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Impact of wastewater irrigation on germination, growth seedling and concentration of heavy metals in soil and Canola (*Brassica napus* L.)

Khosro Estaki Oregani[1*], Zahra Kazemi Esfeh[1], Ali Gholami[2], Hossein Lari Yazdi[1]

[1]*Department of Agronomy, Faculty of Agriculture, Islamic Azad University, Boroujerd, Iran*

[2]*Department of Soil Science, College of Agriculture, Khouzestan Science and Research Branch, Islamic Azad University, Ahvaz, Iran*

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Abstract

The present research was conducted in Boruojerd, Lorestan, Iran to determine the effect of wastewater irrigation treatments on accumulation of some heavy metals (Fe, Mn, Zn, Cu, Pb, Cd and Ni) in root and shoot canola (Brassica napus L.viz., SLM046). The experimental design was random complete blocks with five effluents treatment and 4 replicate lysimeters (20 lysimeters) in the field. Wastewater irrigation application had a significant effect on seed germination, shoot length, length of hypocotyls, length of radical, number of leaves and leaf area. Soil, root and shoot Fe, Mn, Zn, Cu, Pb, Cd, and Ni concentrations increased consistently with increase in the treatment level of wastewater (T_4). From the results of the present study, it was concluded that municipal wastewater of cities containing high levels of heavy metals has inhibitory effect on growth and seed yield of canola.

* **Corresponding Author:** Khosro Estaki Oregani ✉ usaestaki@gmail.com,

Introduction

Rapid growth in human population is one of the major causes of environmental pollution. Increased industrialization and urbanization throughout the world including Iran has resulted in consistent release of toxic effluents, which render both soil and water unfit for crop production (Wahid *et al.*, 2000; Vasudevan *et al.*, 2010). Thus, most agro-industrial wastes are contaminated with a variety of metals which, if applied to crops, may cause inhibitory effects on growth and yield (Al-Nakshabandi *et al.*, 1997; Faryal *et al.*, 2007; Khan and Jones, 2008; Estaki *et al.*, 2014).

In many areas of developing countries untreated wastewater flows through channels into rivers where it is diverted by subsistence farmers to small plots of crop grown for nearby urban markets (Hassanpour *et al.*, 2010). The public risks of using such contaminated streams for irrigation are obvious. Application of wastewater to cropland and forested lands is an attractive option for disposal because it can improve physical properties and nutrient contents of soils; wastewater irrigation provides water, N and P as well as organic matter to the soils, but there is a concern about the accumulation of potentially heavy metals such as Cd, Cu, Fe, Mn, Pb and Zn from both domestic and industrial sources. Heavy metals represent a portion of important environmental pollutants which causes pollution problems by increasing their use in products in recent decades. Recently, one of the issues that attracted the attention of researchers and environmentalists is wastewater. Chemicals and heavy metals especially those which can penetrate into soil, plant and finally food chain (Soumare *et al.*, 2003; Bernala, 2006; Oron *et al.*, 2007). Plants are the major raison of the presence of metals in the food chain. Their concentration into trace elements, in contexts diffuse contamination is highly variable. For a given level of contamination of an environment, the accumulation depends not only on the component, the plant species' family and variety, but also on the body and soil factors such as pH, temperature (Rejeb *et al.*, 2011). In spite of gradual accumulation of heavy metals in the soil, the stability of heavy metals in the environment will cause to pollution since they could not be decomposed like organic pollutants by biological or chemical processes (McBride, 1995).

The family Brassicaceae includes a number of species that have considerable nutritional and economic values and that have been under cultivation since 1500 B.C. These crops are extensively grown as cash crops, fodder and industrial/medicinal crops (Ashraf and McNeilly,2004; Akbar *et al.*, 2008). The objectives of this study were to evaluate the effect of different treatments of irrigation with wastewater on absorption and accumulation of some heavy metals and their possible contaminations in soil and canola crop.

