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Evaluation of Treated Wastewater Irrigation on Growth, Chemical Composition and Essential Oils of Some Woody Trees

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Abstract: Rapid increases in population and industrial growth have led to use low quality water such as drainage and saline water as well as waste water for irrigation. A pot experiment was conducted during 2018/2019 and 2019/2020 seasons in Timber Trees and Forestry Department, Horticulture Research Institute, Agricultural Research Centre, Egypt, to evaluate effects of treated wastewater irrigation on growth, chemical composition and essential oils extracted from seedlings of three woody plants (*Myrtus comunis, Eucalyptus camaldulensis and Cupressus sempervirens*). These seedlings were grown in sandy loamy soil irrigated with two types of irrigation water, tap water and treated waste water. The obtained results revealed that, the use of treated waste water was superior than tap water in improving all growth parameters of the three woody plant species beside its positive effect on their chemical composition. As regard essential oils, data showed that waste water irrigation increased oils quantity reaching, 41.3, 78.6 and 37.5% over control of the three plant species respectively with slight variable changes in its components .Also, data revealed that there is no accumulation of heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in essential oil in spite of its presence in traces in plant leaves. Thus, treated wastewater can be safely used as irrigation water source for these plant species as a timber tree or medicinal plants to partially solve the problem of the shortage in irrigation water and essential oil production.

Key words: Timber tree · Treated wastewater · Irrigation · *Myrtus communis* · *Eucalyptus camaldulensis* · *Cupressus sempervirens* · Essential oil · Heavy metals

INTRODUCTION

Water shortage throughout the world especially in arid regions in the later decades has led to search for alternatives to save potable fresh water. In Egypt, water is becoming scarce resource to consider any source of water, which might be used economically and effectively to promote further development. Recently, the shortage of the fresh water resources is of great concern, especially in the Mediterranean countries with high and increasing needs of water for different applications. The use of primary and secondary effluent in irrigation can improve the quality of the soil and plant growth because it contains nutrient elements and organic matter. The use of waste water for forest irrigation has proven to be a promising and successful practice for reusing wastewater and timber production [1]. Wastewater reuse in forest plays an important role for protecting the environment, because woody tree species uptake heavy metals into their tissues in addition, it also initiates opportunities for wood biomass production [2, 3]. At present, application of wastewater is considered the best solution for disposal problems [4]. Unlike organic contaminants, pathogens and heavy metals are considered stable in soil and cannot be degraded by biological or geochemical means [5]. Although some of these heavy metals are necessary for plant growth (i.e Mn, Cu, Ni, Zn and Co) and many correct nutrients deficiency but, high concentration of these metals can adversely affect the physiological performance of growing plants [6]. The use of wastewater for land irrigation is usually recommended for two main reasons:1-It is an allowable method for the disposal of wastewater, 2-It permits the reclamation and reuse of valuable resource such as water and nutrients [7].

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The use of wastewater in irrigation leads to heavy metals accumulation and this accumulation is mostly abundant in the root system than the aboveground parts which accumulate smaller amount of each metal as mentioned by Lydakis *et al*. [8], also they reported that, the careful analysis of the extracted essential oils from plants grown under heavy metals accumulation showed that neither the quality and the content of the oils were altered significantly, nor detectable amounts of heavy metals were found in these oils. Essential oils are volatile compounds which are formed by some plants as secondary metabolites .They are rich sources of biologically active compounds [9]. The medicinal properties of the plants used in this study have been investigated in the recent scientific developments throughout of the world, due to their potential activity against several diseases, without side effects and economic viability.

Myrtus communis L. (myrtle) is an evergreen shrub belongs to family Myrtaceae growing spontaneously throughout the Mediterranean- region. It is considered one of the important medicinal and aromatic drugs sources being used in human medicine for various therapeutic purposes [10]. The fruits are very astringent and are used as condiment, as a substitute for paper and are considered a rich source of tannins, the plant contains many biological active compounds such as fibers, sugars and antioxidants especially essential oils from plant leaves [11]. Essential oils are gaining remarkable interest for their potential multipurpose such as antioxidant, antibacterial and antiseptic agent [9]. Also, myrtle oil inhibited prostate and breast cancer cells along with sandal wood at very low concentration, through its flavenoide compounds [12].

Eucalyptus camaldulensis is a large genus of aromatic and medicinal trees of the Myrtaceae family. *Eucalyptus* species are used for many purposes including forestry and forest products and in ornamental plantings and horticultural as well as in arts and crafts. *Eucalyptus* species are used as fire wood for the production of mine wood and in the fight against erosion [13]. *Eucalyptus* was chosen for this study because it is fast growing and resistance tree to water logging, drought and salinity. It is common tree for ornamental and commercial planting in different soils. The *Eucalyptus* leaves contain essential oils with different allelo -chemicals, employed in medicine, cosmetic and pharmaceutical industries.

Cupressus sempervirens belongs to family Cupressacae, native to the Mediterranean basin and is distributed in North Africa, Asia, Southern Europe and Northern America .The wood of *Cupressus* has medium texture and shows soft resistance to manual cross-cutting. Its oil has a characteristic odor with a very agreeable smell and durable scent and it is used as antiseptic and an antispasmodic for stubborn coughs [14]. It is also described as deodorant and diuretic to promote venous circulation to the kidneys and bladder area and to improve bladder tone and as a co-adjuvant in therapy of urinary incontinence and enuresis. The parts of the plant used medicinally are the leaves and cones [15].

The aim of this work was to evaluate the effect of irrigation with treated wastewater on growth characters, chemical composition and essential oil quantity and quality as well as the possibility of accumulation of heavy metals in essential oils extracted from *Myrtus communis, Eucalyptus camaldulensis and Curpressus semprevirens*.

