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Application of treated wastewater on yield and heavy metals content of seeds in sunflower cultivars

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Abstract

Treated wastewater could be considered as a new water resource in agriculture especially in arid and semi-arid regions of the world such as Iran. Two one-year field experiments were carried out to determine the effects of municipal treated wastewater on yield and seed heavy metals content of sunflower cultivars, in Research Farm of Yazd Municipal Wastewater Purification Station during 2015 and 2016. The climate of the region was hot and dry according to Koppen climate classification system. Experiment was arranged as split plot based on randomized complete block design with three replicates. Treatments were three irrigation water types as main plot consisted ("WW": irrigation with treated municipal wastewater thoroughly, "WW/FW": irrigation with treated wastewater/fresh water alternatively and "FW": irrigation with fresh water thoroughly) and three sunflower cultivars (Azargol, Record, and Farrokh) as subplots. The following parameters were assessed: plant height (cm), head diameter (cm), seed numbers per head, unfilled seeds per head, seed and biological yield (kg/ha) and seed heavy metals content. Results showed that the highest plant height (194 cm) obtained in Record and the highest head diameter (21.5 cm) obtained in Farrokh cultivars, which were irrigated by WW. Treated municipal wastewater also increased seed yield by 10.7% compared to FW treatment. The highest (5029 Kg/ha) and lowest (3734 Kg/ha) seed yield were obtained in Azargol and Farrokh that irrigated with WW and WW/FW treatment, respectively. However, application of treated municipal wastewater lead to accumulation of trace elements (Fe, Mn, Zn, Cu, Cd, Pb, and As) in seeds; but, the values of all the elements were below the permissible limits recommended by World Health Organization. It is concluded that WW could be used for irrigating sunflower crop without worrying about the accumulation of toxic substances.

Keywords: Accumulation; Irrigation; New water resource; Permissible limits; Trace elements.
Abbreviations: WW_only use of treated municipal wastewater; WW/FW_Use of treated wastewater/fresh water alternatively; FW_only use of fresh water.

Introduction

Water shortages in arid and semi-arid regions like Iran, has inevitably led to use of new sources such as wastewater in agriculture. Treated municipal wastewater may prove a potential economic asset by contribution to saving water resources, irrigating agriculture land and even reducing fertilizer inputs (Fonseca et al., 2005b). In fact, the reuse of treated municipal wastewater in irrigation is an alternative to water scarcity and a means of environmental protection by reducing the discharge of water into rivers and oceans (Zavadil, 2009). It seems that in some cases high yields of the crops could be achieved without deterioration of their quality by using treated wastewater (Munir and Ayadi, 2005). For example Moazzam-Khan et al. (2009) reported that growth and yield of sunflower were significantly affected by using reclaimed municipal wastewater, so that the highest yield were obtained in wastewater treatments. However, application of wastewater generally leads to increased concentrations of heavy metals in the soil and plants (Mojiri and Hamidi, 2011). The accumulation of heavy metals can have middle and long-term health risks, and the strict periodic surveillance of these contaminants is therefore advisable (Etoniho et al., 2013). This concern encouraged the researchers to study the pollution levels of heavy metals in the plant to determine their permissibility for human consumption. It seems that, choice of crops for treated wastewater irrigation is the principal factor for the suitability of effluent irrigation because certain crops could be irrigated without negative implications on yield (Fonseca et al., 2005b). Sunflower (*Helianthus annuus* L.) is an increasingly important source of vegetable oil and biomass, usefully employed for food, chemical, energy, and industrial purposes (Riva and Calzoni, 2004). It is believed that the plant had the capacity to absorb heavy metals (Madejon et al., 2003; Vamerial et al., 2012), but these components probably accumulated in the roots rather than shoot and plant seeds (Madejon et al., 2003; Marchiol et al., 2007; Adesodum et al., 2010). The objectives of this study were to evaluate the effects of treated municipal wastewater on the yield and heavy metals content of the sunflower seed.

Results

Plant height and head diameter

Interaction between irrigation water types and cultivars on sunflower height and head diameter was significant (Table 1). The highest plant height (194.16 cm) and head diameter (21.5 cm) were obtained in Record and Farrokh, which were irrigated by WW, respectively. The lowest plant height was also obtained in Record which irrigated with FW treatment (Table 2). Generally, the WW treatment caused increasing the height of plants and head diameter in compared to the FW and WW/FW (Table 3).

