Terrestrial and Aquatic Environmental Toxicology ©2010 Global Science Books

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

Maria Sidari* • Carmelo Mallamaci • Emilio Attinà • Adele Muscolo

Department of Agriculture and Forest System Management, University "Mediterranea" of Reggio Calabria, Feo di Vito, 89060- Reggio Calabria, Italy

Corresponding author: * msidari@unirc.it

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 Andre *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 (Pèrez *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; Pèrez *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 Mechri *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-

Received: 16 June, 2009. Accepted: 17 August, 2009.

Original Research Paper

Table 1 Chemical characteristics of olive mill wastewater fresh collected (unstored) and 4-months stored in cement task in open air (stored).

Parameters	Un-stored OMW	Stored OMW
pH	4.5 ± 0.2	5.5 ± 0.1
BOD_5 g L^{-1}	8.0 ± 0.5	4.1 ± 0.8
COD g L^{-1}	18.9 ± 0.6	10.5 ± 1.3
EC dS/m	5.1 ± 0.4	4.59 ± 0.03
Organic matter g L^{-1}	11.5 ± 0.7	9.46 ± 1.0
Ash g L^{-1}	5.1 ± 0.7	3.7 ± 0.5
Dry matter g L^{-1}	11.1 ± 0.1	9.80 ± 0.8
WSP mg TAE L^{-1}	25.5 ± 0.1	5.47 ± 0.5
TPP mg TAE L^{-1}	31.6 ± 0.7	11.3 ± 0.7

Each value represents mean of five independent observations ± S.E.

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 m^3 in October 2006 and then taken in February 2007. In this period the meteoric contribution was 0.32 m^3. 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 Firm 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 $CaCO_3$ was carried out using a De Astis Calcimeter. Humic substances were extracted with 0.1 N NaOH and 0.1 N $Na_2P_2O_7$ solution (solid: liquid ratio 1: 10); the suspension was shaken for 16 h at room temperature and centrifuged at 5,000 rpm for 30 min; the extract was dialysed by Wisking tubes against distilled water to pH 6.0. Subsequently, the solution was filtered through a column of Amberlite® IR 120 H^+ form (Sigma-Aldrich Co.). The fractionation of humic substances was carried out as follows: aliquots of extracts were acidified to pH 2.0 with dilute H_2SO_4; the humic acids precipitated and were removed by centrifugation, while the fulvic acids corresponded to the supernatants (Bettany *et al*. 1980). The C content of humic and fulvic acids was determined by dichromate oxidation followed by titration with ferrous ammonium sulphate (Nelson and Sommers 1982).

Humification Ratio (HR%) representing the ratio HA+FA/TOC × 100, where HA and FA are purified humic and fulvic acids on PVP column (Petrussi *et al*. 1988) and TOC the total organic carbon, determined by the Springer and Klee method (Ministero delle Politiche Agricole Alimentari e Forestali 1994). The HR% parameter is proportional to the state of humification of the soil organic matter.

Phenols were extracted with distilled water, 1: 10 (w/v). Soil samples were shaken at 75 rev min^{-1} for 20 h at room temperature and solutions were filtered through Whatman's no 1 paper. Total water-soluble phenols (WSP) were measured by using the Folin-Ciocalteau reagent, following the Box method (1983).Tannic acid was used as a standard and the concentration of water-soluble phenols was expressed as tannic acid equivalents (mg TAE g^{-1} d.w.) (Kuiters and Denneman 1987).

Experimental design

The soil (2 kg) was put in plastic pots and treated with OMW at five different concentrations (10, 25, 50, 75 and 100%). Untreated soil served as a control (0% OMW). All pots were supplemented with 120 mL of OMW at different concentrations or with distilled water (0% OMW). The pots with soil were then moistened up to 50% of water-holding capacity (WHC). Treatments were performed every week. All samples were incubated under controlled conditions of humidity and temperature, in a climatic chamber, using a regime of 16: 8 h light: dark, 24°C and a relative humidity of 70%. Each treatment was replicated five times. At 30 days' incubation, five pots of each treatment were taken and split into sub-samples for all analyses.

