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EFFECT OF DIFFERENT WATER SOURCES AND WATER AVAILABILITY REGIMES ON HEAVY METAL ACCUMULATION IN TWO SUNFLOWER SPECIES

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Abstract: Sunflowers are considered as hyper-accumulators of heavy metals and can be used in the phytoremediation of heavy metal contaminated soils. Wastewater is a valuable irrigation source in peri-urban and urban areas. However, it is contaminated with numerous heavy metals, which can be accumulated and translocated to different plant parts. Unfortunately, limited studies have assessed the accumulation and translocation of heavy metals in ornamental sunflower species. Therefore, the current study was conducted to infer the effects of different water sources (normal and wastewater) and water availability regimes (60% and 35% wetted soil) on the growth and heavy metal (zinc, manganese and chromium) accumulation in two sunflower species (cultivated and ornamental). The cultivated (*Helianthus annuus* L.) and ornamental (*Helianthus giganteus* L.) sunflower species were grown in pots and irrigated with normal and wastewater. Furthermore, the pots were maintained at two different water availability regimes (i.e., 60% and 35% wetted soil). Different growth traits such as root, shoot and total biomass, and achene weight were recorded. Furthermore, accumulation of zinc (Zn), manganese (Mn) and chromium (Cr) was noted in the roots, shoots and achenes of both species. Residual concentration of these metals in the soil was also assessed after the harvest of plants. Nonetheless, bioaccumulation and translocation factors of all metals were computed. The species, water sources and water availability regimes significantly differed for growth traits, heavy metals' accumulation and bioaccumulation and translocation factors. Higher concentration of heavy metals in different plant parts were noted with normal water indicating that the metals were transported from soil rather than wastewater. Nonetheless, ornamental sunflower exhibited significant potential for the phytoremediation of Mn and Cr; thus, it should be explored further with in-depth studies.

Keywords: Sunflowers, wastewater, water availability, heavy metal accumulation,

1. INTRODUCTION

Industrial revolution and rapidly burgeoning population have sharply elevated the environment contamination by heavy metals since the beginning of 20^{th} century, which poses severe risks to environment and human health (Abdelhafez & Li, 2014; Habibi et al., 2019). Different anthropogenic activities, particularly the use of agricultural inputs such as sludge, pesticides, fertilizers, wastewater etc. are among the prevalent heavy metal contamination sources (Abou-Shanab, 2011; Abdelhafez et al., 2012; Anjum et al., 2015, 2016a, 2016b; Horasan & Arik, 2019). Heavy metals are not biodegradable; therefore, they are of serious concern to living organisms and the environment as they possess carcinogenic and mutagenic compounds (Wu et al., 2018). Different cellular components of plant cells are affected by high concentration (more than optimal for plant growth) of heavy metals, which interfere with normal metabolic functioning of plant cells (Tkalec et al., 2014). Therefore, agricultural productivity and growth of the plant species is severely impaired by the presence of heavy metals at high concentration (Roy et al., 2005).

Different wastewaters are a valuable source of irrigation in urban and peri-urban agricultural areas (Mishra et al., 2009; Gupta et al., 2010). Wastewaters are important source of plant nutrients and favor crop growth. However, application of wastewater leaves a burden of heavy metals, which can accumulate in the food chain and cause great hazards for human health and environment (Ghosh et al., 2012). Several studies have concluded that wastewaters carry toxic heavy metals that can be introduced to the soil and aquatic system through various processes and eventually result in prominent accumulation in different crops (Khan et al., 2008; Amin et al., 2013).

Irrigation of agricultural soils with wastewater contributes significant heavy metals to the irrigated soils (Nan et al., 2002; Mapanda et al., 2005). Some heavy metals, such as Zn, Mn and copper (Cu) are regarded as micronutrients, and have different known functions in plant growth, though they become toxic at high concentrations (Pandey, 2008; Tkalec et al., 2014). Consequently, plants grown in heavy metal polluted soil consume significant amount of heavy metals and introduce a substantial amount of potentially toxic metals into the food chain (Pandey & Nautiyal, 2008; Agoramoorthy et al., 2009).

Transfer of heavy metals from soil to various groups of plants are called phytoextraction or bioaccumulation, which is considered as a sub-process of phytoremediation or bioremediation and used as an approach in pollution control (Cay et al., 2019). The tissue metal concentration ratios are extensively studied as efficient and cost-effective approaches to clean-up heavy metal contaminated soils and wastewaters. However, the success of this approach largely depends on type of the substance (soil or water), plant species of organisms and other factors in soil and water (Chandra et al., 2004; Majid et al., 2014). For evaluating heavy metal accumulation in plants irrigated by wastewater, many researchers used the parameters of bioaccumulation factor and translocation factor (Baker, 1981; Connell, 2005; Srivastava et al., 2006; Yoon et al., 2006; Usman & Mohamed, 2009; Badr et al., 2012; Burkhard et al., 2012; Brisebois, 2013; Majid et al., 2014).