MATERIALS AND METHODS

A Pot experiment was conducted during 2018/2019 and 2019/2020 in a sandy loamy soil to investigate the effect of treated wastewater irrigation on the growth parameters, chemical composition, quantity and quality of essential oil as well as the possible accumulation of heavy metals in this oil extracted from seedlings leaves of three woody trees (*Myrtus communis*, *Eucalyptus camaldulensis* and *Cupressus sempervirens*. Soil samples were analyzed to determine their physical and chemical properties according to Page *et al.* [16]. Heavy metals concentrations in the soil were extracted by DTPA and measured by Atomic Absorption Spectrophotometer according to Lindsay and Norvell [17], as shown in Table (1).

One year old seedlings 30 cm height and 3.0 mm diameter at 5 cm from the soil surface of all tree species were used, they were obtained from a private farm at El -Qanater El-Khayria 20-Km northwest of Cairo, Qalioubia Governorate, Egypt.

The seedlings were individually planted on Ist March 2018 and 2019 in plastic pots (30 cm height and 25cm diameter) filled with 12 kg of sandy loamy soil, then all seedlings were irrigated with tap water for one month until adaptation. The two treatments of irrigation sources started using wastewater and tap water (control). All seedlings were irrigated to the field capacity, three times weekly in the summer and twice weekly in the winter. Each treatment comprised 30 seedlings for each plant species arranged in three replicates. Each replicate included 10 seedlings. The secondary treated wastewater used for irrigation was taken from wastewater treatment station at 6th October City, Giza. The analysis of the used water sources in irrigation is shown in Table (1).

Table 1: Physical and chemical analysis of the used soil and water in the experiment comparing with limits for agric. reuse FAO [23]

Properties	Soil	Tap water	Wastewater	Limits for agric.reuse FAO [23]
Practical size				
Distribution				
Sand%	87.00			
Silt%	7.80			
Clay%	5.20			
Soil texture	Sandy loam			
pH	7.40	6.80	7.8 0	6.5-8.4
E.C dSm^{-1}	0.87	0.69	1.55	3.0-7.0
Soluble cations meq/l				
Ca^{+}	3.20	1.15	3.65	0.0-20.0
Mg^{+}	1.54	1.26	2.35	0.0-5.0
K^{+}	0.96	0.31	0.80	-
Na^{+}	1.8 9	0.61	3.70	-
Soluble anion meq/l				
CO_{-3}	-	-	-	-
HCO_3	2.55	1.46	4.60	1.5-8.5
Cl^{-}	2.45	1.40	3.90	-
SO_4	2.59	0.66	2.40	-
*Do mg/l	-	-	2.40	-
*BOD mg/l	-	-	100	40-500
*COD mg/l	-	-	220.0	80-600
*TSSmg/l	-	-	1890	-
(N) (ppm)	8.12	-	12.11	-
(P) (ppm)	1.20	-	3.45	-
Total heavy metals (ppm)				
Cd	n.d	-	0.01	0.01
Cu	0.80	0.001	0.20	0.20
Mn	1.30	0.082	0.18	0.20
Ni	1.15	*Tr	0.03	0.20
Pb	2.11	*Tr	1.13	5.00
Zn	0.78	0.01	1.11	2.00
Fe	2.88	0.31	1.70	5.00
Co	*Tr	-	0.037	0.05
Cr	0.17	-	0.001	0.1

DO (dissolved oxygen), BOD(biochemical oxygen demand)*, COD(chemical oxygen demand)* TSS(Total suspended solids)
*Tr (traces).

After 12 months from starting irrigation treatments in each season, samples of all seedlings were collected and the following measurements were recorded:

Vegetative Growth Characters: Seedling height, root length, stem diameter (cm), fresh and dry weights of shoots and roots (g).

Chemical Composition Characteristic: Total chlorophyll content in fresh leaves was determined with dimethyl formamide as described by Mornai [18].

Total sugars content in fresh leaves was determined using phenol sulphuric acid reagent according to Dubois *et al.* [19].

After 24 month from starting irrigation with treated wastewater of each samples of all seedlings were collected and the following measurements were recorded:

Nitrogen, Phosphorous and Potassium were determined in dry leaves as, nitrogen was determined by Nessler method according to A.O.A.C. [20], phosphorous determination was adopted colorimetrically by using the chlorostannous reduced molybdophosphoric blue colour method according to King [21] and potassium was determined by using the Flame Photometric method according to Piper [22].

Essential Oil Extraction: The essential oil was obtained from leaves of different seedlings species by hydrodistillation using the Clevenger-type apparatus. Distillation was performed with fresh plant materials in 2-5l distilled water for 3-5 hours in order to determine the essential oil % (v/w), according to Egyptian Pharmacopoeia [24]. The oil was stored at-20°C until analyzed according to Paula *et al*. [25].

Gas Liquid Chromatography (GLC) Analysis: The essential oil composition was analyzed by pro-GLC pyeunicam gas chromatograph mass spectrum apparatus in order to investigate the principai components of the oil. The relative percent of each component was estimated according to Guenther and Joseph [26].

Heavy Metals Accumulation: Heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) accumulation in leaves and oils were recorded after two years from starting the experiment and were extracted by DTPA and measured in the solution by Atomic Absorption Spectrophotometer [27].

Statistical Analysis: Data obtained were subjected to ANOVA at 95% confidence according to Snedecor and Cochram [28]. Means of the treatments were compared by T-test ($P \leq 0.05$) for growth parameters.

