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Impact of treated sewage water irrigation on some growth parameters, yield and chemical composition of sunflower, *Helianthus annuus* L. plants

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Abstract: Shortage of irrigation water in the Mediterranean region mandates looking for alternative water resources. A pot experiment was conducted during 2012 and 2013 seasons in Zenein Sanitary Station, Giza, Egypt with sunflower plants (*Helianthus annuus* L.var. Euroflour) grown on sandy soil to study the effect of the station influent and effluent on the growth, yield and yield component of sunflower and the efficiency of sunflower plants in nutrient recovery. The experiment followed the complete randomized block design. The plants were irrigated with primary treated wastewater (P.T.S.) or secondary treated wastewater (S.T.S.). Control plants were irrigated with well water. Results showed that plant height, stem length, root length, number of leaves. Plant^{-1}, total leaf area.plant^{-1}, also fresh and dry weights were increased as the plants were irrigated with treated sewage water compared to control plants. The best growth parameters under these conditions were obtained by using the primary treated sewage water (P.T.S.) at 45 days after sowing. However, the above mentioned growth parameters were declined down below the plants irrigated with well water at 60 days after sowing. Desk diameter was not significantly affected by irrigation sewage water treatments. Desk weight.plant^{-1}, seeds number.plant^{-1}, dry seeds yield.plant^{-1} and dry seed index of the plants irrigated with treated sewage water were less than the values obtained by control plants. Chlorophyll “a”, chlorophyll “b” and carotenoids concentrations in the leaves of the plants irrigated with treated sewage water were significantly higher than those in the leaves of the plants irrigated with well water. Total carbohydrate content of sunflower seeds of the plants irrigated with treated sewage water increased compared to control plants. However, crude protein, oil content, and moisture content were not significantly affected with irrigation treated sewage water. No significant difference in the concentrations of the heavy metals (Pb, Cd and Ni)in the seeds of the plants irrigated with treated sewage water and control plants was detected. Thus, treated sewage water can be safely used as irrigation water for sunflower and similar crops to partially solve the problem of the shortage in irrigation water.
Key words: Sunflower, growth, yield, chlorophyll, total carbohydrate, oil content, heavy metals, treated sewage water.

Introduction

Treated wastewater is one of the promising water resources in the sustainable agricultural development in Egypt. When the wastewater is free from toxic compounds, the nutrients are converted to a valuable biomass, which contains more protein and less fiber than emergent plants[1]. The physical and chemical constituents of wastewater are important parameters in design selection and treatment operations[2,3]. Treated wastewater may prove a potential economic asset by contribution to the water resources and the expansion of irrigated agriculture. It provides solution to the problem associated with indiscriminate disposal, thereby protecting environment and public health[4].

The application of treated wastewater to the soil and plants system may mitigate the scarcity of water resources and the discharge of nutrients to water bodies by using soil and plants as natural filters[5]. In addition, crop irrigation with treated wastewater constitutes ecologically sound method for its disposal to the environment[6]. However, the choice of crops for treated wastewater irrigation is the principal factor for the suitability of effluent irrigation because certain crops can be irrigated without negative implications on yield[4]. Authors [7,8] mentioned that metals uptake was depend on the plant species and the present metals and roots concentrated metals much higher than the stems and leaves.

However, treated wastewaters can still be used for irrigation under controlled conditions which minimize hazard from pathogenic and toxic contaminants to agricultural products, soils, surface, and groundwater[9].

Sunflower is a valuable oil crop from the economic and ornamental viewpoint. Its seed is commonly used as a vegetable oil in many parts of the world including Egypt. Sandy soils have intrinsically least amount in fertility to support in achieving the yield potential of economic crops. Due to high cost and scarcity of chemical fertilizers, the disposal of agricultural, municipal and industrial wastes is widely practiced as a cheaper source of nutrients and organic matter for growing crops.

The present study aimed at investigating the reuse of treated sewage water effluent for agricultural purposes through determining the efficiency of sunflower plants in nutrient recovery and heavy metals removal from the treated sewage water. Chemical compositions of the plant tissues and concentrations of toxic elements in the plant edible parts were also under investigation.

