncus, and Typha as 9.68, 6.37, and 7.68 mg. g⁻¹ of plant dry tissues weight, respectively. Also based on the reported regression coefficients values and the obtained equations, it can be concluded that the variable 'time' was more effective than the 'concentration' variable in terms of adsorbed phosphate by the studied plants. The results of the measurement of phosphate uptake in the terrestrial and aerial parts showed that the maximum uptake was measured as 6.35 and 10.33 mg. g⁻¹ of terrestrial and aerial parts dry weight in Salicornia plant, respectively. Moreover, the results showed that the translocation factor in Salicornia, Juncus, and Typha plants were 1.54, 1.12, and 1.46, respectively, as Salicornia was maximum and Juncus was minimum.

Conclusions: Finally, according to the obtained results from research, all three plants can be known as the hyper-accumulate plants, and they can be used for phytoremediation of phosphate from the agricultural soils according to the high capacity of these plants and prevent these pollutants from entering to the surface waters.

Keywords: Aquatic Plant, Phosphate Uptake, Translocation Factor, Phytoremediation

1. Background

Phosphorous is a vital element for all life forms and a limited nutrient for plants and algae. The human activities and discharge of industrial and agricultural wastewaters to the coastal waters increase phosphate concentrations and pollutes water (1, 2). The high growth of aquatic plants and algae reduces the level of dissolved oxygen, interrupts balance in the ecosystem (eutrophication), and interrupts the environmental adaptation of the creatures inside water (3). Thus, the development of effective technologies to reduce phosphate in input wastewaters to the rivers

and lakes is one of the immediate strategies for controlling eutrophication problem in recent decades. At present, the use of wetland systems and utilization of plant capacity in wastewater treatment or phytoremediation have become popular in developed countries (4). The constructed wetlands and phytoremediation are the studied systems for treatment of urban wastewater with small population and treatment of different types of industrial sewages (5). Many aquatic plants can use more phosphate and nitrogen, and can be used as the highly-consumed and low-cost plants to treat pollutants. Therefore, plant coverage man-

agement must be considered as a pollutant reduction solution for sweet water resources (4). On the other hand, based on its low cost, phytoremediation techniques have been reported to recover polluted soil and water, since the past two decades. Jayaweera and Kasturiarachchi, in a research, studied the phytoremediation potential of water hyacinth to remove phosphorous and nitrogen from industrial wastewater for 15 weeks and thus, they obtained 100% removal of total phosphorous and nitrogen after 9 weeks (6). Haritash et al. (7), in their study on the effectiveness of constructed wetland containing Canna Lily plant in removing phosphate from wastewater, found that the efficiency of phosphate removal in the designed system was about 50%, and plant uptake performance was introduced as the main mechanism of removal. In addition, Angassa et al. showed that using two plants such as Phragmites and Vetiveria removed 80% of phosphate from urban wastewaters and both are good candidates for the constructed wetlands (8). All of these studies have tried to increase phosphate removal from water resources. Since phosphate is one of the nutrient required for plants growth, all plants have a degree of removal or uptake of phosphate. However, plants with higher resistance in aqueous environments can tolerate more than ordinary concentrations and can show maximum efficiency in constructed wetlands.

2. Objectives

According to the items mentioned, the innovation and purpose of this research include: (1) Selection and introduction of three native plants types of Khuzestan province including *Salicornia* (Persica), *Typha* and *Juncus*, which have not been used in previous studies to phosphate phytoremediation from the aqueous solutions, (2) Assessing and comparing the performance of three plants types in phosphate uptake based on the phosphate uptake values in the terrestrial parts and aerial parts of plants, (3) The introduction of the most compatible plant based on its function in phosphate uptake and choosing it as an appropriate and affordable option for cultivating in constructed wetlands.

