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Original Research Article

Olive Mill Wastewater Spreading Effects on the Olive Nutritional Status (*Olea europaea* L. cv. Chemlali)

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Abstract

Olive oil processing leads to the production of huge olive mill wastewater (OMW). In the present work, this rich effluent in minerals and organic compounds was used as amendment for olive tree in the field. The monthly evolution of seven mineral nutrients was studied in the leaves and the olive fruits after two years of OMW spreading at three levels (50, 100 and 200 m^3 ha^{-1} $year^{-1}$). In the plant tissues, the different mineral nutrients concentrations analysed, presented a notable fluctuation during the olive biological cycle. While N, P, Ca, Mg, Na and chloride concentrations were generally unaffected by the three OMW doses agronomic application, in spite of the high OMW amended salt load, the leaves and the fruits potassium concentrations, showed an improvement after the OMW high-levels (100 and 200 m^3 ha^{-1}) spreading, compared to the unamended field.

Keywords

Chemlali cultivar
Mineral status
Olive mill wastewater
Spreading

Introduction

In Tunisia, the oleiculture is the most extended crop, because of its socio-economical impacts, the olive oil health benefits, and its great importance in the green area landscape preservation, noticeably in the arid regions. Also this millenary tree contributes to the desertification limitation, the soil erosion and land degradation prevention. As a result, this dominant species is involved in the maintenance of land durability and it strengthens this tree position at the national socio economical level (Ben Ahmed et al., 2009).

In Tunisian arid regions, soils are globally characterised by low organic matter, phosphorous and nitrogen concentrations. Indeed, the organic matter content is lower than 2%, while that of the nitrogen varies from 0.1 to 0.5%, and that of the phosphorus is ranged between 5 and 40 ppm (Ben Rouina et al., 2002). These values are correlated to the regional fertilization practices lack, and are lower than the limit allowing a suitable olive tree development and an ecological system durability (Tsadilas and Chartzoulakis, 1999). As a consequence, it is urgent to find an adequate solution for improving the olive grove soils fertility which fundamentally determines the plant nutrition. Among the various practices, the olive mill wastewater (OMW) use in agricultural soils fertilization has been extended in the Mediterranean region, especially in Tunisia where OMW production may reach about 7×10^5 m^3 $year^{-1}$, a huge volume generated during few months between November and February (Jarboui et al., 2010).

The OMW high fertilization value is attributed to its richness in water and nutrient resources. In fact, this effluent consists typically of 83-94% water, 4-16% organic compounds, and 0.4-2.5% minerals (Hachicha et al., 2009). Furthermore, OMW is characterized by a high potassium concentration and notable levels of nitrogen, phosphorus, calcium, magnesium and iron (Sellami et al., 2008). Its organic fraction includes sugars, tannins, polyphenols, polyalcohols, pectins, lipids, and proteins (Hachicha et al., 2009; Piotrowska et al., 2011). Consequently, the OMW use for agricultural purpose would be a low cost source for water and nutrients (López-Piñeiro et al., 2007; Belaqziz et al., 2008; Piotrowska et al., 2011), particularly in arid regions, like Tunisia, suffering from serious water and soil organic matter deficiencies (Hachicha et al., 2009; Magdich et al., 2012). Indeed, this waste incorporation into the soil may constitute a valuable approach for C sequestration and reduction in runoff and soil losses (Lozano-Garcia et al., 2011). Furthermore, in agricultural land, OMW application is a simple and relatively inexpensive disposal method, that may contribute to a sustainable agriculture development, particularly under the severe climatic conditions that affect olive oil - producing countries (Sierra et al., 2007). For these reasons, more attention was given for better managing OMW spreading practice to improve olive production with low cost, while considering the product quality and the environment preservation. However, many of the papers focusing on OMW spreading for agricultural purpose were devoted to the effluent impacts on soil physical, chemical and biological properties; and little is known about OMW effect on olive-tree mineral nutritional status. Indeed, leaf nutrient analysis is by far the established method for diagnosing olive trees' nutritional status, and represents a valuable tool for the olive fertilization requirements assessment. However, the leaf analysis remains not well investigated in Mediterranean countries to be used as a guide for olive trees' mineral nutrition (Fernandez-Escobar et al., 1999).

The present study deals with the raw OMW use effects as ferti-irrigation system, on the progress of olive leaves and fruits mineral nutrients content during two successive years, under natural field conditions in an olive orchard.

Materials and methods

Field investigation

The experiments were carried out in an olive-tree (*Olea europaea* L., variety Chemlali) field at the Taous experimental station of the Olive Tree Institute of Sfax, in the South of Tunisia (34°43' N, 10°41' E). This area has a typical Mediterranean climate, with an average annual rainfall of about 200 mm. The field was divided into four plots, three of which were annually dosed in February (from 2005 to 2006). Throughout the experimental period, each of the three latter plots was treated with the same annual dose of raw OMW, spread out on the surface of the corresponding plot at a controlled volume, namely 50, 100, and 200 m^3 ha^{-1}, respectively. The fourth plot was not submitted to the raw OMW treatment and used as a control. Each of the treated plots covered an area of 1 hectare and contained 16 eighty-year old trees, with an inter-tree spacing of 24 m × 24 m. The sandy soil of the experimental orchard (86.63% sand, 13.26% silt and 0.20% clay) was characterized by an organic matter content of 0.32% and a pH of 7.5 (Magdich et al., 2012).

OMW characteristics

The fresh OMW used for olive field applications were collected from a three-phase olive mill plant located near to the experimental station. The OMW effluent main characteristics were as follows: pH: 4.68; electrical conductivity: 12.62 mS cm^{-1}; COD: 122 g L^{-1}; BOD_5: 47.3 g L^{-1}; organic matter: 53 g L^{-1}; total polyphenols: 1.1 g L^{-1}; total nitrogen: 1.61 g L^{-1}; P: 0.71 g L^{-1}; K^+: 5.76 g L^{-1}; Ca^{2+}: 0.92 g L^{-1}; Mg^{2+}: 0.52 g $L^{-1}$and Na^+: 1.47 g L^{-1} (Magdich et al., 2012).

Plant nutrient concentrations

In order to determine the leaves and olive fruits mineral contents, plant samples were over-dried at 70 °C for 48 h and then ground to a fine powder. A 1 g representative mass of the fine powdered sample was dry-ashed in a muffle furnace at 450 °C for 6 h. Then, the ash was dissolved in HNO_3. The N was analysed using the Kjeldahl method. The concentrations of the mineral elements K, Ca, Mg and Na were determined by atomic emission spectrophotometry (JENWAY PFP7, Milan, Italy). The P was determined by a vanado-molybdate colorimetric procedure with a JENWAY 6405 UV/Vis spectrophotometer (Milan, Italy) and Cl was determined titrimetrically with $AgNO_3$.

