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Olive mill wastewater triggered changes in physiology and nutritional quality of tomato (*Lycopersicon esculentum* Mill.) depending on growth substrate

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Abstract

We have studied the changes in the physiology and nutritional quality of *Lycopersicon esculentum* exposed to olive mill wastewater (OMW) with regard to cultivation in sand and soil. Tomato plant performance decreased with increasing concentration of OMW to both substrates. Root was more sensitive to OMW than the upper parts of the plants, grown either in sand or in soil for 10 days and 3 months, respectively, probably due to the direct OMW toxicity on roots as compared to other parts. Significant restriction on uptake and translocation of nutrients (K, Na, Fe, Ca and Mg) under OMW application was found. The decrease in the photochemical efficiency of PSII photochemistry in the light adapted state and the big decrease in photochemical quenching, indicate that OMW resulted in diminished reoxidation of Q_A^- and started to inactivate the reaction centers of PSII. The OMW supply on soil and sand, resulted in leaf water stress and lesser water use efficiency. Plants treated with high OMW concentration, produced fewer but bigger tomatoes as compared to plants treated with lower OMW concentration. Generally, fruit yield and nutritional value was inhibited under OMW application.
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1. Introduction

There is a growing public concern about the environmental impact of industrial development and population expansion in recent decades [1]. The continuous inputs of wastes on agricultural land have, caused imbalance in ecosystems. The olive oil production of Mediterranean countries represents ca. 98% of the entire worldwide production [2]. The industrial olive oil sector generates large quantities of solid and liquid wastes and by-products in these countries during a short period of time (November–February). Around 30 million m^3 of olive mill wastewater (OMW) are produced annually in the Mediterranean

area, causing environmental concern [3]. The disposal and treatment of this liquid waste, are the main problems of the olive oil industry because of its high organic load and content of phytotoxic and antibacterial phenolic substances, which resist biological degradation [4]. OMW has also a high potassium concentration and notable levels of nitrogen, phosphorus, calcium, magnesium and iron, important factors in soil fertility. Various authors have demonstrated that the controlled spreading of OMW on cultivated fields does not induce phytotoxic effects on tree and crop species [5]. However, other authors have observed negative effects on plants and soil properties when OMW is used directly as an organic fertiliser [3,6]. In fact, it is shown that phenolic components were responsible for the phytotoxicity of OMW. The toxic effect on higher plants is especially severe during germination and seedling development [3,7]. It is of paramount importance to evaluate the ecotoxicological risks associated with the release of this waste in the environment.

Much research has been focused on the OMW effect on germinability of several vegetables [3,8,9], but little information

is given on the OMW effect on the whole stages of plant development.

The photosynthetic reactions, both electrons transport as well as CO_2 assimilation are of primary interest in the context of the toxic effects of various stressors on plants. The fluorescence yield of chlorophyll *a*, is dependent on the micro-environment in the thylakoid, therefore indicating structural or organizational changes of the chloroplast membranes and elucidating the physiological and biochemical bases for changes in the ability of leaves to assimilate CO_2 [10,11,12]. Early changes of various tissue characteristics in vegetables have been identified, including respiration, vitamin C and chlorophyll content [13], which are considered sensitive indicators of changes in tissue condition after harvest. Living cells of harvested plant products respire continuously, utilizing stored reserves and oxygen from the surrounding environment, and releasing carbon dioxide [14]. Respiration rate is one of the most important indicators of senescence in fruit, as are weight loss, pigment content, firmness [15].

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetables worldwide because of its high consumption, year around availability and its large content of health-related components. Furthermore, it is an important supplier of nutrients such as folate, potassium, vitamins A and C, flavonoids and carotenoids [16]. In the present study, cherry tomato was chosen as a model plant to monitor the effects of various OMW dilutions because it is often grown in the same areas where olive is widespread.

Two separate experiments were made in order to (a) investigate the differential phytotoxic effects of OMW applied in different growth substrates (sand and soil) and to (b) detect the OMW toxic symptoms on yield and physiology of tomato.

2. Materials and methods

2.1. Plant material and growth conditions

Experiments were conducted on OMW obtained from a three-phase olive mill plant located near the city of Kalamata, Southern Greece. The samples were stored in big PVC vessels tightly closed at 4 °C before use. Their characteristics summarized in Table 1. The cultivated soil exhibited the following characteristics: pH 7.2, water holding capacity (%) 85, electrical conductivity (mS cm^{-1}) 4.23, organic matter (%) 12.0, C.E.C. (mequiv. 100 g^{-1}) 30.4, total salt content (%) 0.28, exchangeable Na (mequiv. 100 g^{-1}) 0.56.