3. Methods

3.1. Preparing Design and Cultivating Plants

This research was conducted for 2 months (April - May 2017) to treat contaminated waters with various concentrations of phosphate using phytoremediation technique in the experimental farm of Shahid Chamran University of Ahvaz. Three aqueous plants, including *Salicornia* (persica), *Typha*, and *Juncus*, were used based on the indicated

characteristics in Table 1. The adaptation with the region climate, quick growth, producing massive biomass, making no color, smell and taste in water, and having proper physiology for participating in this experiments were considered as their advantages.

Plastic vases of 40 cm in diameter and 60 cm height were prepared to execute this research. Then 32 cm sieved gravel from 3.8-inch mesh, which was first washed by ordinary water and finally by distilled water, with 10 cm sand were used to prepare the bed for plants growing in the vases. The experimental design of cultivation of aquatic plants was carried out based on factorial experiment in a completely randomized design with three different plant species and three different levels of phosphate concentration, including 12 treatment and three replications. The simulated wastewater containing 7, 18, and 28 mg. L⁻¹ phosphate (based on common range in agricultural wastewater) were prepared by KH₂PO₄ salt. Figure 1 shows the vase experiment design. In this study, 3 vases for plant and 3 vases for the control sample (without plant) were used as repetitions of the experiment for each pollution level and in each of the studied plant. Totally, 36 vases were executed as the cultivation environment.

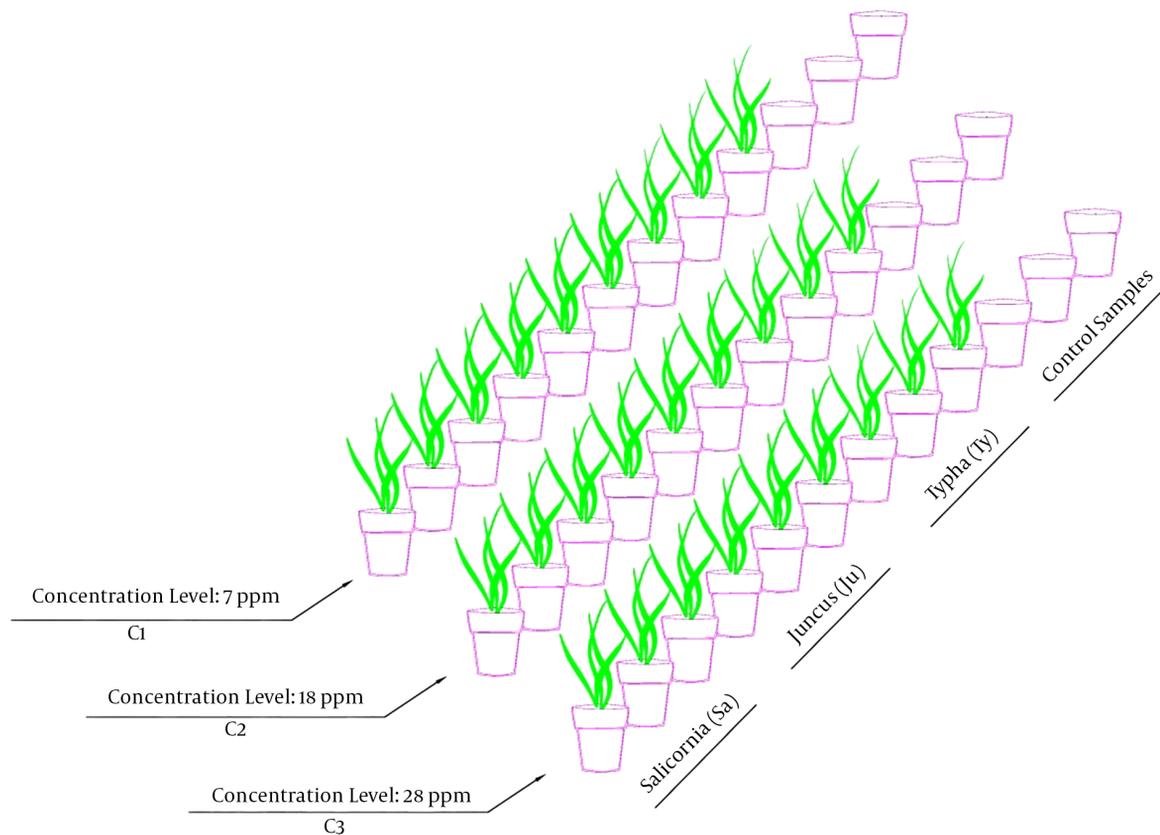
The selected young plant samples were extracted from the margin of the river and natural habitats by shovel and transferred to the to the research center. After preparing the vases and the plant species have been planted (with density of 25 bushes in per m²), they were first irrigated with sweet water for 20 days to adapt them to planting conditions and the environment. Thereafter, irrigation according to schedule was performed using 20 L of simulated wastewater of various phosphate concentrations (in proportion to the measured porosity of the used gravel and sand) and the vases were saturated.

