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# Land Spreading of Olive Mill Wastewater: Effect on Maize (*Zea mays*) Crop

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**Abstract:** This study deals with the evaluation of olive mill wastewater spreading effects on the fertility of soil in the arid area of Marrakech-Morocco. Investigations were performed taking into account agronomic application of OMW (5, 10, 20 and 50 $m^3$ $ha^{-1}$) as compared to chemical fertilisation with a common fertiliser (225 kg $ha^{-1}$ TSP (triple superphosphate) 45%, 200 kg $ha^{-1}$ ammonium sulfate 21%, 100 kg $ha^{-1}$ potassium sulfate 48%) under field conditions of Maize culture. Land spreading of OMW was conducted according to a fractionated application mode (the third of the dose at the beginning, then the rest one month after) during the vegetative stage of maize. Soil moisture, salinity, total organic carbon content, total nitrogen, phosphate and potassium increased proportionally to the amounts of untreated OMW. Plant growth didn't show significant differences when the control was compared to the lower dose treatment by OMW (5 $m^3$ $ha^{-1}$). In parallel, plant growth was efficiently raised by OMW inputs at the rates of 10 and 20 $m^3$ $ha^{-1}$ compared to the control plot. Significant amelioration was obtained in term of shoot height and roots length (12 and 23%, respectively) in plots treated with OMW at 20 $m^3$ $ha^{-1}$ rate. In addition, spikes, stems, fresh and dry weight of maize grown in plot treated with 20 $m^3 ha^{-1}$ of OMW increased by 45, 46, 48 and 55%, respectively. However, leaf area decreased by 10% in the plot amended with 50 $m^3$ $ha^{-1}$ as compared to control. Plants in soil amended with 50 $m^3$ $ha^{-1}$ of OMW accumulated two times phenolics into leaves than did the control. The same tendency was observed in peroxidase activity that constitutes an ubiquitous parameter of stress. Principal Component Analysis (PCA) showed a positive correlation between soil physicochemical properties and treatment in one hand and stress indicators in the other hand. These results suggest a stimulation of plant stress metabolism and this is consistent with the presence of high amounts of ionic salts in soil treated with higher OMW dose (50 $m^3 ha^{-1}$).

**Key words:** Olive mill wastewater, land spreading, soil fertility, stress parameters

## INTRODUCTION

In Mediterranean countries, large amounts of Olive Mill Wastewater (OMW) are produced by traditional and industrial olive mills over a limited time period (usually from October-December). The production was estimated to more than 30 million $m^3$ of OMW annually in the Mediterranean regions, where the culture of the olive-tree has an important socio-economical place (Casa *et al*., 2003). The removal of this waste is a problem for the whole community in general and for the producers and millers in particular. This is due to its toxicity exhibited against microorganisms and plants during seed germination and plant growth (Isidori *et al*., 2005; Fiorentiono *et al*., 2003; Paixao *et al*., 1999). For example, the high percentage of salt, the acidic pH, the presence of phenolics as well as short and long-chain fatty acids are believed to contribute to the phytotoxic (Casa *et al*., 2003; Kistner *et al*., 2004; Isidori *et al*., 2005) and antimicrobial (Gonzalez *et al*., 1990; Paixao *et al*., 1999; Fiorentiono *et al*., 2003; Isidori *et al*., 2005) nature of these wastes. Morocco, with more than 75% of traditional mills, is considered among the principal producers of these wastewaters. These liquid effluents, in the majority are collected in pits located near the mills, or discharged in non adapted wastewaters canalization, which can constitute an environmental pollution for ground water and stations of wastewaters treatment. To this very significant production of OMW and in absence of all adapted treatment processes, controlled spreading can

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constitute a less expensive alternative for the evacuation of a great quantity of these liquid effluents. Some characteristics of this material are favorable for agriculture, since this effluent is rich in organic matter, N, P, K and Mg (Casa *et al*., 2003; Rinaldi *et al*., 2003). To these considerations, controlled spreading of OMW on the ground for soil fertilizing was recommended by Fiestas Ros de Ursinos (1986), Levi-Minzi *et al*. (1992), Bonari *et al*. (1993) and Cabrera *et al*. (1996) in many Mediterranean countries. Furthermore, it was demonstrated that when incorporated or applied as much, OMW could favorably affect the soil balance by reducing evapo-transpiration (Mekki *et al*., 2006), increase soil fertility and crop growth (Vassilev *et al*., 1998; Casa *et al*., 2003; Cereti *et al*., 2004; Paredes *et al*., 2005). The maximum amount of OMW tolerated in the field is 80 and 50 $m^3$ $ha^{-1}$ for OMW obtained by centrifuge and pressure extraction techniques, respectively.

The aim of this study is the evaluation of the OMW spreading effect on the fertility of a marginal soil located in Marrakech region. The impact of these effluents, without preliminary treatments, on some of the soil chemical properties as on the Maize growth and yield was taken into account.