Materials and Methods

A Pot experiment was conducted in Zenein Sanitary Station, Giza, Egypt with sunflower (*Helianthus annuus* L. var. *Euroflour*) to study the effect of the station influent and its effluent on the growth, yield and yield components and chemical compositions of sunflower and the efficiency of sunflower plants in nutrient recovery.

Experimental cultivation:

Table 1. Physical and chemical characteristics of the used soil

Physical characteristics		**Nutrient concentrations**	
		Exchangeable Macronutrients	
Sand (%)	83.0		(mg.100g^{-1} soil)
Silt (%)	13.0	P	0.19
Clay (%)	4.0	K	0.38
Texture	Sandy	Mg	3.30
		Ca	7.00
		Na	3.13
Chemical characteristics			
		Available Micronutrients	
pH	7.50		(mg.Kg^{-1} soil)
E.C. (dS.m^{-1})	1.40	Fe	19.06
OM%	0.2		
$CaCO_3$ (%)	Traces	Mn	6.20
HCO_3	1.50	Zn	7.80
NH_3		Cu	2.40
SO_4	5.06	Heavy Metals	
Cl	3.00	Cd	1.50
		Pb	8.20
		Ni	4.50

Sunflower seeds obtained from the Agricultural Research Centre, Giza, Egypt were sown in April, 15. In black plastic pots, 40 cm in diameter filled with 20.0 Kg sandy soil. Physical and chemical characteristics of the soil are shown in Table1. Twelve seeds were sown in every pot and the seedlings were later thinned to leave five vigorous seedlings per pot.

Fertilization:

Every pot was received 3.0g super-mono-phosphate (15.5% P_2O_5) and 1.0g potassium sulphate (48-52% K_2O) mixed with the soil before sowing. Nitrogen fertilizer was added in two equal doses (one during sowing and the other after thinning) in the rate of 0.6 g N for pot as ammonium sulphate (20.6% N).

Experimental design and treatments:

The experiment followed the complete randomized block design. As treatments, the plants were irrigated with primary treated wastewater (P.T.S) or Secondary treated wastewater (S.T.S.). Control plants were irrigated with well water. Irrigation with desired water quality was fulfilled through the experimental periods by adding equal volume of the desired water quality to each pot until few drops passed through the pot outlet drainage pore. Irrigation started 7 days after sowing and stopped 15 days before harvesting.

Sampling:

Sewage water:

Two monthly water samples from Zenein Sanitary Station were taken during May, June and July for analysis. Average values of the physico-chemical characteristics and element concentrations in the used treated sewage water are shown in tables (2&3), respectively.

Table 2: Physical and chemical characteristics of Zenein treated sewage water (Average values of 4 months during two seasons)

Character	Well water	Influent (P.T.S.)	Effluent (S.T.S)
pH	7.36	8.17	7.50
E.C. (dS.m)	0.41	0.66	0.67
TSS ($mg.l^{-1}$)	9.80	79.30	22.80
TDS ($mg.l^{-1}$)	178	433	220
BOD ($mg.l^{-1}$)	10.30	84.30	18.00
COD ($mg.l^{-1}$)	30.00	392.00	48.00
HCO_3 ($mg.l^{-1}$)	84.49	216.00	155.00

P.T.S. = Primary treated sewage, S.T.S. = Secondary treated sewage

Table 3: Element concentrations in Zenein treated sewage water (Average values of 4 months during two seasons)

Element	Well water	Influent (P.T.S.)	Effluent (S.T.S)
P ($mg.l^{-1}$)	8.04	14.80	13.40
K ($mg.l^{-1}$)	6.81	17.00	18.40
Na ($mg.l^{-1}$)	10.00	19.00	10.80
Ca ($mg.l^{-1}$)	7.90	9.60	6.13
Mg ($mg.l^{-1}$)	9.60	13.40	11.03
Cl ($mg.l^{-1}$)	27.00	90.00	98.00
SO_4 ($mg.l^{-1}$)	26.00	37.20	27.20
Fe (ppm)	0.056	0.197	0.445
Mn (ppm)	0.011	0.017	0.063
Zn (ppm)	0.060	1.457	0.044
Cu (ppm)	0.033	0.030	0.030
Pb (ppm)	0	0	0
Cd (ppm)	0.002	0.013	0.009
Ni (ppm)	0.009	0.085	0.047

P.T.S. = Primary treated sewage, S.T.S. = Secondary treated sewage

Soil:

After preparation and before fertilization, a representative soil sample was taken. The sample was air-dried and passed through a 2.0mm sieve pores.

