

Impact of Olive Mill Wastewater (OMWW) on Young Olive Trees Growth and Soil

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ABSTRACT

This study aimed the using of olive mill wastewater (OMWW) in agriculture to reduce its ecological negative impact by throwing it directly, and the determination of the appropriate quantities of OMWW used in olive grove irrigation. Two types of OMWW derived from traditional discontinuous system pressure (TDSP) and continuous system in three phases (CSTP) were used for irrigating young olive trees (one year old). The young olive trees (Var. *Soury*) were planted in containers (approximate area 1 m²) on two types of soil (sandy and sandy-clay). The OMWW were spreading for three consecutive years at three different doses 5, 10 and 15 liters per young olive tree, with a control treatment. Physico-chemical characteristics of the soil and of OMWW were analyzed before and after its addition. The impact of OMWW has studied by measuring height growth of young olive trees, by the determination of photosynthetic pigments and mineral contents in their leaves. At the end of the experiment foliar diagnosis was promoted. The application of OMWW at different doses does not adversely affect any of the plant parameters measured. At 10 and 15 L.m⁻², the olive mill wastewater has a positive impact on height growth of olive trees, on the total leaf content of photosynthetic pigments especially chlorophyll "a", on leaf content of phosphorus and potassium, but the leaf nitrogen is deficient and its physiological equilibrium is low. The positive effect had observed on phosphorus and potassium contents of sandy-clay soil, but less on nitrogen, organic matter contents hence the need to increase especially nitrogen fertilization and organic matter amendment. The pH, salinity and the C/N ratio of the soils remained stable.

Key words: Chlorophyll, mineral element, olive mill wastewater, organic matter, young olive tree

Introduction

The production of olive oil is located mainly in Mediterranean countries where the extraction and use of olive oil practiced for over 6000 years are connected to the culture of these countries and their history. Its history back to 8000 years since the first cultivation of olive trees in the region of the Middle East. With the increase in the area of olive groves throughout Lebanon and olive production, the number of mills of olive oil has increased in recent years. In Lebanon, a total of 544 oil mills have been identified including 435 traditional oil mills, about 80% of the total number and 67 mills belonging to the continuous systems, the majority are in three phases. Approximately 50% of these mills are located in North Lebanon, 25% in South Lebanon, 20% in Mount Lebanon and 5% in the Bekaa Valley (Daher *et al.*, 2004). This high number of mills is due, perhaps, to increased olive grove surface and consequently the production of olives.

In addition to the olive oil produced by these mills, two by-products are thus generated, solid waste (olive pomace) and liquid effluents (olive mill wastewater- OMWW-). Consequently, there are differences between olive mill wastewater from traditional systems using discontinuous pressure, those produced by continuous systems with two or three phases. The traditional system is a discontinuous system consisting of pressing the paste by means of hydraulic presses. OMWW or vegetation waters are the brown watery liquid residue which has been separated from the oil by centrifugation or sedimentation after pressing (Fedeli and Camurati, 1981).The system for continuous extraction of three phases has major drawbacks that reside in the greater use of water and therefore an abundant production of OMWW and serious pollution.

The improper disposal of olive mill wastes causes serious environmental impacts to soil, water and air (groundwater pollution, soil pollution, toxicity risks for certain crops ...) and related to its high organic content made up largely of simple phenolic compounds (Zenjari *et al.*, 1999). These wastes are an important source of pollution owing to its relatively high content of organic matter in the form of sugars, tannins, polyphenols, polyalcohols, pectins and lipids (Hamdi, 1993). However, the random and uncontrolled disposal of OMWW poses an environmental problem especially in the case of the continuous three- phase system (Le Verge, 2004).

Many researchers have applied OMWW directly on soil and have tested its effect as an organic fertilizer thus revealing positive and negative effects. The advantages are, first, the rapid decomposition of

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phenolic compounds in the soil (Chartzoulakis *et al.*, 2006) and no accumulation trend was observed after subsequent applications (Chartzoulakis *et al.*, 2010). Because of the wealth of OMWW on organic matter, and nutrients especially potassium (Cegarra *et al.*, 1996; De Montpezat and Denis, 1999) they can substitute in part to a conventional fertilizer and used as organic fertilizer (Bricolli-Bati and Lombardo, 1990; Abichou *et al.*, 2003). Negative effects reside in the high levels of salinity or electrical conductivity and acidity, low pH (Morisot and Tournier, 1986). Some authors have reported the inhibitory effect of olive mill wastewater on plant growth due to the presence of phenolic compounds (Cabrera *et al.*, 1997; Paredes *et al.*, 1999).

In order to reduce the effect of pollution of OMWW and solve the environmental problem of their runoff, several processes techniques have been proposed, among other things, the epuration treatment, the lagoon, animal feed, yeast production, irrigation after dilution, production of biogas, physico-chemical and biological treatments. But these solutions have proven expensive, unreliable and ultimately unworkable in terms of technical and economical for mills to medium-sized or small. A rational and economical alternative to the various process has been proposed, the direct application to soils or "spreading" of OMWW (Zenjari and Nejmeddine, 2001; Di Giovacchino *et al.*, 2002) that allows to restore the soil's nutrients (Cegarra *et al.*, 1996). This solution is considered the best option for the oil mills located in rural areas near the land where olive trees are cultivated. Indeed, this application depends on the characteristics of soil and climatic conditions.

The aim of this study is to use an olive mill wastewater (OMWW) in agriculture to reduce environmental pollution. This research involves the study of the effect of the spreading of OMWW from two different extraction systems (traditional discontinuous system pressure-TDSP- and continuous system in three phases-CSTP-) on the development of young olive trees.

Materials and Methods

The experiment was conducted at the station Fanar, Lebanese Agricultural Research Institute (LARI), located at 250-300 m above sea level. The experiment was conducted with 3 groups treated with different doses of OMWW (5, 10 and 15 L.m⁻²) for three consecutive years and a lot used as a control. The first application was performed after 1 year of transplantation of young olive trees; the second was made after two years, and the third last for the three years old of olive trees. The young olive trees (Var. *Soury*), one year old, from the station Tyr-LARI South Lebanon, are developed in two different soil types into containers with an approximate area of 1.3 m². The characteristics of soils before spreading of OMWW are represented in table 1. Texture of each soil was determined using bouyoucos method (Pansu and Gautheyrou, 2003). Total porosity was calculated by determining the bulk and particles densities (Rowell, 1994). Total calcium carbonate (CaCO₃) was determined by Bernard calcimeter method (Pansu and Gautheyrou, 2003). The two soils are characterized as not calcareous soil (1.5%), with density 1.3-1.4 and porosity about 45%. But, they are some differences for the other parameters; one is fine-textured sandy clay with higher content in nitrogen and organic matter than in the other medium-textured sand (Table 1).

Table 1: Characteristics of soils before the spreading of OMWW

Parameters	Medium-textured Sandy soil	Fine-textured Sandy-clay soil
pHwater (1:2.5)	7.88-7.73	7.94-8.05
Electrical Conductivity -EC _{saturated 1:5} - (mS.cm ⁻¹)	0.87	1.13
N (kg.ha ⁻¹)	1173.96	2062.06
P ₂ O ₅ (kg.ha ⁻¹)	269.6	138.11
K ₂ O (kg.ha ⁻¹)	594.14	401.62
Carbon (%)	0.266	0.552
Organic Matter –OM- (%)	0.457	0.952
C/N	5.32	6.4

Physico-chemical analysis of olive mill wastewater (OMWW)

Before the spreading of OMWW untreated in the soil, their physicochemical characteristics have been analyzed. Both types of OMWW came from two olive oil mills located in the region of "Zghorta El Zawiya" in Northern Lebanon. These olive mills waste water came from two different extraction systems, a traditional discontinuous system pressure (TDPS) and a continuous system in 3 phases (CSTP). The OMWW have been with no previous treatment and were stored at 4°C to determine some of their physicochemical characteristics. Analyses were made with respect to the fresh matter. The following parameters were determined: density was determined by simple weighing a certain volume of the crude solution, pH, electrical conductivity (EC), total mineral and organic fractions were determined by evaporation of OMWW raw, total nitrogen (Nt) by Kjeldahl method (Rodier, 1996). Organic matter was determined by calcination at 500°C for 24 hours (Pauwels *et al.*, 1992; Navarro *et al.*, 1993). Total phosphorus and potassium were determined by sulfo-nitro-perchloric

digestion (Beley, 1948; Hamzé *et al.*, 1984). Total phosphorus was determined by spectrophotometry and total potassium by flame emission spectrophotometry (Rodier, 1996). Polyphenolic compounds were extracted by ethyl acetate (Balice and Cera, 1984), and were assayed by spectrophotometry at a wavelength of 765 nm using Folin-Ciocalteu reagent (Maestro Duran *et al.*, 1991; Montedoro *et al.*, 1992; Paredes *et al.*, 1999), total polyphenols are expressed as the equivalent of tyrosol.

After each application, the mineral element as nitrogen, phosphorus and potassium of leaching wastewater for each dose of OMWW from traditional discontinuous system pressure was determined.

Physiological aspects of olive tree

After the second application, height growth of three years old of young olive trees was measured, in November, from the base of the main stem to the top of the longest twig. The percentage annual height growth was calculated by the following formula:

$$\text{Percentage annual height growth} = \frac{[(\text{height growth at } t_2 - \text{height growth at } t_1) / \text{height growth at } t_1] * 100}{}$$

where t_1 and t_2 are the two dates after transplanting.