## MATERIALS AND METHODS

For this study, the field experiment was conducted in Saâda at 15 km to the west of Marrakech-Morocco and OMW were obtained from traditional mill with discontinuous press process, located in Marrakech-Loudaya (Morocco). This extraction unit is considered among the most active mills in the area with an average annual production estimated to more than 80 $m^3$ of OMW.

**Study sites and sampling:** The study area consisted in a field located in Saâda at 15 km to the west of Marrakech-Morocco. An automatic meteorological station was installed during maize crop cycle and data on the main climatic variables (air, temperature, humidity, solar radiation, wind velocity and precipitation) were collected daily. The climate of the experimental area was characterized by: weak and variable rains with an annual average of about 240 mm, for 40 days of rain approximately, a high average temperature, with daily and monthly important variations, the average of maximum (July) is of 37°C, while the minima (January) is of 4°C, a weak hygroscopy whose monthly average varies from 40% (August) to 70% (January) and a very strong evaporation. Annual evaporation is approximately 2.300 mm.

The field experiment was divided in six plots. Four experimental plots P1, P2, P3 and P4 were, respectively amended with 5, 10, 20 and 50 $m^3$ $ha^{-1}$ of untreated OMW. The plot P5 was amended with a common fertiliser (225 kg $ha^{-1}$ TSP (triple superphosphate) 45%, 200 kg $ha^{-1}$ ammonium sulfate 21%, 100 kg $ha^{-1}$ potassium sulfate 48%) while the plot P6 was not amended and served as control. The sowing was carried out with a line space of 70 cm, spacing between seeds of 15 cm and a 4 cm depth. The quantity of olive mill wastewater used for these experiments was supplied progressively with two applications (third of the dose at the beginning then the rest one month after). Soil samples were collected from different parts of each plot from three depth (0-20, 20-40 and 40-60 cm), using a soil auger. All soil samples, taken from each plot were then mixed, air dried, sieved with a mesh size of 450 µm and stored at 4°C prior to use. Physicochemical analysis of soil samples characterized by a clay one (35.7% clay, 25.3% fine silt, 10% coarse silt, 16.7% sand fine, 12.4% coarse sand, 8.5% $CaCO_3$) were done. The parameters analyzed are hydrogen ion concentration by pH meter (WTW-pH meter, 330 $set^{-1}$), Electrical Conductivity (EC) using a conductivimeter (WTW-conductivimeter, F 318 set), soluble contents of potassium using a flame photometer (Jenway PFP7), Available phosphorus (P) was measured by the method of Olsen and Sommers (1982). Organic C was carried out using Walkly- Black method, (Allison, 1965), total nitrogen by Kjeldahl digestion (Keeney and Nelson, 1982) and the soluble phenolics using the method described by El Hadrami *et al*. (2004).

### Physiological response to OMW amendment

**Extraction and analysis of phenolics:** Maize fresh leaves (200 mg) were ground at 4°C with 80% methanol. Total soluble phenolics levels were determined using the Folin-Ciocalteu method (Macheix *et al*., 1990).

**Extraction and analysis of proteins:** Fresh leaves (200 mg) were extracted with 2.5 mL of Tris-maleate buffer (0.1 M, pH 6.5) containing Triton X-100 (0.1 g $L^{-1}$) and centrifuged for 3 min at 7000 g. The supernatant was used as the crude proteins extract. Total proteins content were measured spectrophotometrically at 595 nm according to Bradford (1976) method.

**Extraction and analysis of peroxidases:** Peroxidases were extracted as mentioned above. Peroxidase activity was assayed spectrophotometrically at 470 nm using guaiacol as a substrate. Twenty microliter of enzyme extracts (200 mg FW per 2.5 mL) was added to 2 mL of reaction mixture consisting of a solution of 0.1 M Tris-maleate buffer (pH 6.5) and 25 mM guaiacol. Reactions were initiated with 20 µL of $H_2O_2$ (10 %) and stopped after 3 min.

**Evaluation of phenotypes parameters:** Plant elongation, leaf number, spikes number, leaf area, dry and fresh weight of the six first leaves, stems and spikes were determined to evaluate the effects of OMW treatments. At harvest, grain and straw yield, harvest index (grain yield to total dry matter ratio) and seeds weight were recorded.

**Statistical analysis:** The data obtained with measurement of soil parameters and also maize morphological, agronomic and stress indicators were statistically analysed using the SPSS statistical software Version 12.0 (SPSS Inc., Chicago). The significance of difference between mean values was determined by one-way analysis of variance (ANOVA). Tukey's HSD tests was used to compare means. The significant probability levels of the results are given at the $p<0.05$ level. For correlation testing, the Rho Spearman correlation coefficient was used. In order to determine if these parameters varied with soil treatment, Principal Component Analysis (PCA) was done. PCA was conducted using XLSTAT 2007. The factor loading scores for each parameter measured was used to asses the relative importance of each parameter in the calculation of the principal component axes.

