Effect of short-time irrigation with industrial poultry wastewater on young olive tree (Olea europaea L. cv. Chemlali) growth parameters and the chemical composition of leaves

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Abstract

The purpose of this study was to evaluate the short-term irrigation effect with industrial poultry wastewater on young olive trees (*Olea europaea* L. cv. *Chemlali*). Industrial poultry wastewater can be considered as a bio-fertilizer due to its richness in nutritive elements (SO_4^{2-}, HCO_3^-, total nitrogen and K^+). The physico-chemical analysis of wastewater showed a high concentration of TSS, COD, BOD, COT, NO_3^-, and conductivity. Measurements indicated that poultry wastewater enhanced plant growth, leaves dry matter, and ashes in comparison with tap water, as well as poultry wastewater diluted with tap water, however, a decrease in total soluble sugars (glucose and fructose) was detected in leaves. The determination of fatty acid profile of young olive trees leaves irrigated with poultry wastewater showed richness on saturated fatty acids in comparison with mono- and poly-unsaturated ones. In addition, oleic acid ($C_{18:1}$) presented the lowest content in leaves of trees irrigated with poultry wastewater irrigation. According to those results, poultry wastewater lends itself to being a hydric alternative and at the same time a source of nutrients that can help fill the water deficit in semi-arid countries and avoid costly waste disposal for slaughterhouses.

1. Introduction

The cultivation of olive trees is widespread in the Mediterranean basin, especially in Spain, Italy, and Tunisia. As for Tunisian territory, the cultivated area covers 30% of agricultural land (COI, 2012). The center of Tunisia is the main producer of olive oil with 87% of total national plantations (Ministère de l'Agriculture, 2006), which are represented by *Chemlali* as the major cultivated variety (cv.) (Khlif et al., 2002).

Apart from the importance of olive oil production, a great interest is focusing on olive leaves due to their richness in bioactive molecules (polyphenols) and organic compounds (carbohydrates and lipids). Indeed, olive leaves and their extracts have been considered since old times as a traditional remedy for many diseases (hypertension, inflammatory disorders, bacterial infections, and diabetes) (Boukef, 1986).

In the Mediterranean countries, the olive tree is a rain-fed culture (Lavee, 1996); (Connor and Fereres, 2005). Aside from its tolerance to drought (Wang et al., 2018) and salinity (Chehab et al., 2018), it is considered as one of the most adapted species to the semi-arid climate (Gimenez et al., 1996). To increase olive production, irrigation is recommended by the agricultural authorities, in particular, when the water supply has already shown a favorable response, as in the case of olive culture (Chiraz, 2013). However, the freshwater shortage is one of the main environmental problems, particularly in arid and semi-arid regions, so that wastewater reuse is becoming a recommended alternative to face water scarcity.

In Tunisia, irrigation needs are considered the main source of water consumption, accounting for up to 80% of total national water potential. In recent years, government and research centers have engaged in studies on alternative ways of water supply (Guizani et al., 2019) as well as on wastewater reuse (Horchani, 2007). Fodder (alfalfa, sorghum, berseem, etc.) are the main crops irrigated with treated wastewater (45.3%), followed by fruit trees (olives, citrus, grapes, peaches, pears, apples, pomegranates, etc.) (28.5%), cereals (22.4%), and industrial crops (sugar beet) (3.8%) (Bahri, 2001).

Several studies focused on the effect of wastewater reuse on olive tree irrigation (Batarseh et al., 2011) (Bedbabis et al., 2015). Ayoub et al. (2016) found that irrigation with reclaimed wastewater for four successive years showed a significant increase in annual shoots length as compared to rain-fed treatment. Similar results were observed with short irrigation (four months) of young "*Chetoui*" cv. with treated wastewater, in particular, physiological performances (photosynthesis activity, soluble sugars, and plant growth) showed increasing trends (Ben Hassena et al., 2018). A study conducted in the central-eastern part of Tunisia proved that manual irrigation of *Chemlali* cv. with dairy wastewater showed improvement on leaf area, flowering parameters, and total roots polyphenols, However, shoot elongation, total chlorophyll, and total leaves polyphenols increased significantly under tap-water irrigation regime (Sdiri et al., 2018). According to those authors, drip irrigation with dairy wastewater did not affect morphological growth parameters (plant height, trunk diameter, and shoots number per tree) in comparison with tap water irrigation (Sdiri et al., 2020).

