Terrestrial and Aquatic Environmental Toxicology ©2010 Global Science Books

Raw and Microbiologically Detoxified Olive Mill Waste and their Impact on Plant Growth

Georgia Ouzounidou[1*] • Georgios I. Zervakis[2,3] • Fragiskos Gaitis[4]

[1] Institute of Food Technology, National Agricultural Research Foundation, 1 S. Venizelou str. 14123 Lycovrissi, Greece
[2] Agricultural University of Athens, Department of Agricultural Biotechnology, Laboratory of General and Agricultural Microbiology, Iera Odos 75, 11855 Athens, Greece
[3] National Agricultural Research Foundation, Institute of Kalamata, Lakonikis 87, 24100 Kalamata, Greece
[4] Food Industrial Research and Technological Development Company (ETAT S.A.), 47 Ethnikis Antistaseos Ave, 17237 Dafni-Athens, Greece

Corresponding author: * **geouz@nagref.gr** or **geouz@yahoo.gr**

ABSTRACT

Olive-oil production is of vital importance for the economy and social life of the Mediterranean region; however, the side effect of the olive-oil mill activity is the generation of enormous quantities of biotoxic olive-mill waste within a short period of three months. Numerous physicochemical and biological methods have been used in the past for the treatment of both olive mill wastewater (OMWW, deriving from three-phase extraction systems) and two-phase olive mill waste (TPOMW, deriving from two-phase extraction systems), but these generally failed to come up with a viable solution of wide applicability. On the other hand, since olive mill waste are of purely vegetative origin, their recycling (either raw or after treatment, including composting) into agricultural ecosystems seems environmentally reasonable and financially feasible. The detoxification and biotransformation potential of naturally occurring and other microorganisms is a promising alternative to the previous approach, especially in cases where *in situ* decomposition of such waste can not be readily applied. Raw and detoxified (chemically and microbiologically) olive mill waste application on tree and crop cultivation, showed that there is differentiation of plant responses depending on the plant species, type of the growth substrate and time of exposure. Based on hydroponics, soil and soilless cultures it appears that the higher the organic matter in the growth medium, the lower the waste toxicity. The toxicity is evident in all the plant functions from the germination to organs morphology, physiology, metabolism, ultrastructure, ripening and quality of the final product.

Keywords: biological detoxification, OMWW fertigation, olive pomace compost, phytotoxicity, plant growth, raw OMW application

CONTENTS

INTRODUCTION

The olive tree plays a vital role in the economy, ecology and social life of the Mediterranean countries, where approximately 8.5 million ha are in production, corresponding to 98% of the world olive cultivation. Spain, Italy and Greece account for around 75% of olive oil world production (which is *ca*. 2.8 million tons), while the rest originates mainly from Portugal, Tunisia, Morocco, Algeria, Turkey, the Middle East region, and Australia (IOOC 2008).

In the past, olive oil processing was mainly carried out with the discontinuous (traditional) press-type mills based on mechanical crushing of the olive fruits followed by pressure extraction, but the intensification of production lead to the abandonment of this traditional extraction method, and to the installation of the more efficient and of higher capacity centrifugal mills. Nowadays, there are two basic olive oil extraction technologies which employ a different type of mechanical crushing-homogenisation followed by centrifugal separation through the so-called "three-phase" and "two-phase" processes. The former was introduced in the 1970s for increasing olive-oil yield, and it generates olive oil (*ca*. 20-25 kg out of 100 kg of olives), olive pomace or olive cake or 'orujo' (*ca*. 30-35 kg out of 100 kg of olives; this is commonly further processed for the extraction of residual oil and of olive press-cake) and olive mill wastewater (OMWW, *ca*. 80-100 l out of 100 kg of olives). Hence, the quantities of OMWW, which are produced in the

Received: 22 May, 2009. Accepted: 23 November, 2009.

Invited Review

Mediterranean area alone, exceed 15 Mm^3 per year within a period of 3-4 months (November-February), creating a major environmental problem. On the other hand, the two-phase centrifugation system was introduced in the 1990s in order to deal with the problem of large quantities of water used by the centrifugal extraction process, and consequently for the reduction of the amount of liquid waste produced. Instead, by this system a sludge-like pomace (also called wet husk or 'alperujo', or two-phase olive mill waste, TPOMW; *ca*. 80-100 kg out of 100 kg of olives) is generated in large quantities (four million tons in Spain alone; Alburquerque *et al.* 2004), which is difficult to handle due to its physicochemical and biotoxic properties.

The characteristics of olive mill waste are variable, depending on many factors such as method of extraction, variety and maturity of olives, region of origin, climatic conditions and associated cultivation/processing methods. In general, OMWW is a recalcitrant dark brown effluent, with a distinctive odour, an acidic pH (4.0-5.5), a very complex redox system (conductivity: 6,000-16,000 μs), a high buffer capacity, tension activity and stability. More importantly, OMWW present high values for most pollution parameters: BOD_5: 40-95 g l^{-1}, COD: 50-180 g l^{-1}, LD_{50} toxicity for fish: 8.7%; in addition, they contain large amounts of suspended solids and high concentrations of polyaromatic compounds, e.g. simple phenols and flavonoids, or polyphenols (from 0.5 to 24 g l^{-1}) (Ramos-Cormenzana 1986; Paredes *et al.* 1987; Rouvalis *et al.* 2004; Roig *et al*. 2006). While phenolics are mainly held responsible for the OMWW strong antimicrobial and phytotoxic properties, non-phenolic-related toxicity attributed to long-chain fatty acids and volatile acids was also reported (Capasso *et al.* 1992, 1995; Ramos-Cormenzana *et al.* 1996; Paixao *et al.* 1999; Linares *et al*. 2003; Ouzounidou *et al.* 2008a). Moreover, OMWW possesses considerable amounts of mineral nutrients such as potassium (K_2O: 2.4-10.8 g l^{-1}) and phosphorus (P_2O_5: 0.3-1.5 g l^{-1}), and a wide-range of micronutrients (Tomati and Galli 1992; Cabrera *et al.* 1996; Roig *et al.* 2006; Aqeel and Hameed 2007; Mekki *et al*. 2007). As concerns TPOMW, which practically integrates into one waste-stream the OMWW (though in smaller quantities) and the olive pomace of the three-phase extraction process, it presents a moisture content of about 60-65%, an acidic pH (4.9-6.8), a high content in organic matter (60.3-98.5%) comprising relatively large amounts of lignin, cellulose and hemicellulose, lipids and carbohydrates as well as phenolics (Roig *et al*. 2006).

In the past, various physicochemical approaches have been employed including precipitation and flocculation, adsorption, ultrafiltration and reverse osmosis, chemical oxidation and ion exchange, combustion and pyrolysis, etc., which in general demonstrated satisfactory levels of organic load and COD elimination combined with high reduction of polyphenols content (Fiestas Ros de Urcinos and Borja Padilla 1992; Niaounakis and Halvadakis 2004; Mantzavinos and Kalogerakis 2005; Baldrian *et al.* 2006; Arvanitoyannis *et al*. 2007). However, their scaling-up was often met with serious practical difficulties and long-term economic failure, mainly because of technical complexity, and high installation and operating costs. On the other hand, reduction of OMWW environmental impact was also attempted through the use of combined methodologies (i.e. physicochemical and biological, most often integrating the exploitation of the biochemical potential of selected microorganisms) or only by biological approaches (which are considered to be more 'environmentally-friendly' and less expensive) enabling at the same time some of their primary components to be recovered. In this way, both types of olive mill waste (deriving from two- and three-phase olive-oil extraction processes) formed the base for the generation of a large array of products such as plant fertilizers and/or soil conditioners, substrates for the production of biomass and protein (algae and yeasts), biogas, biopharmaceuticals, biopolymers, feed additives, fodder and edible mushrooms (Codounis *et al.* 1983; Takashi *et al*. 1994; Ramos-Cormenzana *et al*. 1996; Zervakis *et al.* 1996; Ntougias *et al.* 2003; Ehaliotis *et al*. 2005; Mantzavinos and Kalogerakis 2005; Roig *et al*. 2006; Arvanitoyannis and Kassaveti 2007; Ntougias *et al*. 2008b; Morillo *et al.* 2009).

At the moment there is no European Union legislation regulating olive mill waste disposal, and standards are left to be set by individual countries, whereas only in Italy and Portugal among olive-oil producing countries there is legislation (Laws No. 574/1996 and No. 626/2000, respectively) for disposal/application of OMWW and/or TPOMW in agricultural soil. Hence, for example spreading of up to 80 m^3 ha^{-1} yr^{-1} OMWW (generated by the continuous centrifugation systems) is allowed in Italy by taking into account some limitations imposed by the Law. The most common disposal methods include land application, discharge into nearby rivers, lakes or sea and storage in evaporation lagoons. However, environmental problems often associated with such practices are soil contamination, underground seepage, water-bodies pollution and foul odor emissions (Paredes *et al.* 1987; Rinaldi *et al.* 2003). Nevertheless, it seems that the controlled spreading of olive mill waste into agricultural land could offer a significant solution for the disposal of such effluents, especially in areas with soils poor in organic matter. Olive mill waste is a natural product rich in organic compounds, it contains appreciable amounts of sugars, minerals and other plant growth-promoting substances; thus, it could be substantially used as a fertilizer and soil conditioner with a direct and indirect positive effect on plants (Ehaliotis *et al*. 1999; Tamburino *et al*. 1999; Di Giovacchino *et al.* 2001; Ehaliotis *et al.* 2003), provided that its phytototoxic properties and certain adverse effects on soil properties are taken into account and/or eliminated (Cereti *et al.* 2004; Ouzounidou *et al.* 2008a, 2008b).

This review attempts to cover available literature data on the application of raw (untreated) olive mill waste in soil and other plant growth substrates together with their effects on seeds germination, and on plant development, physiology and fruit production. In addition, it summarizes the outcome of numerous studies employing microorganisms for the detoxification and biodegradation of olive mill waste, with particular emphasis on the applications related with plant growth.

EFFECTS OF RAW OLIVE MILL WASTE APPLICATION

Land application of olive mill waste

There is a growing public concern about the environmental impact of industrial development and population expansion in recent decades. The continuous inputs of waste on agricultural land have caused imbalance in ecosystems (Ouzounidou *et al.* 2008a). Land application is the oldest system for the disposal of waste. It is based on the high biodegradative capacity of soils. According to Fuller and Warrick (1985), the land is a gigantic biodigestion system developed over millions of years that is able to biodegrade animal and plant waste to become part of the soil. Land application serves two objectives: (a) waste disposal; and (b) recycling of waste components. Fuller and Warrick (1985) proposed the terms land treatment and land utilization. Land treatment involves the use of soil also as a means of treating waste, while land utilization serves dual objectives: first of waste disposal and second of valuable resource. Land treatment is based on the physical, chemical and microbiological interactions between the components and the microorganisms of soil and waste. In land treatment, the soil is taken as a medium to biodegrade waste, which is applied on the land when no crop is being grown (Cabrera *et al.* 1996).

Since olive mill waste is of purely vegetative origin and contain no xenobiotics, added chemicals, or synthetic pollutants, its recycling into agricultural ecosystems, rather than their exhaustive treatment and disposal into domestic sewage or other water receptors, seems environmentally reasonable and financially feasible (Ehaliotis *et al.* 2003). When soil/land characteristics and climatic conditions are favourable, treatment of specifically designated land using particularly high OMWW rates (>5000 m^3 ha^{-1}) could be performed. Indeed, experiments carried out in lysimeters filled with two types of calcareous clay soils showed that a 2 m layer of soil removed almost completely the organic and inorganic components of OMWW, when applied at annual rates of 5000-10000 m^3 ha^{-1} (Cabrera *et al.* 1996). In field trials, the application of OMWW during three successive years at an annual rate of up to 6000 m^3 ha^{-1} resulted in increased concentrations of organic matter, N, soluble NO_3, extractable phosphorus and exchangeable potassium, and such positive effects on soil fertility were confirmed by other pertinent studies as well (Fiestas Ros de Urcinos 1986; Levi-Minzi *et al.* 1992; Tomati and Galli 1992). Moreover, land application of OMWW improved soil aggregate stability and reduced water evaporation losses (Mellouli *et al.* 1998). On the other hand, soil electrical conductivity and sodium adsorption ratio also increased, but below the levels representing salinization or sodification hazards for the soil; however, leaching of Na and NO_3 ions below the 1 m layer was detected (Cabrera *et al*. 1996).