RESULTS AND DISCUSSION

A-effect of Wastewater Irrigation on Growth Parameters of *Myrtus communis, Eucalyptus camaldulensis* and *Cupressus sempervirens*: Data in Tables (2-4) showed that, all vegetative growth parameters were increased with irrigation by treated wastewater for the three types of seedlings comparing with those irrigated with tap water in the two seasons.

The rate of increasing for *Myrtus communis* reached to 68.09, 52.00, 28.42 , 44.44, 38.13, 52.27 and 88.95% for seedling height, root length, stem dimeter, fresh and dry weights of shoots and fresh and dry weights of roots, respectively due to irrigation with wastewater comparing to tap water. Also, seedlings of *Eucalyptus camaldulensis* recorded an increment in all of the same parameters as following (33.33, 50.00, 72.93, 64.29, 54.84, 52.76 and 43.90 %) respectively. The same increasing (50.00, 21.43, 56.76, 58.33, 56.89, 56.26 and 49.21 %) for *Cupressus sempervirens* seedling. A similar trend obtained in the second season. These increments were significant in all parameters of the three plants species if compared with those irrigated with tap water.

Effect of Treated Wastewater Irrigation on Chemical Composition of *Myrtus communis, Eucalyptus camaldulensis* and *Cupressus sempervirens*

Total Chlorophylls and Sugars, N, P and K% and Oil %: Data in Table (5) showed that, total chlorophylls, total sugars contents, N, P and K% and essential oil% in the leaves of the plants irrigated with treated waste water were higher than those irrigated with tap water in all plants types which represented an increment by (36.36, 45.16 and 16.16 %) for total chlorophylls. (76.92, 82.50 and 24.78 %) for total sugars, concerning the N, P and K % nitrogen increment by (14.55, 11.63 and 8.41%), for phosphorus (3.40, 2.33 and6.50%) and (3.30, 4.96 and 2.00%) for potassium of *M. communis , E.camaldulensis* and *C. sempervirens* , respectively.

Concerning the oil% in leaves it is worth to note that the oil percentages of plants under irrigation with wastewater were higher than that of tap water. Also, plant responses to treated wastewater varied according to plant species science, the highest oil% were 0.75, 0.65 and 0.44 % of *E. camaldulensis*, *M. communis* and *C. sempervirens,* respectively for seedling irrigated with wastewater. This increment if compared with that recorded in tap water irrigation showed a relative increase by 78.6, 41.3 and 37.5% respectively.

Essential Oils Components: Data in Table (6) demonstrated that, the chemical composition of essential oil obtained from the plants was not influenced by the water type used for irrigation. Irrigation with wastewater was beneficial to increase the quantity of the oil, but did not significantly affect the composition of the oil, as there is a fluctuation in some components than others as 1.8 cineol followed by α-pinene then Myrtenyl acetate recorded the highly component appear in *M. communis* oil whether, irrigated by tap water or treated wastewater as (22.50 and 22.90), (21.07 and 22.20) and(20.37 and 21.32), respectively, 1.8 cineol (eucalyptol) followed by α-pinene then Globulol recorded the highly component in *E. camaldulensis* whether irrigated by tap water or treated wastewater as (35.35 and 40.01), (17.25 and18.17) and (6.75 and 7.01) respectively while, á-pinene followed by 3- caren then α-cedrol recorded the highly component in *C. sempervirens* oil whether irrigated by tap water or treated wastewater as (37.14 and 39.20), (7.22 and8.11) and (5.55 and 6.00), respectively.

Heavy Metals Accumulation: The results in this research showed variability in accumulation of heavy metals Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in leaves of the plant seedlings in this study as shown in Table (7). The results revealed more accumulation of all metals in the leaves of seedlings irrigated with wastewater while Cu, Fe, Mn and Zn only slightly accumulated in the leaves irrigated with tap water in all studied seedlings.

Table 2: Effect of treated wastewater irrigation on seedlings height, root length, stem diameter (cm), fresh and dry weights of shoots and roots of *M. communis* seedlings, during 2018/2019 and2019/2020 seasons

Treatments	Seeding height (cm)	Root Length (cm)	Stem diameter (cm)	Shoot F.W (g/plant)	Shoot D.W (g/plant)	Root F.W (g/plant)	Root D.W (g/plant)
				1st season			
Tap water	47.00	25.00	0.95	45.00	16.00	21.10	5.61
Wastewater	79.00	38.00	1.22	65.00	22.10	32.13	10.60
P-Value	0.02	0.03	0.00	0.04	0.04	0.04	0.00
..				2nd season			
Tap water	54.00	29.00	0.99	55.00	20.00	25.14	7.73
Wastewater	85.00	40.00	1.65	70.00	25.00	34.46	11.11
P-Value	0.04	0.04	0.00	0.00	0.02	0.03	0.00

Means were compared by T-test ($P \leq 0.05$)

Table 3: Effect of treated wastewater irrigation on seedlings height, root length, stem diameter (cm), fresh and dry weights of shoots and roots (g/plant) of *E. camaldulensis* seedlings, during 2018/2019 and 2019/2020 seasons

Treatments	Seeding height (cm)	Root Length (cm)	Stem diameter (cm)	Shoot F.W (g/plant)	Shoot D.W (g/plant)	Root F.W (g/plant)	Root D.W (g/plant)
				1st season			
Tap water	135.00	40.00	1.33	210.00	73.30	115.00	41.00
Wastewater	180.00	60.00	2.30	345.00	113.50	175.67	59.00
P-Value	0.00	0.00	0.00	0.00	0.00	0.00	0.00
				2nd season			
Tap water	150.00	45.00	1.40	235.00	78.00	125.00	43.00
Wastewater	190.00	69.00	2.40	380.00	123.50	192.00	63.80
P-Value	0.00	0.00	0.02	0.00	0.04	0.00	0.00

Means were compared by T-test ($P \leq 0.05$).