Poultry industries collect wastewater from various production activities: (i) scalding for feather removal, (ii) bird washing before and after the evisceration process, (iii) chilling, (iv) cleaning and sanitizing equipment and facilities, and (v) cooling of mechanical equipment (Mittal, 2004). On average, these activities consume water as high as 26.5 L per bird (Kosseva and Webb, 2020). The concentrations of poultry wastewater physico-chemical parameters depend on the separation degree of blood, fat, manure, and undigested stomach contents from the effluent stream (Mittal, 2004). The characterization of poultry wastewater shows high macronutrients levels (i.e. nitrogen and phosphorus), suspended solids, oil, and fat (Amorim et al., 2007). Therefore, the direct discharge into the environment is considered as a serious environmental problem that brings about eutrophication phenomenon (Yaakob et al., 2018). On the contrary, these macronutrients could be conveniently used for their fertilizing properties.

The general aim of the project is the valorization of the wastewater coming from an industrial poultry slaughterhouse to ascertain the opportunity for use in the irrigation of olive trees. Due to the abundance of micro- and macro-nutrients present in the poultry wastewater, the specific study was aimed at evaluating the effects of short-period irrigation on the olive trees cv. *Chemlali* growth parameters and the chemical composition of the leaves, as marker organs of the plant physiology and its health state.

2. Materials And Methods

2.1. Area of study and irrigation treatments

The experiment was conducted in the government of Mahdia, in the Middle East of Tunisia (latitude 35° 28' 11" N and longitude 10° 57' 23" E). One-year-old olive trees (*Olea europaea* L.) cv. *Chemlali* were cultivated in clayey-sandy soil from 0 to 40 cm of depth and balanced for 40-60 cm. Olive trees were drip-irrigated for one year from September 2018 to August 2019. The poultry industry was located beside the experimental field.

The field was divided into three lots of 15 olive trees each. Two treatments were established: the first lot was irrigated with poultry wastewater (PWW), the second lot with PWW diluted with tap water 50:50 (PWTWW), while tap water (TW) was used as for control trees. Each tree was irrigated with 14 L of water and the irrigation frequency depended on the pluviometry.

2.2. Wastewater physico-chemical analysis

Once a week over 4 weeks, PWW was sampled each hour from 8:00 to 11:00 from the outlet of the industry. Samples were transported to the laboratory in plastic bottles and immediately analyzed. Total suspended solids (TSS), chemical oxygen demand (COD), biochemical oxygen demand (DOB), nitrates (NO_3^-), total organic carbon (TOC) and absorbable organically bound halides (AOX) were determined using a portable UV analyzer (Pastel UV, Secomam, Alès, France), pH and conductivity were also determined using Eutech instruments and Thermo Scientific™ Orion™ 2-Star Benchtop pH meter, respectively. All the parameters were measured in triplicate. It should be noted that the blood is collected before the effluent is discharged.

2.3. Mineral analysis

Mineral characterization of sulfate (SO_4^{2-}), bicarbonate (HCO_3^-), chloride (Cl^-), calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+) and potassium (K^+), was determined according to methods described by Naanaa and Susini (1988).

The total amount of nitrogen was determined using the Kjeldahl assay according to the standard method (AOAC, 2000).

2.4. Total fat in poultry wastewater determination

The total amount of fat in poultry wastewater was determined according to the method described by Bridoux et al. (1994); briefly, 40 mL of sample were acidified with hydrochloric acid 40 mL of hexane, and 20 mL of methanol were added. The mixture was centrifuged at 4000 rpm for 5 min. The supernatant containing hexane and lipids was recovered, evaporated to dryness using a rotary evaporator, and finally weighed.

2.5. Plant growth parameters

At the beginning (September 2018) and the end (September 2019) of the experiment, plant growth parameters, including height (cm), and trunk diameter (mm), were measured at 10 cm from the ground using a Vernier caliper. Five shoots length (cm) marked at the beginning of the experiment, and total twigs number were measured.

2.6. Olive leaves sampling and pre-treatment

After sampling, fresh olive leaves were transported to the laboratory in ice and dark inside plastic bags. Samples were gently cleaned with deionized water, freeze-dried, milled until fine powder, and finally stored at -20 °C until further analysis.

2.7. Determination of dry matter (DM), moisture, and ashes of leaves

Fresh olive leaves were placed in an oven at 105 °C ± 0.5 for 4 h. After drying, samples were placed in a desiccator for 30 min and finally weighed to determine DM and moisture content. As for ashes, dried samples were placed in a furnace oven at 550 °C for 2 h.

2.8. Carbohydrates extraction and analysis

Exactly-weighed 1 g of finely powdered leaves was introduced into a test tube and extracted with an aqueous formic acid solution (0.125% $v\ v^{-1}$), the suspension was kept in the dark under agitation on a 3D-shaker for 24 h followed by centrifugation at 1854 g for 10 min at room temperature. The extraction was repeated three times for each sample. The extracts were filtered using a syringe filter (0.2 µm, Nylon, Agilent).