After the second and third application, for the three and four year old olive tree, leaves were collected from the sampling foliage for photosynthetic pigments analysis (chlorophylls and carotenoids) and were quantified in June and August. For the extraction of chlorophyll, a dozen leaves, at all levels of olive trees, were taken at random from each young tree in June and August. The leaf blades of each tree were carefully cutting and mixed well. Sub-samples were weighed and placed in Erlenmeyer- flasks containing 30 ml of pure methanol. These Erlenmeyer- flasks were closed, and then placed in a refrigerator at 4°C. After a week, there was total extraction of chlorophyll dissolved in the solvent in which the pieces of leaf samples became whitish. Chlorophyll "a", chlorophyll "b" and carotenoids concentration were determined by spectrometry using the UV-Visible spectrophotometer at different wavelengths (663, 645, 460 nm) which would be the specific absorption of photosynthetic pigments (Lichtenthaler, 1987). The content of pigments was determined based on fresh material. The determination of total chlorophyll concentration (Cchl.) is based on the following formula (Arnon, 1949):

$$Cchl. = A_{652} \times Vchl / 34.5$$

Cchl.: Chlorophyll concentration (mg chl.ml⁻¹)

A₆₅₂: the value of the absorbance when measuring with a spectrophotometer

Vchl.: The volume of the solution of chlorophyll (ml)

At the end of the experiment, for the four year- old olive tree, foliar diagnosis realized on leaves located at the half central twig was promoted in November by analyzing some of the major elements as nitrogen, phosphorus and potassium. According "Lagatu and Maume", "Physiological Balance" or "Physiological Equilibrium"=

$$\% \text{ Element} \times 100 / \text{Global Nutrition (S)} \text{ (Bouat, 1984)}$$

which is considered as percentage was calculated for foliar diagnosis; where "Global Nutrition" = S = N + P₂O₅ + K₂O. The percentages are compared to an "Optimum Experimental Practical" which is used as a basis of comparison for the interpretation of foliar diagnosis of the olive tree.

Yield of dry matter of leaves and twigs were determined by calculated the wet and dry weight of each young olive tree. In the final assessment, concentrations of plant nutrients in the plants were determined. The total aerial parts of the young olive trees were harvested and then separated into leaves, twigs and stems. After being oven dried at 70°C to a constant weight, the dry matter of aerial parts was determined and then ground to pass through 1mm sieve for further analysis. Ash content was determined by ignition at 550-600°C for 24 hours (Cambardella *et al.*, 2001). Total N content was determined by the Kjeldahl method after the digestion of the plant material with a mixture of salicylic acid and sulfuric acid plus sodium thiosulfate to include nitrate and nitrite (Bremner and Mulvaney, 1982). After plant sample mineralization with sulfo-nitro-perchloric acid mixture (Beley, 1948; Hamzé *et al.*, 1984), Total phosphorus content was determined by spectrophotometry using the phospho-vanadomolybdic complex, total potassium content was determined by flame emission spectrophotometry (Rodier, 1996; Pansu and Gautheyrou, 2003).

Soil Analysis

At the end of the experiment and after removing the young four year old olive trees, soil samples were taken from each container after being mixed completely. Then, they were air-dried, mixed and passed through a 2 mm sieve for further analysis. pH of the aqueous suspension extract soil / water (1:2.5; mass:volume) was

measured (Rowell, 1994). Electrical conductivity (EC) was determined on an aqueous extraction solution with a ratio 1:5 (mass:volume) (AFNOR, 1987; Rhoades and Miyamoto, 1990; Rowell, 1994). Total organic carbon was determined by wet digestion after chromic acid oxidation using Walkley and Black Method and Method NF ISO 10694- and organic matter was obtained from the carbon content (Nelson and Sommers, 1982; Navarro *et al.*, 1993; Rowell, 1994; Ryan *et al.*, 1996; Tandon, 1998; Pansu et Gautheyrou, 2003). Total Nitrogen was determined by Kjeldahl method (Rowell, 1994; Pansu and Gautheyrou, 2003). Exchangeable potassium, sodium, calcium and magnesium were extracted using the Schollenberger and Simon method (Schollenberger and Simon, 1945) by acetate ammonium extraction with a ratio (1:10) (mass:volume). Exchangeable potassium and sodium were determined by flame photometry and calcium, Magnesium by compleximetry method (Knudsen *et al.*, 1982; Rowell, 1994). Available phosphorus was determined by Olsen's method with sodium bicarbonate extraction and ratio 1:20 (mass:volume) (Rowell, 1994; Ryan *et al.*, 1996; Tandon, 1998; Pansu and Gautheyrou, 2003)

Statistical Analysis

The distribution of treatments was performed according to the statistical scheme "RCD" (Complete Randomized Design) with 3 replicates. The statistical interpretation was performed by analysis of variance examined (ANOVA), following the procedure of general linear model (GLM) statistical program SAS (Statistical Analysis System, 2004). Differences between the treatment means were measured by calculating the least significant difference (LSD) at 5%. The analysis of the differences between bilateral transactions has been following the protocol of bilateral comparisons analysis (Contrast Analysis).

Results and Discussion

Main features of Olive Mill Wastewater (OMWW)

Variability in the physico-chemical composition of OMWW is usually contributed to genotypic differences in fruit composition, maturity stage at harvest, mechanical damage due to the harvesting technique and modification of the composition of olives during storage, the differences in the different processing units of the mill oil and the extraction technique of olive oil (Cabrera *et al.*, 1996).

The OMWW is a liquid waste acid and organic load salt. Slightly elevated salinity is mainly due to potassium ions. As shown in Table 2, both types of OMWW are characterized primarily by an acidic pH, high salinity and richness especially in potassium. But these levels are more pronounced in the OMWW from discontinuous system pressure than in continuous system of three phases. Nitrogen and phosphorus are also present but at concentrations much lower but considering as significant. The phenolics compounds are considered responsible for producing toxic phenomena (Garcia-Ortiz *et al.*, 1999; Komilis *et al.*, 2005) for plants or in any way to limit biological activity in the root area (Noy and Feinmesser, 1977). However, they are present in these olives mill wastewater but at lower level. In general, the effluent is cloudy, and the dark color (black or brown) of OMWW is due to the presence of phenolic compounds that are formed during the grinding of the olives during the process of oil extraction (Ranalli, 1991). In addition, the color depends on the age, nature of olives pressed and the extraction technique used (Hamdi and Garcia, 1993). According to "Le Verge", (2004), it should be noted that the composition of OMWW varies depending on the year and that the characterization of OMWW remains very difficult because of the presence of several variability factors as the varieties of olives, the stage of maturity, their water content, water washing, and storage of OMWW. This characterization can also be influenced by other factors such as weather's conditions and use of pesticides and fertilizers. Overall, these products from the olive industry can be used as organic fertilizers in agricultural soils in order of their elimination and improving soil fertility (Paredes *et al.*, 1999).

After the applying of OMWW from TDSP to soils, concentrations of nitrogen, phosphorus and potassium are negligible in the leaching wastewater collected (Table 3). These macroelements are fixed by the soil. These results indicate that OMWW can enrich the soil in these elements.

Growth height

The application of OMWW used in this study at different doses does not adversely affect the young olive trees. However, no visual symptoms of toxicity were observed in these olive trees, except for the dose of 15 L.m⁻². At the higher dose (15 L.m⁻²), some of these plants had a browning and falling lower leaves, but after a few days their growth and development become normal. No alterations such as leaf colour or other symptoms due to phytotoxic effects were observed on the leaves of olive trees (Nasini *et al.*, 2006).

Table 2: Characteristics of Olive Mill WasteWater (OMWW) used

Parameters	Traditional discontinuous system pressure -TDSP-	Continuous system in three phases -CSTP-
Color	Brown-black	Brown-reddish
Density (g.cm ⁻³)	1.05	1.01
pH	5.48	5.60
Electrical Conductivity-EC- (mS.cm ⁻¹)	9.15	6.28
Total Polyphenol- (mg.kg ⁻¹ tyrosol)	409	157.89
Total dry residu (g.L ⁻¹)	123.20	45.91
Mineral fraction (g.L ⁻¹)	11.59	4.85
Organic fraction (g.L ⁻¹)	111.61	41.06
Carbon (%)	50.29	49.94
Organic Matter (%)	90.52	89.89
Total potassium (% fresh matter)	0.409	0.203
Total phosphorus (% fresh matter)	0.0441	0.0192
Total Nitrogen (% fresh matter)	0.0149	0.015

Table 3: Characteristics of drained water of treatments received OMWW from traditional discontinuous system pressure-TDSP-

OMWWW from TDSP (L.m ⁻²)	5	10	15
pH	6.69	6.23	6.49
Electrical Conductivity-EC- (mS.cm ⁻¹)	2.87	2.27	3.96
Carbon (%)	43.24	44.46	46.81
Total dry residu (g.L ⁻¹)	9.13	8.09	20.04
Mineral fraction (g.L ⁻¹)	1.99	1.44	3.15
Organic fraction (g.L ⁻¹)	7.14	6.17	16.89
Total potassium (% fresh matter)	0.002	0.001	0.0005
Total phosphorus (% fresh matter)	0.00	0.00	0.00
Total Nitrogen (% fresh matter)	0.00	0.006	0.00

