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Effects of Olive Mill Wastewater on Soil Properties and Seed Germination

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ABSTRACT

This study attempts to address the environmental problems posed by olive mill wastewater (OMW). However, the spreading of OMW seems to be an efficient and economical technique to enhance bio-agro-industrial wastes. This work aims to evaluate the effects of the spreading of OMW on some physico-chemical properties of soil and the development of two cultures that are considered as susceptible as barley (*Hordeum vulgare*) and tomato (*Lycopersicum esculentum*). The experimentation was conducted in a greenhouse under controlled conditions on a sandy loam soil. The doses used of OMW are respectively 0 (as control), 1, 5, 10 and 15 L.m^{-2}. The evolution of physicochemical parameters of the soil was monitored after 1, 15, 30, 50 and 90 days of application. Plantings are made after 1, 15, 50, and 90 days of the application of OMW. The effects of olive mill wastewater on the development of the plants were determined. Phytotoxicity of OMW was determined by the germination test. The results of experimental tests have found no negative indications in terms of application. They found enrichment in potassium, phosphorus and organic matter on soil treated with high doses of OMW. The nitrogen content of soil generally does not change. Slight changes in salinity and acidity are observed, but not proved harmful to crops. For the plants, barley presented a higher sensitivity than the tomato. The inhibitory effects on survival, on height growth, and on dry matter disappeared after 50 and 90 days of application. The germination index is nil for barley. For tomato, this index is above 50% after 9 days.

Key words: Environmental problems, olive mill wastewater (OMW), Seed Germination.

Introduction

In Lebanon, a total of 492 olive mills have been identified including about 87 % use the traditional oil extraction method, while 10 % use three-phase decanters and 3 % use two-phase decanters. The majority of the olive mills (45.73 %) are located in North Lebanon, followed by Mount Lebanon (17.48 %), South Lebanon (16.67 %), Nabatieh (15.45 %) and the Bekaa Valley (4.67 %) (MOE, 2007).This high number of mills is due, perhaps, to increased olive grove surface and consequently the production of olives.

The manufacturing process of olive oil yields three phases; an oily phase, and two by-products a solid residue (olive pomace) and an aqueous phase. The latter, when combined with the washing water process forms an olive mill wastewater (OMW). The amount of this waste depends on the process used for oil extraction. 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 pomace contains the skins, pulp and pit fragments. This separation is most commonly achieved via a horizontal decanter centrifuge or an olive oil press. It is necessary to differentiate the OMW resulted from traditional system and continuous systems. The traditional system is a discontinuous system consisting of pressing the paste by means of hydraulic presses. The continuous three-phase system uses horizontal centrifugation (decanter) to separate the oil from the mass. The three-phase centrifugation system produces the three components; oil, vegetable water, and olive pomace. The discontinuous traditional system uses a decanter that separates the oil and mixes the olive pomace with the olive mill wastewater in one phase called humid olive kernel or watery pomace. The improper disposal of these wastes causes serious environmental impacts to soil, water and air and related to its high organic content made up largely of simple phenolic compounds (Zenjari *et al*., 1999). However, the random and uncontrolled disposal of OMW poses an environmental problem especially in the case of the continuous three- phase system (Le Verge, 2004).

Many researchers have applied OMW directly on soil and have tested its effect as an organic fertilizer thus revealing positive and negative effects. Beneficial effects are related to its high nutrients concentration, especially K, and its potential for mobilizing soil ions, whereas, negative effects are associated with its high mineral salt content, low pH and the presence of phytotoxic compounds, especially of polyphenols (Paredes *et al*., 1999) which are also known to possess antibacterial properties (Pérez *et al*., 1992). Some researchers have

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founded the rapid decomposition of phenolic compounds in the soil, and no accumulation trend was observed after subsequent applications (Chartzoulakis *et al*., 2010).

Furthermore, OMW can substitute in part for a conventional fertilizer and used as organic fertilizer (Fiestas Ros de Ursinos 1981a; Morisot and Tournier, 1986; Bricolli-Bati and Lombardo, 1990; Fiestas Ros de Ursinos and Borja Padilla, 1992; Levi Menzi *et al*., 1992; Garcia-Ortiz *et al*., 1999), due to their high concentration of potassium and to a lesser extent of nitrogen (Albi Romero and Fiestas Ros de Ursinos, 1960 ; Catalano *et al*., 1985; Catalano *et al*., 1986 ; Cegarra *et al*., 1996; Ammar and Ben Rouina, 1999) and of organic matter (Catalano *et al*., 1985). But, other authors have reported the richness of this effluent in organic matter hardly biodegradable (Catalano *et al*., 1986), and its inhibitory effect on plant growth due to the presence of phenolic compounds (Pérez *et al*. 1986; Pérez *et al*., 1992; Rozzi and Malpei, 1996; Cabrera *et al*., 1997; Zenjari *et al*., 1999).

In order to reduce the effect of pollution of OMW and solve the environmental problem of runoff, several processes techniques have been proposed, among other things, the purification of OMW in wastewater treatment plants (Tomati *et al*., 1987), their use for the production of animal feed (Nefzaoui, 1999) ; as substrates for microorganisms and for the production of yeast (Fiestas Ros de Ursinos, 1966; Galli *et al*., 1988); their use in irrigation of soil after dilution (Fiestas Ros de Ursinos, 1981b), the production of biogas and products of special interest generated by the extraction of antioxidants, pectinolytic enzymes and proteins (Hamdi, 1991 ; Hamdi *et al*., 1991; Sayadi et Ellouz, 1993 - 1995); or as physico-chemical and biological treatments (Ranalli, 1991, Paredes *et al*., 1999). But these solutions are expensive, technically and economically, for small and medium-sized mills. Although OMW can be a significant source of pollution if dispose of in streams and lakes. Their controlled direct application to agricultural soils has been proposed as a rational, economical and inexpensive alternative method of disposal and recovery of their mineral and organic components and at the same time to enrich poor soils in organic matter (Ranalli, 1991; Cegarra *et al*., 1996; Paredes *et al*., 1999; Tamburino *et al*., 1999; Zenjari and Nejmeddine, 2001; Di Giovacchino *et al*., 2002; Ben Rouina *et al*., 2006b). This spreading allows nutrients, required for the microflora of soils and plants, to return into the soil. Some studies have showed that the spreading of OMW on the cultivated soil did not induce phytotoxic effects on the cultivations (Marsilio *et al*., 1990; Levi-Minzi *et al*., 1992; Bonari *et al*., 1993; Di Giovacchino *et al*., 2001). This solution is considered the best option for the oil mills located in rural areas. We note that in Lebanon, the mills are mostly distributed in rural areas near the land where olive trees are cultivated.

This research aims to evaluate, under greenhouse, at intervals of time of application and at different doses, the effects of the produced olive mill wastewater, by two different extraction systems: discontinuous traditional press system and continuous three-phase system, on the physical and chemical properties of soil, on the germination and growth of two plants, that are barley "*Hordeum vulgare*" (monocotyledon) and tomato "*Lycopersicum esculentum*" (dicotyledon).This was performed with an adequate time between OMW application and planting.

Materials and Methods

Physico-chemical analysis of olive mill wastewater (OMW):

Both types of OMW derived from two olive oil mills located in the region of Zghorta El-Zawiyeh in North Lebanon. The first type is that of a discontinuous traditional press system (DTP) and the second is that of a continuous 3-phase system (C3Ph). These OMW have been with no previous treatment and were stored at 4°C to determine some physicochemical characteristics. Analyses were made with respect to the fresh matter. The following parameters were determined: pH, electrical conductivity (EC), density (simple weighing of a certain volume of the crude solution), dissolved total matter, mineral and organic fractions, total nitrogen (Kjeldahl method) (Rodier, 1996), organic matter (calcination at 500°C for 24 hours) (Pauwels *et al*., 1992; Navarro *et al*, 1993), total phosphorus and total potassium (sulfo-nitro-perchloric digestion) (Beley, 1948, Hamzé *et al*., 1984), the total phosphorus was measured by UV-absorption spectrophotometry and the total potassium by flame emission spectrophotometry. Polyphenolic compounds were extracted with ethyl acetate (Balice and Cera, 1984) and were assayed by spectrophotometry at 765 nm wavelength using Folin-Ciocalteau reagent (Vazquez Roncero *et al*., 1974; Maestro Duran *et al*., 1991; Montedoro *et al.*, 1992; Paredes *et al*., 1999; Zenjari *et al*., 2001), total polyphenols are expressed as tyrosol.

Effects of different OMW on soil and physico-chemical analysis of soil:

The experiment was conducted in greenhouse under controlled conditions of temperature (20-22°C) and equivalent moisture (70-80%).

The soil was distributed into pots of 113 cm^2 surfaces and capacity of approximately 0.8 kg soil pot^{-1}. The doses of OMW applied at the beginning of the experiment are respectively 0 $L.m^{-2}$ (control), two doses considered as normal in the range of 1 and 5 $L.m^{-2}$ and two doses considered as excessive in the range of 10 et 15 $L.m^{-2}$.

To determine the effects of OMW on soil, we followed the evolution of certain physicochemical properties of soil by sampling in each pot after 1; 15; 30; 50 and 90 days of OMW application. A total of 135 pots were used for soil analysis. The soil samples were air-dried, mixed and sieved through 2 mm diameter sieve. Sub samples were dried in a 105°C oven to determine the dry soil matter. At the end of each period, some physicochemical parameters of soil were determined. pH of the aqueous suspension extract soil/water (w:v ; 1:2.5) was measured (Rowell, 1995). Electrical conductivity (EC) was determined on an aqueous extraction solution with a ratio mass:volume; 1:5 (AFNOR, 1987; Rowell, 1995). Total nitrogen was determined by the Kjeldahl method (Rowell, 1995; Pansu and Gautheyrou, 2003). Available phosphorus was determined by Olsen method with sodium bicarbonate extraction and ratio mass:volume; 1:20 (Olsen and Dean, 1965; Tandon, 1998; Pansu and Gautheyrou, 2003). Exchangeable potassium was determined using the Schollenberger and Simon method (Schollenberger and Simon, 1945) by acetate ammonium extraction with a ratio mass:volume; 1:20. Total organic carbon was determined by wet digestion after chromic acid oxidation (Walkely and Black, 1934), organic matter was obtained from the carbon content (Navarro *et al*., 1993; Tandon, 1998; Rowell, 1995; Ryan *et al*., 1996; Pansu and Gautheyrou, 2003). Total porosity was calculated by determining the bulk and particles densities (Rowell, 1995).

Soil characteristics are presented in table 1. Soil texture is sandy clay loam with alkaline pH, low salinity, rich in phosphorus and potassium, but low in nitrogen and organic matter, however the C/N ratio is adequate.

Table 1: Physicochemical characteristics of the used soil.

Parameters	Value
Clay (%)	21.10
Silt (%)	17.90
Sand (%)	61.00
Texture	Sandy-clay-loam
Total Porosity (%)	43.58
pH_{water} (1:2.5)	8.18
Electrical Conductivity -EC- (1:5) ($mS.cm^{-1}$)	0.22
P_2O_5 ($mg.kg^{-1}$ dry matter)	78.37
K_2O ($mg.kg^{-1}$ dry matter)	275.06
Total N kjeldahl (% dry matter)	0.08
Carbon (% dry matter)	1.13
Organic Matter -OM- (% dry matter)	1.96
C/N	14.45

Effects of different OMW on plants:

To study the effects of OMW on plant, growth test was performed on two plants: one from the dicotyledons family, tomato *"Lycopersicum esculentum*" and the second from the monocotyledons family, barley *"Hordeum vulgare".*

Sowing (10 seeds per pot) was performed after 1; 15; 50; and 90 days of the OMW application. Plants were monitored for 20 days. A total of 204 pots were used. The survival rate of seedlings of different lots was determined. Height growth was estimated by measuring the total height of the plants, the longest leaf in barley and the length of the main stem in tomato. At the end of this period, the dry matter of seedlings was determined.

OMW phytotoxicity was studied as well on barley seeds *"Hordeum vulgare"*, tomato *"Lycopersicum esculentum"* and cress *"Lepidium sativum"*. Phytotoxicity was measured using a modified Zucconi test (Zucconi *et al.*, 1981) by measuring seed germination. Before starting the test, seeds were soaked in water for a period of approximately 24 hours in order to accelerate their development.

Ten seeds were placed in sterilized Petri-dishes (Ø = 8.5 cm) containing filter paper soaked in OMW aqueous solution at the following dilutions: 100%; 75%; 50%; 25%; 15%; 10% and 0%. A volume of 10 ml of each solution were added in each Petri-dish. Distilled water was used as a control and five replicates were set out for each treatment. After incubation at 25°C for 3, 7 and 9 days in the dark, germinated seeds were counted and root length was measured. Germination was then stopped by adding 1 ml 50% (v/v) ethanol to each Petri-dish. A 5 mm primary root was used as the operational definition of germination. Ungerminated seeds were defined as being zero (0) cm long. The germination index was calculated by determining the germination rate (expressed in %) and by measuring root elongation (expressed in mm) in a sample as related to the control (Zucconi *et al.*, 1981; Zucconi *et al*., 1985; Shashi *et al*. 1998; Gariglio *et al*., 2002).
Germination index (GI) was calculated as follows:

GI (%) = (% seed germination x % radicle length) / 100

Where,

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% Germination = Mean germination in OMW solution / Mean germination in distilled water x 100

and

% Radicle Length = Mean radicle length in OMW solution / Mean radicle length in water x 100

Statistical analysis:

The treatment distribution was performed according to the statistical scheme of the Randomized Complete Design (RCD) with 3 replications. The statistical interpretation was performed by the variance analysis with one studied factor (ANOVA) following the procedure of the general linear model (GLM) of the statistical program SAS (Statistical Analysis System, 2004). Comparison of the average of treatments is established by calculating the least significant difference (LSD) at 5%.