Statistical analysis

The cumulative data recorded was subjected to variance analysis. The treatments mean values were compared using Duncan's multiple range test at the 5% ($p = 0.05$) significance level. All the analyses were performed in triplicate. The Principal Component Analysis (PCA), a correlation method - based on the principal component scores - transformed the data of many experimented variables into a set of compound axes; denoted principal components (PC), was adopted to establish the correlation between the studied parameters during the experimental period using SPSS Statistics 20.0 for Windows.

Results

Mineral nutrients content progress in the olive leaves and fruits

Nitrogen

The monthly leaves nitrogen content evolution presented the same tendency for the different treatments (Fig. 1a). In March, the highest nitrogen content was recorded with the highest treatment rate of 200 m^3 ha^{-1} and a maximum value of 1.9%. From April (flowering starting) a progressive decrease was installed which reached a minimum value of 1.1% during August, at the endocarp lignification's stage end. In the fruits, an increase in nitrogen content occurred over the fruit stage growth (Fig. 1b). These contents increased by 50% from June to the autumnal period. This monthly evolution confirmed the nitrogen migration from the leaves to the fruits; this fact may reveal the N importance during the fruit growth.

Phosphorus

The foliar phosphorus content ranged from 0.07 to 0.09% of DM (Table 1). The lowest phosphorus content was recorded during June-July, when the endocarp lignification's occurred. Then, a phosphorus content increase was observed reaching a maximum concentration in February March. For the fruits, phosphorus contents varied between 0.04 and 0.09% of DM (Table 2). The highest phosphorus content was observed during June and July. Then, a progressive phosphorus content decrease was noticed until reaching a minimum value in November.

Fig. 1 a: Leaf nitrogen content evolution after the first and the second OMW spreading at different doses: control (0 m^3 ha^{-1}), 50 m^3 ha^{-1}, 100 m^3 ha^{-1} and 200 m^3 ha^{-1}.

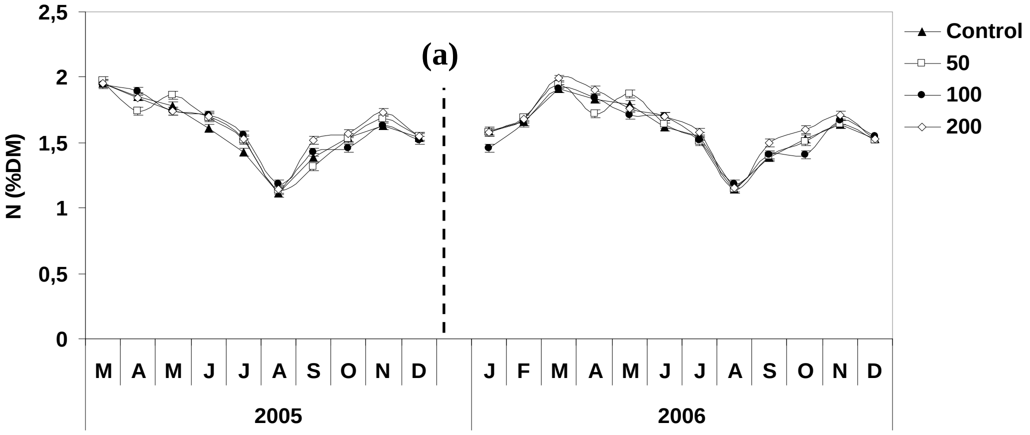


Fig. 1 b: Fruit nitrogen content evolution after the first and the second OMW spreading at different doses: control (0 m^3 ha^{-1}), 50 m^3 ha^{-1}, 100 m^3 ha^{-1} and 200 m^3 ha^{-1}.

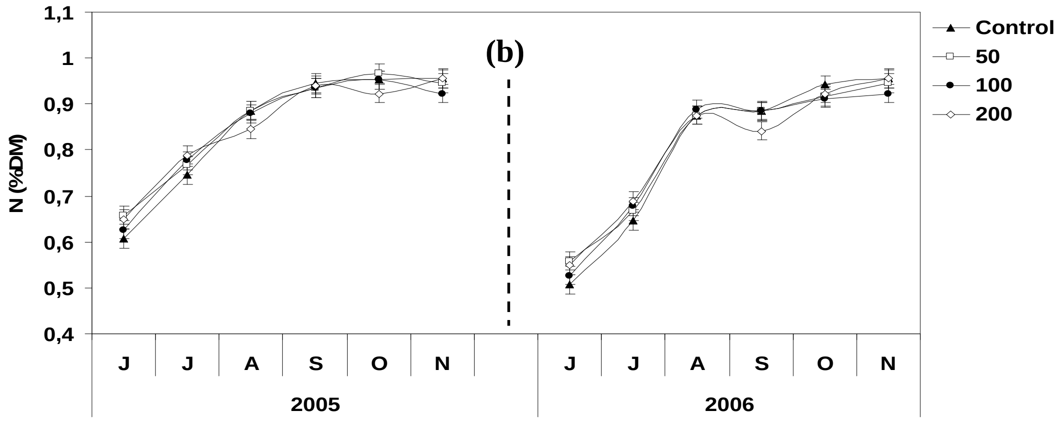


Fig. 2 a: Leaf potassium content evolution after the first and the second OMW spreading at different doses: control (0 m^3 ha^{-1}), 50 m^3 ha^{-1}, 100 m^3 ha^{-1} and 200 m^3 ha^{-1}.