Seed and unfilled seed number per head

Number of seed per head, increased in WW treatment significantly. The highest number of seed per head (560) was obtained in Azargol when irrigated with WW treatment. Farrokh had the lowest number of seed per head (473) where irrigated with WW/FW treatment. Results also show that the highest of unfilled seed number in head (295) was observed in Farrokh which was irrigated by FW treatment, however, there were no significant differences between the cultivars in this treatment (Table 2). The Record produced higher seed number per head in WW and WW/FW treatments than the FW treatment. There was no significant difference between FW/WW and FW treatments in terms of seed number per head in Azargol cultivar. In fact, irrigation with WW treatment improved seed number per head by 6% and the unfilled seed was 20% less than the FW treatment (Table 3). There were no significant differences between FW and WW/FW treatments on the seed number per head; however, the unfilled seed per head in WW/FW treatment was about 12% less than the FW treatment.

Seed yield

Interaction between year and treatments on seed yield were not significant (Table 1). The highest seed yield (5029 Kg/ha) was obtained in Azargol when irrigated with WW treatment. Farrokh produced the lowest seed yield by 3734 Kg/ha in WW/FW treatment (Table 2). Treated municipal wastewater (WW) increased seed yield of Azargol in comparison with the FW and WW/FW treatments. No significant differences were observed between FW and WW/FW on the seed yield of the cultivar. The Record produced higher seed yield when was irrigated by WW and WW/FW compared to FW treatment. Azargol, Record and Farrokh produced more seed yield by 14.55%, 9.5% and 7.7% in WW than the FW treatment, respectively. In addition, seed yield was about 10.7% higher in WW treatment compared with FW treatment (Table 3), although no mineral fertilizer was used WW and WW/FW treatment.

Seed heavy metals content

Results also showed that the effect of treatments on seed heavy metals (Fe, Cu, Zn, Mn, Pb, Cd and As) content was significant (Table 4). The accumulation of Fe, Mn, Zn, Cu, Cd, Pb and As in seed significantly increased in WW treatment compared to WW/FW and FW treatments. There were no significant differences in the concentrations of Fe, Pb, Cd and As of the seeds between FW and WW/FW (Fig 1). Interaction between treatments and cultivars also significantly affected Zn, Cu and Mn contents of seeds (Table 4). The highest Zn and Cu contents observed in cultivars that were irrigated by WW and WW/FW treatments. Azargol had the highest Mn seed content (29 ppm) when irrigated by the WW treatment. The lowest Mn content of seed of the cultivars was obtained in FW treatment. Generally, the lowest Zn, Cu and Mn seed contents of the cultivars was observed in FW treatment (Table 5).

Discussion

The results of the present study showed that all growth parameters were significantly affected by treated wastewater irrigation and the growth parameters in all cultivars increased with treated wastewater. Treated wastewater considered as one of the important sources of nutrients and sunflower growth characteristics substantially increased by the treatment (Moazzam-Khan et al., 2009). In several studies, positive effects of treated municipal wastewater had been reported on the growth of different plants (Fonseca et al., 2007; Fonseca et al., 2005a).

Due to enrichment of the nutrients in WW treatment, generally number of fertilized florets, seeds number per head and number of filled seeds increased compared to WW/FW and FW treatments. These results were in agreement with Safi-naz and Shaaban (2015), who reported that application of treated wastewater enhanced the yield and yield components of sunflower. Similar results were also reported by Andrew et al. (1998), Shahandeh and Hossener (2002), Esmailiyan et al. (2008).

Results also showed that the application of treated wastewater increased seed heavy metals content (Fe, Mn, Zn, Cu, Cd, As and Pb) than the FW treatment; however, seed heavy metals content in WW treatment were lower than the maximum permissible limits. Many investigations, including long- and short- term studies showed that the accumulation of heavy metals in plants increased as a consequence of the application of wastewater (Rusan et al., 2007; Jahantigh, 2008; Jagtab et al., 2010; Masona et al., 2011). The accumulation of heavy metals depends on different factors such as soil pH, percentage of soil organic matters and amount of heavy metals in soil. Furthermore, in presence of organic matter, heavy metals could be found as chelates, which increase the ability of plants to uptake them. The previous study done by Mapanda et al. (2005) on vegetables revealed increasing concentrations of heavy metals (Cu, Zn, Cd, and Pb) in soils irrigated with treated wastewater.