## RESULTS

**Physicochemical properties of OMW and soil:** The sampled OMW used for this study were characterized by their high soluble phenolic contents, acidic pH and high salt values (Table 1). Concerning the soil, the supply of fertiliser increases significantly all physicochemical parameters evaluated excepted for the nitrogen content (Table 2). The pH of the soil decreased significantly ($p<0.05$) after OMW amendment in the two first layers of soil while it increases at 60 cm depth. Application of 50 $m^3$ $ha^{-1}$ of OMW caused a significant increase of EC as

Table 1: Physicochemical characteristics of the OMW used in the present study

| Properties | OMW |
|---|---|
| pH | 4.81±0.10 |
| EC (mS $cm^{-1}$) | 36.70±0.12 |
| SS (g $L^{-1}$) | 0.01±0.001 |
| TDM (g $L^{-1}$) | 0.09±0.001 |
| FDM (g $L^{-1}$) | 0.05±0.003 |
| VDM (g $L^{-1}$) | 0.04±0.0035 |
| COD (g $L^{-1}$) | 139.67±11.24 |
| NaCl (g $L^{-1}$) | 58.50±5.85 |
| $Cl^-$ (g $L^{-1}$) | 35.50±3.55 |
| $P_{total}$ (g $L^{-1}$) | 0.03±0.005 |
| $PO_4^{2-}$ (g $L^{-1}$) | 1.33±0.001 |
| N-$NH_4^+$ (g $L^{-1}$) | 7.95±0.59 |
| $NO^{2-}$ (g $L^{-1}$) | 16.35±0.49 |
| $Na^+$(g $L^{-1}$) | 14.96±0.13 |
| $Ca^{++}$ (g $L^{-1}$) | 0.49±0.00 |
| $K^+$ (g $L^{-1}$) | 7.95±0.59 |
| Soluble phenols (g $L^{-1}$) | 8.51±1.00 |

EC: Electrical Conductivity; COD: Chemical Oxygen Demand; SS: Suspended Solids; TDM: Total Dry Matter; FDM: Fixe Dry Matter; VDM: Volatile Dry Matter

Table 2: Physicochemical characterization of control soil, after application of fertiliser and after irrigation by different amounts from olive mill wastewater

| | | | | OMW concentration ($m^3$ $ha^{-1}$) | | | |
|---|---|---|---|---|---|---|---|
| Soil characteristics | Soil depth (cm) | Control | Fertiliser | 5 | 10 | 20 | 50 |
| pH (21°C) | 20 | 8.30±0.20d | 8.60±0.12c | 8.31±0.12d | 8.20±0.23c | 8.17±0.10b | 8.11±0.11a |
| | 40 | 8.40±0.23b | 8.41±0.20b | 8.65±0.10c | 8.43±0.10b | 8.42±0.16b | 8.31±0.20a |
| | 60 | 8.41±0.11a | 8.72±0.10e | 8.78±0.07f | 8.6 7±0.21d | 8.59±0.18c | 8.48±0.14b |
| Electrical conductivity | 20 | 0.24±0.006a | 0.51±0.011b | 0.46±0.013b | 0.51±0.012b | 0.72±0.02c | 1.47±0.021d |
| (25°C)(mmhos) | 40 | 0.37±0.006c | 0.34±0.011b | 0.31±0.006a | 0.31±0.006a | 0.38±0.01c | 0.50±0.006d |
| | 60 | 0.51±0.010a | 0.62±0.002b | 0.50±0.012a | 0.57±0.006b | 0.78±0.01c | 0.89±0.006d |
| Salinity (g $L^{-1}$) | 20 | 0.76±0.01a | 1.60±0.11b | 1.35±0.04c | 1.48±0.03c | 2.10±0.06d | 4.27±0.07e |
| | 40 | 1.18±0.01c | 1.08±0.01b | 0.90±0.01a | 0.90±0.01a | 1.10±0.04bc | 1.45±0.01d |
| | 60 | 1.63±0.01a | 1.98±0.12c | 1.46±0.03a | 1.67±0.01b | 2.28±0.10e | 2.60±0.01f |
| Total organic carbon (%) | 20 | 0.59±0.11a | 1.67±0.09c | 1.04±0.08b | 0.95±0.08b | 1.01±0.00b | 1.88±0.05d |
| | 40 | 0.55±0.05ab | 1.20±0.09d | 0.41±0.05a | 0.69±0.08bc | 0.75±0.05c | 0.83±0.05c |
| | 60 | 0.29±0.00a | 1.02±0.09c | 0.49±0.05ab | 0.58±0.10b | 0.52±0.05b | 0.68±0.10b |
| Organic matter (%) | 20 | 1.01±0.009a | 2.88±0.16c | 1.79±0.14b | 1.64±0.14b | 1.94±0.00b | 3.25±0.09d |
| | 40 | 0.95±0.091ab | 2.08±0.16d | 0.69±0.08a | 1.19±0.14bc | 1.29±0.08c | 1.39±0.08c |
| | 60 | 0.50±0.000a | 1.76±0.16d | 0.85±0.09ab | 1.01±0.18c | 0.90±0.09c | 1.17±0.18c |
| Total nitrogen (%) | 20 | 0.10±0.006a | 0.10±0.008a | 0.10±0.002a | 0.10±0.003a | 0.14±0.011b | 0.16±0.012b |
| | 40 | 0.06±0.002a | 0.08±0.002b | 0.08±0.002b | 0.09±0.001b | 0.13±0.002c | 0.13±0.001c |
| | 60 | 0.05±0.000ab | 0.06±0.003c | 0.05±0.000a | 0.06±0.001bc | 0.07±0.003d | 0.09±0.004e |
| C/N | 20 | 6.08±0.31a | 17.24±0.48e | 11.13±1.13cd | 9.81±0.50bc | 8.02±0.57b | 11.80±0.79d |
| | 40 | 8.14±1.15b | 14.90±0.84c | 4.52±0.66a | 7.61±1.09b | 5.93±0.59ab | 6.37±0.32ab |
| | 60 | 5.20±0.05a | 15.07±1.84c | 9.59±0.85b | 9.56±1.29b | 6.60±0.59ab | 7.16±1.06ab |
| $K_2O$ (%) | 20 | 566.49±29.10a | 624.70±29.19b | 644.26±16.87bc | 693.04±16.92c | 787.34±1.40d | 1288.87±17.48e |
| | 40 | 216.59±1.82a | 240.99±1.84a | 354.37±16.59b | 469.75±16.70c | 489.05±16.73c | 547.09±16.78d |
| | 60 | 157.35±1.77a | 200.83±1.81b | 223.99±3.17b | 223.99±3.17b | 260.28±1.86c | 363.97±28.76d |
| $P_2O_4$ (%) | 20 | 60.80±0.53a | 75.13±1.20a | 100.31±3.29b | 126.46±7.04c | 134.28±4.71c | 149.59±10.86d |
| | 40 | 61.27±0.96a | 65.64±4.09ab | 74.81±1.26b | 107.46±7.31c | 110.47±1.52c | 111.41±1.07c |
| | 60 | 65.32±1.24ab | 64.07±1.64a | 71.30±0.27b | 95.59±0.28c | 95.59±6.74c | 106.25±1.18d |