Table 4: Effect of treated wastewater irrigation on seedlings height , root length , stem diameter(cm) , fresh and dry weights of shoots and roots(g/plant) of *C.sempervirens* seedlings, during 2018/2019 and2019/2020 seasons

Treatments	Seeding height (cm)	Root Length (cm)	Stem diameter (cm)	Shoot F.W (g/plant)	Shoot D.W(g/plant)	Root F.W(g/plant)	Root D.W (g/plant)
				1st season			
Tap water	52.00	28.00	0.37	36.00	12.20	18.70	6.30
Wastewater	78.00	34.00	0.58	57.00	19.14	29.22	9.40
P-Value	0.00	0.04	0.00	0.00	0.00	0.00	0.00
				2nd season			
Tap water	55.00	29.10	0.45	40.00	14.71	19.71	6.70
Wastewater	84.00	36.20	0.62	62.00	21.34	32.90	10.20
P-Value	0.00	0.00	0.00	0.00	0.00	0.00	0.02

Means were compared by T-test ($P \leq 0.05$).

Table 5: Effect of treated wastewater irrigation on total chlorophylls, total sugars mg/g F.W during 2018/2019 and 2019/2020 and N, P, K and Oil % after two years from establishments of *M.communis, E.camaldulensis* and *C.sempervirens*

Treatments	Total chlorophylls (mg/g) F.W		Total sugars (mg/g) F.W					
	1st season	2nd season	1st season	2nd season	N%	P%	K%	Oil%
				M. communis				
Tap water	0.55	0.57	1.30	1.47	0.1 8	0.05	0.26	0.46
Wastewater	0.75	0.78	2.30	2.52	2.80	0 .22	1.12	0.65
P-Value	0.00	0.00	0.00	0.02	0.00	0.00	0.02	
				E. camaldulensis				
Tap water	0.31	0 .35	1.20	1.28	0.19	0.06	0.2 7	0.42
Waste water	0. 45	0.47	2.19	2.22	2.40	0.20	1.61	0.75
P-Value	0.01	0.01	0.00	0.00	0.00	0.00	0.00	
				C.sempervirens				
Tap water	0.30	0.33	1.13	1.24	0.17	0.04	0.2 6	0.32
Wastewater	0.35	0.37	1.41	1.67	1.60	0.30	0.78	0.44
P-Value	0.01	0.01	0.01	0.01	0.00	0.00	0.01	

Means were compared by T-test ($P \leq 0.05$).

Table 6: The main essential oil components of *M.communis, E. camaldulensis* and *C.sempervirens* due to irrigation by Tap water and treated Wastewater.

	Myrtus communis			*Eucalyptus camaldulensis*			*Cupressus sempervirens*	
Component	Tap water	Waste water	Component	Tap water	Waste water	Component	Tap water	Waste water
1-α-pinene	21.07	22.20	1-α-pinen	17.25	18.17	1-Tricyclene	0.14	0.17
2-β-Pinene	0.41	0.50	2-β-pinene	0.53	0.84	2-α-Thujene	0.14	0.16
3-Limonene	9.53	10.20	3-β-myrcene	0.58	0.15	3-α-pinene	37.14	39.20
4-2-methyl isobutylbutytyrate	1.66	2.40	4-α-phellandrene	2.16	0.17	4-Camphne	0.50	0.53
5-1, 8 cineol	22.50	22.90	5-P-cymene	4.87	5.43	5-Sabinene	0.22	0.45
6-Linalool	3.6 4	4.37	6-D-limonene	4.82	5.24	6-β-pinene	0.88	0.92
7-α-Terpinol	4.53	4.55	7-1-8 cineole(eucalyptol)	35.35	40.01	7-β-myrcene	1.50	1.84
8-Myrtenol	0.51	0.97	8-Gamma-terpinene	0.53	0.89	8-3-caren	7.22	8.11
9-Myrtenyl acetate	20.37	21.32	9-1-Octanol	1.07	1.16	9-1.8 cineole	1.00	1.08
10-Geranle acetate	0.85	0.75	10-Isopinocarveol	1.55	1.72	9-p-cymene	0.21	0.24
11-Mrthyl eugenol	1.55	0.00	11-Terpinene-4.ol	5.5	6.67	10-Limonene	2.85	2.91
12-p-Cymene	0.19	0.70	12-α-terpineol	1.20	1.51	11-γ-Terpinene	0.10	0.20
13-Linalyl acetate	0.35	0.00	13-α-Gurjunene.	0.50	1.11	12-α-terpineol	1.15	1.68
14-α-Humulene	0.08	0.09	14-Aromandendre	1.45	0.91	13-Camphre	0.11	0.11
5-Caroyoxidas	0.84	0.62	15-Globulol.	6.75	7.01	14- isobronyle acetate	0.30	0.40
						15-β-caryophyllene	2.11	2.20
						16- Caryophyllene oxide	0.30	0.30
						17-α-humulene	2.23	2.20
						18-germacrene- D	2.34	2.50
						19-α-cadinene	0.66	0.54
						20-α-cedrol	5.55	6.00

Table 7: Effect of irrigation with treated wastewater on the heavy metals (ppm) accumulation in leaves and essential oil after two years from establishment of *M. communis, E. camaldulensis and C. sempervirens* seedlings