As proved decrease of soil pH could increase the uptake of heavy metals by plant (Fonseca et al., 2005b), so decrease of soil pH in WW treatment (Table 6), increased uptake heavy metals by the sunflowers. These results were consistent with the conclusions by Mojiri and Hamidi (2011). In contrast, due to leaching of the heavy metals in FW and WW/FW treatments, accumulation of them in the root zone could be reduced and their uptake would be reduced by the plant. Contrary to our results, Koottatep et al. (2006) found no significant changes in the concentrations of nutrient

Table 1. Wastewater and cultivars effects on sunflower height, head diameter, seed number per head, unfilled seed per head, and seed yield (2015-2016).

		Mean squares				
S.O.V	df	Plant height	Head diameter	Unfilled Seed/Head	seed/Head	Seed yield
Year	1	50.07 ^{ns}	0.296 ^{ns}	8613.4 **	1075 ^{ns}	3.76 ^{ns}
Block (Year)	4	56.35 ^{ns}	0.962 ^{ns}	128.96 ^{ns}	137.48 ^{ns}	48355 ^{ns}
Water	2	2325 **	80.58 **	15779 **	5157 **	1489606 **
Water (Year)	2	17.01 ^{ns}	0.754 ^{ns}	736.46 ^{ns}	199.41 ^{ns}	17002 ^{ns}
W×B×Y	8	60.52 ^{ns}	1.379 ^{ns}	259.1 ^{ns}	202.09 ^{ns}	28577 ^{ns}
Cultivar	2	129.57 *	2.018 ^{ns}	2288.5 **	5625 **	1260970 **
(C ×W)	4	919.04 **	11.56 **	2651.18 **	1808.6 **	181818 **
Cultivar (Year)	2	10.35 ^{ns}	0.685 ^{ns}	117.85 ^{ns}	62.91 ^{ns}	3727 ^{ns}
(C ×W× Y)	4	72.87 ^{ns}	0.726 ^{ns}	340.07 ^{ns}	109. 96 ^{ns}	10076 ^{ns}
Erorr	24	27.29	0.983	392.388	360.194	36916

*Significant at the 5% level, **Significant at the 1% level and ns. Not significant. W×B×Y = Water × Block × Year; C ×W= Cultivar ×Water; C ×W× Y= Cultivar ×Water× Year.