Means with the same letter(s) in the same line are not significant different at 0.05, using Turkey multiple range test

Table 3: Rho Spearman correlation coefficient between some physicochemical in soil properties

| Variables | pH | Salinity | OM (%) | TN (%) | $K_2O$ (%) | $P_2O_4$ (%) |
|---|---|---|---|---|---|---|
| PH | 1 | | | | | |
| Salinity | -0.600 | 1 | | | | |
| OM (%) | -0.257 | 0.886* | 1 | | | |
| TN (%) | -0.845* | 0.845* | 0.676 | 1 | | |
| $K_2O$ (%) | -0.829* | 0.829* | 0.600 | 0.845* | 1 | |
| $P_2O_4$ (%) | -0.829* | 0.829* | 0.600 | 0.845* | 1.000** | 1 |

*Correlation coefficient differs significantly at 0.05, **Correlation coefficient differs significantly at 0.01, OM: Organic Matter; TN: Total Nitrogen; $K_2O$: Exchange potassium; $P_2O_4$: Extractable phosphorus

well as salinity. A significant difference is also observed between fertiliser and OMW amended soil for conductivity and salinity. Total organic carbon and nitrogen increased at different concentrations of OMW amendment in different layers. The C/N ratio increased proportionally in the soil of the first layer (0-20 cm), remained constant for the other layers and was different comparing to fertiliser. Extractable P and exchange K percentages were significantly greater in soil receiving OMW for all treatments than control and fertiliser soils (Table 2). Correlation between soil properties (pH, salinity, organic matter, nitrogen, potassium and phosphorus) showed that the pH had a negative and significant effect with all others parameters. However, positive correlations were found between salinity and organic matter (r = 0.886*) and with extractable P and exchange K (r = 0.829*). Another positive correlation was observed between extractable P and exchange K (r =1.000**) (Table 3).

**Relationships between soil properties and treatment:** To determine the relationship between soil properties and treatment, PCA was done. The first two components of PAC which represented 94.35% of the total variability separated the six treatments soils into three distinct groups. The first group containing the fertiliser soil was characterized by a highest basic pH and organic matter. The second group contained the control soil and the two lower OMW applications (5 and 10 $m^3$ $ha^{-1}$). This group was characterized by its low content of organic matter, salinity and salts. The third group containing higher OMW applications (20 and 50 $m^3$ $ha^{-1}$) was characterized by its higher content of organic matter, nitrogen salinity, potassium and phosphorous (Fig. 1).