Materials and methods

The experimental protocol had been installed in the field at the Agricultural Experiment Station of Islamic Azad University, Boruojerd Branch at Iran (33°54' N, 48°40' W;1620 m above sea level). The climate in the area is classified as semi-arid. The annual rainfall of 560 mm (mean 1980–2011) falls mostly in the winter. The mean annual temperature ranges from 13.3 to 16.9 °C. The lysimetric experiments were conducted in zero-tension circular lysimeters of 50 (cm) diameter and 150 cm height from the bottom (volume=0.3 m3). The bottom of the lysimeter was covered by geo-textile, overlaid with 10 cm river sand with 0.5-1 mm grain size, then lysimeters filled by soil and in order to prevent water influx from field to lysimeters, those placed on metal legs (height = 45 cm). After filling lysimeters by clay loam soil, fresh lot of certified seeds of canola (Brassica napus L.viz., SLM046) were planted in August 2011 and were irrigated with agronomical water. The experimental design was random complete blocks and 4 replicate lysimeters within each treatment leading to a total of 20 lysimeters.

There were five effluents treatment : T_0 (control or normal agronomical water), T_1 (25% wastewater + 75% well water), T_2 (50% wastewater + 50% well water), T_3 (75% wastewater + 25% well water), T_4 (100% wastewater).

soil analysis

The soil of the experimental grove is a clay loam. The characteristics of the soil used in the experiments are given in table 1. Soil samples were air-dried, crushed, and passed through a 2-mm sieve prior to chemical analysis. Cation exchange capacity (CEC) was determined using sodium acetate (buffered at pH 8.2) and ammonium acetate (buffered at pH 7.0) according to Sumner and Miller (Sumner and Miller, 1996) . The Kjeldahl method was used to determine organic N and plant-available P was determined by using the sodium bicarbonate method of Olsen (Olsen *et al.*, 1954). Electrical conductivity (EC) was measured in saturation extracts according to Rhoades (Rhoades, 1996). Soil pH was termined in 1:2 extracts, and calcium carbonate concentration was determined according to McLean (Mclean, 1982). Soil organic matter was determined using the Smith-Weldon method (Fuentes *et al.*, 2002). Ammonium acetate buffered at pH=7 was used to determine exchangeable cations. For trace elements, samples of soil were digested with a mixture of HCl/HNO3-(McGrath & Cunliffe,1985) and total concentrations were determined by ICP-AES (Inductive Coupled Plasma Atomic Emission Spectrometry, Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA). Some physical and chemical properties of soil are given in Table 2 (Bremner, 1996; Jones, 2001; Horwitz, 2005).

Plant analysis

At maturity shoots and roots of plants were used for chemical analysis. Samples of root and shoot were collected at maturity stage. The root and shoot samples were oven-dried at 68°C for 48 h and ground to pass 1mm sieve. Micro-elements (Fe, Mn, Zn, Cu, Pb, Ni, Cr and Cd) were determined after wet digestion of dried and ground sub-samples using a HNO3 -H2O acid mixture (2:3 v/v) with three steps (first step; 145°C, 75% RF, 5 min; second step; 180°C, 90% RF, 10 min and third step; 100°C, 40% RF, 10 min) in microwave (Bergof Speedwave Microwave Digestion Equipment MWS-2). Tissue Fe, Mn, Zn, Cu, Pb, Ni, Cr and Cd were determined using an inductively coupled plasma spectrophotometer, Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA (Mertens, 2005).

wastewater analysis

Wastewater effluents were collected from the various wastewater channels of Boruojerd city are mixed. These effluents were collected only once before the commencement of each growth season in a sufficient quantity, stored in shade and then its known concentrations were supplied to the plants as a soil drench. Fresh dilutions were made before each irrigation. Some chemical properties of the wastewater used for irrigation were determined by the methods described by American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF) were tested by multiple tube fermentation procedure Table 1(APHA, 2005; APHA, AWWA and WEF, 1985; APHA, 1995).

Statistical analysis

All data obtained from the effects of different wastewater irrigation on soil and plant chemical properties in contrast to non-wastewater irrigated in the present study were subjected to analysis of variance (ANOVA). The Duncan's multiple range tests were performed for mean comparisons using SAS statistical software (SAS .1982).

Results and discussion

Quality of municipal wastewater

The results of well water and wastewater quality analysis are shown in table 1, and they have been compared by suggested standards of Iran environment conservation organization (E.C.O of Iran, 1999) and FAO water qualitative standard. The analysis of wastewater (Table 1) has revealed that it was alkaline. In consequence, available water was found suitable for irrigation and evaluation of wastewater pollution (Table 1) described that magnitude of Zn, Cu and Mn concentrations were below the critical limits (E.C.O. of Iran, 1999).

Regarding metal traces, chemical analysis of wastewater of Boruojerd (Table 1) showed that Fe and Zn were the most represented elements. The average levels found were organized according to the following sequence: Fe > Zn> Pb > Cu > Ni > Cd.

