Response of Soil Properties and Microbial Communities to the Application of Olive Mill Wastewaters

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ABSTRACT

A laboratory trial was carried out to evaluate the short-term influence of different concentrations of olive mill wastewaters (OMW) on soil physico-chemical properties and microbial community. After 30 days incubation, no significant changes occurred in soil pH values and humified organic carbon; soil organic carbon and total water-soluble phenols (WSP) significantly increased. Addition of OMW to the soil caused modification in microbial counts and microbial community structure. Bacteria decreased significantly up to 86% for 100% OMW treatment. No significant differences were found for fungal community in the soil treated up to 50% OMW while a decrease in 50% was found when 100% OMW was applied. The fungal: bacteria ratio increased significantly. The actinomycetes CFU (colony forming units) decreased gradually up to a maximum of 38% when OMW dose increased. Our results evidenced that the storage of OMW in open-air lagoons for a 4-month period before its soil application and the appropriate dilution with irrigation water without any further treatment can be an inexpensive technology to be adopted by small-sized olive mills.

Keywords: microbial counts, organic carbon, physico-chemical properties, phenols, soil fertility, soil quality
Abbreviations: BOD, biochemical oxygen demand; C, carbon; CFU, colony forming units; COD, chemical oxygen demand; EC, electrical conductivity; FA, fulvic acid; HA, humic acid; HR, humification ratio; PVP, polyvinilpyrrolidone; TAE, tannic acid equivalents; TOC, total organic carbon; TPP, total polyphenols; WHC, water-holding capacity; WSP, water-soluble phenols

INTRODUCTION

In Mediterranean countries, over a limited time period (usually from October to December), the olive oil industry produces large amounts of liquid and solid wastes, whose characteristics, particularly moisture and oil contents, depends on a broad variety of factors such as type and maturity of olives, harvesting time, region of origin, climatic conditions, cultivation practices and the extraction process which is employed (Azbar et al. 2004; Neto et al. 2005; Roig et al. 2005).

Olive mill wastewaters (OMW) are formed by the tissue water from the olive fruits, the water added during the oil extraction process, soft tissues from olive pulp and oil, in the form of a very stable emulsion (Lanciotti et al. 2005). The removal of OMW is a crucial problem for the whole community in general and for the producers and millers in particular, because of their polluting effects on soil and water (Cegarra et al. 1996; Paredes et al. 1999; Filippi et al. 2002). This is due to its high organic load (Paredes et al. 1999), phytotoxic properties, (Casa et al. 2003; Kistner et al. 2004; Isidori et al. 2005), antibacterial (Pérez et al. 1992) and antimicrobial (Fiorentino et al. 2003) activities, and relatively low biodegradability (Komilis et al. 2005). In order to find convenient solutions for the using of this by-product without harmful environmental effects, many studies were conduct in several olive-oil producing countries and different disposal methods based on evaporation ponds, thermal concentration, physico-chemical and biological treatments as well as direct application to agricultural soils as organic fertilizers have been proposed (Rozzi and Malpei 1996).

OMW contain substantial amounts of plant nutrients and organic carbon and may also represent a low cost source of water (Cegarra et al. 1996). In countries on the south bank of the Mediterranean with severe water deficient environments and with soils usually characterized by a scarcity of organic matter, the use of such wastes for soil fertigation could be even more beneficial, although toxic effects on microbial growth and plant seed germination have been observed (Capasso et al. 1992). Many study explored the impact of OMW on various soils, showed a temporary decrease in pH, plant available magnesium and hydraulic conductivity, followed by an increase in soil salinity and bulk density (Saviozzi et al. 1991; Pérez et al. 1992; López et al. 1996; Colucci et al. 2002; Saadi et al. 2006).

In contrast, numerous studies have demonstrated that land spreading of OMW increases soil fertility (Martens et al. 1992; Perucci 1992; Albich et al. 2000), improving soil porosity (Pagliai 1996), stabilizing soil conglomerates (Flouri et al. 1988; Saviozzi et al. 1991; Tomati and Galli 1992; Tomati et al. 1996; Colucci et al. 2002; Ferri et al. 2002), increasing soil organic matter, available P and exchangeable K contents (Brunetti et al. 1995; Ferri et al. 2001). In addition, Potenz et al. (1980), Marsilio et al. (1991) and Senette (1991) indicated that OMW had low potentially toxic heavy metal concentrations, so that their application to soil did not lead to environmental pollution risk.