3.2. Sampling and Measurement

All the wastewaters present in the vases were drained by the installed drain valves at the bottom of the vases to study the phosphate uptake ability in the three plants once in every 7 days for 28 days. After measuring the volume of the output wastewaters using graded containers, the vases were again irrigated with wastewater reaching the saturated level. Then, plants uptake was calculated at the end of each week using the mass balance method (amount of input and output phosphate) according to the mass weight of plant dried parts (root and stem). It was calculated by measuring the phosphate concentrations present in the output wastes of vases containing plant species using spectrophotometer (880 nm wavelength) and a comparison with the output wastes analysis of control vases. In addition, all bushes were put out of the vases at the end of

Table 1. Introducing the Some Characteristics of Studied Plant Species

Plant Name	Important Characteristics
Salicornia (persica)	Salicornia is a watery and halophyte plant belonging to the chenopodiacea family and Salicornioideae tribe. Salicornia is from diploid species, yearlong or seldom two-year-old. The natural habitat of this plant is salty, seashores, swamps, and marshes of Europe, South Asia, North America, and South Africa. The use of Salicornia to produce oil, livestock forage, and edible vegetable using the water of sea has been reported (9).
Typha	Typha is the plant species belonging to the Typhaceae family that usually grows in low-depth waters as the perennial herb. This plant has underground stems and velvet-like cylindrical fruits in brown. It is the native plant of marshes, ponds and sweet waters with a height of one to two meters. Typha plant has been extensively used in fabricating wetland to reconstruct the environment and treat the wastewaters. In addition, Typha has the initial raw materials for knitting and paper production (10).
Juncus	Juncus is a plant from Juncaceae species. This plant is yearlong or Perennial with green, straight, long, and sharp peak aerial stems without any branches. Its maximum height is 1 meter and grows in wet places such as flooding rice fields, irrigation water drainage and aquaculture wetlands. The root and stem marrow of this plant is known as the medicinal plant. In addition, the stem of this plant is used to knit basket (11).

**Figure 1.** Schematic representation of vase experiment design, including three different plant species and three different levels of phosphate concentration (12 treatment with 3 replications)

experiments, washed smoothly by water, and dried in an oven. The aerial and terrestrial parts were measured using digital scale and then milled. Finally, the adsorbed phosphate by the terrestrial and aerial parts of plants was measured using wet digestion method. To measure the phosphate concentration using wet digestion method, first and foremost, the plant samples were dried at 65°C in an oven

for 48 hour and then 0.3 g fully dried plant tissue was transferred to 50 mL volumetric flask. Thereafter, 2.5 mL acid mixtures (18 mL distilled water, 100 mL condensed sulfuric acid, and 6 g salicylic acid) were added and mixed completely, and this mixture was kept overnight for 24 hours. In the next step, the sample was heated at 180°C for 1 hour. The sample was removed from the heater and allowed to

cool. Then 5 drops of oxygenated water were added to it, and again, volumetric flask was placed in the heater at 280°C to evaporate the water after 5 - 10 minutes. This action continued until the sample lost its color. After cooling the volumetric flask, the required volume was reached and the sample was filtered after stirring (12, 13). Finally, the translocation factor (TF) of each plant was calculated and examined using Relation 1 (14).

$$TF = \frac{C_s}{C_r}$$

Where, C_s and C_r are the amount of phosphate accumulation in the aerial and terrestrial part (mg. g^{-1}), respectively.

