

GREENHOUSE AND FIELD EVALUATION OF PHYTOREMEDIATION FOR NITROGEN AND PHOSPHORUS IN A RIPARIAN BUFFER STRIP

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Abstract. In this research, greenhouse and field studies were conducted to evaluate the efficiency of Riparian Buffer Strips' (RBS) phytoremediation properties for N and P removal. In the field study, RBSs were investigated according to their perimeter, area, path, and length on the banks of Zayandeh-rood River, Iran. In the greenhouse study, the phytoremediation of dominant species (*Fraxinus rotundifolia*) and proposed species (*Aianthus altissima*) was investigated. The results of measurements done on the concentrations of N and P in soil showed that N and P concentrations in soil increased considerably from the beginning of the farm to the end of the farm and then sharply decreased from the beginning of the RBS to the end of it. Results revealed that in general, the efficiency of phytoremediation in removal of pollution was highest under low concentrations of pollution, lowest in average concentrations, and eventually moderate in highest concentrations (P value < 0.05).

Keywords: *arid and semi-arid basin, eutrication, hyper accumulators non-point pollutants, riparian soil*

Introduction

The shortage and pollution of water are regarded as critical problems in many countries. The contamination of surface and ground waters by nitrogen (N) and phosphorus (P) is a major factor affecting water supplies in many parts of the world (Wang et al., 2002; Boz et al., 2013). Similarly, P is recognized as the most critical nutrient-limiting factor that can induce water pollution (Xiang et al., 2009). Regarding the dangers resulting from the increase of P in water, controlling its input into waters, especially rivers, is very important (Sibrell et al., 2009). High concentrations of N in surface waters can also lead to pollution, and in turn, to instability of the ecosystem (Hefting and Klein, 1998).

Most agricultural soil in Iran is contaminated with high doses of Nitrogen and Phosphorus due to the utilization of Urea and Triple Superphosphate over the last decades. P absorption is fast during initial stages but it decreases over time and eventually the P sediments as mineral Phosphates (with low plant absorption) (Malakouti, 2011).

Eutrication caused by N and P (Stefanidis et al., 2020) negatively affects water quality (Lin et al., 2020), the aquatic ecosystems (Lusiana et al., 2020), and the nutrient cycles (Maciej et al., 2020) resulting in a decrease in species diversity and an increase in the biomass of less desirable species (Boyd, 2020). Therefore, besides controlling the P,

removal of N is also important for solving the problem of water pollution. Agricultural runoff can transport sediment, pesticides and nutrients, especially N and P, to surface water and have an impact on the environment (Otto et al., 2008). Due to the ever-growing population of the world on one hand, and the gradual increase of environmental pollutions and the deterioration of agricultural land on the other, a great challenge has arisen in the field of food security, both quantitatively and qualitatively. However recently, research into the application of buffer zones has provided the grounds for the introduction of an environmentally-friendly technology to reduce the non-spot pollution in not only the soil, but also the runoff. Riparian buffer strips (RBS) are an efficient and economical way to reduce agricultural non-point source pollution (De Souza et al., 2013). The effect is usually highly satisfactory, with abatements of 70–98% for P and 70–95% for N (Borin et al., 2005; Anbumozhi et al., 2005). Phytoremediation of contaminated soils within RBS has proven to be cost effective and environmentally friendly (Wang et al., 2002). Euliss et al. (2008) assess phytoremediation for petroleum contaminants in an RBS and indicated that plants enhanced degradation of target contaminants through their phytoremediation capability. Xiang et al. (2009) suggested that phytoremediation is an effective technology for N and P removal from polluted water. In accordance with the results of Aguiar et al. (2015), buffer zones composed of woody soils were more effective in P (99.9%) and N (99.9%) removal when compared to shrub (66.4% and 83.9%, respectively) or grass vegetation area (52.9% and 61.6%, respectively). Fallahi et al. (2012) showed that plant type is very important regarding the effectiveness of plant properties for phytoremediation. Taheri et al. (2014) indicated that *Fraxinus rotundifolia* is one of the effective species for phytoremediation. Several studies have investigated RBS effectiveness in removing suspended solids, nutrients and pesticides from agricultural runoff (Anbumozhi et al., 2005; Schilling and Jacobson, 2014; Aguiar et al., 2015) or phytoremediation for N and P (Wang et al., 2002; Xiang et al., 2009). Few studies have been done to evaluate the phytoremediation of N and P in RBS in both greenhouse and field conditions. Moreover, studies about them are limited in Middle Eastern countries. Therefore, this research was done with the aim of assessing the use of phytoremediation to reduce N & P contaminants in both greenhouse and RBS. In addition to contaminant quantification, the cumulative effect of roots was evaluated over the 6-month study period.

Materials and methods

Study timeline

The overall timeline of the current study includes five stages and covers a period of two years (24 months) from Mar. 2016 to Feb. 2018. These stages include Buffer Zone sampling and analysis of the RBZs data (Field Study – Mar. to Aug. 2016), Greenhouse Preparation and Deployment phase (3 months – Sep. 2016 to Nov. 2017), Greenhouse Study phase (6 months – Des. to May. 2017), Database Lock (3 months – Jun. to Aug. 2017), and finally, Data Analysis and Report (6 months – Sep. 2017 to Feb. 2018).

Field study

The Zayandeh-Rood River in center of Iran, one of the most polluted sites in this region is an example of the anthropogenic impacts on the environment in the last four

decades that have proven to be extremely deleterious due to the rapid development of agriculture (Sanayei et al., 2009). The semi-arid Zayandeh-Rood River Basin is one of the most strategic and important river basins of Iran (Madani and Marino, 2009). This river basin, with an area of 41,347 km², is located between the 50° 24' to 53° 24' longitudes and 31° 11' to 33° 42' latitudes (Fig. 1).



Figure 1. Buffer zone plots along agricultural land around Zayandeh-Rood – The route with the highest Concentration

The Zayandeh-Rood River Basin covers an area of about 26,917 km² in central Iran. The main traditional staple crops of the basin are wheat, rice, barley, and corn, which are highly water consumptive and polluting. Zayandeh-Rood River, with an average flow of 1400 million m³, including 650 million m³ of natural flow and 750 million m³ of transferred flow, is the main surface water resource of the basin (Gohari et al., 2013). Table 1 summarizes some of the main characteristics of this basin.