Application of OMWW in soils was followed by an increase in microbial activity as expressed by either CO_2 evolution measurements, or by the colony forming units per gram of amended soil, or by higher levels of hydrolytic activity and soil respiration rates (Paredes *et al.* 1986; Moreno *et al.* 1987; Mekki *et al* 2006; Saadi *et al*. 2007). In both control and amended soils, common soil microorganisms dominated; coryneform bacteria were the most abundant in amended soil samples, while *Bacillus* spp. occurrence decreased. Once disposal of OMWW ceases, soil microbiota tends to restore the initial populations profile. On the other hand, the study of Mechri *et al.* (2007) revealed (through analysis of ester-linked fatty acid methyl esters, EL-FAME) decrease of Gram positive bacteria and increase of Gram negative bacteria and fungi 30 days after spreading of OMWW into a field of olive trees at increasing dosages of up to 150 m^3 ha^{-1}; in contrast, the fungal/bacterial ratio increased significantly with increasing quantities of OMWW. Furthermore, the relative abundances of monounsaturated fatty acids increased with OMWW addition, which is consistent with the presence of high substrate availability in OMWW-treated soils. A subsequent series of experiments (Mechri *et al*. 2008) demonstrated that the development of saprotrophic fungi was significantly higher in the OMWW amended soils, whereas the abundance of the soil fatty acid methyl ester (FAME) 16:1ω5 (used as a quantification index of arbuscular mycorrhizal (AM) fungi biomass), root FAME 16:1ω5 (used as index for the development of colonisation of the olive trees roots by AM fungi), photosynthetic rates, and the amount of the total root-soluble carbohydrate decreased significantly after agronomic application of OMWW. The findings suggest that the altering functioning of AM fungi should be considered as a potential factor mediating olive trees responses to agronomic application of OMWW when the OMWW dose applied is higher than 30 m^3 ha^{-1}.

In a recent study (Karpouzas *et al.* 2009) the effects of OMWW applications on the structure of soil fungal groups was investigated, by daily additions of the effluent to pepper plants growing in two types of soil (loamy sand and sandy loam) at two doses for a period of 3 months (total OMWW equivalents 900 and 1800 m^3 ha^{-1}). Nitrogen (N) fertilization alleviated N scarcity and considerably enhanced plant biomass production; however, when applied in combination with the high OMWW dosage, it induced plant stress. OMWW applications resulted in marked changes in the denaturing gradient gel electrophoresis patterns of soil basidiomycete communities, while concurrent N fertilization reduced these effects. In contrast, the ascomycete communities required N fertilization to respond to OMWW addition. Cloning libraries for the basidiomycete communities showed that *Cryptococcus* yeasts and *Ceratobasidium* spp. dominated in the samples treated with OMWW. In contrast, certain plant pathogenic basidiomycetes such as *Thanatephorus cucumeris* and *Athelia rolfsii* were suppressed. The observed changes may be reasonably explained by the capacity of OMWW to enrich soils in organic substrates, to induce N immobilization and to directly introduce OMW-derived basidiomycetous yeasts.

When the effect of untreated and fungal-treated OMWW spreading on the soil microbial communities was examined (Mekki *et al.* 2006), it was found that treated OMWW increased the total mesophilic number whereas the number of fungi and nitrifiers decreased; actinomycetes and spore-forming bacteria were neither sensitive to treated nor to untreated OMWW, and the total coliforms increased with higher doses of treated and untreated OMWW. A toxic effect of the untreated OMWW appeared from 100 m^3 ha^{-1}, while this toxicity was more pronounced with 200 m^3 ha^{-1}, when microflora of total mesophilic, yeasts and moulds, actinomycetes, and nitrifiers were seriously inhibited except for total coliforms and spore-forming bacteria.

In addition to OMWW, the effects of either crude or exhausted olive pomace application on soil and on the yield of durum wheat (*Triticum turgidum* L.) was examined by Brunetti *et al.* (2005). Soil amendment with olive pomaces produced a significant increase in total organic, total extractable, humified and non-humified C forms, and available K contents; increase in O and acidic functional group contents of humic-like acids, C/N ratio and aliphaticity, and decrease in C/H ratio and N and C contents were also observed. Worth mentioning was also the significant increase in wheat grain yield as regards kernel weight and kernel numbers per cultivated surface.

On the other hand, the direct application of TPOMW in agricultural soil has been also proposed on the basis of its high organic matter content (>90%), which could enrich soils poor in organic matter and increase crop yields (Abu-Zreig and Al-Widyan 2002; López-Piñeiro *et al.* 2002). In fact, a two-year greenhouse study using wheat (*Triticum aestivum* L.) and TPOMW or dry TPOMW as a soil conditioner/amendment demonstrated significant increases in organic carbon, aggregate stability, total N, available K, cation-exchange capacity (but with large decrease of available P) combined with much higher grain yields especially after the second year of application (López-Piñeiro *et al.* 2006, 2007, 2008b). Similar results were obtained from applications of TPOMW in irrigated olive grove soil (López-Piñeiro *et al.* 2007): significant increases in total organic carbon, water soluble organic carbon, humic and fulvic acids, and aggregate stability were observed in the treated plots, while the highest humification index was obtained when TPOMW was applied at the lowest rate (i.e. 30 tons ha^{-1}); the increase in aggregate stability correlated positively and significantly with the humic and fulvic acid, and the water soluble organic carbon contents. Further relevant evidence was provided by the five-year field study conducted by López-Piñeiro *et al.* (2008a), who evaluated the potential use of both TPOMW and dried TPOMW as soil amendments on a representative olive grove soil (Cutanic Luvisol); they found significant increases in organic carbon, total N, available P and K, and aggregate stability, while leaf analysis showed significant increases in N, P, and K concentrations in treated plots after the first two years of waste applications, while an increase in olive production (from 10-30%) was observed after five-years of successive amendments. It seems that positive effects from such recycling practices are particularly pronounced in poor, degraded or marginal, semi-arid soils of the Mediterranean basin.

Effect on seeds germinability

Cabrera *et al*. (1996) examined the effect of soil amended with OMWW on ryegrass (*Lolium multiflorum* Lam cv. 'Barwoltra'), and demonstrated that OMW did not have any effect either on seed germination or on seedling emergence. This outcome was attributed to a neutralizing action of Ca, which suggests the possibility of cultivating the land especially with salt-tolerant plants between different periods of treatment. Furthermore, Saadi *et al.* (2007) findings supported the potential of safe controlled OMWW spreading on lands that are not associated with sensitive aquifers by demonstrating that soil phytotoxicity (as expressed by germination and root elongation tests with cress (*Lepidium sativum*) seeds) was short-termed, and the soil was partly or completely recovered between successive OMWW applications; no further phytotoxicity was observed in treated soils as compared with control soil, three months after OMWW application. Such short-term phytotoxicity was not correlated with measured electrical conductivity and total polyphenols in the soil extracts. Colarieti *et al.* (2006) also studied the effect of agricultural soil as a natural catalyst to promote polyphenol oxidation and polymerization and in turn detoxify olive mill wastewaters. A 24-h treatment in soil slurry was sufficient to remove most of the phenolics found in OMWW. Their products were not toxic to the growth of a typical soil bacterium and reduced phytotoxicity in cress seeds germination tests. When OMWW were treated in an aerated, stirred reactor containing agricultural soil, OMWW monomeric phenols decreased by >90% within 24 h. This resulted in a corresponding reduction in phytotoxicity, as measured by germination tests with tomato (*Lycopersicon esculentum* Mill.) and cress seeds, and in microbial toxicity, associating thus the toxicity of OMWW to the presence of monomeric phenols (Greco *et al.* 2006). In addition, germinability of tomato was higher when the soil was irrigated with treated OMW rather than with untreated ones, although it was lower than the control (e.g., soil irrigated with distilled water); at longer incubation times, a complete recovery of the soil germination capability was achieved with treated, but not with untreated, OMWW.

On the other hand, although many experiments evidenced the beneficial effect of controlled OMWW applications on soil fertility, the polluting load of this waste and its inhibition effects on seed germination sometimes led to the avoidance of their use in agriculture (Perez *et al*. 1986). The mineral content, the acidity and the presence of phytotoxic compounds, mainly phenols, in olive mill by-products can induce negative effects on agriculture. Such effects are linked to the quantity of waste supply, soil characteristics and to the nature of the culture. Casa *et al.* (2003) and Asfi *et al.* (2006) using the durum wheat and spinach (*Spinacea oleracea* L.) germinability as a biotest, respectively, have indicated that phenols are the main phytotoxic compounds of raw OMWW. A similar behavior was observed by El Hadrami *et al.* (2004), who reported that crude and undiluted OMWW was lethal when applied to the studied crops, i.e., maize (*Zea mays* L.), chickpea (*Cicer arietinum* L.), tomato and wheat. Significant differences were observed among crops according to their germination ability when treated with OMWW or aqueous phenolic extracts of OMWW solutions. Indeed, maize showed a higher germination index compared to chickpea. By contrast, wheat and tomato germination occurred only when OMWW was diluted to 15 and 5% (of the initial concentration), respectively. Similar results were observed for germination with OMWW aqueous phenolic extracts, suggesting a predominately inhibition effect of seed germinations by OMWW phenolics. Phenolics could be considered as the main compounds implicated in the OMWW germinability suppression or reduction in these crops; thus confirming the toxicity of the phenolic fraction of OMWW as suggested by other studies (Capasso *et al.* 1992; Bonari *et al.* 1993; Aliotta *et al.* 2000). Mekki *et al.* (2007) assessed the phytotoxicity of OMWW phenolic compounds by the determination of the germination index (GI) of a sensitive plant belonging to the family Brassicaceae (*Brassica cernua*). The residual phenolic fraction extracted from the soil treated annually with 200 m^3 ha^{-1} yr^{-1} of OMWW proved to be phytotoxic to the plant *B. cernua*. In the control medium, the GI reached 100% after 24 h of incubation. In the medium containing the soil phenolic residual fraction, the seed GI reached 100% after 72 h of incubation, while in the medium containing diluted fresh OMWW, the GI showed a delay or even a total absence of *B. cernua* germination (Mekki *et al*. 2007). In the same study, it was demonstrated that OMWW spreading, even once per year, may modify the soil structure and composition which could in turn affect olive trees after long-term application. The phenolic fraction extracted from soil one year after OMWW application was not very phytotoxic, and it only increased the time required to obtain 100% germination of *B. cernua* seeds.

OMWW dilution with water is very often used prior to biological treatment to reduce toxicity to the microorganisms responsible for organic matter decomposition. Komilis *et al.* (2005) suggested that not only dilution but also aeration were the primary pretreatment techniques affecting OMWW phytotoxicity. Dilution with water at a high ratio reduced phytotoxicity compared to when dilution was kept at a low ratio. No seed germination (tomato and chicory (*Cichorium intybus* L.)) was observed when raw or settled OMWW, without any prior dilution, was applied to all types of seeds. Aeration of OMWW also resulted in reduced phytotoxicity and was the second most important main effect after dilution. Therefore, reduction of phenols and organic acids is achieved along with reduction of nutrient salts, particulate matter and organic content, minimizing the negative effects on plants and soil. In addition, particulate matter reduction, as achieved through dilution, can prevent clogging of the irrigation piping system. Aeration apparently reduced BOD_5 concentration through biological decomposition – induced by the inherent microbial population present in OMWW – by transforming several phytotoxic compounds to less phytotoxic metabolic organic by products and CO_2. Aeration also resulted in a pH increase but not in an electric conductivity increase. This is probably attributed to the loss of volatile acids and CO_2 to the atmosphere during strirring as well as to the biological decomposition and therefore loss of organic acids (Komilis *et al.* 2005). However, ozonation applied by Andreozzi *et al.* (2008) did not reduce significantly the phytotoxicity of tested OMWW measured through the germination index calculation of *Raphanus sativus* L., *Cucumis sativus* L. and *Lactuca sativa* L seeds. A marked reduction of OMWW inhibition, higher than 50%, was evidenced for 1:8 (v/v) dilution OMWW samples ozonated for 2 h. Better results were obtained on seed germination and root elongation of plantlets of the three species mentioned above, which germinated on OMWW-free solidified medium and were then transferred on a solidified culture medium containing O_3-treated OMWW diluted 1: 2 and 1: 4 (v/v).