Different plant species exhibit varying degree of tolerance to heavy metal pollution; however, all plants are not hyperaccumulators due to adverse impacts of heavy metals on cellular activities (Peixoto et al., 2001; Hall, 2002). Cadmium, Cr, Cu, mercury (Hg), lead (Pb) and Zn represent the most common heavy metal contaminants (Jing et al., 2007). These metals cannot be easily degraded to harmless products, such as carbon dioxide, and the cleanup usually requires their removal (Wu et al., 2018). Different plant species are used to lower the concentration of heavy metals in soil (Chen et al., 2012; Cristaldi et al., 2017). Hyperaccumulator species (e.g. *Brassica juncea*, *Helianthus annuus*, *Festuca arundinacea*, *Populus* spp. etc.) have developed mechanisms that allow them to tolerate high metals concentrations, which could be toxic for other organisms (Lasat, 1999; Ernst, 2006; Kavamura & Esposito, 2011).

Sunflower (*Helianthus annuus* L.) is a multipurpose crop and grown for food, feed and bioenergy production. Sunflower can accumulate Pb, Cu, and Cd in the shoots (Oh et al., 2013). It has been extensively studied for its potential to accumulate heavy metals and proved tolerant and hyper-accumulator of different heavy metals (Chirakkara & Reddy, 2015; Shaheen & Rinklebe, 2015; Rizwan et al., 2016; Govarthanan et al., 2018). Sunflower produces high biomass; it is therefore used for rhyzofiltration and phytoremediation purposes (Mei et al., 2002). Since *H. annuus* L. is a cultivated species, its production on heavy metal contaminated soils could accumulate considerable amount of the metals in achenes, which could pose severe risk to human health. Moreover, plenty of work has been conducted to infer the heavy metal accumulation in cultivated sunflower, whereas ornamental sunflowers have generally been ignored. Thus, the current study was conducted to infer the heavy metal accumulation in different plant parts of cultivated (*H. annuus* L.) and ornamental (*H. giganteus* L.) sunflower irrigated by two different water sources with different water availability regimes.

It was hypothesized that; i) both sunflower species will accumulate considerable amounts of heavy metals in different plant parts as well as achenes, ii) heavy metal accumulation will differ among sunflower species, water sources and water availability regimes and iii) ornamental sunflower will accumulate similar amounts of heavy metals as of cultivated sunflower.

The results of the study will provide empirical information whether irrigation with wastewater to sunflower in urban and peri-urban areas is safe. Furthermore, the result will help to infer the phytoremediation potential of ornamental sunflower. Nonetheless, the result will also help future studies focusing phytoremediation with plant species.

2. MATERIAL AND METHODS

2.1. Study area

The current study was carried out in the greenhouse located at the Forest Nursery, Faculty of

Agriculture, Bingöl University, Bingöl, Turkey during the growing season of 2105-2016. Bingöl is in the upper Euphrates of the Eastern Anatolia region in Turkey. The average annual temperature in Bingöl is 12.1°C. Annual rainfall amounts to 873.7 mm and the number of snowy and frosty days are 24.5 and 94.1 days, respectively.

2.2. Experiment details

The pot experiment was conducted in the greenhouse of Forest Nursery, Faculty of Agriculture, Bingöl University, Bingöl, Turkey during the growing season of 2105-2016. Plastic pots of 30-liter capacity were used in the experiment. Each pot was filled with 23 kg surface soil (0-20 cm). The seeds of sunflower species were sown on April 4, 2016 in germination trays to prepare seedlings. Two-weeks old seedlings were transplanted to the pots. Initially, 3 seedlings were transplanted in each pot and then reduced to one per pot. Plants were harvested after 90 days, i.e., on July 14, 2016. The experiment was laid according to randomized complete block design with four replications. Sunflower species were main plots, water sources were randomized in sub-plots, whereas water availability regimes were regarded as sub-sub-plots.

2.3. Soil collection

Experimental soil was collected from the research field of Agricultural Faculty, Bingöl University. The soil sample was air dried, gently crushed and sieved through 4 mm stainless-steel sieve for the pots. An extra portion of the soil was sieved through 2 mm for physicochemical characterization.