Table 1. General characterizes of the Zayandeh-rood basin (Gohari et al., 2013)

Attribute	Value
<i>Physiographic and hydrologic</i>	
Elevation range (m)	1470-3974
Annual average precipitation range (mm)	50-1500
Average temperature range (°C)	3-30
Annual potential evapotranspiration (mm)	1500
Average humidity (%)	24-57
River length (Km)	350
Average natural flow (MCM)	650
Average transferred flow (MCM)	750
<i>Agricultural</i>	
Irrigation efficiency (%)	34-42
Agricultural water use (%)	73
Irrigated area (ha)	270,000
Rain fed area (ha)	30,000

The river water is used for drinking, industrial and agricultural purposes. In its west-east journey, Zayandeh-Rood River passes several agricultural areas (Gohari et al., 2013). The determination of pollutants throughout the river is important and has a great role in controlling the ecological condition of this body of water. The first step was finding appropriate fields with agricultural lands that are adjacent to RBS along river through field studies, using remote sensing (RS) and data analysis. The area and perimeter of agricultural land was then determined and analyzed. The Polygon centers were determined with a “place mark” and then coordinates were calculated. One buffer zone site was selected for our study.

As agricultural application is the biggest source of non-point pollution on the national scale, samples were collected along 3 parallel transects from the beginning to the end of the farm and beginning to the end of the buffer zone at the depth of 30 cm and then analyzed in laboratory for N and P concentrations. Species biometrics were investigated in the field. Results are shown in *Table 2*. Two of the fast growing and drought-contamination resistant species, the rich “*Fraxinus rotundifolia* Mill” and also the newly proposed “*Aianthus altissima*”, were evaluated in the greenhouse study (Taheri et al., 2014; Li et al., 2015; Yang et al., 2015).

Table 2. Biometrics of tree species at plot 9

Dominant species	Family	Average diameter at breast (cm)	Average height (m)	Diameter standard deviation (cm)	Height standard deviation (m)
<i>Fraxinus rotundifolia</i> Mill	Oleaceae	20.15	11.25	2.68	0.79
<i>Salix alba</i> L.	Salicaceae	29.45	10.55	2.4	0.91
<i>Elaeagnus angustifolia</i> L.	Elaeagnaceae	18.80	9.37	1.50	1.51
<i>Populus nigra</i> L.	Salicaceae	19.10	17.00	1.01	1.01
<i>Morus alba</i> L.	Moraceae	19.85	12.50	1.10	1.45

Greenhouse evaluation

At first, in order to design the greenhouse study, 30 canals were inserted into soil (50 cm × 50 cm × 2 m). In order to avoid water loss, a plastic layer and then a half tube and finally another plastic layer were placed in the soil (*Diagram 1*). Next, an experiment was conducted with ash (*Fraxinus rotundifolia* Mill) as the dominant species in the region, and Heaven tree (*Aianthus altissima*) as the proposed species. Potassium nitrate and Potassium phosphate solutions were used to create concentrations of 5, 10 and 50 mg L⁻¹. To move the water from the beginning to the end of the canal, a slope of 5% was created. Three replicates were included for statistical validity. During early March, 40 young seedlings of *Fraxinus rotundifolia* and 40 young seedlings of *Aianthus altissima* were planted in the canals, so that four plants were placed in each canal to assess cumulative effect of the roots. A total of 10 canals were left un-vegetated as control to consider the role of soil in remediation. To decrease evaporation, soil surface was coated with sand and aluminum foil. Irrigation was done with pure water at the rate of twice per week; after being planted 3 months, plants were irrigated with polluted water. After being planted 4 months (in late spring), water was collected at the end of the canals and the concentrations of N and P were determined in laboratory. One month later, the collected water was circulated in canals and the concentrations of N and P were again determined in laboratory. Finally, after 6 months, the circulated water was again collected and the pollution was determined in laboratory. Therefore, sampling was

done in three stages. Total P (inorganic and organic) was determined by oxidation of unfiltered samples with potassium persulfate, and analyzing at 885 nm wavelength on the spectrophotometer upon color development with an ammonium molybdate-ascorbic acid-antimony method (Genchi, 1990). Total N (inorganic and organic) was determined in unfiltered samples by oxidation with potassium persulfate (Genchi, 1990) upon color development with the salicylic acid method; samples were analyzed at 410-nm wavelength on the spectrophotometer (spectrophotometer UV-Vis- Camspec M350 Double Beam) (Cataldo et al., 1975).

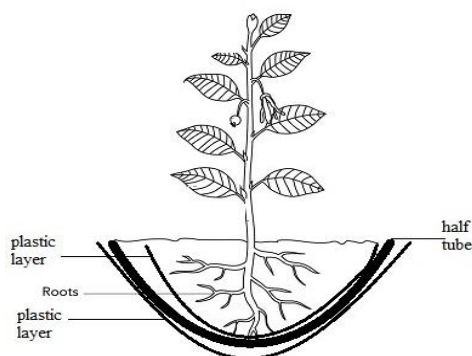


Diagram 1. Canal design to avoid water loss

Statistical analysis

Analysis of the results was done using IBM SPSS version 21. A two-way analysis of variance was conducted to explore the effect of the species and the concentration levels on the reduction of the contamination of N and P. The concentration levels were presented as 5 mg/L, 10mg/L, and 50 mg/L. Based on the results from the initial analysis, multiple one-way ANOVA tests (with Tukey HSD as the Post-hoc test) were conducted to compare the N and P reductions for each concentration level and at different times for each of the species. Homogeneity of Variance was tested using Levene's Test of Equality of Error Variances.

Results

Field evaluation

The total length of Zayande-rood River from Zardkuhe Bakhtiari to Gavkhuni marsh is estimated to be 461.65 Km. The distribution of RBSs in Zayandeh-rood basin with measurements of perimeter, area, length, latitude and longitude along agricultural land are given in *Appendix A*. Coordinates of the starting and the end point of the path are (32.5447° and 51.519°) and (32.6237° and 51.722°), respectively. The largest area of buffer zone is at plot 9 (32.6298 and 51.5614) and the smallest area of buffer zone is at plot 26 (32.5895 and 51.5449). Moreover, the greatest length is located at plot 39 (32.438 and 51.5838) and the shortest length is attributed to plot 41 (32.6416 and 51.5775). Furthermore, the largest perimeter of buffer zone is at plot 33 (32.5731 and 51.5231) and the smallest perimeter is at plot 30 (32.5866 and 51.5375).

The results of measuring the concentrations of N and P in the field RBS with a width of 10 m at plot 9 revealed that the highest concentrations of N and P are related to the

end of the farm and the lowest concentrations are related to the end of the buffer zone. N and P concentrations in soil increased considerably from the beginning of the farm to the end of the farm and then sharply decreased from the beginning of the buffer zone to the end of it, as average concentrations of N and P dropped from 0.33 mg L⁻¹ to 0.035 mg L⁻¹ and from 6.97 mg L⁻¹ to 1.74 mg L⁻¹, respectively (Fig. 2a, b).