Microbial counts

Ten grams of each soil sample were added to 90 mL of 0.1% (w/v) sterile solution of sodium pyrophosphate and 1% glycerol, pH 7.0 (Elliot and Des Jardin 1999). After homogenization for 15 min at 1000 rpm, this solution was decimally diluted (10^{-1} to 10^{-7}) in a 0.85% NaCl sterile solution and aliquots of the resulting solutions plated on appropriate culture media. Tryptone Soya Broth (Oxoid Ltd, UK) diluted (3 g L^{-1}), containing cycloeximide (100 μg mL^{-1}, agent that inhibit fungal growth) and solidified with agar (15 g L^{-1}), adjusted to pH 7 and sterilized at 121°C for 20 min was used for total bacteria count. After incubation at 28°C for 28 days the colony forming units (CFU) were counted. Difco™ Malt Extract Agar containing chloramphenicol (inhibit bacteria growth) was used for fungal estimation (Picci and Nannipieri 2003). The incubation was performed at 28°C for 7 days. Actinomycetes were measured as CFU g^{-1} of soil grown on starch casein medium (Wellington and Toth 1994), after the addition of nystatin (50 μg mL^{-1}, sterilized by filtration at 0.22 μm). The incubation was performed at 28°C for 14 days. Each soil sample was analysed in triplicate and the dilution series were plated in duplicate for each medium.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and treatment means were compared using the Tukey test at $p \leq 0.05$ (Sokal and Rohlf 1969).

RESULTS AND DISCUSSION

The results of selected chemical properties of the olive mill wastewaters stored in cement tanks during a period of 4 months (between October to February) suggest that some degradation and possible solid precipitation may occur during the stored period in open air. The degradation process was associated with the increase in pH (5.5), lower conductivity (4.9 dS m^{-1}), water soluble phenol and polyphenol content (5.47 and 11.33 mg TAE L^{-1}, respectively). The extremely low COD (10.5 g L^{-1}), and BOD (4.1 g L^{-1}) values seems not to be representative but rather reflects solid precipitation of some organic material. These results were confirmed also by the lower content of dry matter and ash in the stored OMW. Alteration of OMW properties are of practical relevance. In a real land application OMW should be stored for a longer period of at least several months before fully utilized. More changes may occur during a long storage period moreover if it is extended into the warm spring and early summer months, where the microbial processes are more intense (García *et al.* 1997; Muscolo and Sidari 2006). OMW storage could represent an efficient low cost pre-treatment before land application (Casa *et al.* 2003; Kistner *et al.* 2004; Lanciotti *et al.* 2005).

The sandy loam soil utilized in this trial had an alkaline

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

	0%	10%	25%	50%	75%	100%
pH (H_2O)	8.10 a*	8.05 a	8.01 a	7.95 a	7.85 a	7.80 a
CO %	0.96 d	1.06 bc	1.04 c	1.11 b	1.26 a	1.31 a
SO %	1.65	1.82	1.78	1.91	2.16	2.25
Sand %	82 a	82 a	82 a	82 a	82 a	82 a
Silt %	2 a	2 a	2 a	2 a	2 a	2 a
Clay %	16 a	16 a	16 a	16 a	16 a	16 a
Texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
$CaCO_3$ %	7 a	7 a	7 a	7 a	7 a	7 a
C.U. tot %	0.23 a	0.23 a	0.23 a	0.23 a	0.22 a	0.22 a
HR %	24 a	23 a	22 b	22 b	17 c	16 c
WSP mg TAE L^{-1}	30 e	35 d	34 d	36 c	38 b	41 a

*values in the same row followed by the same letter are not significantly different ($p \leq 0.05$)

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 ($CaCO_3$ 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 the OMW. The structure of the aromatic compounds in OMW presents obvious similarities to lignin components (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 microorganisms. The results of this study show that OMW applied to soil up to 50% are not harmful to soil microflora in general. Yet, the possibility of inhibition of certain soil bac-