2.4. Water sources

Two different water sources were used for irrigating the sunflower species. The normal water regarded as control was tap water supplied through the pipeline system of Bingöl municipality. The wastewater was domestic wastewater collected from the treatment station of Bingöl municipality. Wastewater was directly taken by using a plastic tank from the storage center of raw wastewater without any chemical and biological treatment. The wastewater was kept in horizontal plastic storage tank (2840 L) which was equipped with tap. The tank was placed inside the greenhouse close to the experiment.

2.5. Soil and water analysis

The soil and water used in the experiment were analyzed before the initiation of the study to assess heavy metal concentration. Electrical conductivity (EC) and reaction (pH) of soil samples were measured in 1:10 soil/distilled water suspension by using a glass electrode and conductance Resistance meter (YSI 34) (Thomas 1996). Organic matter content was determined by Walkley-Black method. Total calcium carbonate content was measured by using the Scheibler calorimeter method (Loeppert & Suarez, 1996). The concentration of Zn, Mn, and Cr in soil samples was determined as recommended by the International Organization for Standardization through digestion of the soil samples in aqua regia ($HCl:HNO_3$, 3:1 V/V) using the digestor of Mars express 6 (CEM corporation model). After digestion, the digested solution was cooled, filtered and transferred to 50 mL volumetric flasks. The clear solutions were then used to determine Zn, Mn and Cr concentrations by AAnalyst 800, Atomic Absorption Spectroscopy, AAS (Perkin Elmer model). Residual concentration of Zn, Mn and Cr in the soil after the experiment was assessed by similar procedure.

The pH, EC and total dissolved solids (TDS) were measured for normal tap water and wastewater according to Eaton et al., (2005). The concentrations of Zn, Mn and Cr were determined by AAnalyst 800, Atomic Absorption Spectroscopy, AAS (Perkin Elmer model) following the procedures of Eaton et al., (2005).

2.6. Chemical properties of soil

Potting soil was slightly acidic with low organic matter and calcium carbonate content (Table 1). The EC was 153.5 µS cm^{-1}, which indicates that the soil was non-saline. Total concentration of Zn, Mn and Cr was 76.9, 315.7 and 160.3 µg g^{-1}, respectively. Both Zn and Mn are considered as essential plant nutrients and plant require them for normal growth (Njinga et al., 2013). The Cr is not required by the plants for normal growth and development; hence, it is not regarded as essential plant nutrients and causes phytotoxicity depending on its concentration (Oliveira, 2012). The concentration of the metals in the soils may vary considerably according to the amount of the input from lithogenic, pedogenic and anthropogenic sources. The concentration of Zn in potting soil was below the optimum level of the adopted regulation by the New Dutch list (140 µg g^{-1}). The Cr concentration exceeded the optimum level (100 µg g^{-1}), but it was lower than the action level (380 µg g^{-1}).

2.7. Chemical properties of water

The pH, EC, TDS and heavy metals exhibited a considerable variation between water sources (Table 2). The differences among water resources are linked to the contamination of wastewater by pollutants.

Table 1. Chemical properties and heavy metal concentrations of the potting soil

Chemical properties						
EC (µS/cm)	pH	$CaCO_3$ (%)	Organic Matter (%)	Total Cr (µg g^{-1})	Total Mn (µg g^{-1})	Total Zn (µg g^{-1})
153.5	6.2	0.58	1.58	160.3	315.7	76.9

The pH of normal water was neutral (7.4) and wastewater was slightly alkaline (8.6) due to the high load of alkaline ions (OH^- and HCO_3^-). The pH of wastewater was higher than the desirable range stated by WHO standards for drinking water (7.0-8.5). The EC of normal water was 107 µS cm^{-1}, while wastewater was 424 µS cm^{-1}. According to FAO standards, water samples that have EC less than 700 µS cm^{-1} are non-saline and safe for irrigation. The TDS values for normal and wastewater were 37 to 226 mg L^{-1}, respectively. Water samples with TDS values less than 500 mg L^{-1} are non-saline (FAO, 2007).

2.8. Sunflower species

Two different sunflower species were included in the study. Cultivated sunflower (*H. annuus* L.) is native to the United States, annual plant and belongs to Asteraceae family. Ornamental sunflower (*H. giganteus* L.) commonly known as giant sunflower or tall sunflower is native to the eastern United States and eastern and central Canada, from Newfoundland west to Alberta south to Minnesota, Mississippi, and South Carolina. It is a perennial herbaceous plant growing up to 4 m tall. Ornamental sunflower is commonly found in valleys with wet meadows or swamps.

2.9. Water availability regimes

Two different water availability regimes were included in the study to assess whether heavy metal accumulation is influenced by the water availability. The water availability regimes were 60% and 30% of soil wetting. The wetting percentage of the soil was determined by following Steadman et al., (2004). Briefly, pots were filled by desired quantity of soil (see above experimental details) and irrigated until water started to percolate from the bottom. The pots were left for 24 hours to drain the extra water and weighed. The differences between saturated and dry soil was taken as wetting percentage. The pots were then maintained at 60% and 35% of soil wetting levels throughout the experiment.