	Myrtus communis				*Eucalyptus camaldulensis*				*Cupressus semprevirens*			
	Leaves		Oil		Leaves		Oil		Leaves		Oil	
`Element (ppm)	Tap water	Waste water	Tap water	Waste water	Tap water	Waste water	Tap water	Waste water	Tap water	Waste water	Tap water	Waste water
Cd	n.d	0.20	n.d	n.d	n.d	0.30	n.d	n.d	n.d	0.18	n.d	n.d
Co	n.d	0.47	n.d	n.d	n.d	0.50	n.d	n.d	n.d	0.25	n.d	n.d
Cr	n.d	0.02	n.d	n.d	n.d	0. 05	n.d	n.d	n.d	0.04	n.d	n.d
Cu	1.40	3.08	n.d	n.d	1.31	3.34	n.d	n.d	1.71	6.20	n.d	n.d
Fe	3.26	7.88	n.d	n.d	4.72	11.24	n.d	n.d	4.18	12.50	n.d	n.d
Mn	0.15	14.28	n.d	n.d	1.08	10.59	n.d	n.d	0.62	11.39	n.d	n.d
Ni	n.d	0.64	n.d	n.d	n.d	0.54	n.d	n.d	n.d.	0.33	n.d	n.d
Pb	n.d	15.51	n.d	n.d	n.d	14.0	n.d	n.d	n.d	13.81	n.d	n.d
Zn	3.18	14.64	n.d	n.d	7.04	9.40	n.d	n.d	3.25	17.15	n.d	n.d

n.d (not detected)

About, the accumulation of heavy metals in seedlings irrigated with wastewater Pb followed by Zn then Mn recorded the highly accumulation content (15.51, 14.64 and 14.28 ppm)respectively in *M. communis* and Pb followed by Fe then Mn recorded the highly accumulation content (14.00, 11.24 and 10.59 ppm) respectively in *E. camaldulensis* while Zn followed by Pb then Fe recorded the highest level of accumulation content (17.15, 13.81 and 12.50 ppm), respectively in *C. sempervirens*.

In spite of the presence of these metals in the leaves of the studied plants the results, showed that these heavy metals Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn content in the volatile oil of the three plants were below the analytical detection limits hence no data were reported.

All vegetative growth parameters were increased with irrigation by treated wastewater.

This increment may be due to the increase in organic matter, macro-and micronutrient in the sewage water where beneficial nutrient improved the metabolic activities and hence the vegetative growth [29]. These results were in agreement with those obtained by Alex *et al*. [30] on *Salix discolor* and *S. eriacephala*.

Similar results were also obtained on growth of ornamental sunflower by Andrade *et al*. [31], who found that the best growth parameters were obtained by the waste water irrigation that, may be due to the higher concentration of essential elements in it, as reported by Zaki and Shaaban [32]. On *Jatropha* plants Luma Abdalah, *et al.* [33] found similar results when treated with wastewater and he attributed this to its nutrient contents (N, P, K) that are necessary for the growth of plants. Also, the results were in agreement with Elsokkary and Aboukila [34] on basil and oregano plants.

Also, the obtained results were in harmony with many investigators, Guo and sims [35] on *Eucalyptus globules*, El-Sayed [36] on *Ceratonia siliqua*, who found that sewage effluent had a stimulatory effect on vegetative growth of the trees, providing the soil with plant nutrients and organic matter and improved the soil physical characteristics, that reflected on the growth by enhancing the cell elongation and division and elongation in the cambium zone and all membranes that increase in stem diameter Harada *et al*. [37] on willow (*Salix* sp).

The increasing in total chlorophylls and total sugars concentration, N, P , K and oil % in the leaves of the plants irrigated with waste water may be due to the higher concentration of nitrogen, magnesium, iron and zinc in the sewage water [38]. Similar results were obtained by Zaki and Shaaban [32] on sunflower. Also, Zeid and Abou El Ghate [39] reported that application of sewage water positively affected the synthesis of photosynthetic pigments. The stimulatory effect of sewage water on chlorophylls content could be attributed to the fact that sewage water enhances the rate of biosynthesis of chlorophylls.

El-Maghraby and Gomaa [29] reported that sewage water application increased number of green leaves and leaf area per plants or it may be increase both of macro and micronutrients elements in soil, which is essential for the plant growth and photosynthetic pigments. Abdel Latef and Sallam [40] indicated that, sewage water irrigation increased the content of sugars in maize plants due to the presence of some mineral ions e.g. Mn and Cu that stimulated the photosynthesis. In the study of Rija *et al.* [41], it was found that sewage irrigation on plants like *Vigna radiata*, *Cicer arietinum* and *Lens culinaris* increased the total protein, carbohydrate and chlorophyll contents, they attributed these increases to the activity of some microbes presented in sewage water which can convert organic matter into by-products like CO_2, NO_3, PO_4, SO_4, NH_3, H_2S and CH_4 and they concluded that the presence of higher amounts of macronutrients in the sewage water provides cofactors to enzymes necessary for the synthesis of protein and carbohydrate. Selahvarzi and Hosseini [42] found that the use of sewage effluent in irrigation resulted in a remarkable change of organic matter available and total soluble N which might have been added to soil. The results were in harmony with Singh and Bahati [43] who found that concentration of N, P and k were greater in foliage compared to the other plant parts. Also, Hassan *et al*. [44] found that irrigation with sewage effluent increased most of the macro elements and organic matter in the soil cultivated with some trees e.g *Acacia saligna*, *Tipuana speciosa* , *Melia azedarach*, *Albizia lebbek* and *Taxiodum distichum*.

Concerning the increase of oil% may be attributed to the sufficient amounts of macro- and micro nutrient and hormons present in wastewater. These results were similar to the findings of Bernstein *et al.* [45] who evaluated the effect of irrigation with secondary treated effluent on essential oil yield, in two commercial cultivated of the aromatic species *Organium vulgar* and *Rosmarinu s officinalis* L.

Also, Khalifa *et al*. [46] found that, the oil yield of five crops were higher under waste water irrigation than that irrigated with fresh water .Similarly, these results agree with Kotb *et al*. [47] on peppermint irrigated by wastewater and freshwater.

