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Effects of treated wastewater of Islamic Azad University, Roudehen Branch irrigation on *Ligustrum vulgare*

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Key words: Irrigation, Wastewater, *Ligustrum vulgare*, plant pigments.

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Abstract

Reusing treated Wastewater for irrigation, depending on its characteristics can be either beneficial or harmful. Therefore, the present study carried out to evaluate the suitability of using the effluent wastewater of the Islamic Azad University of Roudehen for irrigation of the green fields of this area and tis effect on quality of (*Ligustrum vulgare*). Four different treatments of Irrigation water including (1/3 water + 2/3 wastewater, 2 /3 water + 1/3 wastewater, pure water and pure wastewater effluent) used for irrigation during 6 months. Greenhouse experiments have done completely randomized design with three replications on plants separately. Plant samples were collected at the end of each month and chemical properties of irrigation water as well as morphological and physiological of plants were determined. Then the results analyzed by SPSS software. The results showed that, the quality of effluent wastewater is considered suitable for irrigation of green fields expect for MPN and TSS. The amount of nutrients, Na^+ and EC of the wastewater effluent were higher than the water, while DO and Ca^{2+} were less. The maximum Number of branches and plant pigments of *Ligustrum vulgare* observed in irrigation treatment of (2/3 water + 1/3 wastewater). So, mixing of wastewater and well water of field study (2/3 water + 1/3 wastewater) compared with pure water has positive affected on plants growth as well as decrease the costs of irrigation.

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Introduction

The continuous increasing of the world population and the ever increasing water consumption, calls for a better and more management of the water resources. Also, new water sources should be exploited to cover up the existing water deficit (Kalavrouziotis *et al*, 2009). At many countries, treated wastewater has been successfully used for irrigation, in recent years, and many researchers have recognized its benefit (Levine and Asano, 2004; Pedrero and Alarcón, 2009). Treated wastewaters have grate potential for reusing as sources of fresh water, organic matter, nutrients and soil conditioning agents (Cameron *et al*, 1996). The organic matter of wastewater can improve soil aeration, increase water infiltration and soil moisture holding capacity, decrease soil erosion potential, increase soil cation exchange, buffer soil pH and promote the growth of beneficial soil organisms. So, the availability and use of wastewater for irrigation has increased as availability of fresh water becomes more limited and the disposal of sewage into waterways becomes more restricted. The availability of wastewater as well as the nutrients content, would be make it an attractive source for irrigation with potential fertilizer cost saving. However, the use of wastewater can also have negative effects such as increased soil salinity, undesirable pH values, anaerobic conditions in the root zone and excessive leaching of nutrients and heavy metals (Arienzo *et al*, 2009). Also, this practice may severely harm human health and the environment, mainly due to containing pathogens, heavy metals and other unsuitable constituents depending on the source (Qadir *et al*, 2010). Therefore, the planning and managing of agricultural reuse projects need to consider institutional and legal, socio-economic, financial, environmental, technical and psychological aspects. The different reuse applications require different water quality specifications and thus demand different treatments varying from simple processes to more advanced ones. In many cases, the national reuse guidelines of wastewater effluent for irrigation works focus mainly on the quality of wastewater used for irrigation and protection of public health and environment. Considering the studies of other researchers have shown; 24% of wastewater from households and industry is treated in India, while in Pakistan only 2% is treated. Also, less than 10% of the generated wastewater is collected and treated In West African cities, while in many developing countries, large centralized wastewater collection and treatment systems have used to prevent environmental damage (IWMI, 2003; Drechsel *et al*, 2006; Qadir *et al*, 2010). Several studies have carried out to evaluate the effects of treated wastewater irrigation on plants, for example (Pedrero and Alarcón, 2009) evaluated the effects of applying treated wastewater on citrus trees and their results revealed the mix of treated wastewater and water improved the agronomic quality of the reclaimed wastewater and it can be a good solution to avoid the problems associated with wastewater use in agriculture. Also, (Wang *et al*, 2007) used treated wastewater for irrigation of celery, wheat, maize, millet, apples, rapeseed and yellow bean to test its effect on soil. They concluded a slight increase in the organic content of the soil. Also, their results showed that irrigation with treated sewage had no apparent effect on the quality of crops, and to some degree, irrigation can be improved the crop quality. In other study, (Kalavrouziotis *et al*, 2009) chose *Brassica oleracea* var. Italica (Broccoli) as a case study of treated wastewater irrigation effect in Greece and revealed the Broccoli root system, in comparison to leaves and heads accumulated in general, higher levels of nutrients, especially Fe suggesting that this vegetable is a Fe accumulator.In this case, the aim of present study was to evaluate the suitability of using the effluent wastewater of the Azad University of Roudehen Branch for irrigation of the green fields of this area and its effect on quality of *Ligustrum vulgare*. Roudehen is a town located on the east of Tehran province on Tehran-Mazandaran road. The town is just past the fork between Haraz and Firoozkooh roads, at coordinates 51° 55′E, 35° 43′ N. It is 1850 meters above the sea level and has a total area of 50 km^2. The average of annual precipitation of this town is 430 mm. According to the national census in 1976, the population of Roudehen and suburb was 5237 out of which 6.57% were inhabited in the town and the rest in the surrounding villages. Roudehen had a population of 12000 when the Azad University was founded in 1983. Local commuting patterns, housing, and job development increased with the establishment of the Azad University. Municipal wastewater treatment plant of Roudehen Azad University was established in 2001. It is situated in southern east of the campus and treats the wastewater of approximately 350×10^3 (m^3) of all water consumption of the university expect the laboratory wastewater every day. Wastewater treatment plant of Roudehen Azad University consists of a coarse screen and activated sludge process with 4 surface aerators. Aim of present study was to evaluate the suitability of using the effluent wastewater of the Islamic Azad University of Roudehen for irrigation of the green fields of this area, especially *Ligustrum vulgare*.