3.3. Data Analysis Method

According to the obtained measurements, the phosphate uptake rate by plants was investigated with respect to time and concentration and then regression coefficients and polynomial equation was obtained using regression analysis to adsorb phosphate. In addition, the amount of phosphate uptake in the aerial and terrestrial parts of the plants was studied using variance analysis of data and comparing them with Tukey method. Finally, the response surface and the interval plots of the interactions among variables were indicated for each studied plant by Minitab 17 software.

4. Results

As shown in Table 2, the adsorbed phosphate amounts of the three plant species were measured using mass balance method at the end of each week based on mg. g^{-1} of plant dry weight.

Table 3 shows the variance analysis, such as the coded regression coefficient and significant levels of variables, for phosphate uptake by Salicornia, Typha, and Juncus plants.

The obtained coded polynomial equation by software for phosphate uptake by Salicornia, Typha and Juncus are shown in the equations below, respectively.

$$qe \left(\frac{\text{mg}}{\text{g}} DW \right) = 4.313 + 2.907T + 1.567C + 0.999T \times C$$

$$qe \left(\frac{\text{mg}}{\text{g}} DW \right) = 3.002 + 2.165T + 1.366C + 0.967T \times C$$

$$qe \left(\frac{\text{mg}}{\text{g}} DW \right) = 2.377 + 1.684T + 1.375C + 0.921T \times C$$

Table 4 shows the effect of plant species on average of phosphate accumulation in the terrestrial and aerial parts and in various phosphate concentration levels.

Figure 2 shows the effect of time changes (based on the week) and different concentrations of pollutants on the uptake capacity of Salicornia, Typha, and Juncus.

Also, Table 5 shows the effect of various concentrations and translocation factor on phosphate accumulation in aerial and terrestrial parts of various plant species.

5. Discussion

5.1. Statistical Analysis

Based on the results of variance analysis obtained (Table 3), the effect of time (T), concentration (C), and the reciprocal effect of time and concentration ($T \times C$) were within the confidence level of 0.95 for all three studied plants. In addition, the correlation coefficients (R^2) for the obtained phosphate uptake equation for Salicornia, Typha, and Juncus plants were 0.9924, 0.9970, and 0.9949, respectively. This indicates good correlation among the obtained results and that less than 1% of changes is unpredictable by the obtained regression model. Based on the reported regression coefficients values and the obtained equations, it can be concluded that the variable 'time' was more effective than the concentration variable in terms of adsorbed phosphate by the studied plants.

5.2. The Effect of Time and Concentration Changes on Phosphate Uptake

The results showed (Figure 2) that the adsorbed phosphate (mg. g^{-1} of plant dry weight) had incremental procedure in all three plants chronically by increasing the time and the initial concentrations of the pollutant; so that, the maximum uptake capacity was observed on the 28th day (the end of the fourth week) and in high level of phosphate concentration (28 mg. L^{-1}) for each of the plants. Also, the lowest amount of phosphate adsorption was observed at the end of the first week and in low level of phosphate concentration (7 mg. L^{-1}) for each of the plants. By studying the obtained results from the statistical test of variance analysis and comparing them with Tukey method, changes of time (based on the week) and initial concentration than the amount of the adsorbed phosphate in all three plants were significant within the confidence level of 0.95. Thus, the phosphate uptake showed increase in the plants as a result of an increase in the selected plants' root contact with the pollutants, increase in ion exchange between plants and aqueous solution and the high intention of the selected plants to adsorb phosphate.