Results and Discussion

Physicochemical characteristics of the used Olive Mill Wastewater (OMW):

The organic and mineral composition of the olive mill wastewater used is shown in table 2.

Table 2: Physicochemical characteristics of two types of olive mill wastewater (OMW) studied.

Parameters	OMW of (DTP)	OMW of (C3Ph)
Color	Blackish-brown	Reddish-brown
Density ($g.cm^{-3}$)	1.05	1.01
pH	5.24	5.22
EC ($mS.cm^{-1}$)	2.76	2.38
Total polyphenols ($mg.kg^{-1}$ tyrosol)	300.79	184.14
Total dry residue ($g.L^{-1}$)	9.13	5.89
Mineral Fraction ($g.L^{-1}$)	2.55	1.88
Organic Fraction ($g.L^{-1}$)	6.58	4.01
Carbon (%)	40.05	37.76
Organic Matter (%)	72.1	67.97
Total N (% Fresh Weight)	0.014	0.027
Total P ($mg.kg^{-1}$ Fresh Weight)	2.23	1.95
Total K (% Fresh Weight)	0.102	0.06

The OMW that was used throughout the period of the experiment contains appreciable quantities of organic and mineral elements that can replace some of the nutrients provided by conventional fertilization. The OMW is liquid waste acid, rich in organic substances (70 % of organic matter). The slightly elevated salinity is mainly due to potassium ions with values around 0.1 % in the OMW from DTP and 0.06% in those of C3Ph. Nitrogen and phosphorus are also present but at lower concentrations which are considered as significant values. Compared with other organic wastes, in a study realized by Paredes *et al*., (1999), the olive mill wastewater (OMW) had a high potassium concentration, similar organic matter content and notable level of nitrogen, phosphorus. The phenolics 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 our OMW but at lower level. In general, the effluent is cloudy, and the dark color (black or brown) is due to the presence of phenolic compounds that are formed during the grinding of the olives (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 OMW varies depending on the year, and that the characterization remains very difficult because of the presence of several variability factors. Among these factors: the composition of different OMW that depends on the maturity of olives and their water content; the additions of washing water posing a major source of variability, these dilutions reduced the concentration of various compounds already present in the margins; and the impact of OMW storage on reducing the concentration of certain fermentable compounds under the action of micro organisms, on the sedimentation of suspended matter, and on the evolution of the acidity (Tomati, 2001). Overall, these products from the olive industry can be used as organic fertilizers in agricultural soils in order to eliminate them and to improve the soil fertility (Paredes *et al*., 1999).

Effect of application of OMW on the physicochemical properties of soil:

It was noted that the addition of olive mill wastewater modified the soil parameters. They permitted a significant improvement in the soil fertility.

Effect on the rate of organic matter of soil:

The organic matter show insignificant differences between the control soil and soil treated with OMW 1-15-30-50 days after spraying (Figures 1a and 1b). After 90 days of application, the organic matter content increased with the dose administrated. Thus, there are significant differences between the control and treated soils with the highest dose (15 $L.m^{-2}$) of the OMW from DTP on the one hand, and with those treated with doses of 5, 10 and 15 $L.m^{-2}$ of the OMW from C3Ph on the other. These results show that the OMW are beginning to exert their effect after 90 days of application while enriching the soil with organic matter and this from a dose of 5 $L.m^{-2}$ of the OMW from C3Ph and a dose of 15 $L.m^{-2}$ of the OMW from DTP. However, a decrease is noticed in the control soil. This is consistent with the observations of Di Giovacchino *et al*., (2002); Abichou *et al*., (2003); Ben Rouina *et al*., (2006a). 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 OMW were applied at low-dose (3731 $m^3.y^{-1}$) (Cox *et al*., 1996). We should add that according to Le Verge, (2004), it is difficult to assert that the use of OMW in normal doses lower than 5 $L.m^{-2}$ enhances, at long term, the amount of organic matter in soil. However, 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 soil's microorganisms (Tam and Wong, 1996).

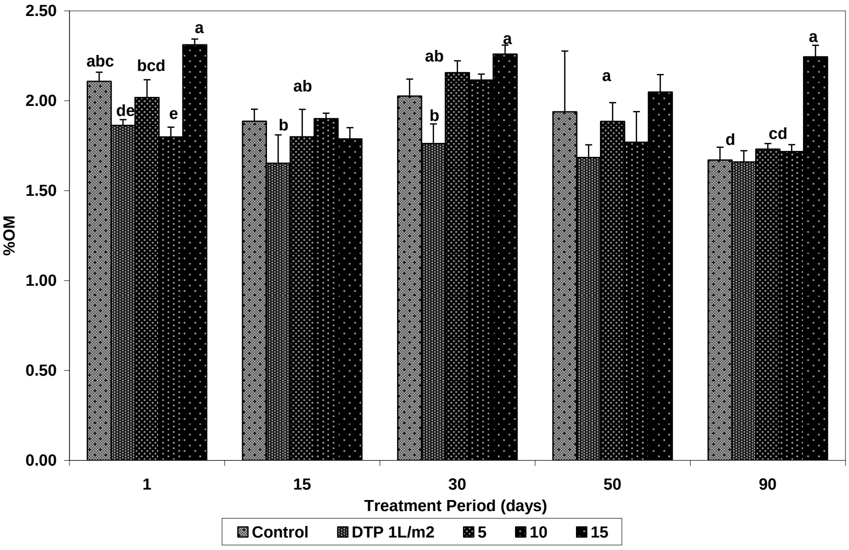

Fig. 1a: Changes of organic matter (% Dry Matter) of soil following various inputs of OMW from DTP.

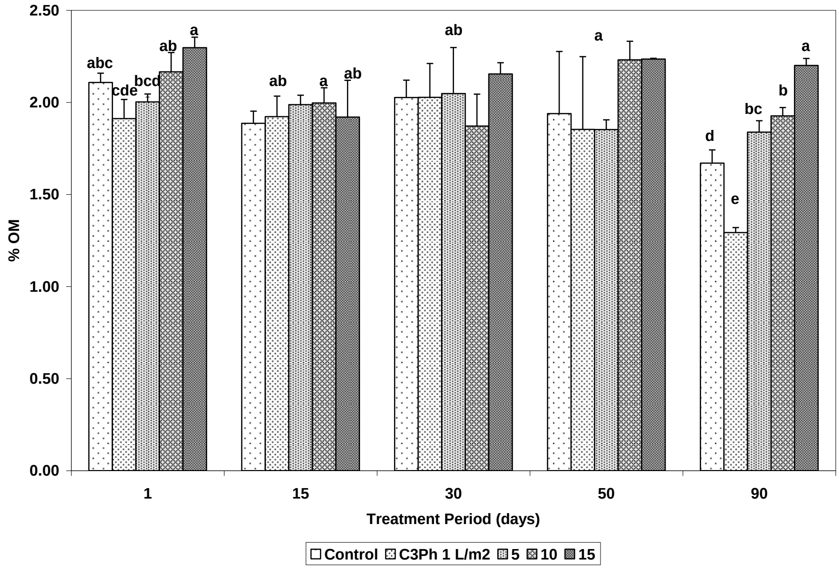

Fig. 1b: Changes of organic matter (% Dry Matter) of soil following various inputs of OMW from C3Ph.
Values with different letters are significantly different at $LSD_{0.05}$ (Fisher's least-significant-difference test)
Bars as Standard Error:

Effect on nitrogen:

For nitrogen, there are no significant differences between control soil and soil treated with OMW 1-15-30-50 and 90 days after spraying (Figures 2a and 2b). We noted, in general, that the OMW from DTP brings a little more nitrogen to the soil than the OMW from C3Ph especially at doses of 5 and 15 $L.m^{-2}$ and this after 90 days of application. Note that the nitrogen present in the OMW is mainly in organic form (Le Verge, 2004). Over time, the levels of nitrogen show a decrease for all treatments. This can be attributed to the mineralization of organic nitrogen to nitrate easily driven by irrigated water. The transformation of organic nitrogen in nitrate nitrogen occurs under the action of microorganisms, whose activity depends on various parameters (temperature, humidity, pH, aeration, exchangeable calcium...). This phenomenon is observed immediately after the addition of OMW (Morisot and Tournier, 1986). These results show that the application of OMW does not provide a very considerable amount of nitrogen to the soil and as showed by Abichou *et al*., (2003). Furthermore, Seferoglu *et al*., (2000), did not observe a significant increase of nitrogen in soils treated with different doses (2.5, 5.0 and 7.5 $L.m^{-2}$). While Ben Rouina *et al*., (2006 a), observed an increase of nitrogen content after a supply of 50 to 100 m^3 per hectare during ten successive years.

According to “Le Verge”, (2004), the highly fermentable organic matter present in the OMW suggests that their use at normal doses does not significantly improve the level of the soil organic nitrogen. Contrariwise, due to excessive intakes of OMW at 10 and 50 $L.m^{-2}$ (Di Giovacchino *et al*., 2002), it was observed an enrichment in the soil organic nitrogen.

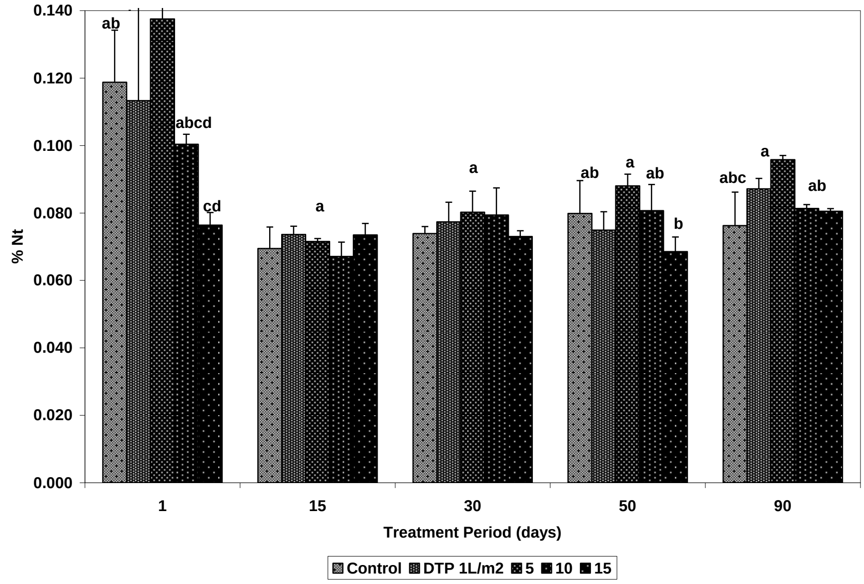

Fig. 2a: Changes of total Kjeldahl nitrogen (% Dry Matter) of soil following various inputs of OMW from DTP.

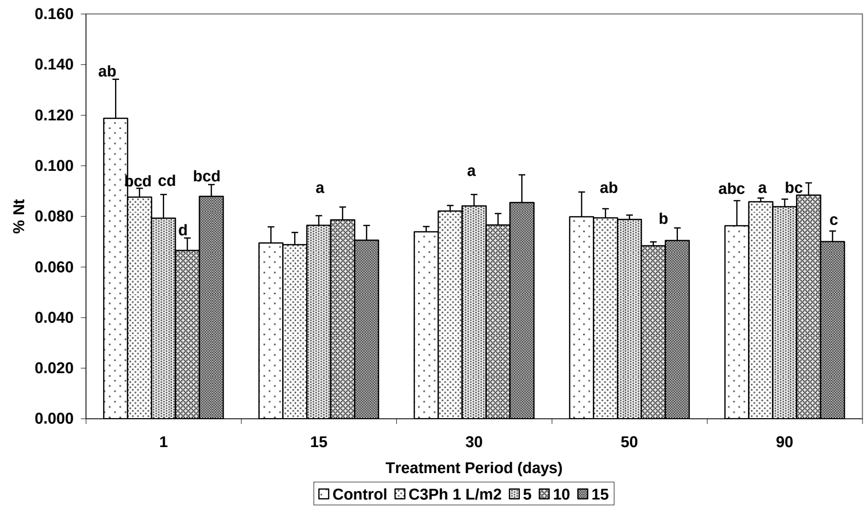

Fig. 2b: Changes of total Kjeldahl nitrogen (% Dry Matter) of soil following various inputs of OMW from C3Ph.

Values with different letters are significantly different at $LSD_{0.05}$ (Fisher's least-significant-difference test).
Bars as Standard Error

Ratio of carbon to nitrogen C/N:

We observed, for the C/N ratio, significant differences between control soil and treated soils and between them (Figures 3a and 3b). The C/N ratio is highest in soil treated with high dose of 15 $L.m^{-2}$ and this immediately after application and for all other periods. 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. These results are consistent with the observations of Di Giovacchino *et al*., (2002), who noted an increase in the C/N ratio for doses of OMW 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. The C/N changes differently according to the amounts of OMW introduced. Introduced in normal doses (less than 50 $m^3.ha^{-1}$), the OMW decrease C/N ratio. Contrariwise, for doses more than $100m^3.ha^{-1}$, vegetable waters increase the C/N ratio (Le Verge, 2004).