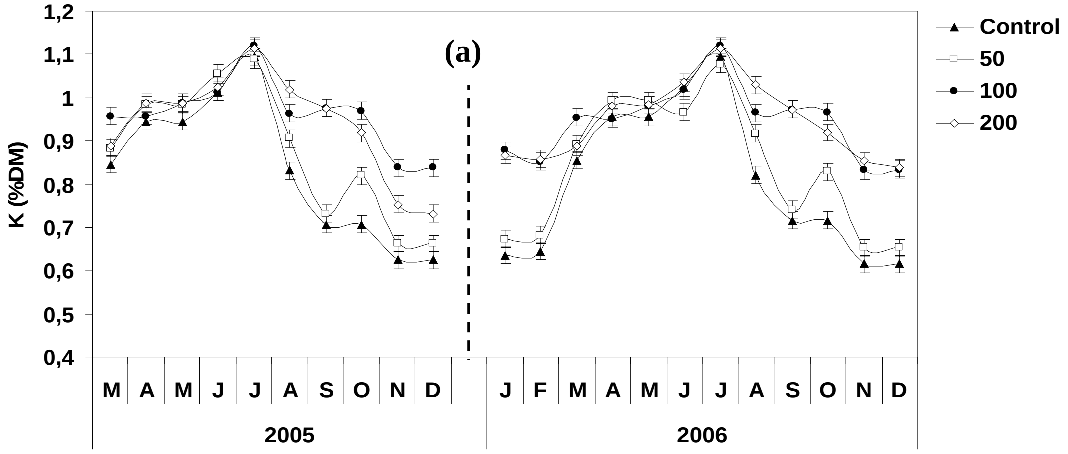


Fig. 2 b: Fruit potassium content evolution after the first and the second OMW spreading at different doses: control (0 m^3 ha^{-1}), 50 m^3 ha^{-1}, 100 m^3 ha^{-1} and 200 m^3 ha^{-1}.

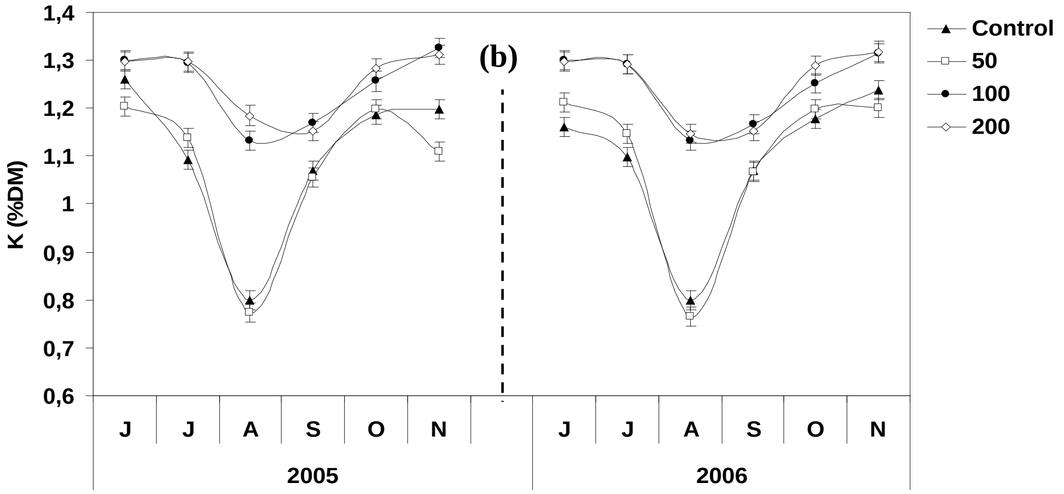


Table 1. Leaf phosphorus and magnesium contents (% in DM) evolution after the first and the second OMW spreading at different doses: control (0 m^3 ha^{-1}), 50 m^3 ha^{-1}, 100 m^3 ha^{-1} and 200 m^3 ha^{-1}.

OMW dose (m^3 $year^{-1}$)		Control		50		100		200	
Spreading	**Month**	**P**	**Mg**	**P**	**Mg**	**P**	**Mg**	**P**	**Mg**
First (2005)	March	0.094 ± 0.003	0.131 ± 0.001	0.098 ± 0.004	0.140 ± 0.008	0.092 ± 0.001	0.152 ± 0.006	0.095 ± 0.001	0.114 ± 0.002
	April	0.081 ± 0.004	0.136 ± 0.002	0.081 ± 0.005	0.121 ± 0.003	0.081 ± 0.003	0.155 ± 0.007	0.081 ± 0.004	0.168 ± 0.004
	May	0.080 ± 0.002	0.159 ± 0.001	0.080 ± 0.003	0.163 ± 0.002	0.081 ± 0.001	0.144 ± 0.001	0.081 ± 0.005	0.169 ± 0.008
	June	0.074 ± 0.001	0.194 ± 0.005	0.081 ± 0.002	0.188 ± 0.001	0.077 ± 0.006	0.162 ± 0.002	0.077 ± 0.007	0.174 ± 0.002
	July	0.073 ± 0.003	0.123 ± 0.006	0.079 ± 0.005	0.140 ± 0.004	0.074 ± 0.007	0.131 ± 0.003	0.072 ± 0.006	0.153 ± 0.009
	August	0.081 ± 0.002	0.122 ± 0.003	0.079 ± 0.003	0.111 ± 0.006	0.076 ± 0.001	0.120 ± 0.004	0.074 ± 0.004	0.122 ± 0.003
	September	0.086 ± 0.004	0.135 ± 0.004	0.083 ± 0.003	0.140 ± 0.007	0.089 ± 0.008	0.158 ± 0.006	0.080 ± 0.003	0.121 ± 0.003
	October	0.087 ± 0.005	0.144 ± 0.007	0.087 ± 0.002	0.152 ± 0.008	0.081 ± 0.001	0.164 ± 0.002	0.081 ± 0.002	0.143 ± 0.001
	November	0.082 ± 0.001	0.112 ± 0.003	0.081 ± 0.001	0.121 ± 0.006	0.085 ± 0.004	0.132 ± 0.004	0.087 ± 0.005	0.114 ± 0.004
	December	0.083 ± 0.003	0.103 ± 0.001	0.082 ± 0.005	0.113 ± 0.002	0.090 ± 0.006	0.131 ± 0.004	0.080 ± 0.003	0.113 ± 0.001
	January	0.088 ± 0.006	0.112 ± 0.002	0.088 ± 0.001	0.102 ± 0.001	0.088 ± 0.002	0.120 ± 0.002	0.088 ± 0.001	0.116 ± 0.005
	February	0.091 ± 0.002	0.124 ± 0.004	0.091 ± 0.003	0.130 ± 0.004	0.091 ± 0.005	0.144 ± 0.003	0.091 ± 0.005	0.154 ± 0.006
Second (2006)	March	0.091 ± 0.001	0.145 ± 0.001	0.091 ± 0.001	0.151 ± 0.004	0.091 ± 0.001	0.162 ± 0.001	0.091 ± 0.001	0.156 ± 0.003
	April	0.081 ± 0.004	0.166 ± 0.006	0.087 ± 0.003	0.155 ± 0.001	0.081 ± 0.006	0.146 ± 0.002	0.084 ± 0.004	0.151 ± 0.006
	May	0.081 ± 0.002	0.182 ± 0.008	0.087 ± 0.004	0.173 ± 0.004	0.081 ± 0.002	0.205 ± 0.006	0.081 ± 0.003	0.183 ± 0.005
	June	0.071 ± 0.003	0.211 ± 0.004	0.081 ± 0.002	0.232 ± 0.002	0.071 ± 0.003	0.244 ± 0.002	0.071 ± 0.006	0.222 ± 0.001
	July	0.077 ± 0.005	0.132 ± 0.001	0.074 ± 0.004	0.141 ± 0.006	0.071 ± 0.004	0.153 ± 0.004	0.077 ± 0.004	0.144 ± 0.007
	August	0.084 ± 0.003	0.114 ± 0.002	0.071 ± 0.004	0.120 ± 0.005	0.074 ± 0.002	0.131 ± 0.005	0.071 ± 0.006	0.136 ± 0.008
	September	0.081 ± 0.001	0.157 ± 0.005	0.087 ± 0.003	0.161 ± 0.003	0.081 ± 0.001	0.173 ± 0.006	0.087 ± 0.007	0.183 ± 0.003
	October	0.084 ± 0.006	0.160 ± 0.006	0.081 ± 0.002	0.182 ± 0.002	0.087 ± 0.005	0.174 ± 0.003	0.081 ± 0.001	0.160 ± 0.007
	November	0.084 ± 0.004	0.169 ± 0.008	0.088 ± 0.004	0.184 ± 0.007	0.086 ± 0.006	0.157 ± 0.002	0.083 ± 0.003	0.172 ± 0.004
	December	0.082 ± 0.002	0.127 ± 0.003	0.081 ± 0.005	0.130 ± 0.001	0.095 ± 0.003	0.122 ± 0.004	0.087 ± 0.002	0.119 ± 0.003
	January	0.082 ± 0.001	0.132 ± 0.005	0.087 ± 0.003	0.127 ± 0.002	0.081 ± 0.003	0.146 ± 0.003	0.088 ± 0.003	0.136 ± 0.008
	February	0.092 ± 0.003	0.119 ± 0.004	0.094 ± 0.006	0.148 ± 0.006	0.092 ± 0.001	0.156 ± 0.005	0.091 ± 0.004	0.148 ± 0.006