Table 2. Calculating the Adsorbed Phosphate in Various Plant Treatments Using Mass Balance Method^a

Treatment ^b	Sa (C1)	Sa (C2)	Sa (C3)	Ju (C1)	Ju (C2)	Ju (C3)	Ty (C1)	Ty (C2)	Ty (C3)
The end of the first week	0.85 ± 0.03 ^A	1.70 ± 0.08 ^A	1.97 ± 0.04 ^A	0.26 ± 0.02 ^A	0.83 ± 0.03 ^A	1.20 ± 0.09 ^A	0.547 ± 0.01 ^A	0.855 ± 0.02 ^A	1.34 ± 0.05 ^A
The end of the second week	1.90 ± 0.05 ^B	3.53 ± 0.11 ^B	4.37 ± 0.13 ^B	0.64 ± 0.08 ^B	1.90 ± 0.07 ^B	2.71 ± 0.12 ^B	1.23 ± 0.05 ^B	2.01 ± 0.06 ^B	3.31 ± 0.08 ^B
The end of the third week	3.12 ± 0.09 ^C	5.59 ± 0.16 ^C	6.91 ± 0.18 ^C	1.15 ± 0.11 ^C	3.09 ± 0.10 ^C	4.51 ± 0.22 ^C	2.06 ± 0.08 ^C	3.50 ± 0.09 ^C	5.49 ± 0.10 ^C
The end of the fourth week	4.56 ± 0.12 ^D	7.83 ± 0.19 ^D	9.68 ± 0.25 ^D	1.76 ± 0.18 ^D	4.32 ± 0.16 ^D	6.37 ± 0.29 ^D	3.02 ± 0.12 ^D	5.11 ± 0.11 ^D	7.68 ± 0.14 ^D

^a Each mean ± standard deviation value was obtained from the three repetitions. Various letters in each specific column indicate significant difference in confidence level of 0.95 ($P < 0.05$).

^b The accumulative amounts of the adsorbed Phosphate (mg.g⁻¹DW).

Table 3. The Predicted Regression Coefficients and Significant Level of Parameters

Parameter	Juncus		Typha		Salicornia	
	Coefficient	P Value	Coefficient	P Value	Coefficient	P Value
Constant	2.377	0.000	3.002	0.000	4.313	0.000
T	1.684	0.000	2.165	0.000	2.907	0.000
C	1.375	0.000	1.366	0.000	1.567	0.000
T × C	0.921	0.000	0.967	0.000	0.999	0.000

Table 4. Effect of Plant Species on Average Phosphate Uptake^a

Different Levels of Concentration	Terrestrial Parts (mg. g ⁻¹ of Terrestrial Parts Dry Weight)	Aerial Parts (mg. g ⁻¹ of Aerial Parts Dry Weight)
Low-level phosphate concentration (7 mg. L⁻¹)		
Salicornia	3.56 ± 0.11 ^A	5.03 ± 0.21 ^A
Juncus	1.92 ± 0.08 ^B	2.01 ± 0.17 ^B
Typha	2.91 ± 0.15 ^C	3.66 ± 0.31 ^C
Medium-level phosphate concentration (18 mg. L⁻¹)		
Salicornia	5.29 ± 0.14 ^A	8.47 ± 0.25 ^A
Juncus	3.95 ± 0.12 ^B	4.59 ± 0.13 ^B
Typha	4.16 ± 0.18 ^B	6.58 ± 0.18 ^C
High-level phosphate concentration (28 mg. L⁻¹)		
Salicornia	6.35 ± 0.10 ^A	10.33 ± 0.29 ^A
Juncus	5.75 ± 0.14 ^B	6.71 ± 0.21 ^B
Typha	6.27 ± 0.13 ^A	9.74 ± 0.25 ^C

^a Each mean ± standard deviation value was obtained from the three repetitions. Various letters in each specific column indicate significant difference in confidence level of 0.95 ($P < 0.05$).