Rahimi *et al*. [48] found also that the essential oil yield, biological yield, flowering shoot yield and essential oil percentage of coriander increased as a result of irrigation with treated wastewater as compared to freshwater. Elsokkary and Aboukila [34] showed that there was an increase in essential oil yield, shoot yield, flowering shoot yield and essential oil percentage % in basil and oregano as a result of irrigation by wastewater as compared to those irrigated by freshwater. Similar results were obtained by Maaloul, *et al.* [49] on *E. camaldulensis*. Also, these results were in harmony with Hossein *et al.* [50] on *Ocimum basilium* L., who indicated that, application secondary treated increased essential oil content of basil, as waste water contains more (N, P, K) which are the major nutrients that influence plant growth and oil yield.

In spite of the presence of these metals in the leaves of the studied seedlings the results, showed that these heavy metals Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn content in the volatile oil of the three plants were below the analytical detection limits. This result confirmed previous results of Yadav *et al.* [51]; Angelova *et al.* [52]; Zheljazkov *et al.* [53] and khalifa *et al.* [46] who stated that plants can be grown in soils enriched with heavy metal without risk for metal transfer into the oils and without significant alteration of essential oil composition that may impair marketability.

Bernstein *et al*. [45] demonstrated that oregano and rosemary were suitable as industrial crops for essential oils and antioxidant production under irrigation with wastewater because their yield quantity and quality were not affected. They affirmed that cultivation of aromatic plants for essential oil is suitable for irrigation with treated effluents because heating during essential oil extraction eliminates human bacterial pathogens originated in the effluents and alleviates health concerns. Additionally, the essential oil which is extracted mainly by steam distillation, will be free of inorganic ion contaminants such as heavy metals originating from the effluents, which may accumulate in the plant tissue and in the soil. These results were in agreement with Zaki and Shaaban [32] and Koottatep *et al.* [54] on sunflower. Also, the results of this study were confirmed in previous results of Zheljazkov *et al.* [53], who concluded that plants irrigated with municipal wastewater, produced volatile oils free from the potential toxic elements and more pronounced when irrigated with secondary sewage effluents. In the same direction, Toze [55] demonstrated that heavy metals in effluents used for irrigation tend to accumulate in the soils where there is a potential that they could became bio available for crops. Similar results obtained by Khalifa *et al.* [46] who stated that no detectable amount of the potential toxic elements was recorded in the essential oils of the tested aromatic plants. From this stand point, treated municipal wastewater can be used for growing aromatic plants in the arid area without mineral fertilizers to produce volatile oils without causing any reduction in its quantity and quality. On the other hand Zaki and Shaaban [32] showed that no significant difference in the concentration of these elements (Pb, Cd and Ni) was detected in plants irrigated with waste water. This means that although the plant absorbed reasonable amounts of heavy metals, they may be accumulated in the shoots, stem and roots [56]. Kootlatep *et al.* [54] found no significant changes in the concentration of nutrient elements or heavy metals in the seeds of sunflower plants irrigated with treated sewage water compared to well water. Also, results agreed with those of Scora and Chang [57] who did not find variation in peppermint oil constituents due to elevated concentration of heavy metals in the medium. Also Zhelijazkov *et al.* [53] showed that peppermint, basil and dill could be grown in soils enriched with Cd, Pb and Cu without risk for metal transfer into the oils and without significant alteration of essential oil composition that may impair its marketability.

CONCLUSION

From the present study it can be concluded that as a part of the solution of the problem of the shortage of water resources, irrigation with treated wastewater can be safely used as irrigation water for *Myrtus communis, Eucalyptus camaldulensis* and *Cupressus sempervirens* seedlings. Results confirmed that the treated waste water could effectively increase all the growth parameters, total chlorophylls and sugars , as well as N, P and K %. On the other hand, it could increase the essential oils without affecting its composition. Finally, it can be concluded that all the volatile oils extracted from the studied plants were free from heavy metals so, the use of the treated waste water in irrigation of these plants may be recommended but, there is a need for continuous monitoring of the concentration of potentially toxic element in soil, plant and ground water.

REFERENCES

1. Sharma, A. and N. Ashwath, 2006. Land disposal of municipal effluents: Importance of choosing agroforestry systems. Desalination, 187: 361-374.
2. Stewart, H.T.L. and D.W. Flinn, 1984. Establishment and early growth of trees irrigated with wastewater at four sites in Victoria, Australia. Forest, Ecol Manage, 8: 243-256.
3. Madejon, P., T. Mara˜non and J.M. Murillo, 2006. Biomonitoring of trace elements in the leaves and fruits of wild olive and holm oak trees. Sci. Total Environ., 355: 187-203
4. Selem, M.M., S. El-Amir, S.M. Abd-Elaziz, M.F. Kandil and S.F. Mansour, 2000. Effect of irrigation with sewage water on some chemical characteristics of soils and plants. Egypt. J. Soil Sci., 40: 49-59.
5. Abdelhafez, A.A. and J. Ali, 2015. Environmental Monitoring of Heavy Metal Status and Human Health Risk Assessment in the Agricultural Soils of the Jinxi River Area, China. Hum Ecol Risk Assess., 21(4): 952-97.
6. Feng, J., Q. Shi, X. Wang, M. Wei, F. Yang and H. Xu, 2010. Silicon supplementation ameliorated the inhibition of photosynthesis and nitrate metabolism by cadmium (Cd) toxicity in *Cucumis sativus* L. Sci. Hortic, 123: 521-30.
7. Wang, H.K., 1984. Sewage irrigation in China. Intern, J. Develop. Tech., 2: 291-301.
8. Lydakis-Simantiris, N., M. Fabian and M. Skoula, 2016. Cultivation of medicinal and aromatic plants in heavy metals-contaminated soils. Global NEST Journal, 18(3): 630-642.
9. Zenebe, H., M. Afework, V.K. Gopalakrishnan Krishna and C. Nagaraju, 2017. Chemical compostion and physicochemical properties of essential oil from *Mytrus communis*. Intern. J. Pharma and Clinical Research, 9(6): 439-443.