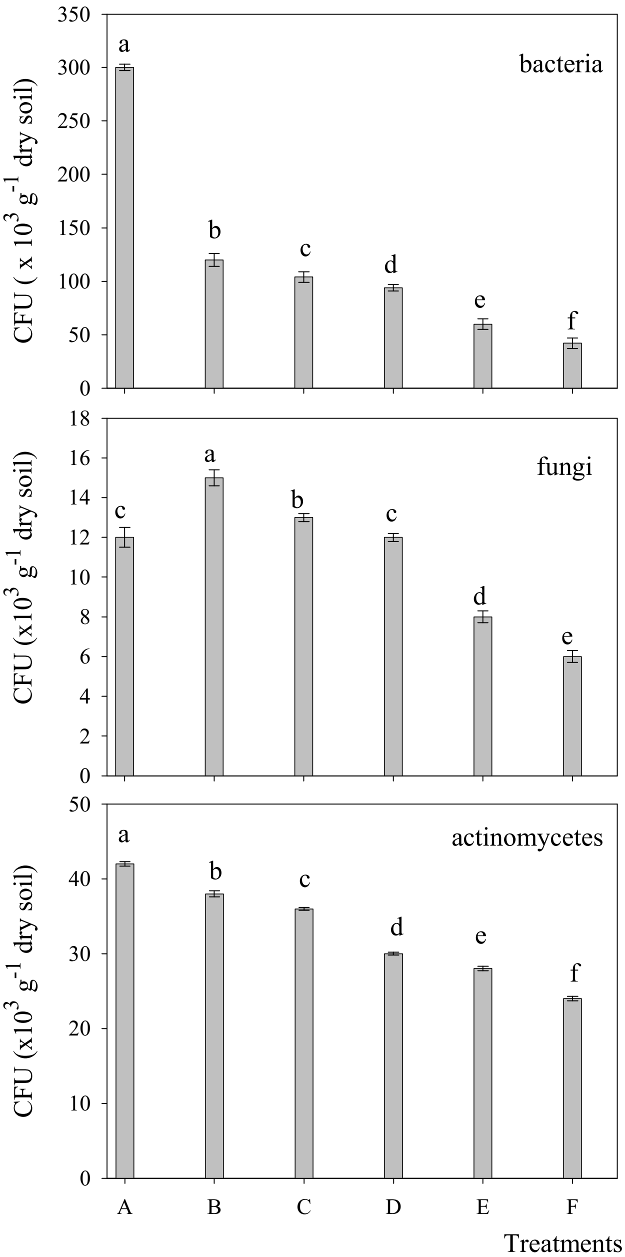

Fig. 1 Variation of CFU number of bacteria, fungi and actinomycetes in soil treated with different concentrations of OMW. A (0%), B (10%), C (25%), D (50%), E (75%), F (100%). Standard error of the mean is marked on the columns by bars. The same letters over bars indicate no significant difference according to Tukey's test ($p \leq 0.05$).

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 (Alianello *et al.* 1998; Bååth and Anderson 2003; Mechri *et al.* 2007) evidencing 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 opportune dilution with irrigation water before soil application may be a suitable inexpensive treatment to be adopted by small-sized olive mills for improving the quality of this material as soil amendment while minimizing the possible negative environmental impact.

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 STAFA, University "Mediterranea" of Reggio Calabria.