5.3. The Effect of Plant Species on Phosphate Uptake

By studying the obtained results (Table 4) by Variance Analysis test and compared using Tukey method, no significant difference was observed in phosphate accumulation values in the terrestrial parts of Juncus and Typha in

medium pollutant concentration levels. In addition, no significant difference (in confidence level of 0.95) was obtained in high concentration levels in terrestrial parts of Salicornia and Typha. Thus, it can be said that plants roots mechanisms had similar performance in same pollutant

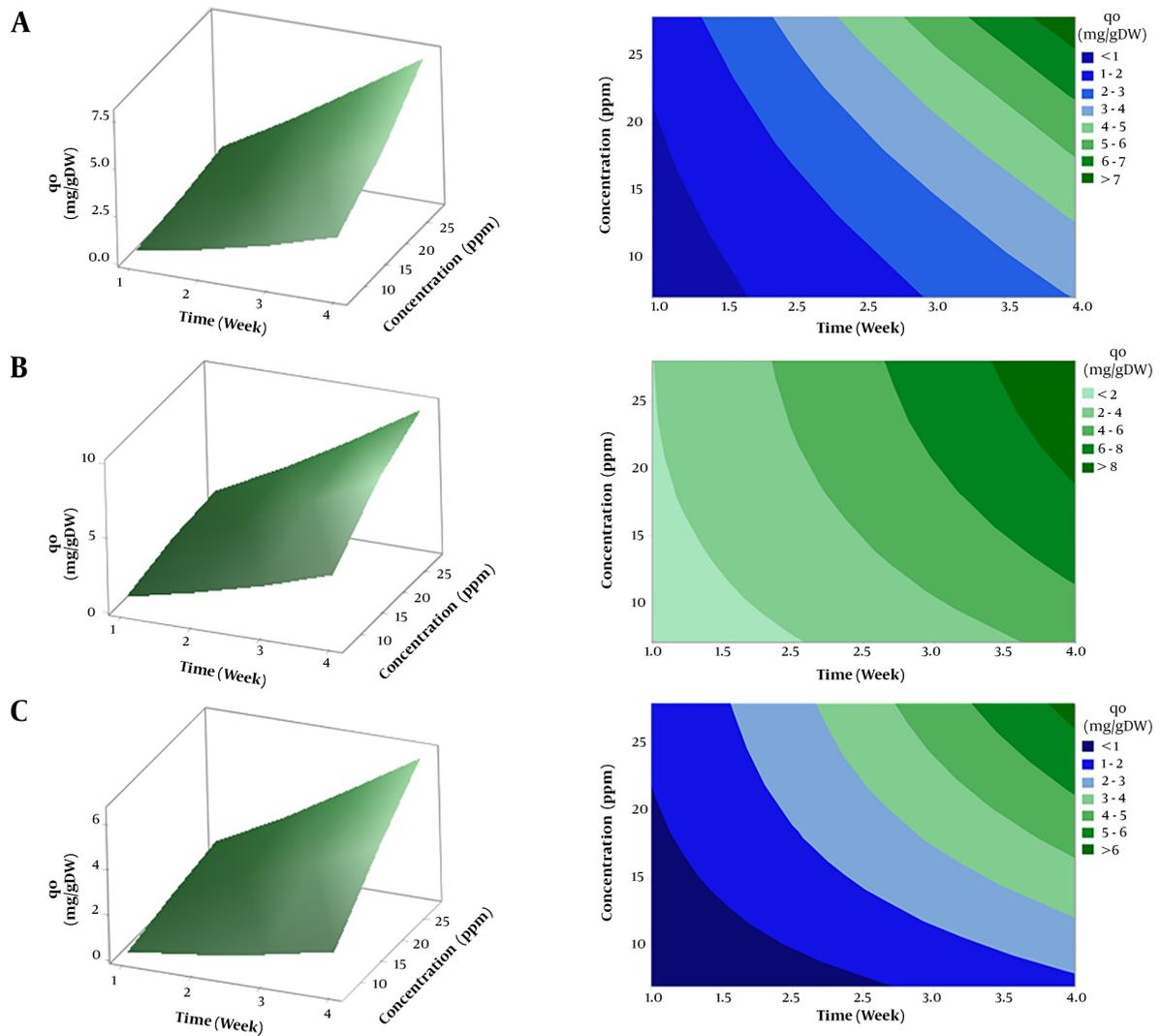


Figure 2. Response surface and the interval plots of the interactive effects of time and concentration variables, A: Typha plant; B: Salicornia plant; and C: Juncus plant (Initial irrigation concentrations: 8, 18, 28 mg/L, Duration of irrigation: 4 weeks)

concentration levels in the phosphate accumulation process, while the obtained results on aerial parts of all the three plants showed more competitiveness and difference in phosphate accumulation values. In this regards, the results showed that phosphate accumulation amounts in aerial parts of plants had 5% significant difference in low, medium, and high pollutant concentration levels, so that the maximum and minimum phosphate accumulations in aerial parts were 10.33 and 2.01 mg. g⁻¹ of aerial parts dry weight related to Salicornia (in concentration of 28 mg. L⁻¹) and Juncus (in concentration of 7 mg. L⁻¹), respectively. Also, the results showed that in all three plants, the most accumulated phosphate was stored in their aerial parts.