Effect on plant growth and development

Published data on OMWW effects on the individual stages of plant growth and development are rather limited and contradictory. A brief illustration of the variety of the OMWW effects on plants is given in **Table 1**. It seems that they depend on plant species, plant developmental stage and mode of plant cultivation. A differentiation of OMWW phytotoxicity depending on the type of the growth substrate of the plants has been also observed; toxic symptoms are more serious in plants grown in hydroponic culture or in the sand than those grown in the cultivated soil (Kistner *et al.* 2004; Ouzounidou *et al.* 2008a). In fact, the higher the soil organic matter, the lower the OMWW toxicity. In order to evaluate the OMWW effects on growth, development and productivity, agronomic tests have been also performed either by greenhouse experiments or by direct land application.

In hydroponic culture of young tomato plants, Kistner *et al.* (2004) showed that addition of diluted raw OMWW led to an increase in internode length, simultaneous decrease in shoot and root length and a big loss of root biomass in comparison to canopy. The substantial impact of raw effluent on tomato roots in static aerated culture became obvious both

Table 1 Impact of olive mill wastes application on plants according to listed literature

Mode of application	Germinability	References
OMWW land application	No affection on germination and on seedling emergence of ryegrass	Cabrera *et al*. 1996
OMWW land application	Inhibition of *Brassica cernua* germination	Mekki *et al.* 2007
OMWW-compost land application	No effect or slight increase of germination index	Paredes *et al.* 2000
Greenhouse raw OMWW	Decreased germinability of wheat, spinach, maize, chickpea, tomato, chicory, *Raphanus sativus*, *Cucumis sativus*, *Lactuca sativa*	Casa *et al.* 2003; El Hadrami *et al.* 2004; Komilis *et al.* 2005; Asfi *et al.* 2006; Andreozzi *et al.* 2008
	Plant growth	
Greenhouse raw OMWW	Decreased biomass, leaf area enhanced flowering, pollen formation of spinach, peas, decreased shoot-root length, elongated internodes, root discoloration of tomato, maize, wheat, chickpea	El Hadrami *et al.* 2004; Asfi *et al.* 2006; Ouzounidou *et al.* 2008a, 2008b
Greenhouse hydroponics	Increased internode, decreased shoot, root length, biomass, root discoloration of tomato	Kistner *et al.* 2004
Greenhouse bio-detoxified OMWW	Increased shoot length, number of leaves, fresh weight shoot-root-stem of tomato	Kistner *et al.* 2004
TPOMW land application	Reduced growth of lettuce and soybean	Martín *et al.* 2002
OMWW land application	Reduced viability and biomass production in spearmint and peppermint	El Hassani *et al*. 2009a, 2009b
OMWW land application	Differential response of *Trifolium repens* growth on first and second crop	Vassilev *et al.* 1998
OMWW land application	Promotion of plant growth, crop yield, increased shoot-root length, total leaves, spike number of maize and wheat, enhancement of date palm growth, shoot weight, trunk height, crown circumference	Elliot and Stevenson 1977; Paredes *et al.* 1987; Tomati and Galli 1992; Brunetti *et al.* 2005; Aqeel and Hameed 2007; Belaqziz *et al.* 2008; Hanifi and Hadrami 2008
OMWW land application	No harmful effects on olive trees, pea and bean growth	Perez *et al*. 1986; Ben Rouina 1994; Ben Rouina *et al.* 1999
	Plant physiology	
Greenhouse raw OMWW	Nutrient deficiencies Ca, Fe, Mg, K of spinach, peas, tomato	Asfi *et al.* 2006; Ouzounidou *et al.* 2008a, 200b
Greenhouse raw OMWW	Loss of photosynthetic pigments of tomato	Ouzounidou *et al.* 2008a
Greenhouse raw OMWW	Reduced vitality index (Rfd) and ΦPSII	Asfi *et al*. 2006; Ouzounidou *et al.* 2008b
Greenhouse raw OMWW OMWW land application	Inhibition of assimilation rate, decreased WUE and photochemical quenching (qP), increased non photochemistry quenching (qN) of tomato and olive trees	Ouzounidou *et al.* 2008a; Mechri *et al.* 2008
OMWW land application	Highest phenolics compound, peroxidase activity of maize	El Hadrami *et al.* 2004; Belaqziz *et al.* 2008; Hanifi and El Hadrami 2008
Greenhouse raw OMWW	Qualitative and quantitative differences of phenolic compounds, peroxidases, chlorophylls on maize, wheat, chickpea	El Hadrami *et al.* 2004
OMWW land application	Deep reduction of essential oil yield and disappearance of essential oil constituents in spearmint	El Hassani *et al*. 2009a
OMWW land application	Reduction of chlorophylls and total proteins and accumulation of phenols in peppermint	El Hassani *et al*. 2009b
Greenhouse raw OMWW	Hormone production by fungi using as growth medium OMWW	Yurekli *et al.* 1999
	Fruit production-quality	
Greenhouse raw OMWW	Reduced fruit yield, lower ascorbic acid and sugars of tomato	Ouzounidou *et al.* 2008a
Greenhouse raw OMWW	Fewer, smaller peas fruits, decreased nutritional value	Ouzounidou *et al.* 2008b

with respect to root length and root discoloration when raw OMWW was supplemented at a rate of 3 and 5%, respectively (Kistner *et al.* 2004). In contrast, addition of bio-detoxified OMWW at a rate of 5% resulted in significantly higher shoot length and number of leaves as compared to the untreated control at maintained internode length. Also, fresh weight of leaves, stems and roots from plants exposed to bio-OMWW increased in comparison to the untreated control plants. Similar results were found by Asfi *et al.* (2006) and Ouzounidou *et al*. (2008b), who reported that the exposure to 1: 10 (v/v) OMWW for one-month period in the soil cultivated peas (*Pisum sativum* L.) and spinach plants, led to a significant decrease in plants shoot biomass and leaf area, while, an enhancement of flowering and pollen formation under the high OMWW concentration was detected in spinach plants. In addition, Ouzounidou *et al.* (2008a) have studied the responses of tomato exposed to olive mill wastewater (OMWW) with regard to cultivation in sand and soil. Soil experiments lasted for 3 months and sand experiments lasted for only 10 days due to intense lethal symptoms. In both cases roots were more sensitive to OMWW than the upper parts of tomato plant grown either in sand or in soil. The reason may be that roots face OMWW toxicity directly, while the toxicity to other parts is indirect. The significant reduction of the growth observed, suggests the susceptibility of tomato to OMWW components such as phenolics and fatty acids. These effects might be due to the lipophilicity of phenolic and fatty acids compounds which could alter the accessibility of nutrients inside the biological membranes as suggested by El Hadrami *et al*. (2004) and Kistner *et al.* (2004). Elongated internodes and concomitantly longer shoots result in a slower development of the crop, less available resources, a deductive delayed entry into the reproductive phase and thus lead to yield reductions as opposed to the control plants. The shorter root length in combination with severe root discoloration of tomato exposed to raw effluent might be an indication for even slower development phase due to impaired potential for nutrient and water uptake. This might be partly explained as a consequence to high content of phenolic compounds in raw waste.

The results obtained by Vassilev *et al.* (1998) clearly demonstrated the phytotoxic nature of untreated OMWW in *Trifolium repens* biomass, which was, however, partly neutralized in the case of mycorrhizal plants. However, when dry TPOMW was administered to soils, the growth of soybean (*Glycine max*) and lettuce (*Lactuca sativa*) plants colonized with the arbuscular fungi *Glomus mosseae* or *G. deserticola* was reduced; moreover, the application of the waste decreased in most cases the percentage of AM colonization of plants and showed that AM fungi increased the phytotoxicity of TPOMW in lettuce and soybean (Martín *et al*. 2002). Furthemore, it has been found that OMWW negatively affected the early growth stage of plants (Perez *et al.* 1980, 1986).

Fertigation of some crops from Mediterranean basin (maize, wheat, chickpea and tomato) by various concentrations of OMWW showed significant different influences as respect to controls both with regard to the germination and growth stages of the plants (El Hadrami *et al.* 2004). High reduction of shoot and root weight, of ramification and leaf extension rates, accompanied with significant reduction of yield, were observed for all the studied crops, especially wheat. The differences observed for some physical and chemical parameters between industrial and traditional OMWW samples could be partly ascribed to the different olive oil extraction procedures used in industrial and traditional mills (El Hadrami *et al.* 2004). For instance, the higher electrical conductivity measured for the traditional OMWW could be due to the high level of sodium and chloride elements. Furthermore, with the traditional method, the milling seems to be less efficient to liberate phenolics in the oil phase (lampante to ordinary quality). By contrast, industrial OMWW was less rich in polyphenols mainly due to the extraction procedure, which liberates more phenols in the oil phase (virgin to extra-virgin quality) than in the OMWW phase.

On the other hand, OMWW was found to promote plant growth in some field experiments. Thus, according to Tomati and Galli (1992) and Aqeel and Hameed (2007) land supplemented with waste, containing humified fraction or organic materials, which can easily be humified, has been considered as positive soil treatment in agriculture. The suitability of olive waste as soil amendment was also suggested by Paredes *et al.* (1987). It was concluded that olive mill by-products undergo processes that lead to production of humic acids. These substances have similar role in soil as other naturally humic fraction usually present in soils. Several experiments confirmed the degradation of organic load in soil within a relatively short time and with a consequent enrichment of soil in nitrogen, phosphorus and potassium (Estaun *et al.* 1985; Aqeel and Hameed 2007). Crop yield improvement, after the administration of different amounts of olive mill by-products to cultures has been recorded by many authors (Elliot and Stevenson 1977; Tomati and Galli 1992). No harmful effects have been reported on trees and some seed legume crops such as pea and bean (*Phaseolus vulgare* L.) (Perez *et al*. 1986). Many cultures may benefit from olive mill by-products when distributed in adequate amounts at a right time. The growth of *Trifolium* during the second crop was higher than that of the control. Similarly, the introduction of untreated OMWW in soil resulted in the lowest value of plant P uptake during the first crop and the greatest further enhancement during the second crop. The enrichment of untreated OMWW with rock phosphate resulted in a slight plant growth increase, which was more pronounced in the mycorrhizal treatment (Vassilev *et al.* 1998). Maize plants grown on soil amended with 10 and 20 m^3 ha^{-1} showed an enhancement of their shoot height and roots length as compared to control (Belaqziz *et al.* 2008). Total leaves and spike numbers of treated plant did not show any significant difference as compared to control. However, plants treated with 20 m^3 ha^{-1} of OMWW presented higher increase for spike, and stems fresh and dry weight. Maize yield was higher in fertilized and amended soils as compared to the control soil. Several chemical and biochemical properties of the investigated soil changed in response to the application of OMWW (Paredes *et al.* 1987; Sierra *et al.* 2001; El Hadrami *et al.* 2004). The increase of nutrient contents, C, N, P and K at the field treated with fertilizer and amended with the highest rate of OMWW (20 and 50 m^3 ha^{-1}), led to a beneficial effect on the soil fertility (Belaqziz *et al.* 2008). Soils treated with 10 and 20 m^3 ha^{-1} of OMWW showed relevant growth stimulation notably with regard to maize leaf area, shoot height, root length, spike, stems, fresh and dry weight. This could be due to nutrient availability, notably nitrogen, organic matter, P and K (Nevens and Reheul 2003; Gavalda *et al.* 2005). This amelioration in plant growth can also be explained by stimulation of soil microbial activity (Tomati *et al.* 1996) and by the amelioration of the physical properties of the soil (Fischler *et al.* 1999). Moreover, the effects of the addition of either crude or exhausted olive pomace at two rates 10 and 20 t ha^{-1} on soil and soil humic acid properties and durum wheat yield were investigated in field by Brunetti *et al.* (2005). Hence, soil application determined a significant increase of total and grain dry matter yields spike and kernel numbers per square meter and kernel weight. These findings suggest that the increase of wheat grain yield in soils amended with olive pomaces may be related primarily to the increased content of both humified and nonhumified soil organic matter fractions. Most probably, the enhanced amount of soil organic matter relieves wheat of drought stress from anthesis to maturity by promoting a good soil structure, thereby reducing water loss by evaporation.

