Beneficial additive values of wastewater irrigation of two aromatic plants grown in low fertile soil

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ABSTRACT

The design of this study was to analyze the influence of irrigation by freshwater (FW) or by treated wastewater (TWW) on the growth and essential oil components in two aromatic plants: basil (*Ocimum basilicum* L.) and oregano (*Origanum marjorum* L.) grown in low fertile calcareous soil. A pot experiment was carried out in the greenhouse. The seeds of basil and oregano were sown and irrigated by either FW or TWW and harvested after 70 days from sowing for analysis. Tests between-subject effects point out there is a significant difference (p < 0.05) between the two forms of irrigation water on shoot yield, the yield of the flowering shoot, the quantity of essential oil, and some element concentrations in leaves of plants (N, P, Cu, Fe, and Mn), while there is no significant difference between essential oil percentage, K and Zn content. The obtained results specified that the essential oil yield significantly increased, as a result of irrigation by TWW from 0.58 to 1.08 in basil and from 2.00 to 3.06 ml 100 g⁻¹ dry matter in oregano. Irrigation by TWW influenced the percentages of the essential oil components, whether positively or negatively, with reference to those produced by FW irrigation. The highest value of relative increase (719%) was that of d-Camphor (oxygenated monoterpene) in basil leaves and the lowest value of relative decrease (83%) was detected in basil leaves for methyl eugenol (oxygenated monoterpenes). Regarding oregano; the highest and lowest values were 82% and 55% for p-Cymene (monoterpene hydrocarbons) and terpinolene (monoterpene hydrocarbons) respectively. These results provide significant beneficial additions due to irrigation of low fertile calcareous soil by treated wastewater to produce high valued essential oils from aromatic plants.

Introduction

Wastewater reuse is considered a favorable substitute for precious freshwater, particularly those in the arid or semi-arid areas of numerous countries. It is valued for its reliability as a water resource and its benefits due to the contained plant nutrients (Darvishi & Farahani, 2010). Several studies showed the agronomical and environmental interest for irrigation of various plant crops by wastewater (Al-Lahham, El Assi, & Fayyad, 2003; Bañón, Miralles, Ochoa, Franco, & Sánchez-Blanco, 2011; Parsons, Wheaton, & Castle, 2001). Treated wastewater has a superior nutritive value which can increase plant growth, lower fertilizer uses, and enhance poor fertile soil productivity (Aiello, Cirelli, & Consoli, 2007; Al-Lahham et al., 2003). Furthermore, wastewater can be used safely for irrigation in controlled environments to minimize the risk to agricultural products, soil, surfaces, and groundwater from pathogens and harmful pollutants (Aiello et al., 2007; Al-Lahham et al., 2003; Bañón et al., 2011). Although there is no all-inclusive worldwide data on wastewater reuse, it is valued that about 20 million hectares use wastewater for irrigation (WHO, 2006), 90% uses raw wastewater without any treatment.