The analysis of soluble sugars (glucose and fructose) was performed using an HPLC system (PU4180, Jasco Europe Srl, Cremella, Italy) equipped with a refractive index detector (RI4030, Jasco). Through an auto-sampler, 5 µL of each sample were injected onto an LC-column for sugars (Rezex RHM-Monosaccharide H^+, 300 × 7.8 mm, Phenomenex, Bologna, Italy) using a mobile phase flow of 0.5 $mL\ min^{-1}$. The elution occurred in isocratic mode using an acid aqueous solution (H_2SO_4 pH = 2.25)-acetonitrile 90:10. The samples were prepared and determined in triplicate. Chromatographic peaks were identified using retention times of pure standards. The quantification was carried out using a series of injections of standard solutions with known concentrations prepared under the same conditions as the samples. The quantification took place through external calibration.

2.9. Fat extraction and analysis

Fat extraction was carried out using the method described by Bahloul et al. (2014), with some minor modifications. Exactly-weighed 3.5 g of finely powdered dried olive leaves samples were introduced into a test tube and extracted twice with hexane (1:5 $w\ vol^{-1}$) under agitation on a 3D-shaker for 4 h at room temperature. The suspension was centrifuged (1465 g for 15 min) and the supernatant was separated and then evaporated to dryness using a rotary evaporator (R-114 Rotavapor, Büchi, Cornaredo, Italy), and finally the percentage yield was calculated.

Base-catalyzed transesterification was carried out on the total amount of lipids to determine FA concentrations using the method described by (Christie, 1982). Lipids were introduced into a glass tube, diluted to 400 µL with hexane and derivatized into fatty acids methyl esters (FAME) with 100 µL of potassium hydroxide in methanol solution (2 M). The tube was tightly closed and shaken on a vortex for 90 sec and then centrifuged at 1752 g for 5 min to speed up the layer separation. The FAME were contained in the upper organic phase.

A gas chromatograph (HP 6890 Series, Hewlett-Packard, Waldbronn, Germany) with a split/splitless injection port and coupled with a mass spectrometer (HP 5973 Mass Selective Detector, Hewlett-Packard, Waldbronn, Germany) was used for FAME determination. The GC system was equipped with a capillary column Mega-10 (100% cyanopropyl polysiloxane, 50 m × 0.25 mm ID, 0.20 mm film thickness, Mega snc, Legnano, Italy). The oven temperature was set at 110 °C, held for 1 min and then brought to 230 °C at 10 °C min^{-1} rate, and finally maintained at this final temperature for 2 min (15 min of analysis). The injector port, operating in split mode (split ratio 50:1), and the detector were both set at 250 °C. The carrier gas (ultrapure helium) was set at a constant flow of 1.5 mL min^{-1}.

The molecular fragmentation was obtained through electron ionization. The data were obtained in full-scan mode and the mass-to-charge ratio (m/z) was recorded between 33 and 450 with an ionization voltage of 70 eV. The ion source temperature was set at 230 °C and the quadrupole temperature at 150 °C. The chromatograms were acquired and processed through the Enhanced Chem Station software (G1701AA Version A.03.00, Hewlett Packard$^{©}$).

The peak identification included (i) the comparison of the peak retention times to those obtained with 37-component FAME Mix (Supelco) and (ii) the matching of the peak mass spectra with those present in the data system library dedicated to FA (Famedb23.l and Famedbwax.l; Agilent Technologies).

2.10. Chemicals

All the reagents and solvents were of AR grade and were purchased from authorized suppliers. Hexane, hydrochloric acid, methanol, potassium hydroxide (KOH), sodium chloride (NaCl), and anhydrous sodium sulfate (an. Na_2SO_4) were purchased from WVR Srl (Milan, Italy). Pure standard fatty acids (FAs) were purchased from Carlo Erba (Milan, Italy). Nonadecanoic acid methyl ester, used as internal standard, was purchased from Fluka (Milan, Italy) and prepared at 1.00% concentration (w vol^{-1}) in hexane. Deionized water was obtained through an $Elix^{3UV}$ purification system (Merck-Millipore, Milan, Italy).

2.11. Statistical analysis

Basic statistical treatments were performed using the Excel 2010 spreadsheet (Microsoft Office 2010 package, Microsoft®, Washington, USA). Differences between methods were evaluated using one-way ANOVA, after the verification of the normal distribution of the data set. When a significant effect ($p \leq 0.05$) was showed, comparative analyses were carried out by the post hoc Tukey’s multiple comparison test using STATISTICA software (v.11, StatSoft Inc., Tulsa, USA).

3. Results

The first aspect that was considered in this study was the assessment of the quality of the poultry company’s wastewater that was focused on the analysis of physico-chemical parameters, minerals and fat content.

3.1. Wastewater analysis

A thoroughly chemical and physico-chemical assessment was carried out over four weeks. The sampling plan included withdrawals taking place once an hour between 8:00 and 11:00 in the morning.