Materials and methods

Experimental conditions

The experiment was based on a randomized block design with three replications, and it was conducted in a greenhouse of the laboratory of Environmental science of Islamic Azad University, Roudehen Branch, Iran, in order to study the comparative effects of the treated wastewater of this university and fresh irrigation water (control), on the interactions of macro nutrients, and morphological and physiological of plants. *Ligustrum vulgare* used as a test plant. Four different treatments of Irrigation water including (1/3 water + 2/3 wastewater, 2 /3 water + 1/3 wastewater, pure water and pure wastewater effluent) used for irrigation during 6 months.

Water and wastewater analysis

The samples were taken during dry weather flow conditions. The composite samples were taken flow proportional during 24 hours and stored in fridge by 4˚C during sampling and transport. In total 60 samples of effluence wastewater and water of the field studied were taken for analyses during 6 months. The different substances were analyzed by a certified laboratory with standard methods (ASTM, 1998). We have determined the values of: pH, EC , DO , BOD ,COD , Ca^{2+} , Mg^{2+} , Cl^- , Na^+, K^+, TS, TSS, PO_4^{3-}, P, NO_2^-, NO_3^-, , NH_4^+ and total coliforms in 30 samples the effluence wastewater during 3 months. The average of these parameters compared with effluent standards published by the environmental protection department of Iran.

Plant analysis

Leaves were sampled before treated wastewater application and after wastewater Irrigation and the fresh weight of leaf (Biomass) was recorded, then oven-dried at 70°C, for dry-weight determination. Leaves samples were Extracted with 80% acetone and analyzed for Chlorophyll a (Chl a), Chlorophyll b (Chl b), Chlorophyll total and Carotenoid by spectrophotometer based on Arnon's(1949) method (Hiscox and Israelstam, 1979). Also, the Number of branches of all samples was counted during the experiments.

Statistical analysis

To determine whether the Islamic Azad University of Roudehen treated wastewater's parameters differ significantly with effluent standards published by the environmental protection department of Iran, One-sample T test was applied. Statistical significance was indicated according to P value of less than 0.05.Then Two- independent samples T tests were done to compare the mean of nutrients between treated wastewater and water of the Islamic Azad University of Roudehen. Also, One-way ANOVA and LSD tests were carried out to determine the plant properties differ significantly upon type of the irrigation treatments using SPSS software.

