# Full Length Research Paper

# Growth of *Elaeagnus rhamnoides* seedlings under wastewater conditions

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#### Abstract

*Elaeagnus rhamnoides* (L.) A. Nelson seedlings were conducted based on completely randomized design in summer season. Non-stress and wastewater (Kashafrood River) treatment were used every two days. Morphological parameters were measured and calculated including: height, diameter, number of leaves, leaf area, shoot and root length, dry weight of roots, stem and leaf and ratios of root to shoot, leaf to shoot, leaf area, specific leaf area and leaf weight, freshly, slenderness index and number of root. Results indicated that diameter growth was significantly different at 1% and diameter mean and height growth reduced significant at the 5% level in comparison with control treatment. Analysis of variance for nonlinear regression in wastewater indicated in the height, diameter and leaf number significant differences of 1% level. Results of repeated measures analysis indicated that effect of time multiplied by treatment interaction for diameter, number of leaves and leaf area was not significant but for height had significant difference at 1% level. Principal component analysis (PCA) showed that in first axis (dry weights leaf and stem, total dry weight, freshly, leaf area, root length and number of root) and second axis (dry weight root, root to shoot ratio, leaf area ratio, specific leaf area, leaf weight ratio and stem length) in wastewater treatment were the most correlated with axis's. Thus, recommend that to be done irrigation with wastewater of Kashafrood River.

Key words: Sea buckthorn, Iran, Kashafrood, Wastewater, Repeated measures, Nursery irrigation

# Introduction

Sea Buckthorn (*Hippophae rhamnoides* L.) from Elaeagnaceae family has become a crop of interest for the food processing industry. Accepted name in the plant list org of this species is *Elaeagnus rhamnoides* (L.) A. Nelson. The exact number of species in the genus Hippophae is still unclear however, there are considered to be seven species and *Hippophae rhamnoides* has nine subspecies (Lu and Ahani, 2013). *Hippophae rhamnoides*, also known as common sea buckthorn is a species of flowering plant, native to the

cold-temperate regions of Europe and Asia. It is a spiny deciduous shrub. The plant is used in the food and cosmetic industry, in traditional medicine (useful for the treatment of skin disorders resulting from bed confinement. stomach and duodenal ulcers cardiovascular diseases and perhaps growth of some tumors), as animal fodder and for ecological purposes. The plants have a very developed and extensive root system, and the roots live in symbiosis with nitrogenfixing Frankia bacteria. The roots also transform insoluble organic and mineral matters from the soil into more soluble states (Lu, 1992). Vegetative reproduction of the plants occurs rapidly via root suckers (Kondrashov and Kuimov, 1987). *E. rhamnoides* has a strong ability to maintain leaf water and can increase chlorophyll content, reduce the photosynthesis and water relations during drought stress (Ahani et al, 2014, 2015). Seed germination at its lowest point of origin China with 32% and the most was in East Azerbaijan with 95% (Ahani et al, 2016). Means of germination percent in seed pretreatments (control, cold, ice water, hot water, lime juice and Gibberellin acid) were 7.5, 23.75, 21.25, 0, 15, and 42.5 in field (Ahani et al, 2014<sup>b</sup>) and 3.75, 43.75, 17.5, 1.25, 15 and 37.5 in greenhouse (Ahani et al, 2014<sup>a</sup>) and in laboratory were 33, 12, 41, 4, 9 and 32, respectively (Ahani et al, 2015).

Many factors, both biotic (pathogens, insects, nematodes) and abiotic (e.g., wounds, pollutants, thermal, water and nutritional imbalances, environmental contaminants) are causes of plant stress and can decrease plant growth and productivity. Plants can react to these stressors through a series of constitutive and/or inductive mechanisms which result in the elimination or the limitation of the negative effects induced by the adverse factors (Vitti et al., 2013). Non-soluble compounds and soluble compounds are the two kinds of pollutants encountered in waste-water. The treatment of the first is achieved easily by mechanical separating processes e.g. sedimentation, flocculation, flotation, hydrocycloning and filtration. On the other hand, the treatment of soluble compounds is more difficult. The conventional solutions used at present are: biological processes (but these are not suitable for toxic pollutants); adsorption (but only for low concentrations); oxida- tion (but this is very expensive for dilute organic compounds); membrane processes (but low cut-off molecular weight is needed for the removal of soluble compounds). The objective of this paper is to present freezing as a new solution for soluble pollutant treatment (Lorain et al., 2001).

The most widely used phytotoxicity test involving terrestrial plants is the seed germination test, which measures germination rate and/or root elongation as the endpoints (Wang and Keturi, 1990; Devare and Bahadir, 1994; Tam and Tiquia, 1994; Pascual et al., 1997). Trees are not good toxicity indicators owing to their lower and more varied sensitivity to most toxic substances (Cheng and Chu, 2007).

Iron and steel-producing industries generate a huge amount of various industrial wastewaters containing toxic organic and/or inorganic compounds (Mo Kim et al., 2008).