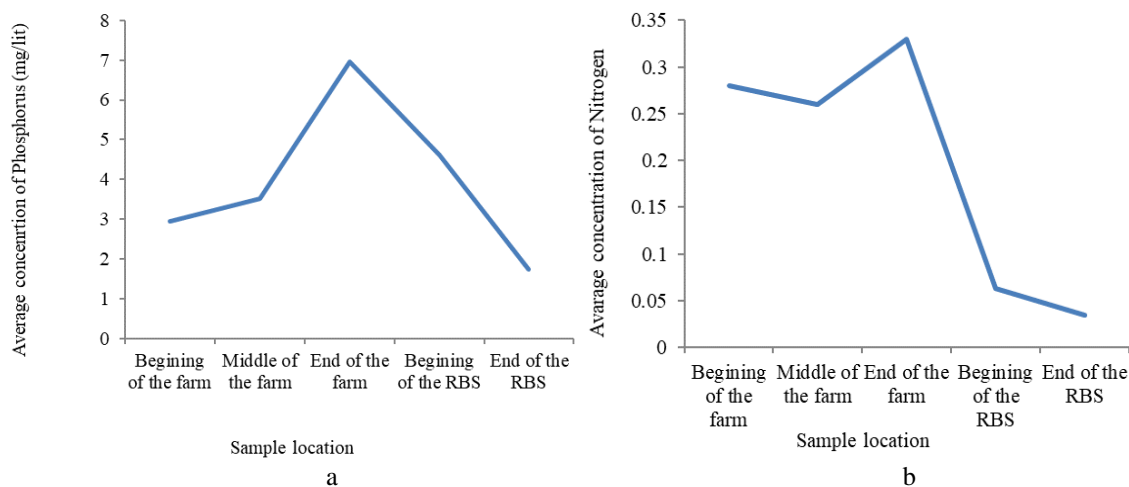


Figure 2. Alterations in average concentration of P (a) and N (b) in the farm and the RBS

According to the results of the Tukey Test presented in Table 3, the changes in the concentration of N from the beginning to the end of the farm is significant ($p < 0.05$), and the same applies to the concentration of P ($p < 0.05$). Likewise, the concentrations of N and P contaminants decrease significantly from the beginning to the end of the RBS ($p < 0.05$ for both).

Table 3. Multiple comparison of field RBS nitrogen and phosphorus by Tukey HSD

Dependent variable: nitrogen						
Tukey HSD						
(I) Point	(J) Point	Mean difference (I-J)	Std. error	Sig.	95% confidence interval	
					Lower bound	Upper bound
Beginning of the farm	Middle of the farm	-.45833	.567240	.925	-2.15573	1.23906
	End of the farm	-2.51000*	.567240	.002	-4.20739	-.81261
	Beginning of the RBS	-.45000	.567240	.930	-2.14739	1.24739
	End of the RBS	1.10500	.567240	.326	-.59239	2.80239
Middle of the farm	Beginning of the farm	.45833	.567240	.925	-1.23906	2.15573
	End of the farm	-2.05167*	.567240	.013	-3.74906	-.35427
	Beginning of the RBS	.00833	.567240	1.000	-1.68906	1.70573
	End of the RBS	1.56333	.567240	.080	-.13406	3.26073
End of the farm	Beginning of the farm	2.51000*	.567240	.002	.81261	4.20739
	Middle of the farm	2.05167*	.567240	.013	.35427	3.74906
	Beginning of the RBS	2.06000*	.567240	.013	.36261	3.75739
	End of the RBS	3.61500*	.567240	.000	1.91761	5.31239
Beginning of the RBS	Beginning of the farm	.45000	.567240	.930	-1.24739	2.14739
	Middle of the farm	-.00833	.567240	1.000	-1.70573	1.68906
	End of the farm	-2.06000*	.567240	.013	-3.75739	-.36261
	End of the RBS	1.55500	.567240	.083	-.14239	3.25239

End of the RBS	Beginning of the farm	-1.10500	.567240	.326	-2.80239	.59239
	Middle of the farm	-1.56333	.567240	.080	-3.26073	.13406
	End of the farm	-3.61500*	.567240	.000	-5.31239	-1.91761
	Beginning of the RBS	-1.55500	.567240	.083	-3.25239	.14239
Dependent variable: phosphorus						
Beginning of the farm	Middle of the farm	.12167	.057171	.247	-.04941	.29274
	End of the farm	-.33333*	.057171	.000	-.50441	-.16226
	Beginning of the RBS	.22833*	.057171	.006	.05726	.39941
	End of the RBS	.28250*	.057171	.001	.11142	.45358
Middle of the farm	Beginning of the farm	-.12167	.057171	.247	-.29274	.04941
	End of the farm	-.45500*	.057171	.000	-.62608	-.28392
	Beginning of the RBS	.10667	.057171	.366	-.06441	.27774
	End of the RBS	.16083	.057171	.072	-.01024	.33191
End of the farm	Beginning of the farm	.33333*	.057171	.000	.16226	.50441
	Middle of the farm	.45500*	.057171	.000	.28392	.62608
	Beginning of the RBS	.56167*	.057171	.000	.39059	.73274
	End of the RBS	.61583*	.057171	.000	.44476	.78691
Beginning of the RBS	Beginning of the farm	-.22833*	.057171	.006	-.39941	-.05726
	Middle of the farm	-.10667	.057171	.366	-.27774	.06441
	End of the farm	-.56167*	.057171	.000	-.73274	-.39059
	End of the RBS	.05417	.057171	.875	-.11691	.22524
End of the RBS	Beginning of the farm	-.28250*	.057171	.001	-.45358	-.11142
	Middle of the farm	-.16083	.057171	.072	-.33191	.01024
	End of the farm	-.61583*	.057171	.000	-.78691	-.44476
	Beginning of the RBS	-.05417	.057171	.875	-.22524	.11691

Greenhouse evaluation (model experiment)

Figure 3 shows the comparison of phytoremediation between *Fraxinus rotundifolia* and *Aianthus altissima* for N and P. According to Tables 4 and 5, efficiency of trees in removal of pollution is the highest in lowest concentrations of pollution, the lowest in average concentration, and finally medium in the highest pollution concentration (P value < 0.05 for both). Figures 4 and 5 show phytoremediation of each species separately and Descriptive Statistics of N and P removal by each species in each time period are given in Appendices D, E, F, G.