REFERENCES

Albich R, Canet R, Pomares F, Ingelmo F (2000) Microbial biomass content and enzymatic activity after the application of organic amendments to a horticultural soil. *Bioresource Technology* **75**, 43-48

Alianello F, Dell'Orco S, Pinzari F, Benedetti A (1998) Effects of olive mill waste water on microbial activity of agricultural soils. *Fresenius Environmental Bulletin* 7, 764-771

Azbar N, Bayram A, Filibeli A, Muezzinoglu A, Sengul F, Ozer A (2004) A review of waste management options in olive oil production. *Critical Reviews in Environmental Science and Technology* **34**, 209-247

Bååth E, Anderson T-H (2003) Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based technique. *Soil Biology and Biochemistry* **35,** 955-963

Bettany JR, Saggar S, Stewart JWB (1980) Comparison of the amount and forms of sulphur in soil organic matter fractions after 65 years of cultivation. *Soil Science Society of America Journal* **44**, 70-75

Bouyoucos GJ (1962) Hydrometer method improved for making particle-size analyses of soils. *Agronomy Journal* **54**, 464-465

Box JD (1983) Investigation of the Folin–Ciocalteau reagent for the determination of polyphenolic substances in natural waters. *Water Research* **17**, 511-525

Brunetti G, Miano TM, Senesi N (1995) Effetti dell'applicazione di reflui da frantoi oleari variamente trattati su alcune proprietà del suolo. *Atti XIII Convegno Nazionale Società Italiana di Chimica Agraria*, pp 36-37

Capasso R, Cristinzio G, Evidente A, Scognamiglio F (1992) Isolation spectroscopy and selective phytotoxic effects of polyphenols from vegetable waste waters. *Phytochemistry* **31**, 4125-4128

Casa R, D'Annibale A, Pieruccetti F, Stazi SR, Giovannozzi Sermanni G, Lo Cascio B (2003) Reduction of the phenolic components in olive-mill wastewater by an enzymatic treatment and its impact on durum wheat (*Triticum durum* Desf.) germinability. *Chemosphere* **50**, 959-966

Cegarra J, Paredes C, Roig A, Bernal MP, García D, Aragon P (1996) Use of olive mill waste water compost for crop production. *International Biodeterioration and Biodegradation* **38**, 193-203

Colucci R, Bari VD, Ventrella D, Marrone G, Mastrorilli M (2002) The effect of oil mill effluents on soil aggregation properties. In: Pagliai M, Jones R (Eds) *Sustainable Land Management – Environmental Protection. A Soil Physical Approach*, Advances in GeoEcology **35**, Geo Science Publisher, Reiskirchen Germany, pp 91-100

Dix NJ, Webster J (1995) *Fungal Ecology*, Chapman & Hall, London, 549 pp

Elliott ML, Des Jardin EA (1999) Comparison of media and diluents for enumeration of aerobic bacteria from bermuda grass golf course putting greens. *Journal of Microbiological Methods* **34**, 193-202

Evelyn H, Michael P, Christina D, Gert B, Sophie ZB (2005) Composition of the microbial communities in the mineral soil under different types of natural forest. *Soil Biology and Biochemistry* **37**, 661-671

Ferri D, Convertini G, Montemurro F, Rinaldi M, Rana G (2002) Olive wastes spreading in Southern Italy: Effects on crops and soil. *Proceedings of 12th ISCO Conference*, May 26-31, 2002, Beijing, China, Vol III, pp 593-600

Ferri D, Convertini G, Montemurro F, Vitti C (2001) Evoluzione di alcune proprietà chimiche di terreni pugliesi ammendati con reflui oleari. *Atti Giornata di Studio "Prospettive di Utilizzazione Agronomica dei Reflui Oleari"*. Bari, 14 February, **24**, pp 81-94

Filippi C, Bedini S, Levi-Minzi R, Cardelli R, Saviozzi A (2002) Co-composting of olive oil mill by-product, chemical and microbiological evaluations. *Compost Science and Utilization* **10**, 63-71

Fiorentino A, Gentili A, Isidori M, Monaco P, Nardelli A, Parella A, Temussi F (2003) Environmental effects caused by olive mill wastewater: Toxicity comparison of low-molecular-weight phenol components. *Journal of Agricultural and Food Chemistry* **51**, 1005-1009