All plants have the ability to accumulate essential metals (Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Se, V and Zn) from the soil solution. Plants need different concentrations for growth and development. This ability also allows plants to accumulate other non-essential metals (Al, As, Au, Cd, Cr, Hg, Pb, Pd, Pt, Sb, Te, Tl and U) which have no known biological function (Djingova and Kuleff, 2000). Forest decline and decay is one of the most significant ecological problems in many countries. Besides biological and chemical causes of forest decline and decay, a special problem is of technological cause, especially the storage of municipal and industry wastewater (Pavlica et al., 2000).

Efficient removal of Cu, Zn, As, Cd, Cr, Fe, Pb, Hg and Ni has been achieved with low-cost adsorbents in bench scale such as: biomass waste from biological wastewater treatment system (Al-Qodah, 2006; Chang et al., 2006); pollens (Ucun et al., 2003); teak leaves powder (King et al., 2006); black gram husk (Saeed and lqbal, 2003); fly ash (Wang and Wu, 2006); wood barks (Genc-Fuhrman et al., 2007; Shin et al., 2007). Tabari and Salehi (2009) showed municipal wastewater could be utilized as an important source of water and nutrients in growing Eldar Pine (*Pinus eldarica* Medw.) trees. Tabatabaei et al. (2014) were founded wastewater of Kashafrood River causes growing *Celtis caucasica* seedlings.

In wastewater irrigation, the criteria often included limits on suspended solids, biological oxygen demand (BOD), chemical oxygen demand (COD) and organic chemicals (oil and grease, trichloroacetylaldehyde, petroleum hydrocarbon, and detergent residues). Numerical limits for these chemicals were included probably because of their potential effects on operation and maintenance (BOD, suspended solids, and oil and grease), groundwater pollution, (petroleum hydrocarbon and benzene), or plant growth (petroleum hydrocarbon, detergent residues, etc.) (Chang *et al.*, 2002).

Wastewater can be considered as both a resource and a problem. Wastewater and its nutrient content can be used extensively for irrigation and other ecosystem services. Its reuse can deliver positive benefits to the farming community, society, and municipalities. However, wastewater reuse also exacts negative externality effects on humans and ecological systems, which need to be identified and assessed (Hussain et al., 2002).

This research was done on the morphological traits of *Elaeagnus rhamnoides* (L.) A. Nelson and the objectives of this study are to determine to investigate the effects of wastewater stress on the morphological traits and growth of these seedlings.

# **Material and Methods**

# Plant materials and salt-stress treatments

Discover River North East Mashhad, Iran is due to runoff and urban wastewaters are contaminated. Phytoremediation or the use of plants to reduce pollution, in addition to the low cost and usability treating a wide range of eco cognition is considered.

In this study the effect of wastewater of Kashafrood River on morphological characteristics Elaeagnus rhamnoides species that seed from accessions were used in Qazvin origin of Iran to produce the mother plants. The four months old seedlings were grown in a natural environment for the rest of the experiment in the field of Torogh nursery of Mashhad city. Seedlings after transfer to pots containing sandy loam, up to two months in terms of the usual nursery of irrigation were in the well water treatment (every two days). Then physical experiments for determining relative particles (percentage of clay, silt and sand) were performed using the hydrometer Baykas on particles smaller than two mm. due to soil laboratory experiment of Research Center of Khorasan Razavi province (Table 1).

Soil	properties	6								
рН	EC (ds/m)	bulk density (g/m³)	Soil texture	San d (%)	Silt (%)	Clay (%)	Organic matter (%)	Moisture (%)	P (mg/kg)	K⁺ (mg/kg)
7.4	6.61	1.42	Sandy Ioam	67	23	10	1.04	9.8	54.8	353

From early summer treatments was conducted on *E. rhamnoides* seedlings to the water pollutant conditions in table 2 and non-stress (were watered to 100% of field capacity by supplying an amount of water by weighting whole pot of each treatment equal to transpiration losses

every other day, each time 150 ml.day<sup>-1</sup>.). This study was conducted in a completely randomized design with twenty replicates in control and thirty replicates in wastewater during the summer.

		Tabl	e 2: Chara	acteristic	s of wast	ewater in	use irrigat	ion		
Constituents	Ca (mg/l)	Mg (mg/l)	Na (mg/l)	TSS (mg/l)	B (mg/l)	As (mg/l)	COD (mg/l)	BOD (mg/l)	Cr <sup>+6</sup> (mg/l)	pН
Kashafrood wastewater	55.54	87.73	947.08	189	0.44	29.58	374.025	122.58	0.054	8.38
EPA*	200	25	3	5	1	0.1	120	30	0.1	6.5-8.4

\*EPA: Environmental Protection Agency

# Plant growth and analysis

Stem growth characteristics were every ten days monitored by measuring length (from substrate surface to the apical meristem), diameter (at the base) and by counting the number of leaves and leaf area per month, so that the base of each treatment, five selection seedling and each seedling five leaf selection of basic randomized to using AutoCAD 2013 software in meters squared size was measured. Three plants were removed from the pots at the end of the experiment in early fall and divided into the aboveground part (leaves and stem) and belowground part (roots), then the fresh weight were taken at electrical balance in grams. Biomass samples were then placed in an oven run at (70°C, 48 h) up to constant weight. (Yin et al. 2005; Sapeta et al. 2013; Howladar, 2014). These dried plants were weighed to record the plant dry mass. After the plants were cut and separated into different parts, the samples were obtained for morphological analyses (table 3).