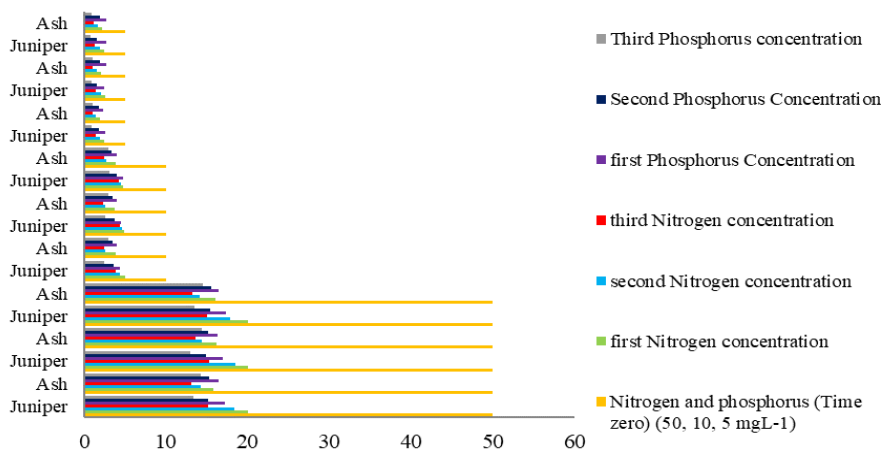


Figure 3. Comparison of phytoremediation between *Fraxinus rotundifolia* and *Aianthus altissima* for N and P in three time periods

Table 4. Multiple comparison of *Aianthus altissima* nitrogen and phosphorus concentration by Tukey HSD

Dependent variable: nitrogen						
(I) Concentration	(J) Concentration	Mean difference (I-J)	Std. error	Sig.	95% confidence interval	
					Lower bound	Upper bound
50 mg/L	10 mg/L	13.3055*	.08107	.000	13.0986	13.5124
	5 mg/L	15.8724*	.08107	.000	15.6655	16.0793
10 mg/L	50 mg/L	-13.3055*	.08107	.000	-13.5124	-13.0986
	5 mg/L	2.5669*	.08107	.000	2.3600	2.7738
5 mg/L	50 mg/L	-15.8724*	.08107	.000	-16.0793	-15.6655
	10 mg/L	-2.5669*	.08107	.000	-2.7738	-2.3600
Dependent variable: phosphorus						
50 mg/L	10 mg/L	11.5133*	.09789	.000	11.2635	11.7632
	5 mg/L	13.5178*	.09789	.000	13.2680	13.7676
10 mg/L	50 mg/L	-11.5133*	.09789	.000	-11.7632	-11.2635
	5 mg/L	2.0044*	.09789	.000	1.7546	2.2543
5 mg/L	50 mg/L	-13.5178*	.09789	.000	-13.7676	-13.2680
	10 mg/L	-2.0044*	.09789	.000	-2.2543	-1.7546

Table 5. Multiple comparison of *Fraxinus rotundifolia* nitrogen and phosphorus concentration by Tukey HSD

Dependent variable: nitrogen						
(I) Concentration	(J) Concentration	Mean difference (I-J)	Std. error	Sig.	95% confidence interval	
					Lower bound	Upper bound
50 mg/L	10 mg/L	11.5535*	.05792	.000	11.4057	11.7013
	5 mg/L	12.9469*	.05792	.000	12.7991	13.0948
10 mg/L	50 mg/L	-11.5535*	.05792	.000	-11.7013	-11.4057
	5 mg/L	1.3935*	.05792	.000	1.2456	1.5413
5 mg/L	50 mg/L	-12.9469*	.05792	.000	-13.0948	-12.7991
	10 mg/L	-1.3935*	.05792	.000	-1.5413	-1.2456
Dependent variable: phosphorus						
50 mg/L	10 mg/L	11.9049*	.04847	.000	11.7812	12.0286
	5 mg/L	13.5380*	.04847	.000	13.4143	13.6617
10 mg/L	50 mg/L	-11.9049*	.04847	.000	-12.0286	-11.7812
	5 mg/L	1.6331*	.04847	.000	1.5094	1.7568
5 mg/L	50 mg/L	-13.5380*	.04847	.000	-13.6617	-13.4143
	10 mg/L	-1.6331*	.04847	.000	-1.7568	-1.5094

Statistical comparison of the two species (using factorial two-way ANOVA) reveals a significant difference in both N and P concentration reduction between the species (Table 6). Considering the homogeneity of Variance (Levene's Test of Equality of Error Variances), it is necessary to perform separate one-way ANOVAs with Tukey HSD as the Post-hoc test to compare the N and P reductions for the different concentration levels and at different times for each of the species.

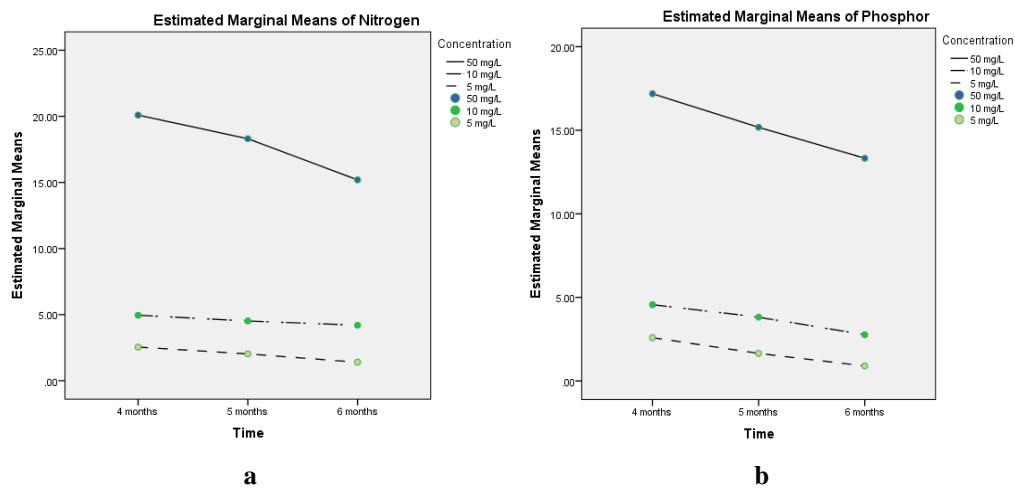


Figure 4. N (a) and P (b) removal by *Aianthus altissima* in three time periods

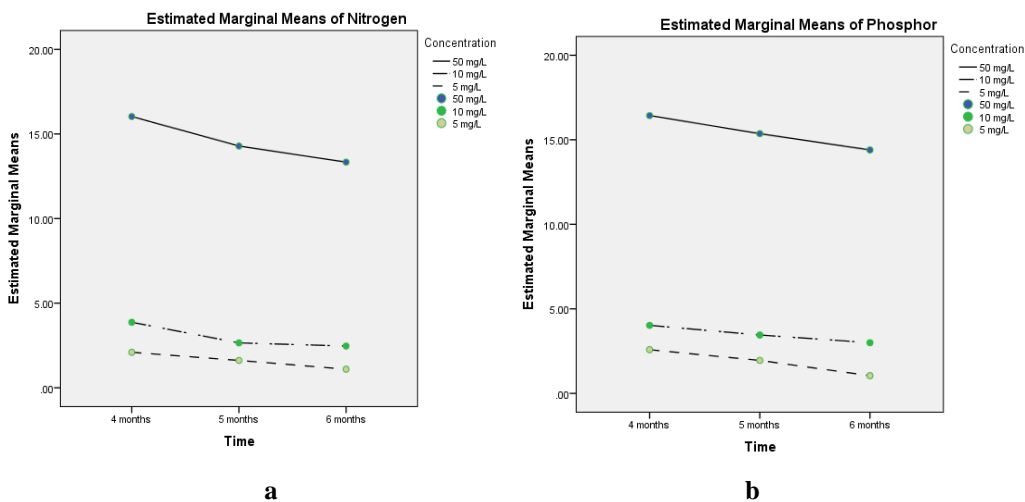


Figure 5. N (a) and P (b) removal by *Fraxinus rotundifolia* in three time periods