The phytotoxic effect of the OMWW phenolic compounds on vegetative production of olive trees was evaluated by Ben Rouina (1994) and Ben Rouina *et al*. (1999). Better olive production yield, but no apparent effects on the physiology and growth of olive trees after 5 successive years of OMWW application, was observed. However, olive trees can not be used for monitoring phytotoxicity since they are very resistant to stresses such as drought, salinity, etc. In Mekki *et al.* (2007) study, the model sensitive plant *B. cernua* was used and demonstrated that spreading OMWW, even once per year, may modify the soil structure and composition which could affect olive trees after long-term application. Hanifi and El Hadrami (2008) evaluated the valorization of soil and OMWW-intrinsic natural degradation potencies as an alternative for safe agronomic management of raw OMWW. In a field experiment, results of three years application of crude OMWW at relatively high dose (150 m^3 ha^{-1} $year^{-1}$) revealed no important perturbations in respect to edaphic parameters notably salinity, pH and phenolic content, thus asserting OMWW biodegradetion in the studied calcareous soil. Date palm (*Phoenix dactylifera* L.) plant growth was efficiently raised by OMWW inputs. Significant amelioration was obtained notably in term of shoots weight, trunk height and crown circumference. Along with the correct choice of convenient soils (notably calcareous ones and tolerant crops such as palm), the storage of OMWW could constitute an efficient approach for avoiding problems attributed to the uncontrolled disposal of these effluents and for efficiently recovering their fertilizing value (Hanifi and El Hadrami 2008).

Effects on plant physiology and metabolism

According to Asfi *et al*. (2006) and Ouzounidou *et al.* (2008b), spinach and peas treated with raw and diluted OMWW revealed nutrient deficiency symptoms, since the uptake and translocation of Ca, Fe, Mg and K were impeded. These findings are in accordance with those of Ouzounidou *et al*. (2008a), who reported that tomato plants grown on sterilized sand, revealed higher loss of the photosynthetic pigments (chlorophylls and carotenoids) than plants grown on cultivated soil; whereas, a remarkable chlorosis of the old leaves was also observed on OMWW treated plants grown on sand. The significant loss of chlorophyll content in the OMWW-treated plants, may be attributed to the interference of the toxic substances present in OMWW in the formation of chlorophyll. Moreover, the decrease in photosynthesis can be related to the significant decrease observed in leaf Fe concentration (Morales *et al.* 1998).

The vitality index (Rfd) of the peas and spinach leaves under OMWW application was significantly suppressed and the photosynthetic pigments were decreased, while the proportion of the light absorbed by the chlorophylls associated with PSII that is used in photochemistry (Φ_{PSII}) was highly reduced, showing functional disturbances in the chloroplasts (Asfi *et al*. 2006; Ouzounidou *et al*. 2008b). Photosynthesis was seriously inhibited by OMWW. The strongly limited photosynthetic rate (P_N) might be due to reduced stomatal aperture. The significantly suppressed water use efficiency (WUE) indicates the induction of water stress by OMWW application. It is worth noting that OMWW application on sand and on soil, displayed different effects on the photosynthesis physiology (Ouzounidou *et al*. 2008a). OMW supply to soil, inhibited the assimilation rate and the WUE, rather than the transpiration and stomata closure. On the contrary, OMWW supply to sand caused a complete closure of stomata with concomitant negative effects on assimilation and transpiration rates. This could be a result of leaf water stress a fact that is confirmed by the high proline concentration in leaves of tomato (Ouzounidou *et al.* 2008a). The insufficiently utilized assimilatory force by Calvin cycle slowed down due to raw OMWW application, may enhance proton gradient formed in chloroplasts and increase non-photochemical dissipation of light energy and decrease photochemical efficiency. The slight decrease in F_v/F_m and the big decrease in photochemical quenching (q_P), indicate that OMWW diminished reoxidation of Q_A^- and started to inactivate the RC of PSII. In addition, the severe increase in non-photochemical quenching (q_N), might be due to the dissociation of light-harvesting complex from PSII core (Ouzounidou and Ilias 2005).

Olive trees amended with 100 and 150 $m^3 ha^{-1}$ resulted in significantly lower photosynthetic rates (Mechri *et al.* 2008) which were measured in the field by a LI-6400 gas exchange system from 10:00 to 13:00 AM. In comparison with the control olive trees, application of OMWW demonstrated a significant increase in P concentration in the roots but not in the leaf. OMWW is known to increase soil organic matter and the concentrations of essential inorganic elements for plant growth resulting in enhanced soil fertility (Paredes *et al*. 1999). Carbohydrates are one of the principal elements of plant growth. Low concentrations of carbohydrates result in reduced plant growth and also in reduced arbuscular mycorrhizal fungi root colonisation (Smith and Read 1997). It was observed that the amount of root-soluble carbohydrate was decreased significantly after agronomic application of OMWW. During the same time, the abundance of saprotrophic fungi was markedly higher in the OMWW amended soils than that in the control soil. The significant difference in photosynthesis between irrigated and non-irrigated olive trees observed in their study, indicates that agronomic application of OMWW can cause changes in some olive trees physiological parameters. To our knowledge the decreased root colonisation observed after agronomic application of OMWW might have decreased the translocation of photosynthates to roots in OMWW amended olive trees which, in turn, could have decline the photosynthetic rate (Douds *et al*. (1988). Wright *et al.* (1998) demonstrated that arbuscular mycorrhizal fungal colonisation stimulated the rate of photosynthesis sufficiently to compensate for the carbon requirement of the fungus.

Yurekli *et al.* (1999) used sterilized and diluted OMWW as growth media for the production of plant growth hormones. Gibberellic acid (GA_3), absisic acid (ABA), indole-3-acetic acid (IAA) and cytokinin (zeatin) were determined in the culture media of the fungi examined. Both organisms produced high levels of all three hormones in the presence of the wastewaters. It is known that higher plants and microorganisms produce plant growth regulators and that wastewaters can be used as growth media for fungi (Yesilada *et al.* 1998), which can diminish the pollutant effects of wastewaters.

Belaqziz *et al.* (2008) reported high contents of phenolic compounds and peroxidase activity in maize plants cultivated in field treated with 20 and 50 $m^3 ha^{-1}$ of OMWW. Positive correlations between peroxidase activity and phenolic contents and peroxidase activity with protein contents were also observed. The neoaccumulation of phenolic compounds is common feature in many species in response to several biotic or abiotic stresses, and phenolic compounds are believed to have antioxidant properties (Cummins *et al.* 2006; Wahid and Ghazanfar 2006). Accumulation of new phenolic compounds, particularly some flavonoids were revealed when plants were treated by OMWW (El Hadrami *et al.* 2004; Hanifi and El Hadrami 2008). Peroxidases are related to OMWW cellular detoxification presumably by catalyzing the phenolics oxidation at the expanse of hydrogen peroxide (Wang and Ballington 2007). Peroxidases play a central role in the detoxification of plant. This biochemical parameter is also involved in lignin biosynthesis as a physical barrier against several stresses (Adam *et al.* 1995; Hegedus *et al*. 2001).

According to El Hadrami *et al.* (2004) significant qualitative and quantitative differences of some stress indicators such as phenolic compounds, peroxidases, chlorophyll contents were also detected between OMWW treated maize, wheat and chickpea plants and controls. Hence, a reduction of chlorophyll contents accompanied by 3 to 5 times stimulation of peroxidases activities and 1.25 to 7 times of phenolic compounds accumulation was observed for OMWW treated plants in comparison to controls. Studies of some stress indicators have shown, in general, a stimulation of secondary metabolites and the peroxidases activity with a deterioration of chlorophyll. Taking into account the role of peroxidases in the scavenging of active oxygen species and free radicals, and in the cell walls cross-linking (Mocquot *et al.* 1996; Prasad *et al.* 1999), it may be suggested that the phytotoxic effects of OMWW on fertirrigated crops results in an oxidative stress as demonstrated in other systems (Gallego *et al*. 1996; Clijsters *et al.* 1999).

Effect on fruit productivity-quality

Few studies have been carried out on changes of fruit quality under OMWW application. Ouzounidou *et al.* (2008b) investigated the effects of raw OMWW supplied in two dilutions (1:20 and 1:10 v/v) on one month old pea plants grown in soil under greenhouse conditions. Despite the OMWW toxicity on peas, fruit production occured. Fruits of control plants were fresh, well formed with bright green color, while those of OMWW were fewer, smaller in size and discolored. Fruits of plants grown with OMWW had decreased nutritional value e.g. decreased glucose and fructose concentration and increased glucose/fructose ratio indicating that fruits produced under OMWW were immature. In addition, a significant loss of ascorbic acid and an enhanced phenol concentration was observed. In parallel, in the experiments conducted by Ouzounidou *et al.* (2008a) on sand and soil growth media using tomato as test plant, it was found that the long-term OMWW application caused reduction on fruit yield measured either as number of fruit per pot or fruit size as a consequence of flower abortion. Generally, plants treated with high OMWW concentration, produced fewer but bigger tomatoes, as compared to plants treated with lower OMWW concentration. Not only the yield but also the quality characteristics of tomatoes have been changed under OMWW application. The reduced sugars and the soluble solids, which have been correlated with sweetness and the fruitiness, were significantly suppressed under OMWW application. Several studies report the allelopathic effects of phenolic compounds of OMWW on higher plants and it has been suggested that such effects arise from alterations of water uptake, or of the metabolism of auxins and/or other phytohormones (Capasso *et al.* 1992; Bonari *et al.* 1993; Casa *et al*. 2003).

MICROBIOLOGICALLY-TREATED OLIVE MILL WASTE AND THEIR EFFECTS ON PLANTS

Microbial olive mill waste detoxification, bioremediation and/or biodegradation was attempted in the past through various approaches, i.e. through the use of indigenous microorganisms, or by inoculating arbitrarily chosen species/strains (which were however known for their ability to bioconvert waste/compounds of similar nature), through the use of suitable enzymes, by anaerobic digestion, or through co-composting with other agricultural waste and by-products, or by exploiting commercial consortia of microorganisms, etc. The detoxified olive mill waste presented an added value since they could be used for enhancing soil fertility and plant growth (e.g. as soil amendments, plant fertilizers and/or pathogens suppressants), for biomass (single cell protein, mycelium, edible mushrooms) production, for the generation of fungal extracellular metabolites, etc. Especially as regards the effect of the microbiologically treated olive mill waste on phytotoxicity and plant growth, particular emphasis has been recently placed on pertinent applications aiming at valorizing olive mill waste for the production of soil amendments, plant fertilizers and/or pathogens suppressants; an overview of such studies is provided below (and the results of the effect of microbiologically-treated olive mill waste on plants are summarized in **Table 2**).