#### **Table 3:** Index of morphological trait specification

Indices	Formula	References			
Root to shoot ratio (R/S)	root dry weight shoot dry weight	Yin et al. 2004			
Leaves to shoot ratio (L/S)	leave dry weight shoot dry weight	Rad et al. 2011			
Leaf area ratio	leaf area total dry weight plant				
Specific leaf area	leaf area total dry weight leaf				
Leaf weight ratio	total dry weight leaf total dry weight plant	Karimi and Azizi, 1994			
freshly	fresh weight up ground-dry weight up ground fresh weight up ground				
Slenderness index	height average diameter average	Taheri Abkenar and Pilehvar, 2009			

#### Statistical analysis and processing

The experiment was arranged in the base of completely The normality randomized design. tested bv (Kolmogorov-Smirnov) using SPSS version 19, data collected were subjected to correlation between various t-independent parameters and comparison of characteristics between the two measurements was used for stress. Pearson correlation coefficients, replication analysis measurements of height, diameter and leaf number in six and leaf area in two times calculated with four different multivariate tests (Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace and Roy's Greatest Root) and ANOVA of nonlinear regression using the statistical software SAS version of the 9.1. Using PC-ORD software version 4.17 to principal component analysis (PCA). Diagrams were drawn using the Excel software for growth average between height, diameter, leaf number and leaf area was conducted by stress condition initiation until end of the experiment.

# Results

Height, Diameter and Number of leaves trait of the end of the season, Height and number of leaves mean and changes in the total number of green leaves an d height growth in two treatments (Control and Wastewater) showed a positive correlation with the diameter mean at one percent level (p=0.0001). Slenderness index average and end of the growth slenderness index for control at five percent level but in wastewater stress is not correlated (p=0.0179-p=0.9387 and p=0.0252-p=0.1535 respectively) (Table 4).

 Table 4: Correlation coefficients among some morphological traits with diameter mean under two treatments

Treatment		h6	h	hm	d6	(h/d)m	(h/d)6	nl6	nl	nlm
Control	Diameter	0.86**	0.83**	0.86**	0.99**	0.52*	0.49*	0.88**	0.82**	0.81**
Wastewater	mean	0.79**	0.57**	0.81**	0.99**	-0.01 <sup>ns</sup>	0.26 <sup>ns</sup>	0.81**	0.71**	0.80**

h6: End of the period height, hm: Height mean, d6: End of the period diameter, (h/d) m: Slenderness index mean, (h/d) 6: End of the slenderness index, nl6: End of the period No. of leaves, nl: No. of leaves, nlm: No. of leaves mean. <sup>ns</sup>: Not significant, \* p<0.05 and \*\* p<0.01.

Results of the t-independent test comparing the two treatments (control- wastewater) was indicated that diameter growth at the one percent level (p=0.006) and diameter mean, height growth and height mean were

significant at the five percent level (p=0.012, p=0.025 and p=0.044 respectively) and other traits not significant (Table 5).

Table 5: Independent samples test comparison; Mean±SE

Morphology traits	Control mean	Wastewater mean	Mean difference	t-value
Diameter growth	0.12±0.02	0.21±0.02	-0.09±0.03	-2.85**
End of period diameter	1.34±0.10	1.58±0.08	-0.25±0.12	-1.91 <sup>ns</sup>
Diameter mean	1.28±0.09	1.48±0.08	-0.19±0.12	-1.57*
Height growth	9.35±1.38	5.43±1.03	3.92±1.69	2.31*
End of period height	19.22±2.24	17.06±1.40	2.16±2.50	0.86 <sup>ns</sup>
Height mean	18.94±2.24	14.46±0.95	4.48±2.16	2.07*
No. of leaves	17.75±3.53	17.43±3.95	0.32±5.64	0.56 <sup>n</sup>
End of period no. of leaves	45.90±7.57	47.03±6.89	-1.13±10.47	-0.11 <sup>ns</sup>
Mean no. of leaves	37.47±6.11	39.87±5.42	-2.39±8.30	-0.28 <sup>ns</sup>
Leaf area growth	4.26×10 <sup>-3</sup> ±1.66×10 <sup>-</sup> <sup>3</sup>	9.99×10 <sup>-3</sup> ±1.99×10 <sup>-3</sup>	-5.73×10 <sup>-3</sup> ±2.59×10 <sup>-</sup> <sup>3</sup>	-2.21 <sup>ns</sup>
End of period leaf area	1.11×10 <sup>-2</sup> ±3.31×10 <sup>-</sup> <sup>3</sup>	1.96×10 <sup>-2</sup> ±3.68×10 <sup>-3</sup>	-8.54×10 <sup>-3</sup> ±4.95×10 <sup>-</sup> <sup>3</sup>	-1.72 <sup>ns</sup>
Leaf area mean	8.96×10 <sup>-3</sup> ±2.66×10 <sup>-</sup> <sup>3</sup>	1.46×10 <sup>-2</sup> ±2.78×10 <sup>-3</sup>	-5.67×10 <sup>-3</sup> ±3.85×10 <sup>-</sup> <sup>3</sup>	-1.47 <sup>ns</sup>