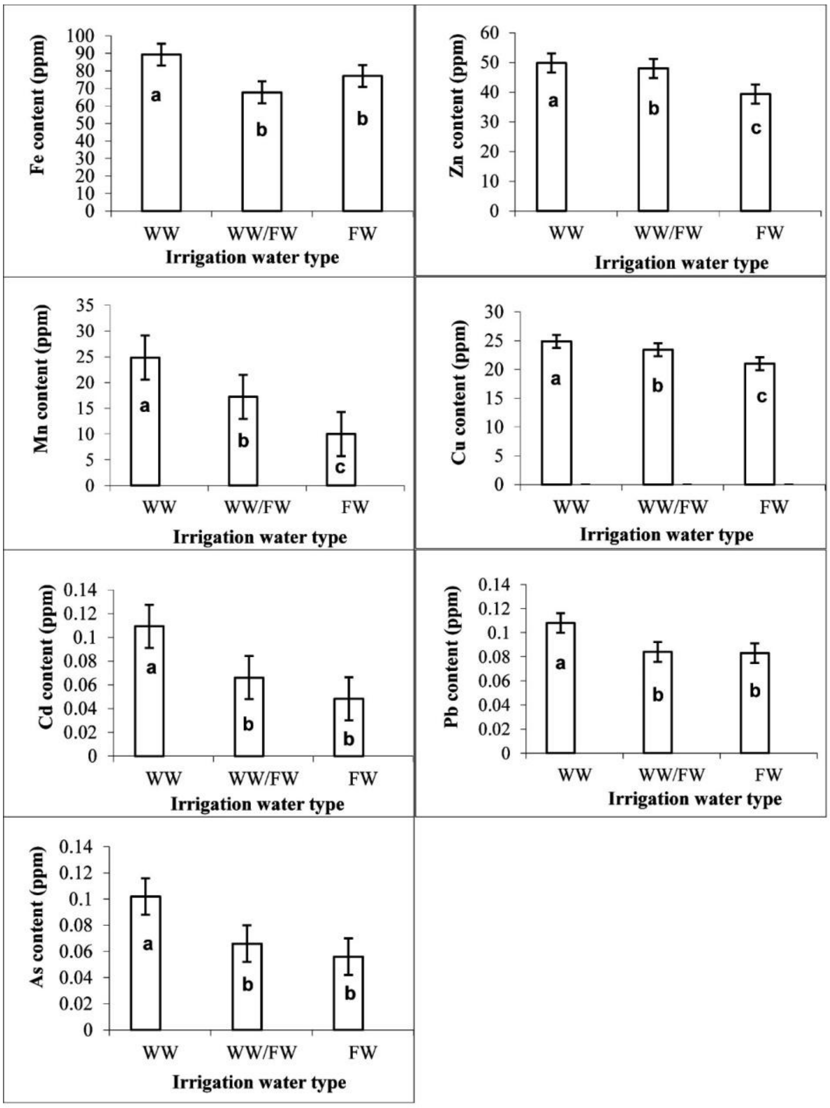

Fig 1. Effects of treated wastewater on accumulation of Fe, Mn, Zn, Cu, As, Cd and As in sunflower seeds (ppm). WW = treated wastewater, WW/FW = treated wastewater/fresh water alternatively and FW= fresh water.

Table 2. Mean comparisons of interaction effects on plant height, head diameter, seed and unfilled seed number per head and seed yield of sunflower.

Irrigation Water type	Cultivar	Means				
		Plant height (cm)	Head diameter (cm)	Unfilled Seed/head	Seed/head	Seed yield (Kg/ha)
	Azargol	166.66 b	19.66 b	254.16 bc	560.83 a	5029 a
WW	Record	194.16 a	18.83 bc	236.66 cd	515 b	4404 b
	Farrokh	165.83 b	21.5 a	213.33 d	508.66 b	4313 b
	Azargol	154 e	17.16 d	278.33 ab	513.33 b	4201 bc
WW/FW	Record	155 de	15.25 e	225.5 d	515.83 b	4194 bc
	Farrokh	155.83 cde	15.66 e	272.5 ab	473 d	3734 d
	Azargol	161.16 bcd	17 d	290.83 a	509.5 b	4390 b
FW	Record	147.5 f	17.75 cd	294.16 a	484.83 cd	4022 c
	Farrokh	162.16 bc	15.5 e	295.83 a	497.5 bc	4005 c

Means within columns followed by the same letters are not significantly different according to Duncan's test ($P \leq 0.05$). WW = treated wastewater, WW/FW = treated wastewater/fresh water alternatively and FW= fresh water.

Table 3. Mean comparisons of manners of water use effects on height, head diameter, seed and unfilled seed number in head, seed yield of sunflower.

Irrigation water type	Means				
	Plant height (cm)	Head diameter (cm)	Unfilled Seed/head	Seed/head	Seed yield (Kg/ha)
WW	175.55 a	20 a	234.72 c	528.16 a	4582.8 a
WW/FW	154.94 b	16.02 b	258.77 b	500.72 b	4043 b
FW	156.94 b	16.75 b	293.61 a	497.27 b	4139 b

Means within columns followed by the same letters are not significantly different according to Duncan's test ($P \leq 0.05$). WW = treated wastewater, WW/FW = treated wastewater/fresh water alternatively and FW= fresh water.

Table 4. Treated wastewater and cultivars effects on Fe, Mn, Cu, Zn, Cd, Pb and As contents in sunflower seed (2015-2016).

S.O.V	df	Mean squares						
		Fe	Mn	Cu	Zn	Cd	Pb	As
Year	1	66.66^{ns}	11.57^{ns}	4.74^{**}	0.296^{ns}	$15 \times 10^{-7\ ns}$	$39 \times 10^{-5\ ns}$	$6 \times 10^{-5\ ns}$
Block (Year)	4	41.42^{ns}	2.074^{ns}	5.703^{ns}	10.907^{ns}	$23 \times 10^{-5\ ns}$	$9 \times 10^{-5\ ns}$	$66 \times 10^{-5\ ns}$
Water	2	2102^{**}	990.35^{**}	69.55^{**}	559.79^{**}	$17 \times 10^{-3\ **}$	$384 \times 10^{-5\ **}$	$107 \times 10^{-4\ **}$
Water (Year)	2	143.72^{ns}	0.351^{ns}	0.074^{ns}	11.129^{ns}	$53 \times 10^{-5\ ns}$	$81 \times 10^{-5\ ns}$	$21 \times 10^{-5\ ns}$
W×B×Y	8	159.14^{ns}	9.962^{ns}	1.897^{ns}	5.574^{ns}	$22 \times 10^{-5\ ns}$	$45 \times 10^{-5\ ns}$	$56 \times 10^{-5\ ns}$
Cultivar	2	872.01^{**}	12.51^{ns}	24.22^{**}	48.35^{*}	$68 \times 10^{-5\ ns}$	$88 \times 10^{-6\ ns}$	$53 \times 10^{-5\ ns}$
(C ×W)	4	231.07^{ns}	39.018^{**}	4.61^{**}	34.18^{*}	$97 \times 10^{-5\ ns}$	$10^{-4\ ns}$	$251 \times 10^{-5\ ns}$
Cultivar (Year)	2	1.5^{ns}	4.518^{ns}	4.96^{ns}	4.018^{ns}	$23 \times 10^{-5\ ns}$	$10^{-4\ ns}$	$118 \times 10^{-5\ ns}$
(C ×W× Y)	4	50.38^{ns}	4.462^{ns}	1.296^{ns}	14.351^{ns}	$57 \times 10^{-6\ ns}$	$83 \times 10^{-6\ ns}$	$63 \times 10^{-5\ ns}$
Erorr	24	103.87	7.222	4.055	9.129	46×10^{-5}	49×10^{-5}	48×10^{-5}