2.10. Observations

The plants were carefully taken off form the pots to avoid any damage to the roots. The roots were carefully washed to remove the potting soil and plants were divided into roots, shoots and achenes. The biomass of roots, shoots and achenes was weighed fresh and then rinsed with distilled water. The rinsed parts were dried in an oven at 65 ± 5 °C to prepare the samples for heavy metal analysis.

2.11. Heavy metal analysis

Plant samples were oven-dried at 65 ± 5 °C and homogenized by reducing the particle size below 0.5 mm with the aid of a stainless-steel grinder. One gram of oven-dried plant tissue was digested by 10 ml HNO_3 and heated between 150-200°C by CEM machine according to Jones (2001). After the completion of digestion, the digested solution was cooled, filtered and transferred into 50 mL volumetric flasks. The clear solutions were used for the analysis of the Zn, Mn and Cr concentrations by AAnalyst 800, Atomic Absorption Spectroscopy, AAS (Perkin Elmer model). All the analysis was taken in compliance with the relevant methods. All reagents were of analytical grade unless otherwise stated.

2.12. Bioaccumulation and translocation factors

Bioaccumulation factor (BAF) refers to the ratio of plant metal concentration in roots to the concentration in soil or polluted environment. A BAF > 1 indicates the potential ability of the growing

Table 2. Chemical properties and heavy metal concentrations of normal and wastewater used in the study

Chemical properties						
	EC (µS/cm)	pH	TDS (µg mL^{-1})	Total Zn (µg L^{-1})	Total Mn (µg L^{-1})	Total Cr (µg L^{-1})
Normal water	107	7.4	37	13	102	3
Wastewater	424	8.6	226	46	307	4

plants for metal accumulation (Yanqun et al., 2005). The BAF was calculated by using the equation 1.

$$Bioaccumulation\, factor = \frac{M_{root}}{M_{soil}} \qquad \text{Equation 1}$$

Here, M_{root} represents metal concentration in roots, whereas M_{soil} represents the metal concentration in soil.

Translocation factor (TF) was determined by the ratio of the metal concentration in the shoots to the metal concentration in roots (Usman & Mohamed, 2009). A TF > 1 indicates the potential ability of the growing plants for metal accumulation. The TF was calculated by using the equation 2.

$$Translocation\, factor = \frac{M_{shoot}}{M_{root}} \qquad \text{Equation 2}$$

Here, M_{shot} represents metal concentration in shoots, whereas M_{root} represents the metal concentration in roots.

2.13. Statistical Analysis

The collected data were tested for normality first, and the variables with non-normal distribution were transformed by Arcsine transformation technique to meet the normality assumption of Analysis of Variance (ANOVA). Three-way ANOVA was then used to test the differences between sunflower species, wastewater sources, water availability regimes and their all possible interactions (Steel et al., 1997). The least significant difference test at 95% probability was used to separate the means where ANOVA indicated significant differences. The three-way interaction, i.e., sunflower species × wastewater sources × water availability regimes was significant; therefore, this interaction was presented and interpreted in the manuscript. The SPSS software version 21 was used for the statistical analysis of the data (IBM, 2012).

3. RESULTS

3.1. Growth traits

The growth traits were significantly affected by sunflower species, wastewater sources, water availability regimes (WARs) and their interactions with some exceptions (Table 3).

The highest shoot weight was recorded for cultivated sunflower under both water sources and WARs, whereas ornamental sunflower irrigated by wastewater at 35% WAR had the lowest shoot weight. Cultivated sunflower irrigated with wastewater at 60% WAR had the highest root weight, whereas the lowest root weight was recorded for ornamental sunflower irrigated with both water sources at 60% WAR.

The highest achene weight was noted for ornamental sunflower irrigated with both water sources at 60% WAR and normal water at 35% WAR, whereas the lowest seed weight was noted for cultivated sunflower irrigated with both water sources at both WARs of the study. Cultivated sunflower with both water sources and WARs produced the highest total biomass, whereas ornamental sunflower irrigated with wastewater at 35% WAR produced the lowest total biomass (Table 4).

3.2. Zn accumulation

Sunflower species, water sources, WARs and their all interactions (with some exceptions) significantly altered the Zn accumulation in different plant parts and BAF and TF (Table 5).