ns: not significant. \* p<0.05 and \*\* p<0.01.

Repeated measures analysis of such statistics, Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace and Roy's Greatest Root of the time multiplied by treatment morphology traits of the measures indicated that the diameter, number of leaves and leaf area of the controlwastewater treatment there is no significant difference (p=0.0564, p=0.3447 and p=0.0754 respectively) but in height showed significant differences at the one percent level (p=0.0001) (Table 6).

Table 6: Effect treatmentxtime morphological traits control-Wastewater treatment

Morphological traits	Wilks' Lambda	Pillai's Trace	Hotelling-Lawley Trace	Roy's Greatest Root
Height	0.46	0.54	1.17	1.17
Height	8.39**	8.39**	8.39**	8.39**
Diameter	0.76	0.24	0.31	0.31
Diameter	2.25 <sup>ns</sup>	2.25 <sup>ns</sup>	2.25 <sup>ns</sup>	2.25 <sup>ns</sup>
No. of loover	0.84	0.15	0.17	0.17
No. of leaves	1.16 <sup> ns</sup>	1.16 <sup> ns</sup>	1.16 <sup> ns</sup>	1.16 <sup> ns</sup>
Lasfaraa	0.66	0.34	0.52	0.52
Leaf area	4.17 <sup>ns</sup>	4.17 <sup>ns</sup>	4.17 <sup>ns</sup>	4.17 <sup>ns</sup>

ns: Not significant and \*\* p<0.01. The number of second rows for each term showed statistics equivalent F.

Repeated measures analysis of such statistics, Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace and Roy's Greatest Root of the time indicated that the all

morphology traits of the measures there is significant difference at the one percent level (p=0.0001) (Table 7).

Morphological traits	Wilks' Lambda	Pillai's Trace	Hotelling-Lawley Trace	Roy's Greatest Root
Hoight	0.22	0.77	3.51	3.51
Height	25.19**	25.19**	25.19**	25.19**
Diamatan	0.28	0.71	2.51	2.52
Diameter	18.12**	18.12**	18.12**	18.12**
No. of looved	0.25	0.74	2.98	2.98
No. of leaves	19.88 **	19.88 **	19.88 **	19.88 **
Lasfaras	0.11	0.88	7.94	7.94
Leaf area	63.53 **	63.53 **	63.53 **	63.53 **

Table 7: Effect time morphological traits control-Wastewater treatment

\*\* p<0.01. The number of second rows for each term showed statistics equivalent F.

Final nonlinear regression model, the coefficient of determination, correlation coefficient, standard deviation error and variance inflation factor without stress and wastewater stresses treatments for morphological traits characters of height, diameter and leaf number has been shown is in Table 8.

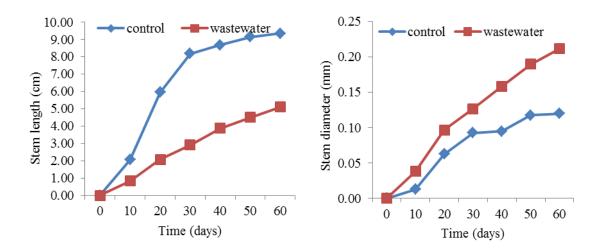
Morphological traits	Treatment	Regression	R <sup>2</sup>	R	Coef var	Std. error	VIF
Height	Control	y =-0.3551x <sup>2</sup> +4.4467x -4.4929	98.17%	0.99	38.96	1.14	1.00
пеідпі	Wastewater	$y = -0.0464x^2 + 1.244x - 1.2952$	99.74%	0.99	49.67	0.65	1.00
Diameter	Control	$y = -0.0029x^2 + 0.0446x - 0.0493$	96.67%	0.98	39.62	0.02	1.00
Diameter	Wastewater	$y = -0.0029x^2 + 0.0446x - 0.0493$	96.67%	0.98	42.48	0.02	1.00
No. of looved	Control	$y = -0.2702x^2 + 5.3298x - 5.6929$	94.04%	0.96	50.79	2.47	1.00
No. of leaves	Wastewater	$y = -0.2349x^2 + 4.9794x - 6.0095$	91.50%	0.95	58.89	2.58	1.00

Table 8: Nonlinear regression model growth for morphological traits

x= Measurement time and y= Height, diameter and No. of leaves traits

# Variance Inflation Factor (VIF)

Polynomial growth curve with a slope showed, reducing the impact of wastewater on growth traits height, diameter and leaf number and increase leaf area compared with control (Figure 1). End of the growth period showed height and diameter growth and leaf numbers (the numbers of leaf were less in treatment than the control) in wastewater stress value of decrease but showed that no significant beginning to end of the experiment between the two treatment groups, But in total height and diameter growth and height means indicated decrease significantly (Fvalue = 5.33, p=0.0253, Fvalue= 8.12, p=0.0064 and Fvalue= 4.29, p=0.0437 respectively).