Effect of irrigation treatment on the soil heavy metals

Municipal wastewater irrigated soils (T_4) showed higher Mn, Zn, Pb, Ni and Cd compared to tape water(T_0). Total heavy metal concentrations, Fe, Mn, Zn, Cu, Ni, Pb and Cd, were less in all soils with treated irrigation water (T_0) than in other treatments (table 2). In general, it seems that heavy metals tend to accumulate in the surface layer of soil and their movement is limited by strong binding with clay minerals and organic matter. Analysis of variance showed the significant (p<0.05) effect of irrigation with wastewater treatments on accumulation rate of Fe. Concentration of iron in the soil is affected by increased wastewater irrigation. Increasing the amount of absorbable iron in the soil of arid regions is important because iron deficiency is considered as the most important problems of plant nutrition in such soil (Lopez *et al.,* 2000). From analysis of variance of data for soil Zn it is evident that soil Zn levels varied significantly in varying treatments of Municipal wastewater (Table 3). The mean soil Zn levels varied from 1.1 to 2.64 mg kg-1 in the wastewater treatments. The soil Zn levels observed in our investigation were much lower than those reported by Cicek, and Koparal, 2004; Abbas *et al.*,2007.

Table 1. Analysis of municipal wastewaters used for irrigation.

Parameters ofwastewater	Treatment (Effluents concentrations)					Standard limit of pollutants	
	T0 (0%)	T1 (25%)	T2 (50%)	T3 (75%)	T4 (100%)	E.C.O Iran	of FAO
pH	7.3	7.5	7.8	8.1	8.6	6-8.5	6-8.5
EC (dSm^{-1})	1.1	2	2.8	3.65	5.3	1.5	3
Calcium	49.4	71	101.2	145.6	158.9	200	75
Magnesium	26	55.3	73.5	88.4	111.7	150	100
Sulfate	31.1	85.5	271.5	303	865.7	1000	1000
Chloride	0.1	0.7	1.8	3.9	6.3	1000	1100
Nitrate	0.9	3.6	8.4	11.3	24.8	--	--
Phosphate	1.08	6.5	14.8	18.6	27.2	15	--
Sodium	19	48.5	121.7	166.8	278	250	--
Potassium	5.6	7.4	14.5	17.4	20.8	--	--
Microelements (mg L^{-1})							
Iron	0.08	0.21	0.49	1.81	3.94	3	5
Zinc	0.07	0.21	0.42	0.64	1.42	10	2
Copper	Nil	0.1	0.24	0.36	0.69	0.2	0.2
Manganese	0.05	0.14	0.23	0.46	1.14	2	2
Lead	Nil	0.1	0.53	0.87	1.45	1	5
Cadmium	Nil	0.06	0.18	0.24	0.31	0.1	0.1
Nickle	Nil	0.08	0.1	0.21	0.49	0.1	0.1
BOD$_5$	Nil	20.5	60	75.6	90.5	100	250
COD	Nil	33	75	141.3	209	200	400
Total Coliform (MPN/100ml)	Nil	Nil	20	165.4	195	1000	1000

BOD$_5$ = Biological oxygen demand; COD = Chemical oxygen demand; SAR = Sodium Adsorption Ratio.

Copper concentration was not affected by irrigation treatments. Ghalavi *et al.*, (2010) believe that in calcareous and high acidity soil, microelements absorption ability (such as copper) is low for plants and hence they have recommended organic fertilizers usage to increase these elements in soil. Soil Cd levels increased consistently with increase in the concentration of municipal wastewater it ranged from 0.01 to 0.17 mg/kg of soil. The toxic Cd level in soil is in the range of 2-50 mg/kg . Thus, all the values of soil Cd reported here are within the lower limit of the toxic range reported by Bergmann (1992).

Soil Ni levels also varied significantly (p<0.05) after treatment with wastewater. The values of Ni in soil ranged from 0.07 to 0.18 mg kg-1 during all treatments (table 2). The mean soil Ni found in our investigation was lower than the critical level of 3.0 mg/kg as reported by Kloke (1980). Thus, according to this criterion the soil Ni levels in our investigation were below the toxic level of Ni.

Accumulation of heavy metals in shoots and roots of the Canola

As expected, the concentration of heavy metals measured in the tissues of plants grown on soil irrigated with wastewater was higher than in the controls. The increase rate was often 10-fold or more. Root heavy metal uptake was greater than shoot heavy metal uptake. In all wastewater application rates, heavy metal concentrations in roots were about 4–8 times higher than in shoots. Among the wastewater doses were the most effective for Cu, Cd, Zn and Pb uptake in plants (Table 3).