The annual height growth of olive trees treated with the OMWW-TDSP- is faster than those treated with the OMWW-CSTP-. For this parameter, significant differences were observed between treatments of OMWW of traditional discontinuous system pressure (10, 15 L.m⁻²) on the one hand and those that received the OMWW of continuous system in three phases (5, 10, 15 L.m⁻²) and controls on the other hand. In sandy-clay soil, and for olive trees treated with the OMWW- TDSP-, the differences are significant between olive trees that received 10 L.m⁻² and those treated with 15 L.m⁻². Height growth is more important for the dose of 10 L.m⁻². It appears that the higher dose of 15 L.m⁻² has affected the height growth of olive trees (Table 4). In other studies, the growth of young olive trees was not reduced with doses of 16 L.m⁻² (Marsilio *et al.*, 1990). However, many studies, consistent with the results obtained in this study showed that the application of OMWW at adequate periods and doses would improve the growth, the development, and yield cultures. Many studies have shown the positive effects of the spreading of OMWW, either directly or after treatment, on land planted with cereals or other annual crops (maize, wheat, tomato, ryegrass) (Garcia-Ortiz *et al.*, 1999; Ammar and Ben Rouina, 1999; Hanifi and El Hadrami, 2006), and on soil planted with grapes and olive trees (Marsilio *et al.*, 1990; Ben Rouina, 1994; Chartzoulakis *et al.*, 2006; Bedbabis *et al.*, 2006). In addition, the use of OMWW at doses above 10 L.m⁻², had achieved a higher total biomass from 30 to 40% of that obtained on control plots and prevent or reduce the use of chemical fertilizers (Di Giovacchino *et al.*, 2002). Furthermore, the application of OMWW did not have a negative effect on the production of olive plants. However, total production of olives for 3 year old plants in all treatments was approximately 2158 grams, which equates to an average production of 65 grams per plant. The average production was approximately 53 grams at the previous year for 2 year old plants.

Photosynthetic Pigments contents

On June, for the 3 years-old olive trees developed on the two types of soils and received OMWW for two years, the content of total chlorophyll pigments (Chl. "a" + Chl. "b") of leaves show significant differences between the controls of olives trees developed on the two types of soils (Table 5). The total chlorophyll content is higher in the olive trees developed on sandy - clay soil than that found in those developed on a sandy soil. Total chlorophyll content show a significant differences between olives trees that have received the different doses of TDSP and are developed on the sandy soil and their controls. The amounts of total chlorophylls are higher in the treated olive trees than in the controls. By contrast, there were no significant differences between different treated olive trees whatever the doses used. For the olive trees developed on sandy- clay soil, only 15 L.m⁻² of TDSP had a positive effect by increasing the content of chlorophyll "a" while showing significant differences with those control and those treated by doses 5 and 10 L.m⁻² of TDSP. For olive trees that have received CSTP and are developed on sandy soil, chlorophylls a and b contents show significant differences between treated olive trees and those of controls. For olive trees developed on sandy clay soil these chlorophyll

pigments did not show significant differences between the treated trees and those of controls. The carotenoids were not affected by the addition of OMWW.

Table 4: Comparison of annual growth in height (cm) of young olive trees treated with different olive mill wastewater

Treatment by OMWW 2 times (1 time.year ⁻¹)	Growth in height (cm)	
	Sandy soil	Sandy-clay soil
Control	11.61 ¹ ef ²	8.83 ef
OMWW-TDSP- (L.m⁻²)		
5	33.87 abcd	29.7 abcde
10	48.88 a	38.03 abc
15	41.49 ab	15.96 def
OMWW- CSTP- (L.m⁻²)		
5	15.21 def	16.16 def
10	24.14 bcdef	7.20 f
15	18.77 cdef	20.12 cdef
Signification³	**	
Least Significant Difference- LSD-	21.18	

¹Each value is a mean of 3 samples

²Values followed by different letter differ significantly at the 5% level; values followed by the same letter do not differ significantly at the 5% level

³Significant at the probability levels of p<0.05: NS not significantly; * significant; **highly significant; ***very highly significant

Table 5: Comparison of chlorophylls (Chl.) and carotenoids (Car.) contents (mg.g⁻¹ Fresh Matter) of 3 year old olive trees treated 2 times (once per year) with olive mill wastewater (OMWW) measured on June

	3 years after plantation (measured on June)							
	Chl. "a"	Chl. "b"	Total Chl.	Car	Chl. "a"	Chl. "b"	Total Chl.	Car.
	Sandy soil				Sandy clay soil			
Control	0.442 ¹ c ²	0.141 c	0.600 c	0.123 c	0.811 b	0.267 ab	1.120 b	0.175 abc
OMWW-TDSP (L.m⁻²)								
5	0.818 b	0.271 ab	1.127 b	0.184 abc	0.757 bc	0.254 ab	1.053 b	0.172 abc
10	0.829 b	0.274 ab	1.143 b	0.183 abc	0.767 b	0.256 ab	1.061 b	0.168 abc
15	0.861 b	0.284 ab	1.196 b	0.186 abc	1.194 a	0.310 a	1.588 a	0.231 a
OMWW- CSTP(L.m⁻²)								
5	0.730 bc	0.234 ab	0.995 b	0.164 abc	0.710 bc	0.233 ab	0.979 bc	0.164 abc
10	0.781 b	0.251 ab	1.063 b	0.177 abc	0.703 bc	0.223 bc	0.962 bc	0.157 bc
15	0.764 b	0.245 ab	1.116 b	0.160 bc	0.952 ab	0.315 a	1.311 ab	0.217 ab
Signification³	NS	NS	*	NS	NS	NS	*	NS
LSD	0.32	0.08	0.38	0.07	0.32	0.08	0.38	0.07

¹Each value is a mean of 3 samples

²Values followed by different letter, in the same column, differ significantly at the 5% level; values followed by the same letter do not differ significantly at the 5% level

³Significant at the probability levels of p<0.05: NS not significantly; * significant; **highly significant; ***very highly significant

On August, for the 3 years old olive trees developed on the two types of soils and received OMWW for two years, olive trees that have received 10 to 15 L.m⁻² of the TDSP and are developed on the sandy soil showed significant differences with those of controls for the chlorophylls (Table 6). The total chlorophyll content is higher in the olive trees developed on sandy - clay soil than that found in those developed on a sandy soil. The amounts of the total chlorophyll are higher in the treated lots especially on those treated with 15 L.m⁻². For these trees developed on sandy clay soil, only 15 L.m⁻² had a positive effect by increasing the content of chlorophylls "a" and "b" while showing significant differences with those of control and those treated by doses of 5 and 10 L.m⁻². For olive trees treated with CSTP and are developed on sandy soil, only the dose 15 L.m⁻² had showed a positive effect on the content of total chlorophylls and chlorophylls "a" and "b" compared to those of the control. For olive trees developed on sandy clay soil, significant differences are observed between the olive trees treated by 15 L.m⁻² and those of control concerning the chlorophyll "b" and total chlorophyll. The carotenoids were not affected by the addition of OMWW.

In general, the amount of total chlorophylls (Chl "a" + Chl "b") is higher on leaves of olive trees treated with both types of OMWW than in controls, particularly for doses of 10 and 15 L.m⁻². Note that all olive trees of all treatments showed the amounts of chlorophyll "a" higher than those of chlorophyll "b" and carotenoids, which indicated that chlorophyll assimilation and plant growth are normal. In addition, between June and August, the evolution of chlorophyll pigments is decreasing and the decrease in quantity is almost the same between treatment and control groups. For treated olive trees, the amount of carotenoids is not affected by the spreading of the two types of OMWW. By cons, no significant differences were noted for carotenoids between control olive trees on both soil types and those treated with both types of OMWW.

Table 6: Comparison of chlorophylls (Chl.) and carotenoids (Car.) contents (mg.g⁻¹ Fresh Matter) of 3 year old olive trees treated 2 times (once per year) with olive mill wastewater (OMWW) measured on August

	3 years after plantation (measured on August)							
	Chl. "a"	Chl. "b"	Total Chl.	Car.	Chl. "a"	Chl. "b"	Total Chl.	Car.
	Sandy soil				Sandy clay soil			
Control	0.373 ¹ d ²	0.128 d	0.521 d	0.100 b	0.600 bcd	0.209 c	0.853 c	0.157 ab
OMWW-TDSP- (L.m⁻²)								
5	0.579 bcd	0.202 c	0.813 cd	0.124 b	0.633 bc	0.220 bc	0.889 c	0.149 b
10	0.666 bc	0.227 bc	1.004 bc	0.133 b	0.694 bc	0.244 bc	0.977 bc	0.160 ab
15	0.685 bc	0.240 bc	0.962 bc	0.149 b	0.942 a	0.323 a	1.318 a	0.194 ab
OMWW- CSTP- (L.m⁻²)								
5	0.550 cd	0.197 cd	0.785 cd	0.130 b	0.686 bc	0.219 bc	0.863 c	0.148 b
10	0.584 bcd	0.201 cd	0.818 cd	0.135 b	0.641 bc	0.226 bc	0.906 bc	0.149 b
15	0.652 bc	0.223 bc	0.900 c	0.159 ab	0.803 ab	0.283 ab	1.210 ab	0.177 ab
Signification ³	*	*	**	NS	*	*	**	NS
LSD	0.23	0.07	0.31	0.11	0.23	0.07	0.31	0.11

¹Each value is a mean of 3 samples

²Values followed by different letter, in the same column, differ significantly at the 5% level; values followed by the same letter do not differ significantly at the 5% level

³Significant at the probability levels of p<0.05: NS not significantly; * significant; **highly significant; ***very highly significant