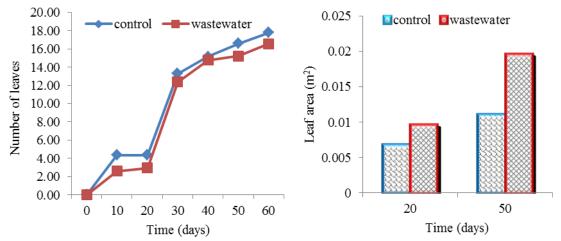


Figure 1: Average growth rates over time for stem length and diameter, leaf number and leaf area of (*Elaeagnus rhamnoides* (L.) A. Nelson) subjected to varying effect of salinity stress. Day 0 represents the first day the experimental treatments were applied

Analysis of variance for nonlinear regression in measurement two months in seven times indicated that height, diameter and number of leaves trait in control and wastewater stress significant differences of one percent (p=0.0028-p=0.0001, p=0.0006-p=0.0001 and p=0.0006-p=0.0011 respectively) (Table 9).

Table 9: ANOVA results of nonlinear regression model growth for morphological traits

Treatment	Mean Square	F-value
Control	72.24	29.77**
Wastewater	21.33	443.48**
Control	0.01	55.35**
Wastewater	0.03	198.40**
Control	280.98	57.76**
Wastewater	269.14	44.68**
	Control Wastewater Control Wastewater Control	Control72.24Wastewater21.33Control0.01Wastewater0.03Control280.98

First and second axis in treatments, Eigen value treatments is greater than the statistics Broken-Stick a result of the first and second axis of variation accounted

for a significant share of the mean and of the use is analysis and other axes are not significant (Table 10).

Table 10: Variance extracted of control- Waste	water treatment
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Treatment	Axis	Eigen value	Percent of Variance	Broken-stick Eigen value
Control	1	10.158	72.556	3.252
Control	2	3.842	27.444	2.252
Montowator	1	9.118	65.129	3.252
Wastewater	2	4.882	34.871	2.252

Variables that have the greatest equity without positive and negative symptoms are known as the most important variable that is correlated with the axis and so are the next variables. According to table 7 associated with non-stress the variables (dry weight leaf and stem, leaf area, leaf to shoot ratio, leaf area ratio, specific leaf area and leaf weight ratio) have most correlated with the first axis (PC<sub>1</sub>) as a result, the largest share of mining this axis and (dry weight root, total dry weight, freshly and root length) variables were the most correlated with the second axis (PC<sub>2</sub>). In wastewater stress the variables (dry weights leaf and stem, total dry weight, freshly, leaf area, root length and number of root) in the first axis (PC<sub>1</sub>) and (dry weight root, root to shoot ratio, leaf area ratio, specific leaf area, leaf weight ratio and stem length) variables were the most correlated with the second axis (PC<sub>2</sub>). Positive and negative symptoms value of special, to show the axis of extraction relationship between variable of interest positive and negative, respectively (Table 11).

Table 11: Principle Component Analysis coefficients of morphological trait

Morphological trait	Control		Wastewate	ər
Morphological trait	PC <sub>1</sub>	PC <sub>2</sub>	PC1	PC <sub>2</sub>
dry weight leaf	-0.3119*	-0.0552	-0.3312*	0.0012
dry weight stem	0.3127*	-0.0428	-0.3156*	-0.1369
dry weight root	0.1846	-0.4125*	-0.2242	-0.3331*
total dry weight	0.0932	-0.4872*	-0.3092*	-0.1622
freshly	0.0615	0.5003*	-0.3107*	0.1568
leaf area	0.3096*	-0.0825	-0.3196*	0.1187
root to shoot ratio	-0.2611	-0.2828	-0.2096	-0.3504*
leaf to shoot ratio	-0.3126*	0.0438	-0.2901	0.2183
leaf area ratio	0.3137*	-0.0062	-0.1211	0.4212*
specific leaf area	0.3137*	0.0048	-0.1759	0.3835*
leaf weight ratio	-0.3091*	0.0875	-0.0536	0.4466*
stem length	0.2918	-0.1874	-0.2465	-0.3023*
Root length	-0.1888	-0.4075*	-0.3288*	-0.0539
No. of root	-0.2888	-0.1994	-0.3174*	0.1291

\*Eigen value more than 0.3

#### Discussion

Tabatabaei et al. (2014) showed *Celtis caucasuca* Willd seedlings in wastewater of Kashafrood river treatment was causes reduce root length and dry weight root, freshly, total dry weight, number of root, root to shoot ratio, leaf are ratio, specific leaf area and stem length increase significantly. Analysis of variance for nonlinear regression that indicated height, diameter and leaf number showed in wastewater stress significant differences at the 1% level. The application of wastewater had positive influence on the growth of *Celtis caucasuca* trees.