Results from the follow-up one-way ANOVAs show that the highest N reduction occurs at the early stage (i.e. 4 months) and much lesser reduction occurs in the second and third stages (i.e. 5 and 6 months, respectively), as N reduction in *Aianthus altissima* and *Fraxinus rotundifolia* is about 60% and 68% at the first, and then reaches to almost 63% and 71% at the second, eventually reaching 69% and 74% at the third stage, respectively (P value < 0.05) (Tables 7 and 8). Similarly, P declines sharply at the early stage and reduction in the second and third stages are much lesser, whereas P reduction in *Aianthus altissima* and *Fraxinus rotundifolia* is about 65% and 67% at first and then reaches to almost 70% and 69% at the second and 74% and 71% at the third stages, respectively (P value < 0.05) (Tables 7 and 8). It seems that *Fraxinus rotundifolia* is more effective in N reduction than *Aianthus altissima*, and vice versa, P declines more than N in *Aianthus altissima* (P value < 0.05). P reduction in *Fraxinus rotundifolia* is almost equivalent to the reduction of N (a little more) (Tables 7 and 8) (P value < 0.05).

Table 6. Tests of between-subjects effects for the two-way ANOVA– *Aianthus altissima* and *Fraxinus rotundifolia*

Dependent variable: nitrogen						
Source	Type III sum of squares	df	Mean square	F	Sig.	Partial eta squared
Corrected MODEL	777.985 ^b	5	155.597	6264.251	.000	1.000
Intercept	942.359	1	942.359	37938.845	.000	1.000
Type	19.845	1	19.845	798.949	.000	.985
Concentration	748.262	2	374.131	15062.301	.000	1.000
Type * concentration	9.879	2	4.939	198.852	.000	.971
Error	.298	12	.025			
Total	1720.642	18				
Corrected total	778.283	17				
Dependent variable: phosphorus						
Corrected model	725.008 ^b	5	145.002	5926.186	.000	1.000
Intercept	1122.527	1	1122.527	45877.453	.000	1.000
Type	.842	1	.842	34.429	.000	.742
Concentration	723.722	2	361.861	14789.197	.000	1.000
Type * concentration	.443	2	.222	9.055	.004	.601
Error	.294	12	.024			
Total	1847.829	18				
Corrected total	725.302	17				

^aTime = 4 months

^bR squared = 1.000 (Adjusted R squared = .999)

Table 7. Multiple comparison of *Aianthus altissima* nitrogen and phosphorus time by Tukey HSD

Dependent variable: nitrogen						
(I) Time	(J) Time	Mean difference (I-J)	Std. error	Sig.	95% confidence interval	
					Lower bound	Upper bound
4 months	5 months	.9100*	.08107	.000	.7031	1.1169
	6 months	2.2645*	.08107	.000	2.0576	2.4714
5 months	4 months	-.9100*	.08107	.000	-1.1169	-.7031
	6 months	1.3544*	.08107	.000	1.1475	1.5613
6 months	4 months	-2.2645*	.08107	.000	-2.4714	-2.0576
	5 months	-1.3544*	.08107	.000	-1.5613	-1.1475
Dependent variable: phosphorus						
4 months	5 months	1.2333*	.09789	.000	.9835	1.4832
	6 months	2.4511*	.09789	.000	2.2013	2.7009
5 months	4 months	-1.2333*	.09789	.000	-1.4832	-.9835
	6 months	1.2178*	.09789	.000	.9680	1.4676
6 months	4 months	-2.4511*	.09789	.000	-2.7009	-2.2013
	5 months	-1.2178*	.09789	.000	-1.4676	-.9680

Table 8. Multiple comparison of *Fraxinus rotundifolia* nitrogen and phosphorus time by Tukey HSD

Dependent variable: nitrogen						
(I) Time	(J) Time	Mean difference (I-J)	Std. error	Sig.	95% confidence interval	
					Lower bound	Upper bound
4 months	5 months	1.1461*	.05792	.000	.9983	1.2939
	6 months	1.6993*	.05792	.000	1.5515	1.8472
5 months	4 months	-1.1461*	.05792	.000	-1.2939	-.9983
	6 months	.5532*	.05792	.000	.4054	.7010
6 months	4 months	-1.6993*	.05792	.000	-1.8472	-1.5515
	5 months	-.5532*	.05792	.000	-.7010	-.4054
Dependent variable: phosphorus						
4 months	5 months	.7584*	.04847	.000	.6347	.8822
	6 months	1.5331*	.04847	.000	1.4094	1.6568
5 months	4 months	-.7584*	.04847	.000	-.8822	-.6347
	6 months	.7747*	.04847	.000	.6510	.8984
6 months	4 months	-1.5331*	.04847	.000	-1.6568	-1.4094
	5 months	-.7747*	.04847	.000	-.8984	-.6510

In addition, results revealed that soil has an important role in N and P reduction (Fig. 6). Canals without trees indicate significant reduction. Generally, P reduction is about 32% and N reduction is about 30% (Appendices F and G) (P value < 0.05). It seems that P is more available than N in soil (Tables 9 and 10), which could be related to stronger P bonds than those of N with soil clay particles as well as little movement of P through soil clay particles.

Table 9. Multiple comparison of soil nitrogen and phosphorus time by Tukey HSD

Dependent variable: nitrogen						
(I) Time	(J) Time	Mean difference (I-J)	Std. Error	Sig.	95% confidence interval	
					Lower bound	Upper bound
4 months	5 months	1.5343*	.14963	.000	1.1524	1.9161
	6 months	2.7576*	.14963	.000	2.3757	3.1395
5 months	4 months	-1.5343*	.14963	.000	-1.9161	-1.1524
	6 months	1.2233*	.14963	.000	.8415	1.6052
6 months	4 months	-2.7576*	.14963	.000	-3.1395	-2.3757
	5 months	-1.2233*	.14963	.000	-1.6052	-.8415
Dependent variable: phosphorus						
4 months	5 months	1.4822*	.17440	.000	1.0371	1.9273
	6 months	2.9422*	.17440	.000	2.4971	3.3873
5 months	4 months	-1.4822*	.17440	.000	-1.9273	-1.0371
	6 months	1.4600*	.17440	.000	1.0149	1.9051
6 months	4 months	-2.9422*	.17440	.000	-3.3873	-2.4971
	5 months	-1.4600*	.17440	.000	-1.9051	-1.0149