Flouri CH, Chatzipavlidis L, Balis K, Servis D, Tzerakis S (1988) Olive mill wastewater application to soil. In: Ministry of the Aegean (Ed) *Proceedings of Scientific Conference on Aegean Olive Trees*, Eleourgiki PE, Mytilene, 234 pp (in Greek)

García C, Roldán A, Hernández T (1997) Changes in microbial activity after abandonment of cultivation in a semiarid Mediterranean environment. *Journal of Environmental Quality* **26**, 285-291

Isidori M, Lavorgna M, Nardelli A, Parrella A (2005) Model study on the effect of 15 phenolic olive mill wastewater constituents on seed germination and *Vibrio bischeri* metabolism. *Journal of Agricultural and Food Chemistry* **53**, 8414-8417

Kistner T, Nitz G, Schnitzler WH (2004) Phytotoxic effects of some compounds of Olive Mill Wastewater (OMW). *Fresenius Environmental Bulletin* **13**, 1360-1361

Komilis DP, Karatzas E, Halvadakis CP (2005) The effect of olive mill wastewater on seed germination after various pretreatment techniques. *Journal of Environmental Management* **74**, 339-348

Kuiters AT, Denneman CAJ (1987) Water-soluble phenolic substances in soils under several coniferous and deciduous tree species. *Soil Biology and Biochemistry* **19**, 765-769

Lanciotti R, Gianotti A, Baldi D, Angrisani R, Suzzi G, Mastrocola D, Guerzoni ME (2005) Use of *Yarrowia lipolytica* strains for the treatment of olive mill wastwwater. *Bioresource Technology* **96**, 317-322

López R, Martínez-Bordiú A, Dupuy de Lome E, Cabrera F, Sánchez MC (1996) Soil properties after application of olive oil mill waste water. *Fresenius Environmental Bulletin* **5**, 49-54

Marsilio V, Di Giovacchino L, Di Giacomi F (1991) Contenuto e dinamica di metalli veicolati nel terreno con lo spargimento di acque di vegetazione delle olive. NIA Ricerche, 2 November 1991, pp 39-41

Martens DA, Johanson JB, Frankenberger Jr. WT (1992) Production and persistence of soil enzymes with repeated addition of organic residues. *Soil Science* **153**, 53-61

Mechri B, Echbili A, Issaoui M, Braham M, Ben Elhadj S, Hammami M (2007) Short-term effects in soil microbial community following agronomic application of olive mill wastewaters in a field of olive trees. *Applied Soil Ecology* **36**, 216-223

Mekki A, Dhouib A, Sayadi S (2006) Changes in microbial and soil properties following amendment with treated and untreated olive mill wastewater. *Microbiological Research* **161**, 93-101

Ministero delle Politiche Agricole Alimentari e Forestali (1994) *Metodi Ufficiali di Analisi Chimica del Suolo*. ISPREA, Rome, 207 pp

Muscolo A, Sidari M (2006) Seasonal fluctuations in soil phenolics of a coniferous forest: effects on seed germination of different coniferous species. *Plant and Soil* **284**, 305-318

Nelson DW, Sommers LE (1982) Total carbon, organic carbon and organic matter. In: Page AL, Miller RH, Keeney DR (Eds) *Methods of Soil Analysis*, American Society of Agronomy, Madison, pp 539-579

Neto Andre R, Pinto F, Franco C, Dias M, Gulyurtlu I, Matos MAA, Cabrita I (2005) Fluidised bed co-gasification of coal and olive oil industry wastes. *Fuel* **84**, 1635-1644

Pagliai M (1996) Effetti della somministrazione di acque reflue di frantoi oleari sulle caratteristiche fisiche del suolo. *Proceedings of International Congress "Trattamento e Riciclaggio in Agricoltura dei Sottoprodotti dell'industria Olearia"*, Lecce, 8-9 March, pp 43-47

Paredes C, Monteoliva-Sánchez M, Moreno E, Pèrez J, Ramos-Cormenzana A, Martìnez J (1986) Effect of waste waters from olive oil extraction plants on the bacterial population of soil. *Chemosphere* **16**, 659-664