*Significant at the 5% level, **Significant at the 1% level and NS. Not significant. W×B×Y = Water × Block × Year; C ×W= Cultivar ×Water; C ×W× Y= Cultivar ×Water× Year.

Table 5. Mean comparisons of interaction effects on Mn, Cu and Zn contents of sunflower seeds.

Irrigation water type	Cultivar	Means (ppm)		
		Mn	Cu	Zn
	Azargol	29 a	25 a	50.16 a
WW	Record	22.33 b	25 a	49.16 a
	Farrokh	23.16 b	24.66 a	50.16 a
	Azargol	16 c	24.16 ab	48.66 a
WW/FW	Record	17.66 c	24.33 ab	48.33 a
	Farrokh	18 c	22.83 ab	47 a
	Azargol	9.5 d	21.83 b	43.33 b
FW	Record	9.d	21.33 b	34.83 c
	Farrokh	11 d	18.83 c	40 b

Means within columns followed by the same letters are not significantly different according to Duncan's test ($P \leq 0.05$). WW = treated wastewater, WW/FW = treated wastewater/fresh water alternatively and FW= fresh water.

Table 6. Maximum permissible limits for WW application in agriculture according to World Health Organization (WHO) and Iranian Department of Environment (IRNDOE) and averages of selected properties of fresh water and treated wastewater.

			Treated wastewater		Wastewater standards for agriculture	
Parameters	Unit	Fresh water	2015	2016	WHO	IRNDOE
EC	ds/m	0.8	1.6	1.67	< 3	-
pH	-	7.2	7.5	7.6	6 – 8.4	6 – 8.5
Nitrate	mg/l	7.21	14.5	12.5	5-30	10
Nitrite	mg/l	0.003	1.2	1.5	5-30	10
Ammonium	mg/l	-	8.5	5.7	-	1
P	ppm	-	2	2.1	4	6
K	ppm	-	0.42	0.44	-	-
Fe	ppm	<0.02	0.186	< 0.1	5	3
Zn	ppm	<0.01	0.157	< 0.1	2	2
Cu	ppm	<0.01	< 0.1	< 0.1	0.2	0.2
Mn	ppm	<0.01	< 0.1	< 0.1	0.2	1
As	ppm	Not detected	0.17	< 0.1	0.1	0.1
Pb	ppm	Not detected	0.11	< 0.1	5	1
Cd	ppm	Not detected	0.023	< 0.01	0.01	0.05

Table 7. Chemical characteristics of soil due to irrigation with WW, WW/FW and FW at the beginning and end of growing seasons.

		(Irrigation water type)					
	Beginning of growing season	FW		WW/FW		WW	
		Year		Year		Year	
Parameters		2015	2016	2015	2016	2015	2016
pH	7.9	7.7	7.8	7.6	7.7	7	6.8
Organic carbon (%)	0.101	0.11	0.13	0.165	0.145	0.257	0.31
N (%)	0.009	0.008	0.009	0.014	0.016	0.022	0.027
P (ppm)	13	11	12.01	12.1	13.3	15.3	14.33
K (ppm)	92	92	80	100	121	221	242
Fe (ppm)	6.14	5.55	5.2	6.12	6.3	7	7.21
Cu (ppm)	0.66	0.6	0.65	0.81	0.9	1.11	1
Zn (ppm)	1.73	1.65	1.38	1.88	1.92	2.2	2.73
Mn (ppm)	1.32	1.31	1.25	1.65	1.45	2.81	2.77

elements or heavy metals in the seeds of sunflower plants irrigated with WW compared to the plants irrigated with FW treatment.