Table 10. Multiple comparisons of soil nitrogen and phosphorus concentration by Tukey HSD

Dependent variable: nitrogen						
Tukey HSD						
(I) Concentration	(J) Concentration	Mean difference (I-J)	Std. error	Sig.	95% confidence interval	
					Lower bound	Upper bound
50 mg/L	10 mg/L	30.2731*	.14963	.000	29.8912	30.6549
	5 mg/L	33.5341*	.14963	.000	33.1522	33.9160
10 mg/L	50 mg/L	-30.2731*	.14963	.000	-30.6549	-29.8912
	5 mg/L	3.2610*	.14963	.000	2.8792	3.6429
5 mg/L	50 mg/L	-33.5341*	.14963	.000	-33.9160	-33.1522
	10 mg/L	-3.2610*	.14963	.000	-3.6429	-2.8792
Dependent variable: phosphorus						
50 mg/L	10 mg/L	30.7044*	.17440	.000	30.2593	31.1495
	5 mg/L	33.2800*	.17440	.000	32.8349	33.7251
10 mg/L	50 mg/L	-30.7044*	.17440	.000	-31.1495	-30.2593
	5 mg/L	2.5756*	.17440	.000	2.1305	3.0207
5 mg/L	50 mg/L	-33.2800*	.17440	.000	-33.7251	-32.8349
	10 mg/L	-2.5756*	.17440	.000	-3.0207	-2.1305

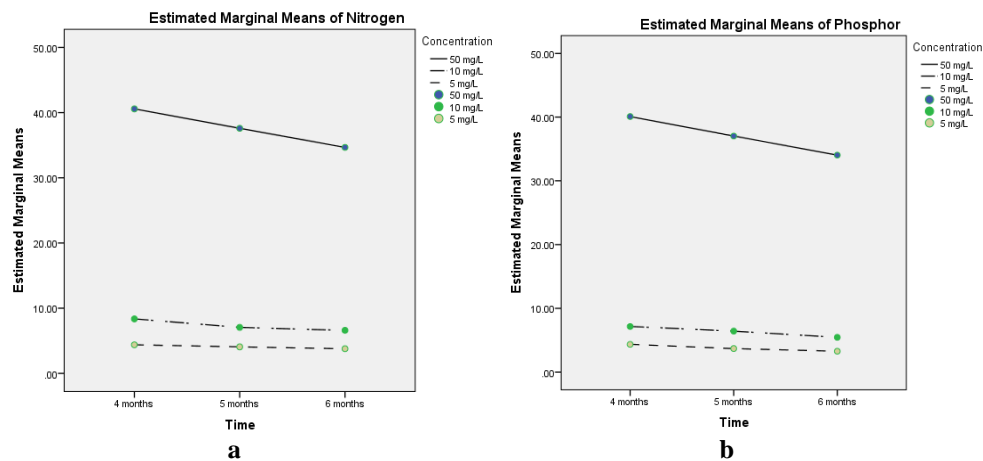


Figure 6. N (a) and P (b) removal by soil in three time periods

Discussion

Medium to long term monitoring is necessary to fully understand environmental sustainability and efficiency of RBSs. Therefore, measurements of perimeter, area, length, latitude and longitude of RBS plots along agricultural land on the banks of Zayandeh-rood River are such a helpful tool for future studies and for understanding the RBS efficiency. In this research, our focus was on plot 9 as it encompasses the largest area of buffer zone (total area = 4.3 ha). Results in this plot suggest that N and P removal occurs through an RBS. RBS seems to be more efficient in removal of P compared to N. Generally, results obtained from the RBS showed that N and P were accumulated from the beginning to the end of the farm, as there was a 0.12% increase per meter for N and a 0.50% increase per meter for P. Subsequently, at the beginning of

the root system in the mentioned trees in the RBS, a 1.38% reduction per meter for N concentration and a 0.59% reduction per meter for P concentration were observed at the beginning of the RBS. Eventually, due to the final completion of phytoremediation and the integral effect of root system in the trees in the RBS, a 0.3% reduction in N concentration and a 1.47% reduction in P concentration were measured at the end of the RBS.

It should be noted that significant reductions of these two pollutants can be due to the composition of ash, willow, poplar, buckthorn and mulberry, based on their effective phytoremediation properties (*Table 2*). Woody vegetation has deep rooting systems and is more effective in P and N removal than shrubs and grass areas (Aguiar et al., 2015). They have a significant energy advantage over grassy plants (Glibovyt'ska et al., 2019). They cover broader surface and volume of soil that make them able to accumulate higher amounts of pollutant in their bigger organs. Additionally, tree species are suggested as the most affordable vegetation type for phytoremediation with profounder root system, being appropriate to be planted in less fertile sites with a frail structure and a higher rate of transpiration (Haroni et al., 2019). The costs of phytoremediation would be reduced if other regarded vegetation are replaced with tree species (EPA, 2000). The ability of a RBS to remove N and P from water or soil is influenced by the type and quantity of existing vegetation and by soil condition (Mikkelsen and Vesho, 2000; Wang et al., 2002) Indeed, the effectiveness of phytoremediation is also affected by different contamination levels of wastewater (Assif and Saeed, 2020).

Previous studies of N and P attenuation in RBSs have indicated that they reduce suspended soil particulates considerably (Schilling and Jacobson, 2014; Aguiar et al., 2015) because of their flatter slopes and high surface roughness and in doing so, they often reduce P concentrations in streams (Mikkelsen and Vesho, 2000).

Since the greatest reduction occurs after 4 months, it could be concluded that phytoremediation is very active in early spring, and that after this period, the trees' capacity will decrease. Perhaps, it is because the root system achieves its highest function in this time period. According to Mali et al. (2019) growth stage significantly influenced the nutrient uptake, and during the winter dormant period, plant uptake is generally very low or nonexistent in RBSs (Haycock and Pinay, 1993). Groffman et al. (1992) showed that plant uptake was the dominant sink for N during growing season in RBSs. Ebrahimi et al., 2019 showed that Plant species and the growth season influence the efficiency of plant uptake that is associated with root systems properties.