The seeds of *L. esculentum*, cv. Principe Borghese were sown in sterilized sand and left for 10 days germination. Then the seedlings were transferred either into cultivated soil filled pots or into sterilized sand filled pots (one plant per pot). The pots (5 l each) were daily irrigated with full strength Hoagland nutrient solution [17]. A total of 30 pots (15 pots for soil experiments and 15 pots for sand experiments) were placed in a randomized block designed in a growth chamber programmed for 16 h photoperiod with photosynthetic photon flux density of 500 μmol m^{-2} s^{-1} at plant level, temperature 21/19 °C day/night and relative humidity 65/75%. One-month-old plants cultivated either in soil or in

Table 1
Physicochemical properties of raw OMW used as nutrient solution

Parameters	Three-phase OMW
pH	4.8 ± 0.2
Total proteins (g l^{-1})	2.6 ± 0.75
COD (mg l^{-1})	36800 ± 170
TOC (mg l^{-1})	26600 ± 670
Total phosphorus (mg l^{-1})	9.51 ± 1.2
Total phenols (mg l^{-1})	27.5 ± 3.5
Potassium as K$_2$O (mg l^{-1})	3840 ± 310
Total nitrogen (mg l^{-1})	370 ± 58
Tannins (g l^{-1})	2.2 ± 0.8
Total suspended solids (TSS, %)	1.1 ± 0.5
Fatty acids (%)	
Myristic acid (C 14:0)	2.82
Palmitic acid (C 16:0)	10.75
Palmitoleic acid (C 16:1)	0.84
Stearic acid (C 18:0)	3.10
Oleic acid (C 18:1)	69.70
Linoleic acid (C 18:2)	9.40
Arachidic acid (C 20:0)	0.49
Linolenic acid (18:3)	1.6

Values are the mean of three measurements ± S.E.

sand were subjected to OMW treatments. Three types of solutions were used: control-Hoagland, 1:20 OMW dilution and 1:10 OMW dilution adjusted to a pH 5.2 ± 0.1.

Soil experiments lasted for 3 months and were terminated when all plants had fruits, while sand experiments lasted for only 10 days due to intense lethal symptoms.

2.2. Metal accumulation

Plants were uprooted from the pots with the help of fine jet of water, causing minimum damage to the roots, were washed thoroughly with running deionized water, and were blot dried. Different parts of the plant were separated manually, cut in small pieces and oven dried (100 °C for 24 h). The contents of K, Na, Fe, Ca and Mg were determined by atomic absorption spectrophotometry after wet-digesting in 4:1 (v/v) HNO$_3$–HClO$_4$ [18].

2.3. Photosynthetic pigments analysis

Chlorophylls ($a + b$) and total carotenoids ($x + c$) of the youngest fully expanded leaf, were quantitatively measured in the same 100% acetone extract by spectrophotometry using the re-determined extinction coefficients [10,12].

2.4. Gas exchange–chlorophyll fluorescence measurements

Leaf gas exchange measurements were coupled with measurements of chlorophyll fluorescence using an open gas exchange portable system (LI-6400; LI-COR, Inc., Lincoln, NE) with an integrated fluorescence chamber head (LI-6400-40 leaf chamber fluorometer; LI-COR, Inc.) [19]. Measurements on the youngest mature leaves were conducted between 9:00 and 12:00 h. Leaf temperature inside the cuvette maintained between 25 and 27 °C and cuvette relative humidity was about 60%.

The CO_2 concentration at the reference infrared gas analyzer (IRGA) was maintained at $400\ \mu mol\ mol^{-1}$ by means of a 12 g CO_2 cylinder and the 6400-01 CO_2 injector, with the airflow rate through the chamber maintained at $400\ \mu mol\ s^{-1}$. Leaf gas exchange measurements were calculated by the LI-6400 operating software, according to the method of von Caemmerer and Farquhar [20].

In vivo chlorophyll fluorescence was measured on the upper surface of the fully expanded younger leaves after they were left for 30 min to dark adaptation, at room temperature. Different values were selected in order to determine any structural and functional changes of the photosynthetic apparatus under OMW dilutions: the F_v/F_m (maximal photochemical efficiency of PSII photochemistry in the dark adapted state), the F'_v/F'_m (photochemical efficiency of PSII photochemistry in the light adapted state), q_P (photochemical quenching) and q_N (non-photochemical quenching) [17].

2.5. Respiration rate measurements

A sample of three fruits was used to measure respiration. Gas exchange measurements (CO_2) were made on individual fruits in glass jars. These jars were placed at $20\ ^\circ C$. One millilitre of the gas sample was removed using a special syringe, after the jars had been closed for 1 h, and injected into a gas-chromatograph PerkinElmer 8700 with a thermal conductivity (TC) detector. All results are expressed as mg $CO_2\ kg^{-1}\ h^{-1}$.

2.6. Chemical analysis

The ascorbic acid content of tomato fruits was estimated by macerating the fruit sample mechanically with a stabilising agent (5% metaphosphoric acid) and titrating the filtered extract with 2,6-dichlorophenolindophenol. An automatic digital refractometer of the firm Index Instruments UK, type GPR 12-70, was used to determine the Soluble Solids of the fruit samples. Results were expressed as Brix at $20\ ^\circ C$. Glucose and fructose were determined with an HP 1100 Series High Performance Liquid Chromatograph refractive index detector (RID) using a reverse phase column 250 mm × 4 mm of Lichrosphere NH_2 bonded to microparticulate silica of 5 μm diameter maintained at $37\ ^\circ C$. Injection of 20 μl of sample solution into a mobile solvent of H_2O/AcCN: 25:75 (v/v) with a flow rate of 1.1 ml min^{-1} gave the optimum result [21]. Cellulose was determined by the enzymatic method of Englyst FIBERZYM Kit.