In this study it was observed that the diameter and leaf area in wastewater compared to the control increase significantly; but height growth and number of leaves is decrease.

Shoot seedling length was reduced after metal treatments. This could be because of reduction in meristematic cells present in the region and some cotyledonary and endosperm enzymes which became active and began to digest the stored food. This resulted in not proper supply of food to the radical and plumule (Madhvi et al., 2014). The increased growth may be linked to sufficient availability of water and better status of nutrients in soil (Tabari and Salehi, 2009). Shoots and leaves when water is supplied to the roots, as they continue to grow. Also materials provide the root from the shoot, as they continue to grow (Jalili Marandi, 2010).

While wastewater and sewage sludge provide water and/or nutrients for plant growth, they are also reservoirs of potential pollutants (Chang et al., 2002). The amounts of pollutant extracted by the plants are considerably smaller, relative to inputs from irrigation. The trace elements will accumulate in soils with irrigation, unless their concentrations are considerably lower that the numerical limits or they are leached below the root zone (Chang and Page, 2000).

The size, morphology, or architecture of a root system may control the relative size and growth rate of the shoot. Optimum root systems throughout the plant life cycle can ensure optimum shoot growth, shoot development, and subsequent yields (Leskovar and Stoffella, 1995).Understanding the relationships of root and shoot components to stand establishment, particularly as influenced by abiotic stresses, is essential for developing optimum cultural and management techniques for field planting systems. Detecting differences in root growth patterns and architecture between genotypes may offer unique genetic selection criteria for tolerance to root diseases and pests, lodging, drought, flooding, stressful root-zone temperatures, or edaphic adaptations (Leskovar and Stoffella, 1995). Seedlings have a high demand for mineral nutrients, which, in part, is thought to be a result of their fast growth rates relative to mature plants (Widders and Lorenz, 1982). A strategy used to facilitate root growth, minimize transplant shock, and increase seedling survival is to enhance the seedling nutrient content before transplanting (Dufault, 1986).

The increase in root length can be attributed to absence of nutrient in rainwater leading to the plants rooting deeper into soil for nutrient (Ekanayake et al., 1985). Overall, the nutrient availability has positive influence on the plant survival, height, numbers of leaf and leaf area. The wastewater also increased the organic matter and exchangeable nutrient contents in soil enhancing the growth of plants (Thapliyal et al., 2011). Wastewater is a rich source of plant food nutrients (Hussain et al., 2002).

# Conclusion

To water pollution which discharge industrial wastewater, causing a large amount of nutrients, toxic, pathogens causing even suspended matter in the fields of water use, the can create a thin layer on the water surface, in addition to unsightly consume dissolved oxygen in the water and fish in the river disappear. The study showed that despite of decrease in morphological characteristics, this species has been able to tolerance this amount of wastewater and no died that showed comparative tolerance this species however need to do other tests because the morphological indicators studied here were not able to clearly explain the differences in water pollutant tolerance and need to analyze Physiology parameter (i.e. Water Use Efficiency (WUE), Relative Water Content (RWC), Water Potential (WP), Water Saturation Deficit (WSD) Chlorophyll content, Photosynthetic of leaves, moisture, ash, fat, protein, fiber and carbohydrate).