On August, for the 4 years old olive trees developed on the two types of soils and received OMWW for three years, the differences concerning the photosynthetic pigments are likely as for the 3 years old olive trees but less pronounced. The differences are significant between the controls and the olive trees that have received only 15 L.m⁻² of the TDSP and CSTP concerning total chlorophyll, chlorophyll a, chlorophyll b and carotenoids, especially in the sandy soil (Table 7). The amounts of total chlorophyll are higher in the lots treated with 15 L.m⁻². In generally, the amounts of chlorophyll "a" are higher in the lots especially treated with 15 L.m⁻². After 4 years, the differences between the controls of the two soils become insignificant. Olive trees developed on control soils show significant differences between them only at the level of carotenoids. Olive trees developed on sandy soil and have received 10 to 15 L.m⁻² of TDSP have showed significant differences with those of control especially for chlorophyll "a". Only the dose of 15 L.m⁻² had showed a positive effect on chlorophylls "a" and "b" compared to the olive trees treated with 5 and 10 L.m⁻². These differences were noted for carotenoids between 10 and 15 L.m⁻² on the one hand, and the dose of 5 L.m⁻² on the other hand. For olive treated with CSTP, doses of 5 and 15 L.m⁻² showed a positive effect compared to those of the control concerning the chlorophyll "a". By cons, significant differences were not observed between different treatments for chlorophyll a and b, but the carotenoids are higher in treated than in the controls. Olive trees developed on sandy clay soil and treated by TDSP does not show significant differences with those of the controls for chlorophyll 'a', but significant differences are noted, especially for chlorophyll b and at least for carotenoids. The amounts of chlorophyll "b" are greater in treated lots more than in the control lots, but the amount of carotenoids are less than in olive treated with 5 L.m⁻² compared to other lots. For olive trees treated with CSTP, the dose of 15 L.m⁻² showed significant differences with the controls and those treated with 10 L.m⁻² for chlorophyll "a". For other pigments there no significant differences between different treatments.

Table 7: Comparison of chlorophylls (Chl) and carotenoids (Car) contents (mg.g⁻¹ Fresh Matter) of 4 year old olive trees treated 3 times (once per year) with olive mill wastewater (OMWW) measured on August

	4 years after plantation (measured on August)							
	Chl. "a"	Chl. "b"	Total Chl.	Car.	Chl. "a"	Chl. "b"	Total Chl.	Car.
	Sandy soil				Sandy clay soil			
Control	0.398 ¹ e ²	0.177 f	0.603 d	0.083 e	0.517 cde	0.186 ef	0.737 cd	0.131bcd
OMWW-TDSP- (L.m⁻²)								
5	0.523bcde	0.195cdef	0.750bcd	0.116cde	0.549bcde	0.269 ab	0.807bcd	0.087 e
10	0.597 bcd	0.218abcde	0.854bcd	0.137bcd	0.651abcd	0.262abc	0.931abc	0.132bcd
15	0.781 a	0.280 a	1.11 a	0.180 a	0.691 abc	0.258abcd	0.995 ab	0.155 ab
OMWW- STP- (L.m⁻²)								
5	0.586 bcd	0.209 bcdef	0.831bcd	0.136bcd	0.578bcde	0.218abcde	0.834bcd	0.128bcd
10	0.488 de	0.190 def	0.713 cd	0.115 de	0.505 de	0.186 ef	0.724 cd	0.126bcd
15	0.612abcd	0.225abcde	0.879abc	0.141bcd	0.701 ab	0.255 abcde	1.002 ab	0.154abc
Signification ³	*	*	*	***	*	*	*	***
LSD	0.18	0.07	0.25	0.04	0.18	0.07	0.25	0.04

¹Each value is a mean of 3 samples

²Values followed by different letter, in the same column, differ significantly at the 5% level; values followed by the same letter do not differ significantly at the 5% level

³Significant at the probability levels of p<0.05: NS not significantly; * significant; **highly significant; ***very highly significant

The amount of total chlorophyll is higher on the olive trees treated with OMWW-TDSP- in the sandy soil and on those treated with OMWW-CSTP- in the sandy-clay soil especially by 15 L.m⁻² than on those of

control lots. The olive mill wastewater has a positive effect on the formation of photosynthetic pigments and especially on chlorophyll "a" and especially for the doses of 15 L.m⁻². The husk did not substantially affect photosynthesis (Nasini *et al.*, 2006). Christenson *et al.*, (2000), have showed no significant difference in chlorophyll composition of compost-treated plants (sugar beet, soybean, corn, field bean, and wheat). It could be concluded that there was enough N and Mg for the chlorophyll synthesis. Chlorophylls a and b are generally found associated with each other in the green leaves and the ratio (chlorophyll "a": chlorophyll "b") is usually (2:1), but not invariably (Jackson, 1976). The chlorophyll "a": chlorophyll "b" ratio of the various treatments in our study was approximately (2.7:1) and (3:1) and there was no significant difference between treatments regarding this ratio. Chang and Tai, (1979), reported that the chlorophyll "a": chlorophyll "b" ratio of the leaf blades of rice plants at various growth stages varied between "2.4 - 3.4".

Aerial parts analysis

The following results concerned comparison treatments of four years old olive trees that have treated with OMWW during three years (once time every year). For the production of the foliar dry matter, there are no significant differences between the treatments control of the two types of soil and other transactions. The foliar dry matter was higher in treatment of soil sandy-clay that was added to it 10 to 15 liters.m⁻² of OMWW- CSTP- than in treatment of the sandy soil which was added to it 15 liters.m⁻² of OMWW-TDSP-. The differences are insignificant between the two controls of soil for the production of branches and twigs (woody parts as dry matter), and as well as between the treatments of sandy soils that received the two types of OMWW. However, a significant difference was observed between the treatments of sandy-clay soil, where it came from their production is higher in the treatment of OMWW-TDSP- (10 L.m⁻²) than on the treatment with OMWW-CSTP- (15 L.m⁻²). The latter one showed significant differences with the control. Comparing the production of total aerial dry matter (foliar + wood as leaves + branches and twigs) of young olive trees, we noted the absence of significant differences between the two controls of soils and between the treatments of sandy soils which received the two types of OMWW and their control. There are significant differences between the control of sandy-clay soil and the treatments of both OMWW at 15 L.m⁻², and especially among the OMWW-TDSP- of 10 liters.m⁻² and the other treatments. It appears that the dose of 15L.m⁻² has affected the production of total aerial dry matter. Nevertheless these differences come from those governing the production of wood (Table 8).

For the foliar diagnosis realized by analysis the major elements as nitrogen, phosphorus and potassium, it is found that foliar nitrogen content showing the absence of significant differences between the various treatments (Table 9). For phosphorous, there are no significant differences between the control treatments of soils, but the presence of significant differences is showed between the two controls on the one hand and the treatment of the sandy soil with quantity of 5 liters.m⁻² of OMWW-TDSP- and the treatments of sandy-clay soil with quantities of 5 and 15 liters.m⁻² of OMWW-CSTP- on the other hand. Nevertheless, the percentage of phosphorus content is higher in all treatments than in the two controls. For potassium, the absence of significant differences between the two controls of soils was showed, but with significant differences between the treatments of OMWW -TDSP- 10 and 15 L.m⁻² of sandy soil. In the latter two the proportion of potassium is higher than in the control, the treatment of 5 L.m⁻² and the other transactions. But for the OMWW -CSTP- treatments, the proportion of potassium is higher in the treatment of 15 L.m⁻² than in the treatment of 10 L.m⁻². There are also significant differences in the transactions of the sandy-clay soil, where the proportion of potassium is highest in the treatment of -TDSP- OMWW 5 and 15 L.m⁻² than in the control, as though the percentage is higher in the treatment of 5 L.m⁻² of OMWW -TDSP- than in other treatments, including OMWW -TDSP- and -CSTP-. It can be said that the proportion of foliar potassium content vary varies between the different treatments, but it is the lowest in the two controls.

Overall, the estimated optimum balance of the olive tree through foliar diagnosis is for nitrogen (N) between 45-66%, for phosphorus (P₂O₅) 9-11% and for potassium (K₂O) between 27-33% (Bouat,1984) By comparing the "Physiological Equilibrium" to "Optimum Experimental Practical", it appears to almost all treatments, that overall nutrition is not good and incomplete, especially for nitrogen balance and ranges between 33-48%, while the balance of phosphorus (14-23%) and potassium (35-48%) is within an optimal balance and sometimes higher. And thus to increase the percentage of the total nutrition in this experiment, it should increase the proportion of nitrogen fertilization either increase the amount of OMWW or another source of nitrogen. Furthermore, the physiological equilibrium of different elements must be follow-up (Table 10).

Effects of OMWW on the physicochemical properties of soils

As mentioned before (Table 1), the two soils are characterized as not calcareous soil (1.5%), with density 1.3-1.4 and porosity about 45%. But, they are some differences for the other parameters. The one is medium-textured sand, the other fine-textured sandy-clay.

Before adding OMWW to the two types of soil in each container, we can observe no significant differences of the different physico-chemical parameters (nitrogen, phosphorus, potassium, calcium, magnesium, sodium,

cation exchange capacity (CEC), organic matter, pH and electrical conductivity). Only the proportion of phosphorus content is lower in the sandy-clay than in the sandy soil (Tables 11 and 12).