Results and discussions

The mean results of analysis of Islamic Azad University of Roudehen treated wastewater and well water of the field study are presented in table.1. The analysis of both types of irrigation water showed clear differences in their composition (table.1). According the results of this table and comparing the average of these parameters with effluent standards published by the environmental protection department of Iran, indicated that the treated wastewater's parameters such pH, EC , DO , BOD ,COD , Ca^{2+} , Mg^{2+} , Cl^- , Na^+, K^+, PO_4^{3-}, P, NO_2^-, NO_3^- and NH_4^+ are suitable for irrigation. So that, the results of One- sample T test for BOD, COD, Mg^{2+}, Cl^-, have been revealed the significant different ($p<0.05$) between means of these parameters in the treated wastewater effluent and the standards published by the environmental protection department of Iran (table.2). The average of total Coliform and TSS in the effluence (table.1) were 3.8×10^5(MPN/100 ml) and 205 (mg/L), respectively. Whereas, the amounts of both of them are higher than the standards, and these differences were significant, according to P value of less than 0.05 (table.2), they can reduce the quality of using the wastewater for irrigation. Because of the concerning of Coliforms on human health, they are used as indicator of wastewater contamination. Also, the wastewater effluent irrigation can potentially transport microorganisms to groundwater. The health concern is in proportion to the degree of human contact with wastewater, and the municipal wastewater treatment plants can reduce microbial concentrations by many orders of magnitude. However, protective measures such as wearing boots and gloves, and changing irrigation methods can reduce farmer exposure and risk of wastewater contamination(USEPA and USAID, 2004; WHO, 2006; Qadir *et al*, 2010). Also, Drip irrigation, especially with sub-surface drippers, can effectively protect farmers and consumers by minimizing crop and human exposure, but irrigation kits with appropriate planting density and pre-treatment of wastewater is needed to avoid clogging of emitters (Qadir *et al*, 2010).Based on table.1 and comparing the Biochemical characteristic of the fresh water with standards of irrigation indicated that, all of the water's parameters are suitable for irrigation. Furthermore, the differences were significant ($p<0.05$) (table. 2). Then, Two-independent samples T tests were carried out to compare the mean of nutrients between treated wastewater and water of the Islamic Azad University of Roudehen. These results are shown in table.3 and confirm the significant different ($p<0.05$) between them (table.3). So that, the mean concentrations of NO_3^-, PO_4^{3-}, $Mg^{2+}$and K^+ in the wastewater were higher than water, significantly. However, these parameters are considered essential nutrients for improving plant growth and soil fertility and productivity levels (Feigin *et al*, 1991; Singh and Bhati, 2005). In this case, (Bamniya *et al*, 2010) in their study on the effect of wastewater on biochemical contents of *Brassica oleracea* and *Spinacia oleracea* found similar result. Also, fig.1 Shows the maximum of Chl a (0.78 mg/g Biomass) related to treatment of (2/3water + 1/3 wastewater) and minimum of it (0.47 mg/g Biomass) related to treatment of (pure wastewater).Chl a content of (pure water) and (1/3 water + 2/3 wastewater) treatments were measured 0.64 and 0.65 (mg/g Biomass) respectively, and there is no significant different between them. According fig.2 indicated, the maximum of total Chl (1.18 mg/g Biomass) related to treatment of (2/3water + 1/3 wastewater) and minimum of it (0.68 mg/g Biomass) related to treatment of (pure wastewater).Total Chl content of (pure water) and (1/3 water + 2/3 wastewater) treatments were measured 0.91 and 1.02 (mg/g Biomass) respectively, and these differences were no significant, according to P value of less than 0.05. Based on fig.3 revealed, the maximum of carotenoid (0.33 mg/g Biomass) related to treatment of (2/3water + 1/3 wastewater) and minimum of it (0.21 mg/g Biomass) related to treatment of (pure wastewater).Carotenoid content of (pure water) and (1/3 water + 2/3 wastewater) treatments were measured 0.24 and 0.26 (mg/g Biomass) respectively, and these differences weren't significant, according to P value of less than 0.05. Finally, comparing the results of the (carotenoid/ total Chl) ratio in different treatments showed its maximum (0.32) at the samples irrigated with (pure wastewater) and this ratio was significantly higher than other treatments. Also, Based on Fig.4 revealed, minimum of the (carotenoid/ total Chl) ratio (0.25) related to samples irrigated with (pure water). In any case, Carotenoid is photosynthetic pigment, also functions as non-enzymatic antioxidant protecting plants from oxidative stress by changing the physical properties of photosynthetic membranes with involvement of xanthophyll cycle (Gruszecki and Strzatka, 1991). An increase in carotenoid content is suggested a defense strategy of the plants to combat metal stress (Sinha *et al*, 2007; Guo *et al*, 2007). Fig.5 shows the mean number of branches of *Ligustrum vulgare* in different treatments. According Fig.5 indicated, the most numbers of branches of *Ligustrum vulgare* (11) related to treatment of (2/3water + 1/3 wastewater) and the least of them (3) related to treatment of (pure wastewater). The numbers of plant branches of (pure water) and (1/3 water + 2/3 wastewater) treatments were 9 and 7 respectively, and these differences were no significant. Sum up, the maximum Number of branches and plant pigments of *Ligustrum vulgare* observed in irrigation treatment of (2/3 water + 1/3 wastewater).So, mixing of the wastewater and fresh water in comparison with pure water has positive affected on plants growth ,increasing Number of branches and plant pigments of *Ligustrum vulgare*. In this case, the results of previous researchers (Wang *et al*, 2007; Kalavrouziotis *et al*, 2009; Singh *et al*, 2009) showed; the plants were irrigated with wastewater produced taller plants and more heads per unit area, than samples were irrigated with water alone. Consequently, the results of present study confirm this behavior. Also, photosynthetic rate and producing of oxygen would be increased by increasing of plant pigments. So, using treated wastewater not only saves water resources but also increases useful oxygen. Consequently, mixing of the wastewater and well water (2/3 water + 1/3 wastewater) compared with pure water has positive affected on plants growth as well as decrease the costs of irrigation.