Treated wastewater may carry harmful significant concentrations of salts, heavy metals, or pathogenic organisms based on its source and treatment (Bañón et al., 2011; Elsokkary & Abukila, 2014; Qadir et al., 2010). Thus, the possibilities of transferring the toxic contaminants from wastewater to the agricultural ecosystem would adversely influence human health during the network of the nutrition chain. However, few investigations have been published for wastewater irrigation of ornamental, aromatic and medicinal plants (Bañón et al., 2011) and thus, it is significant to consider several factors regarding the potential for treated wastewater in irrigation of these plants. This approach converts unconventional low-grade water to productive virtual water for producing commodities of high added values such as essential oils. The using rank of essential oils from plants has gone up over the past two decades, a trend that is expected to continue growing at a brisk pace. Medicinal plants today are realized not only as an origin of reasonable healthcare in developing countries but also as an essential module of a variety of medicinal and non-medicinal uses and applications in the developed world as well. Over 80 percent of the world’s population relies on
traditional medical systems, primarily based on aromatic plants, to meet their main healthcare needs (Nagpal & Karki, 2004). To avoid the risk from edible crops contamination under using treated wastewater for irrigation, it is suggested to using the treated wastewater for growing oil crops and fiber (Angelova, Ivanova, Delibaltova, & Ivanov, 2004; Bañón et al., 2011; Bernstein, Chaimovitch, & Dudai, 2009, 2012), where the oil extracted will contain lowest or free heavy metals, or for the cultivation of cut flowers such as roses (Bernstein et al., 2006) or for cultivating aromatic plants (Bernstein et al., 2012). Perennial aromatic plants irrigate with substantial amounts of water to appropriate concentrated harvesting. In arid and semiarid regions, where scarcity of fresh-water, irrigation with treated wastewater is an inevitable practice (Bernstein et al., 2012). Bernstein et al. (2012) monitored for three years a variety of aromatic crops, as a source of essential oil, for their yield quantity and quality under irrigation with treated wastewater. The results demonstrate that treated wastewater effluent is appropriate for growth and quality products in all species tested. Another study by Bernstein et al. (2009) found that oregano and rosemary are ideal for the production of antioxidants of essential oil under irrigation with treated wastewater since their yield and quality have not been influenced. Replacement of freshwater by treated wastewater for irrigation of oregano and rosemary promoted the expansion of large production of biomass and essential oils (Bernstein et al., 2009). It has been found also that secondarily treated wastewater improved the growth and quality production of essential oils of basil (Darvishi, Manshouri, Sedghi, & Jahromi, 2010). The lemongrass grown in soil irrigated by wastewater showed normal growth characters without any significant morphological and physiological symptoms and Cd, Pb, and Ni were the most absorbed heavy metals by the plant are accumulated in the root a (Lal et al., 2013). Studies carried by (Zheljaskov, Craker, Xing, Nielsen, & Wilcox, 2008; Affholder et al., 2013; Lal et al., 2013) showed that the aromatic plants grown in heavy metals contaminated soils, neither the quality and the contents of the essential oils were significantly altered, nor detectable concentrations of toxic elements were measured in these oils. Chamomile, sag, and thyme plants grown in heavy metals contaminated soil accumulated high amount of Cd, Pb, and Ni in their roots whereas the above-ground parts of the plants demonstrated lower accumulation magnitude for these metals. Regardless of the levels of Cd, Pb, and Ni accumulated in plant root, the quantities of essential oils from these three-plant species were not affected and, in all cases, the extracted essential oils were free from heavy metals (Lydakis-Simantiris, Fabian, & Skoula, 2016). The quantity of the volatile oil of peppermint irrigated by diverse sources of treated wastewater increased significantly and no detectable concentration of the potentially toxic elements was recorded in the essential oils (Kotb, Moursy, & Noby, 2012).

The objective of this study, therefore, was to assess the influence of irrigation of aromatic plants (basil and oregano) grown in poorly fertile calcareous soil by treated wastewater on their growth performance and essential oils percentage.

**Materials and methods**

**Experimental layout**

Pot experiments were executed in the greenhouse of the Research Laboratory of Saline and Alkaline Soils at Abis, Ministry of Agriculture and Reclamation, Alexandria Egypt. The used soil is calcareous (typic calcorthids) taken from Bangar EL-Sokkar Region, 65 km west Alexandria City. This region was chosen considering the Egyptian government strategy focused on the horizontal land expansion in western Alexandria City. The principal chemical and physical quality of this soil are presented in Table 1. Two sources of irrigation water were used; freshwater (FW) from the El-Mahmoudiya canal in the western area of Nile Delta, and secondary treated wastewater (TWW) from Alexandria West Sewage Treatment Plant that collects mixed domestic-industrial influent and discharges into Lake Mariut. Table 2 displays the most critical chemical parameters of each water.

Twenty-five kg soil were placed in a clay-fired glazzy pot of 25 cm inside diameter and 45 cm depth with five holes in the bottom for drainage excess water. The soil in the pot was watered every day with FW for one week before seed sowing.

Seeds of basil (Ocimum basilicum L.) or oregano (Origanum marjoram L.) were sown in each pot and

**Table 1. The main chemical and physical characteristics of the used calcareous soil as an average value.**

| Soil parameter       | value | Unit  |
|----------------------|-------|-------|
| pH                   | 8.2   |       |
| EC                   | 3.1   | dS m⁻¹|
| Total CO₂³⁻         | 28.4  | %     |
| OM                   | 0.9   | %     |
| Total N              | 0.1   | %     |
| Available P          | 6.7   | mg kg⁻¹|
| Available K          | 20.0  | mg kg⁻¹|
| Available Cu         | 0.5   | mg kg⁻¹|
| Available Fe         | 4.0   | mg kg⁻¹|
| Available Mn         | 1.0   | mg kg⁻¹|
| Available Zn         | 0.2   | mg kg⁻¹|
| Available Cd         | nd    | mg kg⁻¹|
| Available Cr         | nd    | mg kg⁻¹|
| Available Pb         | nd    | mg kg⁻¹|

**Particle size distribution:**

| Sand     | 76   | %    |
| Silt     | 11   | %    |
| Clay     | 13   | %    |

**Soil texture**

Sandy loam

**Bulk density**

1.65 g cm⁻³

**WHC**

45.5 %
irrigated daily to about 60% of the soil Water Holding Capacity (WHC) by FW for two weeks (the normal field soil WHC is 60%. When the WHC falls below 55-60% plants could suffer from dryness and when the WHC is over 80% plants begin to suffer from a diminution of soil oxygen (Brady & Weil, 1996)). The plants were then thinned to five seedlings per pot. The plants in each pot were irrigated by either FW or by TWW according to experimental design. The experimental layout was splitting design and each treatment was repeated in four replicates.

