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Dose and frequency dependent effects of olive mill wastewater treatment on the chemical and microbial properties of soil

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HIGHLIGHTS

- Spreading olive mill wastewater (OMW) on olive-trees field was investigated.
- Three levels of OMW were applied to an olive trees field over three successive years.
- Organic matter, total nitrogen and potassium soil contents increased with OMW levels.
- Electrical conductivity, sodium and chloride contents increased with OMW applications.
- Aerobic bacteria and fungi raised with OMW successive applications.

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ABSTRACT

Olive mill wastewater (OMW) is a problematic by-product of olive oil production. While its high organic load and polyphenol concentrations are associated with troublesome environmental effects, its rich mineral and organic matter contents represent valuable nutrients. This study aimed to investigate the valorization of this waste biomass as a potential soil conditioner and fertilizer in agriculture. OMW was assayed at three doses 50, 100, and 200 $m^3\ ha^{-1}\ year^{-1}$) over three successive years in olive fields. The effects of the effluent on the physico-chemical and microbial properties of soil-layers were assessed. The findings revealed that the pH of the soil decreased but electrical conductivity and organic matter, total nitrogen, sodium, and potassium soil contents increased in proportion with OMW concentration and frequency of application. While no variations were observed in phosphorus content, slow increases were recorded in calcium and magnesium soil contents. Compared to their control soil counterparts, aerobic bacteria and fungi increased in proportion with OMW spreading rates. The models expressing the correlation between progress parameters and OMW doses were fitted into a second degree polynomial model. Principal component analysis showed a strong correlation between soil mineral elements and microorganisms. These parameters were not related to phosphorus and pH.

1. Introduction

The olive tree (*Olea europaea* L.) is a multipurpose plant species native to the Mediterranean region. It is particularly valued for its fruit and the oil it produces. The olive oil production process is, however, associated with the generation of large amounts of a liquid waste by-product known as olive mill wastewater (OMW), which, if improperly managed, presents major environmental problems. In fact, OMW production reaches about $7 \times 10^5\ m^3$ - $year^{-1}$ in Tunisia, an amount which is generated during few months between November and February (Hachicha et al., 2006; Jarboui et al., 2010; Ntougias et al., 2012). OMW might have deleterious impacts on soil and water environments (López-Piñeiro et al., 2007). The latter are often attributed to the presence of antimicrobial and phytotoxic compounds, namely polyphenols (Perez et al., 1992; Isidori et al., 2005; Quaratino et al., 2007; Sellami et al., 2008; Hachicha et al., 2009).

Various proposals have been suggested to overcome the inadequacies and negative impacts associated with OMW management. The fertilisation effects of the direct application of OMW were extensively investigated in agricultural soils (Rinaldi et al., 2003; Sierra et al., 2007; Belaqziz et al., 2008). This practice has been extended in the Mediterranean region particularly where olive grove soils presented very low levels of organic matter and are exposed to progressive degradation processes (López-Piñeiro

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et al., 2007). The high fertilisation value attached to OMW is attributed to its richness in water and nutrient resources. In fact, OMW typically consists of 83–94% water, 4–16% organic compounds, and 0.4–2.5% minerals (Ammar et al., 2005). It is also reported to have high levels of potassium, nitrogen, phosphorus, calcium, magnesium, and iron (Paredes et al., 1999; Ammar et al., 2005). Its organic fraction includes sugars, tannins, polyphenols, polyalcohols, pectins, lipids, and proteins (Lesage-Meessen et al., 2001; Mulinacci et al., 2001).

Accordingly, several researchers have established that OMW improves soil fertility (Di Giovacchino et al., 2002; Cereti et al., 2004) and soil microflora (bacteria, yeasts and fungi) by inducing changes in microbial communities (Paredes et al., 1986; Moreno et al., 1987; Saadi et al., 2007; Mechri et al., 2008). Nevertheless, and due to problems associated with dark colour, strong odour, and acidity, controversial opinions have emerged with regard to the OMW application as a fertilizer for agricultural soils. In fact, the regulations against the spreading out of OMW on agricultural fields are increasing worldwide, except in Italy (Rinaldi et al., 2003). In Tunisia, an exceptional authorisation has exceptionally been granted for research purposes to an international project (CFC/IOOC/04 Olive Tree Institute of Sfax) that started in 2004.

Considering the promising opportunities that this natural biomass might open with regards to the alleviation of the current growing concerns over soil poverty and lack of nutrients, further research is needed to investigate its potential valorization and ultimately legitimize the reconsideration of its banned use in agriculture. Accordingly, the present study was undertaken to investigate and evaluate the effects of raw olive mill wastewater, applied at different concentrations and frequencies during three successive years under natural field conditions in an olive orchard, on the fertility and physico-chemical and microbial properties of soil.