Plant tissues:

Three plant samples were randomly collected from the different treatments at 45, 60 days after sowing and at harvest.

Growth characteristics:

Plant growth characteristics recorded were: plant height, leaf number.plant^{-1}, total leaf area.plant^{-1} and fresh and dry weight of leaves and stems.

Yield and yield components:

Yield was assessed as desk diameter, desk weight.plant^{-1} seeds number.plant^{-1}, dry seeds yield.plant^{-1} and dry seed index.

Chemical analysis:

Photosynthetic pigments

Photo synthetic pigments were determined as chlorophyll "a", chlorophyll "b" and carotenoids. Leaf discs from the fourth upper leaf on the main stem were taken according to the method of Wettstein[10]. Pigment contents were calculated using the following equation:

Chl. "a" = 9..78 * E662 – 0.99 * E644 (mg.l^{-1})
Chl. "b" = 21.426 * E644 – 4.65 * E662 (mg.l^{-1})
Cartenoids = 4.695* E440.5 – 0.268 * Chl "a" + Chl. "b" (mg.l^{-1})
Where E is the spectrophotometer reading at the specified wavelength.

Soil analysis:

Soil mechanical analysis was carried out using hydrometer method[11]; pH and E.C (electric conductivity) were determined in soil/water extract (1:2.5)[12]; Calcium carbonate ($CaCO_3$) content was determined using Calcimeter method[13]; Organic matter (O.M.) was determined using potassium dichromate method[14]. Soil phosphorus (P) was extracted using sodium bicarbonate[15]. Potassium (K) and magnesium (Mg), were extracted using ammonium acetate[16], while Fe, Mn, Zn, Cu, Cd, Pb and Ni were extracted using DTPA[17].

Plant Tissues analysis:

Vegetative samples of every replicate were washed with tap water, 0.01 N HCl and bidistilled water. Plant tissues and the remaining seed tissues after oil extraction were oven dried at 70°C for 24 hours and ground.

Exactly 0.5g of the dry powder was wet digested using 5ml Conc. H_2SO_4 at 180-200°C on a sand bath until the dense white fumes were evolved. Then, 1.0ml perchloric acid was added and the process was continued until the acid liquid was mostly volatilized. The remaining dense solution was diluted to known volume by distilled water. This stock solution was used for mineral ion estimation.

Treated sewage water analysis:

Samples of treated sewage water were analyzed by Zenein Sanitary Station according to the Standard Methods for the Examination of Water and Wastewater[18].

Macroelements:

Total nitrogen (N) was determined using the modified micro-kjeldahl method).Phosphorus (P) was determined spectrophtometrically as described by A.O.A.C.[19]. Potassium (K), calcium (Ca) and sodium(Na) were determined using flame Photometer according to Eppendorf[20]. Magnesium(Mg) was determined using Atomic Absorption Spectrophotometer Zeiss FMD3.

Microelements and heavy metals:

Iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), lead (Pb), cadmium (cd) and nickel (Ni) were determined in the digested solution using Atomic Absorption Spectrophotometer Zeiss FMD3 according to Jackson[12].

Total carbohydrates:

Seed total carbohydrates content was determined as (%) according to the method described by Dubois[21].

Total protein:

Seed total crude protein was determined as total nitrogen multiplied by 6.26 according to Tripathi[22].

Oil content:

Oil content of the seed was determined according to A.O.A.C.[19] using the Soxhlet extraction apparatus using Hexan (60-80° boiling point) as a solvent.

Statistical analysis:

Data were subjected to the statistical analysis of variance (ANOVA) for complete randomized block design (CRBD) according to Snedecor and Cochran[23] using 0.05 values L.S.D level for means comparison.