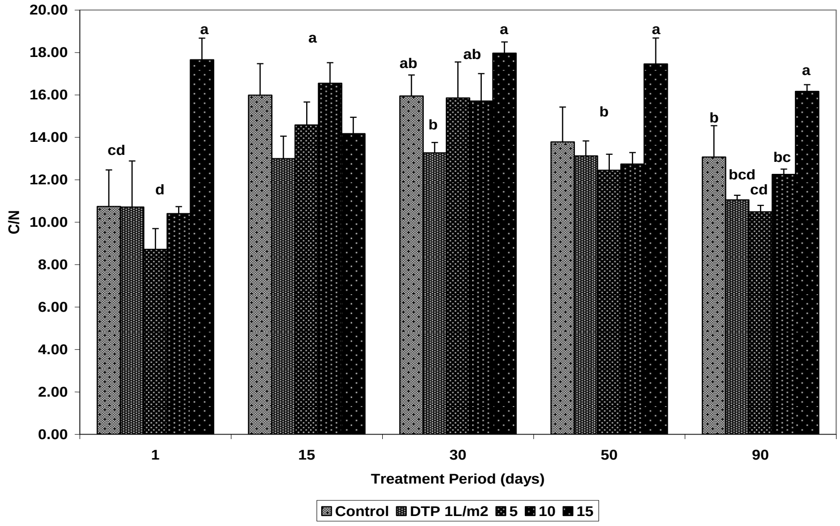

Fig. 3a: Changes in the ratio of carbon to nitrogen (C/N) following various inputs of OMW from DTP.

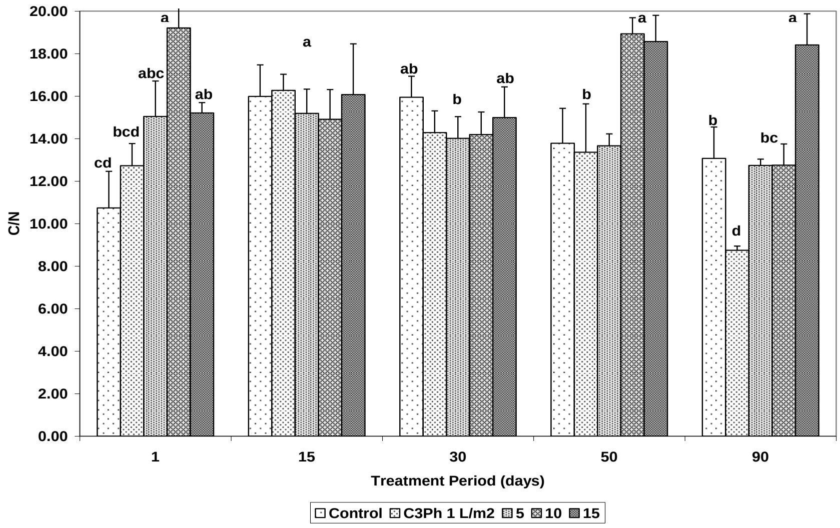

Fig. 3b: Changes of the ratio of carbon to nitrogen (C/N) due to the different inputs of OMW from C3Ph.
Values with different letters are significantly different at $LSD_{0.05}$ (Fisher's least-significant-difference test). Bars as Standard Error

Effect on available phosphorus:

Figures 4a and 4b do not show significant differences after 90 days of application between the treated group and the control. The available phosphorus content is higher in the treated groups at higher doses than the control. We note, in general, that phosphorus in soils treated with OMW from DTP is higher than soil treated with OMW from C3Ph, regardless of the applied dose. However, significant differences between the different doses are more marked in the OMW from DTP. Phosphorus levels are higher in soils treated with higher doses than in those treated with low doses. However, these results show that OMW don't increase the content of the soil phosphorus after 90 days of application. But, the improvement from available phosphorus is observed in case of fertilization by OMW (Zenjari and Nejmeddine, 2001). Moreover, at the 135th day after the intake of OMW, phosphorus is more available in soils enriched with OMW applied at different doses (8, 16 and 32 $L.m^{-2}$) (Levi-Minzi *et al*., 1992). This is probably due to the mineralization of soil organic matter, or to the phenomena of dissolution and mineralization of phosphorus contained in OMW and involved in the degradation of the organic matter (Della Monica *et al*., 1978; Levi-Minzi *et al*., 1992). Consequently, inputs of organic matter can increase the proportion of phosphorus in the soil. Therefore, the application of OMW reduces phosphorus inputs.

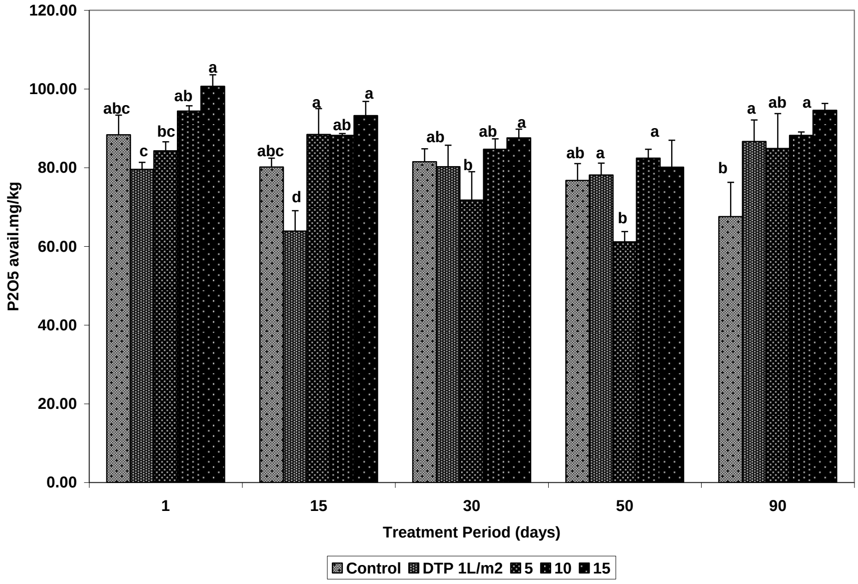

Fig. 4a: Changes of available phosphorus ($mg.kg^{-1}$ Dry Matter) following various inputs of OMW from DTP.

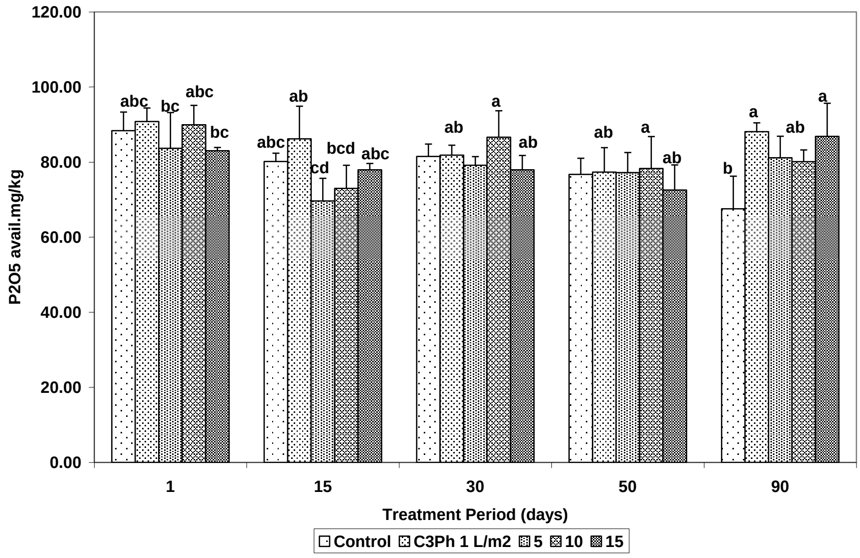

Fig. 4b: Changes of available phosphorus ($mg.kg^{-1}$ Dry Matter) following various inputs of OMW from C3Ph.
Values with different letters are significantly different at $LSD_{0.05}$ (Fisher's least-significant-difference test). Bars as Standard Error

Effect on exchangeable potassium:

Figures 5a and 5b show significant differences between the control and treated groups and between them. The groups treated with the OMW from DTP showed potassium content greater than the comparable control, and also than the lots treated with the OMW from C3Ph. In addition, doses greater than 1 $L.m^{-2}$ provide more potassium to the soil treated with the two types of OMW. These results indicate that the potassium of OMW was available from the first day of application for doses above 1 $L.m^{-2}$. So, potassium, as the major mineral element of OMW, causes the soil enrichment. These results are consistent with those obtained in other studies (Levi-Minzi. *et al*., 1992; Ammar and Ben Rouina, 1999; Zenjari and Nejmeddine, 2001; Abichou *et al*., 2003; Ben Rouina *et al*., 2006a; Chartzoulakis *et al*., 2010). However, Levi-Minzi *et al*., (1992), found a net decrease in potassium levels after 53 days of the application of OMW, which is probably due to adsorption of this element on the clay-humus complex and its integration within organic compounds. 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).

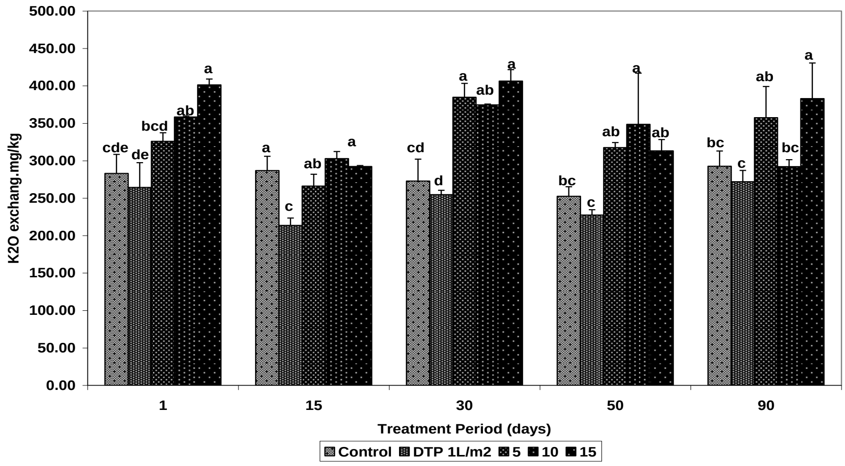

Fig. 5a: Changes of exchangeable potassium (mg.kg^{-1} Dry Matter) following various inputs of OMW from DTP.

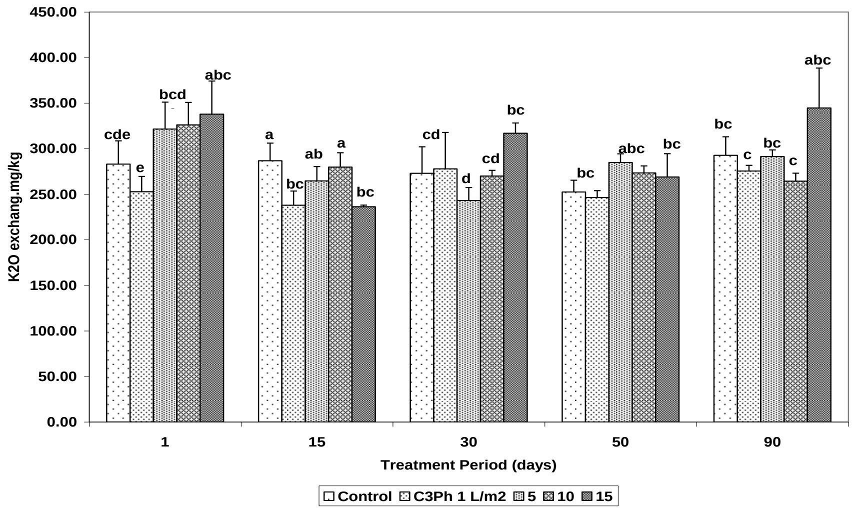

Fig. 5b: Changes of exchangeable potassium (mg.kg^{-1} Dry Matter) following various inputs of OMW from C3Ph.

Values with different letters are significantly different at $LSD_{0.05}$ (Fisher's least-significant-difference test).
Bars as Standard Error

Effect on soil pH:

The pH values of treated groups were lower than those obtained for the control soil throughout the period of our observations (Figures 6a and 6b). The soil used was alkaline, the application of OMW led to slight decreases in pH for all doses applied, but especially for doses of 10 and 15 L.m^{-2} of the two types of OMW. The effect of OMW from DTP is higher than that of the OMW from C3Ph and the highest doses lower the pH values more than the lowest doses. However, the OMW, while acids (pH 4-5), modify slightly the pH, probably due to the buffering capacity of the soil. This was also observed by Levi-Minzi *et al*., (1992), Marsilio *et al*., (1990), Tam and Wong, (1996). But Tomati, (2001), showed that normal doses of OMW do not changes soil acidity. Tests conducted by several researchers (Della Monica *et al*., 1978; Morisot and Tournier, 1986; Levi-Minzi *et al*., 1992; Seferoglu *et al*., 2000) have shown that the exaggerated input of OMW only modify temporarily the acidity of the alkaline soil and returns to its original pH after 15 days of application. This slight acidification is also beneficial in alkaline soils because it makes the phosphorus and trace elements more similar. An increase in pH over time can be observed 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).