Microbial treatment of olive mill waste by indigenous microorganisms

Several research groups have drawn their attention on the exploitation of microorganisms isolated directly from OMWW or from substrates receiving OMWW for long periods. Characteristic is the case of a *Azotobacter vinelandii* strain originating from soil treated with OMWW, which was subsequently used as inoculum for the aerobic biodegradation of OMWW in a biowheel-type reactor, since it was found to reduce significantly OMWW phytotoxicity by reducing COD (70% after three days) and phenolic compounds (up to 100% within seven days) and enhance soil fertility (Ehaliotis *et al.* 1999; Piperidou *et al.* 2000). Its use was based on experimental evidence that OMWW lead to enrichment of soils in nitrogen-fixing bacteria (Paredes *et al.* 1987; Balis 1994), whereas an increase of *ca*. 600-fold was recorded in OMWW amended soils from where several diazotrophs were isolated (Balis 1994). Apart from the nitrogen gain, free-living nitrogen bacteria benefit soils by producing plant growth regulators, and by enriching them in polysaccharides which further improve stability of soil aggregates (Balis 1994; Balis *et al.* 1996). Such beneficial effects were also confirmed by Fiorelli *et al.* (1996), who evidenced active nitrogen fixation by the same *A. vinelandii* strain grown in OMWW accompanied by a two- to three-fold increase in IAA biosynthesis when tryptophan was added in the growth medium. Such bacterial nitrogen-fixing ability by growing on OMWW and produce hormone-like compounds was previously made evident for *Arthrobacter* strains as well (Tomati *et al.* 1990). The capacity for exopolysaccharides (EPS) synthesis was also notable among *Paenibacillus jamilae* strains initially isolated from a compost of corn treated with OMWW; the strain with the highest EPS yield presented a more than 50% reduction in OMWW toxicity (Aguilera *et al.* 2001, 2008).

In another pertinent study (Ntougias *et al.* 2006a), 119 strains of indigenous microorganisms were isolated from OMWW and TPOMW; 28 of them (one bacterium, eight yeasts and nineteen filamentous fungi) decreased phytotoxicity up to 17 times as compared to the control, while some of the strains also reduced phenolic content (up to 43%) and/or colour (up to 85%). For all isolates, total content in polyphenolics and germination index were not correlated; ten of the screened strains failed to reduce polyphenolics, although they decreased phytotoxicity by 66.3-126.9%. OMWW indigenous bacterial strains of the genera *Pseudomonas*, *Sphingomonas* and *Ralstonia* were characterized by Di Gioia *et al.* (2001, 2002), and were found capable of aerobically degrading hydroxylated and methoxylated monocyclic aromatic compounds present in this waste. Further interest in such indigenous bacterial isolates led to the description of new genera and species, which were subsequently studied for their efficacy in biodegradation and detoxification of olive mill waste (Ntougias and Russell 2001; Ntougias *et al.* 2006b, 2007a, 2007b).

Particularly pronounced were the effects presented by a *Phanerochaete chrysosporium* strain isolated from Moroccan OMWW, which reduced phenolics content, COD and colour by at least 60% within nine days of growth under optimized conditions (Kissi *et al*. 2001). From Morocco again, yeasts isolated from olive oil mills demonstrated significant reduction of total phenols concentration and of COD (44 and 63%, respectively), whereas an indigenous *Candida holstii* strain presented high germination rate (80%) of barley seeds in undiluted OMWW (Ben Sassi *et al.* 2008). On the other hand, *Penicillium* spp. isolated from OMWW disposal ponds demonstrated also reduction in the effluents COD value and phenolics content (*ca*. 45%), which was accompanied by a decrease in its antimicrobial effects (Robles *et al.* 2000). Similar results have been previously obtained by several species of the genus *Aspergillus* isolated from this waste and found to be particularly ef-

Table 2 Direct and indirect effects of microbiologically-treated (excl. composts) olive mill wastes on plants.

Waste	Microorganism	Outcome/Effect	References
OMWW	*Azotobacter vinelandii*[a]	active N fixation, IAA production	Fiorelli *et al.* 1996
OMWW	*Aspergillus niger*	beneficial effect in the growth of Trifolium plants	Vassilev *et al.* 1998
OMWW	*Azotobacter vinelandii*[a]	active N fixation, polysaccharide production	Ehaliotis *et al.* 1999
OMWW	*Lentinula edodes*[b]	increase in germination of durum wheat seeds	Casa *et al.* 2003
OMWW	Consortium (anaerobic digestion)	decreased phytotoxicity on cress seeds	Filidei *et al.* 2003
TPOMW	*Phanerochaete flavido-alba*	increase (by >40%) in the shoot length of tomato seeds	Linares *et al.* 2003
OMWW	*Aspergillus niger*	increase in seed biomass, spike number, and kernel weight of durum wheat plants	Cereti *et al.* 2004
OMWW	*Lentinula edodes*	increase in germination of durum wheat seeds	D'Annibale *et al.* 2004b
TPOMW[c]	saprotrophic fungi	decrease of phytotoxicity in tomato and soybean plants	Sampedro *et al.* 2004a
TPOMW[c]	*Fusarium lateritium*	elimination of phytotoxicity and increased shoot dry weight of tomato plants	Sampedro *et al.* 2005
TPOMW	*Coriolopsis rigida*	increase in tomato shoot dry weight	Aranda *et al.* 2006
OMWW	*Panus tigrinus* (± laccase)	elimination of phytotoxicity and mean germination times of maize seeds	Quaratino *et al.* 2007
TPOMW[c]	*Fusarium oxysporum*	decrease of phytotoxicity in seedlings of tomato, soybean and alfalfa plants	Sampedro *et al* 2007a
TPOMW[c]	*Poria subvermispora* *Coriolopsis rigida*	decreased (by 60.3% and 57.4% respectively), inhibition of tomato plant growth	Sampedro *et al* 2007b
OMWW	*Candida holstii*[a]	increase in germination (80%) of barley seeds	Ben Sassi *et al*. 2008
TPOMW[c]	*Paecilomyces farinosus*	decrease of phytotoxicity, and increase of shoots and roots biomass in alfalfa plants	Sampedro *et al* 2009

[a] Microorganisms isolated directly from olive mill wastes or from substrates receiving OMWW.
[b] Experiments were performed with laccase deriving from *L. edodes* (without the presence of the fungus).
[c] Dried TPOMW (dry olive-mill residue, DOR) was used.

ficient at its degradation: strains of *A. niger* yielded large amounts of biomass with the simultaneous elimination of COD and improved the filtration kinetics of the effluent (Raimbault and Mazard 1980; Hamdi *et al.* 1991; Hamdi and Ellouz 1992), while *A. flavus*, *A. versicolor* and *A. terreus* were reported to grow well and decrease the OMWW content in polyphenols (Saiz-Jiménez and Gómez-Alarcón 1986; Martínez-Nieto *et al*. 1992). Furthermore, *Candida tropicalis* strain isolated from OMW decreased COD values of OMW in batch cultures by 62.8%, whereas polyphenols removal reached 51.7% and it was accompanied by significant decolorization of the effluent (Fadil *et al.* 2003). Similarly, it was determined that yeasts (*Candida boidinii* and *Saccharomyces* sp.) isolated from TPOMW were able to reduce its COD and phenolic content (Giannoutsou *et al.* 2004). In addition, significant decrease in the polyphenolic content of the TPOMW by its indigenous microbiota (and especially after stimulation of its fungal fraction) was also assessed through the use of laboratory-scale bioreactors (Morillo *et al*. 2008).

Detoxification and biodegradation of OMWW through the use of fungi

Vassilev *et al*. (1997) used a passively immobilized, acid-producing strain of *A. niger* for the detoxification of OMWW supplemented with N and Mg, and achieved 59% reduction in the initial phenolic content of the waste with a simultaneous decrease in its pH. When five types of OMWW with or without the addition of rock-phosphate (RP) (receiving fungal treatment or not), were tested in a soil-*Trifolium* system for their fertilizing ability, the beneficial effect of fungal treated OMWW was more evident during the first crop cycle. The best plant growth response and phosphorus uptake were found in mycorrhizal plants grown in soil amended with fungal treated OMWW+RP. The growth of *A. niger* on OMWW supplemented with RP was also studied by Cereti *et al*. (2004) in an air-lift bioreactor in batch and repeated-batch processes. The fungus grew well and reduced the waste COD by 35 and 64% in the batch and repeated-batch (fourth batch) processes, respectively; on the other hand reduction of total phenols was minimal. In a subsequent greenhouse experiment, it was found that fertilizing effects of OMWW application on a soil-wheat (*Triticum durum* Desf.) model system were most pronounced when OMWW was previously treated by the repeated-batch process; plants which received such effluents showed an increase in seed biomass, spike number, and kernel weight, which were accompanied by the relatively highest harvest index. Several other microorganisms (mainly fungi) were selected for the biodegradation and detoxification of olive mill waste. For example, *Candida tropicalis* strain YMEC14 demonstrated 69.7, 69.2, and 55.3% reduction of COD, monophenols and polyphenols, respectively, within a 24 h fermentation cycle of OMWW supplemented with hexadecane by immobilizing yeast cells in calcium alginate beads (Ettayebi *et al.* 2003). Similarly, strains of *Yarrowia lipolytica* grown in OMWW achieved reduction of COD and polyphenols, which was accompanied with production of citric acid and lipase (Scioli and Vollaro 1997; Lanciotti *et al.* 2005). In addition, two strains of *Geotrichum* sp. and *Aspergillus* sp. decreased COD values by 55.0 and 52.5%, and polyphenols by 46.6 and 44.3%, respectively, and achieved a high decolorization of OMWW (Fadil *et al.* 2003).

White-rot fungi, on the other hand, constitute a rather physiological group comprising organisms capable of extensively degrading lignin by excreting one or more of three potent non-specific extracellular enzymes; many of the compounds found in olive mill waste are identical, or very similar to the enzymatic degradation products of lignin by white-rot fungal ligninases, peroxidases and lacasses/phenoloxidases. These microorganisms have been preferably used either for the pre-treatment of olive mill waste or directly for the bioremediation of its notorious environmental effects achieving depolymerization of high molecular mass aromatics combined with the mineralization of a wide range of monoaromatics leading to COD reduction and decolourization through the contribution of their enzymatic systems (Sayadi and Ellouz 1993; Martirani *et al*. 1996; Sayadi *et al.* 2000). Hence, one of the most commonly used species *Ph. chrysosporium* demonstrated a high OMW decolourization efficiency (by 73%), phenolics reduction (by 90%), and COD decrease (by 45%) (Dias *et al.* 2004). When fungal treatment of OMWW was used as a pre-treatment stage prior to anaerobic digestion, COD of the effluent was reduced by 50%; however, subsequent biomethanation was inhibited by the remaining high biotoxicity (Gharsallah *et al.* 1999). In contrast, OMWW pre-treatment with *Ph. chrysosporium* followed by anerobic digestion and ultrafiltration resulted in complete detoxification of the effluent (Dhouib *et al.* 2005). At a further step, when *Ph. chrysosporium* or *Trametes versicolor* were tested for their OMWW biodegradation efficacy in the presence of activated sludge (and the resulting competition with other microorganisms), they showed high removal of organic matter, reduced COD/BOD_5 ratio and toxicity; the subsequent anaerobic digestion of OMWW pretreated with activated sludge and white-rot fungi showed higher biomethanization yields than that pre-treated with activated sludge only (Dhouib *et al.* 2006).

Similarly effective was the use of *Pleurotus ostreatus*, since OMWW pre-treated with this fungus was more amenable to a subsequent anaerobic digestion (Fountoulakis *et al.* 2002). This result is a consequence of the significant OMWW polyphenolics reduction achieved primarily by the *P. ostreatus* laccases (Tsioulpas *et al.* 2002; Aggelis *et al.* 2003); its capacity to remove phenols from the OMWW-based culture medium reached 78% of the initial concentration for sterilized (50% diluted) OMWW, whereas the respective values could exceed 60% for the thermally processed (pasteurized) OMWW even without dilution (Fountoulakis *et al*. 2002). In other cases, polyphenols abatement was shown to be controlled by the availability of nutrients and reached levels of 95% of the initial concentration, while bioconversion of non-sterilized OMWW did not result into appreciable decolourization of the liquid medium (Olivieri *et al*. 2006). Noteworthy is the use of this species for producing edible mushrooms when raw OMWW is applied on a suitable mycelium support, e.g. wheat straw, olive press cake, etc. (Sanjust *et al*. 1991; Zervakis *et al.* 1996; Kalmis *et al*. 2008).

