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ORIGINAL ARTICLE

Physiological and Morphological Responses of Barley and Rape Plants Grown in Wastewater

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ABSTRACT
Experiments were conducted to compare some physiological parameters of barley (Hordeum vulgar L.) CV Nosrat and rape seedlings (Brassica napus L.), CV Licord grown in refined wastewater (RWW) of Shiraz Water and Waste Water Treatment Plant with those grown in standard nutrient solution (half strength Hoagland solution). The experiments were designed as randomized complete block design with 6 replications in petri dishes (germination stage) and in solution culture (seedling stage). Seedling and vegetative parameters studied included, seed germination percent, radicles length, shoot axes length, number of lateral roots, seedlings stem, and root length, both roots and shoots fresh and dry weight. Besides, biochemical parameters such as chlorophyll, carotenoids, Total N, P, K, Na, Fe and Pb contents were studied. Data were analyzed by SPSS 16 software at α≤0.05 level. The differences between plants grown in standard nutrient solution and those grown in RWW were much less in barley than in rape seedlings. Barley seedlings (a monocot) performed more or less the same way in the both solution cultures. However, rape seedlings (a dicot) performed poorly in RWW as compared to standard nutrient solution. It was concluded that to be used as irrigation water, the refined waste water should be supplemented with some biological or synthetic fertilizers to ensure maximum yield especially for dicotyledon plants such as rapes. Further studies with other dicot plants are recommended.
***Keywords**: Standard nutrient solution, refined sewage effluent, Rape and barley seedlings performance, Vegetative and biochemical parameters*

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INTRODUCTION

Nowadays, water resources in most parts of the world are diminishing due to drought therefore the use of wastewater released from water treatment plants is considered as an alternative solution for the shortage of irrigation water in agriculture. Using municipal waste water effluent is considered to be a necessity for the development of agriculture and the surrounding urban areas, both in arid and semiarid regions of the world [26, 57].

The use of RWW as irrigation water will help to reduce the discharge of both unrefined sewage effluent and chemical fertilizers which are one of the main sources of environmental contaminations. Municipal wastewater from food processing plants, slaughterhouses and soda factories can be used for irrigation after being refined. However, wastewater from hospitals, petrochemical and chemical plants, and oil refineries are not suitable for this purpose [25, 39]. Due to the short life span of some pathogens, crops with a long growing period, such as corn, wheat, and rice are more suitable to be irrigated by RWW than crops with short growing period, such as vegetables [27]. In sandy soils and in areas with high groundwater levels, the use of treated waste should be avoided to prevent groundwater contamination [3, 59]. Treated wastewater has been successfully used as irrigation water for corn, sorghum, and sunflower [29].

Smart [28] studied changes in soil chemical properties in North Adelaide, Australia irrigated with water and wastewater separately and reported that irrigation with wastewater has resulted in an increase in the amount of soil sodium and bromine. Although the increases in the amount of these two elements were not high enough to affect agricultural crops, the reported increase in soil sodium and Sodium Absorption Ratio (SAR) content is a warning for the future soil structural degradation and poor drainage capacity. Irrigation with RWW has resulted in both reduction and increase in soil pH [41].
Studies on *Azolla* species grown in sewage media in Japan have revealed that *Azolla* is able to fix nitrogen and remove phosphorous from the environment effectively resulting in both plants high proteins and biomass contents [5, 44, 55]. A study made on physiological, biochemical and growth characteristics of leafy vegetable spinach irrigated with wastewater and ground water, separately, has shown that continuous use of wastewater has caused an increase in the amount of soil micronutrients and heavy metals [40, 47].
In the present study, the physiological and biochemical responses of barley and rape seedlings grown in RWW supplied by Shiraz Water Processing Plant and in half strength Hoagland nutrient solution were compared during March 2013.

MATERIALS AND METHODS

Experiments were designed as a randomized complete block with 6 replications using two culture media, half strength Hoagland solution and RWW separately. Rape seeds CV Licord and barley seeds CV Nosrat, were kindly supplied by Fars Seeds and Seedlings Improvement Centre near the city of Shiraz. Samples of RWW were collected once a week during the experimental period at an external station of Fars Water and Sewage Plant The chemical compositions of the refined wastewater and those of half strength Hoagland solution (Control) are given in table1 and 2, respectively (mg L^{-1}).

Table1- Chemical compositions of refined wastewater supplied by Shiraz Water Processing Plant.

Amount	Contents	Amount	Contents	Amount	Contents
Detergent	0.5 mg L^{-1}	**Ca**	55-110 mg L^{-1}	**TKN**	20-30 mg L^{-1}
P	2.2-3 mg L^{-1}	**BOD**	30-35 mg L^{-1}	**K**	5-6 mg L^{-1}
Na	110-200 mg L^{-1}	**COD**	50-80 mg L^{-1}	**EC**	1800-2000 µS cm^{-1}
Cl	150-250 mg L^{-1}	**Turbidity**	5-7 NTU	**Pb**	4-7µgL^{-1}
Mg	40-70 mg L^{-1}	**TSS**	55 mg L^{-1}	**Fe**	0.67 mg L^{-1}

Table 2-Amounts of macro and micro elements in half-strength Hoagland's solutionin terms of milligrams per liter(mg L^{-1})

MACRO elements	Amounts (mg L^{-1})	MICRO elements	Amounts (mg L^{-1})
K	118.25	B	0.25
P	16.01	Mn	0.39
N	136.26	Cl	0.51
Ca	144.71	Zn	0.045
Mg	50.07	Cu	0.064
S	66.09	Mo	0.006
		Fe	5.00

Germination stage

To evaluate the germination stage barley and rape seeds were disinfected by 10% bleach water and then were washed several times by distilled water. Hoagland and RWW Culture medium were prepared and petri dishes were used for planting the seeds. Twenty five seeds were placed in each petri dish among three layers of filter paper. After dropping 12.5 ml of the treatment (effluent or solution of half strength Hoagland), a filter paper was placed on the seeds to keep their moisture. Plates containing the seeds were put in Memmert ® incubator (model; INE500) at 25 ± 1 ˚C. After a week, seed germination percentage, root length, shoot length, and number of lateral roots measured in each case [13].