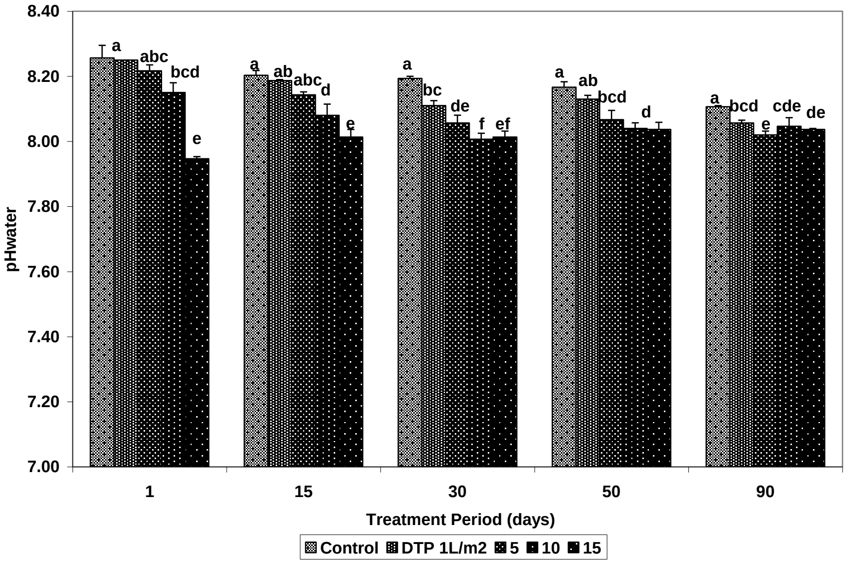

Fig. 6a: Changes of pH_{water} following various inputs of OMW from DTP.

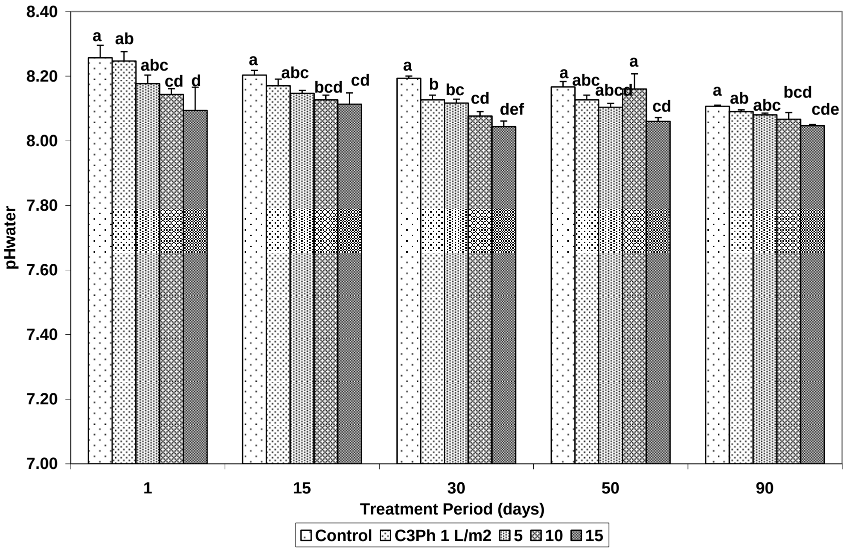

Fig. 6b: Changes of pH_{water} following various inputs of OMW from C3Ph.
Values with different letters are significantly different at $LSD_{0.05}$ (Fisher's least-significant-difference test). Bars as Standard Error

Effect on electrical conductivity or salinity of the soil:

The electrical conductivity was measured to determine the amount of salts present in the soil solution. Figures 7a and 7b show significant differences between different treatments immediately after one day of application and extending to the 90th day. This setting has many variations between the different treatment groups and the control. But, in general, the electrical conductivity in the treated groups at high doses is higher than the control and the dose of 1L.m^{-2}. However, these values remain low and below the harmful limit to the development of certain plants. The addition of OMW causes an increase in soil salinity. This can be attributed to the phenomena of mineralization that enrich the soil with salts (especially potassium). However, the OMW (electrical conductivity about 3 mS.cm^{-1}) has slightly changed the salinity. The values around 0.15 - 0.3 mS.cm^{-1} are considered very low and not harmful for either the soil or to crops. The increase in salinity was also 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 OMW (Morisot and Tournier, 1986). Tomati, (2001), showed that OMW used in normal doses do not alter the soil salinity. However, Levi-Minzi *et al*., (1992), showed that for excessive doses of OMW 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*., 2006a).

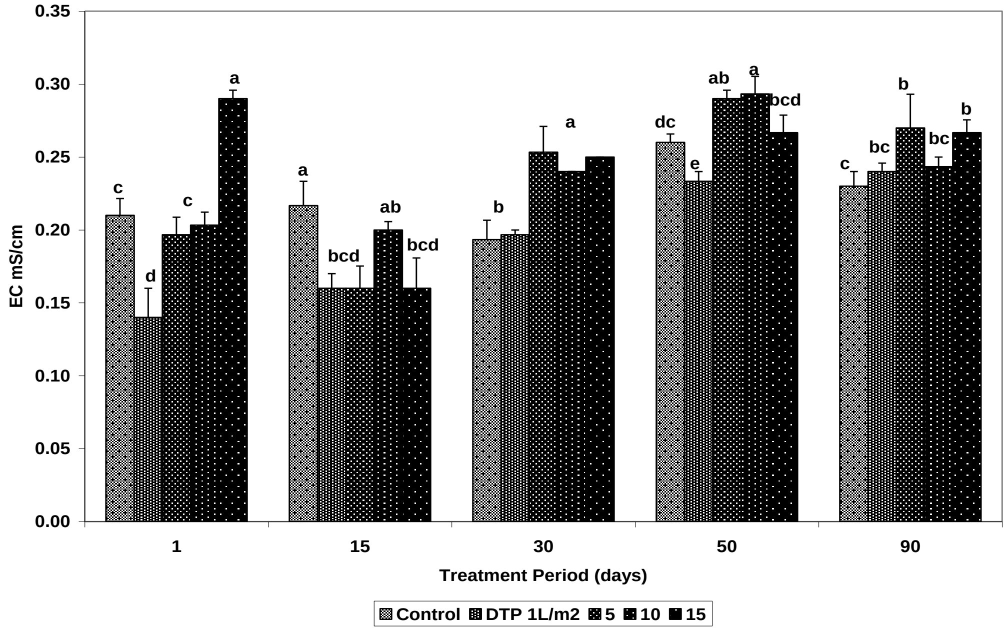

Fig. 7a: Changes of electrical conductivity (EC $mS.cm^{-1}$) following various inputs of OMW from DTP.

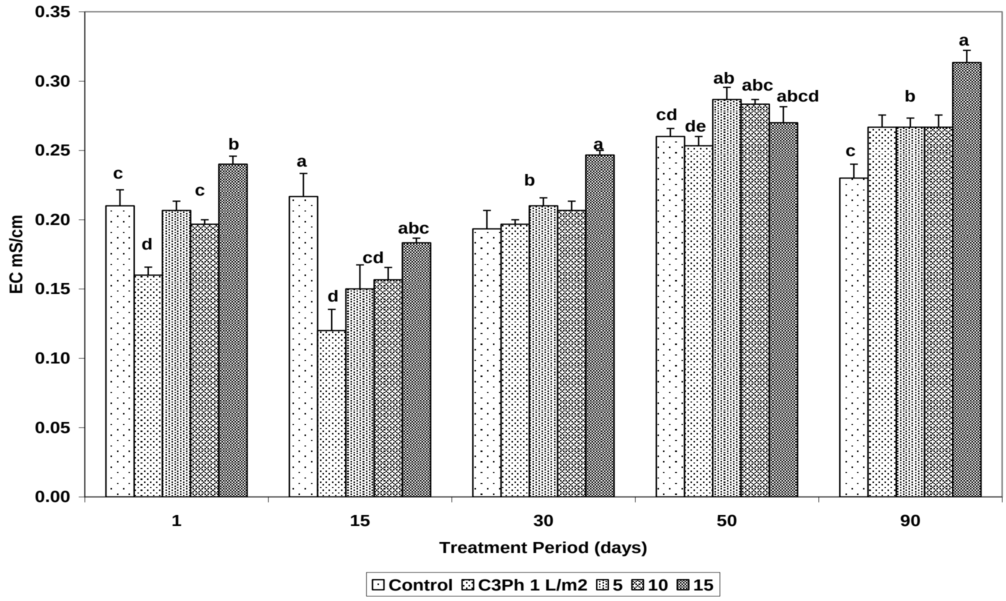

Fig. 7b: Changes of the electrical conductivity (EC $mS.cm^{-1}$) following various inputs of OMW from C3Ph.
Values with different letters are significantly different at $LSD_{0.05}$ *(Fisher's least-significant-difference test).*
Bars as Standard Error

Effect on porosity of soil:

Figures 8a and 8b show non-significant differences between the first and the 50th day after application. The effect of OMW on the porosity appears only after 50 and 90 days of application. The porosity of the treated soil has increased compared to control soil and this especially at higher doses. But, in general, the increases are not remarkable. Studies conducted by Pagliai and Vittorio Antisari, (1993), Pagliai, (1996), and Tomati, (2001), on the microporosity and macroporosity, showed significant increases proportionally with the amounts of OMW introduced and compared to untreated soil. However, excessive doses (200 m^3. ha^{-1}) can cause structural damage accompanied by a decrease in porosity, particularly in clay soils. The benefits of OMW on the porosity persist only for a period less than one year (Pagliai and Vittorio Antisari, 1993; Tomati, 2001).

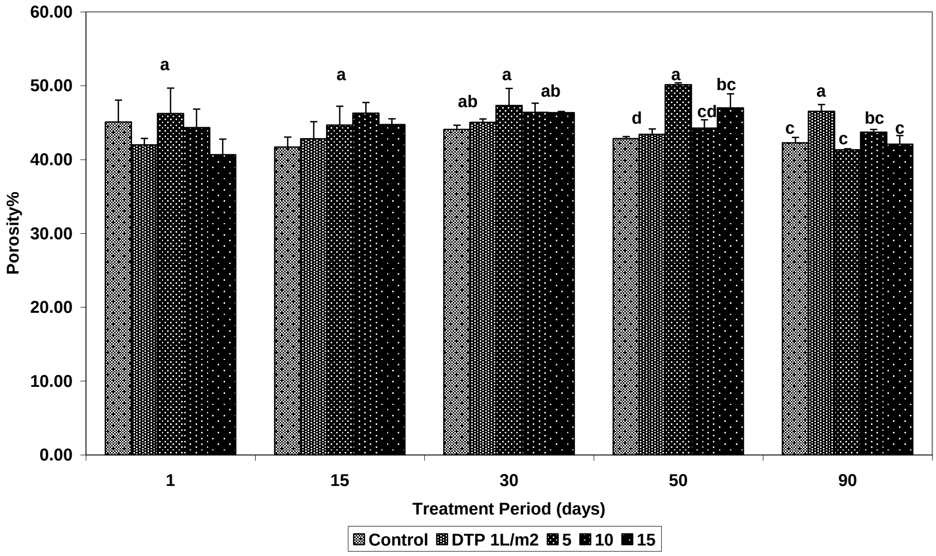

Fig. 8a: Changes of soil total porosity (%) following various inputs of OMW from DTP.

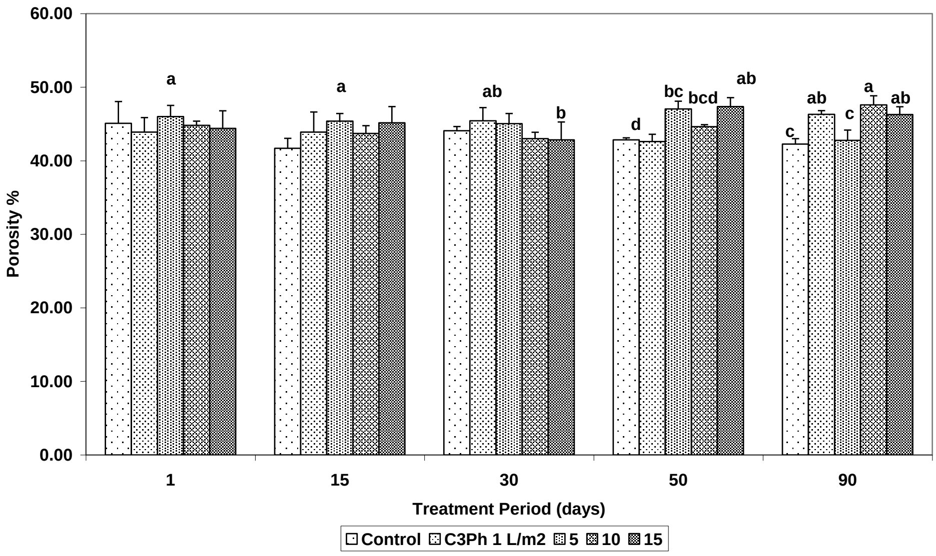

Fig. 8b: Changes of soil total porosity (%) following various inputs of OMW from C3Ph.
Values with different letters are significantly different at $LSD_{0.05}$ (Fisher's least-significant-difference test).
Bars as Standard Error

Effects of OMW on the growth of plants:

The effects of OMW are studied on two different plants; one is barley the other tomato. The presence of salts and phenolic compounds in the OMW is likely to inhibit the growth of plants. However, numerous studies have shown that the application of OMW at adequate periods and doses would enhance the growth and crop yields. Biological tests are used to define the period required for the disappearance of phytotoxicity of OMW.

Effect of OMW intake on survival rate:

For growing barley, Figures 9a and 9b show significant differences between the treated group and the control especially for sowing after the first day of application. The survival rate is still higher in the control than in the treated group and especially at high doses. These differences become insignificant between the various lots for the other periods of sowing. We note that the inhibitory effect is more pronounced in the case of OMW from DTP on the survival rate. In addition, the dose of 15 $L.m^{-2}$ has the inhibitory effect on the survival of plants than other doses. This effect extends to 50 days after sowing spreading. The tests performed on the culture of barley indicate that phytotoxicity tests, after one day of application, disappeared at the end of the period of the test. The seedling emergence of barley is a period of 2 to 3 days, for against that of the tomato is more; it did last for a period of a week or more.