Table 1. The means concentration of investigated parameters in effluence wastewaters and water.

Parameter	COD (ppm)	BOD (ppm)	total Coliform MPN	Cl^- (ppm)	Mg^{2+} (ppm)	Ca^{2+} (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	TSS (ppm)	NH_4^+ (ppm)	PO_4^{3-} (ppm)	P (ppm)	DO (ppm)	EC (µs/cm)	pH	K^+ (ppm)	Na^+ (ppm)
Water	32	20	380	106.5	28.8	120	0.108	12	10	0.02	0.57	0.18	3.2	113.1	7.33	19.7	65.3
Treated wastewater	108	75	4500	387	51.6	104	8.23	35	140	0.03	20.9	6.8	1.8	1301.8	7.12	32	238.1
Irrigation standards	200	100	1000	600	100	-	-	-	100	-	-	-	2	-	6-8.5	-	-

Table 2. One- sample T test analysis of Treated Wastewater and Waters' parameters.

Parameter	Test value	Sig.(2-tailed) Treated Wastewater	Sig.(2-tailed) Water
DO	2	0.251	0.040
BOD	100	0.001	0.001
COD	200	0.000	0.000
Cl^-	600	0.000	0.001
Mg^{+2}	100	0.000	0.000
TSS	100	0.001	0.000
Total Coliform	1000	0.003	0.001

Table 3. Two- Independent samples T tests analysis of Treated Wastewater and Waters' Nutrients.