Table 2. Chemical properties of the rhizosphere area irrigated after the harvest of canola.

	Irrigation treatment				
Parameters	To	T1	T2	T3	T4
pH	7.8 a	7.7 a	7.62 a	7.58 a	7.05 b
EC (dSm^{-1})	1.15 c	1.92 bc	2.8 b	3.45 ab	4.52 a
O.C (%)	0.261 b	0.3 b	0.364 b	0.41 ab	0.68 a
SAR	7.7 b	8.1 b	9.8 ab	10.6 ab	12.36 a
CEC ($cmol_c\ kg^{-1}$)	24.6 a	25.2 a	27.3 a	28.1 a	30.4 a
Total nitrogen (%)	0.04 a	0.05 a	0.05 a	0.06 a	0.08 a
K ($mg\ kg^{-1}$)	180 a	181 a	187 a	193.5 a	200.6 a
Ca + Mg (me/L)	23 a	24 a	26.8 a	27.1 a	27.5 a
Na (ppm)	21.3 b	21.5 b	25.6 ab	27.4 a	29.8 a
Fe ($mg\ kg^{-1}$)	1.2 b	2.7 a	3.1 a	3.2 a	3.28 a
Zn ($mg\ kg^{-1}$)	1.14 c	1.28 bc	1.48 b	1.68 ab	2.64 a
Mn ($mg\ kg^{-1}$)	1.62 c	1.72 c	1.92 c	2.56 b	3.18 a
Cu ($mg\ kg^{-1}$)	1.29 a	1.37 a	1.41 a	1.5 a	1.55 a
Cd ($mg\ kg^{-1}$)	ND	0.01 b	0.06 b	0.12 a	0.17 a
Pb ($mg\ kg^{-1}$)	ND	0.05 c	0.05 c	0.14 b	0.21 a
Ni ($mg\ kg^{-1}$)	ND	0.07 c	0.1 b	0.18 b	0.48 a

* Means with different letters within rows are significantly different ($p < 0.05$), ND: Non detectable, O.C= Organic Carbon; CEC= Cation Exchange Capacity.

In the present research, Fe concentration increased significantly by wastewater treatments (Table 4). Furthermore, Ghalavi *et al.* (2010) in a research on heavy metal uptake by sorghum and accumulation in soil which has been irrigated by wastewater have reported significant increase for Fe, Cu, Zn, Mo and Pb content of sorghum. These results also indicates that the addition of fertilizer helps in reducing the Fe concentration due to it phytostabilizing effect mainly from DAP (Khan & Jones, 2008).

The Zn is accumulated at the root and shoot (table 3). The highest amounts of Zn were obtained for T_4 including 100% wastewater in root (189.52 ppm). For example, shoot analysis revealed a maximum of 138.8 ppm obtained T_4 while with T_1, values were recorded 46.8 ppm.

The analysis of the plant showed that Cd levels are only detectable for treatments with and this in shoots and roots, some Cd levels exceeded the acceptable threshold according to Mench & Baize (2004).Irrigation with wastewater led to create optimal conditions for copper uptake and increased absorption by the plant rather the control treatment. Organic matter increased by the use of wastewater in soil. Environmental factors such as temperature and light affect plants growth; these two factors effectively can increase copper absorption (Akinola *et al.*, 2006 ; Ghalavi *et al.*, 2010). The mean lead levels ranged from 0.06 to 1.28 mg/kg (0.1 – 1.28 in root and 0.06 – 0.9 mg/kg in shoot) in various treatments, although there was an inconsistent pattern of increase during different wastewater doses. The forage lead levels detected in the present investigation were lower than the acceptable Pb limit level, 3.0 ppm reported for plants (Yang *et al.,* 2003). Forage Cd levels ranged from 1.26 to 3.15 mg/kg in the present study. The adequate level of Cd in plants has been suggested to be around 3 mg/kg. So the forage Cd levels reported in the present study show no potential threat for livestock if such sewage water treated forages are fed to it. In the present study Ni concentrations were equally increased during all the wastewater treatments (Table 3). The forage Ni levels ranged from 0.1 to 2.24 mg/kg in shoot and 0.3 – 3.35 mg/kg in root. The Ni levels found in our study were in the range of Ni in other plants level previously reported by Deya *et al.*, (2005) in Vieques, Puerto Rico.The forage Ni level is less than the toxic level so it is safe for livestock use.