Materials and methods

Experimental site and weather description

Field experiment was conducted during 2015 and 2016 at Research Farm at Yazd Municipal Wastewater Purification Station. Urban wastewater resources were mostly residential, commercial, institutional and recreational. The research area is located at geographical coordinates of 31° 96´N, 54° 30´E, and 1145 meters above sea level. The climate is hot and arid according to Koppen climate classification system (Dastorani et al., 2011). Annual precipitation averages for 2014-2015 and 2015-2016 were 51 and 25 mm, respectively.

Experimental design and plant materials

Experiment was arranged as split plot based on randomized complete block design with three replicates. Treatments were three irrigation water types under surface irrigation system comprising "WW": irrigation with treated municipal wastewater throughly, "FW/WW": was done by using irrigation with fresh water and treated municipal wastewater alternatively during growing seasons and "FW": irrigation with fresh water throughly as main plots. Sub plots consisted of three sunflower cultivars: Azargol, Record, and Farrokh. The cultivars of sunflower used in this study, provided by seed and plant improvement institute of Karaj, Iran. Seeds were stored in the dark in a cold room at 5°C in a resealable bag until required.

Fertilization and cultivation

Fertilizer was applied according to soil test recommendations. In FW plots, plants were received 70 kg/ha triple superphosphate (46% P_2O_5) and 70 kg/ha potassium sulphate (48-52% K_2O) at sowing date. Nitrogen fertilizer was top-dressed in two equal splits at sowing and just after thinning at the rate of 75 kg N/ha. Due to WW treatment contained plenty of nutrients, and application of WW sequentially or alternatively can lead to increase of nutrients in the soil, no mineral fertilizer was used in WW and WW/FW treatments. In both years, sunflower seeds were planted on 1st April, in plots having 6 rows × 5 m long with 0.5 m interrow spacing and 0.2 m interplant spacing within rows.

Soil and irrigation water analysis

To determine the physical and chemical properties of the soil at planting, samples were taken from 0- to 30-cm depth. Samples were air-dried and passed through a 2.0 mm sieve. physical analysis was carried out using hydrometer method. Electrical conductivity (EC) and pH were determined in soil/water extract. Soil heavy metals content i.e. Fe, Zn, Cu, Mn, Cd, Pb, and As were measured using inductively coupled plasma mass spectrometry (ICP-MS). Physical characteristics and heavy metals content of the soil are shown in Table 7. Two monthly water samples, were taken during May and July from Yazd Purification Station. Average values of the physical characteristics and element concentrations in WW and FW treatments is shown in Table 6. Quality standards for treated wastewater application in agriculture according to World Health Organization (WHO), Food and Agriculture Organization (FAO) and Iranian Department of Environment (IRNDOE) are also shown in Table 6.

Yield and yield components

In both years, the plots were harvested on 5^{th} September. samples were taken from the center of the plot on an area of 2.25m² (1.5 x1.5 m). The following parameters were assessed: plant height (cm), head diameter (cm), seed numbers per head, unfilled seeds per head, seed yield, biological yield (kg/ha) and seed heavy metals content.

Heavy metals content of sunflower seeds

To measure Heavy metals content of sunflower seeds, samples were digested using a 4-acid digestion procedure. This digestion was carried out in open vessels on a hot plate. The method uses a combination of nitric, hydrochloric, hydrogen peroxide and perchloric acids. Heavy metals analysis was carried out after mineralization using acids for seed samples. Heavy metal contents (Fe, Zn, Cu, Mn, As, Cd and Pb) were measured by the inductively coupled plasma mass spectrometry (ICP_MS) technique (Avula et al., 2010).

Statistical analysis

All data were subjected to combined analysis of variance (ANOVA) using the General Linear Models (GLM) procedures of the Statistical Analyses System (SAS, 9.2). Treatment Means were also compared by Duncan's Multiple Range Test ($P<0.05$).

Conclusion

The present investigation showed that due to rich nutrients and increasing soil organic matter, irrigation with treated municipal wastewater, increased growth, yield, and yield components of sunflower. On the other than; irrigation with WW increased heavy metals content in sunflower seeds, but these values were below the permissible limits recommended by World Health Organization (WHO), European Union (EU), and Institute of Standards and Industrial Research of Iran (ISIRI). It was concluded that treated municipal wastewater could be used for irrigating sunflower crop without worrying about the accumulation of toxic substances. In turn, this approach could reduce consumption of fertilizers as well as saving freshwater.

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