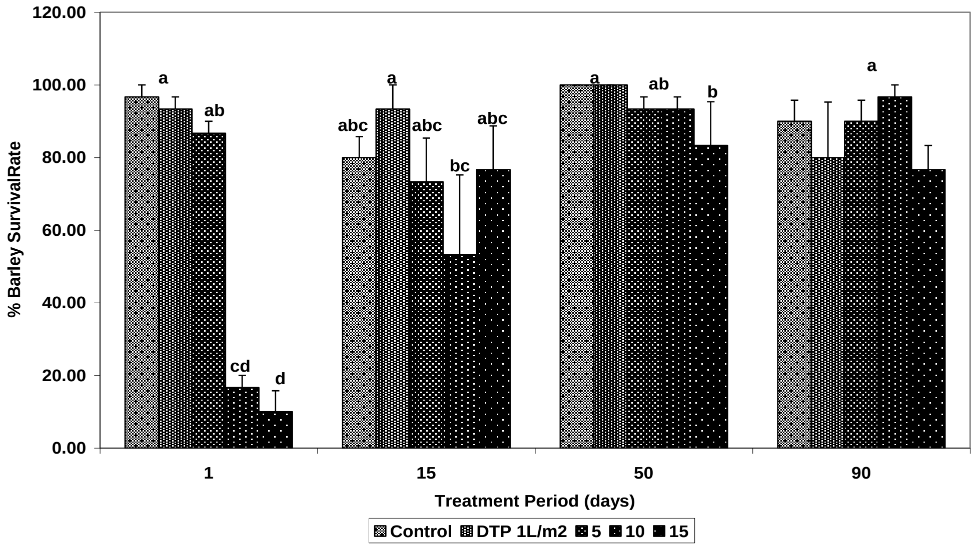

Fig. 9a: Effect of OMW from DTP on the survival rate (%) of barley (*Hordeum vulgare*).

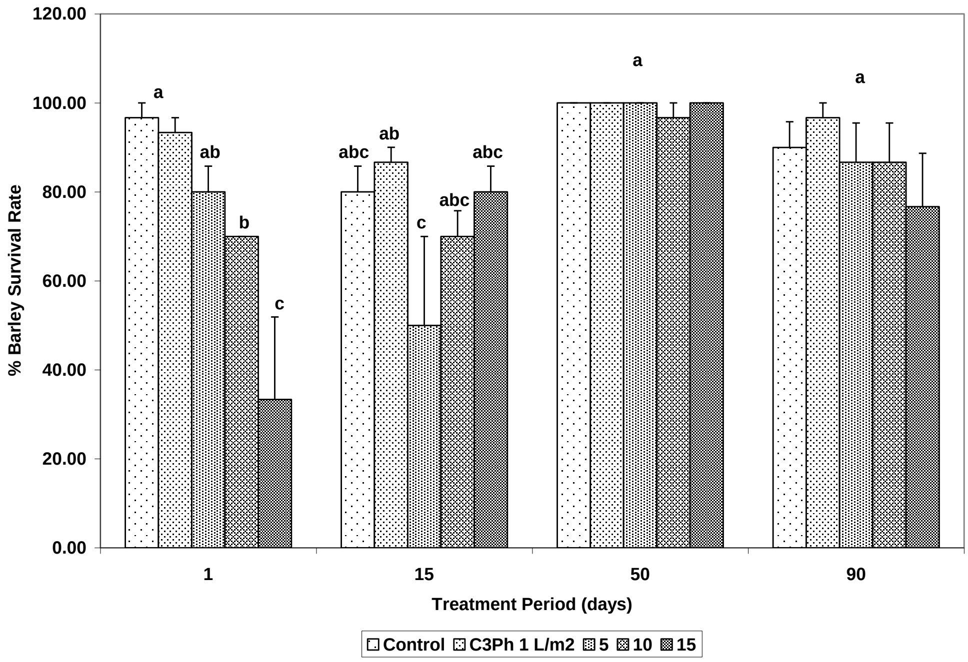

Fig. 9b: Effect of OMW from C3Ph on the survival rate (%) of barley (*Hordeum vulgare*).
Values with different letters are significantly different at $LSD_{0.05}$ (Fisher's least-significant-difference test).
Bars as Standard Error

For growing tomatoes, statistical analysis showed no significant differences between treatments for different seeding performed after 1, 15, 50 and 90 days of application (Figures 10a and 10b). The survival rate in tomato was not affected by increasing doses of OMW. Similarly, over time, the seedlings, after 90 days, showed an improvement in the survival rate for high doses of OMW. These results indicate that tomato crops have shown no great sensitivity to the use of OMW. For other plants**,** Levi-Minzi *et al.*, (1992), have confirmed the inhibitory effect of OMW on the germination of herbaceous plants after application of excessive doses (16 and 32 $L.m^{-2}$). In addition, they noted an increase in germination of corn seedlings after 135 days over the control for the dose of 32 $L.m^{-2}$. Other studies conducted on maize plantings have set the time for disappearance of toxicity at approximately 45-60 days (Garcia-Ortiz *et al.*, 1999; Di Giovacchino *et al.*, 2002). The results obtained by De Simone and De Marco, (1996), indicate that doses of 8 $L.m^{-2}$ of OMW had no negative impact either on the germination nor on the emergence time of crops such as barley and corn, with one condition that to allow a time interval of 50 - 60 days between application of OMW and planting of vegetable.

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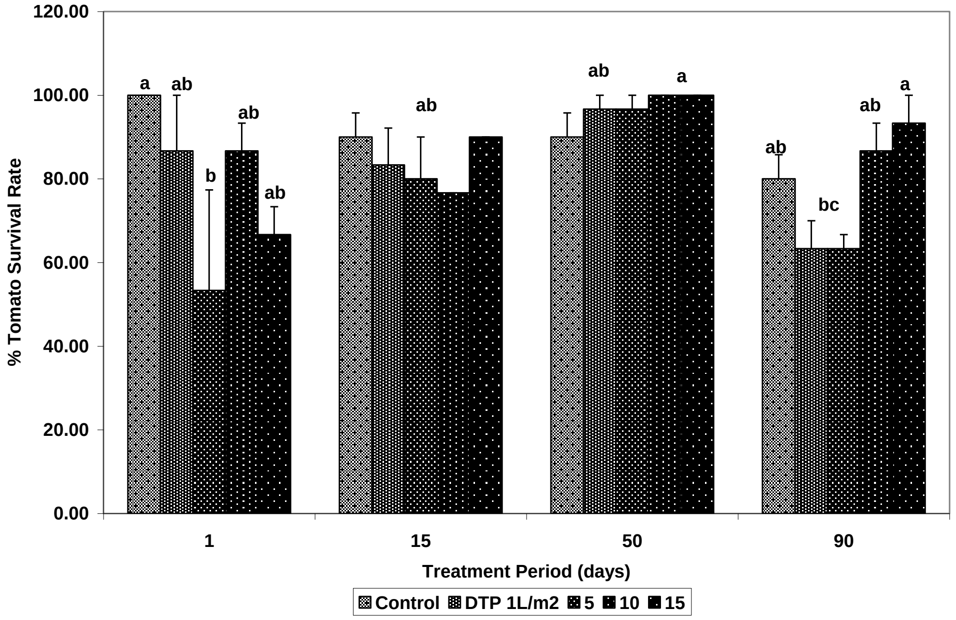

Fig. 10a: Effect of OMW from DTP on the survival rate (%) of tomato plants (*Lycopersicon esculentum*).

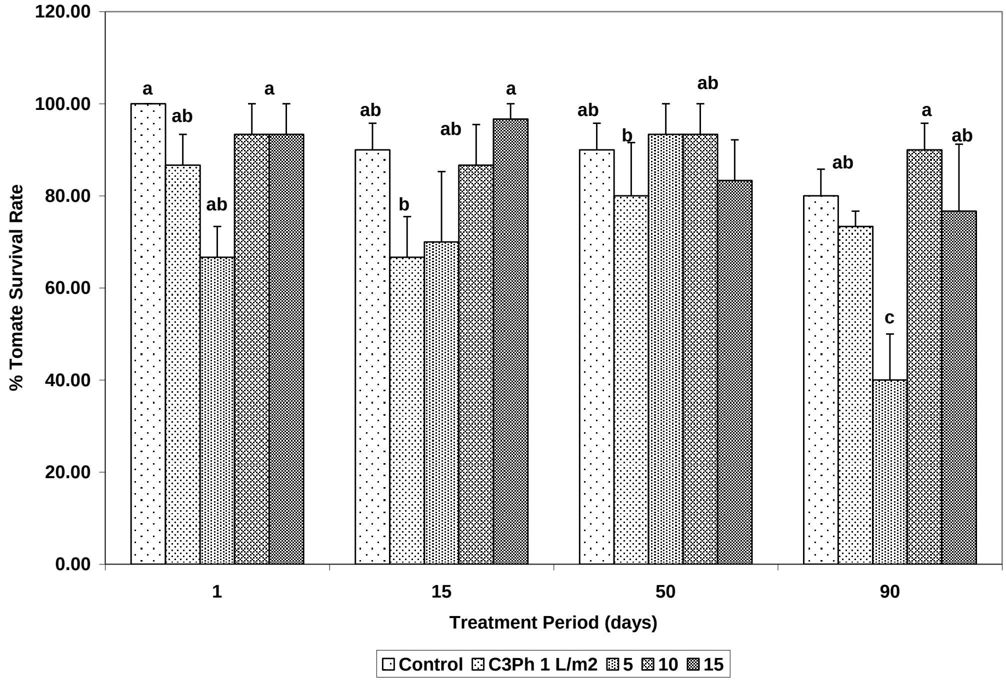

Fig. 10b: Effect of OMW from C3Ph on the survival rate (%) of tomato plants (*Lycopersicon esculentum*).
Values with different letters are significantly different at $LSD_{0.05}$ (Fisher's least-significant-difference test). Bars as Standard Error

Effect of OMW on height growth:

For barley, Figures 11a and 11b show significant differences between treated groups and the control. Twenty days after planting, height growth of treated groups was still lower than the control for both OMW. We note that the length of plants treated with the higher dose is less than that of plants treated with the lowest dose, for sowing 1 and after 15 days of application. These differences become insignificant between the various lots and disappear for sowing after 90 days of application.

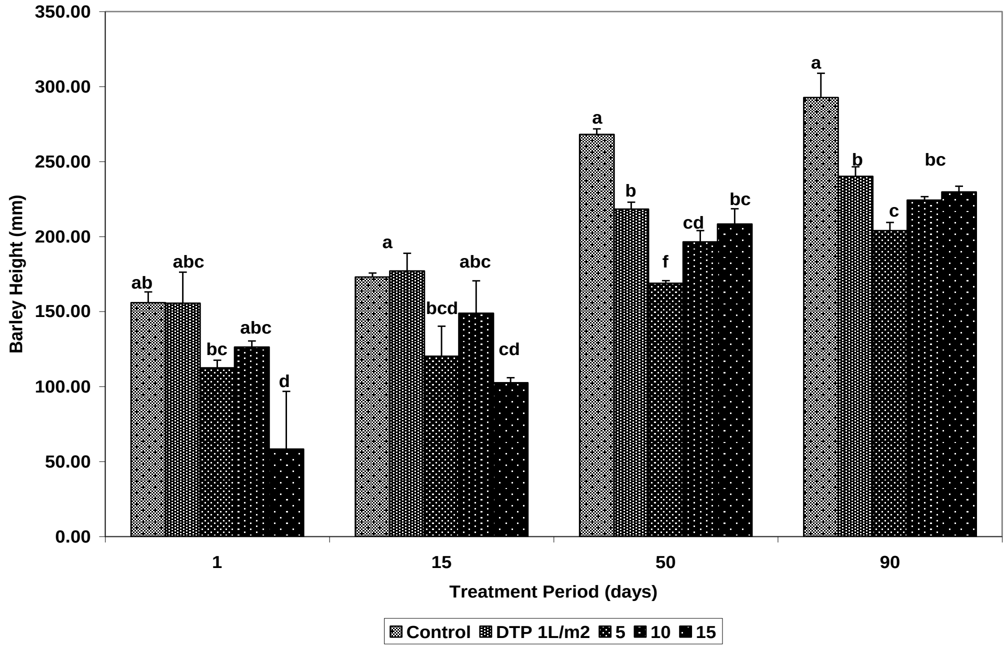

Fig. 11a: Effect of OMW from DTP on growth in height (mm) of barley (*Hordeum vulgare*).