Values are the means of three replications ± SE.

Table 2. Fruit phosphorus and magnesium contents (% in DM) evolution after the first and the second OMW spreading at different doses: control (0 m^3 ha^{-1}), 50 m^3 ha^{-1}, 100 m^3 ha^{-1} and 200 m^3 ha^{-1}.

OMW dose (m^3 $year^{-1}$)		Control		50		100		200	
Spreading	**Month**	**P**	**Mg**	**P**	**Mg**	**P**	**Mg**	**P**	**Mg**
First (2005)	June	0.089 ± 0.003	0.180 ± 0.004	0.087 ± 0.002	0.160 ± 0.006	0.088 ± 0.004	0.170 ± 0.006	0.087 ± 0.004	0.180 ± 0.003
	July	0.083 ± 0.002	0.200 ± 0.005	0.080 ± 0.001	0.190 ± 0.005	0.082 ± 0.003	0.180 ± 0.004	0.083 ± 0.003	0.210 ± 0.006
	August	0.075 ± 0.003	0.240 ± 0.006	0.081 ± 0.004	0.220 ± 0.004	0.077 ± 0.001	0.123 ± 0.008	0.064 ± 0.002	0.240 ± 0.007
	September	0.065 ± 0.002	0.160 ± 0.003	0.072 ± 0.002	0.150 ± 0.003	0.072 ± 0.005	0.170 ± 0.006	0.064 ± 0.005	0.160 ± 0.005
	October	0.058 ± 0.001	0.150 ± 0.002	0.068 ± 0.003	0.170 ± 0.004	0.066 ± 0.004	0.160 ± 0.003	0.061 ± 0.003	0.150 ± 0.003
	November	0.062 ± 0.002	0.140 ± 0.001	0.063 ± 0.004	0.13 0± 0.005	0.061 ± 0.002	0.120 ± 0.003	0.059 ± 0.002	0.140 ± 0.004
Second (2006)	June	0.084 ± 0.001	0.160 ± 0.002	0.089 ± 0.006	0.190 ± 0.003	0.086 ± 0.003	0.160 ± 0.003	0.086 ± 0.003	0.170 ± 0.003
	July	0.083 ± 0.003	0.240 ± 0.006	0.088 ± 0.005	0.230 ± 0.005	0.083 ± 0.003	0.220 ± 0.005	0.087 ± 0.004	0.220 ± 0.007
	August	0.085 ± 0.005	0.250 ± 0.005	0.082 ± 0.003	0.230 ± 0.005	0.077 ± 0.005	0.240 ± 0.006	0.074 ± 0.003	0.230 ± 0.008
	September	0.065 ± 0.001	0.170 ± 0.003	0.061 ± 0.004	0.150 ± 0.004	0.082 ± 0.003	0.160 ± 0.003	0.064 ± 0.006	0.180 ± 0.006
	October	0.048 ± 0.003	0.160 ± 0.002	0.068 ± 0.003	0.170 ± 0.003	0.056 ± 0.002	0.170 ± 0.005	0.051 ± 0.004	0.160 ± 0.004
	November	0.062 ± 0.003	0.150 ± 0.004	0.053 ± 0.002	0.160 ± 0.002	0.061 ± 0.003	0.160 ± 0.003	0.049 ± 0.005	0.150 ± 0.005

Values are the means of three replications ± SE.

Potassium

In the leaves, potassium accumulation occurred in July, and the highest potassium content was noticed at the biological cycle end. Then, it decreased until a minimum value of 0.61% in November-December (Fig. 2 a). In the fruits, potassium content varied inversely with that of the leaves, with an important decrease during the summer, followed by an autumnal accumulation phase (Fig. 2 b). Potassium content ranged between 0.7 and 1.3% of the DM, the highest concentration was recorded in June. But an important decrease was observed during July and August reaching a minimum value of 0.7%. Then, an increase of these contents during the autumnal period was noticed reaching the maximum value in November, at the olive harvesting period.

Calcium

The four treatments applied showed similar trends of monthly foliar calcium content (Fig. 3 a), where the calcium concentration increased progressively from March to reach a maximum of 2.3% of the DM in July. Then, rapid decrease and stabilisation were noticed at the biological cycle end. In the fruits, the highest Ca contents were registered form July until October, revealing Ca importance for the olive development and maturation (Fig. 3b).