Results and Discussion

Effect of treated sewage water on sunflower growth

Table 4: Effect of treated sewage irrigation water on growth criteria of sunflower plants in different growth intervals (Average values of two seasons)

Irrigation treatments	Plant height (cm)	Stem length (cm)	Root length (cm)	Leaves No./plant	Total leaf area/Plant (dm^2)	Total plant weight (gm)	
						Fresh	Dry
45 days after sowing							
Control	53.00	43.17	9.83	8.33	0.54	8.34	2.45
P.T.S.	61.50	47.67	13.83	11.33	1.61	15.33	4.27
S.T.S.	61.33	48.67	12.67	10.33	1.08	13.05	2.81
LSD at 0.05	7.78	N.S.	3.91	1.99	0.617	N.S.	N.S.
60 days after sowing							
Control	90.67	72.83	17.83	14.67	2.62	40.66	9.31
P.T.S.	77.67	63.00	14.67	15.33	1.67	27.74	7.46
S.T.S.	82.00	66.67	15.33	17.00	2.12	27.66	6.73
LSD at 0.05	12.93	N.S.	2.88	N.S.	0.92	12.80	N.S.

P.T.S = Primary treated sewage, S.T.S. = Secondary treated sewage

Growth parameters of sunflower plants irrigated with treated sewage water compared to those irrigated with well water are shown in table (4). It was observed that all growth parameters were significantly affected with treated sewage water irrigation compared to those irrigated with well water. Plant height, Stem length, root length, number of leaves per plant, total leaf area per plant and fresh and dry weights were increased as the plants were irrigated with treated sewage water The best growth parameters under these conditions were obtained by the primary treated sewage water (P.T.S.) at 45 days after sowing. However, the above mentioned growth parameters were declined down below the plants irrigated with well water at 60 days after sowing. Similar results were obtained on sunflower and on growth of ornamental sunflower genotypes [24,25]. Freitas et al.[1] found that all growth parameters of sunflower plants were increased linearly as a function of the irrigation water levels and the parameter values obtained for treated sewage water were consistently higher than those obtained with well water. The better growth obtained by the P.T.S at the early growth stage may be due to treated sewage water contains higher concentrations of essential elements and less heavy metals than the secondary treated sewage water (S.T.S.). The declined down of the growth parameters as the plants reached 60 days age may be attributed to the heavy metals accumulation by the plant tissues.

Effect of treated sewage water on leaf pigments concentration

Leaf pigments concentration of sunflower plants as affected by irrigation water is shown in Table (5). Chlorophyll a, chlorophyll b and carotenoids concentrations in the leaves of the plants irrigated with treated sewage water were significantly higher than those in the leaves of the plants irrigated with well water. On the other hand, Chlorophyll “a” concentration in the leaves of the plants irrigated with the primary treated sewage water (P.T.S.)was higher than that in the leaves of the plants irrigated with the secondary treated sewage water (S.T.S.). The increase of pigments concentrations in the leaves of the plants irrigated with treated sewage water may be due to the higher concentrations of nitrogen, magnesium, iron and zinc in the sewage water[26]. Pigments concentration increase in the leaves of the plants irrigated with sewage water may also explain the higher fresh and dry weight accumulation in these plants compared to the control plants irrigated with well water (Table 4).While the findings of the present work proved the increase of pigments concentrations in the leaves of sunflower plants irrigated with treated sewage water, however, the chlorophyll “a” and chlorophyll “b” contents of sunflower plants irrigated with sewage water remained constant[26].

Table 5: Effect of treated sewage irrigation water on pigment concentration of sunflower plants leaves (mg/g Fw) (Average values of two seasons)

Irrigation treatments	Chl. (a)	Chl. (b)	Carotenoids	Chl. (a+b)	Chl. (a/b)
45 days after sowing					
Control	5.93	2.10	2.91	6.03	1.87
P.T.S.	7.35	2.53	3.48	9.88	2.90
S.T.S.	6.72	2.65	3.49	9.37	2.53
LSD at 0.05	N.S.	N.S.	N.S.	1.63	-
60 days after sowing					
Control	8.65	2.66	2.97	11.35	3.20
P.T.S.	10.79	2.89	3.94	13.68	3.73
S.T.S.	10.07	2.89	3.25	12.93	3.47
LSD at 0.05	0.91	N.S.	0.72	0.64	-
90 days after sowing					
Control	3.31	1.64	1.48	4.95	2.01
P.T.S.	6.56	2.96	2.53	9.52	2.21
S.T.S.	6.77	2.62	2.71	9.34	2.56
LSD at 0.05	1.27	0.46	0.91	0.82	-