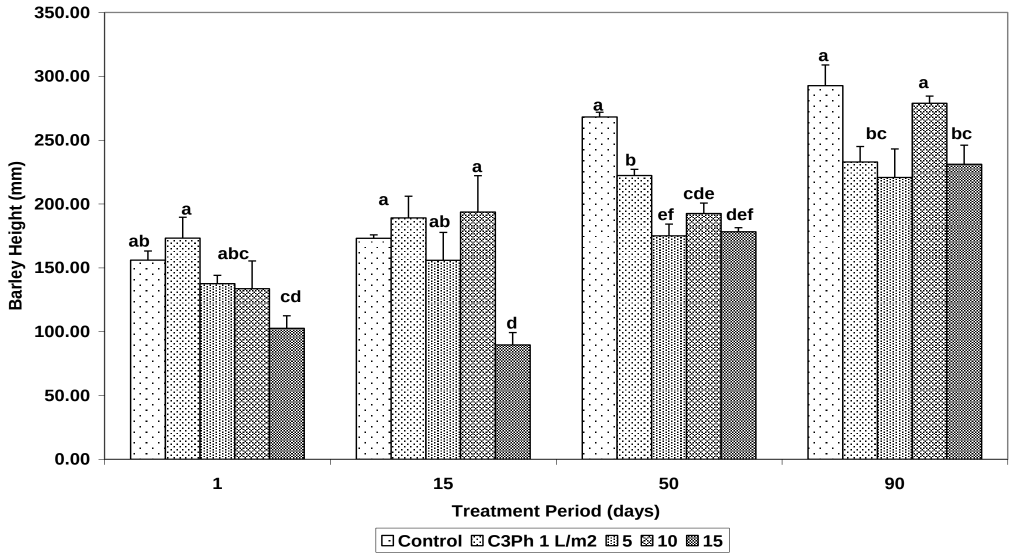

Fig. 11b: Effect of OMW from C3Ph on growth in height (mm) of barley (*Hordeum vulgare*).
Values with different letters are significantly different at $LSD_{0.05}$ (Fisher's least-significant-difference test).
Bars as Standard Error

For tomato, we note that there are no significant differences between the treated group and the control for the OMW from DTP with the exception at low doses after 50 and 90 days of application (Figure 12a). In the case of OMW from C3Ph, significant differences are noticed with the control group especially after 1, 30 and 50 days of application (Figure 12b). After 90 days of application, these differences become insignificant. On average, in the case of OMW from DTP, plant height after 50 and 90 days of application, is superior in lots at higher doses than those at lower doses. In the case of OMW from C3Ph, the height of plants treated with the higher doses is less than or equal to that of other doses, at 1, 30 and 50 days of application. This effect is reversed after 90 days of application. But, in general, the OMW did not inhibit growth of tomatoes. On the other hand, during a trial performed by Ammar and Ben Rouina, (1999), the growth of tomato plants grown in pots of 16 liters was significantly improved following the intake of 0.5 L OMW. Also, the OMW increased growth of ryegrass when sowing was carried out 48 days after the addition, despite the application of excessive doses of 40 and 80 $L.m^{-2}$ (Morisot and Tournier, 1986).

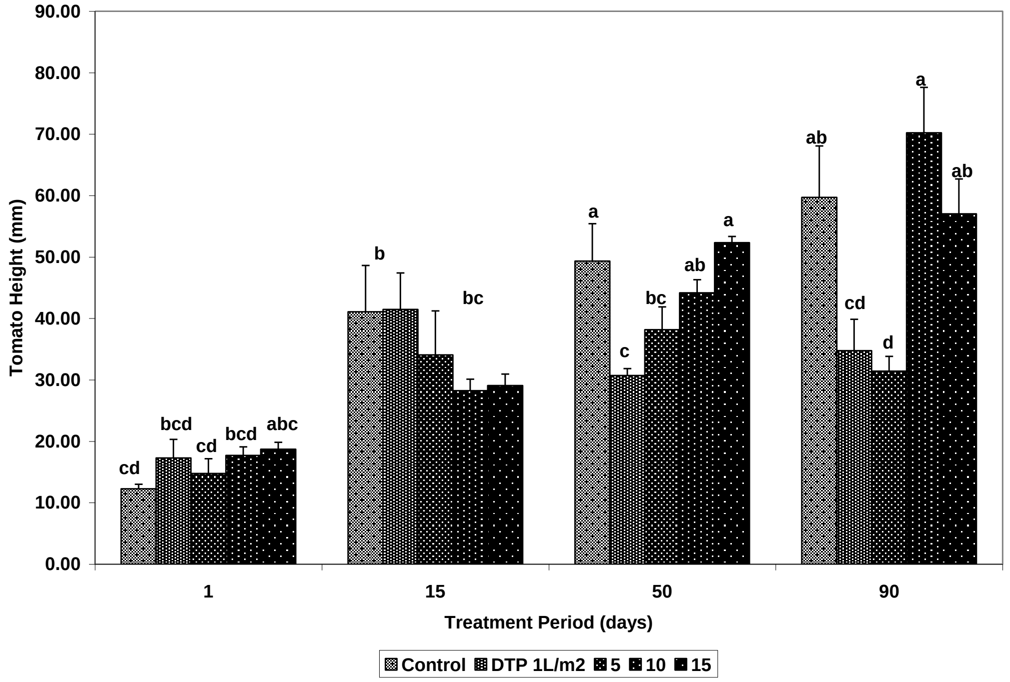

Fig. 12a: Effect of OMW from DTP on growth in height (mm) of tomato plants (*Lycopersicon esculentum*).

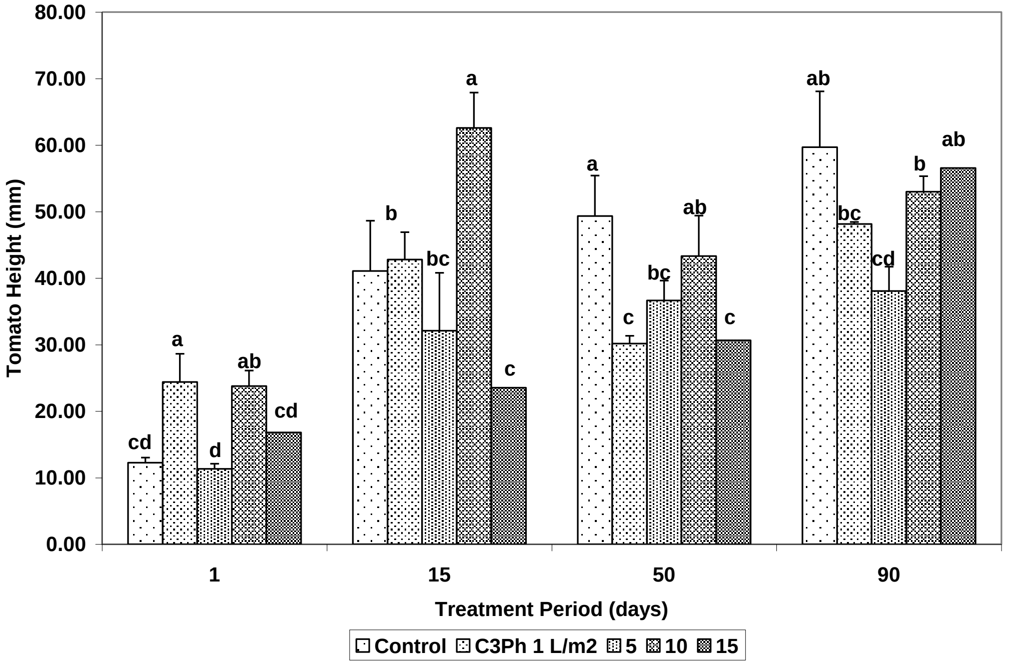

Fig. 12b: Effect of OMW from C3Ph on growth in height (mm) of tomato plants (*Lycopersicon esculentum*).
Values with different letters are significantly different at $LSD_{0.05}$ (Fisher's least-significant-difference test). Bars as Standard Error

Effect of OMW on the dry matter:

For barley seedlings, Figures 13a and 13b show that at 1 and 15 days after application, the dry matter of the control is higher than other treatments especially at the most important dose 15 $L.m^{-2}$ OMW from DTP. Over time, an improvement is observed for this highest dose. However, after 50 and 90 days of application, an improvement was observed and that is relative to the control for all doses of OMW. The beneficial effect of OMW for 50 days after sowing is likely related to a part the disappearance of phytotoxicity with the time, and partly to the enrichment of OMW on nutrients and organic matter. Contrariwise, Rinaldi *et al.*, (2003), showed that plant dry matter accumulation was not significantly influenced by OMW, obtained from the traditional extraction method, that was directly applied at 50 t ha^{-1} to durum wheat (*Triticum durum Desf.*).

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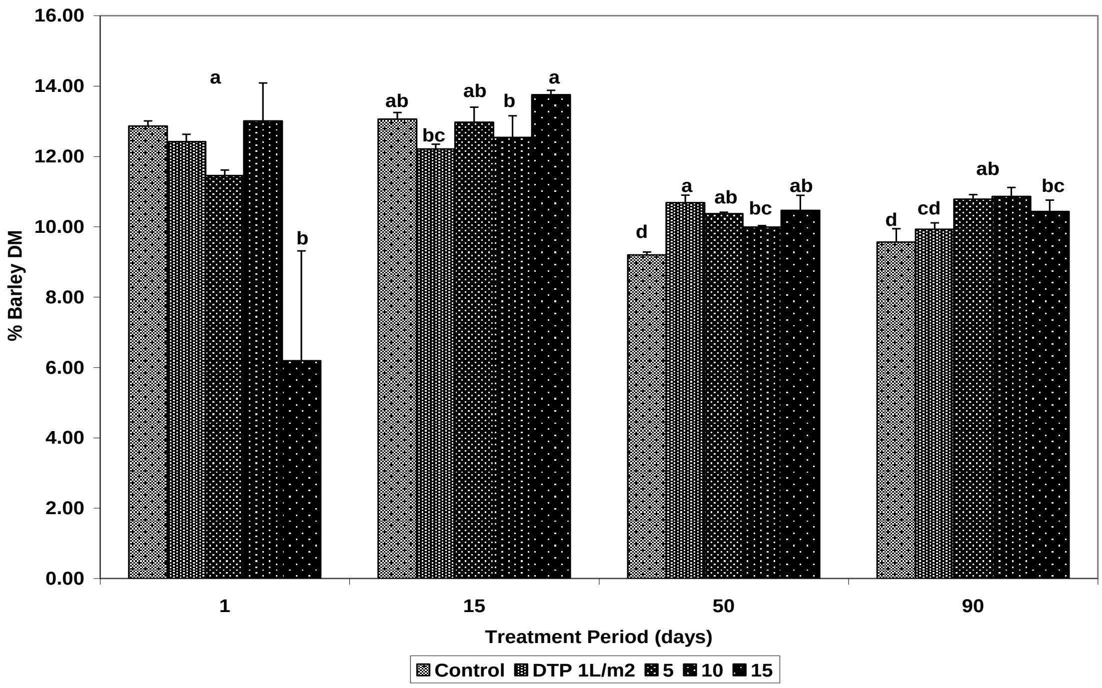

Fig. 13a: Effect of OMW from DTP on the dry matter (% DM) of barley (*Hordeum vulgare*).

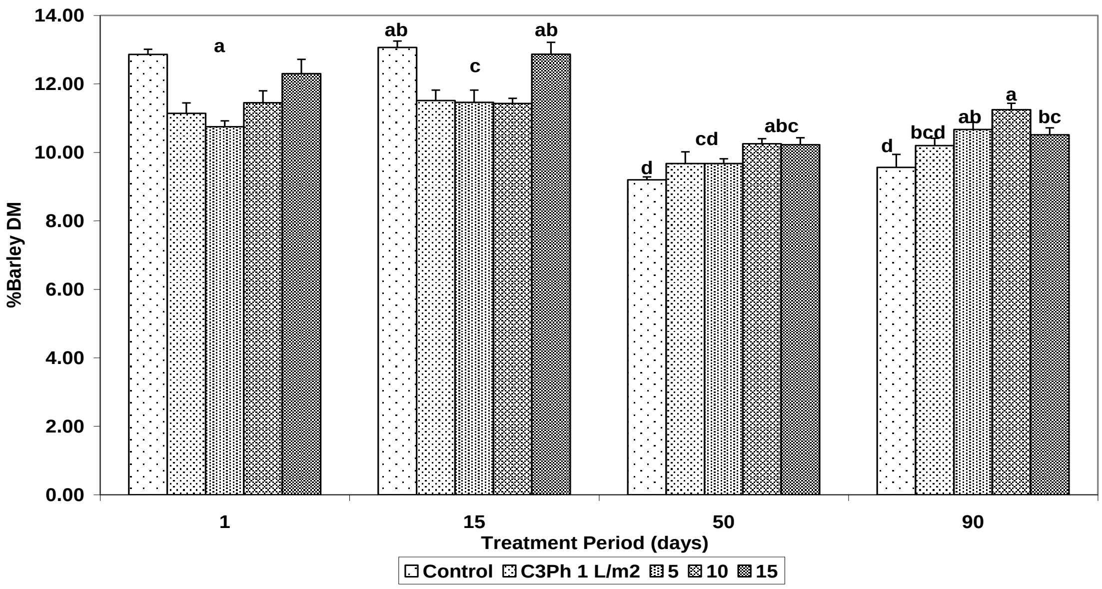

Fig. 13b: Effect of OMW from C3Ph on the dry matter (% DM) of barley (*Hordeum vulgare*).
Values with different letters are significantly different at $LSD_{0.05}$ (Fisher's least-significant-difference test). Bars as Standard Error

For tomato, statistical analysis showed no significant differences between treatments for sowing after 1, 15 and 30 days of application (Figures 14a and 14b). For the tomato seedlings developed after 50 days of application, an improvement of dry matter was noted for all doses of OMW used with respect to the control. This is probably due to the enrichment of soil by OMW after 50 days of application. These results show that OMW affect dry matter production of barley and tomato. However, the inhibitory effect of OMW, marked for planting out after 1 and 15 days of application, disappear after 50 and 90 days of application.