The mean values registered for PWW and PWTWW showed that all parameters by-far exceeded the thresholds imposed by the Tunisian law, except for pH and conductivity (Table 1). One-way ANOVA showed significant differences among the three water qualities (TW, PWW, and PWTWW). As expected, the lowest values were observed in TW for TSS, COD, BOD, TOC, and conductivity, while PWW showed the highest ones, and, finally, PWTWW presented intermediate values. As for pH, TW presented slight alkaline values (7.95), while PWW pH values shifted to slight acidity.

As for PWW, results showed that TSS, COD, BOD, TOC, and conductivity values were not significantly different between 08:00 AM and 09:00 AM. In addition, samples collected at 09:00 AM had lower concentrations of pollution’s chemical markers in comparison with the samples collected at different times. However, the same parameters measured only one hour later (10:00 AM) showed the highest concentrations for TSS, COD, BOD, TOC, and conductivity (Table 1).

3.2. Mineral and fat content in wastewater samples

PWW showed higher values of SO_4^{2-}, HCO_3^{-}, Na^{+}, Cl^{-}, total nitrogen and K^{+} in comparison with TW and PWTWW, while in TW samples water hardness (represented by the sum of Ca^{2+} and Mg^{2+}) was the only parameter with higher concentrations in comparison with PWW ones (table 2).

According to Tunisian discharge standards, total values by-far exceeded the threshold imposed by the national regulation, except Cl^{-}.

3.3. Plant growth parameters

The measurement of vegetative growth parameters after one year under drip system irrigation showed significantly different values for all parameters (table 3), except shoots length where the highest average value (12.14 cm) obtained with PWW irrigation system was associated to a very high standard deviation.

As for plant height and trunk diameter, significant differences were registered between the TW and PWW irrigation treatments. Olive trees irrigated with PWTWW showed intermediate values mostly closer to TW. Newly emerged shoots also increased significantly in trees irrigated with PWW as compared to PWTWW irrigated trees (table 3).

3.4. Physiological parameters content

The determination of physiological parameters content in young olive tree leaves showed a significant increase in DM and ashes in plants irrigated with PWW as compared to plants irrigated with TW and PWTWW (Table 4).

3.5. Carbohydrates analysis

The concentration of glucose in olive tree leaves showed significant statistical differences related to the different qualities of irrigation water (Table 5). Olive trees irrigated with TW presented the highest glucose concentration (99.09 g kg^{-1}), while plants irrigated with PWW showed the lowest one (46.73 g kg^{-1}). As for PWTWW irrigated plants, values were intermediate (64.76 g kg^{-1}). Regarding fructose, the highest concentration was registered in control lot (55.46 g kg^{-1}) as well, while PWW and PWTWW did not show significant statistical differences with each other.

3.6. Total lipid content and fatty acids profile

Olive leaves showed an abundance of saturated FA in comparison with monounsaturated and polyunsaturated FA (Table 6), and the major representatives were palmitic acid ($C_{16:0}$) (33.71 - 34.10 mg 100 mg_{FAT}^{-1}), α-linolenic acid ($C_{18:3}$) (25.52 - 27.97 mg 100 mg_{FAT}^{-1}), and oleic acid ($C_{18:1}$) (17.39 - 19.60 mg 100 mg_{FAT}^{-1}).

The FA composition of the leaf extracts did not show any significant statistical difference related to the different qualities of irrigation water, except for caproic acid ($C_{6:0}$), lauric acid ($C_{12:0}$), myristic acid ($C_{14:0}$), oleic acid ($C_{18:1}$), and linoleic acid ($C_{18:2}$). As for lauric acid ($C_{12:0}$) content, plants irrigated with PWTWW showed a very high statistical difference as compared to plants irrigated with TW and PWW.

Leaves samples from trees irrigated with TW showed higher content on linoleic acid ($C_{18:2}$) than PWW and PWTWW samples while regarding oleic acid ($C_{18:1}$), the lowest content was observed in PWW irrigated plants (17.39 mg 100 mg_{FAT}^{-1}).

4. Discussion

4.1. General strategy of the study

Wastewater represents a problem for the food industry to manage. Indeed, whenever wastewaters exceed the limits imposed by the into-force law for critical parameters, they must be subjected to reclamation treatment or be destined to specialized companies for proper disposal. However, these treatments are very expensive, therefore the food companies try and seek alternative and safe ways to use them without being obliged to resort to remediation. Furthermore, in a worrying scenario of climate change, the agriculture practiced in arid and semi-arid areas, such as Tunisia, represents a serious concern. The olive tree currently needs irrigation treatment, therefore the opportunity of using wastewater coming from food-chain plants would represent valuable support for cultivation. For this purpose, it is strictly necessary to verify that these wastewaters do not cause problems to the growth of plants and the plant physiology.