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#### References

- Ahani, H., Jalilvand, H., Vaezi, J., Sadati, SE., Jia, D., Bai, X.T. and Bagheri, H. (2015). DNA quality and quantity of Sea Buckthorn (*Hippophae rhamnoides*) subspecies in Iran and China, 7th conference of International Seabuckthorn Association, India.-Ahani, H., Jalilvand, H. and Vaezi, V. (2014<sup>a</sup>). Greenhouse Treatments on *Elaeagnus rhamnoides* Seed Germination, Research Journal of Environmental Sciences, 8(4): 215-224, DOI: 10.3923/rjes.
- Ahani, H., Jalilvand, H., Vaezi, J. and Sadati, S.E. (2014<sup>b</sup>). Investigation of nursery treatments on sea buckthorn (*Elaeagnus rhamnoides* (L.) A. Nelson) seed germination in the field, Journal of Biodiversity and Environmental Sciences., (6)4: 18-8.
- Ahani, H., Jalilvand H., Vaezi J. and Sadati S.E., (2014). Effects of different water stress on photosynthesis and chlorophyll content of *Elaeagnus rhamnoides*, Iranian Journal of Plant Physiology,5(3): 1403-1409.
- Ahani, H., Jalilvand H., Vaezi J. and Sadati S.E., (2014). Physiological Response of Sea Buckthorn (*Elaeagnus rhamnoides* (L.) A. Nelson) to Water-Use Strategie. ECOPERSIA 2(3). 681-695.
- Ahani, H., Jalilvand H., Vaezi J. and Sadati S.E., (2015. Studying the seed germination traits of Seabuckthorn (*Elaeagnus rhamnoides*) of Iran, China and Tibet, Journal of forest and wood products (Iranian journal of Natural resources), 69(2): 225-235.
- Ahani, H., Jalilvand H., Vaezi J. and Sadati S.E., (2016). Effect of different treatments on *Hippophae rhamnoides* seed germination in laboratory, Iranian Journal of Forest, 7(1)3:45-56.
- Al-Qodah, Z. 2006). Biosorption of heavy metal ions from aqueous solutions by activated sludge. Desalination 196, 164–176.
- Chang, A. C. and A. L. Page. (2000). Trace Elements Slowly Accumulating, Depleting in Soils. California Agriculture 54(2):49 – 55.
- Chang, A.C., Pan, G., Page, A.L. and Asano, T. (2002). Developing Human Health-related Chemical Guidelines for Reclaimed Water and Sewage Sludge Applications in Agriculture, Prepared for World Health Organization, 105p.
- Chang, W.C., Hsu, G.S., Chiang, S.M. and Su, M.C. (2006). Heavy metal removal from aqueous solution by wasted biomass from a combined AS-biofilm process. Bioresour. Technol. 97, 1503–1508.
- Cheng, C.Y. and Chu, L.M. (2007). Phytotoxicity data safeguard the performance of the recipient plants in leachate irrigation. Environmental Pollution, 145:195-202. doi:10.1016/j.envpol.2006.03.020.
- Devare, M. and Bahadir, M. (1994). Biological monitoring of landfill leachate using plants and luminescent bacteria. Chemosphere 28, 261-271.
- Djingova, R. and Kuleff, I. (2000). Instrumental techniques for trace analysis. In: Trace elements: Their distribution and effects in the environment. Vernet, J.P. (eds) Elsevier Science Ltd., United Kingdom, pp. 146.
- Dufault, R.J. (1986). Influence of nutritional conditioning on muskmelon transplant quality and early yield. J. Amer. Soc. Hort. Sci. 111:698–703.
- Ekanayake, I.J., Toole, J.C.O., Garrity, D.P. and Jan, T.M.M. (1985). Inheritance of root characters and their relations to drought resistance in rice. Crop Sci., 25, 927-933.

- Genc-Fuhrman, H., Mikkelsen, P.S. and Ledin, A. (2007). Simultaneous removal of As, Cd, Cr, Cu, Ni, and Zn from storm water: experimental comparison of 11 different sorbents. Water Res. 41, 591–602.
- Howladar, SM. (2014). A novel Moringa oleifera leaf extract can mitigate the stress effects of salinity and cadmium in bean (*Phaseolus vulgaris* L.) plants. Ecotoxicology and Environmental Safety 100, 69–75. http://dx.doi.org/10.1016/j.ecoenv.2013.11.022.
- Hussain I., Raschid, L., Hanjra, M.A., Marikar; F. and van der Hoek, W. (2002). Wastewater use in agriculture: Review of impacts and methodological issues in valuing impacts. (With an extended list of bibliographical references). Working Paper 37. Colombo, Sri Lanka: International Water Management Institute.
- Jalili Marandi, R. (2010). Environmental stress physiology and mechanisms of resistance in horticultural plants. Urumie, Jahad University Press, 636 pages.
- Karimi, M. and Azizi, M. (1994). Analysis of crop growth. Jahad Mashhad University Press, pp. 111.
- King, P., Srinivas, P., Prasanna Kumar, Y. and Prasad, V.S.R.K. (2006). Sorption of copper (II) ions from aqueous solution by *Tectona grandis* L.F. (teak leaves powder). J. Hazard. Mater. 136, 560–566.
- Kondrashov, V.T. and Kuimov, V.N. (1987). Vegetative propagation of *Hippophae rhamnoides*. Sadovodstvo. 6: 13–16.
- Leskovar, D.I. and Stoffella, P.J. (1995). Vegetable Seedling Root Systems: Morphology, Development, and Importance. HortScience, 30(6): 1153-1159.
- Lorain, O., Thiebaud, P., Badorc, E. and Aurelle, Y. (2001). Potential of Freezing in Wastewater Treatment: Soluble Pollutant Applications. Wat. Res. 35(2): 541-547.
- Lu, R. (1992). Sea buckthorn: A multipurpose plant species for fragile mountains. ICIMOD Occasional Paper No. 20. International Centre for Integrated Mountain Development, Katmandu, Nepal.
- Lu, R. and Ahani, H. (2013). The Genetic Resources of *Hippophae* genus and its utilization, Adv. Agr. Biol., 1(2): 27-31.
- Madhvi, S., Sharma, K.C. and Manju, S. (2014). Study of Morphological Characteristics of Spinach Irrigated with Industrial waste water of Bhiwadi, Rajasthan, India. International Research Journal of Environment Sciences, 3(3): 31-38.
- Mo Kim, Y., Park, D., Ok Jeon, Ch., Sung Lee, D. and Moon Park, J. (2008). Effect of HRT on the biological predenitrification process for the simultaneous removal of toxic pollutants from cokes wastewater. Bioresource Technology, 99: 8824–8832. doi:10.1016/j.biortech.2008.04.050.
- Pascual, J.A., Ayuso, M., Garcia, C. and Herna ndez, T. (1997). Characterization of urban wastes according to fertility and phytotoxicity parameters. Waste Management and Research 15, 103-112.
- Pavlica, M., Besendorfer, V., Rosa, J. and Papes, D. (2000). The cytotoxic effect of wastewater from the phosphoric gypsum depot on common oak (*Quercus robur* L.) and shallot (*Allium cepa* var. *ascalonicum*). Chemosphere, 41:1519-1527.
- Rad, M.H., Assare, M.H., Meshkat, M.A. and Soltani, M. (2011). Effects of drought stress on biomass, several growth parameters and water use efficiency of eucalyptus (*Eucalyptus camaldulensis* Dehnh) in response to drought stress. Iranian Journal of Rangelands and Forests Plant Breeding and Genetic Research, 19(1): 13-27.
- Saeed, A. and Iqbal, M. (2003). Bioremoval of cadmium from aqueous solution by black gram husk (*Cicer arientinum*). Water Res. 37, 3472–3480.
- Sapeta, H., Costa, J.M., Lourenco, T., Maroco, J., van der Linde, P. and Oliveira, M.M. (2013). Drought stress