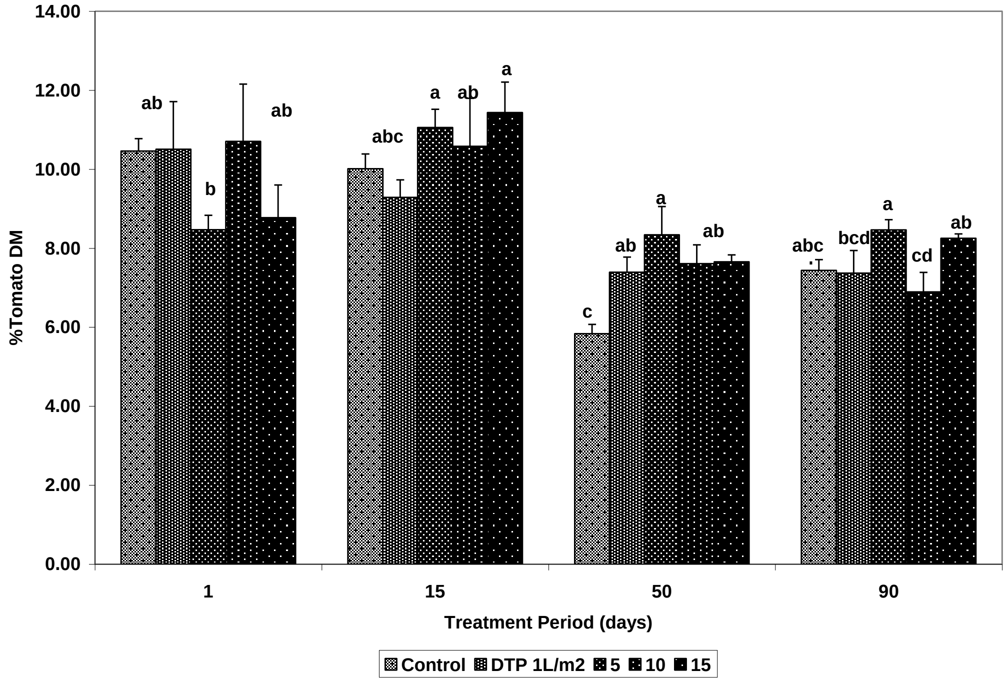

Fig. 14a: Effect of OMW from DTP on the dry matter (% DM) of tomato plants (*Lycopersicon esculentum).*

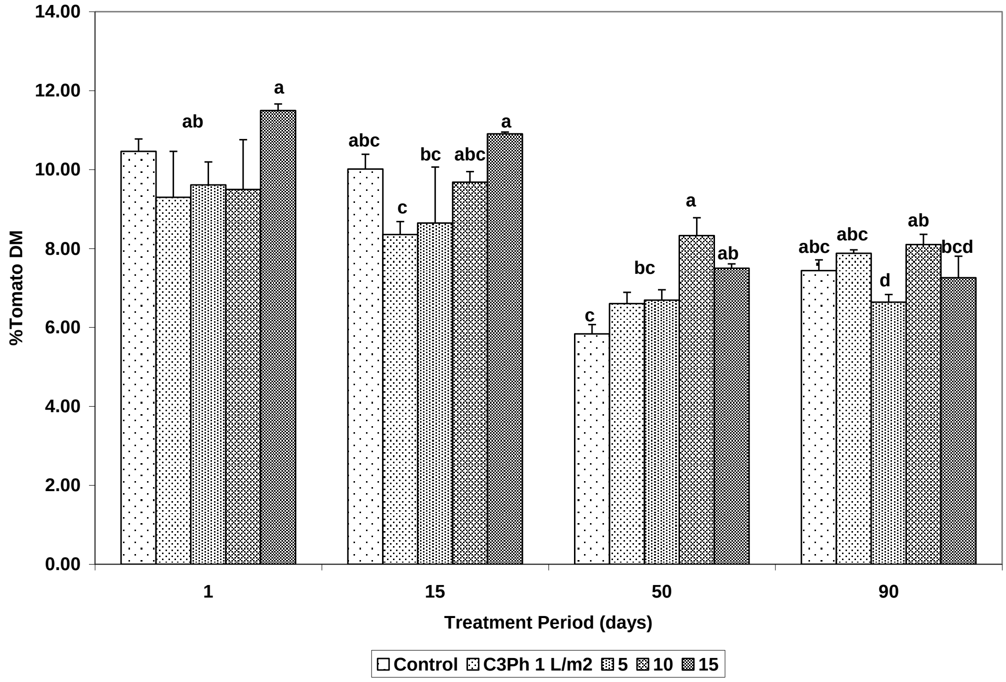

Fig. 14b: Effect of OMW from C3Ph on the dry matter (% DM) of tomato plants (*Lycopersicon esculentum).*
Values with different letters are significantly different at $LSD_{0.05}$ (Fisher's least-significant-difference test).
Bars as Standard Error

Effect of OMW on the germination index:

The magnitude of phytotoxicity can be determined indirectly by reducing the phytotoxicity germination when the index rises. For barley and watercress (Figure 15a), the index of germination is nil for all dilutions. The seeds of these plants have not germinated in solutions of vegetation water. The inhibition of germination is complete. Both types of plants are considered sensitive and detect direct phytotoxicity of OMW. For tomato (Figure 15b), after seven days, the germination index is below the value of 50 % which is the limit that indicating the absence of phytotoxicity. The inhibitory effect is noticeable for all solutions. After 9 days, the inhibitory effect is diminished especially for the OMW from C3Ph and for dilutions ranging from 10 to 25%. As a result, the germination index is higher than 50%. But, Komilis *et al*., (2005), have shown that the direct application of OMW has an inhibitory effect on seed germination of tomato and watercress. Using wheat (*Triticum aestivum*) and radish (*Raphanus sativus*) as indicators, Garcia-Ortiz *et al*., (1999), showed a strong decrease in the phytotoxicity after 15 days of OMW application in pot assays. The main effect of its application was not only a decrease in the germination but also a delay in the emergency of plants; but in order to lose the phytotoxic effects, at least 45 days must pass before planting the seedlings.

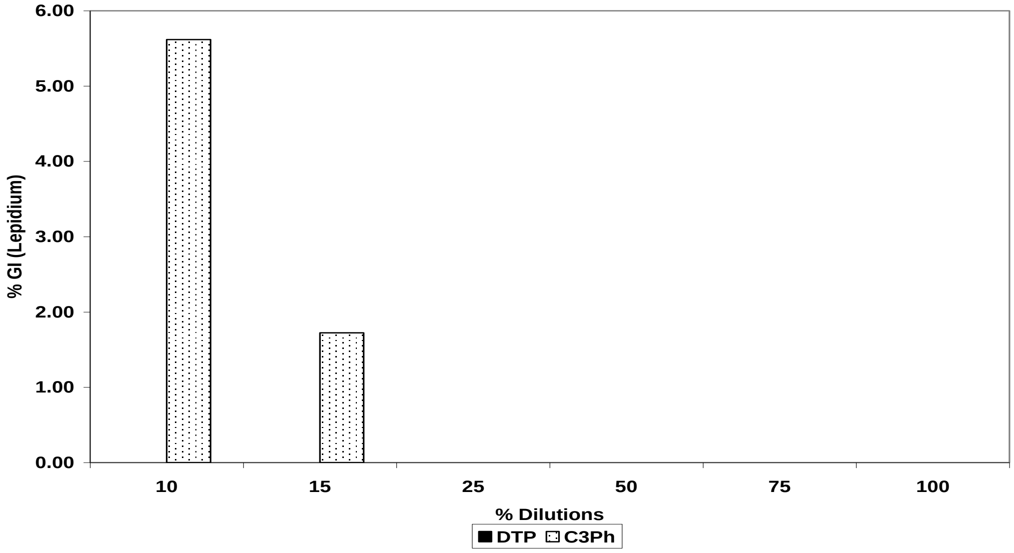

Fig. 15a: Effect of different aqueous dilutions of OMW from DTP and from C3Ph on the germination index (GI%) of seedlings of cress (*Lepidium sativum)* after 3 days of incubation.

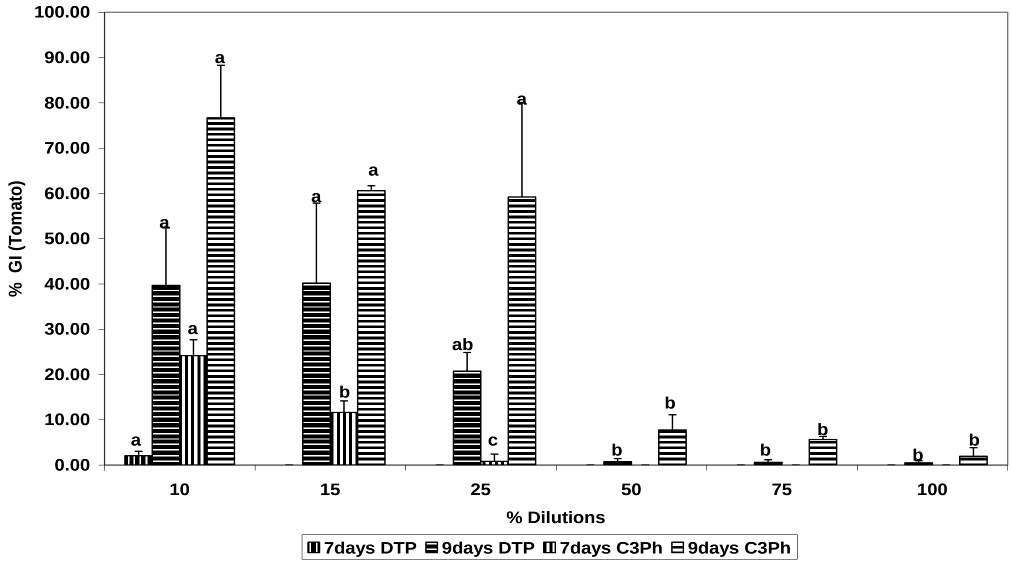

Fig. 15b: Effect of different aqueous dilutions of OMW from DTP and from C3Ph on the germination index (GI%) of tomato seedlings (*Lycopersicon esculentum*) after 7 and 9 days of incubation.
Values with different letters are significantly different at $LSD_{0.05}$ *(Fisher's least-significant-difference test).*
Bars as Standard Error

Conclusion:

Under our experimental conditions, our results suggest that the olive mill wastewater (OMW) can reduce the use of chemical fertilizers as potassium and phosphorus, and can improve soil fertility and the bioavailability of potassium and phosphorus. On the other hand, the controlled use of OMW adjusts the slight changes of acidity and salinity. Improvements were observed for organic matter, the C/N ratio. On the other side, application of OMW has not provided considerable nitrogen to the soil.

The OMW has relatively affected the development of the plant according to the doses and times of sowing after a certain time of application. Despite the low levels of phenolic substances in our OMW and salts, high doses and clearly those of the vegetation water press decrease the survival rate of barley after one day of application. However, this inhibitory effect disappears after 50 days. The tomato crops did not show great sensitivity to the use of OMW even for increasing doses and after one day of application. On the dry matter production, the negative effect of OMW obtained for planting out after 1 and 15 days of application tend to disappear after 50 and 90 days of application with the best production of the dry matter.

The relatively positive results from the application of OMW, obtained for sowing in the soil, are not observed in the Petri-dishes in which the inhibition of germination is almost complete especially for barley and watercress.

Ultimately, the spreading of OMW on agricultural soils may be the most economical and most useful technique to use, in order to solve the problem of the olive mill wastewater.

References

Abichou, M., B. Ben Rouina, H. Taamallah and K. Gargouri, 2003. Essais de valorisation des margines par epandage en oliveraies. Revue Ezzaitouna, 9, 1 et 2, 1-21.

AFNOR., 1987. Qualité des sols, méthodes d'analyse. 1ere édition, Edt. Afnor, 135 pp.

Ammar, E. and B. Ben Rouina, 1999. Potential horticultural utilization of olive oil processing waste water. Acta Horticulturae, 474(2): 741-744.

Albi Romero, M.A. and J.A Fiestas Ros De Ursinos, 1960. Study of alpechin for industrial use. Trials for possible utilization as fertlizer. Grasas Aceites, 11: 123-124.

Balice, V. and O. Cera, 1984. Acidic phenolic fraction of the olive vegetation water determined by a gas chromatographic method. Grasas Aceites, 35: 178-180.

Beley, J., 1948. Dosage des éléments minéraux chez les végétaux., 391-450. Dans: "Traité pratique de chimie végétale", tome II, A.BRUNEL, Ed., Georges frère, Tourcoing, 515pp

Ben Rouina, B., K. Gargouri, M. Abichou and H. Taamallah, 2006a. Mill wastewater as an ecological fertilizer for olive tree orchards. Olivebioteq, Vol II, November 5th-10th –Mazara del Vallo, Marsala, Italy, 139-141.

Ben Rouina, B., K. Gargouri, M. Ayadi, M. Abichou, A. Rhouma, H. Bentaher, et H. Taamallah, 2006b. La gestion des effluents liquides des huileries: Etude de l'effet de l'épandage direct des margines sur le sol et la plante en oliveraie. Conférence Internationale MEDAWATER. Marrakech, Maroc, pp 10.

Bonari, E., M. Macchia, L.G. Angelici and L. Ceccarini, 1993. The waste water from olive oil extraction: Their influence on the germinative characteristics of some cultivated and weed species. Agricoltura Mediterranea, 123: 273-280.

Bricolli-Bati, C. and N. Lombardo, 1990. Effects of olive oil waste water irrigation on young olive plants. Acta Horticulturae, 286: 489-491.

Cabrera, R., A. Lopez and J. M. Murillo, 1997. Land treatment of olive mill wastewater. Inter. Biodeter. & Biotechnol., 215-245.

Catalano, M., T. Gomez, M. De Felice and T. De Leonardis, 1985. Disposal of wastewaters from oil mills. What alternatives to treatment? Inquinamento, 27(2): 87-90.