Vegetative stage

Firstly, barley and rape seeds were surface sterilized and spread on perforated plastic plates in contact with tap water and allowed to germinate in 7 days at room temperature. Germinated seeds were transferred to 1 litre black painted plastic containers (3 seedlings per container) containing either half strength Hoagland or RWW. Plastic pots were placed under fluorescent light with a photoperiod of 14/10 hours of light/dark periods of 20 to 25 °C and aerated for 5 minutes every 30 minutes [32]. Before changing the Hoagland and RWW solutions every week, the EC and pH of the media were also measured. Six replicates were used for each treatment and seedlings were allowed to grow for 35 days. Traits measured in the growth stage were included: Shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight. At this point 35 days plants were harvested from 24 dishes. Then the shoot and root length of samples (three plants per dish) were measured by a ruler in centimeter unit. In the next step roots and stems were separated and to remove the adsorbed cations and anions from the root surface, roots were soaked in 100 mM $CaCl_2$ solution for 2 minutes followed by 2 minutes rinse in distilled water and their damp were quite taken by tissue. The process of preparation followed by weighting the fresh root and shoot samples. To determine chlorophyll and carotenoid contents of leaves and shoots 200 mg of each replicate were randomly isolated. Samples related to each repetition were put in separate paper envelope and the type of treatments was specified. Finally, the vegetative samples were put within oven at 75 °C for 48 hours and treatment plants were weighed and recorded [38].

Chlorophyll and Carotenoid analyses

Leaf chlorophyll and carotenoid contents were determined according to Arnon [4] and Eijckelhoff and Dekker [17], respectively.

Biochemical analysis

After wet digestion of dried samples (0.2 g of each sample) in a H_2SO_4–Selenium–salicylic acid mixture, following methods were used for chemical analysis of samples: total nitrogen by Microkejeldahl method [24], Sodium and potassium by flame photometer (Model; Sherwood M410), Iron and lead by atomic absorption spectrophotometer (Model; Analytik Jena VARIO6), and P was measured spectrophotometrically (Model; HACH DR5000) due to the indophenol-blue method after its reaction with ascorbic acid [7].

Statistical analysis

Data were analyzed with SPSS 16 software by T-test at $\alpha \leq 0.05$ level. The Excel 2007 software was used to draw diagrams and standard curves.

RESULTS AND DISCUSSION

3.1. Effects of RWW and standard nutrient solutions on germination parameters of barley and rape seeds

The results of seed germination percentage, radicles length, shoot axes length, and number of lateral roots is indicated in Table 3.

Table 3- Amount of germination parameters included: germination percentage, root length, shoot length and number of lateralroots of barley and rape seeds grown in standard nutrient and refined waste water solutions.

Medium	**Standard nutrient solution**	**Refined wastewater**	**Standard nutrient solution**	**Refined wastewater**
Seeds	**Barley**	**Barley**	**Rape**	**Rape**
Germination percentage	76.66b	46.66a	75.00b	46.66a
Shoot axes length(cm)	10.95b	6.01a	6.89a	5.16a
Radicles length(cm)	9.81a	7.93a	4.75a	4.66a
Number of lateral roots	3.37a	3.44a	4.50a	4.35a

The longer shoots (10.95 cm versus 6.01cm) and more germination percentage (76.66% versus 46.66%) of barley plants grown in control medium in compare with those grown in RWW is remarkably noticeable. However, there were not remarkable differences in other parameters. Probably one of the crucial factors influencing the observed differences between the plants grown in two mentioned media is in connection with accumulation of heavy metals in RWW. It is completely documented that after leaving the radicle and shoot axes existence of inhibitory elements such as heavy metals, will prevent the development of these organs (fig.1 and 2) [60, 30].

On the other hand, effects of different pathogenic microorganisms existing in RWW on plants cause them many fungal diseases and also rapid inhibitory effects of detected amounts for Na (110 to 200 mg L^{-1}) and K (6 mg L^{-1}) due to influencing on the enzyme activated with K can be considered as some other important parameters limiting the growth of germination stage (Oliveira et al., 2014). Besides, small amounts of Fe and P in the RWW compared to control (Tables 1 and 2) should not be ignored [30]. Evaluated amount of SAR for RWW is higher than what defined by FAO for irrigation water in agriculture which is equal to 3μg L^{-1} [18].