According to the results obtained from soil, it is suggested that soil has an important role in N and P reduction, as well. Boz et al. (2013) claimed that soil microbial community composition and activity could be affected by suitable manipulation of the environment they live in. If correctly applied, such an approach could become a very effective way to remediate excess of chemicals. Furthermore, we could suggest that in this regard, tree root systems could provide macro-pores and create rapid inflow routes which will be used for transmission of N and P into the soil profile. Therefore, it could decrease the efficiency of Phytoremediation. Another possible explanation for the effectiveness of the root system in these two species, as they are necessarily mycorrhizal, is that the Arbuscular Mycorrhizal fungi have boosted the uptake of the N and P contaminants through the roots. According to (Bücking and Kafle, 2015), mycorrhizal fungi play a crucial role in the uptake of nutrients, such as phosphate and nitrate, in many plant species. This means that this N and P contaminant removal could have been improved by the mycorrhizal fungi and through the roots of the species

studied here. Mycorrhizal fungi could have affected the results of the current study in yet another way. According to (Kebrabadi et al., 2014), the highest colonization percentage of mycorrhizal fungi is in spring, which corresponds with the timing of this current study. The findings of the current study revealed that the highest rate of contaminant reduction was in early spring, which corresponds with the results of (Kebrabadi et al., 2014; Mali et al., 2019). According to the results of the current study, *Aianthus altissima* was more efficient than *Fraxinus rotundifolia* in reduction of P (Li et al., 2015; Yang et al., 2015), and as stated in Taheri et al. (2014), *Fraxinus rotundifolia* is an effective species for phytoremediation of N. Thus, combining these two species can be more helpful to remove excess N and P and avoid water eutrophication.

Systematic evaluation of the greenhouse studies conducted based on the results from the field study suggests that RBZs with emphasis on the phytoremediation applications are beneficial environmentally friendly technique with the potential to rectify the contamination issue due to the eutrophication caused by the agricultural runoff. Therefore, investigating the existing plant species in RBZs to determine species with higher phytoremediation capabilities, and the utilization of such results in the design of the RBZs, is of prime importance to future studies. Utilization of phytoremediation properties in buffer zones necessitates the integration of the results from background studies with agricultural skills and technique. Successful contamination remediation programs can be achieved through correct integration of the field conditions and the planting of the proper species to increase the access of the plant to the contamination, which in turn, emphasizes the need for bilateral field and greenhouse studies.

Conclusion

According to the results of the current study, when runoff passes through the buffer zone, P and N contaminations decrease significantly. Generally, the average N and P contamination reaches its highest levels at the end of the farm and the beginning of the RBS; however, the average contamination falls down to its minimum by the end of the RBS, which proved our first hypothesis, stating that RBSs are effective tools to reduce N and P contamination. As a result, we can conclude that the best approach to prevent the non-spot contamination is to create RBSs on the banks of rivers and other water resources which are vulnerable to contamination. The results of the current study revealed that an RBS, based on phytoremediation properties, may be a helpful and friendly technique to deal with the problem of agricultural runoff pollution in the process of purifying eutrophicated water.

Moreover, the findings of the current study revealed that, as Aguiar et al. (2015), Lou et al. (2017) and Seo et al. (2017) claimed, plants with deep root systems, like *Fraxinus rotundifolia* and *Aianthus altissima* are more effective in reducing N and P contaminants and Mixed cultivation can enhance the effects of phytoremediation. Due to the existing inter-species differences in phytoremediation of different contamination, biofilters using a mixture of plants are proposed (Irga et al., 2019).

Therefore, it is concluded that in creating RBSs for the purpose of contaminant removal, combining the two species of *Fraxinus rotundifolia* and *Aianthus altissima* could be an ideal and will result in effective removal of N and P contaminants. It is worth mentioning that *Fraxinus rotundifolia* is one of the most common species in this region and numerous ecological and geographical ranges are allocated to it. Moreover, it is available and inexpensive and, as the results of the current study also showed, has

effective properties in remediation of N and P. Noteworthy, *Aianthus altissima* is a species which is resistant to drought and pollution, so it could be considered as an applicable species to be planted in RBSs.

Recommendations for further research

The selection of appropriate plants for phytoremediation is an open field for study, as different combinations of woody plants with deep root systems could have different effects on N and P contaminant removal. In our current study, we examined the beneficial effect of two species of woody plants which were commonplace to the region. However, examining the N and P contaminant reduction capability of other woody species can be subject to further research.

Another area for research could be the investigation of the role of mycorrhizae fungi on the reduction or removal of N and P contaminants. The findings of this study revealed that there might be a close direct relationship between the mycorrhizae activity and the reduction of the N and P contaminants.

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APPENDIX

Appendix A. Distribution of RBS in Zayandeh-rood basin with measurements of perimeter, area, length, latitude, and longitude along agricultural land

Plot number	Perimeter (Km)	Area (Ha)	Path length (Km)	Latitude	Longitude
1	0/99	0/5	0/48	32/6414	51/5758
2	0/45	0/18	0/19	32/6421	51/5744
3	0/29	0/07	0/13	32/6413	51/5702
4	0/24	0/11	0/11	32/6424	51/572
5	0/98	0/52	0/48	32/6381	51/5644
6	0/6	0/54	0/27	32/6348	51/5622
7	0/46	0/24	0/22	32/6333	51/5628
8	0/22	0/09	0/1	32/631	51/5617
9	1/47	4/31	0/28	32/6298	51/5614
10	0/47	0/36	0/16/	32/6334	51/5628
11	2/19	2/04	0/94	32/6272	51/5656
12	0/63	0/56	0/3	32/6206	51/5672
13	0/68	0/89	0/2	32/6198	51/5665
14	0/74	1/6	0/29	32/6108	51/5668
15	0/4	0/38	0/16	32/6091	51/5661
16	0/21	0/28	0/16	32/6077	51/5664
17	0/88	0/61	0/19	32/6064	51/5661

18	3/17	3/84	1/38	32/5963	51/5645
19	0/51	0/24	0/24	32/5977	51/5653
20	0/51	0/25	0/24	32/5952	51/5599
21	0/72	0/27	0/36	32/5942	51/5596
22	0/6	0/26	0/27	32/5937	51/5554
23	0/52	0/17	0/25	32/5903	51/5252
24	0/82	0/24	0/4	32/5895	51/5486
25	0/21	0/14	0/15	32/5889	51/5459
26	0/19	0/05	0/09	32/5895	51/5449
27	0/5	0/24	0/19	32/5902	51/5422
28	0/45	0/22	0/19	32/5869	51/5387
29	1	1/05	0/32	32/5854	51/5362
30	0/13	0/09	0/06	32/5866	51/5375
31	0/22	0/07	0/1	32/586	51/5355
32	0/36	0/14	0/17	32/5854	51/534
33	4/65	2/53	2/06	32/5731	51/5267
34	0/86	0/6	0/41	32/5777	51/5267
35	1/01	0/8	0/44	32/5687	51/5206
36	3/06	3/02	1/24	32/5662	51/5223
37	0/97	1	0/36	32/5662	51/5223
38	0/21	0/2	0/09	32/5626	51/5197
39	4/27	2/09	2/1	32/6438	51/5838
40	0/46	0/24	0/2	32/6429	51/5781
41	0/19	0/9	0/04	32/6416	51/5775
42	1/15	0/86	0/53	32/6446	51/5851
43	2/04	1/27	0/91	32/6424	51/5945
44	0/29	0/25	0/13	32/6429	51/5898
45	0/53	0/38	0/25	32/6434	51/5917
46	0/66	0/53	0/3	32/6394	51/6023
47	0/81	0/41	0/4	32/6398	51/6053
48	0/94	0/61	0/42	32/6402	51/6078
49	0/53	0/44	0/23	32/6373	51/6354
50	0/4	0/22	0/16	32/6393	51/6369
51	0/35	0/23	0/14	32/6399	51/638