Table 8: Comparison of aerial parts production (leaves and woods) (as % Dry Matter) of 4 years old olive trees treated 3 times (once per year) with olive mill wastewater on two different soils

4 years old olive trees	Leaves parts % DM	Woody parts % DM	Total Aerial parts % DM
Sandy soil			
Control	56.33 ¹ ab ²	59.73 abc	58.68 abc
OMWW-TDSP- (L.m⁻²)			
5	54.92 ab	58.97 abc	57.40 abcd
10	54.22 ab	55.88 bcd	55.13 bcd
15	52.34 b	62.06 abc	58.35 abc
OMWW- CSTP- (L.m⁻²)			
5	54.94 ab	54.09 bcd	54.41 cd
10	55.96 ab	57.29 abcd	57.10 abcd
15	54.92 ab	54.93 bcd	55.02 bcd
Sandy clay soil			
Control	55.33 ab	66.99 ab	62.42 ab
OMWW-TDSP- (L.m⁻²)			
5	53.62 ab	56.82 abcd	55.28 bcd
10	54.69 ab	69.09 a	63.37 a
15	55.00 ab	54.05 bcd	54.20 cd
OMWW- CSTP- (L.m⁻²)			
5	54.42 ab	54.82 cd	53.45 cd
10	57.66 a	54.03 bcd	55.57 abcd
15	57.27 a	44.38 d	50.27 d
Signification³	NS	NS	NS
LSD	4.12	13.06	7.82

¹Each value is a mean of 3 samples

²Values followed by different letter, in the same column, differ significantly at the 5% level; values followed by the same letter do not differ significantly at the 5% level

³Significant at the probability levels of p<0.05: NS not significantly; * significant; **highly significant; ***very highly significant

Table 9: Comparison of foliar nitrogen, phosphorus, potassium, calcium and magnesium contents (as % Dry Matter) of 4 years old olive trees treated three times (once per year) with OMWW

	Foliar Content (% DM) 4 years old olive trees treated 3 times (once per year)				
	N	P	K	Ca	Mg
Sandy soil					
Control	0.737 ¹ a ²	0.124 b	0.652 cde	1.934 bc	0.323 abc
OMWW-TDSP- (L.m⁻²)					
5	0.797 a	0.210 a	0.742 bc	1.711 cde	0.442 a
10	0.752 a	0.192 ab	0.896 a	1.984 bc	0.128 c
15	0.864 a	0.167 ab	0.957 a	1.374 de	0.216 bc
OMWW- CSTP- (L.m⁻²)					
5	0.992 a	0.186 ab	0.658 cde	1.903 bc	0.314 abc
10	0.774 a	0.192 ab	0.557 e	2.0957 bc	0.203 bc
15	0.864 a	0.178 ab	0.736 bc	1.695 cde	0.159 c
Sandy clay soil					
Control	0.916 a	0.118 b	0.585 de	2.689 a	0.414 ab
OMWW-TDSP- (L.m⁻²)					
5	0.948 a	0.157 ab	0.849 ab	2.057 bc	0.336 abc
10	0.975 a	0.178 ab	0.659 cde	2.017 bc	0.252 abc
15	0.954 a	0.182 ab	0.737 bc	1.277 e	0.284 abc
OMWW- CSTP- (L.m⁻²)					
5	0.938 a	0.229 a	0.678 cde	2.271 ab	0.257 abc
10	1.097 a	0.178 ab	0.693 cd	2.237 ab	0.342 abc
15	1.084 a	0.227 a	0.695 cd	1.839 bcd	0.403 ab
Signification³	NS	NS	***	***	NS
LSD	0.37	0.083	0.14	0.50	0.23

¹Each value is a mean of 3 samples

²Values followed by different letter, in the same column, differ significantly at the 5% level

³Significant at the probability levels of p<0.05: NS not significantly; * significant; **highly significant; ***very highly significant

Table 10: Global Nutrition and Physiological Equilibrium of 4 years old of olive trees treated with olive mill wastewater

	Global Nutrition = S	Physiological Equilibrium = % Element * 100 / S			Quantity of element = % Element * dry weight / 100			
4 years old olive trees treated 3 times (once per year)								
Sandy soil	N + P₂O₅ + K₂O	% N	% P₂O₅	% K₂O	Q N	QP₂O₅	QK₂O	QTotal
Control	1.80 ¹ b ²	40.69 bc	15.93 bc	43.77 ab	0.88 d	0.33 h	0.92 fg	2.13 e
OMWW-TDSP- (L.m⁻²)								
5	2.17 ab	36.850 cd	22.23 a	40.92bcd	1.26 cd	0.76cdefg	1.43cdefg	3.44 bcde
10	2.27 ab	33.27 d	19.12 abc	47.62 a	1.76 abcd	0.93abcde	2.54 ab	5.23 abcd
15	2.39 a	36.08 cd	15.97 bc	47.95 a	1.81 abcd	0.80 bcdef	2.42 abc	5.02 abcd
OMWW- CSTP- (L.m⁻²)								
5	2.21 ab	45.35 ab	18.92 abc	35.73 cd	1.21 cd	0.50 fgh	0.96 efg	2.67 e
10	1.88 ab	41.28 bc	23.10 a	35.62 cd	0.99 d	0.57 efgh	0.86 g	2.42 e
15	2.16 ab	39.86 bcd	18.60 abc	41.55 abc	1.30 cd	0.65 defgh	1.41cdefg	3.37 cde
Sandy clay soil								
Control	1.89 ab	48.30 a	14.09 c	37.61 bcd	1.45 bcd	0.41 gh	1.15 defg	3.01 de
OMWW-TDSP- (L.m⁻²)								
5	2.33 ab	40.65 bc	15.58 bc	43.77 ab	2.52 ab	0.94abcde	2.70 a	6.16 a
10	2.17 ab	44.678 ab	18.64 abc	36.68 cd	2.69 a	1.08 abc	2.17 abcd	5.93 a
15	2.25 ab	42.22 abc	18.49 abc	39.29 bcd	1.69 abcd	0.74 cdefg	1.58 bcdefg	4.02abcde
OMWW- CSTP- (L.m⁻²)								
5	2.28 ab	41.08 bc	22.40 a	36.52 cd	2.16 abc	1.14 ab	1.99 abcdef	5.29 abcd
10	2.34 ab	44.99 ab	18.32 abc	36.69 cd	2.56 a	0.98 abcd	1.99 abcde	5.53 abc
15	2.44 a	44.20 ab	21.16 ab	34.64 d	2.52 ab	1.22 a	1.97 abcdef	5.72 ab
Signification³	NS	**	NS	**	*	***	**	**
LSD	1.80	6.85	5.62	6.43	1.10	0.38	1.07	2.31

¹Each value is a mean of 3 samples

²Values followed by different letter, in the same column, differ significantly at the 5% level

³Significant at the probability levels of p<0.05: NS not significantly; * significant; **highly significant; ***very highly significant

Table 11: Chemicals characteristics (nitrogen, phosphorus, potassium, calcium, magnesium, sodium, cation exchange capacity) of two different soils before the plantation of olives plants and the spreading of olive mill wastewater (OMWW)

Before plantation and spreading of OMWW							
	N (ppm)	P₂O₅ (ppm)	K₂O (ppm)	CaO (ppm)	MgO (ppm)	Na₂O (ppm)	CEC (cmolc.kg⁻¹)
Sandy soil							
Control	638.2 ¹ bcde ²	107.47abcd	200.23 ab	3434.6 def	540.6cdef	62.48 ef	5.36 d
OMWW-TDSP- (L.m⁻²)							
5	601.0 cde	127.08 ab	277.66 ab	3549.2 de	470.9 ef	63.13 ef	6.58 c
10	599.2 cde	131.80 a	262.09 ab	3306.6 def	578.8cdef	63.48 ef	6.66 c
15	388.0 e	83.15 abcd	193.63 ab	3430.9 def	525.6 def	66.49 de	6.58 c
OMWW- CSTP- (L.m⁻²)							
5	540.9 de	133.68 a	296.27 ab	3662.7 d	411.8 f	56.90 ef	6.88 c
10	412.5 e	137.94 a	265.78 ab	3210.9 ef	449.0 ef	52.54 f	6.54 c
15	488.7 e	121.39 abc	361.02 a	3036.9 f	348.4 f	52.39 f	6.24 cd
Sandy clay soil							
Control	942.8 ab	74.94 abcd	210.95 ab	7308.3 abc	871.8 ab	83.93abc	9.55 b
OMWW-TDSP- (L.m⁻²)							
5	998.6 a	62.69 bcd	175.78 ab	7478.9 ab	773.3 abc	79.15 bc	10.42 ab
10	828.7abcd	61.48 bcd	205.84 ab	7376.6abc	815.7 ab	79.26abc	10.61 a
15	931.7 ab	63.69 bcd	183.43 ab	6988.1 c	948.1 a	80.92abc	10.43 ab
OMWW- CSTP- (L.m⁻²)							
5	919.3 abc	61.41 bcd	156.17 b	7202 bc	898.8 ab	76.96 cd	10.21 ab
10	841.6 abcd	56.6 cd	178.32 ab	7668.7 a	672.5bcde	90.39 a	10.19 ab
15	981.2 a	50.77 d	144.57 b	7273.0 abc	734.9abcd	88.60 ab	10.02 ab
Signification³	**	*	NS	***	***	***	***
LSD	322.41	66.90	189.19	440.61	234.22	11.18	0.91

¹Each value is a mean of 3 samples

²Values followed by different letter, in the same column, differ significantly at the 5% level

³Significant at the probability levels of p<0.05: NS not significantly; * significant; **highly significant; ***very highly significant

Soil pH

For pH, there are no significant differences between the different containers of two soils before and after the addition of OMWW (Tables 12 and 14).