Furthermore, several other selected white-rot fungi proved to be efficient at the detoxification of OMWW. Hence, an adapted *T. versicolor* strain was able to remove 78% of total phenolics in shake flask experiments and 39% in static culture using undiluted OMWW medium, while in continuously stirred tank reactor (CSTR) conditions, 70% of total phenolics removal was achieved, and subsequent analysis revealed that no simple phenolics were present in the medium after the 8^{th} day of cultivation (Ergul *et al.* 2009). Furthermore, when *Panus tigrinus* degradative capability was investigated in shaken cultures using two different OMWWs, it was shown that only when the effluent was diluted by 50% (v/v to water, initial COD: 43,000 mg l^{-1}) the fungus could readily and efficiently degrade the phenolics and decolorize OMWW, while in undiluted OMWW the fungus demonstrated a significant delay in removal of color, organic load and phenolics, which was associated with delayed onset of laccase and Mn-dependent peroxidase (D'Annibale *et al.* 2004a). When *Phanerochaete flavido-alba* was tested, it was found that nitrogen-limited cultures containing 40 μmg l^{-1} Mn(II) were the most efficient not only at decolorizing OMWW but to produce a 90% decrease in the OMWW phenolic content as well (Ben Hamman *et al*. 1999). The same fungus was also involved in the degradation of aromatic compounds (51.7%) and decolourization (70.3%) of OMWW in a bioreactor experiment (Blanquez *et al*. 2002), whereas when it was incubated for 48 h achieved 60% OMWW decolourization combined with detoxification and phenolics degradation (Ruiz *et al.*

2002). In another study (Assas *et al.* 2002), the use of aerated batch bioreactor with *Geotrichum candidum* led to significant colour removal (75%) and COD reduction (50%) in fresh OMWW. Optimisation of different factors controlling *G. candidum* cultures in OMWW showed that dilution, carbon and ammonium concentrations significantly affected decolourisation and activities of ligninolytic peroxidases (LiP and MnP), while batch and continuous OMWW treatments in settler or bubble column bioreactors showed high COD and colour removal efficiencies of 60 and 50%, respectively (Asses *et al.* 2009). These data confirm the outcome of a previous work (García García *et al.* 2000) reporting that *G. candidum* was able to grow and decrease the OMWW organic load but did not affect the effluent total phenol concentration; in contrast, a *Ph. chrysosporium* strain demonstrated a particularly high (exceeding 85% within 3 days of incubation) decrease in OMWW phenolics content accompanied by an up to 75% COD reduction. In another study, *Pycnoporus coccineus*, *Pleurotus sajor caju*, *Coriolopsis polyzona* and *Lentinus tigrinus* proved to be very active in decolourisation and COD removal of OMWW at 50 and 75 g l^{-1} COD, while at 100 g l^{-1} COD only the first two fungi were effective; in particular, *P. sajor caju* and *C. polyzona* showed levels of decolourisation as high as 75% when COD of the effluent was 50 g l^{-1}, and both mono and polyaromatics were considerably reduced after 20 days incubation with *C. polyzona* (Jaouani *et al.* 2003). Furthermore, germinability experiments on durum wheat showed that OMWW phytotoxicity was significantly reduced through the use of *Lentinus edodes* cultures by 34 ± 5% and 57 ± 6% in undiluted and two-fold diluted effluent, whereas it was almost completely suppressed in the respective control treatments (D'Annibale *et al.* 2004b).

On the other hand, when dry TPOMWW (or dry olive mil residue, DOR) was treated with selected lignin-degrading fungi such as *Phlebia radiata*, *Coriolopsis rigida*, *Ph. chrysosporium*, *Pycnoporus cinnabarinus*, *Poria subvermispora* and *Pleurotus pulmonarius*, its chemical composition and phytotoxicity was not seriously affected after 2 weeks of incubation; in contrast, both a significant depletion of phenolic compounds and a partial removal of phytoxicity towards tomato plants were generally obtained after 20 weeks, while the most effective fungus in degrading lignin, total phenols and in removing phytotoxicity was *C. rigida* (Sampedro *et al.* 2007b). Similar results on dry TPOMW, but this time by using the non-pathogenic fungus *Fusarium lateritium*, were obtained after 20 weeks of incubation with a significant (80%) reduction in ethylacetate extractable phenols (through the production of several lignin-modifying oxidases, including laccase, Mn-peroxidase and Mn-inhibited peroxidase) combined with complete removal of the waste phytotoxicity and a higher shoot dry weight of tomato plants than that obtained in the absence of the treated waste (Sampedro *et al.* 2005). In a pertinent previous work (Sampedro *et al.* 2004b), it was found that most of the saprotrophic fungi tested first eliminated *o*-diphenols and then non-*o*-diphenols from dry TPOMW (with the exception of *Ph. chrysosporium* and *Paecylomyces farinosus*, which first removed hydroxytyrosol and tyrosol and then eliminated all monomeric phenols; as concerns waste decolorization, *C. rigida* showed the highest capacity, followed by *Phlebia radiata*, *Py. cinnabarinus*, and *Ph. chrysosporium*. In addition, incubation of dry TPOMW for 20 weeks by these fungi could significantly increase the biomass production of tomato plants (Sampedro *et al.* 2004a). Similar results on dry TPOMW, but this time by using the non-pathogenic strains of *Fusarium lateritium* and *F. oxysporum*, were obtained after 20 weeks incubation with a significant reductions of ethylacetate extractable phenols (through the production of several lignin-modifying oxidases) combined with complete removal of the waste phytotoxicity with a higher shoot dry weight of tomato plants presented by the former fungus, and of tomato, soybean and alfalfa seedlings by the latter (Sampedro *et al.* 2005, 2007a).

TPOMW and pine chips mixtures have been treated by *Ph. flavido-alba* in solid-state cultures, and their resulting aqueous extracts contained about 70% less phenolic compounds; hence, inhibition of tomato seed germination was eliminated to a significant degree (Linares *et al.* 2003). In addition, the use of two other white-rot fungi, *Py. cinnabarinus* and *C. rigida*, reduced the phytotoxicity of water-soluble substances from dry TPOMW on tomato plants through the production of laccase; a close relationship was found between the amount of laccase produced versus the decrease in phenol content and phytotoxicity of TPOMW, and the increase in dry weight of tomato plants (Aranda *et al.* 2006).

In other cases, where fungal enzymes (laccases) were directly applied for OMWW treatment, results were particularly promising: germinability and mean germination times of maize seeds in soil spread with laccase-treated OMWW (up to volumes of 120 m^3 ha^{-1}) did not significantly differ from those observed in soil irrigated with tap water (control), although the highest phenol reduction (*ca.* 81%) was obtained by the sequential use of laccase and the white-rot fungus *Panus tigrinus* (Quaratino *et al.* 2007); in addition, OMWW treatment with laccase resulted in a 65 and 86% reduction in total phenols and ortho-diphenols, respectively (due their polymerization as revealed by size-exclusion chromatography), and germinability of durum wheat seeds was increased by 57% at a 1: 8 dilution and by 94% at a 1: 2 dilution as compared to the same dilutions using untreated OMWW (Casa *et al.* 2003). Similarly efficient was also the use of laccase deriving from *Pycnoporus coccineus* and from *L. edodes* to decrease the phenolic content, COD and colour of OMWW (D'Annibale *et al.* 1998; Jaouani *et al.* 2005). Earlier, Greco *et al.* (1999) study demonstrated that over 90% of low-molecular phenolics were removed through the use of polyphenol-oxidase and laccase.

Other microbial processes for the treatment of olive mill waste

Aerobic bacterial consortia (commercial formulations or not) from different sources have also been used for the treatment of OMWW (Zouari and Ellouz 1996; Benitez *et al.* 1997), demonstrating efficient removal of the organic load (by 85%) and phenols (by 67%) accompanied with reduced toxicity for rotifers and for crustaceans (by 43 and 83%, respectively) (Isidori *et al.* 2004). Similar results were found by a combined approach (chemical oxidation and commercial bacterial consortium), where biological treatment of OMWW led to significant reduction of COD (up to 62.8%) and phenols (60.2%) in two activated sludge pilot plants (Fiorentino *et al.* 2004).

Very scarce is the information obtained from the treatment of OMWW through anaerobic digestion and the evaluation of the end product as regards its effect on plants. Filidei *et al.* (2003) demonstrated that anaerobic digestion of OMWW first reduced the organic load by 78-89% and the content of polyphenols by 33–43%, and secondly produced biogas (mean value of methane 83-85%). Phytotoxicity tests carried out on *Lepidium sativum* seeds showed that the anaerobic treatment considerably reduced the phytotoxic character of OMWW. Anaerobic digestion of OMWW was successfully carried out for both the reactors, even if there were differences between reactor R1 (prepared with treated filtered OMWW) and reactor R2 (containing treated OMWW without separation of the sludge). The depuration efficiency was better for reactor R1 (total carbon removal of 60.38%, polyphenol removal of 20%) than for reactor R2 (total carbon removal of 34%, polyphenol removal of 12%). Both the anaerobic effluents were much less phytotoxic than untreated OMWW; in particular the effluent of reactor R1 allowed a higher germination of *Lepidium sativum* seeds than that of reactor R2.

Composting of olive mill waste and assessment of the end-product

Since composting is an aerobic decomposition process that efficiently degrades and bioconverts (mainly through the use of microorganisms) complex organic compounds into a humus-like product, co-composting of OMWW with various materials of agricultural origin results at detoxification of the waste and generation of a value-added product, which can successfully support plant growth and protection (**Table 3**). In order to achieve the proper conditions for OMWW composting, suitable material(s) should be selected for regulating the optimal starting C/N and moisture values. In the past, several research groups studied OMWW co-composting and the applications of the final product: Tomati *et al.* (1996) used OMWW and wheat straw for obtaining an organic amendment with a high degree of humification and without phytotoxicity, Galli *et al.* (1997) studied the microbiological aspects of OMWW-wheat straw co-composting, while other studies used only olive mill by-products (olive press cake, olive leaves) for the co-composting of OMWW for obtaining a soil amendment and fertilizer (Papadimitriou *et al.* 1997; Mari *et al.* 2003; Ntougias *et al.* 2003). On the other hand, Paredes *et al.* (2001) reported that the addition of OMWW in piles composed of various agricultural residues and sewage sludge resulted in greater degradation of organic matter, higher pH and electrical conductivity values and greater losses of total N than compost pile formed without OMWW. Such type of processes led to significant degradation of complex organic compounds which is accompanied by the elimination of the phytotoxicity presented by the initial waste streams. Hence,

Table 3 Composts deriving from olive mill wastes, and their principal properties and applications.