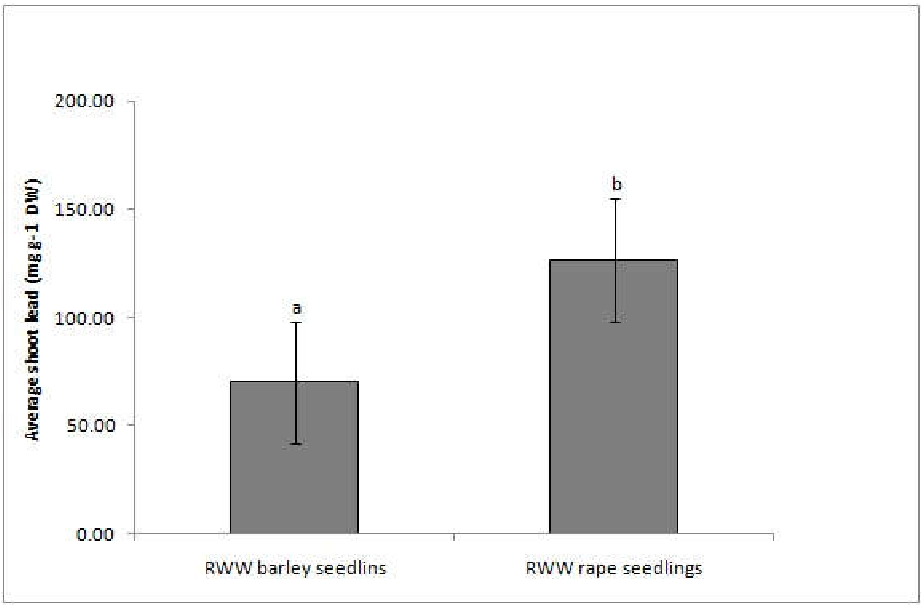

Figure 1- Pb^{+2} accumulation (mg g^{-1} DW) in shoots of barley and rape seedlings grown in refined wastewater effluent.

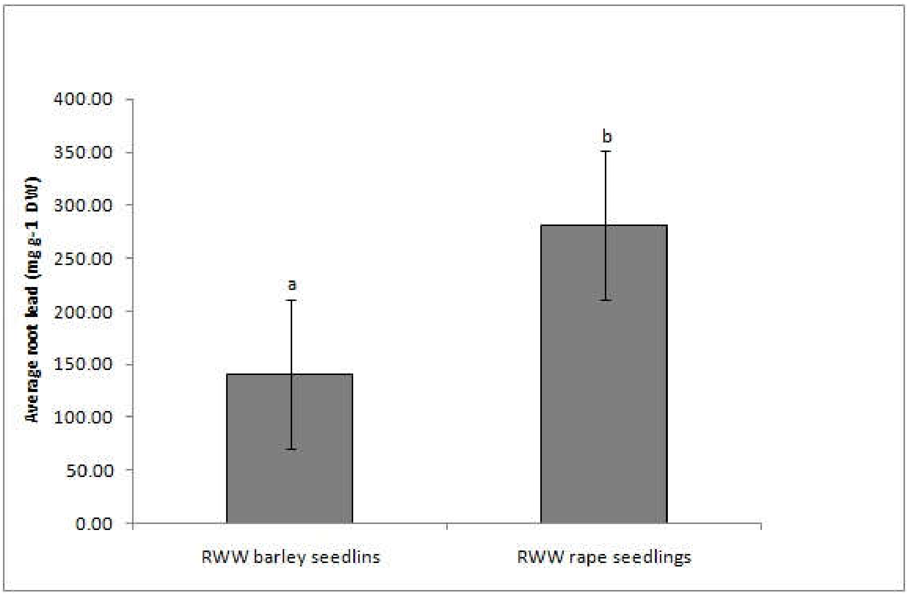

Figure 2- Pb^{+2} accumulations (mg g^{-1} DW) in roots of barley and rape seedlings grown in refined wastewater effluent.

In the experiment, impacts of the different levels of industrial effluents were examined on seed germination and seedling growth in *Brasicca napus* L. The results showed that the concentration of industrial effluents had no considerable effect on percentage and seed germination speed of *Brasicca napus* L. However, sewage concentrations of 20 to 100 percent is statistically significant at the one percent level on root and shoot length, seedling length and seed vigor index of treatments effect [31]. Panaskar and Pawar (2011) reported that *Vigna unguiculata* and *Pisums sativum* indicated an obvious decrease in germination percentage with expansion in accumulation of effluent salinity. In the other study the stress effect of heavy metals (Fe, Cu, Zn, and Mn) in industrial effluents was investigated on seed germination of barley plant. The results showed that treatment of industrial wastewater at concentrations above 50% caused to a significant reduction in the shoot length, root length and dry weight (root and shoot) compared to the control (distilled water) [45]. Studies show that heavy metals such as lead and cadmium are easily absorbed in to the skin of root and then readily enter to xylem from Symplasty or Apoplastmy [48, 43]. These elements affect the division and cell growth, overall plant growth, cell division of meristematic region, regulation, and plant growth [14].

Effect of RWW and standard nutrient solutions on vegetative parameters of barley and rape seedlings

Obtained data (Table 4) obviously indicates that there is no significant difference between vegetative parameters of barley seedlings grown in wastewater and those of control. However, all vegetative parameters of rape seedlings grown in controlled environments are considerably more than those grown in sewage wastewater. (shoot length in control 30.07cm and in RWW 19.44cm, root length in control l27.25 cm and in RWW 18.41 cm, fresh weight of rape plant 17.07 g in control and 3.92 g in RWW, root fresh weight in control, 1.01 g and in RWW 0.32 g, shoot weight in control 1.17 g and in RWW 0.31 g, root dry weight in the control 0.08 g and in RWW is 0.03g).

Table 4- Amount of vegetative parameters included: stem and root length, both roots and shoots fresh and dry weight of barley and rape seedlings grown in standard nutrient and refined waste water solutions

Medium	Standard nutrient solution	Refined wastewater	Standard nutrient solution	Refined wastewater
Seedlings	**Barley**	**Barley**	**Rape**	**Rape**
Stem length(cm)	52.29a	54.61a	30.07b	19.44a
Root length(cm)	44.84a	48.00a	27.25b	18.42a
Shoot fresh weight(gr)	8.31a	8.74a	17.07b	3.92a
Root fresh weight(gr)	2.93a	2.78a	1.01b	0.32a
Shoot dry weight(gr)	0.80a	0.87a	1.17b	0.31a
Root dry weight(gr)	0.27a	0.25a	0.08b	0.03a

Thus compounds in RWW have had a negative impact on all vegetative parameters of rape seedlings. It seems that one of the reasons for the recorded decrease of rape seedlings growth in RWW particularly in comparison to wastewater barley seedling is due to the higher accumulation of lead in the shoots and roots of rape plants (fig.1 and 2). In addition, according to figure 3 and 4, while barley plant grown in refined wastewater has stored more sodium in its root, the strong accumulation of the element was detected in shoots and photosynthetic cells (more sensitive parts to sodium) of the rape plant has stored sodium. Furthermore, the mechanism adopted by barley to protect the plant from different damages caused by sodium is storing it in the vacuoles and endodermal of root [52].