**Comparison of the soil amendment by chemical fertiliser and OMW on maize growth:** In order to determine the effect of the chemical fertiliser and OMW amendment on plant growth, some agronomical parameters of maize were determined under field conditions. Fertiliser had no effect on the development of maize as compared to control. Indeed, no significant difference was observed between fertiliser and control regarding FW (Fresh Weight) and

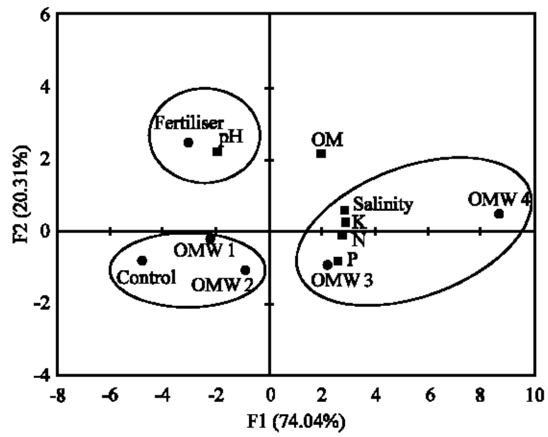


Fig. 1: PCA showing the effect of different soil treatments (●)(control, fertiliser and OMW) on some soil parameters (■) (pH, salinity, organic matter (OM), total nitrogen (N), total potassium (K), available phosphorus (P)). OMW1: 5 $m^3$ $ha^{-1}$, OMW2: 10 $m^3$ $ha^{-1}$; OMW3: 20 $m^3$ $ha^{-1}$; OMW4: 50 $m^3$ $ha^{-1}$

DW (Dry Weight) while leaves area (fifth and sixth leaves) presented a significant difference. No effect of OMW concentrations was observed for FW, DW and leaves area (Table 4). In addition, no significant differences were found in terms of shoots height, roots length, total leaves number per plant, spike number per plant comparing the control soil and the fertilizer soil (Table 4).

Plants grown on soil amended with 10 and 20 $m^3$ $ha^{-1}$ showed an enhancement of their shoot height and roots length with 12 and 23%, respectively as compared to control. Total leaves and spike numbers of treated plant didn't show any significant difference as compared to control. However, plants treated with 20 $m^3$ $ha^{-1}$ of OMW presented higher value with an increase of 45, 46, 48 and 55% for spike, stems fresh and dry weight, respectively. The yield of maize was more important in fertilizer and amended soils compared to the control soil (Table 5).

**Effect of OMW spreading and soil fertiliser on phenolics, peroxidases and proteins:** Some significant differences of stress indicators evaluated (phenolic compounds, peroxidases and protein contents) were found between OWM, fertiliser and control soils (Table 6). Highest contents of phenolic compounds and peroxidase activity were observed in plot treated with 20 and 50 $m^3$ $ha^{-1}$ of OMW. Indeed, plants in soils amended with 50 $m^3$ $ha^{-1}$ of OMW were two fold more concentrated in phenolic compounds and present peroxidase activities two times higher than that of control soil (Table 6). In addition,

Table 4: Fresh and dry weight, area of the six first plant leaves (control, fertiliser and OMW applications)

| | | | | OMW concentration ($m^3$ $ha^{-1}$) | | | |
|---|---|---|---|---|---|---|---|
| Leaves No. | Parameters | Control | Fertiliser | 5 | 10 | 20 | 50 |
| L1 | Leaves fresh weight (g) | 3.27±0.33ab | 2.88±0.85a | 3.51±0.26ab | 2.45±0.18a | 4.22±1.42b | 2.42±0.23a |
| | Leaves dry weight (g) | 0.86±0.5a | 0.70±0.57a | 0.59±0.39a | 1.09±0.6a | 0.94±0.42a | 0.96±0.46a |
| | Leaves area ($cm^2$) | 104.60±35.13ab | 128.48±22.59bc | 70.73±21.16a | 159.17±31.25c | 92.46±27.23ab | 68.87±21.36a |
| L2 | Leaves fresh weight (g) | 4.17±0.22a | 5.55±1.72ab | 6.08±0.23b | 5.49±0.21ab | 6.41±0.14b | 5.05±0.62ab |
| | Leaves dry weight (g) | 1.74±0.7a | 1.58±0.68a | 1.61±0.77a | 1.84±0.72a | 1.69±0.16a | 1.95±0.54a |
| | Leaves area ($cm^2$) | 192.40±54.65ab | 217.97±43.23ab | 170.61±46.48a | 250.74±49.77b | 200.65±58.83ab | 163.23±41.03a |
| L3 | Leaves fresh weight (g) | 6.30±0.26a | 9.46±0.14cd | 8.37±0.31bc | 7.85±0.26b | 10.18±1.75c | 8.84±0.75bcd |
| | Leaves dry weight (g) | 2.04±0.73a | 2.20±0.64a | 2.27±0.7a | 2.53±0.65a | 2.66±0.45a | 2.18±0.69a |
| | Leaves area ($cm^2$) | 253.50±59.9abc | 326.72±63.92c | 240.28±50.18ab | 302.44±62.94bc | 281.10±55.93abc | 208.83±61.07a |
| L4 | Leaves fresh weight (g) | 7.76±0.31a | 10.35±2.29ab | 8.57±0.33ab | 10.20±1.98ab | 11.10±2.4c | 9.67±0.29ab |
| | Leaves dry weight (g) | 2.43±0.88a | 2.65±0.69a | 2.68±0.93a | 2.53±1.01a | 2.95±0.48a | 2.71±0.65a |
| | Leaves area ($cm^2$) | 282.89±39.29a | 292.98±30.59a | 276.18±53.68a | 331.37±58.47a | 324.75±40.96a | 263.32±62.01a |
| L5 | Leaves fresh weight (g) | 8.34±0.41a | 10.09±0.83ab | 11.27±1.97b | 10.87±0.97b | 10.79±1.02b | 9.54±0.21ab |
| | Leaves dry weight (g) | 2.77±0.74a | 2.97±0.59a | 3.13±0.68a | 3.16±0.57a | 3.04±0.55a | 2.95±0.58a |
| | Leaves area ($cm^2$) | 311.07±42.8ab | 400.28±13.91c | 285.71±50.52ab | 360.18±44.06bc | 329.70±46.5abc | 273.40±74.53a |
| L6 | Leaves fresh weight (g) | 9.69±0.41a | 9.53±0.37a | 11.10±1.44b | 10.28±0.65ab | 10.67±0.22ab | 9.52±0.3a |
| | Leaves dry weight (g) | 2.70±0.71a | 3.11±0.66a | 2.85±0.84a | 3.17±0.72a | 3.01±0.83a | 2.86±0.58a |
| | Leaves area ($cm^2$) | 307.82±57.35a | 412.15±19.16b | 290.10±40.18a | 349.38±34.09ab | 317.59±31.87a | 276.27±81.74a |