Each pot was fertilized by (i) superphosphate fertilizer (15.5%P2O5) with a rate of 300 kg ha−1 in one dose before seed sowing; (ii) ammonium nitrate fertilizer (52%K2O) with a rate of 200 kg ha−1 in two equal doses after 30 and 45 days from plants thinning; and (iii) potassium sulfate (52% K2O) at a rate of 60 kg ha−1 in one dose after 30 days of plant thinning. The previous fertilization process is recommended by FAO (2005).

### Sampling

#### Soils

Composite soil samples were collected from Bangar El-Sokkar Region, West Alexandria City, ground, passed through 2 mm sieve, and kept for analysis and pot experiment. Table 1 demonstrated that the soil is characterized by the valuable concentration of total CO2− (28.4%) and low concentration of both OM (0.9%) and total N (0.1%). The quantities of available P and K are in the deficient domain (Chapman, 1966). This is also concerning available Cu, Fe, Mn, and Zn. The concentrations of available hypothetically toxic trace elements (Cd, Cr, and Pb) were below the detected limit of the AAS unit. This is also identified for Ni. The soil texture is coarse and relatively has low water holding capacity. Also, the soil had a slightly alkaline reaction and relatively low salinity (ECe = 3.10 dS m−1). These data point out, in general, that the used soil is infertile.

### Irrigation water

Samples of water were collected monthly, during the plant growth period from El-Mahmoudiya Canal (FW) and from the Western Wastewater Treatment Plant of Alexandria Governorate (TWW).

#### Plants

After 70 days from seed sowing, basil and oregano plants were harvested one cm the above soil surface, washed with tap water then by distilled water. The fresh weight was measured. Samples of plants were oven-dried at 65°C for 24 hrs., ground in a stainless-steel mill and preserved for analysis (Chapman & Pratt, 1961). Another plant samples were kept fresh and preserved for essential oils determinations.

### Analysis

#### Soils

The soil was analyzed for the determination of pH, EC, total carbonate, OM, total N, and the amount of available P, K, Cu, Fe, Mn, Zn, Cd, Cr, Pb, and Ni, and for the determination of particle size distribution (sand, silt, and clay), bulk density, and water holding capacity (Black, 1965).

#### Water

The major quality values of irrigation waters were determined to agree with APHA (1998). The concentrations of Mg2+ and Ca2+ were measured by Na2EDTA method and those of K+ and Na+ by flame photometer (Chapman & Pratt, 1961) and the value of SAR was calculated (Ayers & Westcot, 1989). Total Coliform (TC) was counted by the Lauryl Tryptose Broth Technique and the Multiple Tube Fermentation approach was utilized for counting coliform bacteria (APHA, 1998).

#### Plants

One-gram oven-dried plant material was subjected to wet digestion in H2SO4/H2O2 (Jones, 1989) and the concentration of N was measured by the Kjeldahl method, P was estimated colorimetrically, and K by the flame photometer (Chapman & Pratt, 1961). Perkins Elmer 2380 Atomic Absorption Spectrometer quantitatively quantified Cu, Fe, Mn, Zn, Cd, Cr, Pb, and Ni concentrations (Chapman & Pratt, 1961; Black, 1965). To determined essential oils contents and composition; fifty grams of fresh leaves and 250 ml distilled water were placed in one-liter volume round bottom boiling flask (modified Clevenger type apparatus) and boiled using an electric sand path for 3 hrs. (Asta, 1968; Charles & Simon, 1990). The quantity of oil was measured and expressed as ml 100 g−1 dry matter. The obtained oil was then kept in a silica vial in the cooler at 2°C in the dark for chromatographic analysis. The resolution and
identification of essential oil constituents were achieved using the GLC technique according to Charles and Simon (1990). The oil was analyzed at isothermal and programmed temperature. The fragments were identified by matching the relative retention time (calculated to linalool) with those of the authentic samples analyzed under similar conditions and by enrichment technique. The working conditions were: Pu 4500 GC "Philips" column, 10% DEGC "s-st" packed column, flame ionization detector (FID), column temperature from 70 to 190 °C programmed as follows: isothermal increase at the rate of 4 °C min⁻¹ and 15 min constant at 190 °C, injection port temperature was 240 °C, detector temperature was 280 °C, the carrier gas is N flow at a rate of 30 ml min⁻¹. The flame gas was H flow at a rate of 30 ml min⁻¹, the airflow rate was 300 ml min⁻¹. Chart speed was 0.5 cm min⁻¹; the sample size was 5 μl essential oil. The attenuation was 64 × 10⁻². For the quantitative analysis of the essential oil, the peak wave method was employed. Computing integrator Philips system (Pu 4815) computing was used for measuring the peak area.