Fig. 3 a: Leaf calcium content evolution after the first and the second OMW spreading at different doses: control (0 m^3 ha^{-1}), 50 m^3 ha^{-1}, 100 m^3 ha^{-1} and 200 m^3 ha^{-1}.

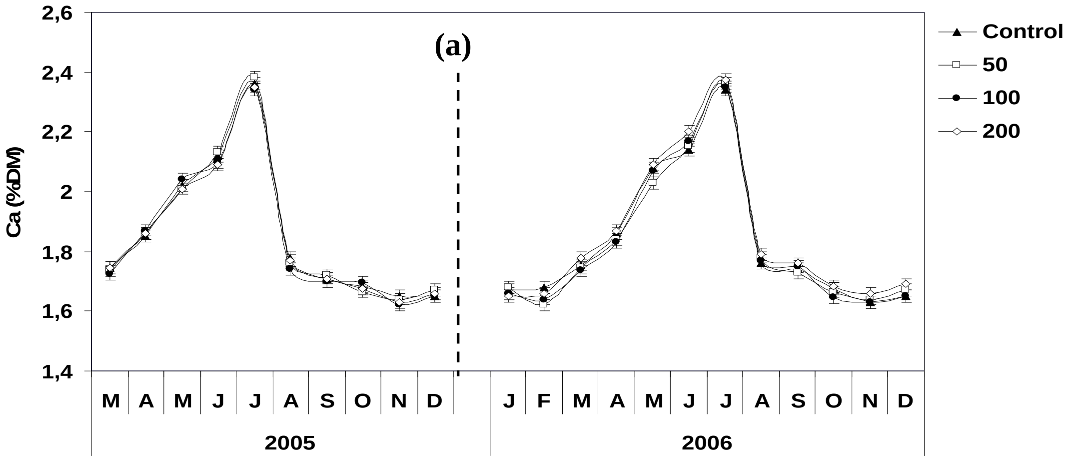
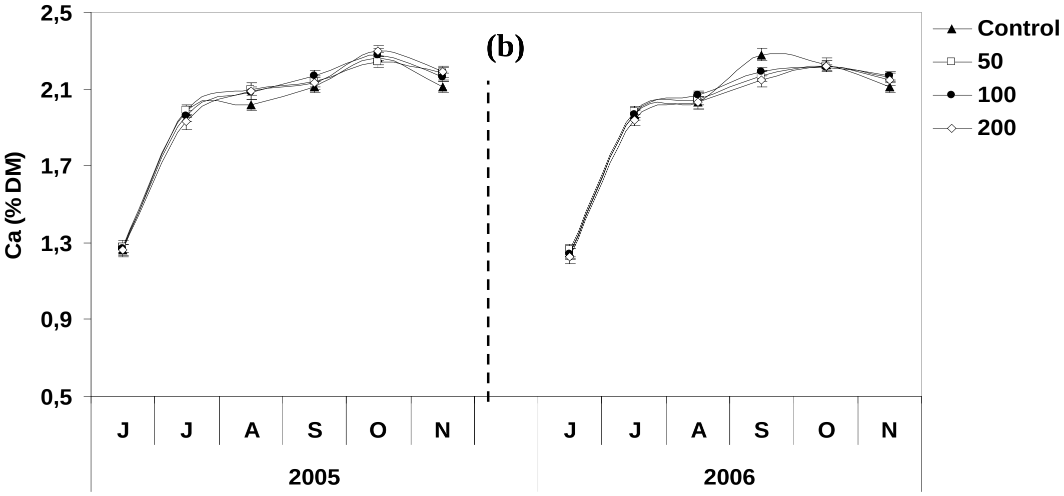


Fig. 3 b: Fruit calcium content evolution after the first and the second OMW spreading at different doses: control (0 m^3 ha^{-1}), 50 m^3 ha^{-1}, 100 m^3 ha^{-1} and 200 m^3 ha^{-1}.



Magnesium

During the study period, the leaf magnesium content increased beginning in March until reaching a maximum value (0.24%) in June (Table 1). From July a continuous decrease was noted until reaching a minimal content of 0.1% in the winter period. The Mg fruit concentrations were ranged between 0.12 and 0.25 % of the DM (Table 2). The magnesium olive fruit highest concentrations were observed in August. Then, a decrease during the autumn phase occurred.

Sodium

The sodium foliar content presented a notable fluctuation during the olive biological cycle (Table 3). For fruits, the sodium contents ranged between 0.01 and 0.08% (Table 4). These contents decreased during the summer period and then increased in the autumn to reach maximal values in October-December with fruit ripeness.

Chloride

The leaf chloride content ranged between 0.06 and 0.13% of DM (Table 3). The lowest concentrations were observed during the springer and autumnal periods. In contrast, the highest concentrations are recorded during the summer. The harvested olive fruits, issued from trees treated with various OMW doses, were characterized by chloride contents varying from 0.01 to 0.07% (Table 4).

PCA analysis

A principal component analysis was performed to examine different parameters studied evolution under investigation (N, P, K, Ca, Mg, Na and Cl) after the two successive OMW spreading years. In fact, PCA showed the presence of an anti-correlation between the N, P, K, Ca, Mg leaf and fruit concentrations. At the leaves and the fruits, this anti-correlation between these 5 mineral nutrients confirmed the monthly mineral changes in concentrations to provide the olive tree nutrients requirements over the biological cycle. In addition, Na and Cl contents showed a correlation at the two organs studied. This correlation affirmed less needs of these two elements during the olive cycle (Fig. 4).

Discussion

Nitrogen is considered to be the most commonly applied nutrient in olive orchards (Therios, 2009). During this experiment, foliar nitrogen contents were adequate for growth since N concentration in leaves ranged between 1.1 and 2% of DM. In August, the leaves nitrogen decrease could be explained by its migration to the fruits. In the leaves, nitrogen content increase occurred in autumn, this fact could be explained by nitrogen recovery needed by the olive tree to perform the physiological process. This nitrogen concentration increase depended on the tree biological cycle; and it allowed a vegetal reserve recuperation that compensates the losses following the strong fruit size development. These results are in agreement with several previous reports (Fernandez-Escobar et al., 1999; Fabbri and Benelli, 2000). During the two experimented crop seasons, the OMW agronomic application didn't induce significant differences between the treated trees by the different OMW doses and the untreated (control) soil ($p > 0.30$). These similarities were recorded despite the highest total nitrogen level content recorded at 0-20 cm depths for the three OMW treatments. This supply was of 7.43, 11.57 and 16.79% respectively for the doses 50, 100 and 200 m^3 ha^{-1} after three successive years (Magdich et al., 2013). Indeed, Chartzoulakis et al. (2010) didn't affirmed that high OMW dose application (252 m^3 ha^{-1}), affected the leaves nitrogen concentration after two successive years of application. However, López-Piñeiro et al. (2008) observed a foliar nitrogen content amelioration after OMW crude application, and explained this increase by the nitrogen availability in the soil solution favourable, for trees N important assimilation.