The highest Zn accumulation in shoot was noted for both species irrigated with wastewater at 60% WAR, whereas the lowest Zn accumulation was noted for both species irrigated with wastewater at 35% WAR. The highest Zn concentration in root was noted for cultivated sunflower irrigated with normal water at 60% WAR, while the lowest was recorded for ornamental sunflower irrigated with wastewater at 35% WAR accumulated the lowest Zn in achenes. The highest residual Zn concentration was noted for ornamental sunflower irrigated with normal water and 60% WAR, and wastewater with 35% WAR. The lowest residual Zn was recorded for cultivated sunflower with both water sources and WARs (Table 6).

Table 3. Analysis of variance of different sunflower species, water sources and water availability regimes for growth traits

	P values			
Source	**Shoot weight**	**Root weight**	**Seed weight**	**Total biomass**
Species (S)	0.0001*	0.0001*	0.0001*	0.0001*
Water Sources (WS)	0.0020*	0.5828^{NS}	0.0166*	0.0010*
Water Availability Regimes (WAR)	0.4850^{NS}	0.4111^{NS}	0.0001*	0.9663^{NS}
S×WS	0.0832^{NS}	0.0084*	0.0123*	0.0185*
S×WAR	0.1267^{NS}	0.0001*	0.0032*	0.5703^{NS}
WS×WAR	0.0011*	0.0627^{NS}	0.0132*	0.0003*
S×WS×WAR	0.0001*	0.0387*	0.0090*	0.0001*

*= Significant ($p<0.05$), NS = non-significant ($p>0.05$)

Table 4. The influence of different water sources and water availability regimes on growth traits of two sunflower species

	H. annus		*H. giganteus*		*H. annus*		*H. giganteus*	
	Shoot weight (g plant^{-1})				**Seed weight (g plant^{-1})**			
	NW	**WW**	**NW**	**WW**	**NW**	**WW**	**NW**	**WW**
60% WAR	81.97 a	76.46 a	49.17 c	55.66 c	14.70 bc	14.69 bc	19.36 a	19.43 a
35% WAR	83.48 a	82.77 a	63.47 b	38.34 d	14.09 c	14.18 c	18.49 a	15.47 b
LSD 0.05	7.13				1.13			
	Root weight (g plant^{-1})				**Total biomass (g plant^{-1})**			
60% WAR	17.87 ab	18.56 a	13.42 d	13.44 d	114.54 a	109.70 a	81.95 c	88.53 c
35% WAR	15.54 c	16.06 c	17.14 b	15.38 c	113.11 a	113.01 a	99.11 b	69.19 d
LSD 0.05	1.03				7.43			

NW = normal water, WW = untreated wastewater, WAR = water availability regime, NS = non-significant, Means followed by similar letters within a column or a row are statistically non-significant (p>0.05) for each measured variable

The highest TF was noted for ornamental sunflower irrigated with wastewater at 60% WAR, whereas the smallest was observed for cultivated sunflower with normal water at both WARs. The largest BAF was recorded for cultivated sunflower irrigated with normal water at 60% WAR, whereas the smallest was noted for ornamental sunflower with both water sources and WARs (Table 6).

3.3. Mn accumulation

The Mn accumulation, its TF and BAF were significantly affected by sunflower species, water sources, WARs and their interactions (Table 7). The highest and the lowest Mn accumulation in shoot was noted for ornamental sunflower irrigated with normal water at 35% WAR, and wastewater at 60% WAR, respectively. Similarly, the highest Mn concentration in root was noted for ornamental sunflower irrigated with normal water at 60% WAR, while the lowest Mn accumulation in root was recorded for cultivated sunflower irrigated with wastewater at 35% WAR.

Ornamental sunflower irrigated with wastewater at 35% WAR accumulated the highest amount of Mn in achenes, whereas ornamental sunflower with normal water and 60% WAR accumulated the lowest Mn in achenes. The highest residual Mn was noted for cultivated sunflower irrigated with normal water and 35% WAR and ornamental sunflower irrigated with wastewater at 60% WAR, whereas the lowest was recorded for ornamental sunflower with normal water and both WARs (Table 8). The highest TF was noted for ornamental sunflower irrigated with wastewater at 35% WAR, whereas the smallest was observed for ornamental sunflower with both water sources and 60% WAR. The largest BAF was recorded for ornamental sunflower irrigated with normal water at 60% WAR, whereas the smallest was noted for cultivated sunflower with both water sources and WARs (Table 8).

3.4. Cr accumulation

The Cr accrual, its TF and BAF were significantly altered by sunflower species, water sources, WAR and their all interactions with some exceptions (Table 9). Sunflower species × water sources × WAR interaction was significant for all measured variables of Cr accumulation.