Substrate/Source	Properties/Applications	References
OMWW (with various agricultural residues)	Organic soil amendment and/or fertilizer for plant growth	Cegarra *et al*. 1996
OMWW (with straw)	Organic soil amendment and/or fertilizer for ryegrass, maize and horticultural plants	Tomati *et al*. 1996
OMWW with olive mill solid residue	Soil conditioner	Vlyssides *et al*. 1996
OMWW with extracted olive press cake and olive leaves	Organic soil amendment and/or fertilizer for plant growth	Papadimitriou *et al*. 1997
OMWW (with cotton waste or maize straw)	Organic soil amendment and/or fertilizer for plant growth	Paredes *et al*. 2000
OMWW (with sewage sludge, cotton gin and orange peels)	Organic soil amendment and/or fertilizer for plant growth	Paredes *et al*. 2001
OMWW (solid fraction) and olive leaves	Organic soil amendment and/or fertilizer for ornamental plants	Garcia-Gomez *et al*. 2002
OMWW sludge (plus cotton gin and maize straw)	Organic soil amendment and/or fertilizer for plant growth	Paredes *et al*. 2002
OMWW with extracted olive press cake	Organic soil amendment and/or fertilizer for plant growth	Mari *et al*. 2003
OMWW with olive leaves and/or pomace and/or extracted press cake	Organic soil amendment and/or fertilizer for plant growth	Ntougias *et al.* 2003
OMWW with olive pomace (plus wheat straw)	Organic soil amendment and/or fertilizer for plant growth	Baddi *et al*. 2004
TPOMW (plus sheep litter)	Organic soil amendment and/or fertilizer in olive orchards	Cayuela *et al*. 2004
Olive tree branches and extracted press cake (plus other wastes)	Effective bulking agent for use in composting	Manios 2004
OMWW with olive pomace (plus poultry manure and wheat straw)	Organic soil amendment and/or fertilizer for ryegrass	Montemurro *et al*. 2004
Olive pomace (plus rice husk)	Limited nematicidal activity	Nico *et al.* 2004
OMWW with olive leaves and extracted press cake	Organic soil amendment and/or fertilizer for poinsettia	Papafotiou *et al*. 2004
TPOMW (plus sheep litter and sulphur)	Organic soil amendment and/or fertilizer for plant growth	Roig *et al.* 2004
TPOMW (plus cotton waste)	Organic soil amendment and/or fertilizer for pepper	Alburquerque *et al.* 2005
TPOMW (plus grape stalks)	Organic soil amendment and/or fertilizer for plant growth	Baeta-Hall *et al*. 2005
Olive press cake (plus digested biosolids from sewage sludge)[a]	Reactivation of C, P and N-cycles in degraded soils for regeneration purposes	Benitez *et al*. 2005
TPOMW (plus goat manure and grape stalks)	Organic soil amendment and/or fertilizer in olive orchards	Cayuela *et al.* 2005
Olive pomace and olive leaves	Organic soil amendment and fertilizer for organic greenhouse production of cucumber, increase in rhizosphere temperature	Ehaliotis *et al*. 2005
OMWW with olive leaves and extracted press cake	Organic soil amendment and/or fertilizer for foliage potted plants	Papafotiou *et al.* 2005
OMWW (with sewage sludge and cotton gin waste)	Organic soil amendment and/or fertilizer for Swiss chard crop	Paredes *et al*. 2005
TPOMW (plus cotton waste, grape stalks, olive leaves, cow bedding)	Organic soil amendment and/or fertilizer for plant growth	Alburquerque *et al.* 2006
TPOMW (plus sheep litter and grape stalks)	Organic soil amendment and/or fertilizer for plant growth	Cayuela *et al.* 2006
TPOMW (plus cotton gin waste)	Organic soil amendment and/or fertilizer for ryegrass	Alburquerque *et al.* 2007
Olive pomace (plus cotton gin trash)	Suppressiveness against *Botrytis cinerea*	Segarra *et al.* 2007
TPOMW and olive leaves	Organic soil amendment and/or fertilizer for plant growth	Alfano *et al.* 2008
TPOMW (plus animal manure, rice straw or almond shells)	Organic soil amendment and/or fertilizer for plant growth	Canet *et al*. 2008
TPOMW (plus sheep litter and grape stalks)[b]	Phytotoxicity against weeds, suppressiveness against plant-pathogenic fungi, and nematicidal activity	Cayuela *et al.* 2008
OMWW with extracted olive press cake (plus poultry manure)	Organic soil amendment and/or fertilizer for potato plants	Hachicha *et al*. 2008
OMWW with olive leaves and/or pomace and/or extracted press cake	Suppressiveness against *Phytophthora nicotianae* and *Fusarium oxysporum* f.sp. *radici-lycopersici* in tomato plants	Ntougias *et al.* 2008a
TPOMW (with olive leaves and OMWW)	Suppressiveness against *F. oxysporum* f.sp. *radici-lycopersici* in tomato plants	Ntougias *et al.* 2008b
TPOMW (plus sheep litter and grape stalks)	C source for soil C sequestration	Sánchez-Monedero *et al.* 2008
TPOMW (plus cotton gin trash, and mixed with rice husk)	Suppressiveness against *F. oxysporum* f. sp. *dianthi* in carnation plants	Borrero *et al.* 2009
TPOMW (plus cotton waste, grape stalks, olive leaves, cow bedding)	Organic soil amendment and/or fertilizer for plant growth	Alburquerque *et al.* 2009
OMWW sludge (plus sesame bark)	Organic soil amendment and/or fertilizer for plant growth	Hachicha *et al*. 2009a
OMWW sludge (plus poultry manure)	Organic soil amendment and/or fertilizer for plant growth	Hachicha *et al*. 2009b

[a] compost obtained through vermicomposting process
[b] compost's water extract

when a piled mixture of olive-mill waste (OMWW and olive pomace) with wheat straw was composted for one year, at the end of the process lignin, hemicellulose and cellulose were reduced by 44, 76 and 58%, respectively, the fat and water-soluble phenol contents decreased by 97 and 66%, respectively, while no phytotoxicity was detected in the mature compost (Baddi *et al.* 2004). Later, Paredes *et al.* (2005) co-composted OMWW with a mixture of cotton gin waste and sewage sludge, through the Rutgers static pile system; the end product presented lower organic matter and nitrate concentrations, higher electrical conductivity, and a stabilised and humified organic matter similar to that of the compost produced without OMWW. The OMWW-compost application to soil did not present any adverse effects on plants (Swiss chard, *Beta vulgaris* L. var. *cicla*), and their yields were comparable for both treatments examined (composts with and without OMWW and inorganic fertiliser). When co-composting of exhausted olive press-cake and poultry manure was performed with continuous OMW applications throughout the composting period (which lasted four months in three aerated windrows), electrical conductivity was relatively high in OMW-composts (5.46–5.48 S m^{-1}) as compared composts prepared without OMW addition, while phenol contents were reduced by more than 49% (Hachicha *et al.* 2008). Mature composts were then used as an amendment for potato production in a field, where no inhibitory effect on plant growth and no noticeable negative impact on the soil system were observed; on the other hand, potato productivity increased 10–23% as a result of compost application. In a recent study (Hachicha *et al.* 2009a), co-composting of OMW with sesame bark demonstrated large reductions in total organic matter (53%) and water-soluble phenol degradation (72%) after seven months of processing accompanied with its gradual detoxification towards compost maturation.

García-Gómez *et al.* (2002) examined the use of composts deriving from the solid fraction of OMWW plus olive leaves in mixtures with sphagnum peat or commercial substrates in ratios (of the compost) and arrived at the conclusion that such composts are suitable for the growth of two ornamental plants (calendula and calceolaria), since they act as slow-release organic fertilisers providing mainly nutrients such as N and K. However, they advised that their content in the final mixture should not exceed 50% (or 25% for salt sensitive plants) since electrical conductivity values might prove to be the principal limiting factor for the cultivation of sensitive plants. Moreover, Papafotiou *et al.* (2004) demonstrated the adverse effects of increased additions of compost prepared by co-composting of olive leaves and exhausted olive press-cake with OMWW on the growth of rooted cuttings of the pot ornamental *Euphorbia pulcherrima* cv. 'Peterstar'. Increasing replacement of the peat in the medium by the compost (up to 75%) caused analogous increase of the electrical conductivity that was however rapidly reduced during the culture period. Increasing replacement of peat by compost induced a gradual decrease in the plant height, bract number and node number where the first bract was initiated. The restriction of vegetative growth occurred only during the first month of culture in media with 25 and 50% peat replacement, while in 75% replacement there was growth restriction all over the culture period. Media with 50 and 75% peat replacement caused delayed pigmentation of the bracts and flowering, while plants in the medium with 25% peat replacement showed colour and flowered simultaneously with the control. In a subsequent study (Papafotiou *et al.* 2005), the same compost was also evaluated in foliage potted plant production: when up to 75% of peat in the control medium was replaced by the compost, *Codiaeum variegatum* foliage and root growth were similar to the control, while a 50% peat replacement produced the best growth. *Syngonium podophyllum* was more sensitive to peat replacement compared to *C. variegatum*, as only 25% of peat could be replaced by the compost without any reduction in foliage growth. In *Ficus benjamina*, peat could be replaced up to 75% without effects on plant height and lateral shoot number, while the length of the laterals, main stem diameter, foliage fresh weight, and root dry weight were progressively reduced with increasing compost addition. A considerable decrease in total porosity and readily available water were measured in media where 50 or 75% of peat was replaced by the compost. The pH of the medium with the highest compost level was high during the first 4 months of culture compared to the other media. The electrical conductivity was initially related to the compost values level, but it subsequently decreased to values similar to that detected in the control medium during the first month of cultivation.

In general, the use of various agricultural and husbandry by-products (e.g. maize straw, cotton waste, grape stalks, olive leaves, cow bedding, poultry manure, etc.) as bulking agents in the composting of both OMWW and TPOMW resulted in well-matured products presenting high organic matter mineralization and rather complete humification combined with decrease in phytotoxicity (Paredes *et al.* 2000, 2002; Alburquerque *et al.* 2006). Especially as regards TPOMW, its evaluation as a composting substrate demonstrated that composting performance was mainly influenced by the type of bulking agent added, and by the number of mechanical turnings performed (Alburquerque *et al.* 2009). Among the bulking agents examined in this particular study olive leaves presented the worse performance as compared with cotton waste, grape stalk, and fresh cow bedding. The composting process involved a substantial degradation of the organic substrate with average losses of 48, 29, 54 and 57% for total organic matter, lignin, cellulose and hemicellulose, respectively. Both organic matter biodegradation and humification were greatly influenced by the lignocellulosic nature of the starting material; the end products were of good quality in terms of nutrient content, stabilised and nonphytotoxic organic matter. In a previous study (Alburquerque *et al.* 2006), preparation of TPOMW and cotton waste compost involved a relatively low level of organic matter biodegradation, an increase in pH, and a clear decrease in the C/N ratio, fat, water-soluble organic carbon and phenol contents. The resulting compost, which was rich in organic matter and free of phytotoxicity, had a high potassium and organic nitrogen content but was low in phosphorus and micronutrients. When the TPOMW-based compost was compared with cattle manure and sewage sludge composts for use as organic amendment on a calcareous soil for supporting a commercial pepper crop in a greenhouse using fertigation, the marketable yields of pepper obtained with all three organic amendments were similar, thus confirming the composting performance of the raw TPOMW. In addition, for cattle manure and sewage sludge composts the soil organic matter content was significantly reduced after cultivation, while it remained almost unchanged in the TPOMW amended plots (Alburquerque *et al.* 2006). When a pot experiment was conducted on a low-fertility calcareous soil in order to evaluate the effect on ryegrass growth and nutrient uptake of an organic fertiliser obtained by composting TPOMW and cotton gin waste, it was demonstrated that the compost application enhanced plant growth in the first and third harvest (Alburquerque *et al.* 2007). However, additional nitrogen fertilisation clearly improved soil productivity due to the scarce availability of this nutrient in the compost examined. On the other hand, an increase in the plant contents of phosphorus and potassium in the first two harvests was recorded, while treatments with the maximum compost rate showed the highest plant content of copper in the last two harvests; in contrast, reduction in calcium, magnesium, iron and manganese concentrations were detected in one or more harvests.

The possibility of using dry TPOMW as an organic fertilizer has been also examined through the use of certain saprotrophic and AM fungi (Sampedro *et al.* 2008): the application of 25 g kg^{-1} of dry TPOMW to soil decreased the shoot and root dry weight of tomato and alfalfa. Plants were more sensitive to the toxicity of the waste when colonized with the arbuscular mycorrhizal (AM) fungi, while this sensitivity varied also according to whether they were colonized by *Glomus deserticola* or by indigenous AM fungi. The phytotoxicity of the waste towards tomato and alfalfa was decreased by its incubation before planting with saprotrophic fungi for a period of 20 weeks. The contribution of AM fungi to the beneficial effect of the waste incubated with saprotrophic fungi varied according to the type of the plant and AM fungi; for example, *G. deserticola* increased the shoot and root dry weight of plants when they were grown in the presence of waste incubated with saprotrophic fungi. The beneficial effect of the latter on the dry weight and the level of AM mycorrhization of plants seem to be related to the decrease caused by these fungi in the waste polyphenolics. Improvement in microbial activity and reduction in the phenolic content was also achieved by inoculating the white-rot fungus *Ph. chrysosporium* during the maturation phase of OMWW co-composting, which resulted in the enhancement of this whole process and in the increase in the germination index of the final product (Taccari *et al.* 2009).