In the fruits, nitrogen evolution affirmed the principal role of this element over the fruits maturation, as previously indicated by Marcelo and Jorado (1994). In addition, in perennial plant organs nitrogen storage, including the leaves, and its remobilization throughout the plant is well-known in evergreen (Klein and Weinbaum, 1984) and deciduous trees (Marschner, 1995; Smith, 2009). No significant difference was observed among the four applied treatments during the two experimented campaigns ($p > 0.40$). These results reflected the lack of different OMW spreader effects (50, 100 and 200 m^3 ha^{-1} $year^{-1}$) on the fruits nitrogen contents in olive orchard.

Table 3. Leaf sodium and chloride contents (% in DM) evolution after the first and the second OMW spreading at different doses: control (0 m^3 ha^{-1}), 50 m^3 ha^{-1}, 100 m^3 ha^{-1} and 200 m^3 ha^{-1}.

OMW dose (m^3 year^{-1})		Control		50		100		200	
Spreading	**Month**	**Na**	**Cl**	**Na**	**Cl**	**Na**	**Cl**	**Na**	**Cl**
First (2005)	March	0.012 ± 0.003	0.081 ± 0.006	0.012 ± 0.004	0.080 ± 0.003	0.015 ± 0.001	0.084 ± 0.002	0.014 ± 0.001	0.086 ± 0.004
	April	0.013 ± 0.004	0.074 ± 0.003	0.014 ± 0.002	0.075 ± 0.004	0.015 ± 0.002	0.079 ± 0.003	0.016 ± 0.003	0.076 ± 0.004
	May	0.013 ± 0.002	0.065 ± 0.002	0.014 ± 0.002	0.063 ± 0.006	0.015 ± 0.003	0.064 ± 0.007	0.013 ± 0.002	0.062 ± 0.003
	June	0.015 ± 0.001	0.111 ± 0.001	0.016 ± 0.003	0.112 ± 0.007	0.018 ± 0.004	0.113 ± 0.006	0.019 ± 0.003	0.114 ± 0.007
	July	0.012 ± 0.002	0.125 ± 0.005	0.012 ± 0.002	0.126 ± 0.003	0.014 ± 0.002	0.126 ± 0.005	0.015 ± 0.001	0.127 ± 0.008
	August	0.016 ± 0.005	0.132 ± 0.004	0.015 ± 0.001	0.133 ± 0.004	0.016 ± 0.004	0.132 ± 0.008	0.014 ± 0.002	0.134 ± 0.007
	September	0.018 ± 0.003	0.110 ± 0.003	0.019 ± 0.005	0.120 ± 0.004	0.016 ± 0.003	0.130 ± 0.003	0.017 ± 0.004	0.110 ± 0.006
	October	0.015 ± 0.003	0.094 ± 0.002	0.014 ± 0.001	0.092 ± 0.002	0.013 ± 0.001	0.093 ± 0.006	0.015 ± 0.005	0.094 ± 0.003
	November	0.019 ± 0.002	0.083 ± 0.004	0.018 ± 0.002	0.085 ± 0.005	0.020 ± 0.002	0.081 ± 0.004	0.018 ± 0.004	0.080 ± 0.005
	December	0.015 ± 0.001	0.082 ± 0.006	0.016 ± 0.004	0.084 ± 0.003	0.017 ± 0.001	0.086 ± 0.003	0.015 ± 0.001	0.085 ± 0.004
	January	0.016 ± 0.005	0.073 ± 0.003	0.017 ± 0.001	0.075 ± 0.006	0.018 ± 0.003	0.074 ± 0.005	0.017 ± 0.003	0.076 ± 0.002
	February	0.015 ± 0.001	0.074 ± 0.001	0.016 ± 0.001	0.075 ± 0.003	0.015 ± 0.002	0.071 ± 0.002	0.014 ± 0.002	0.072 ± 0.003
Second (2006)	March	0.012 ± 0.003	0.084 ± 0.005	0.013 ± 0.001	0.082 ± 0.003	0.012 ± 0.001	0.083 ± 0.003	0.013 ± 0.002	0.085 ± 0.003
	April	0.012 ± 0.002	0.074 ± 0.003	0.011 ± 0.004	0.076 ± 0.001	0.013 ± 0.002	0.077 ± 0.002	0.012 ± 0.002	0.074 ± 0.003
	May	0.014 ± 0.004	0.065 ± 0.005	0.016 ± 0.004	0.067 ± 0.005	0.015 ± 0.003	0.063 ± 0.005	0.014 ± 0.001	0.068 ± 0.002
	June	0.016 ± 0.005	0.113 ± 0.008	0.016 ± 0.002	0.114 ± 0.004	0.017 ± 0.001	0.112 ± 0.006	0.016 ± 0.003	0.115 ± 0.004
	July	0.017 ± 0.004	0.124 ± 0.006	0.017 ± 0.003	0.122 ± 0.007	0.016 ± 0.003	0.125 ± 0.007	0.017 ± 0.004	0.126 ± 0.007
	August	0.013 ± 0.001	0.132 ± 0.006	0.015 ± 0.001	0.134 ± 0.008	0.014 ± 0.002	0.136 ± 0.007	0.013 ± 0.003	0.135 ± 0.006
	September	0.015 ± 0.004	0.110 ± 0.002	0.014 ± 0.002	0.130 ± 0.007	0.015 ± 0.002	0.120 ± 0.005	0.016 ± 0.001	0.110 ± 0.006
	October	0.015 ± 0.002	0.096 ± 0.003	0.016 ± 0.002	0.097 ± 0.005	0.017 ± 0.001	0.092 ± 0.005	0.016 ± 0.005	0.093 ± 0.003
	November	0.018 ± 0.003	0.083 ± 0.004	0.018 ± 0.002	0.086 ± 0.005	0.018 ± 0.003	0.082 ± 0.004	0.017 ± 0.004	0.084 ± 0.005
	December	0.018 ± 0.004	0.081 ± 0.005	0.017 ± 0.001	0.083 ± 0.004	0.016 ± 0.002	0.083 ± 0.003	0.017 ± 0.003	0.082 ± 0.002
	January	0.017 ± 0.001	0.073 ± 0.006	0.017 ± 0.003	0.075 ± 0.003	0.015 ± 0.001	0.074 ± 0.002	0.016 ± 0.004	0.076 ± 0.003
	February	0.016 ± 0.003	0.075 ± 0.005	0.015 ± 0.002	0.076 ± 0.003	0.014 ± 0.001	0.073 ± 0.003	0.015 ± 0.003	0.074 ± 0.003