The Cr accumulation in shoot was similar for all treatments except for cultivated sunflower irrigated with normal water and 60% WAR. The highest Cr concentration in root was noted for cultivated sunflower irrigated with normal water at both WARs and ornamental sunflower irrigated with normal water at 60% WAR, while the lowest Cr accumulation in root was recorded for both sunflower species irrigated with

Table 5. Analysis of variance of different sunflower species, irrigation water sources and water availability regimes for zinc accumulation in roots, shoots, leaves, soil and translocation and bioaccumulation factors of zinc

	P values					
Source	**Shoot Zn**	**Root Zn**	**Seed Zn**	**Soil Zn**	**TF**	**BF**
Species (S)	0.0080*	0.0001*	0.0003*	0.0001*	0.0001*	0.0001*
Water Sources (WS)	0.5445^{NS}	0.0082*	0.9587^{NS}	0.0909^{NS}	0.0858^{NS}	0.0249^{NS}
Water Availability Regimes (WAR)	0.0001*	0.4117^{NS}	0.0043*	0.1232^{NS}	0.0001*	0.1435^{NS}
S×WS	0.0009*	0.0010*	0.1979^{NS}	0.1000^{NS}	0.0005*	0.0002*
S×WAR	0.0928^{NS}	0.0030*	0.0872^{NS}	0.1887^{NS}	0.0994^{NS}	0.0094*
WS×WAR	0.0001*	0.0031*	0.0176*	0.0005*	0.0001*	0.1337^{NS}
S×WS×WAR	0.0028*	0.0015*	0.0081*	0.0003*	0.0033*	0.0079*

*= Significant (p<0.05), NS = non-significant (p>0.05), TF = translocation factor, BF = bioaccumulation factor

Table 6. The influence of different water sources and **water availability regimes** on zinc accumulation in roots, shoots, leaves, soil and translocation and bioaccumulation factors of zinc of two sunflower species

	H. annus		***H. giganteus***		***H. annus***		***H. giganteus***	
Shoot Zn (ppm)					**Soil Zn (ppm)**			
	NW	**WW**	**NW**	**WW**	**NW**	**WW**	**NW**	**WW**
60% WAR	17.41 b	20.87 a	18.67 b	20.49 a	57.99 c	55.50 c	62.63 a	56.46 c
35% WAR	14.86 c	14.75 c	18.67 b	14.38 c	55.72 c	58.13 bc	61.07 ab	62.24 a
LSD 0.05	1.51				2.98			
Root Zn (ppm)					**Translocation Factor**			
60% WAR	35.94 a	27.97 bc	23.48 de	22.05 e	0.49 c	0.75 b	0.80 b	0.93 a
35% WAR	29.93 b	27.71 bc	23.24 de	26.12 cd	0.50 c	0.54 c	0.81 b	0.55 c
LSD 0.05	3.07				0.10			
Seed Zn (ppm)					**Bioaccumulation Factor**			
60% WAR	33.06 a	30.47 abc	30.37 abc	29.11 bc	0.62 a	0.50 bc	0.38 d	0.39 d
35% WAR	30.39 abc	30.99 ab	24.52 d	27.62 c	0.54 b	0.48 c	0.38 d	0.42 d
LSD 0.05	3.02				0.05			

NW = normal water, WW = untreated wastewater, WAR = water availability regimes, NS = non-significant, Means followed by similar letters within a column or a row are statistically non-significant (p>0.05) for each measured variable

Table 7. Analysis of variance of different sunflower species, irrigation water sources and WAR for manganese accumulation in roots, shoots, leaves, soil and translocation and bioaccumulation factors of manganese

	P values					
Source	**Shoot Mn**	**Root Mn**	**Seed Mn**	**Soil Mn**	**TF**	**BF**
Species (S)	0.0001*	0.0001*	0.5597^{NS}	0.0025*	0.4758^{NS}	0.0001*
Water Sources (WS)	0.0001*	0.0001*	0.0050*	0.0397*	0.0046*	0.0001*
Water Availability Regimes (WAR)	0.0001*	0.0001*	0.0030*	0.2766^{NS}	0.0001*	0.0001*
S×WS	0.0005*	0.0001*	0.0129*	0.0002*	0.0001*	0.0001*
S×WAR	0.0001*	0.0001*	0.0199*	0.0086*	0.0001*	0.0001*
WS×WAR	0.0681^{NS}	0.0001*	0.1733^{NS}	0.3841^{NS}	0.0001*	0.0022*
S×WS×WAR	0.0001*	0.0176*	0.0071*	0.0050*	0.0092*	0.0031*

*= Significant (p<0.05), NS = non-significant (p>0.05), TF = translocation factor, BF = bioaccumulation factor

Table 8. The influence of different water sources and water availability regimes on manganese accumulation in roots, shoots, leaves, soil and translocation and bioaccumulation factors of manganese of two sunflower species