**Statistical analysis**

The objective of the statistical analysis was to investigate whether the evaluated parameters across different levels of irrigation water qualities were similar or varied significantly. The analyses started with using Shapiro tests’ statistics with a Lilliefors significance level for testing normality, then multivariate analysis of variance (MANOVA) was used for the parameters that fulfilled its basic assumptions (normality and homogeneity of variances). These parameters are shoot yield, flowering shoot yield, essential oil yield, essential oil percentage, and some element contents in leaves of basil and oregano (N%, P%, K%, Cu mg kg⁻¹, Fe mg kg⁻¹, Zn mg kg⁻¹).

**Results and discussion**

**Water characteristics**

The analysis of the two categories of irrigation water types revealed significant variations in their composition (Table 2). The analyzed elements in TWW showed a higher concentration of TDS, NH₄⁻-N, NO₃⁻-N, Total N, Total P, Total Cu, Fe, Mn, Zn, and Fecal Coliform than in FW. The high concentration TDS of treated wastewater was due primarily to the high contents of chloride, sulfate, sodium, calcium, magnesium (not mentioned in Table 2) and the K also assisted in raising the TDS but to a minor amount (Bahón et al., 2011). In general, as total dissolved solid increased in the TWW, the probability of the problems for certain soils, and cropping due to continuous irrigation by this water increased. Under such environments, adequate drainage is essential to allow a continuous penetrate of saltwater under the root zone. Thus, long-period reuse of wastewater for irrigation is not generally possible without satisfactory drainage (Oster & Grattan, 2002; Rhoades, 1999).

Applying TWW, the mean SAR (Table 2) was within the limits of the FAO-approved water quality restriction on agriculture (Ayers & Westcott, 1989). These depositions on plants can damage the structure of the soil by decrease both percentage of soil water and soil aeration. If the percentage of infiltration is significantly reduced, crops cannot be fed for healthy growth. The permeability difficulty usually occurs within a few centimeters of the soil surface and is mainly linked to a relatively high concentration in sodium or very low calcium in this soil area. (Ayers & Westcott, 1989). In the SAR monitoring, the rate of infiltration increases as salinity increases or decreases as salinity decreases. SAR and TDS should, therefore, be used for grouping to test the potential permeability issue (Pedroso, Kalavrouziotis, Alarcon, Koukoulakis, & Asano, 2010). In both, TWW and FW the pH was slightly alkaline (Table 2). Partly treated wastewater for crop irrigation is a source of many pathogens, such as bacteria, viruses, protozoa, and nematodes, which is the root of numerous diseases. (Elsockary & Abukila, 2014). In the current study, use Fecal Coliform enumeration was employed to scale the microbiological quality of the used TWW. Both Fecal Coliforms and E. Coli are demonstrators of effluent contamination (Salgot, Huertas, Weber, Dott, & Hollender, 2006). E. Coli is the fragment of the cluster of Fecal Coliform and the most prevalent species of the Fecal Coliform group. The E. Coli predominates in the human digestive tract and on this basis, it can be considered one of the best available fecal contamination pointers (Molleda, Blanco, Ansola, & de Luis, 2008). Worldwide, many guidelines are mentioning to wastewater reuse that recommend E. Coli as a fecal indicator (NRMM-EPHC-AHMC, 2006), while others recommend Fecal Coliforms to be the characteristic fecal contamination indicator (Marcos Do Monte, 2007; USEPA, 2004). Meanwhile, E. Coli is over 90% of Fecal Coliforms (Dufour, 1977). The Fecal Coliforms concentration in the used TWW was 470 MPN 100 ml⁻¹, which did not surpass the bounds of use restriction recommended by the World Health Organization (>1000 MPN 100 ml⁻¹) (Cairncross & Mara, 1989), while the counting in FW was 10 MPN 100 ml⁻¹ (Table 2).

**Plant growth characters**

The analysis of variance applied to the growth parameters and quality index pointed to a significant interdependence between the irrigation water (FW, TWW) and the basil and oregano indicating that the response to water treatments differed between species. Four
statistics parameters are listed in Table 3 (Pillai’s Trace; Wilks’ Lambda, Hotelling’s Trace, and Roy’s Largest Root) to accomplish the multivariate test. As all P values (value in the “Sig.” column, Table 3) are below 0.05 of the four previous statistics parameters, therefore there was a significant effect of the irrigation water (FW and TWW) and plant species (basil and oregano) on one or more tested variable of plant growth characters (shoot yield, essential oil yield, flowering shoot yield, and essential oil percentage; and element contents in plant leaves).