One of the mentioned reasons is that the aerial parts have more water and larger vacuoles and as such, more phosphate is accumulated in this part. Another reason is that in adequate quantities light, the amount of photosynthesis increases and this can increase the uptake of pollutants (15). Finally, it can be said that the maximum phosphate accumulation among these three plants was obtained in the terrestrial and aerial parts of Salicornia plant.

5.4. The Effect of Various Concentration Levels on Phosphate Accumulation

According to variance analysis results (Table 5) and comparison means based on Tukey test showed significant

Table 5. Effect of Various Concentration Levels on the Uptake of Phosphate and Translocation Factor in the Studied Plants^a

Different Levels of Concentration	Terrestrial Parts (mg. g ⁻¹ of Terrestrial Parts Dry Weight)	Aerial Parts (mg. g ⁻¹ of Aerial Parts Dry Weight)	TF	TF Mean
Salicornia				1.54
Low level (7 mg. L ⁻¹)	3.56 ± 0.11 ^A	5.03 ± 0.21 ^A	1.41	
Medium level (18 mg. L ⁻¹)	5.29 ± 0.14 ^B	8.47 ± 0.25 ^B	1.60	
High level (28 mg. L ⁻¹)	6.35 ± 0.10 ^C	10.33 ± 0.29 ^C	1.62	
Juncus				1.12
Low level (7 mg. L ⁻¹)	1.92 ± 0.08 ^A	2.01 ± 0.17 ^A	1.04	
Medium level (18 mg. L ⁻¹)	3.95 ± 0.12 ^B	4.59 ± 0.13 ^B	1.16	
High level (28 mg. L ⁻¹)	5.75 ± 0.14 ^C	6.71 ± 0.21 ^C	1.16	
Typha				1.46
Low level (7 mg. L ⁻¹)	2.91 ± 0.15 ^A	3.66 ± 0.31 ^A	1.25	
Medium level (18 mg. L ⁻¹)	4.16 ± 0.18 ^B	6.58 ± 0.18 ^B	1.58	
High level (28 mg. L ⁻¹)	6.27 ± 0.13 ^C	9.74 ± 0.25 ^C	1.55	

^a Each mean ± standard deviation value was obtained from the three repetitions. Various letters in each specific column indicate significant difference in confidence level of 0.95 ($P < 0.05$).