Means with the same letter(s) in the same line are not significant different at 0.05, using Turkey multiple range test

Table 5: Effect of chemical fertiliser and OMW on some agronomic parameters of maize

| | | | Rate of OMW ($m^3$ $ha^{-1}$) | | | |
|---|---|---|---|---|---|---|
| Parameters | Control | Fertiliser | 5 | 10 | 20 | 50 |
| Shoots height (cm) | 167.10±25.07ab | 152.25±13.02a | 178.11±20.44ab | 190.89±16.69b | 190.50±25.19b | 180.00±7.81ab |
| Roots length (cm) | 14.60±3.15a | 17.25±2.59ab | 16.80±3.53ab | 20.25±2.23b | 19.15±3.99ab | 17.90±1.77ab |
| Total leaves number $plant^{-1}$ | 10.70±1.25a | 11.50±1.91a | 10.56±1.59a | 10.33±1.32a | 10.78±1.72a | 10.60±1.43a |
| Spike number $plant^{-1}$ | 2.40±0.52a | 2.75±0.50a | 2.87±0.35a | 2.67±0.50a | 2.44±0.73a | 2.30±0.73a |
| Spike fresh weight (g) | 172.36±17.34a | 249.20±32.03b | 191.00±31.76a | 255.19±30.77b | 316.26±31.98c | 161.95±20.35a |
| Spike dry weight (g) | 99.59±19.01a | 179.75±16.23c | 133.85±29.85b | 153.64±27.66bc | 183.50±14.37c | 137.35±27.36b |
| Stems fresh weight (g) | 141.63±34.55a | 239.68±48.47bc | 174.63±66.25ab | 215.48±38.89abc | 270.70±28.40c | 174.60±26.42ab |
| Stems dry weight (g) | 35.42±10.25a | 76.96±17.88b | 37.50±6.45a | 53.33±17.51ab | 78.75±15.94b | 45.00±16.95a |
| Straw yield (kg $ha^{-1}$) | 2020.00±72.11a | 4446.00±350.05d | 3326.00±236.92bc | 3500.00±200.00c | 3500.00±435.89c | 2634.67±165.85ab |
| 1000 seeds weight (g) | 189.33±6.09a | 213.76±6.35b | 224.36±6.80b | 222.33±5.85b | 228.56±9.30b | 222.26±9.15b |

Means with the same letter(s) in the same line are not significant different at 0.05, using Tukey's HSD test, Shoots height (cm), roots length (cm), total leaves and spike number, spike fresh and dry weigh (g), stems fresh and dry weight (g), straw yield and 1000 seeds weight of the control plants, after application of fertilizer and after soil amendment by different rate of olive mill wastewater (5 $m^3$ $ha^{-1}$, 10 $m^3$ $ha^{-1}$, 20 $m^3$ $ha^{-1}$ and 50 $m^3$ $ha^{-1}$)

Table 6: Comparison of some stress indicators in leaves from control plants, after application of fertilizer and after soil amendment by different rate of olive mill wastewater (5, 10, 20 and 50 $m^3 ha^{-1}$)

| | | | Rate of OMW ($m^3$ $ha^{-1}$) | | | |
|---|---|---|---|---|---|---|
| Parameters | Control | Fertiliser | 5 | 10 | 20 | 50 |
| Phenolic levels (mg equivalent (+)- catechin/g FW) | 36.06±1.15a | 47.52±1.29b | 37.92±0.36a | 49.66±1.51bc | 52.12±0.41c | 67.53±0.75d |
| Total peroxidase activity/g fresh weigh | 637.50±56.25a | 652.08±81.14a | 845.83±55.58b | 853.12±84.60b | 986.45±43.33bc | 1130.20±15.72c |
| Specific peroxidase activity/(mg protein) | 14.23±1.08b | 12.84±1.44b | 13.41±1.84b | 9.14±0.76a | 8.66±0.20a | 15.33±0.73b |
| Protein contents (mg $g^{-1}$ fresh weigh) | 8.95±0.11a | 10.14±0.29a | 12.91±0.96b | 18.64±0.37d | 22.75±0.50e | 14.76±0.76c |