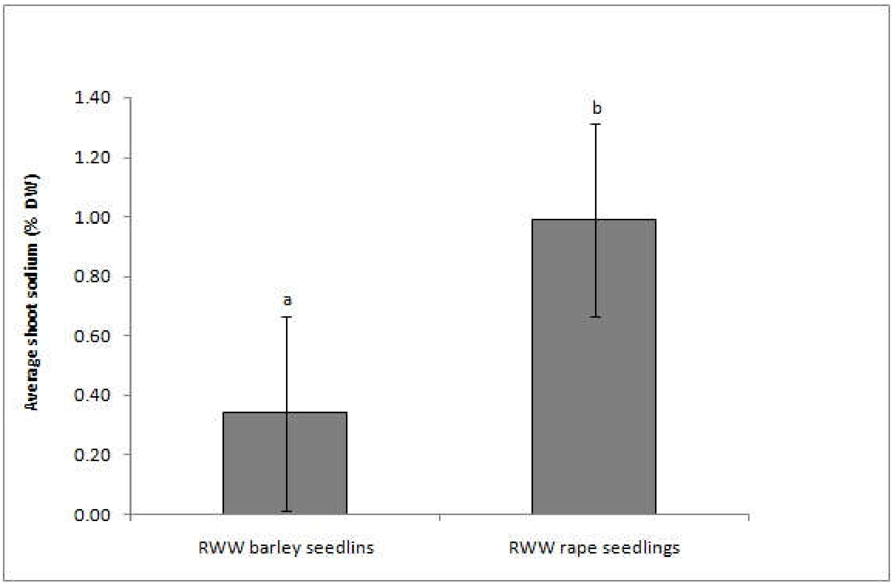

Figure 3- Sodium accumulation of (% DW) in shoots of barley and rape seedlings grown in refined wastewater effluent.

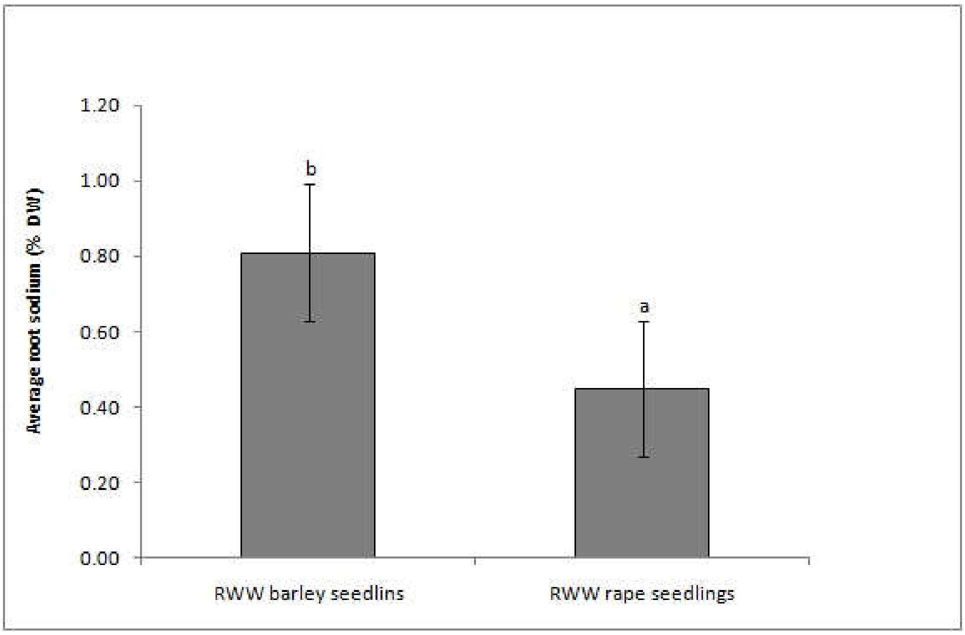

Figure 1- Sodium accumulation (% DW) in roots of barley and rape seedlings grown in refined wastewater effluent.