2.7. Statistical analysis

Soil and sand experiments were set up in a randomized design with five replications each. One-way analysis of variance (ANOVA) and Duncan's multiple comparison test were performed to compare means at significance level $p = 0.05$. Sample variability is given as the standard error of the mean values.

3. Results

3.1. Plant growth performance

Non-treated tomato plants showed the best vegetative growth when compared to all other treatments. Plant biomass was more affected than plant height during all OMW dilutions, in both sand and cultivated soil substrate (Table 2). The strongest reduction was found for the treatments with the lowest rate of OMW dilution (1:10). Plants grown on sterilized sand, expressed a more significant inhibition of their mass and length, by over

Table 2
Effect of three OMW dilutions on root, shoot and leaf characteristics ($n = 12$, ±S.E.) of *Lycopersicon esculentum* plants grown in cultivated soil (1) and sterilized sand (2)

Morphological parameters	OMW dilutions		
	Control (0)	1:20	1:10
Root biomass (g FW)			
1	42.2 ± 2.2a	34.6 ± 1.3b	16.57 ± 2.3c
2	4.5 ± 0.5a	2.6 ± 0.3ab	1.5 ± 0.6b
Shoot biomass (g FW)			
1	426.5 ± 19.2a	332.5 ± 16.0b	258.5 ± 24.3c
2	6.6 ± 0.9a	3.2 ± 0.5b	2.2 ± 0.4b
Root length (cm)			
1	45.0 ± 2.1a	38.0 ± 1.6b	31.1 ± 1.8c
2	18.4 ± 1.4a	11.6 ± 2.3b	9.6 ± 1.5b
Shoot length (cm)			
1	124.0 ± 12.0a	116.0 ± 10.4a	103.0 ± 5.8b
2	30.1 ± 3.5a	17.1 ± 2.3b	13.5 ± 1.9b
Leaf area (LA, cm^2)			
1	30.3 ± 2.2a	23.4 ± 1.3b	19.3 ± 0.9c
2	17.0 ± 1.5a	9.4 ± 1.1b	5.0 ± 0.6c

Means followed by a different letter within a row are significantly different at $p < 0.05$ according to Duncan's multiple comparison test.

Table 3
Accumulation of K, Na, Fe, Ca and Mg ($\mu g\,g^{-1}$ DW) in different parts of *L. esculentum* grown on different dilutions of OMW

OMW dilutions	Metal accumulation ($\mu g\,g^{-1}$ DW)				
	K	Na	Fe	Ca	Mg
Control					
Shoot					
1	20500 ± 400a	4838 ± 310a	123 ± 4.5a	37050 ± 705a	8175 ± 189a
2	26200 ± 560a	4475 ± 290a	132 ± 6a	36000 ± 670a	6250 ± 210a
Root					
1	24300 ± 370a	2700 ± 190a	300 ± 8a	38150 ± 660a	6050 ± 220a
2	17100 ± 328a	2425 ± 86a	230 ± 16a	19700 ± 450a	3400 ± 170a
1:20					
Shoot					
1	18325 ± 185b	3538 ± 240b	90 ± 9b	25425 ± 410b	7775 ± 90b
2	22000 ± 150b	2875 ± 310b	83 ± 11b	25150 ± 536b	4250 ± 236b
Root					
1	17225 ± 220b	1875 ± 68b	249 ± 7b	30525 ± 430b	5638 ± 160b
2	15550 ± 256b	1600 ± 146b	188 ± 13b	15100 ± 270b	2300 ± 280b
1:10					
Shoot					
1	17725 ± 87c	2300 ± 100c	84 ± 6b	25200 ± 380b	6400 ± 85c
2	21050 ± 129c	2025 ± 94c	52 ± 9c	19850 ± 230b	2750 ± 195c
Root					
1	15150 ± 115c	1825 ± 57b	206 ± 12c	17250 ± 620c	3350 ± 96c
2	10050 ± 213c	1056 ± 85c	161 ± 11c	10750 ± 310c	1300 ± 165c

All the values are means of three replicates ± S.E, (1) plants grown in cultivated soil, (2) plants grown in sterilized sand. Means followed by a different letter within a row are significantly different at $p < 0.05$ according to Duncan's multiple comparison test.

65% for root and shoot mass and about 50% for root and shoot length, as compared to the control, than plants grown on cultivated soil (Table 2, $p < 0.05$). Furthermore, a more severe decrease (70% as compared to the control) of leaf area of plants grown on sand and under lower OMW dilution (1:10) was revealed, in comparison to those grown on cultivated soil (Table 2, $p < 0.05$).

3.2. Metal accumulation

Increasing ratio of OMW caused a progressive decrease in the accumulation of metals (K, Na, Fe, Ca, Mg) in roots and shoots of tomato. Generally, plants grown on sterilized sand, lost higher amount of metals in comparison to the plants grown on cultivated soil substrate (Table 3). Moreover, the roots lost more metals than the shoots, at the two cultivation substrates, but the magnitude differed among the metals. Under 10 d application of 1:10 OMW dilution, the roots of tomato grown on sand lost more than half of Na and Mg concentration ($p < 0.05$), while, the shoots lost more than half of Na, Fe and Mg concentration ($p < 0.05$), as compared to the respective controls (Table 3). On exposure to the higher OMW concentration for 3 months, significant decreases of all the tested metals were found in plants grown on cultivated soil. More precisely, Ca accumulation in roots was significantly reduced by 55% of the control ($p < 0.05$), while Na content in tomato shoots dramatically decreased by more than 50% of the control (Table 3, $p < 0.05$).