Factor	t	df	Sig.(2-tailed)	Mean Difference	Std. Difference	95% Confidence Interval of the Difference	
Nutrient						Lower	Upper
PO_4^{-3}	-348.655	19	0.000	-20.230	0.05802	-20.390110	-20.6890
NO_3^-	-29.394	19	0.000	-24.000	0.81650	-26.26696	-21.73304
Ca^{+2}	-112.677	19	0.000	-92000	0.75120	-94.16803	-89.72068
Mg^{+2}		19	0.000	-22.800	0.9651	-25.0696	-20.53304
K^+		19	0.000	-12.300	1.3680	-14.5782	-10.03304

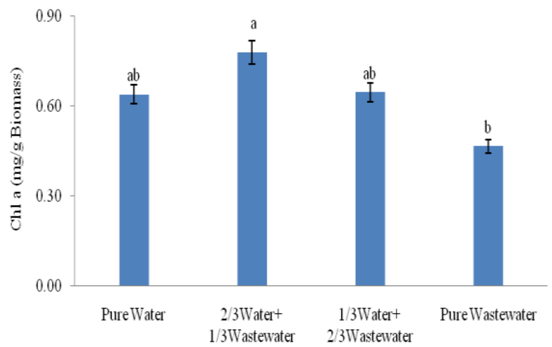

Fig.1. Compare the average of Chl a of *Ligustrum vulgare* grown at different irrigated treatments.

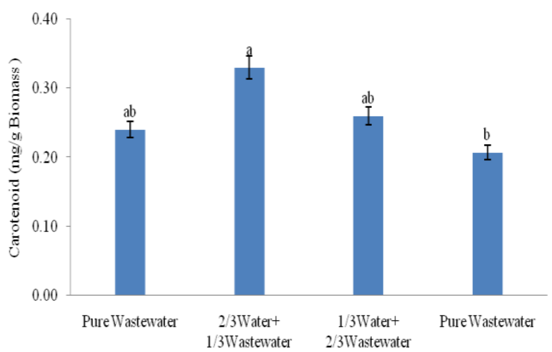

Fig.3. Compare the average of Carotenoid of *Ligustrum vulgare* grown at different irrigated treatments.

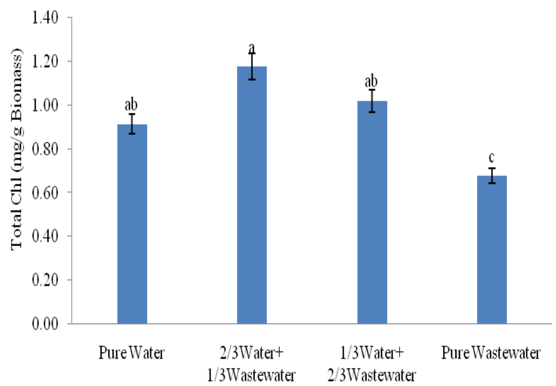

Fig.2. Compare the average of Total Chl of *Ligustrum vulgare* grown at different irrigated treatments.

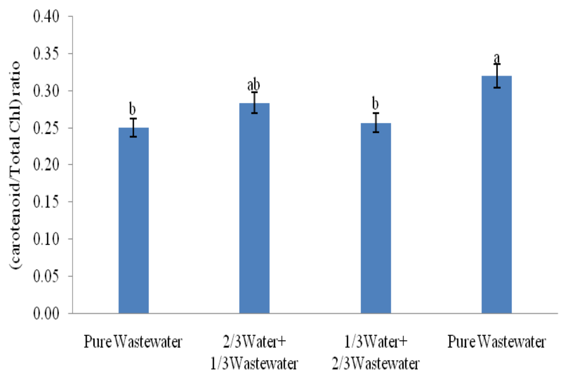

Fig.4. Compare the average of Carotenoid / Total Chl ratio of *Ligustrum vulgare* grown at different irrigated treatments.

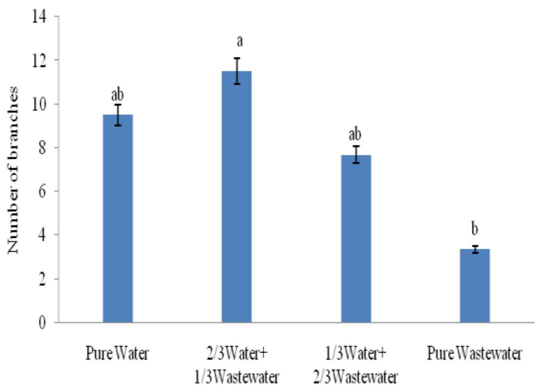

Fig.5. Compare the mean number of branches of *Ligustrum vulgare* grown at different irrigated treatments.

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