	H. annus		***H. giganteus***		***H. annus***		***H. giganteus***	
Shoot Mn (ppm)					**Soil Mn (ppm)**			
	NW	**WW**	**NW**	**WW**	**NW**	**WW**	**NW**	**WW**
60% WAR	6.68 c	3.93 ef	5.57 d	4.53 e	55.94 abc	52.71 cd	49.23 d	58.33 a
35% WAR	3.08 fg	3.03 g	14.35 a	8.98 b	59.03 a	57.33 ab	49.93 d	54.13 bc
LSD 0.05	0.88				3.96			
Root Mn (ppm)					**Translocation Factor**			
60% WAR	8.39 de	11.83 d	50.90 a	44.90 b	0.81 b	0.33 cd	0.11 e	0.10 e
35% WAR	11.78 d	6.93 e	33.58 c	8.10 de	0.26 d	0.44 c	0.43 c	1.11 a
LSD 0.05	4.48				0.12			
Seed Mn (ppm)					**Bioaccumulation Factor**			
60% WAR	8.07 b	7.73 b	5.89 c	8.81 ab	0.15 c	0.22 c	1.04 a	0.77 b
35% WAR	7.86 b	8.44 ab	8.82 ab	9.24 a	0.20 c	0.12 c	0.68 b	0.15 c
LSD 0.05	1.17				0.12			

NW = normal water, WW = untreated wastewater, WAR = water availability regime, NS = non-significant, Means followed by similar letters within a column or a row are statistically non-significant (p>0.05) for each measured variable

wastewater at 60% WAR. Ornamental sunflower irrigated with both water sources at 60% WAR accumulated the highest amount of Cr in achenes, whereas cultivated sunflower with normal water and 60% WAR, and ornamental sunflower with normal water and 35% WAR accumulated the lowest concentration of Cr in the seeds. The highest residual Cr concentration in soil was noted for ornamental sunflower irrigated with wastewater and 60% WAR, whereas the lowest residual Cr concentration in soil

Table 9. Analysis of variance of different sunflower species, irrigation water sources and water availability regimes for chromium accumulation in roots, shoots, leaves, soil and translocation and bioaccumulation factors of chromium

	P values					
Source	**Shoot Cr**	**Root Cr**	**Seed Cr**	**Soil Cr**	**TF**	**BF**
Species (S)	0.0001*	0.0222*	0.0001*	0.0001*	0.0001*	0.0001*
Water Sources (WS)	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
Water Availability Regimes (WAR)	0.0010*	0.9883^{NS}	0.0001*	0.0001*	0.8713^{NS}	0.0001*
S×WS	0.0002*	0.0088*	0.0092*	0.0001*	0.0002*	0.0001*
S×WAR	0.0001*	0.1299^{NS}	0.0001*	0.0001*	0.0047*	0.0001*
WS×WAR	0.0001*	0.0001*	0.2942^{NS}	0.0001*	0.0001*	0.0001*
S×WS×WAR	0.0001*	0.0010*	0.0001*	0.0001*	0.0060*	0.0001*

*= Significant ($p<0.05$), NS = non-significant ($p>0.05$), TF = translocation factor, BF = bioaccumulation factor

was recorded for cultivated sunflower with normal water and 60% WAR (Table 10).

4. DISCUSSION

The solubility, mobility and bioavailability of heavy metals are controlled by plant species, type of root system and plants' response to specific elements in relation to seasonal cycles, genotype, forms or chemical species and sequestration (Hooda, 2010; Damian F. et al., 2019, Damian G. 2019). Clemens et al., (2002) pointed that bioaccumulation of heavy metals depend on mobilization and uptake from the soil, speciation and sequestration within the root, efficiency of xylem loading and transport, distribution between metal sinks in the aerial parts as well as isolation and storage in leaf cells.

The accumulation of heavy metals was significantly affected by sunflower species, water sources and WARs. The heavy metal accumulation, as hypothesized, significantly differed among sunflower species, water sources and WARs. Similarly, ornamental sunflower accumulated comparable amount of heavy metals to cultivated sunflower. Nonetheless, considerable amounts of heavy metals were accumulated by different plant parts and achenes of both species. However, different plant parts of both species exhibited affinity for different metals. Sunflower can accumulate Pb, Cu, and Cd in shoots (Oh et al., 2013). Sunflower has been extensively studied for its potential to accumulate heavy metals and proved hyper-accumulator (Chirakkara & Reddy, 2015; Shaheen & Rinklebe, 2015; Rizwan et al., 2016; Govarthanan et al., 2018). The results of the current study agree with the findings of these earlier studies.