By comparing each transaction separately, the degree of pH had not changed in all treatments. It can be said that the pH of soil was not affected by the OMWW added (spreading) (Table 15). This phenomenon may be explained by the buffering capacity of the soil. These results are consistent with those obtained by several other

authors (Chartzoulakis *et al.*, 2006; Gargouri *et al.*, 2013). However, contrary results were obtained by Di Giovacchino, (2005), and Mechri *et al.*, (2007). There was a trend for compost addition and the application of organic matter to decrease soil pH (Das *et al.*, 1991; Simard *et al.*, 1998). However, an increase in soil pH after the addition of organic matter has been reported (Chung and Wu, 1997) and attributed to the production of ammonia due to degradation of organic matter added with the olive mill wastewater (Della Monica *et al.*, 1978; Potenz *et al.*, 1985; Levi-Minzi *et al.*, 1992). The increase in soil pH resulted from the application of organic matter was not simply due to the higher pH and higher application rate of the organic matter itself.

Table 12: Characteristics (organic matter, pH water, electrical conductivity and C/N ratio) of two different soils before the plantation of olives plants and the spreading of olive mill wastewater

Before plantation and spreading of OMWW				
	Organic Matter (%)	C/N	pH water	EC (mS.cm ⁻¹)
Sandy soil				
Control	0.504 ¹ b ²	4.86 ab	7.91 ab	0.130 bc
OMWW-TDSP- (L.m⁻²)				
5	0.393 b	3.83 b	7.94 ab	0.132 bc
10	0.507 b	4.89 ab	7.85 ab	0.134 bc
15	0.402 b	6.11 ab	7.81 ab	0.130 c
OMWW- CSTP- (L.m⁻²)				
5	0.468 b	5.01 ab	7.90 ab	0.134 bc
10	0.515 b	7.28 a	7.55 b	0.142 bc
15	0.407 b	5.24 ab	8.10 ab	0.147 abc
Sandy clay soil				
Control	0.911 a	5.6 ab	8.04 ab	0.164 abc
OMWW-TDSP- (L.m⁻²)				
5	0.969 a	6.85 ab	7.73 ab	0.198 a
10	0.899 a	6.75 ab	7.82 ab	0.195 a
15	0.980 a	6.46 ab	7.88 ab	0.182 ab
OMWW- CSTP- (L.m⁻²)				
5	0.957 a	6.06 ab	8.05 ab	0.163 abc
10	0.989 a	7.24 a	7.91 ab	0.175 abc
15	0.954 a	5.86 ab	8.14 a	0.153 abc
Signification³	***	NS	NS	NS
LSD	0.185	3.35	0.58	0.052

¹Each value is a mean of 3 samples

²Values followed by different letter, in the same column, differ significantly at the 5% level

³Significant at the probability levels of $p < 0.05$: NS not significantly; * significant; **highly significant; ***very highly significant

Electrical conductivity (EC) or salinity

The electrical conductivity was measured to determine the amount of salts present in the soil solution. The salinity of the OMWW was usually posed as limiting factor. Before the addition of OMWW, there are no significant differences between the two soils in all containers concerned electrical conductivity or salinity (Table 12). After the addition of OMWW, and by comparing the treatments between them, the differences are significant between the treatments 10 liters.m⁻² of the two types of OMWW in sandy-clay soil and the control (Table 14). The EC has decreased in the control. By comparing the same treatment before and after the addition of OMWW, there are no significant differences were observed. The addition of OMWW doesn't change the salinity in the soils (Table 15). However, the increase in salinity was observed by other researchers (Ammar and Ben Rouina, 1999; Seferoglu *et al.*, 2000; Zenjari and Nejmeddine, 2001). In addition, salinity had progressed slightly after two and a half months, while still acceptable, for trials with 20 L.m⁻² of OMWW (Morisot and Tournier, 1986). However, Levi-Minzi *et al.*, (1992), showed that for excessive doses of OMWW on sandy clay loam soil, the increased salinity was only momentary. In other studies, salinity of the amended soil increased noticeably but not proportionally with sprayed quantities (Ben Rouina *et al.*, 2006). The values around 0.14 - 0.2 mS.cm⁻¹ are considered very low and not harmful for either the soil or to crops.

Organic matter

Before the addition of OMWW, it is clear, for the organic matter, that there are no significant differences between all containers with the same soil. Note the existence of significant differences between the two types of soils; the content of organic matter is the highest in the sandy-clay soil (Table 12).

After the application of OMWW, there has been significant differences between the various treatments on the one hand, the controls on the other hand in both soils (Tables 14 and 16). The amount of organic matter increased in the treatments of sandy soil in which added 5 and 10 liters of the two types of OMWW as well as in all treatments of sandy-clay soil. This enrichment in organic matter on soil treated with 15 L.m⁻² of OMW was

observed by Haddad *et al.*, (2014). The increase of organic matter ranged between twice and four times. Despite the increasing amount of organic matter in the soil, it is generally considered as low. However, to have good soil functioning and conservation, Seguin, (1980), recommended an organic matter content in the topsoil of 20 g kg⁻¹. These results are consistent with those obtained by Gargouri *et al.*, (2013). For Zenjari and Nejmeddine, (2001), olive mill wastewater induced an increase in organic matter in upper soil horizons. The immobilization of the organic matter facilitates the mineralization when the OMWW were applied to low-dose (3731 m³.y⁻¹) (Cox *et al.*, 1996). According Le Verge, (2004), it is difficult to assert that the use of OMWW in normal doses lower than 5 L.m⁻² enhances, at long term, the amount of organic matter in soil. As against, the disappearance of the added carbon was observed by Della Monica *et al.*, (1979), and Levi-Minzi *et al.*, (1992), on plots planted with corn and vines, which could increase the use of soil organic matter. It is possible that the added organic carbon is biologically degraded by aerobic and anaerobic microorganisms soil (Tam, 1996), and this organic matter decomposition can be explained by an increase of microbial mineralization activities during a period of several months following the amendments (Perucci, 1990).

Table 13: Chemicals characteristics (nitrogen, phosphorus, potassium, calcium, magnesium, sodium, cation exchange capacity) of two different soils after the spreading of olive mill wastewater (OMWW) 3 times (once per year)

After spreading of OMWW							
	N (ppm)	P ₂ O ₅ (ppm)	K ₂ O (ppm)	CaO (ppm)	MgO (ppm)	Na ₂ O (ppm)	CEC (cmolc.kg ⁻¹)
Sandy soil							
Control	491.3 ¹ d ²	29.18 d	110.5 b	3642.9 e	482.1 g	61.35 bcd	5.55 d
OMWW-TDSP- (L.m⁻²)							
5	1283.3 abc	78.22 abcd	335.5 ab	4333.6 c	523.1 efg	45.19 de	7.15 c
10	1465.8 abc	97.88 abc	708.9 ab	3740.5 de	634.2 defg	53.03 de	7.14 c
15	1228.5 abc	107.54 ab	297.9 ab	3696.6 e	569.8 defg	56.62 cde	7.40 c
OMWW- CSTP- (L.m⁻²)							
5	1186.5abcd	84.80 abcd	143.1 ab	4411.2 c	800.1bcdef	40.15 e	7.24 c
10	1106.5abcd	85.41 abcd	154.4 ab	3863.0 cde	604.1 defg	51.29 de	7.06 c
15	945.2 bcd	56.80 bcd	410.8 ab	4324.7 cd	506.0 fg	61.05 bcd	6.94 c
Sandy clay soil							
Control	841.1 cd	35.22 cd	231.7 ab	7179.0 ab	1098 ab	72.98 abc	9.80 b
OMWW-TDSP- (L.m⁻²)							
5	1375.4 abc	88.35 abcd	823.0 ab	7410.8 a	1057.5 ab	61.50 bcd	10.66 ab
10	1707.3 a	127.02 a	867.4 ab	6811.1 b	814.9 bcde	72.73 abc	10.85 a
15	1456.7 abc	111.89 ab	811.4 ab	7679.4 a	983.3 abc	61.99 bcd	10.62 ab
OMWW- CSTP- (L.m⁻²)							
5	1356.5 abc	54.07 bcd	392.6 ab	7611.6 a	1169.3 a	80.63 a	10.41 ab
10	1759.0 a	67.91 abcd	902.1 a	7405.4 a	862.3 bcd	77.84 ab	10.29 ab
15	1578.6 ab	69.79 abcd	790.1 ab	7587.3 a	710.0cdefg	85.12 a	10.45 ab
Signification³	NS	NS	NS	***	***	***	***
LSD	716.14	66.08	774.67	589.22	303.96	17.62	0.91

¹Each value is a mean of 3 samples

²Values followed by different letter, in the same column, differ significantly at the 5% level

³Significant at the probability levels of $p < 0.05$: NS not significantly; * significant; **highly significant; ***very highly significant

Kjeldahl nitrogen

Before the addition of OMWW, it is clear, for nitrogen, that there are no significant differences between all containers with the same soil (Table 11). However, the amount of nitrogen is considered higher in sandy - clay soil than in sandy soils.

After the addition of OMWW, there have been significant differences between the various treatments and the control in both soils. These differences concerned the treatments of sandy soil on which has added 5,10 and 15 L.m⁻² of OMWW-TDSP- , as well as the treatments of sandy-clay soil on which added 10 liters of OMWW-TDSP- and 10, 15 L.m⁻² of OMWW-CSTP-(Table 13).