3.3. Leaf pigment concentration

Total chlorophyll and carotenoid concentrations of tomato leaves progressively decreased with the decreasing OMW dilution in both substrates (Table 4). Plants grown on sterilized sand, revealed higher loss of the photosynthetic pigments (by more than 50% and 80%, respectively of the control in 1:10

Table 4
Effect of three OMW dilutions on photosynthetic pigments ($mg\,g^{-1}$ FW) and water use efficiency (Pn/E, $\mu mol\,CO_2\,m^{-2}\,s^{-1}/mmol\,H_2O\,m^{-2}\,s^{-1}$), of the leaves of *L. esculentum* plants grown in cultivated soil (1) and sterilized sand (2)

Parameters	OMW dilutions		
	Control	1:20	1:10
Total chlorophyll			
1	3.74 ± 0.10a	2.22 ± 0.08b	1.86 ± 0.03c
2	2.70 ± 0.05a	1.80 ± 0.12b	1.30 ± 0.06c
Carotenoid			
1	1.20 ± 0.11a	0.76 ± 0.08b	0.58 ± 0.03c
2	1.94 ± 0.07a	1.40 ± 0.04b	0.30 ± 0.05c
WUE			
1	4.30 ± 0.2a	2.68 ± 0.1b	2.13 ± 0.8c
2	3.21 ± 0.4a	1.85 ± 0.3b	1.21 ± 0.3c

All the values are means of three replicates ± S.E, means followed by a different letter within a row are significantly different at $p < 0.05$ according to Duncan's multiple comparison test.
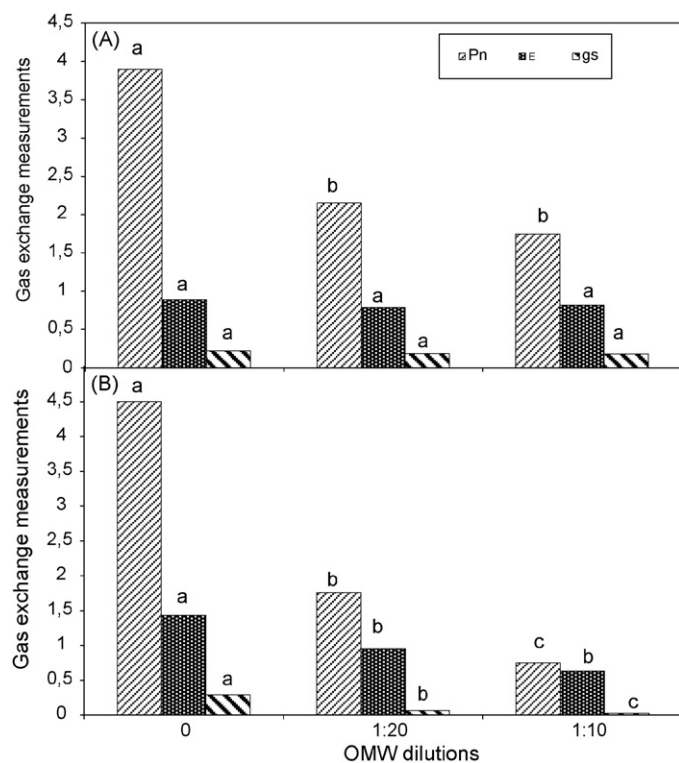
Fig. 1. Changes of the assimilation (Pn, $\mu mol\, CO_2\, m^{-2}\, s^{-1}$), transpiration (E, $mmol\, H_2O\, m^{-2}\, s^{-1}$) rate and stomatal conductance (gs, $mmol\, H_2O\, m^{-2}\, s^{-1}$) of the youngest fully expanded tomato leaf after 3 months and 10 days exposure to OMW dilutions on soil (A) and sand (B), respectively. Data are means of three replications. Means with different letter in the same parameter for each treatment differ significantly ($p < 0.05$).

dilution) than plants grown on cultivated soil (Table 4, $p < 0.05$). A remarkable chlorosis of the old leaves was also observed on OMW-treated plants grown on sand.