The wastewater coming from the poultry industry was first analyzed to assess the profile of those physico-chemical parameters that indicate the abundance of organic matter (COD, BOD, TOC, AOX), nitrates, aside from pH and conductivity. Then, the contents of specific ions and fat in the wastewater were analyzed. After these first evaluations overtime on the wastewater, the vegetative parameters of the plants subjected to the different irrigation regimes were assessed and finally, leaf samples were collected to evaluate their composition in primary constituents, as the leaves are representative of the physiological activity and state of health of the whole plant.

4.2. Considerations on wastewater composition and relationship with soil and tree requirements

PWW physico-chemical parameters showed variation overtime in organic and inorganic loads, whose trends follow the chronology of the different production chain operations. According to (Zhang et al., 1997), industrial poultry wastewater is mainly composed of proteins (35%), lost from the carcass, that bring about high levels of COD and BOD. The slaughtering process and the periodic cleaning of residual particles are the main sources of pollution, with the organic matter being the main pollutant (Awang et al., 2011).

Many authors studied the physico-chemical parameters of poultry effluents. Poultry effluents show common values of pH and BOD that range between 6.1-7.1 and 4,500-12,000 mg L^{-1}, respectively (Mercado, 1995). In the present study, pH values of PWW were around 6.0, thus falling within the limits for crop irrigation ranged from 6.5 to 8.0 (Pescod, 1992). The wastewater collected from a poultry slaughterhouse factory in Malaysia showed average values (mg L^{-1}) of TSS, COD, BOD, NO_3^-, and pH of 589, 1,301, 875, 116, 6.6, respectively (Aziz et al., 2018). The values of TSS, COD, and BOD were lower than those obtained in the present study. This may be attributed to the partial physical treatment (the screening of internal organs and feathers) before discharging.

A study conducted in a Mexican poultry slaughterhouse showed that only pH values were similar to the results obtained in the present study. However, mean values of COD and BOD (7333 and 5500 mg L^{-1}, respectively) were by-far higher as compared to the present results, while conductivity average values (117 µS cm^{-1}) were very much lower (Chávez et al., 2005).

Several studies reported concentration of oils and fat in poultry slaughterhouse effluents between 125 and 306 mg L^{-1} (Chávez et al., 2005) (De Nardi et al., 2008) (Del Nery et al., 2016). These values are much lower than those found in the present study. However, the amount of fat in the wastewater coming from a turkey meat processing plant was 950 mg L^{-1}, which is comparable to the fat quantity in the wastewater (Makhlay et al., 2018).

Few studies evaluated the impact of poultry wastewater irrigation on soil characteristics. Zhang et al. (2018) concluded that the single time irrigation with poultry wastewater during the growth season of maize increased soil nitrogen concentration, however, N levels remained within safety limits, with no effect on the concentration of phosphorous. Furthermore, the single time irrigation regime stimulated the decomposition of organic matter by increasing soil C storage. In addition, this practice did not present any environmental risk on the quality of groundwater.

Like most other species of the vegetal kingdom, the olive tree is composed of about 20 mineral elements that are essential for plant development and production (Marschner, 2011). As far as our knowledge, there are only a few data concerning the mineral analysis of poultry wastewater. According to Yaakob et al. (2018) and Maizatul et al. (2018), chicken wastewater showed important amounts in total nitrogen and phosphorus. Olive tree fertilization is recommended during the three first years to boost root growth (Palese et al., 1997). Aside from amino acids and protein synthesis, nitrogen compounds play an important role in the physiological processes of the olive tree, such as the increase of photosynthetic activity and chlorophyll levels, vegetative growth, and the enhancement of shoots length. The presence of nitrogen compounds increases the ability of absorption of other mineral elements (Stan and David, 2007). Nitrogen deficiency causes a reduction in growth (Scollo et al., 2018), similarly to phosphorus which is considered as a plant growth regulator, which contributes in mitotic and meristematic tissues activities (Scollo et al., 2018), sugars and starch catabolism (Stan and David, 2007). Benlloch-González et al. (2008) and Arquero et al. (2006) both concluded that potassium plays a very important role in the vegetative growth of the olive tree. It acts as a promoter for carbohydrates accumulation, water consumption, and transpiration regulation.

Aside from NPK macronutrients, calcium and magnesium are very essential to olive tree metabolism due to their respective contribution in the constitution of cell walls and chlorophyll molecules (Briccoli Bati et al., 2012).

4.3. Considerations on plant growth parameters and leaves composition

The reuse of PWW in agriculture for short period has been shown to provide essential nutrients for plant growth compared to control plants irrigated with TW. This important vegetative development can be explained by the richness of PWW in mineral nutriments A recent study conducted in Tunisia over one year on *Chetoui* cv. irrigated with treated wastewater coming from biological processes (TWW) and diluted TWW during four months showed enhancement on plant height and leaf area (Ben Hassena et al., 2018).