By a binary comparison between the same treatment before and after the addition, there are no significant differences between the controls. However, the amount of nitrogen increased in the treatments of sandy soil on which has added 5,10 and 15 L.m⁻² of OMWW-TDSP- and on the treatments with 5 and 10 liters of OMWW-CSTP- and also in the treatments of sandy-clay soil on which has added 10 liters of OMWW-TDSP- and 10-15 L.m⁻² of OMWW-CSTP-(Table 16)

Nitrogen content is considered as low in all treatments in the beginning of the experiment, but after the addition of OMWW the amount of nitrogen increased, but overall it is considered as a medium proportion. These results allow us to see that the application of OMWW does not provide a very considerable amount of nitrogen to the soil and as observed by Haddad *et al.*, (2014). Seferoglu *et al.*, 2000, did not observe a significant increase of nitrogen in soils treated with different doses (2.5, 5 and 7.5 L.m⁻²), also Gargouri *et al.*, (2013), don't observe a modified on the soil N content. According to "Le Verge", (2004), the highly fermentable organic matter present in the OMWW suggests that the use of OMWW in normal doses does not significantly

improve the level of soil organic nitrogen. Contrariwise, due to excessive intakes of OMWW at 10 and 50 L.m⁻² (Di Giovacchino *et al.*, 2002), it was observed an enrichment of soil organic nitrogen.

Table 14: Characteristics (organic matter, pHwater, electrical conductivity and C/N ratio) of two different soils after the spreading of olive mill wastewater 3 times (once per year)

After spreading of OMWW				
	Organic Matter (%)	C/N	pH water	Electrical Conductivity (mS.cm ⁻¹)
Sandy soil				
Control	0.573 ¹ f ²	7.02 a	8.02 a	0.116 d
OMWW-TDSP- (L.m⁻²)				
5	1.593 bcde	7.61 a	7.96 a	0.157 abcd
10	1.308 de	6.72 a	7.95 a	0.147 bed
15	1.174 ef	6.38 a	8.02 a	0.159 abcd
OMWW- CSTP- (L.m⁻²)				
5	1.250 e	6.99 a	8.14 a	0.139 cd
10	1.505 cde	9.13 a	7.95 a	0.137 cd
15	1.181 ef	7.28 a	8.19 a	0.142 bcd
Sandy clay soil				
Control	1.033 ef	7.14 a	7.99 a	0.149 bcd
OMWW-TDSP- (L.m⁻²)				
5	2.367 a	9.97 a	8.02 a	0.174 abc
10	2.007 abc	6.97 a	8.10 a	0.198 a
15	2.346 a	9.42 a	8.05 a	0.176 abc
OMWW- CSTP- (L.m⁻²)				
5	2.187 ab	9.28 a	8.00 a	0.165 abc
10	2.323 a	7.71 a	8.01 a	0.200 a
15	1.967 abcd	7.90 a	8.08 a	0.188 ab
Signification³	***	NS	NS	*
LSD	0.676	4.36	0.39	0.048

¹Each value is a mean of 3 samples

²Values followed by different letter, in the same column, differ significantly at the 5% level

³Significant at the probability levels of p<0.05: NS not significantly; * significant; **highly significant; ***very highly significant

Table 15: Comparison of pHwater and electrical conductivity (EC) of different soils before and after spreading of OMWW 3 times (once per year)

	pH water		CE (mS.cm ⁻¹)	
	Before spreading	After spreading	Before spreading	After spreading
Sandy soil				
Control	7.91 ¹ ab ²	8.02 ab	0.130 ef	0.116 f
OMWW-TDSP- (L.m⁻²)				
5	7.94 ab	7.96 ab	0.132 ef	0.157 abcdef
10	7.85 ab	7.95 ab	0.134 def	0.147 bedef
15	7.81 ab	8.02 ab	0.130 ef	0.159 abcdef
OMWW- CSTP- (L.m⁻²)				
5	7.90 ab	8.14 a	0.134 def	0.139 def
10	7.55 b	7.95 ab	0.142 cdef	0.137 def
15	8.10 a	8.19 a	0.147 bcdef	0.142 cdef
Sandy clay soil				
Control	8.04 a	7.99 ab	0.164 abcdef	0.149 bcdef
OMWW-TDSP- (L.m⁻²)				
5	7.73 ab	8.02 ab	0.198 a	0.174 abcde
10	7.82 ab	8.10 a	0.195 ab	0.198 a
15	7.88 ab	8.05 a	0.182 abcd	0.176 abcde
OMWW- CSTP- (L.m⁻²)				
5	8.05 a	8.00 ab	0.163 abcdef	0.165 abcde
10	7.91 ab	8.01 ab	0.175 abcde	0.200 a
15	8.14 a	8.08 ab	0.153 abcdef	0.188 abc
Signification³	NS		*	
LSD	0.487		0.049	

¹Each value is a mean of 3 samples

²Values followed by different letter, in the same row, differ significantly at the 5% level

³Significant at the probability levels of p<0.05: NS not significantly; * significant; **highly significant; ***very highly significant

Ratio of carbon to nitrogen C/N

Before the addition of OMWW, there are no significant differences between the two soils in all containers concerned the C/N ratio (Table 12).

By comparing each transaction separately, the C/N did not change in all treatments. Consequently, the C/N ratio was not affected by adding OMWW. However, the range of this ratio is between “4 – 7” before the addition, and become “7 – 10” after the addition of OMWW (Tables 14 and 16). Di Giovacchino *et al.*, (2002),

noted an increase in the C/N ratio for doses of OMWW than 10 L.m⁻². On the other hand, Marsilio *et al.*, (1990), noted a decrease in the C/N ratio due to the slight increase of nitrogen in the soil. This ratio is a parameter that tells us about the evolution of organic matter and soil biological activity. The carbon is used as energy source for the microflora, while nitrogen is involved in the proliferation of microorganisms.

Table 16: Comparison of nitrogen kjeldahl, organic matter and C/N ratio of different soils before and after spreading of OMWW 3 times (once per year)

	N (ppm)		MO (%)		C/N	
	Before spreading	After spreading	Before spreading	After spreading	Before spreading	After spreading
Sandy soil						
Control	638.2 ¹ hijk ²	491.3 ijk	0.504 ijk	0.573 ghijk	4.86 de	7.02 abcde
OMWW-TDSP- (L.m⁻²)						
5	601.0 hijk	1283.3 abcdefg	0.393 k	1.593 bcd	3.83 e	7.61 abcde
10	599.2 hijk	1465.8 abcd	0.507hijk	1.308 def	4.89 de	6.72 abcde
15	388.0 k	1228.5 abcdefg	0.402 k	1.174 def	6.11 bcde	6.38 abcde
OMWW- CSTP- (L.m⁻²)						
5	540.9 ijk	1186.5 bcdefg	0.468 k	1.250 def	5.01 de	6.99 abcde
10	412.5 jk	1106.5 cdefgh	0.515 hijk	1.505 cde	7.28 abcde	9.13 abc
15	488.7 ijk	945.2 defghij	0.407 k	1.181 def	5.24 de	7.28 abcde
Sandy clay soil						
Control	942.8 defghij	841.1 fghijk	0.911fghij	1.033 efg	5.6 cde	7.14 abcde
OMWW-TDSP- (L.m⁻²)						
5	998.6 defghi	1375.4 abcdef	0.969 fghi	2.367 a	6.85 abcde	9.97 a
10	828.7 ghijk	1707.3 ab	0.899 fghij	2.007 ab	6.75 abcde	6.97 abcde
15	931.7 defghij	1456.7 abcde	0.980 fghi	2.346 a	6.46 abcde	9.42 a
OMWW- CSTP- (L.m⁻²)						
5	919.3 efghijk	1356.5 abcdefg	0.957 fghi	2.187 a	6.06 bcde	9.28 abc
10	841.6 fghijk	1759.0 a	0.989 fgh	2.323 a	7.24 abcde	7.71 abcd
15	981.2 defghi	1578.6 abc	0.954 fghi	1.967 abc	5.86 bcde	7.90 abcd
Signification³	***		***		NS	
LSD	543.1		0.485		3.80	

¹Each value is a mean of 3 samples

²Values followed by different letter, in the same row, differ significantly at the 5% level

³Significant at the probability levels of p<0.05: NS not significantly; * significant; **highly significant; ***very highly significant

Available phosphorus

Before the addition of OMWW, there are no significant differences between all containers with the same soil. But there are significant differences between the soils, as the amount of phosphorus is considered higher in the sandy soil. There are no significant differences between the two controls (Table 11). For the treatments of the two soils, the amount of phosphorus is higher in the treatments of 10 and 15 L.m⁻² of OMWW -TDSP- than in the two controls. But, as regards the comparison between the same treatment before and after the addition, there are some significant differences only in the treatment of sandy-clay soil in which added 10 L.m⁻² of OMWW traditional where the amount of phosphorus is increased. The content of phosphorus in the soil is considered high before and after the addition of OMWW (Tables 13 and 17). The improvement of available phosphorus is observed in case of fertilization by OMWW (Zenjari and Nejmeddine, 2001). The phosphorus uptake by plants is often problematic because only the fraction dissolved in the soil solution is actually available for cultures. Phosphorus, attached to the clay-humus complex and hydroxides of iron and alumina, is partially exchangeable with the soil solution. As for the small fraction of soluble and insoluble, it is essential and is evolving slowly exchangeable forms. In general, inputs of organic matter can increase the proportion of phosphorus in the soil. Therefore, the application of OMWW reduces fertilizer phosphorus on the plots. The enrichment in phosphorus on soil treated with 15 L.m⁻² of OMW was observed by Haddad *et al.*, (2014).