3.4. Photosynthesis and fluorescence response

CO_2 fixation rates, transpiration, stomatal conductance and chlorophyll fluorescence parameters were measured on attached leaves under 1:20 and 1:10 OMW dilutions of plants grown either on soil or on sand. The rate of CO_2 assimilation of plants grown on soil was significantly decreased under both the OMW dilutions, showing the maximum reduction of 83% ($p < 0.05$) at 1:10 dilution (Fig. 1A). No significant changes were observed on transpiration and stomatal conductance under both the OMW dilutions (Fig. 1A). However, the two OMW dilutions induced a large drop of WUE, which reached its minimum values under 1:10 OMW dilution (about 50% and 40% of the control plants grown on soil and sand, respectively, Table 4, $p < 0.05$). Plants grown on sterilized sand revealed more severe reductions of gas exchange parameters at both OMW dilutions, compared to plants grown on soil. More precisely, the exposure to 1:10 OMW dilution resulted in significant ($p < 0.05$) decrease in CO_2 assimilation, transpiration and stomatal conductance by 83%, 56% and 91% of the control, respectively, (Fig. 1B). The photochemical efficiency of PSII photochemistry (F_v'/F_m') of tomato leaves affected less than the photochemical (q_P) and non-
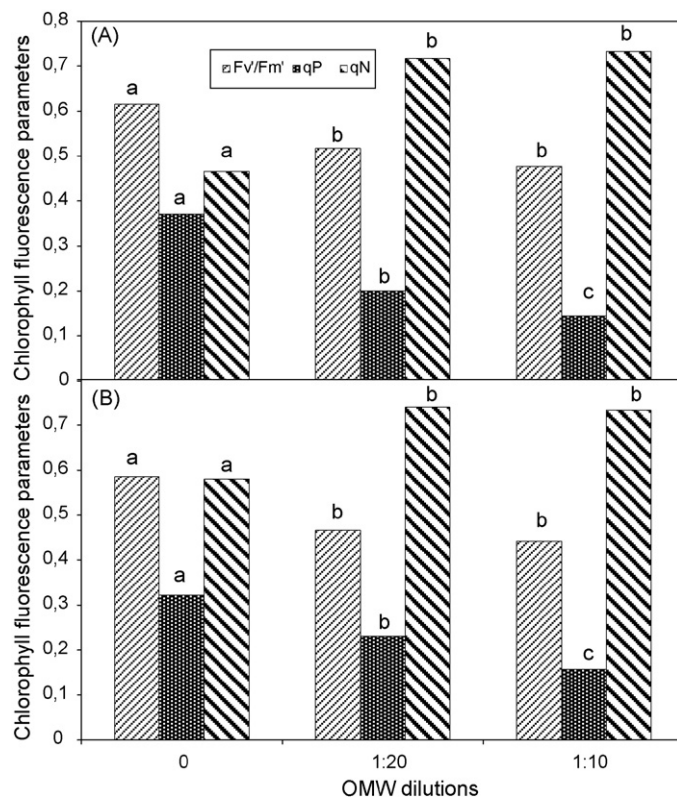

Fig. 2. Changes of the F_v'/F_m' (photochemical efficiency of PSII photochemistry in the light adapted state), q_P (photochemical quenching) and q_N (non-photochemical quenching) of the youngest fully expanded tomato leaf after 3 months and 10 days exposure to OMW dilutions on soil (A) and sand (B), respectively. Data are means of three replications. Means with different letter in the same parameter for each treatment differ significantly ($p < 0.05$).

photochemical quenching (q_N) under both the OMW dilutions and under the two growth substrates (Fig. 2A and B). Significant decreases of q_P under 1:20 (by 46% of control) and 1:10 (by over 60% control) OMW dilutions were observed, for plants grown on cultivated soil (Fig. 2A, $p < 0.05$). Growth on sand substrate resulted in more severe suppress of q_P in both OMW dilutions, by 28% and 51% of the control, respectively (Fig. 2B, $p < 0.05$). In contrast, q_N was significantly ($p < 0.05$) enhanced on exposure to OMW dilutions, showing an increase by 57% and 26% of their respective controls, for plants grown on soil and sand substrate (Fig. 2A and B).

3.5. Yield, quality and respiration of tomato fruit

From the two experiments, only in the first one, that took place on cultivated soil, the plants achieved mature fruits. Fruit production significantly decreased by 43% of the control, under 1:10 OMW dilution. The size of the mature fruits also displayed severe loss, that was more pronounced under 1:20 OMW dilution (by 40% of the control) (Table 5, $p < 0.05$). Exposure of plants to OMW dilutions, for 3 months promoted decreases in soluble sugars (glucose and fructose) and soluble solids by 48%, 32% and 20% of control, respectively in 1:10 dilution (Table 5, $p < 0.05$). No significant alterations were found for total cellulose of tomato fruit. A dramatic drop in ascorbic acid content,

Table 5
Effect of three OMW dilutions on fruit yield, size of mature fruits as well as on some quality characteristics and respiration rate of the fruits of *L. esculentum* grown on cultivated soil ($n = 3$, $\pm$S.E.)

Parameters	Control (0)	1:20	1:10
Yield (number pot^{-1})	14a	12b	8c
Fruit size (mm)	$32 \pm 1.9a$	$19 \pm 2.2b$	$26 \pm 1.8c$
Glucose (%, FW)	$3.36 \pm 0.23a$	$2.78 \pm 0.14b$	$2.03 \pm 0.20c$
Fructose (%, FW)	$2.59 \pm 0.12a$	$2.70 \pm 0.20a$	$1.76 \pm 0.16b$
Soluble solids (%, FW)	$6.0 \pm 0.17a$	$5.5 \pm 0.15b$	$4.8 \pm 0.17c$
Ascorbic acid (mg 100g^{-1})	$16.7 \pm 0.68a$	$4.2 \pm 0.27b$	$3.9 \pm 0.31b$
Cellulose (%, FW)	$3.2 \pm 0.10a$	$3.3 \pm 0.14ab$	$3.5 \pm 0.16b$
Respiration (mg CO$_2$ kg^{-1} h^{-1})	$120 \pm 2.5a$	$146 \pm 3.1b$	$178 \pm 2.9c$

Means followed by a different letter within a row are significantly different at $p < 0.05$ according to Duncan's multiple comparison test.

even under the higher dilution (1:20) by 75% of the control, was also detected. On the contrary, the CO_2 production which expresses the respiration rate of the fruit (mg CO_2 kg^{-1} h^{-1}), was approximately 50% higher than that of the control under 1:10 OMW dilution (Table 5, $p < 0.05$).