Means with the same letter(s) in the same line are not significant different at 0.05, using Tukey's HSD test

when compared to control soil, specific peroxidase activity of plants decreased 36 and 39%, respectively in soil amended with 10 and 20 $m^3$ $ha^{-1}$. Plants obtained in OMW soil had 2.5 fold higher protein contents as compared to control. The protein contents and peroxidase activity did not show significant differences between fertiliser and control soils. Positive correlations were found between peroxidase activity and phenolic contents (r = 0.943**) in one hand and peroxidase activity with protein contents (r = 0.829*) in the other hand. There was no significant correlation between proteins and phenolic contents.

**Relationship between the soil, biochemical and agronomical parameters:** In order to determine the relationships between the stress plant indicators and agronomical parameters with the physicochemical soil properties, Rho Spearman correlation coefficient was used. Phenolic contents as well as peroxidase activity correlated positively with salinity, nitrogen, extractable P and exchange K (r values raging from 0.84* to 1**). There was no significant correlation between pH and phenolic contents as well as peroxidase activity. No significant correlations were found between yield (straw yield, 1000 seeds weight) and soil properties (Table 7).

Table 7: Rho Spearman correlation between some stress indicators and soil physico-chemical parameters

| | Rho Spearman correlation coefficient (r) | | | | |
|---|---|---|---|---|---|
| Soil variables | Phenols | Peroxidases | Proteins | Straw yield | 1000 seeds yield |
| pH | -0.771 | -0.829* | -0.657 | 0.348 | -0.314 |
| Salinity | 0.943** | 0.829* | 0.600 | 0.319 | 0.314 |
| OM (%) | 0.714 | 0.600 | 0.257 | 0.319 | 0.143 |
| TN (%) | 0.845* | 0.845* | 0.541 | -0.189 | 0.304 |
| $K_2O$ (%) | 0.943** | 1.000** | 0.829* | 0.058 | 0.600 |
| $P_2O_4$ (%) | 0.943** | 1.000** | 0.829* | 0.058 | 0.600 |

*Correlation coefficient differs significantly at 0.05, **Correlation coefficient differs significantly at 0.01; Stress indicators: phenols, peroxidases, proteins, straw yield and 1000 seeds yield; Soil physico-chemical parameters: pH, salinity, Organic Matter (OM), Total Nitrogen (TN), Exchange Potassium ($K_2O$) and available phosphorus ($P_2O_4$)

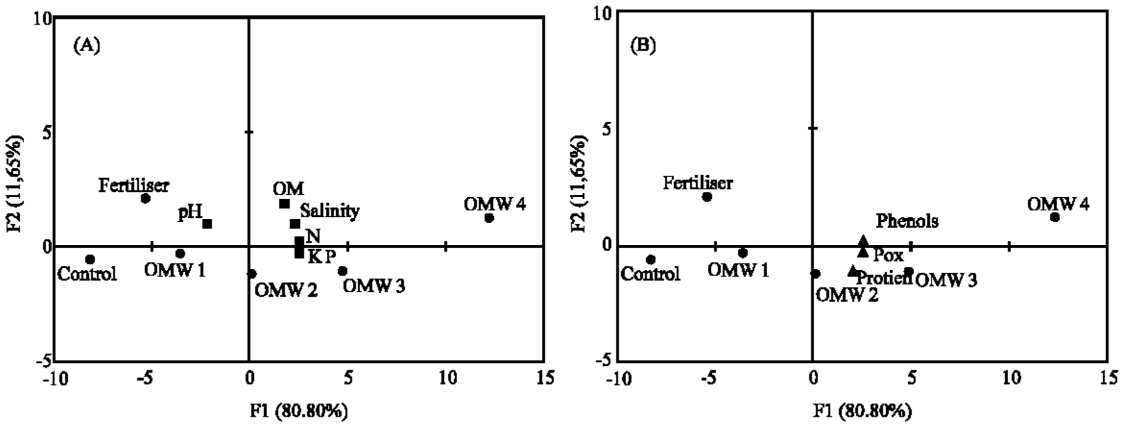


Fig. 2: Effect of soil treatment on the physicochemical properties and stress indicator. (A) PCA showing the effect of different soil treatments (●)(control, fertiliser and OMW) on some soil parameters (■) (pH, salinity, organic matter (OM), total nitrogen (N), total potassium (K), available phosphorus (P)). (B) PCA showing the effect of soil treatments (●)(control, fertiliser and OMW) on some stress indicators (▲) (phenols, proteins, peroxidase). OMW1: 5 $m^3 ha^{-1}$;OMW2: 10 $m^3 ha^{-1}$; OMW3: 20 $m^3 ha^{-1}$; OMW4: 50 $m^3 ha^{-1}$