Iron deficiency in root cells of rape plant grown in RWW has caused to disrupt metabolic processes, respiratory enzymes, cytochromes, and other enzymes, which as results has its negative effects in the activity and development of shoot. As one of the major elements iron plays a catalytic and structural role in influencing the growth of shoots. Among the elements that exist in plant N has the most important and the most aboundant after C, H, and O. Therefore, reduction in Canola shoot growth is due to reduction of this element and may also be due to iron deficiency in the RWW (Table 2) [30]. Another reason for reduction in the growth of Canola in effluent medium is a sharp decline in the amount of chlorophyll in addition to the lack of nitrogen and sodium influence on shoot. Moreover, the limited content of potassium in the effluent (5 to 6 mg L^{-1}) (table 1) is another reason for observed phenomenon. Thus, high amount of sodium in compete with potassium has stopped many critical reaction of cells. One reason for the good growth of barley in RWW could possibly be related to plant ability at ideal using of wastewater organic matters. In this case experiment showed effective absorption of nitrogen and organic matters by corn plant enhanced the production of whole plant dry weight [22]. In this regard, during research with different concentrations of sugar mill effluents, It was understood that for lower concentrations of irrigated effluent (20% and 40%), the root and shoot length of radish plants were higher than control plants, which may be taken as an indication of beneficial range, while for higher concentrations of effluents (60%, 80% and 100%) a decreasing trend was observed, which confirms the toxic effect of this effluents to radish plants [56]. In a related study various wastewater portions was evaluated on Maize plant. The results showed that plant height increased by increase in wastewater amount to 75 percent and stem diameter and operation of dry Forage Maize increased by increase in wastewater amount to 100 percent. However, in treatments of 75 and 100, there was no great difference between plants in terms of plant height, stem diameter, and dry Maize operation. But the number of tillers per plant decreased with increasing the amount of wastewater. Also the highest dry Maize operation was obtained in wastewater treatment 100%, 23.1 T ha [19]. Data released from a study concerning with the stem length of wheat plant treated by wastewater (10% and 20%) and control environment (irrigated by well water) proved that there was a significant difference between their impact on the plant. By and large, there was no significant differences between fresh and dry weight of stem and spike in wastewater and in control in terms of tons per acre. But the average yield of wheat in terms of tons per acre over two years of wheat cultivation showed that the control treatment had the lowest level in the first year and it had significant difference with other treatments and this means that increase in yield operation is due to the use of wastewater for irrigation. Also between10 and 20 percent of sewage treatment did not observe significant difference [15]. During the study the effect of different portions of RWW, fertilizer and manure were examined on yield agronomic characteristics and components of wheat yield. The results showed that the highest grain yield and biological yield was related to 75% treated RWW with grain yield 48.4 and biological yield 21.7 ton per acres and The highest yield of protein was in the treated wastewater

50% which compared to control treatment (pure water) increased 33 percent [21].

Effect of RWW on accumulation of lead in shoots and roots of barley and rape seedlings

A comparison of the highest detected amounts for lead accumulation in the shoots (70.09 μg g^{-1} DW versus 126.56 μg g^{-1}DW) and roots (140.25 μg g^{-1} DW versus 281.26 μg g^{-1} DW) of the rape plants versus barley plants (fig. 1 and 2, respectively) implies the significant differences between them. The more lead accumulation in the rape roots rather than barley roots can be easily explained by the absorption positions of bivalent cations in roots of dicotyledons which are more than monocots. As an example, higher amounts of calcium in dicotyledons compared with monocots is well documented. Moreover, since the amount of pecticacids in dicotyledon plants is more than monocots, calcium bounds to these acids and increases the wall consistence [30]. The entrance of lead to dicotyledon plants from the exchange position of two capacity cations can cause some damages to the plants due to bonding with pectic acids instead of calcium or substitution in metabolic pathways for two capacity cations playing crucial roles as cofactor or have structural role in organic compounds. [16]. Plants adopt various mechanisms, such as protein binding, prevention of entering into cells, excretion to wall, or accumulation in their vacuoles to reduce the toxic effect of lead. The availability of lead to plants will be increased with the enhancement of Phosphate and Nitrate fertilizers. Lead is absorbed easier and faster by the roots of tree plants in the form of Phosphate leaded or Nitrate leaded salts by soil tampon property, however the free form of lead (Pb^{2+}) can be absorbed by the roots of bushes and shrub plants. Proline accumulation in plants exposed to heavy metal stress reduces the cause damage to membrane sand proteins [54]. During a study investigated the effect of sewage on crops irrigation, achieved results signified that use of wastewater for irrigation increased the contents of cadmium, nickel, lead, and chromium in the soil and it was found out that by increasing the amount of lime and soil organic matters, the accumulated lead significantly increased in the soil however the other elements showed no strong correlation with the amount of lime and organic matters [2]. In a study implemented by Paliwal *et al.* (1998) was found that in irrigation of *hardwickia binatu* plant under greenhouse conditions by different concentrations of effluent (0,25, 50, 70, and 100%), the highest concentrations of heavy metals in the plant were as follows: Mn>Zn>Pb>Cu. Also the highest growth rate was observed in the effluent concentration of 50%. In the study conducted by Singh and Agarawal (2012) physiological, biochemical, and growth characteristics of spinach (*Beta Vulgaris* L.) irrigated by wastewater were compared with samples irrigated by groundwater. Results showed that the uptake portion and transport of heavy metals were higher in plants grown by wastewater. Manganese showed the maximum after zinc, copper, lead, nickel, chromium, and cadmium. Plants generated more secondary metabolites and antioxidants to resistance against the negative impact of heavy metals in land irrigated with wastewater. A Research was done on the effects of sewage sludge on concentration of Zn and Cd in organs of barely. The results indicated that the application of sewage sludge during the 7 years has increased the amount of Zn and Cd in roots and shoots of plants by 40 tons of treated sewage sludge and also accumulation of Cd in the roots was more than shoots [53].

Effect of RWW on accumulation of sodium in shoots and roots of barley and rape seedlings

The results demonstrates that the differences between the accumulation of sodium in shoots and roots of barley and rape seedlings grown in effluent are noticeable (fig.3 and 4, respectively) as the amount of sodium percent in barley and rape shoots are 0.34% versus 0.99%, respectively and for barley and rape roots are 0.81% versus 0.45%, respectively. In fact the sodium retention in roots and prevention of its transfer to the shoots due to condensation of the element in vacuoles of root cells has been the main strategy adopted by barley plants grown in wastewater. But due to most transpiration the more transfer of sodium to shoot has done and its negative effect ton plant growth will be clear [30]. Thus one limiting and harmful compositions of effluents is their high sodium content [51]. In the study on the effects of different sewages on Eucalyptus showed that high concentration of sodium in sewages of textile factories reduced concentrations of magnesium and micronutrients that are essential in stimulating the growth of roots and leaves. So, plants grown in sewage textile factories have lower average growth biomass [8]. In an experiment, Wang *et al.* [57] have reported that by application of treated wastewater in three different wheat irrigations, the maximum amount of sodium has been observed in root, stem, and leaves, respectively. Al Lahham *et al.* [1] suggested that sodium concentration in grass shoots increased from 0.27% in the control (well water) to 0.54% and 0.6% in treated effluent. Also the amount of sodium in tomato fruit shoots increased in treated effluent due to the high sodium content of sewage effluent.