Tests between-subject effects (Table 4) specify a significant difference (p < 0.05) between the two types of irrigation water due to the difference between shoot yield, flowering shoot yield, essential oil yield, essential oil percentage; and some element contents (N %, P%, Cu mg kg⁻¹, Fe mg kg⁻¹, Mn mg kg⁻¹), while there is no significant difference (p > 0.05) in essential oil percentage, K%, and Zn mg kg⁻¹. On the other hand, there is a significant difference (p < 0.05) between the two types of plants on shoot yield, flowering shoot yield, essential oil yield, essential oil percentage; N% and Zn mg kg⁻¹ contents in plant leaves and no significant difference on P%, K%, Cu mg kg⁻¹, Fe mg kg⁻¹ and Mn mg kg⁻¹ contents in plant leaves.

No mortality was detected in basil or oregano in any irrigation treatment. There is a significant increase in both the shoot yield and flowering shoot yield of basil and oregano plants as a result of irrigation by TWW as compared to those irrigated by FW with values of the relative increase of 23.89 and 19.00% for shoot yield of basil and oregano, respectively. The flowering shoot yield also increased due to irrigation by TWW as compared to that of FW irrigation (Table 5) with values of the relative increase of 18.18 and 14.58% for basil and oregano, respectively (Figure 1).

The essential oil percentages in leaves of basil and oregano were higher in plants irrigated by TWW than in those irrigated by FW with values of the relative increase of 8.33 and 7.14% for basil and oregano, respectively. These results indicated a high growth performance of plants irrigated by TWW than those irrigated by FW. This could be explained as a result of the presence of higher concentrations of N, P, and micronutrients in TWW than in FW, which indicates that TWW has higher nutrients supply than FW. In the same sense, irrigation low or poor fertile soil with TWW can significantly improve the growth of the aromatic or medicinal plant when grown in such soil. In concern of essential oil yield, there is a parallel trend with the data of both shoot yield and flowering shoot yield (Table 5), with relative increases of 86.21 and 53% for basil and oregano, respectively. A similar trend could be observed with values of essential oil percentage for both basil and oregano, which were 8.33 and 7.14% respectively (Figure 1).

These data show the significant positive effect on the Shoot yield, flowering shoot yield, and essential oil yield in those two aromatic plants when irrigated by TWW as compared to irrigation by FW. It is also clear that the essential oil percentage in leaves of oregano was higher than in those of basil, whether irrigated by FW or TWW. It was reported that the levels of essential oil percentage in leaves of oregano varied between 0.9 and 1.0% (Bernstein et al., 2009), while it was recorded as 0.66% in leaves of basil (Darvishi et al., 2010). The use of secondary treated wastewater for

| Effect                  | Test           | F       | Sig. |
|-------------------------|----------------|---------|------|
| Irrigation treatment    | Pillai’s Trace | 2.811E3*| 0.000|
|                         | Wilks’ Lambda  | 2.811E3*| 0.000|
|                         | Hotelling’s Trace | 2.811E3*| 0.000|
|                         | Roy’s Largest Root | 2.811E3*| 0.000|
| Plant species           | Pillai’s Trace | 1.393E3*| 0.000|
|                         | Wilks’ Lambda  | 1.393E3*| 0.000|
|                         | Hotelling’s Trace | 1.393E3*| 0.000|
|                         | Roy’s Largest Root | 1.393E3*| 0.000|

a = Exact statistic

| Parameters              | Irrigation Water | Plant species |
|-------------------------|------------------|---------------|
|                         | Sig.     | F       | Sig.     | F       |
| Shoot yield             | 0.000    | 46.797  | 0.000    | 25.566  |
| Flowering shoot yield   | 0.005    | 11.481  | 0.005    | 11.481  |
| Essential oil yield     | 0.000    | 166.620  | 0.000    | 166.497  |
| Essential oil percentage| 0.151   | 2.328   | 0.000    | 2.81698  |
| N%                      | 0.014    | 8.030   | 0.000    | 151.701  |
| P%                      | 0.001    | 18.511  | 1.000    | 0.000    |
| K%                      | 0.107    | 3.006   | 0.445    | 0.621    |
| Cu mg kg⁻¹              | 0.037    | 5.420   | 0.509    | 0.461    |
| Fe mg kg⁻¹              | 0.001    | 18.349  | 0.238    | 1.531    |
| Mn mg kg⁻¹              | 0.013    | 8.325   | 0.032    | 0.015    |
| Zn mg kg⁻¹              | 0.273    | 1.309   | 0.023    | 6.627    |

| Irrigation water | Shoot yield | Flowering shoot yield | Essential oil yield | Essential oil percentage |
|------------------|-------------|-----------------------|---------------------|--------------------------|
| Basil plant      |             |                       |                     |                          |
| FW               | 3390        | 330                   | 0.58                | 0.48                     |
| TWW              | 4200        | 390                   | 1.08                | 0.52                     |
| Oregano plant    |             |                       |                     |                          |
| FW               | 3000        | 288                   | 2.00                | 1.12                     |
| TWW              | 3570        | 330                   | 3.06                | 1.20                     |

Table 3. Multivariate Test.

