



# Long-term field evaluation of the changes in fruit and olive oil chemical compositions after agronomic application of olive mill wastewater with rock phosphate



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## ABSTRACT

The objectives of this study were to determine the long-term effects of agronomic application of olive mill wastewater (OMW) with rock phosphate (RP) in a field of olive trees, on olive fruits and oil quality. The results revealed that olive fruits of OMW-RP amended plants had higher contents of polyphenols and mannitol indicating that agronomic application of OMW with RP generated an oxidative stress. Land spreading of OMW with RP altered the relative proportions of individual sugars in leaves and fruits. Consequently, the oil content decreased significantly, and a marked decrease in the contents of carotenoids and chlorophylls was also observed. Changes also took place in the composition of fatty acids, particularly by the increase of linoleic acid and the decrease of oleic acid. Our results suggested that the use of OMW in combination with RP is expected to have a major negative impact on olive fruit and oil quality.

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## 1. Introduction

The olive mill wastewater (OMW) is an important environmental problem that is disturbing olive oil producing countries (Di Serio et al., 2008). This problem results from the large quantities of OMW that are produced yearly during a short period of time. The extraction process and the oil organoleptic characteristics have been significantly ameliorated by the increased use of continuous automated systems. However, these systems produced higher volumes of wastewater because of the addition of large amounts of water during oil extraction (El Hadrami et al., 2004). The chemical composition of OMW depends mainly on olive tree variety, fruit ripening, climatic conditions at harvesting and, in particular, on the processing method that strongly influences the water content (Aviani et al., 2012). OMW is characterised by its richness in mineral nutrients, and thus may constitute a low-cost source of nutrients and water for agricultural purposes (Angelakis, Marecos Do Monte, Bontoux, & Asano, 1999). Consequently, the reuse of

OMW as soil amendment is potentially doubly useful, especially in the Mediterranean regions which are facing to serious water and soil organic matter deficiencies. OMW amendment to soils is a simple and relatively inexpensive technique for disposal that may contribute to the progress of sustainable agricultural systems (El Hassani, Zinedine, Mdaghri Alaoui, Merzouki, & Benlemlih, 2010). In addition, there are other interesting proposed ways of valorisation of OMW. In fact, OMW is a natural source of valuable antioxidants, particularly phenolic compounds (such as oleuropein, hydroxytyrosol, tyrosol and caffeic acid) characterised by a wide array of biological activities which could be used in pharmaceutical, cosmetic and food industries (Roig, Cayuela, & Sánchez-Monedero, 2006; Shadabi, Ghiasvand, & Hashemi, 2013). Other less common, but also very attractive ways of valorisation, have been proposed: its use as heavy metal sorbent material for the treatment of aqueous solutions (Pagnanelli, Toro, & Vegliò, 2002); and its use for soil sanitation against certain pathogens due to its high antimicrobial capacity (Kotsou et al., 2004).

Plant mineral nutrition depends generally on the soil phosphorus level which can be assimilated only as soluble phosphate. In

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developing countries, soluble fertilizer phosphates are usually too expensive. Rock phosphates (RP) are inexpensive, abundantly found and easily mined. Consequently, their use as fertilizers for phosphorus-deficient soils has received increased attention in recent years. However, RP is relatively insoluble and not available to plants when the soil pH is superior to 5.5–6 (Reddy, Kumar, & Khosla, 2002). It is well recognized that RP application is not economically feasible, particularly at soil conditions characterised by a high pH, low cation exchange capacity, high P-sorption capacity, low organic matter content, low microbial activity and low rainfall (Simpson, Sale, & Tennakoon, 1997). Kpombekou and Tabatabai (1994) indicated that phosphorus-solubilizing capacities of organic acids are greatly correlated with the amount of hydroxyl and carboxyl groups and their relative positions on the main carbon chain. Thus, given that OMW is characterised by an acidic pH (ranging between 3.5 and 5.5), a high load of organic matter (COD of 45–220 g O<sub>2</sub>/l) and phenolic compounds (0.5–24 g/l) (Paraskeva & Diamadopoulos, 2006), its use in combination with RP may represent a new perspective in RP research and a promising solution for OMW reuse. This report suggests that agronomic application of OMW with RP elevates the fertilizer value of RP especially in alkaline soils where solubilization of RP as such is not possible.

Recently, we have reported that agronomic application of OMW with RP changed rhizosphere microbial functional groups (Mechri, Attia, Tekaya, Cheheb, & Hammami, 2014). In view of the above background, the following question was addressed: Did the specific environment created after agronomic application of OMW with RP cause beneficial or detrimental effects on the quality of olive fruits and olive oil? To our knowledge, this is the first report which studies the effects of agronomic application of OMW with RP on olive fruit and olive oil quality.

## 2. Materials and methods

### 2.1. OMW and RP used for experimentation

In the present study, samples of 3-phase OMW were collected from the city of Sidi Bou Ali (Sahel of Tunisia). The OMW physico-chemical characteristics were provided as [supplementary material](#) and revealed an acidic pH with a medium conductivity as well as an important organic load.