Effects of RWW and standard nutrient solutions on shoots and roots of barley and rape seedlings mineral contents

The amount of total nitrogen, potassium, phosphorus, and iron in the roots and shoots of barley and rape seedlings grown in standard and RWW solutions are given in table 5. The total amount of nitrogen in barley shoots and roots grown in RWW is dramatically different from those grown in standard nutrient solution, 1.83% and 2.30%, respectively. With respect to rape seedlings, these values are 1.83% and 3.89% on dry weight basis, respectively. The nitrogen content is completely different in the rape shoots for seedlings grown in standard nutrient solution or in RWW (1.25% versus 1.89% dw). In roots, the values are 1.88% and 3.53%, respectively.

Table 5- Amount of total nitrogen, potassium, phosphorus and iron in the roots and shoots of barley and rape seedlings grown in standard nutrient and refined waste water solutions

Nutrient	Standard nutrient solution		Waste water solution		Standard nutrient solution		Waste water solution	
	Barley seedling		Barley seedling		Rape seedling		Rape seedling	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
N(%)	2.30	3.89	1.83	1.83	1.89	3.53	1.25	1.88
K(%)	7.56	2.40	4.81	2.04	4.59	2.44	2.22	2.23
P(%)	NS	0.93	NS	0.68	NS	1.31	NS	0.62
Fe(μg g^{-1}DW)	NS	4579	NS	779	NS	6705	NS	313

The higher amount of nitrogen in both shoots and roots of seedlings grown in Hoagland nutrient solution are most probably due to higher amount of nitrogen in Hoagland solution (table 2) than in RWW (table 1). In the experiments, different effluents (industrial effluents, effluents from fertilizer treated distillery and sugar factory mixed effluent) were used as irrigation water for wheat, garden pea, black gram, and mustard. It was found that the macro elements; nitrogen, phosphorus, and potassium were mostly accumulated in fruit, stems, pedicels, and roots of these plants. By contrast, the minimum amounts of these elements were observed in plants treated with water mixed with fertilizer and wastewater which were used in rotation with water. The maximum amount of nitrogen and phosphorus have been observed in the leaves and roots of black gram plants grown in treated industrial effluents and the least amount of nitrogen and phosphorus have been observed in treated well water mixed with sugar factory mixed effluent which was used in rotation with well water [34]. Paliwal *et al*. [36] stated that protein content of *Hardwickia binata* seedlings irrigated by more than 50% wastewater effluent is more than 50% below than control situation. In contrast, Asgharipour and Azizmoghaddam [6] declared that foxtail millet plants irrigated by diluted municipal sewage supplemented with some micronutrients spray stimulated the crop efficiency parameters. They concluded that the high nitrogen, phosphorus, and organic substances presence in sewage effluent have increased the plants efficiency parameters. The amount of potassium in both barley and rape shoots and in roots grown in wastewater and in standard Hoagland nutrient solutions are not comparable. The elements content in barley shoots and roots growing in wastewater and Hoagland solution are 4.81% versus 7.56% in shoots and 2.04% versus 2.40% in roots, respectively. Besides this parameter is changing between 2.22% versus 4.59% in shoots and 2.23% versus 2.44% in roots of rape seedlings, respectively. Potassium is an essential element which besides activating more than 50 enzymes in plants, it is also involved in stomatal movement, control of cellular pH, and phloem loading. Rape plants growing in refined effluents media showed potassium deficiency symptoms which can be related to the low potassium content in their media (table 1). On the other hand, this phenomenon could be interconnected with the high sodium content in the effluent (table 1) which interferes with potassium uptake (30;42).There are no considerable differences between the amount of iron in barley shoots for plants growing in refined wastewater and those of controls. However, when it comes to roots the differences are detectable. The differences between the amounts of iron in the roots of rape seedlings growing in both media are significant, but the situation is completely different for the shoots ($\alpha \leq 0.05$). In our study, iron deficiency symptoms were evident in the leaves of rape plants growing in refined wastewater effluent indicating that refined wastewater effluent should be supplemented with iron fertilizer used as irrigation water for dicotyledon plants such as rape. In spite of rape plants, barley plants as monocotyledon did not show any sign of iron deficiency which could be related to differences in efficient use of iron between these two plants. With respect to iron in refined effluent wastewater and its effects on rape seedling growth, our results are in contrast to reports showed higher amounts of iron and some other elements in the shoots and roots of tomato and lettuce plants [10, 12]. The amounts of phosphorus in the roots of barley seedlings growing in refined wastewater effluent were significantly lower than those of control (0.68% versus 0.93%, respectively). However, in the shoots, the differences are not significant. The differences between the amount of phosphorous in the roots of rape seedlings grown in refined wastewater and in those grown in standard nutrient solution are significant (0.62% and 1.31%, respectively). However, in the seedlings shoots the differences are not significant. The phosphorous content of refined wastewater used in this study is relatively low as compared to standard nutrient solution (table 2), thus, barley and rape seedlings growing in these media preferably accumulated this metabolically important nutrient in their roots as the first site of ion uptake in plants in order to maintain their metabolic activities. Some studies regarding alfalfa (*Medicago sativa* L.) seedlings grown in treated wastewater have shown that the plant had accumulated more phosphorous in its roots than the shoots as compared to plants irrigated by well water [9, 11].