Carbohydrates are produced by the photosynthetic activity. These compounds serve as precursors for lipids, protein, and polysaccharides biosynthesis and energy source for plant growth. In addition, soluble sugars play a crucial role in the modulation of osmotic potential (Getnet et al., 2015) (Zelalem et al., 2015), and their metabolism is affected by environmental factors (Pelleschi et al., 1997).

Young olive trees accumulate soluble carbohydrates under water stress conditions (Arji and Arzani, 2004). An earlier report mentioned that the increase of total sugars content in leaves is a stress indicator of nutrient deficiency (Peuke, 2010) (Achuba, 2006).

In the present work, irrigation with PWW and PWTWW caused a significant decrease in leaves soluble sugars (glucose and fructose) content. This decrease may be attributed to the richness of PWW in nutrients which may stimulate carbohydrates metabolism in response to growth requirements. Many researchers demonstrated also a direct relationship between some minerals and carbohydrates metabolism (Marschner, 1995) (Perica et al., 2001) (Tranavičienė et al., 2007). A study on the effect of slaughterhouse wastewater on the metabolic state of cowpea seedling found a significant decrease in total sugars content accompanied by a significant increase of α-amylase (polysaccharides hydrolytic enzyme) (Achuba and Ja-anni, 2018). A similar reduction in sugar concentration was observed under olive tree foliar fertilization with boron (B), manganese (Mn), magnesium (Mg), sulfur (S), and potassium (P) (Tekaya et al., 2016).

Fatty acids biosynthesis was demonstrated to be almost exclusively localized in plastids (Harwood, 1988). Great interest is given today to fatty acids due to their important role in plants development (structural role, activity of membrane enzymes, molecular signaling) (Truchet et al., 1991). Only a few bibliographical references provided a linkage between the irrigation water quality and olive tree leaves' FA composition. Previous studies showed that palmitic acid was the most abundant FA in olive leaves (Aranda et al., 2004), accordingly to the results obtained in the present study. Linolenic acid is a structural component of membrane lipids and intervenes as a signaling molecule precursor for plant growth and stress response (Ohlrogge and Browse, 1995) (Weber, 2002). A study conducted in Tunisia on own-rooted cuttings of 3-month-old *O. europaea Chemlali* cv. and *Chetoui* plants subjected to drought stress showed an increase in linolenic acid and a decrease in stearic acid while in the present study no difference among the lots was detected (Guerfel et al., 2008).

5. Conclusions

The high levels of organic material present in the industrial poultry wastewater preclude the company from discharging it into the environment. On the other hand, the richness in these compounds combined with the presence of minerals makes industrial poultry wastewater an interesting resource for the irrigation of some crops. Due to the richness of poultry wastewater in important nutrients for the plants' metabolism, short term irrigation with industrial poultry wastewater resulted in a significant increase in young olive tree growth parameters (plant height, trunk diameter, and shoots number). Furthermore, results showed that poultry wastewater reduced the amounts of soluble sugars in leaves, thus showing a state of the comfort of the plant. However, no effect was detected on the main fatty acids compounds. The reuse of industrial poultry wastewater offers the advantage of saving freshwater and, in parallel, of reducing the use of fertilizers. The proximity of the olive grove to industrial poultry company is a fundamental factor to avoid the expensive and not-sustainable transport of wastewater. Further analyzes are needed to assess the effect of this wastewater on leaves' secondary metabolites as well on fruit and oil quality.

Declarations

Ethical Approval

Authors commit to upholding the integrity of the scientific according to the COPE guidelines. Authors declare refrain from misrepresenting research results, which could damage the trust in the journal.

Consent to Participate

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Consent to Publish

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors Contributions

All authors contribute to this work:

Amira Oueslati : Effect of short-time irrigation with industrial poultry wastewater on young olive tree (*Olea europaea* L. cv. Chemlali) growth parameters of leaves

Giuseppe Montevecchi: Study of chemical composition of leaves

Andrea Antonelli: The determination of fatty acid profile of young olive trees leaves

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Availability of data and materials