Table 4. Summary of two-way MANOVA statistics for parameters studied.

Table 5. The average values of shoot, flowering shoot (kg ha⁻¹) and essential oil yield (ml 100 g⁻¹ DM) and essential oil percentage of basil and oregano plants.
irrigation of coriander (Coriandrum sativum L) had increased the biological yield, flowering shoot yield and essential oil percentage as compared to irrigation by well or canal water (Rahimi, Farahani, & Darvishi, 2010). The volatile oil concentration in peppermint grown in sandy soil significantly increased as a result of irrigation by sewage effluent than irrigation by freshwater (Kotb et al., 2012) and this increase in oil percentage in plant leaves may be attributed to the sufficient amounts of macro-and micronutrients in sewage effluent as compared to those in FW.

**Element contents in plant leaves**

The concentrations of N and P in leaves of plant irrigated by TWW were significantly higher than in those irrigated by FW (Table 6). The relative increases in the concentrations of N, P, and K in leaves of basil were 10.83, 20.00, and 10.53%, respectively, and in those of oregano was 11.92, 20.00, and 9.09%, respectively (Figure 2). The highest values of the relative increase were that of P, which was 20.00% for both basil and oregano, while lower values were recorded for N and K with the two plants. Also, the values of the relative increase in the concentrations of Cu, Fe, Mn, and Zn in leaves of basil due to TWW irrigation were 9.52, 18.25, 15.77 and 5.47%, respectively and in those of oregano were 11.71, 17.92, 14.29 and 6.09%, respectively (Figure 2). These data specify that the concentrations of micronutrient elements in both basil and oregano leaves are dependent on their concentrations in both soil and irrigation water. Therefore, both basil and oregano leaves had been enriched with Fe and Mn when irrigated by TWW as compared to FW irrigation. The accumulations of Cd, Cr, Pb, and Ni in the leaves of both plants were extremely low to the extent that they cannot influence the growth of these two-plant species. That due to the accumulations of Cd, Cr, Pb, and Ni in the plant leaves are dependent on their available amount in the soil. Since the used soil is calcareous, of high pH and high carbonate percentage (Table 1) the Cd, Cr, Pb, and Ni are subjected to precipitation due to combination with carbonate and hydroxyl anions forming carbonate and hydroxyl complexes and precipitate, which are unavailable to be taken up, by plant roots and translocation to plant leaves (Blaylock & Huang, 2000).

It has been concluded that aromatic plants can grow normally without any significant morphological and physiological symptoms as they are almost tolerant of Cd, Pb, and Ni concentrations in soil and most of the accumulated elements are stored in the roots with low translocation rate to the leaves (Affholder et al., 2013; Lal et al., 2013). WHO (1998) recommended the concentration levels of 0.3 mg kg⁻¹

![Figure 1. The relative increase of plant growth character of basil and oregano plants due to irrigation by TWW.](image-url)

| Element | basil | oregano |
|---------|-------|---------|
| FW | TWW | FW | TWW |
| N% | 5.54 | 6.14 | 3.44 | 3.85 |
| P% | 0.25 | 0.30 | 0.25 | 0.30 |
| K% | 2.85 | 3.15 | 2.75 | 3.00 |
| Cu mg kg⁻¹ | 11.55 | 12.65 | 11.10 | 12.40 |
| Fe mg kg⁻¹ | 55.52 | 65.65 | 53.00 | 62.50 |
| Mn mg kg⁻¹ | 34.55 | 40.00 | 35.00 | 40.00 |
| Zn mg kg⁻¹ | 25.60 | 27.00 | 28.75 | 30.50 |
| Cd mg kg⁻¹ | nd | 0.02 | nd | 0.02 |
| Cr mg kg⁻¹ | nd | 0.01 | nd | 0.01 |
| Pb mg kg⁻¹ | nd | 0.02 | nd | 0.01 |
| Ni mg kg⁻¹ | nd | 0.03 | nd | 0.03 |

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DM for Cd and 10 mg kg\(^{-1}\) DM for Pb in leaves aromatic plants (WHO, 1998).