4. Discussion

OMW phytotoxicity is a complex matter, since more than one compound can be responsible for it and possible negative synergism could be forecasted. Phenolic and fatty acids are the main responsible compounds, considering OMW as an environmentally hazardous material [2]. According to Komilis et al. [9], dilution was found to be the principal pre-treatment technique affecting OMW phytotoxicity among all pre-treatment techniques tested, however, water supply in most of the olive oil producing countries is a big and increasing problem and this technique is not applicable. Other methods based on chemical and biological treatments able to remove toxic substances by the OMW have been suggested [22–24]. In our study, we used two dilutions of OMW, in order to determine and explain OMW toxicity in the physiology and quality of tomato. Plant performance decreased with increasing concentration of OMW, to both growth substrates. Similar results were obtained by Kistner et al. [25] in hydroponically grown tomatoes under various OMW concentrations. The application of 50% OMW in maize, wheat and chickpea resulted in a severe reduction of plant height, while germination percentages were also reduced [8]. Our present research showed that root was more sensitive to OMW than the upper parts of tomato plant grown either in sand or in soil. The reason may be that root face OMW toxicity directly, while the toxicity to other parts is indirect. The highly significant reduction of the growth observed, suggests the susceptibility of tomato to components as phenolics and fatty acids of OMW. These effects might be due to the lipophilicity of phenolic and fatty acids compounds which could alter the accessibility of nutrients inside the biological membranes as suggested by El-Hadrami et al. [8] and Kistner et al. [25]. The analysis of our data showed that tomato plants grown in sand, lost higher concentrations of metals than those grown in the soil under OMW dilutions, while roots suffered much more than shoots from nutrient deficiency. An explanation of these results may be found most likely in a general structure alteration of membranes and modifica-

tion of their metabolic functions, including membrane efficiency and stability, during OMW application, as it also happens during other stressors [26]. The nitrogen supplied with the OMW was "organic" and not completely available for the plants. This could easily explain poor plant growth, reduction of chlorophyll content and low photosynthetic activity. In addition, by applying OMW with such high organic matter, we can anticipate oxygen deficiency in the rhizosphere resulting in growth inhibition.

Lesser inhibition of growth and also greater tomato productivity after long-term exposure to OMW in the soil, as compared to short-term experiments in the sterilized sand was observed. It seems likely that the high organic matter in combination with the micro-organisms present in the soil are able to detoxify OMW. The inhibitory effects of OMW might be modified by chemical and biological processes, influencing their fate in the soil. In fact, it is evident that soil has the potential to reduce the phenolic load of OMW as reported in several studies [5,27]. This soil capability is ascribable both to its biotic components (microbial cells, free enzymes or associated to the organic or organo-mineral component in soil) and to its abiotic constituents [3].

The significant loss of chlorophyll content in the OMW-treated plants, may be attributed to the interference of the toxic substances present in OMW in the formation of chlorophyll. The reduction of tomato photosynthesis observed was in good correlation with the chlorophyll reduction. Moreover, the decrease in photosynthesis can be related to the significant decrease observed in leaf Fe concentration [28]. In addition, carotenoids, which are a part of photosynthetic pigment, were more sensitive to OMW application. Thus, they were not able to protect chlorophylls under the stress conditions. Our results are similar with those found by El-Hadrami et al. [8] for some crops treated with OMW and quite different to Singh et al. [29] who found an increase of chlorophylls and carotenoids under tannery sludge applications in sunflower.

The insufficiently utilized assimilatory force by Calvin cycle slowed down due to OMW stress, may enhance proton gradient formed in chloroplasts and increase non-photochemical dissipation of light energy and decrease photochemical efficiency. In parallel, the reduced chlorophyll content is one of the reasons for the decreased photosynthetic efficiency [18]. The slight decrease in F_v/F_m (data not shown) and the big decrease in q_P,

indicate that OMW diminished reoxidation of Q_A^- and started to inactivate the RC of PSII. In addition, the severe increase in q_N might be due to the dissociation of light-harvesting complex from PSII core [30].

It is worth noting that OMW application on sand and on soil, displayed different effects on photosynthetic parameters. Concerning OMW supply on soil, it seems that OMW preferentially inhibits the assimilation rate and the WUE, rather than the transpiration and stomata closure. On the contrary, OMW supply on the sand caused a complete closure of stomata with concomitant negative effects on assimilation and transpiration rates. This could be a result of leaf water stress a fact that is confirmed by the high proline concentration in leaves of tomato (unpublished data). The modification of gs by OMW may be also related to an alteration in the K^+/Ca^{2+} ratio in the guard cells, or with the alteration in the abscisic acid concentration, which controls stomatal movement [31].