Paredes C, Cegara J, Roig A, Sánchez-Monedero AM, Bernal MP (1999) Characterization of olive mill wastewater (alpechin) and its sludge for agricultural purposes. *Bioresource Technology* **67**, 111-115

Paul EA, Clark FE (1996) *Soil Microbiology and Biochemistry*, Academic Press Inc., San Diego, 340 pp

Pèrez J, de la Rubia T, Moreno J, Martìnez J (1992) Phenolic content and antibacterial activity of olive oil waste waters. *Environmental Toxicology and Chemistry* **11**, 489-495

Perucci P (1992) Enzyme activity and microbial biomass in a field soil amended with municipal refuse. *Biology and Fertility of Soils* **14**, 54-60

Petrussi F, De Nobili M, Viotto M, Sequi P (1988) Characterization of organic matter from animal manures after digestion by earthworms. *Plant and Soil* **105**, 41-46

Picci G, Nannipieri P (2003) *Metodi di Analisi Microbiologica del Suolo*, Franco Angeli Editore, Milano, Italy, 224 pp

Potenz D, Righetti E, Volpicella M (1980) Effetto inquinante delle acque reflue della lavorazione delle olive su terreno agrario. Nota. *Inquinamento* **2**, 65-68

Roig A, Cayuela ML, Sánchez-Monedero AM (2005) An overview on olive mill wastes and their valorization methods. *Waste Management* **26**, 960-969

Rozzi A, Malpei F (1996) Treatment and disposal of olive mill effluents. *International Biodeterioration and Biodegradation* **38**, 135-144

Saadi I, Laor Y, Raviv M, Medina S (2006) Land spreading of olive mill wastewater: effects on soil microbial activity and potential phytotoxicity. *Chemosphere* **66**, 75-83

Sayadi S, Ellouz R (1992) Decolourisation of olive mill wastewaters by *Phanerochaete chrysosporium*: involvement of the lignin degrading system. *Applied Biochemistry and Biotechnology* **37**, 813-817

Sanjust E, Pompei R, Rescigno A, Augusto R, Ballero M (1991) olive milling wastewaters as medium for growth of four *Pleurotus* species. *Applied Biochemistry and Biotechnology* **31**, 135-148

Saviozzi A, Levi-Minzi R, Riffaldi R, Lupetti A (1991) Effetti dello spandimento di acque di vegetazione sul terreno agrario. *Agrochimica* **35**, 135-148

Senette C, Melis P, Uscidda C (1991) Indagine sull'impatto dei reflui oleari a seguito del loro smaltimento nel terreno. *Annali Facoltà di Agraria, Università di Sassari* **34**, 187-197

Sokal RR, Rohlf FJ (1969) *Biometry* (1st Edn), Freeman & Co., San Francisco, 340 pp

Stenström J, Svensson K, Johansson M (2001) Reversible transition between active and dormant microbial states in soils. *FEMS Microbiology Ecology* **36**, 93-104

Tardioli S, Bànnè ETG, Santori F (1997) Species-specific selection on soil fungal population after olive mill waste-water treatment. *Chemosphere* **34**, 2329-2336

Tomati U, Galli E (1992) The fertilizing value of waste water from the olive processing industry. In: Kabat J (Ed) *Humus, its Structure and Role in Agriculture and Environment*, Elsevier, Barking, pp 117-126

Tomati U, Galli E, Fiorelli F, Pasetti L (1996) Fertilizers from composting of olive-mill wastewaters. *International Biodeterioration and Biodegradation* **38**, 155-162

Tuomela M, Vikman M, Hatakka A, Itavaara M (2000) Biodegradation of lignin in a compost environment: a review. *Bioresource Technology* **72**, 169-183

Wellington EMH, Toth IK (1994) Actinomycetes. In: Mickelson SH, Bigham JE (Eds) *Methods of Soil Analysis. Part 2. Microbial and Biochemical Properties*, Soil Science Society of America, Madison, pp 269-290