**Essential oil components**

Table 7 showed wide variations between the percentages of essential oil components in leaves of basil plant irrigated by FW and TWW. Irrigation by TWW increased the percentage of α-Pinene, Camphor, β-Pinene, Myrcene, 1,8 Cineole, p-Cymene, d-Camphor, Bornyl acetate, and Eugenol, in the same times; Linalyl acetate, β-Caryophyllene, Methyl chavicol, α-Terpineol, and Methyl eugenol had been decreased. The highest values of relative variation, due to TWW irrigation, belonged to p-Cymene, d-Camphor and Bornyl acetate with values of the relative increase of 425.00, 719.44 and 209.52 %, respectively, while those of Linalyl acetate and Methyl eugenol with values of relative decrease of 61.57 and 83.42% respectively (Figure 3). Elsokkary, Saleh, Abdel-Salam, and Abdel-Salam (1998) found that irrigating basil plant with nutrient solution containing 4 mg Cd/l increased the percentage of Linalol from 10.14 to 11.89% and Eugenol from 5.04 to 6.60% and decreased the percentage of Methyl chavicol from 13.48 to 11.17% concerning the control treatment (Elsokkary et al., 1998).

Table 8 showed wide variations between the percentages of the different constituents of the essential oil in leaves of the oregano plant as influenced by FW and TWW irrigation. As a result of TWW irrigation, the percentages of Sabinene, Myrcene, α-Terpineol, Limonene, δ- Terpinene, p-Cymene, Linalyl acetate, and Terpinen-4-ol had increased while those of α-Pinene, β-Phellandrene, Terpinolene, Linalool, β-Caryophyllene, Methyl

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**Figure 2.** The relative increase of element contents in leaves of basil and oregano leaves due to irrigation by TWW.

| Peak No. | Component       | Group                  | FW | TWW |
|----------|-----------------|------------------------|----|-----|
| 1        | Unknown         |                        |    |     |
| 2        | α-Pinene        | Monoterpene hydrocarbons | 2.10 | 3.16 |
| 3        | Camphene        | Monoterpene hydrocarbons | 0.72 | 1.00 |
| 4        | β-Pinene        | Monoterpene hydrocarbons | 0.49 | 0.66 |
| 5        | Myrcene         | Monoterpene hydrocarbons | 1.10 | 1.65 |
| 6        | 1,8 Cineole     | Oxygenated monoterpenes | 0.65 | 0.95 |
| 7        | P-Cymene        | Monoterpene hydrocarbons | 9.25 | 10.35 |
| 8        | d-Camphor       | Oxygenated monoterpenes | 0.40 | 2.10 |
| 9        | Unknown         |                        | 0.16 | 0.95 |
| 10       | Linalool        | Oxygenated monoterpenes | 2.10 | 4.50 |
| 11       | Bornyl acetate  | Oxygenated monoterpenes | 2.55 | 0.98 |
| 12       | Linalyl acetate | Oxygenated monoterpenes | 7.36 | 6.98 |
| 13       | β-caryophyllene | Sesquiterpene hydrocarbons | 2.85 | 2.10 |
| 14       | Unknown         |                        | 11.84 | 9.56 |
| 15       | Methyl chavicol | Oxygenated monoterpenes | 14.25 | 9.50 |
| 16       | α-Terpineol     | Oxygenated monoterpenes | 5.55 | 0.92 |
| 17       | Methyl eugenol  | Oxygenated monoterpenes | 4.95 | 5.54 |
| 18       | Eugenol         | Oxygenated monoterpenes | 4.70 | 6.10 |
Pinene, 

Figure 3. The relative variation of essential oil components of basil leaves due to irrigation by FW or TWW.

Table 8. The main essential oil components of oregano due to irrigation by FW or TWW.

| Peak No. | Component     | Group               | FW    | TWW    |
|----------|---------------|---------------------|-------|--------|
| 1        | α-Pinene      | Monoterpene hydrocarbons | 1.88  | 1.75   |
| 2        | β-Pinene      | Monoterpene hydrocarbons | 0.44  | 0.40   |
| 3        | Sabine        | Monoterpene hydrocarbons | 7.36  | 9.50   |
| 4        | Myrcene       | Monoterpene hydrocarbons | 2.00  | 2.05   |
| 5        | α-Terpineol   | Monoterpene hydrocarbons | 3.89  | 5.70   |
| 6        | Limonene      | Monoterpene hydrocarbons | 2.00  | 2.40   |
| 7        | β-Caryophyllene | Monoterpene hydrocarbons | 1.80  | 2.10   |
| 8        | δ-terpinene   | Monoterpene hydrocarbons | 8.10  | 9.50   |
| 9        | P-cymene      | Monoterpene hydrocarbons | 1.10  | 2.00   |
| 10       | Terpinolene   | Monoterpene hydrocarbons | 7.46  | 3.36   |
| 11       | Unknown       |                     | 12.85 | 8.55   |
| 12       | Linalool      | Oxygenated monoterpenes | 2.90  | 2.10   |
| 13       | Linalyl acetate| Oxygenated monoterpenes | 8.85  | 14.15  |
| 14       | Terpinen-4-ol | Oxygenated monoterpenes | 15.38 | 15.50  |
| 15       | β-Caryophyllene | Sesquiterpene hydrocarbons | 7.35  | 3.85   |
| 16       | Methyl chavicol | Oxygenated monoterpenes | 1.23  | 1.10   |
| 17       | α-Terpineol   | Oxygenated monoterpenes | 1.52  | 1.25   |