The duration of OMW application on sand experiments was too short (10 days) in order to study the generative performance of the crop. However, long-term OMW application on soil experiments, caused reduction on fruit yield measured either as number of fruit per pot or fruit size as a consequence of flower abortion during OMW application. It is noticeable that despite the reduced number of tomatoes under 1:10 OMW dilution, the fruit size was bigger than that under 1:20 OMW dilution. Generally, plants treated with high OMW concentration, produced fewer but bigger tomatoes, as compared to plants treated with lower OMW concentration. In parallel, not only the yield but also the quality characteristics of tomatoes have been changed under OMW application. The reduced sugars and the soluble solids, which have been correlated with sweetness [32], and the fruitiness, which has been correlated with reducing sugars, particularly glucose, were suppressed significantly at the lower OMW dilution. Several studies report the allelopathic effects of phenolic compounds of OMW on higher plants and it has been suggested that such effects arise from alterations of water uptake, or of the metabolism of auxins and/or other phytohormones [3,7,33,34].

Based to our findings we can conclude that there is differentiation of OMW phytotoxicity depending on the type of the growth substrate of the plants. The toxic symptoms were more serious in plants grown in the sand than those grown in the cultivated soil, since lethal symptoms were observed after 10 days. In fact, the higher the organic matter, the lower the OMW toxicity. Despite the OMW toxicity in tomato grown in soil, fruit production was achieved. However, retardation in fruit maturity and yield was observed. The fruits were of reduced quality and nutritional value (ascorbic acid and brix). Nevertheless, the use of diluted OMW for fertilization/irrigation purposes at least in the case of tomato cultivation is not recommended.

Our future studies will focus in the utilization of detoxified OMW in order to determine possible supply of OMW for plant fertilization. Moreover, the combination between the use of pre-treated OMW to eliminate harmful effects and soils with different characteristics should be addressed to design and plan the most suitable strategy of OMW utilization.

Acknowledgements

References

[1] J. Xu, L. Yang, Z. Wang, G. Dong, J. Huang, Y. Wang, Toxicity of copper on rice growth and accumulation of copper in rice grain in copper contaminated soil, Chemosphere 62 (2006) 602–607.

[2] C. Paredes, J. Cegarra, M.P. Bernal, A. Roig, Influence of olive mill wastewater in composting and impact of the compost on a Swiss chard crop and soil properties, Environ. Int. 31 (2005) 305–312.

[3] R. Casa, A. D'Annibale, S.R. Stazi, G. Giovannozzi Sermanni, B. Lo Cascio, Reduction of the phenolic components in olive-mill wastewater by an enzymatic treatment and its impact on durum wheat (*Triticum durum* Desf.) germinability, Chemosphere 50 (2003) 959–966.

[4] E.S. Aktas, S. Imre, L. Ersoy, Characterization and lime treatment of olive mill wastewater, Water Res. 35 (2001) 2336–2340.

[5] P. Proietti, A. Palliotti, A. Tombesi, G. Cenci, Chemical and microbiological modifications of two different cultivated soils induced by olive oil wastewater administration, Agric. Med. 125 (1995) 160–171.

[6] C.F. Cereti, F. Rossini, F. Federici, D. Quaratino, N. Vassilev, M. Fenice, Reuse of microbially treated olive mill wastewater as fertilizer for wheat (*Triticum durum* Desf.), Biores. Technol. 91 (2004) 135–140.

[7] R. Capasso, G. Cristinzio, A. Evidente, F. Scognamiglio, Isolation, spectroscopy and selective phytotoxic effects of polyphenols from vegetable wastewater, Phytochemistry 31 (1992) 4125–4128.

[8] A. El Hadrami, M. Belaqziz, M. El Hassni, S. Hanifi, A. Abbad, R. Capasso, L. Gianfreda, I. El Hadrami, Physico-chemical characterization and effects of olive oil mill wastewaters fertirrigation on the growth of some Mediterranean crops, J. Agronomy 3 (2004) 247–254.

[9] D.P. Komilis, E. Karatzas, C.P. Halvadakis, The effect of olive mill wastewater on seed germination after various pretreatment techniques, J. Environ. Manage. 74 (2005) 339–348.

[10] H.K. Lichtenthaler, C. Buschmann, Chlorophylls and carotenoids—measurement and characterization by UV–vis, in: Current Protocols in Food Analytical Chemistry (CPFA), Supplement 1, John Wiley, New York, 2001, pp. F4.3.1–F4.3.8.

[11] G.H. Krause, E. Weis, Chlorophyll fluorescence and photosynthesis: the basics, Annu. Rev. Plant Physiol. Plant Mol. Biol. 42 (1991) 313–349.

[12] G. Ouzounidou, M. Moustakas, L. Symeonidis, S. Karataglis, Response of wheat seedlings to Ni stress: effects of supplemental calcium, Arch. Environ. Contam. Toxicol. 50 (2006) 346–352.

[13] J.R. DeEll, P.M.A. Toivonen, Chlorophyll fluorescence as a nondestructive indicator of broccoli quality during storage in modified-atmosphere packaging, HortScience 35 (2000) 256–259.