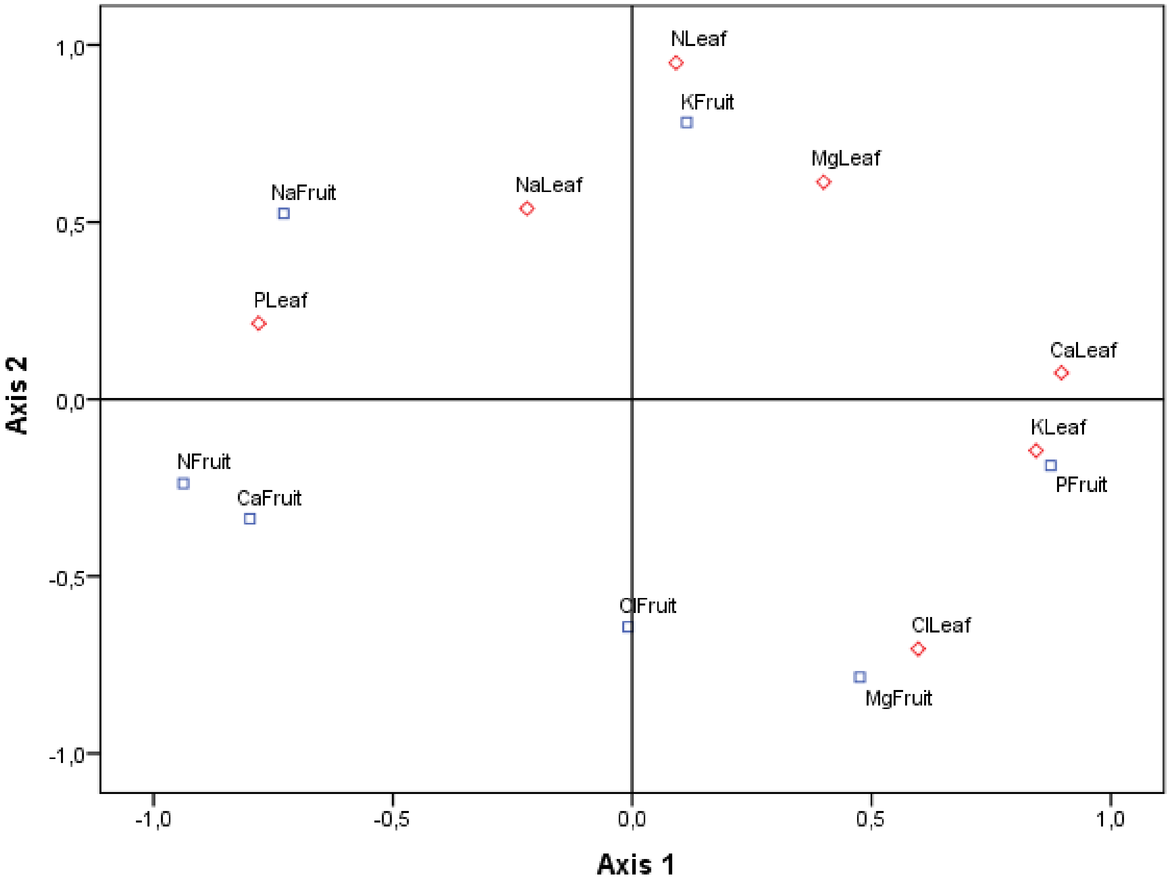
Values are the means of three replications ± SE.

Table 4. Fruit sodium and chloride contents (% in DM) evolution after the first and the second OMW spreading at different doses: control (0 m^3 ha^{-1}), 50 m^3 ha^{-1}, 100 m^3 ha^{-1} and 200 m^3 ha^{-1}.

OMW dose ($m^3 year^{-1}$)		**Control**		**50**		**100**		**200**	
Spreading	**Month**	**Na**	**Cl**	**Na**	**Cl**	**Na**	**Cl**	**Na**	**Cl**
First (2005)	June	0.032 ± 0.003	0.030 ± 0.002	0.044 ± 0.002	0.020 ± 0.004	0.032 ± 0.002	0.010 ± 0.002	0.021 ± 0.002	0.040 ± 0.003
	July	0.040 ± 0.002	0.020 ± 0.002	0.020 ± 0.003	0.040 ± 0.002	0.030 ± 0.002	0.030 ± 0.003	0.040 ± 0.003	0.020 ± 0.002
	August	0.010 ± 0.001	0.060 ± 0.004	0.020 ± 0.001	0.050 ± 0.003	0.010 ± 0.001	0.070 ± 0.004	0.010 ± 0.001	0.060 ± 0.005
	September	0.052 ± 0.003	0.010 ± 0.001	0.061 ± 0.004	0.020 ± 0.001	0.052 ± 0.003	0.030 ± 0.002	0.061 ± 0.005	0.010 ± 0.001
	October	0.070 ± 0.004	0.020 ± 0.002	0.070 ± 0.005	0.030 ± 0.002	0.070 ± 0.004	0.020 ± 0.002	0.060 ± 0.005	0.020 ± 0.001
	November	0.060 ± 0.002	0.040 ± 0.003	0.080 ± 0.003	0.040 ± 0.002	0.070 ± 0.003	0.030 ± 0.004	0.060 ± 0.003	0.030 ± 0.002
Second (2006)	June	0.020 ± 0.003	0.030 ± 0.003	0.030 ± 0.002	0.030 ± 0.004	0.020 ± 0.002	0.040 ± 0.002	0.030 ± 0.002	0.040 ± 0.003
	July	0.010 ± 0.001	0.020 ± 0.002	0.020 ± 0.002	0.030 ± 0.003	0.030 ± 0.002	0.020 ± 0.001	0.010 ± 0.001	0.020 ± 0.003
	August	0.020 ± 0.004	0.060 ± 0.003	0.030 ± 0.003	0.060 ± 0.005	0.020 ± 0.003	0.070 ± 0.006	0.010 ± 0.001	0.050 ± 0.004
	September	0.042 ± 0.003	0.030 ± 0.004	0.035 ± 0.004	0.020 ± 0.002	0.046 ± 0.004	0.030 ± 0.003	0.046 ± 0.003	0.040 ± 0.005
	October	0.050 ± 0.002	0.020 ± 0.001	0.060 ± 0.004	0.040 ± 0.003	0.040 ± 0.003	0.040 ± 0.005	0.050 ± 0.002	0.020 ± 0.003
	November	0.060 ± 0.002	0.040 ± 0.003	0.070 ± 0.003	0.040 ± 0.005	0.060 ± 0.005	0.030 ± 0.004	0.050 ± 0.003	0.030 ± 0.002

Values are the means of three replications ± SE.