Furthermore, even if many authors investigated the short-term effects of spreading OMW on soil microbial biomass, only limited information is available on the effects of OMW application on soil microbial community structure, which in turn may influence the viability of agriculture soils. Paredes et al. (1986) found decrease in the number of Bacillus sp., Tardioli et al. (1997) evidenced changes in soil fungal compositions: these findings were confirmed by Mecra et al. (2007) that found an alteration in the microbial community structure after agronomic application of OMW.

Because of the existing contradictory results, not always useful to draw practical conclusion, and the limited infor-
mation on the effects of OMW application on soil microbial community structure, the present work was aimed at assessing, under laboratory conditions, the direct application of raw OMW to soil. To this end, the short-term influence of different OMW concentrations on several chemical soil properties and microbial community structure, reflecting soil ecosystem quality and functioning was studied.

**MATERIALS AND METHODS**

**Olive mill wastewater**

The OMW samples were collected from an olive oil mill located in the city of Lametia Terme (Italy), which employs a three-phase decanter centrifuge process for oil separation, and stored in cement tanks of 200 m³ in October 2006 and then taken in February 2007. In this period the meteoric contribution was 0.32 m³. The characteristics of OMW are given in Table 1. The OMW were refrigerated at 4°C until used.

**Sampling and physico-chemical analyses of soil**

Composite soil samples (0-20 cm) were taken from the Agrarian Farm of the Agriculture Faculty of Reggio Calabria. According to USDA classification, the soil can be classified as sandy loam soil (clay 16%, sand 82%, silt 2%). Soil samples were stored at 4°C until the analysis.

Soil analyses were performed on air dried and sieved (<2 mm) soil samples. Particle-size analysis was carried out by the hydrometer method using sodium hexametaphosphate (SHMP) as a dispersant (Bouyoucos 1962); pH was measured in distilled water (solid: liquid ratio 1: 2.5) with a glass electrode; organic C was estimated by the Walkley-Black procedure (Nelson and Sommers 1982) and was converted to organic matter by multiplying the percentage of C by 1.72. The total CaCO₃ was carried out using a De Astis Calcimeter. Humic substances were extracted with 0.1 N NaOH and 0.1 N Na₂P₂O₇ solution (solid: liquid ratio 1: 2.5) with a glass electrode; organic C was estimated by the Walkley-Black procedure (Nelson and Sommers 1982) and was converted to organic matter by multiplying the percentage of C by 1.72. The total CaCO₃ was carried out using a De Astis Calcimeter. 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character (pH 8.1) (Table 2), and few content of organic carbon (0.96%). The results presented in this study demonstrate that the chemical properties of the soil amended with different concentrations of OMW were in part modified. After 30 days' incubation, OMW acidity (pH 5.5) has been neutralised by soil carbonate alkalinity (\(\text{CaCO}_3\) 7%) and intrinsic buffering capacity of soil.

Soil organic carbon and water-soluble phenols increased (26 and 27%, respectively) after 100% OMW addition, no variations were recorded on humified organic carbon, probably for the short period of trial; consequently, a significant decrease in the humification ratio (HR%) was observed. These results are consistent with the results of Paredes et al. (1999) and Mekki et al. (2006). They have demonstrated that OMW increase soil organic matter, resulting in enhanced soil fertility.

The addition of OMW to the soil induced significant modifications in the soil microbial community. Bacteria decreased significantly up to 86% for 100% OMW treatment compared to control. This reduction is presumably due to the slight lowering soil pH caused by the addition of OMW (Fig. 1). The growth of fungi was influenced by OMW treatment differently: no significant differences were found in the soil treated up to 50% OMW, a decrease in 50% was found in 100% OMW compared to control (Fig. 1). The fungal: bacteria ratio increased significantly. This suggests that the presence of OMW caused a shift between the proportion of fungi and bacteria, OMW represent an adequate substrate for fungi. The increase in fungi after application of OMW can help to degrade the phenolic and non phenolic aromatic pollutants in OMW (Sanjust et al. 1991), and it is generally known that the ligninolytic enzymes are involved in the fungal degradation of polyphenols in OMW (Sayadi and Ellouz 1992). Fungal populations are known for their considerable diversity of depolymerising enzymes and for their resistance to recalcitrant substances (Dix and Webster, 1995). Evelyn et al. (2005) showed that fungi are the organisms principally responsible for lignin degradation in soil.

Under natural conditions, rarely are substances transformed by a single microbial species, rather a mixed flora is usually responsible for the conversions that occur. Because lignin is highly resistant, it protects cellulose against attack by most microbes, and it must be degraded by biological means before the cellulose can be utilized. A part some higher fungi such as the basidiomycetes, the actinomycetes are also capable of degrading substances with high resistance to microbial attack (Paul and Clark 1996), as some cellulose and lignin (Tuomela et al. 2000).