10. Sabiha, S., A. Aflab Asif, M. Mohd, A. and S. Ibne, 2012. Physicochemical and photochemical standardization of berries of *Myrtus communis* l.m. Orginal Artivle, 4(4): 322-326.
11. Hayder, N., A. Abdelwahed, S. Kilania, S. Ben Ammer, A. Mahmoud and K. Ghedirab, 2004. Anti-genotoxic and free-radical scavenging activities of extract from (Tunisian) *Myrtus communis*. Mutat Res., 564: 89-95.
12. Sedat, S., E. Sezai, S. Memnune, G. Kazim and O. Emine, 2010. Antioxidant activity and fatty acid composition of wild grown myrtle. Pharmacogn Mag., 6(21): 9-12.
13. Verdeguer, M., M.A. Blázquez and H. Boira, 2009. Phytotoxic effects of *Lantana camara, Eucalyptus camaldulensis* and *Eriocephalus africanus* essential oils in weeds of Mediterranean summer crops. Biochemical Systematics and Ecology, 37(4): 362-369.
14. Rawat, P.O., M.F. Khan, M.A.K. Tamarkar and A.K. Srivastava, 2010. Constituents from fruits of *Cupressus sempervirens*. Fitoterapia, 81: 162-166.
15. Mahmood, Z., I. Ahmed, M. Saeed and M.A. Sheikh, 2013. Investigation of physico-chemical composition and antimicrobial activity of essential oil extracted from lignin-containing *Cupressus sempervirens*. BioResources, 8(2): 1625-1633.
16. Page, A.L., R.H. Miller and D.R. Keeny, 1982. Methods of Soil Analysis, Part II. 2nd (ed.) Agronomy Monogr. ASA and SSSA, Medison, WI.3.
17. Lindsay, W.L. and W.A. Norvell, 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Sci. Soc. Am. J., 42: 421-428.
18. Mornai, R., 1982. Formula for determination of chlorophyllus pigments extracted with N.N dimethyl fomamide. Plant Physiol., 69: 1371-1381.
19. Dubois, M., F. Smith, K.A.Gilles, J.K. Hammilton and P.A. Robers, 1956. Colorimetric method to determination of sugars and related substances. Anal. Chem., 28(3): 350-356.
20. A.O.A.C., 1990. Official Methods of Analysis (15th ed.). Association of Official Analytical Chemists. Washington, D.C., U.S.A.
21. King, E.J., 1951. Micro-Analysis in Medical Biochemistry. 2nd Ed. Churchil. London.
22. Piper, C.S., 1950. Soil and Plant Analysis. Inter. Sci., Pulb, New York, pp: 368.
23. FAO, Food and Agriculture Organization of United Nations, 1992. Wastewater treatment and use in agriculture. In: Pescod, M.B. (Ed.), Irrigation and Drainage, Paper 47, FAO, Rome.
24. Egyptian Pharmacopoeia, 1984. General Organization for Governmental Printing Office, Ministry of Health, pp: 31-33. Cairo, Egypt.
25. Paula, C.P., J.C. Maria M. Gabriela and G. Bernardo, 2009. Evolution of the yields and composition of essential oil from Portuguese myrtle (*Myrtus communis* L.) through the vegetative cycle Molecules, 14: 3094-3105.
26. Guenther, Z. and S. Joseph, 1978. Handbook Series in Chromatography. CRC Press, Inc. New York.
27. Lindsay, W.L. and W.A. Norvell, 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Sci. Soc. Am. J., 42: 421-428.
28. Snedecor, G.W. and G.W. Cochran., 1981. Statistical Methods, 7th ed. The Iowa State University Press, Ames, Iowa.
29. El-Maghraby, S.S. and M.A. Gomaa, 1992. Effect of sewage sludge as a fertilizer on syrup yield and some agronomic characters of sweet sorghum. Egypt J. Applied Sci., 7: 300-317.
30. Alex, M. and J.E. Major, 2017. Phytoremediation efficacy of *Salix discolor* and *S. eriocephela* on adjacent acidic clay and shale overburden on a former Mine Site: Growth, Soil and foliage traits. Forests., 8: 475.
31. Andrade, L.O., H.R. Gheyi, R.G. Nobre Dias, E.C.S. Nascimento, 2012. Growth of ornamental sunflower irrigated with treated wastewater under organic production. IRRIGA; Special Issue, pp: 69-82.
32. Zaki, S.M. and M.M. Shaaban, 2015. Impact of treated sewage water irrigation on some growth parameters, yield and chemical composition of sunflower, *Helianthus annuus* l. Plants, 8: 114-122.
33. Luma Abdalah, S., W. Alabadi, A. SahibABood, M. Hussein Khaeim and H. Al-Azam Hadithy, 2018. Utilization of treated wastewater in irrigation and growth of *Jatropha* plant to protect the environment from pollution and combating desertification. Plant Archives, 18(2): 2429-2434.
34. Elsokkary, I.H. and A.F. Aboukila, 2020. Beneficial additive values of wastewater irrigation of two aromatic plants grown in low fertile soil. Water Science, 34(1): 132- 142.
35. Guo, L.B. and R.E.H. Sims, 2000. Effect of meat works effluent irrigation on soil, tree biomass production and nutrient uptake in *Eucalyptus globulus* seedlings in growth cabinets. Bioresour. Technol., 72: 243-251.