Conclusions

This study aimed to evaluate the responses of two aromatic plants (basil and oregano) grown in calcareous soil to irrigation by treated wastewater (TWW) concerning growth characters and essential oil contents. The obtained results showed higher biomass yield (Table 5), macro- and micronutrient contents in plant leaves (Table 6) oil yield, and percentage (Table 7 and 8) in plants irrigated by TWW than in those irrigated by FW. This is due to the highest nutritive value of TWW than that of FW (Table 2). Basil and oregano differed in their response to TWW irrigation since the relative increase in shoot yield was higher in basil (23.89%) than in oregano (19.00%), and also those of flowering shoot yield was 18.18% and 14.58 respectively. Our result showed an increase in oil percentage in leaves of plants irrigated by TWW more than in those irrigated by FW, which may be
attributed to the sufficient amount of macro-and micro-nutrients in TWW as compared to those in FW. These results agree with those found by Kotb et al. (2012) on peppermint irrigated by wastewater or freshwater. Rahimi et al. (2010) found also that the essential oil yield, biological yield, flowering shoot yield, and essential oil percentage increased from 10.2 to 12.0 kg ha⁻¹, 4900 to 5400 kg ha⁻¹, 410 to 500 kg ha⁻¹, and 0.48 to 0.66% as a result of irrigation coriander by treated wastewater as compared to FW. In the present study essential oil yield, shoot yield, flowering shoot yield and essential oil percentage increased from 0.957 to 2.107-liter ha⁻¹, 3390 to 4200 kg ha⁻¹, 330 to 390 kg ha⁻¹ and 0.48 to 0.52% in basil and from 2.880 to 5.049-liter ha⁻¹, 3000 to 3500 kg ha⁻¹, 288 to 330 kg ha⁻¹ and 1.12 to 1.20% in oregano, respectively, as a result of irrigation by TWW as compared to those irrigated by FW.

The relative increase of essential oil yield was higher in basil (86.21%) than in oregano (53.00%) and also that of essential oil percentage was higher in basil (8.33%) than in oregano (7.14%). These data indicate the higher response of basil to TWW irrigation than oregano under the used experimental conditions.

The percentage of essential oil components showed a different response to both TWW irrigation and plant species. While α-Pinene, Camphor, β-Pinene, Myrcene, 1,8 Cineole, p-Cymene, δ-Camphor, Bornyl acetate and Eugenol in basil showed the positive response, Linalyl acetate, β-Caryophyllene, Methyl chavicol, α-Terpineol, and Methyl eugenol showed the negative response (Figure 3). In oregano, the percentages of Sabinene, Myrcene, α-Terpine, Limonene, δ-Terpine, p-Cymene, Linalyl acetate, and Terpinen-4-ol had been increased while those of α-Pinene, β-Phellandrene, Terpinolene, Linalool, β-Caryophyllene, Methyl chavicol, and α-Terpineol had been decreased (Figure 4). The variations in the percentage of components of essential oil could be explained based on both the nutritional value of TWW and plant species (Bensabah, Lamiri, & Naja, 2015; Khalifa at al., 2011; Kotb et al., 2012; Rahimi et al., 2010). These data indicate additions of significant beneficial values from the treated wastewater to the low fertile calcareous soil to produce the valued aromatic plants.

The water shortage, combined with the growing demand due to ongoing urban growth and the high demand from intensive agriculture have necessitated the irrigation of treated wastewater. It must therefore also ensure that concerns relating to the environment and public health are fully addressed. We systemati-cally analyzed the benefits and risks associated with TWW irrigation by combining results from field research. The study has shown that TWW can provide a major economic advantage by using the treated wastewater for growing oil crops and heavy metals uptake by root systems be influenced by the bioavailability of the metal in the water phase. The heavy metals absorption mechanism by plant roots can be affected by various factors. The roots’ uptake performance can be significantly reduced with knowledge of these factors: (i) The Plant Species. Plants species or varieties are screened, and those that have a hyperaccumulating property to heavy metals must be ignored; (ii) Soil properties. High pH and high carbonate percentage heavy metals are subjected to precipitation due to combination with carbonate and hydroxyl anions forming carbonate and hydroxyl

![Figure 4](image-url) The relative variation of essential oil components of oregano leaves due to irrigation by FW or TWW.
complexes and precipitate, which are unavailable to be taken up, by plant roots and translocation to plant leaves (Blaylock & Huang, 2000).

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Disclosure statement

No potential conflict of interest was reported by the authors.

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