response in *Jatropha curcas*: Growth and physiology. Environmental and Experimental Botany 85: 76-84. - doi:10.1016/j.envexpbot.2012.08.012.

- Shin, E.W., Karthikeyan, K.G. and Tshabalala, M.A. (2007). Sorption mechanism of cadmium on juniper bark and wood. Bioresour. Technol. 98, 588–594.
- Tabari, M. and Salehi, A. (2009). The Use of Municipal Wastewater in Afforestation: Effects on Soil Properties and Eldar Pine Trees. Polish J. of Environ. Stud., 18(6): 1113-1121.
- Tabatabaei, S.A.H., Jalilvand, H. and Ahani, H. (2014). Study of Morphological Characteristics of Caucasian Hackberry Irrigated with Wastewater of Kashafrood River. International Journal of Scientific Research in Environmental Sciences, 2(11): 389-396.
- Taheri Abkenar, K. and Pilehvar, B. (2009). Silviculture with a dictionary of silviculture (French-English to Persian Equivalents). Haghshenass publication. 223p.
- Tam, N.F.Y. and Tiquia, S. (1994). Assessing toxicity of spent pig litter using a seed germination technique. Resources Conservation and Recycling 11, 261-274.
- Thapliyal, A., Vasudevan, P., Dastidar, M.G., Tandon, M. and Mishra, S. (2011). Irrigation with domestic wastewater: Responses on growth and yield of ladyfinger *Abelmoschus esculentus* and on soil nutrients. Journal of Environmental Biology, 32, 645-651.
- Ucun, H., Kemal Bahyan, Y., Kaya, Y., Cakici, A. and Faruk Algur, O. (2003). Biosorption of lead (II) from aqueous solution by cone biomass of *Pinus sylvestris*. Desalination 154, 233–238.
- Vitti, A., Nuzzaci, M., Scopa, A., Tataranni, G., Remans, T., Vangronsveld, J. and Sofo, A. (2013). Auxin and Cytokinin Metabolism and Root Morphological Modifications in Arabidopsis thaliana Seedlings Infected with Cucumber mosaic virus (CMV) or Exposed to Cadmium. International Journal of Molecular Sciences, 14, 6889-6902. doi: 10.3390/ijms14046889.
- Wang, S. and Wu, H. (2006). Environmental-benign utilization of fly ash as low-cost adsorbents. J. Hazard. Mater. 136 (3), 482–501.
- Wang, W. and Keturi, P.H. (1990). Comparative seed germination tests using ten plant species for toxicity assessment of a metal engraving effluent sample. Water, Air, and Soil Pollution 52, 369-376.
- Widders, I.E. and Lorenz, O.A. (1982). Potassium nutrition during tomato plant development. J. Amer. Soc. Hort. Sci. 107:960–964.
- Yin, C., Wang, X., Duan, B., Luo, J. and Li, C. (2005). Early growth, dry matter allocation and water use efficiency of two sympatric *Populus* species as affected by water stress. Environmental and Experimental Botany, 53: 315–322.
- Yin, C., Duan, B., Wang, X. and Li, C., (2004). Morphological and physiological responses of two contrasting Poplar species to drought stress and exogenous abscisic acid application. Plant Science, 167: 1091-1097.