P.T.S = Primary treated sewage, S.T.S. = Secondary treated sewage

Effect of treated sewage water on yield and yield components

Table 6: Yield and yield components of sunflower plants as affected by treated sewage irrigation water (Average values of two seasons)

Irrigation treatments	Desk diameter (cm)	Desk weight/plant (gm)	Seed No./plant	Dry seed yield/plant (gm)	Dry seed index (gm)
Control	7.24	21.55	278	13.85	4.98
P.T.S.	6.61	11.58	231	8.11	3.51
S.T.S.	7.83	13.84	251	9.37	3.71
LSD at 0.05	N.S.	2.15	9.37	1.836	1.06

P.T.S. = Primary treated sewage, S.T.S. = Secondary treated sewage

Yield and yield components of sunflower plants as affected by irrigation water are shown in Table (6). Desk diameter was not significantly affected by irrigation water. However, desk weight.plant^{-1}, seeds number.plant^{-1}, dry seeds yield.plant^{-1} and dry seed index were significantly affected by irrigation water. Values obtained by the plants irrigated with secondary treated sewage water (S.T.S.) were better than those irrigated by the primary treated sewage water (P.T.S.), but both were less than the values of the plants irrigated with well water[27]. The same author found also that yield and yield components of sunflower plants irrigated with treated sewage water were drastically reduced. Reduction of yield and yield components of the plants irrigated with sewage water due to higher microbial load[28] and heavy metal accumulation in the plant tissues which negatively affected synthesises translocation and accumulation in the storage tissues[7].

Effect of treated sewage water on seed chemical composition

Irrigation with treated sewage water found to significantly affected total carbohydrates content of sunflower seeds (Table 7). However, crude protein, oil content, moisture content and iodine number were not significantly affected with irrigation water. Increase of carbohydrate content in the seeds produced by the plants irrigated with sewage water may attributed to the higher nutrient content of the sewage water[26] and the higher pigment concentrations in the leaves of these plants.

Table 7: Effect of treated sewage irrigation water on major chemical compositions of sunflower seeds (dry matter basis) (Average values of two seasons)

Irrigation treatments	Total carbohydrate %	Crude protein %	Oil content %	Moisture content %
Control	25.49	19.95	39.59	5.93
P.T.S.	30.44	21.23	43.75	5.36
S.T.S.	27.82	20.20	41.18	5.91
LSD at 0.05	1.307	N.S.	N.S.	N.S.

P.T.S. = Primary treated sewage, S.T.S. = Secondary treated sewage

Effect of treated sewage water on heavy metals accumulation in the seeds

Heavy metals (Pb, Cd and Ni) concentrations in the seeds of sunflower irrigated with treated sewage water compared to control plants irrigated with well water are shown in Table (8). No significant different in the concentrations of these elements was detected. This means that although the plants absorbed reasonable amounts of heavy metals, they may be accumulate in the shoots, stems and roots[29], but not accumulated in the seeds. Authors[30, 31] found no significant changes in the concentrations of nutrient elements or heavy metals in the seeds of sunflower plants irrigated with treated sewage water compared to the plants irrigated with well water.

Table 8: Heavy metals concentration (ppm) in sunflower seeds as affected by treated sewage irrigation water (Average values of two seasons)

Irrigation treatments	Pb	Cd	Ni
Control	20	2.60	2.53
P.T.S.	20	2.60	2.40
S.T.S.	20	2.60	2.40
LSD at 0.05	N.S.	N.S.	N.S.

P.T.S. = Primary treated sewage, S.T.S. = Secondary treated sewage

Conclusions

From the present work it can be concluded that as a part of the solution of the problem of the shortage of water resources, treated sewage water can be safely used as irrigation water for sunflower plants and similar crops.

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