Fig. 4: PCA analysis carried out using the studied parameters over the experiment investigation.



Considering the phosphorus, all the P concentrations were within the normal to over-sufficient range (0.07-0.14 % of DM) mentioned by Panagiotopoulos (2001), and no P deficient concentrations were recorded. The lowest foliar phosphorus content was recorded in July and met the hardening nucleus phase. This low P content was explained by the olive-tree requirement for the oil synthesis (Chesworth et al., 1998). This content was significantly unaffected by the three OMW-doses experimented during the two crop seasons ($p > 0.29$).

During the fruit maturation, the phosphorus was not used, despite its important role (Erel et al., 2011). The OMW spread didn't present any significant difference between the two experimented-years ($p = 0.40$). In addition, the three OMW amended plots didn't show any significant difference among them and with the control treatment ($p > 0.47$). Consequently, the different OMW-rates application did not influence the olive tree phosphorus nutrition. This was due to the soil contents amelioration absence in available phosphorus, following the different OMW-doses ferti-irrigation used during the experimental period (Magdich et al., 2013).

In the case of 100 and 200 m^3 ha^{-1} OMW doses, leaf potassium concentration fluctuated during the experimental period and did not drop below the lowest K concentration of 0.7% of DM for the olive growth optimum value (Panagiotopoulos, 2001). However, the leaves collected from trees subjected to a 50 and 0 m^3 ha^{-1} OMW doses presented concentrations inferior to the lowest K concentration during the winter period. The autumnal foliar K content decrease could be explained by its mobilization from the leaves to support fruit growth and maturity (Fernandez-Escobar et al., 1999). Statistical analyses showed a significant difference between the control K content and those of the treatments with 100 and 200 OMW m^3 ha^{-1} applied during the experimental period ($p < 0.04$). However, no significant difference was noted between the control and the treatment 50 m^3 ha^{-1}. Accordingly, in the olive orchard, 100 and 200 OMW m^3 ha^{-1} spread could affect the foliar K nutrition during the two successive years of OMW spreading. This result would be attributed to the soil layers K content improvement after OMW application (Magdich et al., 2013), that is reverberate on plant potassium assimilation.

In the autumnal period, the potassium fruits accumulation implied the importance of this nutritional element in the fruit maturity process, particularly in the lipogenesis (Braham, 1999), and confirmed the potassium migration from leaves to fruits. In fact, in the mature fruits, 60% of the tree potassium is located in the fruit; this content remains constant until the harvesting period (Lavée, 1997). Indeed, the potassium enhances the organic acids transformation in to fatty acids, by activating more than 60 enzymes (Marschner, 1995). In addition, K is the most abundant macroelement in the olive tree and plays various functions i.e., in osmoregulation, carbohydrate translocation, protein synthesis, enzyme activation, cell expansion, and stomatal regulation (Pallardy, 2008). Recently, Bustan et al. (2013) mentioned that a high fruits K content reflected plant requirement to storage the K excess. Statistically, the OMW high-levels (100 and 200 m^3 ha^{-1}) showed a significant difference of K content when compared to the control ($p < 0.02$). Accordingly, OMW agronomic application enhanced the fruits K accumulation when the applied doses exceeded 50 m^3 ha^{-1}.

Concerning leaf calcium concentration, it was above the 1%, which is suggested to be a deficiency threshold (Therios, 2009). In addition, a different distribution was noted for leaves and fruits during olive tree biological cycle; this result is related to the olive tree requirements. The statistical analysis between the three OMW treatments and the control didn't show any significant difference ($p > 0.41$). Thus, the yearly and successively different OMW-levels spread in olive field didn't affect the Ca content in the leaves and fruits through the experimented period. Likewise, monthly evolution of the leaves and the fruits magnesium contents has almost the same trend in the control and treatments received OMW effluent, without any significant difference ($p > 0.05$). Our results were in concordance with those obtained by Chartzoulakis et al. (2010), whom concluded that OMW application didn't affect the leaf Ca and Mg concentrations of 'Kalamata' olive trees.

The foliar sodium and chloride concentrations recorded for the different treatments were lower than those of the foliar toxicity values equalled to 0.2 and 0.5% of olive tree respectively for sodium and chloride (Freeman et al., 2005). In the leaves and fruits, the sodium and chloride contents didn't show any significant difference between the three OMW-doses and the control ($p > 0.05$), while, the soil sodium and chloride contents were noted to undergo significant increments in all the soil-layers and these mineral rates were proportional to the OMW concentrations and the spreading operations number (Magdich et al., 2013). This result may be attributed to the olive roots role in inhibiting salt ions transport to the leaves, a mechanism allowing salt ions exclusion from organs in which it could promote toxic responses (Bustan et al., 2013).

Conclusion

The monthly evolution of most leaf and fruit mineral nutrients contents were not considerably influenced by the three OMW doses applied in olive orchard. As well as that no significant differences were recorded in N, P, Ca, Mg, Na and Cl tissues nutrient contents between the four treatments, with the exception of K element concentration that showed an increase in the two organs collected from the trees subjected to 100 and 200 m^3 ha^{-1} OMW treatment. Therefore, during the two-year experiment, olive nutritional status was not affected negatively by the application of increasing doses of OMW in the olive groves. This result presented an important interest in the Mediterranean basin when olive groves are implemented in marginal lands (dry and nutrient poor soils) and where extended periods of drought during the summer are common. Consequently, OMW agronomic application may substitute the chemical fertilizer and give the K required for the olive tree, especially when potassium is considered as one of the most important minerals in olive nutrition.

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