difference in 5% level among all the phosphate accumulated means in terrestrial parts in the three plant species. Moreover, the result of phosphate accumulation in the aerial parts showed that various pollutant concentration levels had significant effect on phosphate accumulation in the aerial parts of the three plant species. Comparison and evaluation of the results showed that Salicornia plant had more adaptability and ability to adsorb phosphate in its terrestrial parts and roots. It seems that Salicornia plant, due to the adsorption and the need for more water and greater distribution and development of the roots, has a higher uptake capacity than the two other species. In addition, translocation factor was calculated which showed phosphate uptake ratio in aerial and terrestrial parts. The higher values of this index show more capacity of plant in uptake and accumulation of phosphate in the aerial parts. According to the obtained results shown in Table 4, the mean value of translocation factor in Salicornia, Juncus, and Typha plants was obtained as 1.54, 1.12, and 1.46, respectively, as its maximum and minimum value was observed in Salicornia and Juncus, respectively. Moreover, based on the obtained values which were higher than one for translocation factors, all three studied plants can be introduced as the hyper-accumulate plants. Furthermore, studying the translocation factors showed that increasing concentration in Salicornia plant increases the translocation factor, while translocation factor in Juncus plant was constant and in Typha plant reduced in high concentrations of phosphate which shows a reduction in the phosphate transfer rate from terrestrial parts to aerial parts in

high concentration of phosphate in these two plants. Ahmadpoor et al. reported that the plant has the adjusting mechanism in high concentration of phosphate, preventing extra adsorption and translocation of phosphate in aerial parts (16).

5.5. Conclusion

In this research, changes of the pollutant initial concentration and time had significant effect in 5% level on uptake and accumulation of phosphate by the studied plants, so that increase in various concentrations levels of phosphate and passing time increased the phosphate uptake in plants and the maximum uptake capacity was observed on the 28th day (the end of the fourth week) and in high level of phosphate concentration (28 mg. L⁻¹) for each of the plants. The results showed that the maximum phosphate accumulation in root was found in Salicornia plant with 6.35 mg. g⁻¹ of terrestrial parts dry weight in high phosphate concentration levels, and the minimum value was found in Juncus plant with 1.92 mg. g⁻¹ of terrestrial parts dry weight obtained in low concentration levels. Moreover, the results of the measurement of phosphate uptake in the aerial parts showed that the maximum uptake was measured as 10.33 mg. g⁻¹ of aerial parts dry weight in Salicornia plant and the minimum was measured as 2.01 mg. g⁻¹ of aerial parts dry weight in Juncus plant. Thus, these results showed more adaptability of Salicornia to phosphate uptake. Moreover, results of the present study showed that translocation factor in Salicornia, Juncus, and Typha plants was obtained 1.54, 1.12, and 1.46, respectively, indicating

that the maximum translocation factor was found in *Salicornia* plant and the minimum was in *Juncus* plant. Generally, by inferring the obtained results from the translocation factor, all three plants can be referred to as hyper-accumulate and they can be used to adsorb phosphate from agricultural soils according to their high capacity in phosphate biological uptake and can prevent the entry of nutrients into free waters. Therefore, two main roles can be considered for these plants: first, their role as a bio-filter and phosphate uptake from the soil, surface waters, or wastewaters to prevent their leaching and consequently prevent their further pollution transmission in the environment and second as the salable and usable by-product.

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Footnotes

Authors' Contribution: Study concept and design: Ehsan Derikvand, Saeb Khoshnavaz, Saeed Boroomand Nasab and Mohsen Solimani Babarsad; analysis and interpretation of data: Sadegh Ghasemi; drafting of the manuscript: Sadegh Ghasemi and Ehsan Derikvand; critical revision of the manuscript for important intellectual content: Sadegh Ghasemi, Ehsan Derikvand, Saeb Khoshnavaz, Saeed Boroomand Nasab and Mohsen Solimani Babarsad; and Statistical analysis: Sadegh Ghasemi.

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