**Relationship between the soil treatments, physicochemical parameters and stress indicators:** To determine the extent to which the soil treatment were related to physicochemical properties of soil and stress indicators (peroxidase activity, phenolic and protein contents), PCA was done. The first two factors represented 92.44% of the total variability with 80.80% of the variance for the first factor and 11.65% for the second factor. Soils rich in organic matter, salinity and salts (OMW3 and OMW4) contained more phenolics and protein contents and high peroxidase activity. Fertiliser soil with highest pH and high amount of organic matter was characterized by lower peroxidase activity. The control soil and the soil amended with lower amount of OMW (OMW1 and OMW2) were characterized by lower amount of physicochemical contents (salinity, N, K+ and P) and are negatively correlated to stress parameters (Fig. 2).

## DISCUSSION

The purpose of this study as compared to what it has been described by the team and others studies was to complete the evaluation of the influence of different application rates of OMW on the soil physicochemical properties and plant growth under natural field conditions.

Results obtained in this study showed that several chemical and biochemical properties of the investigated soil changed in response to the application of OMW. The acidity of the untreated OMW was compensated by the soil carbonate alkalinity. The soil carbonate at the same time became bicarbonates, moved and accumulated in deeper horizons as was proposed by Sierra *et al.* (2001). The high electrical conductivity measured for soil amended by 50 $m^3 ha^{-1}$ of OMW can be explained by the high level of sodium and chloride content in traditional OMW. This is in line with earlier studies done by El Hadrami *et al.* (2004), Hanifi and El Hadrami (2008), Paredes *et al.* (1987) and Sierra *et al.* (2001). Hence, in long-term applications, replacement of the soil $Ca^{++}$ by the $Na^+$, $K^+$ and $Mg^{++}$ could lead to the degradation of the soil structure and the formation of saline soils as was suggested earlier by Zenjari and Nejmeddine (2001). Indeed, the management of big olive stokes in small olive mill locally known as Maasras could not be made without addition of high quantities of salt to prevent olives from deterioration. It should be pointed out that there was considerable enrichment in available K after OMW addition, thought mainly at the 50 m$^3$ ha$^{-1}$ treatment level. The fresh input of easily degradable C and N substrates in OMW increased organic C concentration in all soil treatments and resulted in high microbial biomass levels (measured at total fatty acid methyl ester content) (Zelles *et al*., 1997). The increase of nutrient contents, C, N, P and K at the plot treated with fertiliser and amended with the highest rate of OMW (20 and 50 m$^3$ ha$^{-1}$), lead to a beneficial effect on the soil fertility. Despite their known phytotoxicity, OMW have a significant amelioration in plants growth. Soils treated with 10 and 20 m$^3$ ha$^{-1}$ of OMW show relevant growth stimulation notably with regard to leaf area, shoot height, root length, spike, stems, FW and DW. This could be due to nutrient availability, notably nitrogen, organic matter, P and K (Nevens and Reheul, 2003; Gavalda *et al*., 2005). This amelioration in plant growth can also be explained by stimulation of soil microbial activity (Tomati *et al*., 1996) and by the amelioration of the physical properties of the soil (Fischler *et al*., 1999). Consistent with these findings, growth assessment also reflects maize tolerance to OMW amendment even at high amounts. The yield of maize was more important in fertilizer and amended soils compared to the control soil. However, the evaluation of certain indicators of stress is essential for better understanding of the impact of OMW on the physiological state of plants.

Phenolic compounds are widespread in plant and tissue and could be more or less accumulated depending on the stress conditions. General tendency indicates relevant activation of phenolic metabolism proportionally to OMW doses. Phenolic contents as well as peroxidase activity were correlated positively with an increase of soil salinity, nitrogen, extractable P and exchange K. The neoaccumulation of phenolic compounds is common feature in many species in response to several biotic or abiotic stresses and phenolic compound are believed to have antioxidant properties (Cummins *et al*., 2006; Wahid and Ghazanfar, 2006). Accumulation of new phenolic compounds, particularly some flavonoids was revealed when plants are treated by OMW (El Hadrami *et al*., 2004; Hanifi and El Hadrami, 2008). Peroxidases are related to OMW cellular detoxification presumably by catalyzing the phenolics oxidation at the expanse of hydrogen peroxide (Jouili and Ferjani, 2003; Wang and Ballington, 2007). Peroxidases played a central role in the detoxification of plant. This biochemical parameter is also involved in lignin biosynthesis as a physical barrier against several stresses (Hegedüs *et al*., 2001; Adam *et al*., 1995).

## CONCLUSION

The recycling of OMW and its use as water for irrigation in agriculture is an attractive prospect for the Mediterranean countries in which water resources have been severely decreased in the past years. For this reason, a rational re-use coupled to a choice of the type of soil, such as the soil in Marrakech and of the cultures to be fertirrigated with can be regarded as an inexpensive solution to limit the impact of the seasonal discharge of these liquid effluents.

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