Table 3. Comparing the averages of heavy metals concentration in Canola.

Part of canola	Wastewater irrigation treatments					
	Elements (mg/kg dm)	T_0	T_1	T_2	T_3	T_4
Shoot	Fe	87.8 d	139.3 c	186.6 b	202.8 b	251.4 a
	Zn	46.83 e	91 d	103.4 c	123.5 b	138.8 a
	Cu	2.1 d	6.8 c	14.2 a	11.4 b	7.4 c
	Cd	0.06 d	0.48 c	0.77 bc	1.38 a	1 b
	Pb	0.06 d	0.33 c	0.55 b	0.7 b	1.9 a
	Ni	0.1 e	0.43 d	0.76 c	1.05 b	2.24 a
Root	Fe	97.3 d	232.1 c	247.8 c	273.1 b	324.3 a
	Zn	60.3 d	113 cd	128.82 c	140.25 b	189.52 a
	Cu	4.2 d	10 c	18.45a	15.7 ab	14.83 b
	Cd	0.2 d	0.61 c	0.84 c	1.43 b	1.78 a
	Pb	0.1 c	0.63 bc	0.8 b	1.08 ab	2.88 a
	Ni	0.3 e	0.71 d	1.1 c	2.02 b	3.35 a

*Means with different letters within rows are significantly different ($p < 0.05$), ND: Non detectable.

Effects of wastewater treatments on seed germination and seedling growth

Analysis of variance showed that wastewater treatments had significant effects on seed germination. The data presented in table 4, revealed that seed germination of canola was seriously affected by varying doses of applied wastewater. The greater the concentration of wastewater, the lesser the rate of germination observed. A maximum germination percentage was noted in T_1 (98.8%) and a minimum in T_4 (90.5%). Increasing the concentration of different wastewater treatments resulted dramatically in reduction in seed germination. Seed germination is a good topic for scientific exploration since it is easy to observe and there are so many obvious and not so obvious environmental factors that can affect the germination (Bazai and Achakzai, 2006). In general, there was a reduction in seed germination as metal concentrations in the growing media increased. Shoot length in table 4 was significantly affected by municipal wastewater treatment as there was a consistent decrease in this growth attribute with increase in the level of municipal wastewater. The same trend was observed for length of hypocotyls, length of radical, number of leaves and leaf area including mature leaves of canola plants. It was also noted that in the initial doses of polluted water, the length of hypocotyls, length of radical and leaf area were slightly increased as compared with their respective treatment (T1). This reduction in seedling growth was highly marked in T_4, T_3, T_2, T_0 and T_1, respectively . Decreased growth in different plants due to metal contaminated waters has been earlier reported by other researchers (Meagher, 2000; Andaleeb *et al*., 2008).

Table 4. Effect of wastewater on percentage of germination, length of hypocotyls, length of radical , number of leaves, leaf area, shoot length in canola (Brassica napus L.,).

Physical parameters	Wastewater irrigation treatments				
	T_0	T_1	T_2	T_3	T_4
Germination (%)	97.6 b	98.8 a	98.6 a	97.5 b	89.5 c
Length of hypocotyls(cm)	7.5 ab	8.1 a	7.6 ab	6.5 b	6.0 b
Length of radical (cm)	9.3 ab	10.4 a	10.0 a	9.1 ab	8.6 b
Number of leaves	11.7 b	14.9 a	13.0 ab	12.3 ab	11.45 b
Leaf area (cm^2)	96.4 bc	119.4 a	104.8 b	94.2 bc	90.45 c
Shoot length (cm)	70.24 ab	79.75 a	80.22 a	72.3 ab	68.5 b

* Means with different letters within rows are significantly different ($p < 0.05$), ND: Non detectable.

Municipal wastewater of cities having high concentrations of salinity and heavy metals proved to have a potential retarding effect on growth, development and yield affecting parameters of Canola crop. Research further revealed that salinity and heavy metals not only affects seed germination but also significantly reduced the subsequent growth of seedlings. This reduction in seedling growth is either due to accumulation of mineral ions in their cell sap or because of the failure of sub-cellular organelles to adjust high osmotic potential of cell sap. Based on findings of our investigation, the significant reduction in Physical parameters in the Canola, level of treatment with municipal wastewater was found.

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