2. Materials and methods

2.1. Field investigation

The experiments of the present study were carried out in an olive-tree (*O. europaea* L., variety Chemlali) field at the Taous experimental station of the Olive Tree Institute of Sfax, South of Tunisia (34°43′N, 10°41′E). This area of the country 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 2004 to 2007). Throughout the experimental period, each of the three latter plots was treated with the same annual dose of raw OMW, which was spread out on the surfaces of the corresponding plots at controlled volumes, namely 50, 100, and 200 $m^3\ ha^{-1}$, respectively. The fourth plot was not submitted to raw OMW treatment and served 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. Soil samples were collected yearly from the different plots at 0–20, 20–40, 40–60, and 60–80 cm depths using a soil auger. The sample taken for each layer consisted of a homogenized mixture of six sub-samples that were randomly from six different locations across the whole plot in accordance with standard procedures (ISO 8358, 1991). All samples were taken 10 days after OMW application during the three successive years of treatment. One plot application was experimented per dose. All the collected soil samples were air-dried and filtrated through a 2 mm sieve, and then stored at 4 °C prior to analysis. The soil texture basic characteristic was sandy, with 86.63% of sand, 13.26% of silt and 0.20% of clay.

2.2. OMW used for experiments

The fresh OMW used for field applications was collected from a three-phase olive mill plant located near the Taous experimental station of Sfax.

The pH and electrical conductivity (EC) of the effluent were measured using pH and conductivity meters, respectively. The dry weight and moisture content were determined by weighing the sample before and after drying overnight at 105 °C. Chemical oxygen demand (COD) was determined according to the Knechtel method (1978). Biochemical oxygen demand (BOD) was measured according to the respirometric method. Organic matter content was determined after heating the samples for 4 h at 550 °C in a muffle furnace. Phenol content was estimated by the Folin–Ciocalteu method (Box, 1983). Total nitrogen was determined by the Kjeldahl method. Total P was measured by colorimetric analysis, K and Na by flame photometry, and Ca and Mg by atomic absorption spectrophotometry (Hitachi U-2000).

The main characteristics of the raw OMW effluent spread were: pH 4.52; EC: 12.52 $mS\ cm^{-1}$; COD: 121.60 $g\ L^{-1}$; BOD: 44.96 $g\ L^{-1}$; Organic matter: 51.80 $g\ L^{-1}$; Total phenols: 1.06 $g\ L^{-1}$; N: 1.65 $g\ L^{-1}$; P: 0.63 $g\ L^{-1}$; K: 5.93 $g\ L^{-1}$; Ca: 0.78 $g\ L^{-1}$; Mg: 0.57 $g\ L^{-1}$; Na: 1.32 $g\ L^{-1}$.

2.3. Soil chemical analysis

Measurements of pH and electrical conductivity (EC) were determined on a mixture of soil/water (1:2.5 and 1:5, respectively). The soil organic matter was analysed according to the Walkley–Black method (Nelson and Sommers, 1996). Total nitrogen content was determined by the Kjeldahl method. Available phosphorus was analysed following the method of Olsen and Sommers (1982). Available K^+, Na^+, Ca^{++}, and Mg^{++} were extracted with ammonium acetate at pH 7. K^+ and Na^+ were then measured by flame photometry and Ca^{2+} and Mg^{2+} by atomic absorption spectrophotometry (Hitachi U-2000).

2.4. Microbial estimation

Ten g of each soil sample were suspended in 90 mL of a sterile peptone water solution and stirred at 200 rpm for 2 h. The suspensions were then used for microbial counts, which were expressed as total colony forming units (cfu). For each suspension, serial decimal dilutions were plated in triplicate on different agar media; Plate Count Agar (PCA) for total aerobic bacteria incubated for 3 days at 30 °C, and Sabouraud medium for total fungi incubated for 5 days at 25 °C. Microbial counts were expressed as colonies forming units (cfu) per gram of dried soil (AFNOR, 1995).

2.5. Statistical analysis

Principal Component Analysis (PCA), a multivariate method, was used to examine the multivariate relationship and explain the variance of data whilst reducing the variable number to groups of individuals, based on principal component scores. PCA is a correlation method transforming the data of many experimental variables into a set of compound axes denoted as principal component (PC). This analysis was based on twelve variables per treatment. For all parameters, the sampling number was: $n = 48$ (4 plots × 4 layers × 3 applications) and corresponded to correlation coefficient of r P|0.28| and the Pearson value inferior to 0.05 ($p < 0.05$). The cumulative data recorded was submitted to variance analysis using SPSS software (version 11). The mean values of the treatments were compared using the Duncan's multiple range test at 5% level of significance ($p = 0.05$). All analyses were performed in triplicate.