[14] S.J. Kays, Postharvest Physiology of Perishable Plant Products, Van Nostrand Reinhold, New York, 1991, p. 78.

[15] F.F. Calegario, R.G. Cosso, F.V. Almeida, A.E. Vercesi, W.F. Jardim, Determination of the respiration rate of tomato fruit using flow analysis, Postharvest Biol. Tech. 22 (2001) 249–256.

[16] A.B. Wold, H.J. Rosenfeld, K. Holte, H. Baugerod, R. Blomhoff, K. Haffner, Colour of post-harvest ripened and vineripened tomatoes (*Lycopersicon esculentum* Mill.) as related to total antioxidant capacity and chemical composition, Int. J. Food Sci. Technol. 39 (2004) 295–302.

[17] G. Ouzounidou, M. Moustakas, R. Strasser, Sites of action of copper in the photosynthetic apparatus of maize leaves: kinetic analysis of chlorophyll fluorescence oxygen evolution, absorption changes and thermal dissipation as monitored by photoacoustic signals, Aust. J. Plant Physiol. 24 (1997) 81–90.

[18] G. Ouzounidou, H.A. Constantinidou, Changes in growth and physiology of tobacco and cotton under Ag exposure and recovery: are they of direct or indirect nature? Arch. Environ. Contam. Toxicol. 37 (1999) 480–487.

[19] C.J. Bernacchi, A.R. Portis, H. Nakano, S. von Caemmerer, S.P. Long, Temperature response of mesophyll conductance. Implications for the determination of rubisco enzyme kinetics and for limitations to photosynthesis in vivo, Plant Physiol. 130 (2002) 1992–1998.

[20] S.V. Von Caemmerer, G.D. Farquhar, Some relationships between the biochemistry of photosynthesis and gas exchange of leaves, Planta 153 (1981) 376–387.

[21] H. Manolopoulou, P. Papadopoulou, A study of respiratory and physicochemical changes of four kiwi fruit cultivars during cool storage, Food Chem. 63 (1998) 529–534.

[22] R. Capasso, A. De Martino, G. Cristinzio, Production, characterization and effects on tomato of humic acid-like polymerin metal derivatives from olive oil mill waste waters, J. Agric. Food Chem. 50 (2002) 4018–4024.

[23] A. Ben Sassi, N. Ouazzani, G.M. Walker, S. Ibnsouda, M. El Mzibri, A. Boussaid, Detoxification of olive mill wastewaters by Moroccan yeast isolates, Biodegradation 19 (2008) 337–346.

[24] G. Aggelis, C. Ehaliotis, F. Nerud, I. Stoychev, G. Lyberatos, G. Zervakis, Evaluation of white-rot fungi for detoxification and decolorization of effluents from the green olive debittering process, Appl. Microbiol. 59 (2002) 353–360.

[25] T. Kistner, V. Jung, E. Olsson, G. Nitz, H. Heubergre, B.W. Aslanius, W.H. Schnitzler, Effect of olive mill wastewater on young hydroponically grown tomato plants, J. Appl. Bot. 78 (2004) 25–31.

[26] M. Ciamporova, Morphological and structural responses of plant roots to aluminium at organ, tissue, and cellular levels, Biol. Plant 45 (2002) 161–171.

[27] C. De Simone, A. De Marco, M. Raglione, C. D'Ambrosio, P. Pitoni, Utilizzazione agronomica delle acque di vegetazione. Tossicita ed influenza su alcune caratteristiche pedologiche, Riv. Agr. 33 (1999) 8–13.

[28] F. Morales, A. Abadia, J. Abadia, Photosynthesis, quenching of chlorophyll fluorescence and thermal energy dissipation in iron-deficient sugar beet leaves, Aust. J. Plant Physiol. 25 (1998) 403–412.

[29] S. Singh, R. Saxena, K. Pandey, K. Bhatt, S. Sinha, Response of antioxidants in sunflower (*Helianthus annuus* L.) grown on different amendments of tannery sludge: its metal accumulation potential, Chemosphere 57 (2004) 1663–1673.

[30] G. Ouzounidou, I. Ilias, Hormone-induced protection of sunflower photosynthetic apparatus against Cu toxicity, Biol. Plant 49 (2005) 223–228.

[31] W.E. Pereira, D.L. de Siqueira, C.A. Martinez, M. Puiatti, Gas exchange and chlorophyll fluorescence in four citrus rootstocks under aluminium stress, J. Plant Physiol. 157 (2000) 513–520.

[32] M. Anza, P. Riga, C. Garbisu, Effects of variety and growth season on the organoleptic and nutritional quality of hydroponically grown tomato, J. Food Qual. 29 (2006) 16–37.

[33] E. Bonari, M. Macchia, L.G. Angelini, L. Ceccarini, The wastewaters from olive oil extraction: their influence on the germinative characteristics of some cultivated and weed species, Agric. Med. 123 (1993) 273–280.

[34] S.W. Lyu, U. Blum, Effects of ferulic acid, an allelopathic compound, on net P, K and water uptake by cucumber seedlings in a split-root system, J. Chem. Ecol. 16 (1990) 2429–2439.