Effects of RWW and standard nutrient solution on chlorophyll and carotenoid contents of barley and rape plants

Results indicate that the differences between the amount of chlorophyll and carotenoid pigments of barley seedlings grown in RWW and those grown in standard nutrient solution are significant. The average amount of chlorophyll pigments in plants grown in Hoagland nutrient solution is 2.32 mg g^{-1} FW and for those grown in RWW is 1.82 mg g^{-1} FW (fig. 5), and in the same plants, the average amount of carotenoid pigments are 3.36 and 2.31 mg mL^{-1}, respectively (fig. 6).

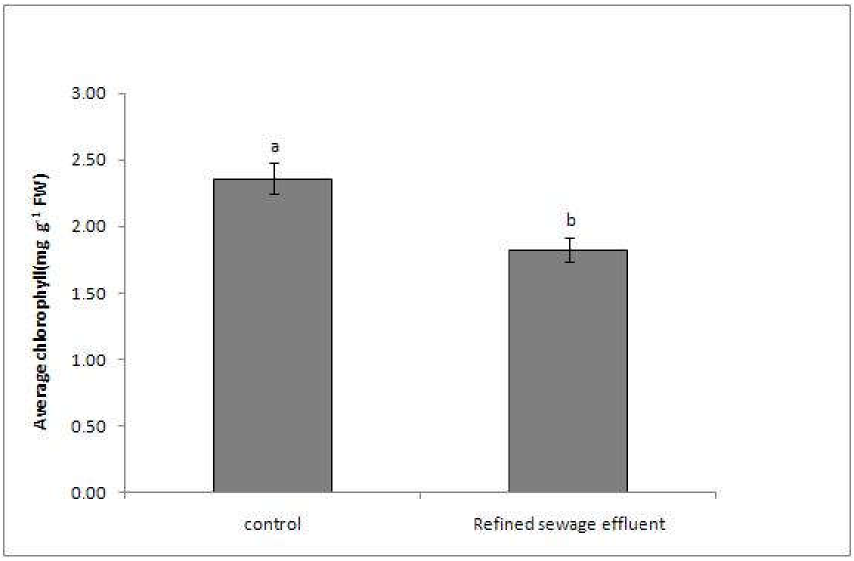

Figure 5- The amount of leaf chlorophyll content (mg g^{-1} FW) of barley seedlings grown in refined wastewater effluent and half-strength Hoagland's solution.

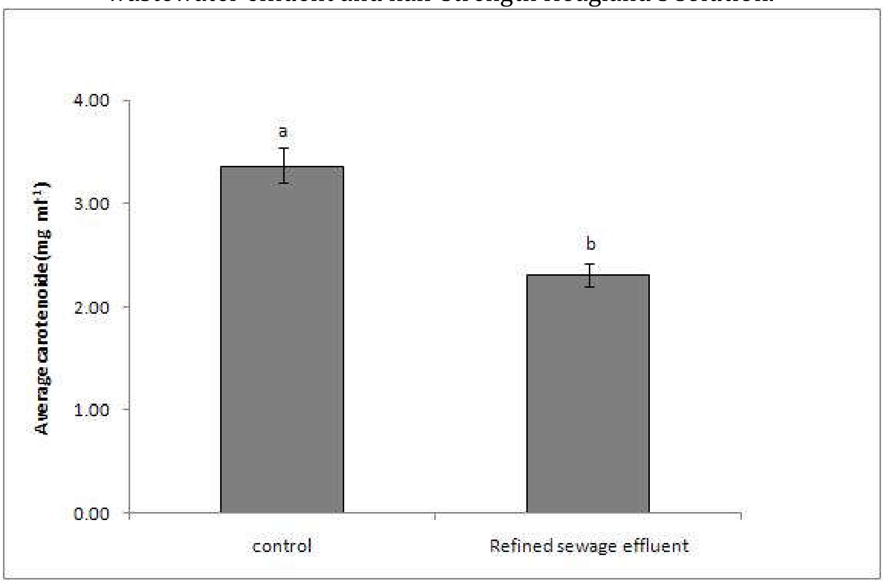

Figure 6- The amount of leaf carotenoid content (mg g^{-1} FW) of barley seedlings grown in wastewater effluent and half-strength Hoagland's solution.

There are remarkable differences between the chlorophyll and carotenoid pigments contents of results show that the amount of rape seedlings grown in Hoagland and RWW solutions (fig.7 and 8).

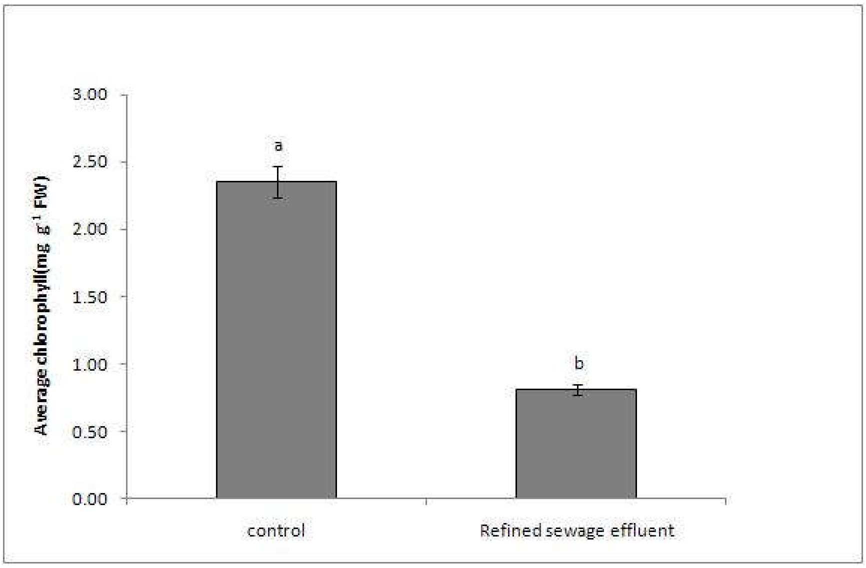

Figure 7- The amount of leaf chlorophyll content (mg g^{-1} FW) of rape seedlings grown in refined wastewater effluent and half-strength Hoagland's solution.