36. El-Sayed, N.A.A., 2005. The impact of irrigation with treated wastewater effluent on soil bio-physicochemical properties and on growth and heavy metals content of some fodder trees grown on calcareous soil. Ph.D. Thesis, Fac. Agric., Tanta Univ, Egypt.
37. Harada, E., A. Hokura and I. Nakai, 2011. Assessment of willow (*Salix sp*.) as a woody heavy metal accumulator: field survey and in vivo X-ray analyses. Metallomics., 3: 1340-1346.
38. Khan, M.A., S.S. Shaukatand and M.A. Khan, 2009. Growth, yield and nutrient content of sunflower (*Helianthus annuus* L.) using treated wastewater from waste stabilization ponds. Pakistan Journal of Botany, 41(3): 1391-1399.
39. Zeid, I.M. and H.M. Abou El-Ghate, 2007. Effect of sewage water on growth, metabolism and yield of bean. J. Biol. Sci., 7: 34-40.
40. Abdel Latef, A.A. and M.M. Sallam, 2015. Changes in growth and some biochemical parameters of maize plants irrigated with sewage water. Austin J. Plant Biol., 1(1): 1004-1009.
41. Rija, H., Z.S. Siddigui and A.U. Zaman, 2005. Changes in chlorophyll, protein and carbohydrate contents of *Vigna radiate*, *Cicer arietinum* and *Lens culinaris* after irrigation with sewage water. Int. J. Biol. Biotech., 2: 981-984.
42. Selahvarzi, B. and S.M. Hosseini, 2012. Survival, growth and mineral accumulation in ash *Fraxinus excelsior* L.seedling irrigated with water treatment effluent. Foliar For. Pol., Ser. A., 54: 159-168.
43. Singh, G. and M. Bhati, 2005. Growth of Dalbergia sissoo in desert regions of western India using municipal effluent and the subsequent changes in soil and plant chemistry. Bioresource Technology, 96(9): 1019-1028.
44. Hassan, F.A., L.I. El-Juhany, A.A. El-Settawy and M.S. Shehata, 2002. Effects of irrigation with sewage effluent on the growth of some forest trees species, physical and chemical properties of the soil. In: Proceeding of the second Conference ''Sustainable Agricultural Development'' 8-10 May, Fayoum, pp: 300-311.
45. Bernestein, N., D. Chaimovitch and N. Dudai, 2009. Effect of irrigation with secondary treated effluent on essential oil, antioxidant activity and phenolic compounds in oregano and rosemary. Agronomy Journal, 101(1): 10.19.
46. Khalifa, R.K.M., H. Abouziena, R. El-Mergawi and A. Youssef, 2011. Nutritional status of some aromatic plants grown to produce volatile oils under treated municipal wastewater irrigation. Australian Journal of Basic and Applied Sciences, 5(12): 2999-3007.
47. Kotb, E.A., A.A. Moursy and M.F.A. Noby, 2012. Response of peppermint (*Mentha piperita* L.) grown on different sources of wastewater. Third Inter. Conf. on Radiation Sci Application, Nov. 12-16, Hurghada, Egypt.
48. Rahimi, T., H.A. Farahani and H.H. Darvishi, 2010. Effect of irrigation by treated domestic wastewater instead of well water on essential oil content in coriander (*Coriandum sativum* L) varieties. Indian J. Environ. Sci., 5(5): 306-310.
49. Maaloul, A., M. Verdeguer, M. Oddo, E. Saadaoui, M. Michalet, D. Franca, M. Mohamed and R. Mehrez, 2019. Effect of short and long term irrigation with treated wastewater on chemical composition and herbicidal activity of *Eucalyptus camaldulensis* Dehn. essential oils. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 47(4):
50. Hossein, H.D., M. Mohammad, S. Hossein, H. Sayed and J. Mousavui, 2010. Irrigation influence by treated domestic wastewater instead of agronomical water on essential oil yield of basil *Ocimum basilium* L.African Journal of Microbiology Reaseach, 4(6): 475-479.
51. Yadav, R.K., B.R.K. Goyal, S.K. Sharma Dubey and P.S. Minhas, 2002. Post-irrigation impact of domestic sewage effluent on composition of soil, crop and ground water-A case study. Environment International, 28(6): 481-486.
52. Angelova, V., R. Lvanov, V. Delibaltova and K. Lvanov, 2004. Bio-accumulation and distribution of heavy metals in fiber crops (flax, cotton and hemp).Ind. Crop Pro., 19: 197-205.
53. Zheljazkov, V.D., L.E. Craker and B. Xing, 2006. Effects of Cd, Pb and Cu on growth and essential oil contents in dill, peppermint and basil. Environ. and Experim. Bot., 58(3): 9-16.
54. Koottatep, T., C. Polprasert and S. Hadsoi, 2006. Integrated faecal sludge treatment and recycling through constructed wetlands and sunflower plant irrigation. Water Science and Technology, 11(12): 155-164.
55. Toze, S., 2006. Reuse of effluent water-benefits and risks. Agric. Wat. Manag., 80: 147-159.
56. Mostafa Zadeh-Fard, B., S.A.M.M. Meibody and M.Y. Kopaei, 2005. A comparison of sunflower, corn and sugarbeet characteristics under different irrigation systems using wastewater. Iranian Journal of Agricultural Sciences, 36(5): 215-222.
57. Scora, R.W. and A.C. Chang, 1997. Essential oil quality and heavy metal concentrations of peppermint grown on a municipal sludge-amended soils. J. Environ. Qual., 26: 975-979.