Extractable potassium

There are no significant differences between the various treatments for the same soil before and after the addition of OMWW, so that all the containers contain almost the same amount of potassium (Tables 11 and 13).

When compared the transactions with each other after the addition, we see the existence of significant differences in the transactions of sandy-clay soil in which was added both of OMWW (Table 17). The proportion of potassium has increased in all treatments except in the treatment of 5 L.m⁻² of OMWW -CSTP-. The amount of potassium remains stable in the controls. The content of potassium in the soil is considered as low before the addition of OMWW, which improved and increased after the addition and considered as a high

percentage, which appeared evident by the texture of the soil, especially in all transactions of sandy – clay soil. So, potassium, major mineral element of OMWW, causes soil enrichment (Haddad *et al.*, 2104). These results are in accord with those obtained by Levi-Minzi *et al.*, (1992); Zenjari and Nejmeddine, (2001); Ammar and Ben Rouina, (1999). Like phosphorus, potassium used by plants or as free ions present in the soil solution (0.1% of total potassium) is related to organic matter. In general, the contribution of organic matter influences the bioavailability of potassium in plants (Le Verge, 2004).

Table 17: Comparison of available phosphorus and extractible potassium of different soils before and after spreading of OMWW 3 times (once per year)

	P ₂ O ₅ (ppm)		K ₂ O (ppm)	
	Before spreading	After spreading	Before spreading	After spreading
Sandy soil				
Control	107.47 ¹ abcdef ²	29.18 h	200.23 ef	110.5 f
OMWW-TDSP- (L.m⁻²)				
5	127.08 abcd	78.22 abcdefgh	277.66 cdef	335.5 bcdef
10	131.80 abc	97.88 abcdefg	262.09 def	708.9 abcde
15	83.15 abcdefgh	107.54 abcdef	193.63 ef	297.9 cdef
OMWW- CSTP- (L.m⁻²)				
5	133.68 ab	84.80 abcdefgh	296.27 cdef	143.1 f
10	137.94 a	85.41 abcdefgh	265.78 def	154.4 f
15	121.39 abcde	56.80 efgh	361.02 abcdef	410.8 abcdef
Sandy clay soil				
Control	74.94 abcdefgh	35.22 gh	210.95 ef	231.7 ef
OMWW-TDSP- (L.m⁻²)				
5	62.69 defgh	88.35 abcdefgh	175.78 ef	823.0 abc
10	61.48 efgh	127.02 abcd	205.84 ef	867.4 ab
15	63.69 defgh	111.89 abcdef	183.43 ef	811.4 abcd
OMWW- CSTP- (L.m⁻²)				
5	61.41 efgh	54.07 fgh	156.17 f	392.6 abcdef
10	56.6 efgh	67.91 cdefgh	178.32 ef	902.1 a
15	50.77 fgh	69.79 bcdefgh	144.57 f	790.1 abcd
Signification³		*		*
LSD		65.03		551.44

¹Each value is a mean of 3 samples

²Values followed by different letter, in the same row, differ significantly at the 5% level

³Significant at the probability levels of p<0.05: NS not significantly; * significant; **highly significant; ***very highly significant

Exchangeable calcium and magnesium

Before the addition of OMWW, for the calcium, that there are significant differences between some containers (Table 11). On sandy soil, the calcium content is higher on the container that will receive 5 Liter of OMWW three phase than on these that will receive 10 and 15 L.m⁻². On sandy-clay soil, the calcium content is higher on the containers that will receive 5 L.m⁻² of OMWW press or three phases than on these that will receive 15 L.m⁻² and 10 L.m⁻² respectively.

Before the addition of OMWW, for the magnesium, that there are no significant differences between all containers with the same soil (Table 11). But, in general all the containers contain almost the same amount of these elements. Note the existence of significant differences between the two types of soils; the content of calcium and magnesium is higher in the sandy-clay soil than in the sandy soil. The content of calcium in the soil is considered as low in the sandy soil, before the addition of OMWW, and high in the sandy-clay soil. Before the addition of OMWW, the content of magnesium is considered as medium and high in the two types of soil.

After addition of OMWW, the differences become significant between all treatments and the controls (Table 13). The calcium content is higher in all treatments than in the controls. The differences observed before application are eliminated after application. After addition of OMWW, the amount of calcium and magnesium remains stable in the controls. When compared the transactions with each other after the addition, the existence of significant differences were observed, for calcium, in the transactions of sandy soil especially in which was added OMWW-CSTP (Table 18). The proportion of calcium has increased in treatments of sandy soil which had received OMWW-CSTP- and in the treatment which had received 5 L.m⁻² of OMWW-TDSP- and also in the treatment of sandy-clay soil which had received 15 L.m⁻² of OMWW-TDSP-. It considered that the calcium content was improved especially in the sandy soil. For magnesium, the proportion of magnesium has increased especially in treatments of the two soils which had received 5 L.m⁻² of both OMWW.

Before and after the addition of OMWW, for the sodium, there are no significant differences between all containers with the same soil. After application of OMWW, a decrease in sodium content was showed in some treatments.

Before and after the addition of OMWW, the soil cation exchange capacity (CEC) doesn't show significant differences between all treatments (Table 18).

Table 18: Comparison of exchangeable calcium, magnesium, extractible sodium and cation exchange capacity of different soils before and after spreading of OMWW three times (once per year)

	CaO (ppm)		MgO (ppm)		Na ₂ O (ppm)		CEC (cmole.kg ⁻¹)	
	Before spreading	After spreading	Before spreading	After spreading	Before spreading	After spreading	Before spreading	After spreading
Sandy soil								
Control	3434.6 ¹ fghi ²	3642.9 fgh	540.6 ijklmn	482.1 klmn	62.48 fgh	61.35 fgh	5.36 f	5.55 f
OMWW-TDSP- (L.m⁻²)								
5	3549.2 fgh	4333.6 de	470.9 klmn	523.1 jklmn	63.13 efgh	45.19 ij	6.58 de	7.15 d
10	3306.6 ghi	3740.5 fg	578.8 hijklmn	634.2 fghijklm	63.48 defgh	53.03 ghij	6.66 de	7.14 d
15	3430.9 fghi	3696.6 fgh	525.6 jklmn	569.8 hijklmn	66.49 cdefg	56.62 ghi	6.58 de	7.40 d
OMWW- CSTP- (L.m⁻²)								
5	3662.7 fgh	4411.2 d	411.8 mn	800.1 cdefghi	56.90 ghi	40.15 j	6.88 de	7.24 d
10	3210.9 hi	3863.0 ef	449.0 lmn	604.1 hijklmn	52.54 ghij	51.29 hij	6.54 de	7.06 de
15	3036.9 i	4324.7 de	348.4n	506.0klmn	52.39 ghij	61.05 fgh	6.24 ef	6.94 de
Sandy clay soil								
Control	7308.3 abc	7179.0 abc	871.8 bcdef	1098.0 ab	83.93 ab	72.98 bcdef	9.55 c	9.80 bc
OMWW-TDSP- (L.m⁻²)								
5	7478.9 ab	7410.8 ab	773.3 efghij	1057.5 abc	79.15 abc	61.50 fgh	10.42 abc	10.66 ab
10	7376.6 ab	6811.1 c	815.7 defgh	814.9 cdefgh	79.26 abc	72.73 bcdef	10.61 a	10.85 a
15	6988.1 bc	7679.4 a	948.1 abcde	983.3 abcd	80.92 ab	61.997 fgh	10.43 abc	10.62 ab
OMWW- CSTP- (L.m⁻²)								
5	7202 abc	7611.6 a	898.8 bcdef	1169.3a	76.96 abcde	80.63 abc	10.21 abc	10.41 abc
10	7668.7 a	7405.4 ab	672.5 fghijklm	862.3 bcdefg	90.39 a	77.84 abcd	10.19 abc	10.29 abc
15	7273.0 abc	7587.3 a	734.9 efghijk	710.0 efghijkl	88.60 a	85.12 ab	10.02 abc	10.45 ab
Signification₃	***		***		***		***	
LSD	508.22		265.36		14.43		0.889	

¹Each value is a mean of 3 samples

²Values followed by different letter, in the same row, differ significantly at the 5% level

³Significant at the probability levels of p<0.05: NS not significantly; * significant; **highly significant; ***very highly significant

Conclusion

The agronomic valorization of olive mill wastewater was realizable with important beneficial effects on soil and plants and with specific doses. Under the conditions of this experiment, adding different types of olive mill wastewater is possible to young olive trees at doses of 10 and 15 L.m⁻². The addition of OMWW has a positive impact on plant growth in terms of height growth and chlorophyll assimilation.

The foliar content of phosphorus and potassium increased by the addition of OMWW, but the foliar nitrogen is deficient and its physiological equilibrium is low. The positive effect has observed on phosphorus and potassium contents of sandy-clay soil, but less on nitrogen, organic matter contents hence the need to increase especially nitrogen and organic matter fertilization. The pH, salinity and the C/N ratio of the soils remains stable. Before adding the OMWW, the physical and chemical characteristics of OMWW and soil must be analyzed.

Ultimately, the spreading of olive mill wastewater on agricultural land may be the most economical and useful to solve the problem of this by-product pollutant, which is considered as a natural resource.

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