.

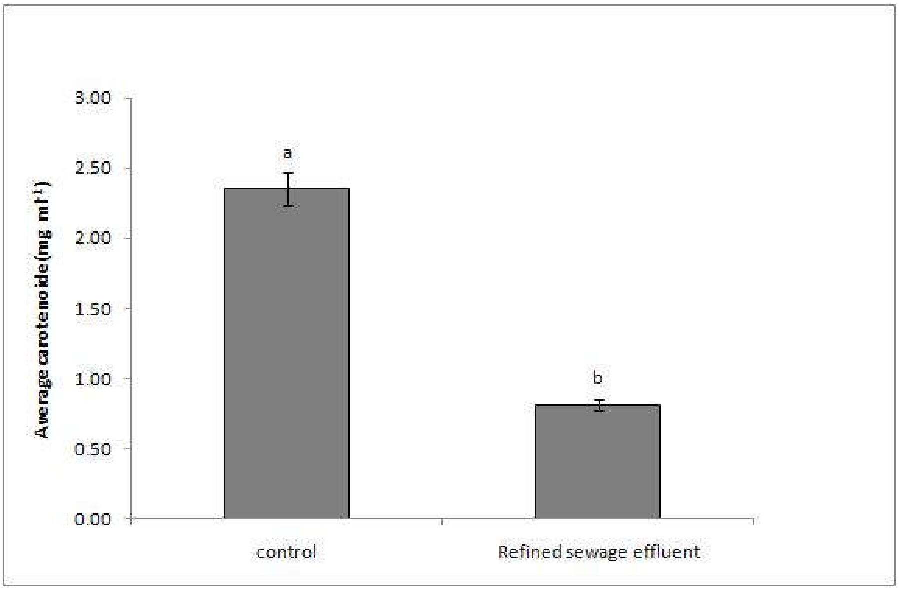

Figure 8- The amount of leaf carotenoid content (mg g^{-1} FW) of rape seedlings grown in refined wastewater effluent and half-strength Hoagland's solution.

The average amount of chlorophyll pigments for plants grown in Hoagland nutrient solution and in RWW media are 1.82 mg g^{-1} FW and 0.39 mg g^{-1} FW, respectively and in terms of carotenoid pigments, the values are changing between 2.35 mg $mL^{-1}$and 0.80 mg mL^{-1},respectively.Since iron is required at two stages in the metabolic pathway leading to chlorophyll synthesis [33, 50], the decrease in the amount of chlorophyll in plants grown in RWW could be affected by low iron content in their media (0.67 versus 5.00 mg L^{-1} in Hoagland solution).The low carotenoid and chlorophyll amount in plants growing in RWW can be due to low nitrogen and phosphorus contents in effluent solution (table 1). Although nitrogen and phosphorus are not the constituents of carotenoid molecules, these elements are required for the synthesis of enzymes involved in carotenoid synthesis [30]. The protective effect of carotenoid against oxidants such as singlet oxygen produced in the light reactions of photosynthesis is well established. Generally, the poor performance of rape seedlings grown in RWW media could be justified by the low phosphorus and nitrogen contents of these media. Similar findings have been reported by Paliwal *et al*. [36] who noted that *Hardwickia binata* grown in media containing more than 50% wastewater produced less photosynthetic pigments. Also Vijavaragavan *et al*. [46] revealed that radish plants (*Raphanus sativus*

L.) grown in different concentrations of sugar mill effluents contained less chlorophylls "a" and "b", total chlorophyll, and carotenoids.

CONCLUSIONS

Data presented in this study indicates that when barley plants (a monocotyledon plant) and rape plants (a dicotyledon plant) grown in petridish and hydroponically in RWW of Shiraz Water and Waste Water Treatment Plant. They respond differently with respect to some essential nutrient contents as compared to standard Hoagland nutrient solution. Barley germination factors including root length and number of lateral roots in the effluent were not comparable with those of the control. In case of rape plants grown in RWW the germination percentage was reduced but other parameters were functioned properly. All vegetative factors of barley seedlings in wastewater (shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight) showed a good performance in the competition with control plants. All vegetative parameters revealed poor performance without suitable growth signs in refined wastewater for rape seedling. The main reason probably is further concentration of lead in roots and shoots of rape plants grown in sewage effluent. More accumulation of sodium in the roots of barley plants grown in RWW and shoots of rape plants in the same medium indicates the resistance mechanism of barley RWW in order to sodium retention in roots and prevention of its transmission to shoots of the plants. Due to the existence of the more bivalent cation absorption positions in dicotyledon plants the more lead entered and concentrated and finally have made its negative effects on the roots and shoots of rape plants grown in RWW in comparison with barley plants grown in the RWW. In order to use the RWW as irrigation water, it should be supplemented with some nutrients such as iron, phosphorus, and to some extent nitrogen. However, further study regarding other essential macro and micro elements is recommended.

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