The addition of OMW to the soil caused a gradual decrease in actinomycetes CFU compared to control, until a maximum of 38% when the dose of OMW increased (Fig. 1).

The impact of OMW on soil microflora may be due to a temporary enrichment of the soil with nutritive solution, carbon source, and inhibiting components to some micro-

### Table 2 Some chemical and physical properties of soil treated with different stored OMW concentrations.

<table>
<thead>
<tr>
<th>%</th>
<th>0%</th>
<th>10%</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (H2O)</td>
<td>8.10 a</td>
<td>8.05 a</td>
<td>8.01 a</td>
<td>7.95 a</td>
<td>7.85 a</td>
<td>7.80 a</td>
</tr>
<tr>
<td>CO %</td>
<td>0.96 d</td>
<td>1.06 bc</td>
<td>1.04 c</td>
<td>1.11 b</td>
<td>1.26 a</td>
<td>1.31 a</td>
</tr>
<tr>
<td>SO %</td>
<td>1.65</td>
<td>1.82</td>
<td>1.78</td>
<td>1.91</td>
<td>2.16</td>
<td>2.25</td>
</tr>
<tr>
<td>Sand %</td>
<td>82 a</td>
<td>82 a</td>
<td>82 a</td>
<td>82 a</td>
<td>82 a</td>
<td>82 a</td>
</tr>
<tr>
<td>Silt %</td>
<td>2 a</td>
<td>2 a</td>
<td>2 a</td>
<td>2 a</td>
<td>2 a</td>
<td>2 a</td>
</tr>
<tr>
<td>Clay %</td>
<td>16 a</td>
<td>16 a</td>
<td>16 a</td>
<td>16 a</td>
<td>16 a</td>
<td>16 a</td>
</tr>
<tr>
<td>Texture</td>
<td>Sandy loam</td>
<td>Sandy loam</td>
<td>Sandy loam</td>
<td>Sandy loam</td>
<td>Sandy loam</td>
<td>Sandy loam</td>
</tr>
<tr>
<td>CaCO3 %</td>
<td>7 a</td>
<td>7 a</td>
<td>7 a</td>
<td>7 a</td>
<td>7 a</td>
<td>7 a</td>
</tr>
<tr>
<td>C.U. tot %</td>
<td>0.23 a</td>
<td>0.23 a</td>
<td>0.23 a</td>
<td>0.23 a</td>
<td>0.22 a</td>
<td>0.22 a</td>
</tr>
<tr>
<td>HR %</td>
<td>24 a</td>
<td>23 a</td>
<td>22 b</td>
<td>22 b</td>
<td>17 c</td>
<td>16 c</td>
</tr>
<tr>
<td>WSP mg TAE L-1</td>
<td>30 e</td>
<td>35 d</td>
<td>34 d</td>
<td>36 e</td>
<td>38 b</td>
<td>41 a</td>
</tr>
</tbody>
</table>

*values in the same row followed by the same letter are not significantly different (p<0.05)
teria need to be further investigated. Possible changes in soil bacterial and fungal communities are not necessary detrimental to soil fertility. These changes may be the results of different fresh input of degradable carbon supplied with wastewater. Such changes are expected also with many types of soil organic additive as already reported by Stenström et al. (2001). Our results confirm previous findings (Allanello et al. 1998; Bäß and Anderson 2003; Mechri et al. 2005) assuming that the microbial community responds to agronomic application of OMW with shift in key components of the biomass, and suggest that the changes in microbial structure may positively affect soil ecosystem leading to changes in the patterns of C, nutrient cycling and soil fertility.

CONCLUSIONS

Although the experiments presented here are limited by the laboratory controlled conditions, they may be suitable for assessing the short-term response of soil to an applied OMW. These results seem to confirm that the impact of OMW on soil properties and soil microbial communities depend on the relative amounts of beneficial and toxic compounds present, and soil had an intrinsic buffering capacity to resist to the applied perturbation.

The results of the present study indicate that the storage of OMW in open-air lagoons for a 4-month period and their capacity to resist to the applied perturbation.

ACKNOWLEDGEMENTS

This research was supported by University “Mediterranea” of Reggio Calabria. Program PRIT “Gestione sostenibile delle acque reflue olearie e dei sottoprodotti di industrie agro-alimentari”. Project Coordinator: Prof. Santo Marcello Zimbone, Department ISTAFA, University “Mediterranea” of Reggio Calabria.

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