Appendix B. Descriptive statistics of N removal by *Aianthus altissima* in three time periods

Time	Concentration	Mean	Std. deviation	N
4 months	50 mg L ⁻¹	20.0901	.03499	3
	10 mg L ⁻¹	4.9536	.15663	3
	5 mg L ⁻¹	2.5430	.08154	3
	Total	9.1956	8.23779	9
5 months	50 mg L ⁻¹	18.3067	.33262	3
	10 mg L ⁻¹	4.5200	.09849	3
	5 mg L ⁻¹	2.0300	.07211	3
	Total	8.2856	7.59484	9
6 months	50 mg L ⁻¹	15.1933	.16803	3
	10 mg L ⁻¹	4.2000	.26458	3
	5 mg L ⁻¹	1.4000	.10000	3
	Total	6.9311	6.31631	9
Total	50 mg L ⁻¹	17.8634	2.15441	9
	10 mg L ⁻¹	4.5579	.36517	9
	5 mg L ⁻¹	1.9910	.50129	9
	Total	8.1374	7.19743	27

Appendix C. Descriptive statistics of P removal by *Aianthus altissima* in three time periods

Time	Concentration	Mean	Std. deviation	N
4 months	50 mg L ⁻¹	17.1867	.16623	3
	10 mg L ⁻¹	4.5633	.22502	3
	5 mg L ⁻¹	2.5900	.14000	3
	Total	8.1133	6.86022	9
5 months	50 mg L ⁻¹	15.1767	.25325	3
	10 mg L ⁻¹	3.8167	.17786	3
	5 mg L ⁻¹	1.6467	.12897	3
	Total	6.8800	6.29528	9
6 months	50 mg L ⁻¹	13.3233	.30436	3
	10 mg L ⁻¹	2.7667	.28746	3
	5 mg L ⁻¹	.8967	.05033	3
	Total	5.6622	5.80644	9
Total	50 mg L ⁻¹	15.2289	1.68705	9
	10 mg L ⁻¹	3.7156	.80761	9
	5 mg L ⁻¹	1.7111	.74139	9
	Total	6.8852	6.17158	27

Appendix D. Descriptive statistics of N removal by *Fraxinus rotundifolia* in three time periods

Time	Concentration	Mean	Std. deviation	N
4 months	50 mg L ⁻¹	16.0300	.16823	3
	10 mg L ⁻¹	3.8695	.05367	3
	5 mg L ⁻¹	2.0955	.09425	3
	Total	7.3317	6.56958	9
5 months	50 mg L ⁻¹	14.2867	.07767	3
	10 mg L ⁻¹	2.6533	.05508	3
	5 mg L ⁻¹	1.6167	.12014	3
	Total	6.1856	6.09288	9
6 months	50 mg L ⁻¹	13.3333	.25166	3
	10 mg L ⁻¹	2.4667	.05774	3
	5 mg L ⁻¹	1.0970	.07515	3
	Total	5.6323	5.80768	9
Total	50 mg L ⁻¹	14.5500	1.19454	9
	10 mg L ⁻¹	2.9965	.66149	9
	5 mg L ⁻¹	1.6031	.44078	9
	Total	6.3832	5.96662	27

Appendix E. Descriptive statistics of P removal by *Fraxinus rotundifolia* in three time periods

Time	Concentration	Mean	Std. deviation	N
4 months	50 mg L ⁻¹	16.4333	.08622	3
	10 mg L ⁻¹	4.0253	.02397	3
	5 mg L ⁻¹	2.5833	.20232	3
	Total	7.6807	6.59506	9
5 months	50 mg L ⁻¹	15.3633	.16653	3
	10 mg L ⁻¹	3.4533	.05508	3
	5 mg L ⁻¹	1.9500	.06000	3
	Total	6.9222	6.36489	9
6 months	50 mg L ⁻¹	14.3967	.08622	3
	10 mg L ⁻¹	3.0000	.04359	3
	5 mg L ⁻¹	1.0460	.05011	3
	Total	6.1476	6.24466	9
Total	50 mg L ⁻¹	15.3978	.88830	9
	10 mg L ⁻¹	3.4929	.44652	9
	5 mg L ⁻¹	1.8598	.67785	9
	Total	6.9168	6.18496	27

Appendix F. Descriptive statistics of N removal by soil in three time periods

Time	Concentration	Mean	Std. deviation	N
4 months	50 mg L ⁻¹	40.5748	.40448	3
	10 mg L ⁻¹	8.3589	.32265	3
	5 mg L ⁻¹	4.3858	.09633	3
	Total	17.7731	17.18955	9
5 months	50 mg L ⁻¹	37.5933	.46972	3
	10 mg L ⁻¹	7.0567	.05033	3
	5 mg L ⁻¹	4.0667	.05033	3
	Total	16.2389	16.06984	9
6 months	50 mg L ⁻¹	34.6667	.57735	3
	10 mg L ⁻¹	6.6000	.26458	3
	5 mg L ⁻¹	3.7800	.02646	3
	Total	15.0156	14.79225	9
Total	50 mg L ⁻¹	37.6116	2.59314	9
	10 mg L ⁻¹	7.3385	.81786	9
	5 mg L ⁻¹	4.0775	.26834	9
	Total	16.3425	15.46038	27

Appendix G. Descriptive statistics of P removal by soil in three time periods

Time	Concentration	Mean	Std. deviation	N
4 months	50 mg L ⁻¹	40.0833	.60575	3
	10 mg L ⁻¹	7.1567	.14844	3
	5 mg L ⁻¹	4.3467	.14572	3
	Total	17.1956	17.21188	9
5 months	50 mg L ⁻¹	37.0267	.08622	3
	10 mg L ⁻¹	6.4233	.56012	3
	5 mg L ⁻¹	3.6900	.41869	3
	Total	15.7133	16.03263	9
6 months	50 mg L ⁻¹	34.0367	.46199	3
	10 mg L ⁻¹	5.4533	.28572	3
	5 mg L ⁻¹	3.2700	.17349	3
	Total	14.2533	14.87032	9
Total	50 mg L ⁻¹	37.0489	2.64625	9
	10 mg L ⁻¹	6.3444	.80737	9
	5 mg L ⁻¹	3.7689	.52679	9
	Total	15.7207	15.48488	27