Data and Materials are available

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Tables

Table 1

Wastewater physicochemical analysis

			8 h		9 h		10 h		11 h	
	Unit of measure	**TW***	**PWW****	**PWTWW*****	**PWW****	**PWTWW*****	**PWW****	**PWTWW*****	**PWW****	**PW**
TSS	mg L^{-1}	7.13±8.22	$1434^{d,e}$±269	$762^{d,e}$±165	$1017^{d,e}$±158	650^{d}±129	3656^{g}±1183	$1662^{e,f}$±541	2524^{f}±1026	114
COD	mg L^{-1}	10±6	$2593^{e,f,g}$±643	$1563^{d,e,f}$±303	$2048^{e,f}$±385	$1451^{d,e}$±321	6329^{h}±1629	3383^{g}±843	2745^{g}±598	180
BOD	mg L^{-1}	2.24±1.73	$837^{e,f,g}$±137	$494^{d,e}$±85	$686^{e,f}$±89	$468^{d,e}$±108	2023^{h}±436	$1029^{f,g}$±210	1237^{g}±490	719
TOC	mg L^{-1}	2.89±1.89	$497^{f,g}$±38	$247^{d,e}$±38	$384^{e,f}$±58	$222^{d,e}$±51	1043^{h}±267	$533^{f,g}$±93	713^{g}±226	363
NO_3^-	mg L^{-1}	5.59±2.55	10.38^{a}±9.00	$<0.50^{a}$±0.00	36.8^{a}±9.0	1.15^{a}±1.00	2.69^{a}±3.00	4.69^{a}±6.00	6.08^{a}±10.00	<0.5
AOX	mg L^{-1}	<0.50±0.00	18.91^{b}±18.41	$<0.50^{a}$±0.00	19.12^{b}±19.00	$<0.50^{a}$±0.00	0.65^{a}±0.00	$8.96^{a,b}$±13.00	$11.87^{a,b}$±17.00	<0.5
pH		7.95±0.05	6.29^{g}±0.06	$6.54^{d,e}$±0.10	6.52^{d}±0.17	$6.71^{e,f}$±0.09	$6.55^{d,e}$±0.11	6.77^{f}±0.07	$6.55^{d,e}$±0.15	6.75
EC	µS. cm^{-1}	1979±169	$2388^{d,e}$±169	2187^{d}±166	$2340^{d,e}$±190	2168^{d}±179	3174^{g}±491	$2719^{e,f}$±249	$2933^{f,g}$±169	245

TSS: total suspended solids; COD: chemical oxygen demand; BOD: biochemical oxygen demand; TOC: total organic Carbone; NO_3^-: nitrates; AOX: Adsorbable Organic Halides

EC: Electrical Conductivity

Means with common letters are not significantly different at $p \leq 0.05$, according to Tukey's HSD test.

[1] Limit values set by the Tunisian Republic for effluent discharged from the meat industry and slaughterhouses in the public hydraulic sector.

[*] Significant difference between TW, PWW and PWTWW.

Table 2

Mineral and total fat analysis. Data are reported in mg L^{-1}

	TW	PWW	PWTWW	Tunisian limits[1]
SO_4^{2-}	1708^{a}	3054^{c}	2726^{b}	-
HCO_3^-	211^{a}	1376^{c}	768^{b}	-
Na^+	452^{a}	844^{c}	650^{b}	-
Cl^-	426^{a}	674.5^{c}	586^{b}	700
$Ca^{2+} + Mg^{2+}$	375^{c}	270^{b}	255^{a}	-
Total Kjeldahl nitrogen	4^{a}	286^{c}	140^{b}	5
K^+	7^{a}	74^{c}	52^{b}	-
Total fat	75^{a}	825^{c}	475^{b}	10

[1] Limit values set by the Tunisian Republic for effluent discharged from the meat industry and slaughterhouses in the public hydraulic sector.

Table 3

Effect of PWW irrigation on vegetative plant growth parameters.

	TW	PWW	PWTWW
Plant height (cm)	24.53^{a} ± 11.23	40.18^{b} ± 18.89	26.81^{ab} ± 8.26
Trunk diameter (mm)	1.46^{a} ± 0.76	5.24^{b} ± 1.31	$2.54^{a,b}$ ± 1.13
Shoots' number	8^{ab} ± 7	19^{b} ± 15	8^{a} ± 4
Shoots length (cm)	3.80^{a} ± 2.89	12.14^{a} ± 14.09	5.51^{a} ± 3.72

Means with common letters are not significantly different at $p \leq 0.05$, according to Tukey's HSD test.

Table 4

Olive leaves physiological parameters content

	Dry matter (%)	**Moisture (%)**	**Ashes (%)**
TW	$50^{a} \pm 1$	$49^{b} \pm 1$	$2.85^{a} \pm 0.06$
PWW	$56^{b} \pm 2$	$43^{a} \pm 2$	$3.68^{ab} \pm 0.41$
PWTWW	$50^{a} \pm 1$	$49^{b} \pm 1$	$3.20^{a} \pm 0.18$

Means with common letters are not significantly different at $p \leq 0.05$, according to Tukey's HSD test

Table 5

Concentrations of sugars (g kg^{-1} DW) in olive leaves extracts

	TW	**PWW**	**PWTWW**	**ANOVA (*F value*)**
Glucose	$99.09^{a} \pm 0.94$	$46.73^{c} \pm 3.87$	$64.76^{b} \pm 12.60$	55.83***
Fructose	$55.46^{a} \pm 1.97$	$32.65^{b} \pm 3.81$	$38.19^{b} \pm 7.41$	27.83***

Results of one-way ANOVA are reported as F values (*** $p \leq 0.001$), and results of Tukey's test are reported as lowercase letters, where different letters identify samples that are significantly different ($p \leq 0.05$).