The largest and the smallest TF was noted for cultivated sunflower irrigated with wastewater and normal water at 60% WAR, respectively. Similarly, the largest BAF was recorded for ornamental sunflower irrigated with normal water at 35% WAR, whereas the smallest BAF was noted for ornamental sunflower with wastewater and both WARs (Table 10).

The variations in metal concentrations in soil, root, shoot and achenes can be attributed to the treatment combinations of water sources and available moisture depletion levels (Kurt, 2018). The solubility of many heavy metals is reduced at high soil moisture due to low redox potential, reducing condition, and formation of sparingly soluble sulfides for the metal (Marschner, 1995; Damian et al., 2013). Residual heavy metal concentration in the soil after the harvest were much lower compared to the original concentrations in the soil. This finding indicated that the soil is the main source for Zn, Mn and Cr accumulation in the current study. The result could be attributed to the fact the both Cr and Mn were more mobile and higher in plant available form. The variation in metal concentrations is in agreement with the results of Babincev (2017), who studied bioaccumulation and phytoremediation of lettuce (*Lactuca sativa*), common onion (*Allium cepa*), legumes: bird's- foot trefoil (*Lotus corniculatus* L.), red clover (*Trifolium pretense* L.), grasses (weed plants), zubach (Cynodondactylon), and tall fescue (*Festuca arundinaceous* Schreb).

The growth traits of both sunflower species were suppressed by wastewater compared to normal irrigation water. The wastewater had higher amount of Zn and Mn, which impaired the functioning of plant cells; thus, hampered growth traits. Different cellular components of plant cells are affected by high concentration of heavy metals, which interfere normal metabolic functioning of plant cells (Tkalec et al., 2014). Thus, the growth suppression in the current study can be linked with disturbed metabolic functions of plants due to high metal concentration in wastewater.

Ornamental sunflower exhibited high affinity for Zn and Mn, whereas cultivated sunflower better accumulated Cr. Overall, higher metal accumulation was noted for wastewater and 60% WAR. Plant species show variable response to available heavy metals and water in soil depending on their ability to accumulate and detoxify various heavy metals (Lee et al., 2008). The results are consistent with the findings of Majid et al., (2014), who studied the uptake and bioaccumulation of Cr, Mn, Cu and Pb from wastewater by two macrophyte species. Some plants can accumulate high concentration of specific metals in roots rather than the shoots, while others bio-accumulate high concentration of the same metals in shoot than roots (Bech et al., 2012). Ornamental sunflower is a perennial species; thus, could produce higher amount of biomass compared with cultivated sunflower. Therefore, the higher affinity of ornamental sunflower to specific heavy metals could be explained with its high biomass production potential and perennial nature.

Bioaccumulation and translocation factor values for the investigated metals significantly varied for sunflower species, WARs and water sources. These values were used to evaluate the suitability of sunflower species for phytoremediation processes and to measure their abilities for bioaccumulation of Cr, Mn and Zn. Bioaccumulation factor value should be greater than 1.0 to consider a plant species as a hyper-accumulator for a metal (Badr et al., 2012). The bioaccumulation factor values (soil-to-root transfer) indicated that the ornamental sunflower has the potential to bio-accumulate Mn and Cr in roots. However, these values are for normal irrigation water indicating that the interaction among wastewater and soil heavy metal concentration are complex and need further exploration. The results show that bioaccumulation rates of heavy metals in plants are affected by the soil environment in the rhizosphere through exudation of compounds that are involved in the uptake mechanism. Ma et al., (2001) indicated that the translocation factor values higher than 1.0 are considered high efficiency for translocation of metals from their roots to the shoots. Translocation factor values of ornamental sunflower for Mn and Cr under normal water were >1 indicating that enough amounts of these metals were translocated. Thus, ornamental sunflower could be used for phytoremediation of heavy metals in addition to cultivated sunflower.

Considerable amounts of heavy metals were accumulated in the achenes of both sunflower species, which indicate that these heavy metals could enter food chain if cultivated sunflower is grown on heavy metal contaminated soils. Therefore, cultivation of sunflower on heavy metal contaminated soils should be carefully monitored to avoid any negative effect on human health.

5. CONCLUSION

The current study concluded that heavy metal accumulation was significantly affected by sunflower species, water sources and WARs. Higher amounts of studied metals were accumulated under high water availability. Similarly, higher concentration of heavy metals in different plant parts were noted with normal water indicating that the metals were transported from soil rather than wastewater. Nonetheless, ornamental sunflower exhibited significant potential for the phytoremediation of Mn and Cr; thus, it should be explored further with in-depth studies.

The results obtained from future studies with crops grown in the field will be decisive in explaining this complex process, and only after field experiments firm conclusions could be drawn.

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