Catalano, M., T. Gomez, M. De Felice and T. De Leonardis, 1986. Disposing of vegetation water mills. Inquinamento, 27(2): 199-202.

Cegarra, J., C. Paredes, A. Roig, M.P. Bernal and D. Garcia, 1996. Use of olive mill wastewater compost for crop production. International Biodeterioration Biodegradation, 38: 193-203.

Chartzoulakis, K., G. Psarras, M. Moutsopoulou and E. Stefanoudaki, 2010. Application of olive mill wastewater to a Cretan olive orchard: Effects on soil properties, plant performance and the environment. Agriculture, Ecosystems & Environment, 138, Issues 3–4, 15 August, 293-298.

Cox, L., A. Becker, R. Celis, R. Lopez, N.R.C. Hermosin and J. Cornejo, 1996. Movement of clopyralid in a soil amended with olive oil mill wastewater as related to soil porosity. Fresenius Environ., 3-4: 167-171.

Della Monica, M., D. Potenz, E. Righetti and M. Volpicella, 1978. Polluting effect of wastewater from the olive processing on agricultural land.1: Evolution of pH, the compound of nitrogen and phosphate. Inquinamento, 22(10): 81-87.

Della Monica, M., D. Potenz, E. Righetti and M. Volpicella, 1979. Polluting effect of wastewater from the olive processing on agricultural land. 2: Evolution of lipids, polyphenols and organic matter in general. Inquinamento, 21(1): 27-30.

De Simone, C. and A. De Marco, 1996. Toxicity and genotoxicity of wastewaters. International Seminar on "Treatment and recycling in agriculture by-products of the oil industry", Lecce, 8-9 March.

Di Giovacchino, L., C. Basti, N. Costantini, M.L. Ferrante and G. Surricchio, 2001. Effects of olive vegetable water spreading on soil cultivated with maize and grapevine. Agricoltura Mediterranea, 131: 33-41.

Di Giovacchino, L., C. Basti, N. Costantini, G. Surricchio, M. Ferrante, et D. Lombardi, 2002. Effets de l'épandage des eaux de végétation des olives sur des sols complantés de maïs et de vigne. Olivae, 91, avril, 37-43.

Fedeli, E. and F. Camurati, 1981. Valorisation des margines et des grignons épuises par récupération de quelques composants. Séminaire International sur la valorisation des sous-produits de l'olivier PNUD/FAO/COI. Monastir, Tunisia, December 1981.

Fiestas Ros de Ursinos, J.A., 1966. Study of alpechin for industrial use. VII. Commercial installation for obtaining yeast-feed. Grasas y Aceites, 17: 41.

Fiestas Ros de Ursinos, J.A., 1981a. Water residues purification of olives and olive oil. Grasasyaceites, 28: 113-121.

Fiestas Ros de Ursinos, J.A., 1981b. Différentes utilisations des margines : recherches en cours, résultats obtenus et applications. In : Séminaire International sur la Valorisation des Sous-Produits de l'Olivier. PNUD/FAO. Monastir, Tunisie, 93-110.

Fiestas Ros de Ursinos, J.A. and R. Borja Padilla, 1992. Use and treatment of olive mill wastewater: Current situation and prospects in Spain. Grasas Aceites, 43: 101-106.

Galli, E., U. Tomati, A. Grapelli and R. Buffone, 1988. Recycle of olive oil waste waters for Pleurotus mycelium production in submerged culture. Agrochim., 32: 451.

Garcia-Ortiz, A., G. Beltran, P. Gonzalez, R. Ordoñez and J.V. Giraldez, 1999. Vegetation water (alpechin) application effects on soils and plants. Acta Horticulturae, 474: 749-752.

Gariglio, N.F., M.A. Buxatti, R.A. Pilatti, D.E. Gonzalez Rossia and M.R. Acosta, 2002. Use of a germination bioassay to test compost maturity of willow (salix sp.) sawdust. New Zealand Journal of Crop and Horticultural Science, 30: 135-139.

Hamdi, M., 1991. The effect of pretreatment and agitation on the batch anaerobic digestion of olive mill waste waters. Bioresource technology, 36: 173-178.

Hamdi, M., A. Khadir and J.L. Garcia, 1991. The use of Aspergillus niger for bioconversion of olive mill waste water. Appl. Microl. Biotech., 34(6): 249-268.

Hamdi, M. and L.J. Garcia, 1993. Anaerobic digestion of olive mill wastewaters after detoxification by prior culture of Aspergillus niger. Process Biochemistry, 28: 155-159.

Hamzé, M., M. Nimah and M. Zaabout, 1984. Effectiveness of six digestion procedures to evaluate the states of major elements (Ca, K, Mg and Na) in citrus leaves. Commun. Soil Sci. Plant Anal., 15: 1135-1145.

Komilis, D., E. Karatzas and C.P. Halvadakis, 2005. The effect of olive mill waste water on seed germination after various pretreatement techniques. Journal of Environmental Management, 74: 339-348.

Le Verge, S., 2004. La fertilisation à partir des résidus de la trituration des olives. Le nouvel Olivier, 42: 5-21.

Levi-Minzi, R., A. Saviozzo, R. Riffaldi, Falzo, 1992. L'épandage au champ des margines: effets sur les propriétés du sol. Olivae, 40, Février, 20-25.

Maestro Duran, R., R. Borja Padilla, A. Martin Martin, J.A. Fiestas Ros de Ursinos, J. Alba Mendoza, 1991. Biodegradation of phenolic compounds present in the alpechin. Grasas Aceites, 42: 271-276.

Marsilio V., L. Di Giovacchino, M. Solinas, N. Lombardo and C. Briccoli-Bati, 1990. First observations on the disposal effects of olive oil mills vegetation waters on cultivated soil. Acta Horticulturae, 286: 493-496.

MOE, 2007. Integrated waste management for the olive oil pressing industries in Lebanon, Syria and Jordan (IMOOPW). Http://Olivepress.MOE.Gov.Lb- Ministry of Environment-Lebanon, 1(1): 4.

Montedoro, G., M. Sevili, M. Baldini and E. Miniati, 1992. Simple and hydrolysable phenolic compounds in virgin olive oil.1. Their extraction, separation, and quantitative & semi quantitative evaluation by HPLC. Journal of Agricultural Food Chemistry, 40: 1571-1576.

Morisot, A., J.P. Tournier, 1986. Répercussions agronomiques de l'épandage d'effluents et déchets de moulins à huile d'olive. Agronomie, 6(3): 235-241.

Navarro, A.F., J. Cegarra, A. Roig and D. Garcia, 1993. Relationships between organic matter and carbon contents of organic wastes. Bioresource Technology, 44: 203-207.

Nefzaoui, A., 1999. Olive tree by-products. The Development of Integrated Crop/Livestock Production in Low Rainfall Areas of West Asia and North Africa (Mashreq/Maghreb Project). International Center for agricultural Research In the Dry Areas (ICARDA), October, 1999. 123 pp.

Noy, J. and A. Feinmesser, 1977. The use of waste water for agricultural irrigation. In: Water Renovation and Reuse (H.I. Shuval Ed.), Academic Press, New York, 73.

Olsen, S.R. and L.A. Dean, 1965. Phosphorus Methods of soil Analysis. Part 2. Ed. C.A. Black. American Society of Agronomy. Inc. Publisher Madison Wisconsin, USA.

Pagliai, M. and L. Vittori Antisari, 1993. Influence of waste organic matter on soil micro and macrostructure. Bioresource Technology, 43.

Pagliai, M., 1996. Effects of administration of wastewater from oil mills on the physical characteristics of the soil. International Seminar on "Treatment and recycling in agriculture by-products of the oil industry." Lecce, 8-9 March.

Pansu, M. and J. Gautheyrou, 2003. L'analyse du sol minéralogique, organique et minérale. Springer-Verlag France, 993 pp.

Paredes, C., J. Cegarra, J. Roig, M.A. Sanchez-Monedero and M.P. Bernal, 1999. Characterization of olive mill wastewater (alpechin) and its sludge for agricultural purposes. Bioresource Technology, 67: 111-115.

Pauwels, J.M., E. Van Rust, M. Verloo and Z.E. Mvoudo, 1992. Manuel de laboratoire de pédologie : Méthodes d'analyses des sols et des plantes. Publications Agricoles, 28: 265.

Pérez, J.D., E. Esteban, M. Gomez and F. Gallardo-Lara, 1986. Effects of waste water from olive processing on seed germination and early plant growth of different vegetable species. J. Environ. Sci. Health, Part B, 21: 349-357.

Pérez, J., T. de la Rubia, J. Moreno and J. Martinez, 1992. Phenolic content and antibacterial activity of olive oil waste waters. Environ. Toxicol. Chem., 11: 489-495.

Potenz, D., E. Righetti, A. Bellettieri, F. Girardi, P. Antonacci, L.A. Calianno and G. Pergolese, 1985. Evolution of phytotoxicity in a soil treated with sewage mills. 2. Application of the test "Germination of Lepidium sativum" and comparative study of some chemical and physico-chemical parameters. Inquin., 29: 49.

Ranalli, A., 1991. L'effluent des huileries d'olive: Propositions en vue de son utilisation et de son épuration. Références aux normes italiennes en la matière. I[ere] partie, Olivae, 37: 30-39.

Rinaldi, M., G. Rana and M. Introna, 2003. Olive-mill wastewater spreading in southern Italy, effects on a durum wheat crop. Field Crop Res., 84: 319-326.

Rodier, J., 1996. L'analyse de l'eau, eaux naturelles, eaux résiduaires, eau de mer. 8[ème] édition, DUNOD, 1384 pp.

Rowell, D.L., 1995. Soil Science: methods and applications. Longman Scientific & Technical. 329 pp.

Rozzi, A. and F. Malpei, 1996. Treatment and disposal of olive mill effluents. International Biodeterioration Biodegradation, 38: 135-144.

Ryan, J., S. Garabet, K. Harmsen and A. Rashid, 1996- A Soil and Plant Analysis Manual Adapted for the West Asia and North Africa Region. International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, 140pp.

Sayadi, S. and R. Ellouz, 1993. Screening of white rot fungi for the treatment of olive mill wastewaters. J. Chem. Tech. Biotechnol., 57: 141-146.

Sayadi, S. and R. Ellouz, 1995. Roles of lignin peroxidase and manganese peroxidase from Phanerochaete chrysosporium in the decoloration of olive mill wastewaters. Appl. Environ. Microbiol., 60: 1098-1103.

Schollenberger, C.J. and R.H. Simon, 1945. Determination of exchange capacity and exchangeable bases in soil-ammonium acetate method. Soil Sci., 59: 13-24.

Seferoglu, S., G. Aydin and M. Aydin, 2000. Effects of vegetation water of oil mills on some physical and chemical characteristics of soils. In "Proceedings of International Symposium on desertification" Konya, Turkey, 13-17 June 2000, 247-251.

Shashi, S.R., E. Hidekazu, S. Kazunori and I. Kazuyuki, 1998. Stabilization of chemical and biochemical characteristics of grass straw and leaf mix during in-vessel composting with and without seeding material. Soil Sci. Plant Nutr., 44(4): 485-495.

Statistical Analysis System, 2004. Stat Software, SAS Institute Inc., Cary, North Carolina, USA.

Tam, M.N.F.Y. and Y.S. Wong, 1996. Retention of wastewater-borne nitrogen and phosphorus in mangrove soils. Environ. Technol., 117: 851-859.

Tamburino, V., S.M. Zimbone and P. Quattrone, 1999. Storage and land application of olive-oil wastewater. (Accumulation et écoulement des margines par épandage sur le sol agricole.) Olivae, 76: 36-45.

Tandon, HLS., 1998. Methods of Analysis of Soils, Plants, Waters and Fertilizers. Tandon HLS (Ed.). Fertiliser Development and Consultation Organisation, New Delhi, India, 144pp.

Tomati, U., E. Gaslli and A. Grappelli, 1987. Prospects for the purification of waste water from oil mills. L'Inf. Agr., 48: 73.

Tomati, U., 2001. Programma Nazionale di Ricerca sui Reflui del Sistema Agricolo-Industriale, Sottoprogretto Reflui Oleari. Stato dell'arte sul problema dell'utilizzanione dei reflui oleari. (National Research Program on Agricultural and Industrial Wastewater System, Wastewater from oil mills. State of the art on the problem of utilization of oil mill wastes.) Http://users. Unimi.it/~fsangio/cnr/olio1.htm.

Vazquez Roncero, A., E. Graciani Constante and R. Maestro Duran, 1974. Components olive phenolics. I-pulp Polyphenols. Grasas y Aceite, 25: 269-279.

Walkley, A.J. and C.A. Black, 1934. Estimation of soil organic carbon by the chromic acid titration method. Soil Sci., 37: 29-38.

Zenjari, B., M. Hafidi, I. El Hadrami, J.R. Bailly and A. Nejmeddine, 1999. Traitement aérobie des effluents d'huileries par les micro-organismes du sol. Agrochimica, XLIII: 276-285.

Zenjari, B. and A. Nejmeddine, 2001. Impact of spreading olive mill wastewater on soil characteristics: laboratory experiments. Agronomie, 21: 749-755.

Zucconi, F., M. Pera, M. Forte and M. De Bertoldi, 1981. Evaluating toxicity of immature compost. Biocycle, 22: 54-57.

Middle East J. Agric. Res., 3(3): 425-447, 2014

Zucconi, F., A. Monaco, M. Forte and M. De Bertoldi, 1985. Phytotoxins during the stabilization of organic matter. In : Grasser, J.K.R. ed. Composting of agricultural and other wastes. Elsevier Applied Science Publishers, London, 73-86.