Table 6

Fatty acids profile (mg 100 mg_{FAT}^{-1}) of olive leaves "*Chemlali* cv"

Fatty acids compounds	**Common name**	**TW**	**PWW**	**PWTWW**	**ANOVA**
$C_{6:0}$	Caproic acid	$0.03^{ab}\pm0.01$	$0.05^{b}\pm0.02$	$0.01^{a}\pm0.02$	
$C_{8:0}$	Caprylic acid	$0.03^{a}\pm0.01$	$0.05^{b}\pm0.01$	$0.01^{a}\pm0.01$	
$C_{10:0}$	Decanoic acid	$0.02^{a}\pm0.02$	$0.05^{a}\pm0.05$	$0.01^{a}\pm0.1$	n.s.
$C_{12:0}$	Lauric acid	$0.09^{a}\pm0.02$	$0.17^{a}\pm0.08$	$0.33^{b}\pm0.07$	
$C_{14:0}$	Myristic acid	$3.35^{a}\pm1.23$	$4.58^{a}\pm2.62$	$2.08^{a}\pm0.61$	n.s.
$C_{15:0}$	Pentadecanoic acid	$0.26^{a}\pm0.03$	$0.32^{a}\pm0.09$	$0.20^{a}\pm0.02$	n.s.
$C_{16:0}$	Palmitic acid	$34.10^{a}\pm2.04$	$35.66^{a}\pm3.52$	$33.71^{a}\pm0.33$	n.s.
$C_{16:0}$ **isomer1**		$0.42^{a}\pm0.03$	$0.42^{a}\pm0.06$	$0.37^{a}\pm0.04$	n.s.
$C_{16:0}$ **isomer2**		$0.45^{a}\pm0.07$	$0.54^{a}\pm0.14$	$0.55^{a}\pm0.14$	n.s.
$C_{16:1}$	Palmitoleic acid	$0.66^{a}\pm0.18$	$1.04^{a}\pm0.30$	$0.88^{a}\pm0.23$	n.s.
$C_{17:0}$		$2.10^{a}\pm0.18$	$1.87^{a}\pm0.06$	$1.90^{a}\pm0.12$	n.s.
$C_{17:1}$		$0.48^{a}\pm0.03$	$0.43^{a}\pm0.07$	$0.42^{a}\pm0.08$	n.s.
$C_{18:0}$	Stearic acid	$4.27^{a}\pm0.31$	$3.66^{a}\pm0.43$	$3.85^{a}\pm0.02$	n.s.
$C_{18:1}$	Oleic acid	$18.72^{a,b}\pm0.22$	$17.39^{a}\pm1.16$	$19.60^{b}\pm0.49$	
$C_{18:2}$	Linoleic acid	$4.29^{b}\pm0.19$	$3.13^{a}\pm0.32$	$3.39^{a}\pm0.15$	
$C_{18:3}$ **(gamma)**	γ-Linolenic acid	$0.27^{a}\pm0.04$	$0.34^{a}\pm0.08$	$0.29^{a}\pm0.03$	n.s.
$C_{18:3}$ **(alpha)**	α-Linolenic acid	$25.52^{a}\pm1.53$	$26.49^{a}\pm3.06$	$27.97^{a}\pm2.01$	n.s.
$C_{20:0}$		$2.05^{a}\pm0.80$	$1.09^{a}\pm0.54$	$1.33^{a}\pm0.44$	n.s.
$C_{20:1}$	Eicosenoic acid	$0.32^{a}\pm0.13$	$0.23^{a}\pm0.05$	$0.37^{a}\pm0.03$	n.s.
$C_{21:0}$	Heneicosylic acid	$0.43^{a}\pm0.18$	$0.22^{a}\pm0.09$	$0.28^{a}\pm0.09$	n.s.
$C_{22:0}$	Behenic acid	$0.73^{a}\pm0.28$	$0.36^{a}\pm0.21$	$0.54^{a}\pm0.21$	n.s.
$C_{23:0}$	Tricosylic acid	$0.38^{a}\pm0.12$	$0.20^{a}\pm0.06$	$0.30^{a}\pm0.11$	n.s.
$C_{24:0}$	Lignoceric acid	$0.24^{a}\pm0.07$	$0.15^{a}\pm0.06$	$0.23^{a}\pm0.06$	n.s.
DHA $C_{22:6}$	Docosahexaenoic acid	$0.64^{a}\pm0.04$	$1.41^{a}\pm0.47$	$1.32^{a}\pm0.48$	n.s.
Saturated fatty acids		49.11	49.53	45.76	
Monounsaturated fatty acids		20.18	19.09	21.27	
Polyunsaturated fatty acids		30.71	31.37	32.97	