Phenolic composition of the fresh OMW ethyl acetate extracts (De Marco, Savarese, Paduano, & Sacchi, 2007) was analyzed by HPLC using a Hewlett-Packard system comprising a Rheodyne model 7725 injector (Cotati, CA, USA, loop volume 20 µl), a HP-1100 pump equipped with a C18 Technochrom Eurosphere 100 analytical column (4.6 × 250mm, 5 µm). Acetonitrile (Solvent A) and sulfuric acid/water (2:98) (Solvent B) were used as mobile phases. The flow rate was 0.5 ml min<sup>-1</sup> and the absorbance was detected at 280 nm. Chromatographic analysis was conducted following a linear gradient: from 15% (A) to 40% during 12 min, it changed to 60% in 2 min, after 4 min it changed to 80% and then to 90% after 2 min. At 24 min it became 100% (A) for 4 min (Tekaya et al., 2016). Phenolic compounds were identified comparing their retention times with the corresponding standards. Analysis by HPLC showed a predominance of hydroxytyrosol, phenyl acetic acid, tyrosol and *m*-Coumaric acid in the OMW used in this study (see [supplementary material](#)).

The RP used in this study came from Gafsa (Tunisia, West sector). The physico-chemical characteristics of RP were provided as [supplementary material](#).

### 2.2. Field site and sampling

The present study was performed in an orchard located at Sidi Bou Ali (Sahel of Tunisia), on mature olive trees planted at

12 m × 12 m apart. The climate of this region is typical Mediterranean with a mean annual temperature of about 19 °C and a mean annual rainfall of 300 mm. A composite sample soil taken from the soil of the experimental farm was analyzed, and physico-chemical characteristics were provided as [supplementary material](#).

In this experiment, 68 'Chemlali' olive trees were selected to be similar in canopy and potential yield. The experiment included three levels of OMW and RP application. Treatments were made in triplicate and distributed as a complete randomized block:

- M0: 0 m<sup>3</sup>/ha of OMW + 0 kg/ha of RP (for 20 trees).
- M1PN: 30 m<sup>3</sup>/ha of OMW + 150 kg/ha of RP (for 24 trees).
- M2PN: 60 m<sup>3</sup>/ha of OMW + 150 kg/ha of RP (for 24 trees).

Leaves and olive fruit samples were collected at harvest, approximately after 5 years of agronomic application of OMW and RP. Once in the laboratory, leaves were rinsed with 0.03% Triton X-100 and then with deionized water. Fruits (3 kg) were transported to the laboratory on the same day for oil extraction and to analyse olive fruits and oil quality.

### 2.3. Soluble carbohydrate determination in leaves and fruits

The extraction of soluble carbohydrates of leaves and fruits was performed as described by Bartolozzi, Bertazza, Bassi, and Cristoferi (1997). A silylation mixture (pyridine, hexamethyldisilazane and trimethylchlorosilane) was added to the dried extracts before gas chromatography (GC) analysis. Olive and leaf samples were analyzed using a Hewlett-Packard 5890 series II gas chromatograph equipped with a flame ionization detection (FID) system and a HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm). Injector and detector temperatures were 280 °C and 300 °C, respectively. The following temperature programme was set: 80 °C for 1 min, it increased to 170 °C (at 10 °C/min), then to 200 °C (at 15 °C/min), then to 315 °C (at 25 °C/min) and finally it stayed at 315 °C for 8 min (Mechri, Tekaya, Cheheb, & Hammami, 2015). The identification of individual sugars was performed by comparing their retention times with those of standard compounds.

### 2.4. Extraction and determination of total phenols in olive fruits

0.5 g of M0, M1PN and M2PN fresh olive fruits were extracted in 10 ml of methanol on a shaker at 200 rpm for 24 h. After centrifugation (5000×g for 10 min), all obtained extracts were then transferred to vials and kept in the dark at –20 °C (Taamalli et al., 2012). Total phenolic content was determined according to the method of Montedoro, Servili, Baldioli, and Miniati (1992). The absorbance was measured at 765 nm and total phenol concentration was expressed as µg/mg of hydroxytyrosol.

### 2.5. Oil analyses

#### 2.5.1. Oil extraction

Oil content was determined using a Soxhlet apparatus by extracting dry material with 40–60 °C petroleum ether. The olive oil used for the qualitative analyses was extracted using a bench hammer mill: fruits were crashed and then mixed for 30 min at 25 °C. The oil was separated by centrifugation.

#### 2.5.2. Physico-chemical analyses of extracted olive oil

Free acidity (Acidity, given as a percentage of oleic acid), peroxide index (PV, expressed as milliequivalents of active oxygen per kilogram of oil (meq of O<sub>2</sub>/kg)), and extinction coefficients (K<sub>232</sub> and K<sub>270</sub>) were determined following analytical methods described

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