Another characteristic case of valorizing residues and by-products of the olive-mill agro-industry (leaves and pomace) was provided in the study of Ehaliotis *et al.* (2005); composting of the above materials below the root-zone of cucumber (*Cucumis sativus*) plants, resulted in an optimal 20–30°C ambient root-zone temperature during the whole cultivation (winter) period, while no phytotoxicity was observed on any of the four different cucumber cultivars tested. The mature compost derived from the same initial materials, when assessed as a soil amendment (in relatively high application rates) for providing nutrients in organic greenhouse production of cucumber, demonstrated yields comparable to sheep manure treatments.

Several compost types suppress a large range of soil-borne plant pathogens, and disease suppressiveness is clearly linked with its degree of maturity, although excessively stabilized composts with low content of organic matter have lower suppressiveness capacity (Raviv 2008). Composts contribute to disease suppression in a complex manner, involving microbial competition for nutrients and ecological niches, antibiosis, parasitism, changes in nutrient availability and induction of host resistance (Erhart *et al.* 1999; Hoitink and Boehm 1999; Perez-Piqueres *et al.* 2006); however, since sterilization often eliminates compost suppressiveness, its mode of action usually relates to biological factors. Indicative results from pertinent studies with composts prepared from olive mill waste are presented in **Table 3**.

Of particular interest are the results of studies evaluating the effect of OMW and its compost products in the suppression of plant pathogens and nematodes. Hence, it was demonstrated that damping-off due to the soil-borne fungus *Rhizoctonia solani* in lettuce plants grown in soil previously treated with OMW was significantly reduced compared to the control (soil receiving water); results suggest that addition of OMW to soil creates a nutrient-rich environment that is dominated by r-strategists, which confer to the creation of unfavourable growth conditions for *R. solani* (Kotsou *et al.* 2004). Similarly, when OMW and some of its indigenous bacterial strains were tested *in vitro* and *in vivo* for their efficacy against damping-off caused *R. solani* and *Fusarium solani*, it was observed that OMW and polyphenols displayed a high level of antifungal activity especially against *R. solani*. In pot experiments, the percentage of tomato plants showing symptoms of damping-off was significantly reduced with different doses of OMW (0.5, 1 and 2%) as compared to the control (Yangoui *et al.* 2008). Furthermore, fungicidal properties of TPOMW compost extracts, assayed in a microwell assay format, showed that the growth of *Phytophthora capsici* was consistently and strongly inhibited by all TPOMW extracts diluted 1: 10 (w/v). In contrast, suppression of *Pythium ultimum* and *Botrytis cinerea* by the extracts was not as effective and depended on the specific TPOMW sample. Mature compost extracts inhibited *P. capsici* and *B. cinerea* at dilutions as much as 1: 50 (w/v); however, none of them were able to inhibit the growth of the *R. solani* (Cayuela *et al.* 2008).

As far as composts are concerned, Ntougias *et al.* (2008a) evidenced high levels of suppressiveness against *Phytophthora nicotianae* in tomato plants by composts prepared from olive mill waste (i.e. OMW, olive pomace, extracted olive press-cake and olive leaves; used alone or in different combinations), especially when they were applied directly after curing, indicating the occurrence of a "general suppression phenomenon" (92-100% decrease in plant disease incidence); on the other hand, suppressiveness against *Fusarium xysporum* f.sp. *radicis-lycopersici* was relatively lower (57-75% decrease in plant disease incidence for composts used directly after the curing stage). In another pertinent study (Kavroulakis *et al.* 2005), it was demonstrated that a compost prepared with grape marc waste and extracted olive press cake consistently induced a tomato plant defence reaction against the root-infecting fungal pathogen *F. oxysporum* f.sp. *radicis-lycopersici*; plants exhibited an enhanced defensive capacity against the pathogen as compared with control plants grown on peat, while the pathogen was not able to penetrate and colonize the root tissue although its viability and growth in the potting mixes (as determined by GUS activity measurements and by colony forming unit enumeration) was not affected. Induction of systemic resistance by the compost was also confirmed using the foliar pathogen *Septoria lycopersici* and by PR gene expression analysis carried out in leaves of tomato plants grown on the same compost. As it was revealed in subsequent experiments, this supressive compost was able to elicit consistent and increased expression of certain PR genes in the roots of tomato plants, even in the absence of any pathogen (Kavroulakis *et al.* 2006); furthermore, it was found that an endophytic *Fusarium solani* strain, obtained from root tissues of tomato plants grown on the same suppressive compost was able to colonize root tissues and subsequently protect plants against *F. oxysporum* f.sp. *radicis-lycopersici*, and was responsible for eliciting induced systemic resistance against the *S. lycopersici*; interestingly, attenuated expression of certain pathogenesis-related genes, i.e. PR5 and PR7, was detected in tomato roots inoculated with the *F. solani* strain compared with non-inoculated plants (Kavroulakis *et al.* 2007). The use of TPOMW was also studied as regards its potential suppressiveness on plant pathogens; when composts were produced by TPOMW with olive leaves (co-composted or not with OMWW throughout the composting process) and then mixed with peat at 1: 3 (w/w) ratios in pot experiments, they demonstrated high levels of disease reduction (as low as 15-34%) caused by *F. oxysporum* f.sp. *radicis-lycopersici* in tomato plants (Ntougias *et al.* 2008b). Similarly, significant suppressiveness against carnation wilt (disease reduction of 80% with respect to peat), caused by *F. oxysporum* f.sp. *dianthi*, was demonstrated by a substrate prepared from TPOMW and cotton gin trash (1: 1 v/v, composted and mixed with rice husk), which was accompanied with satisfactory support of carnation plant growth (Borrero *et al.* 2009). When a compost prepared by olive pomace (plus cotton gin trash) was examined, it was shown to suppress disease incidence (to 32% as compared to the peat control) by *Botrytis cinerea* on cucumber plants (Segarra *et al.* 2007).

On the other hand, TPOMW extracts strongly inhibited egg hatch and second-stage juvenile (J2) motility of the root-knot nematode *Meloidogyne incognita* (Cayuela *et al.* 2008). Similar encouraging results were obtained in a tomato field infested by *M. incognita*, where different dosages (10, 20 and 40 t/ha) of two composts prepared with either exhausted or fresh olive pomace or raw extraction sewage were assessed at rates of 40 or 80 m^3/ha (Sasanelli *et al.* 2003). All compost treatments statistically increased tomato yields with respect to the control; their application contributed at significantly reducing the root gall index and the populations of the root-knot nematode declined, except in the soil treated with the lowest doses of raw sewage or exhausted pomace compost. However, the use of composted 1: 1 mixture of dry-olive pomace and dry-rice husk as an amendment to potting mixtures demonstrated limited reduction in root galling in tomato plants and insignificant decrease in *Meloidogyne* spp. nematode populations (Nico *et al*. 2004).

CONCLUDING REMARKS

In conclusion, it could be stated that:

- Land application of olive mill waste was demonstrated to be a viable solution for the disposal of both OMWW and TPOMW. In most of the pertinent studies conducted so far, results evidence significant increase in organic carbon, aggregate stability, available K, and cation-exchange capacity, which are usually accompanied with increase in the crop production (particularly as concerns olive tree cultivation). The positive outcome of such application is more pronounced in poor and marginal soils. On the other hand, possible phytotoxicity hazards are significantly lower in organic-rich soils; such substrates could also better support plant growth and productivity. Horticultural crops have different sensitivities to olive mill waste, larger seeds seem to be more sensitive. This could be a good predictive tool for the choice of cultures to be irrigated with OMWW.

- Composting of olive mill waste and by-products (mixed with a relatively large variety of agricultural and agro-industrial residues abundant in the Mediterranean) seem to provide efficient soil amendments, fertilizers and/or plant-pathogens suppressants, which could perform well in the market and complement and/or replace relevant chemical compounds. Of course, there are still issues that need addressing, and these mostly are mostly related to certain adverse effects of the end product (e.g. high salinity, phytotoxicity), its fertilizing value and market price as compared with similar commercial compounds, etc.

- Last, but not least, the fact that no olive mill waste treatment methodology has been universally adopted is not only an indication of the possible drawbacks that each one of them possesses, but it is mostly a result of the large range of particular conditions met at the local small or medium-scale level of olive oil extraction. Hence, individual approaches need to be considered and applied to fit local requirements; they should combine two or more relatively simple and cost efficient methodologies (i.e. physicochemical and/or biological, land application, composting, etc.) depending on the particular characteristics of the olive mill, the generated waste and the region (incl. local agriculture priorities and needs). Hence, the environmental issue could be solved together with the generation of specific value-added products ranging from soil amendments to fine chemicals.

ACKNOWLEDGEMENTS

This work has been performed in the frame of the project "*Biological treatment and valorization of olive mill wastewater: mechanisms and integrated applications*" (Code: FP66), which is co-funded from the European Union by 75% and from the Hellenic State by 25%, through the Operational Programme 'Competitiveness'. The authors would like to thank all three reviewers for their constructive comments and suggestions.

REFERENCES

Abu-Zreig M, Al-Widyan M (2002) Influence of olive mills solid waste on soil hydraulic properties. *Communications in Soil Science and Plant Analysis* **33**, 505-517

Adam AL, Bestwick CS, Barna B, Mansfield JW (1995) Enzymes regulating the accumulation of active oxygen species during the hypersensitive reaction of bean to *Pseudomonas syringae* pv. *phaseolicola*. *Planta* **197**, 240-249

Aggelis G, Iconomou D, Christou M, Bokas D, Kotzailias S, Christou G, Tsagou V, Papanikolaou S (2003) Phenolic removal in a model olive oil mill wastewater using *Pleurotus ostreatus* in bioreactor cultures and biological evaluation of the process. *Water Research* **37**, 3897-3904

Aguilera M, Monteoliva-Sánchez M, Suárez A, Guerra del Águila V, Lizama C, Bennasar A, Ramos-Cormenzana A (2001) *Paenibacillus jamilae* sp. nov., an exopolysaccharide-producing bacterium able to grow in olive-mill wastewater. *International Journal of Systematic and Evolutionary Microbiology* **51**, 1687-1692

Aguilera M, Quesada MT, Guerra del Águila V, Morillo JA, Rivadeneyra MA, Ramos-Cormenzana A, Monteoliva-Sánchez M (2008) Characterisation of *Paenibacillus jamilae* strains that produce exopolysaccharide during growth on and detoxification of olive mill wastewaters. *Bioresource Technology* **99**, 5640-5644

Alburquerque JA, Gonzálvez J, García D, Cegarra J (2004) Agrochemical characterisation of "alperujo", a solid by-product of the two-phase centrifugation method for olive oil extraction. *Bioresourse Technology* **91**, 195-200

Alburquerque JA, Gonzalvez J, Garcia D, Cegarra J (2005) Composting of a solid olive-mill by-product (alperujo) and the potential of the resulting compost for cultivating pepper under commercial conditions. *Waste Management* **26**, 620-626

Alburquerque JA, Gonzálvez J, García D, Cegarra J (2006) Measuring detoxification and maturity in compost made from "alperujo", the solid by-product of extracting olive oil by the two-phase centrifugation system. *Chemosphere* **64**, 470-477

Alburquerque JA, Gonzálvez J, García D, Cegarra J (2007) Effects of a compost made from the solid by-product ("alperujo") of the two-phase centrifugation system for olive oil extraction and cotton gin waste on growth and nutrient content of ryegrass (*Lolium perenne* L.). *Bioresourse Technology* **98**, 940-945

Alburquerque JA, Gonzálvez J, Tortosa G, Baddi GA, Cegarra J (2009) Evaluation of "alperujo" composting based on organic matter degradation, humification and compost quality. *Biodegradation* **20**, 257-270

Alfano G, Belli C, Lustrato G, Ranalli G (2008) Pile composting of two-phase centrifuged olive husk residues: Technical solutions and quality of cured compost. *Bioresource Technology* **99**, 4694-4701

Alliota G, Cafiero G, De Feo V, Di Blasio B, Iacovino R, Oliva A (2000) Allelochemicals from rue (*Ruta graveolens* L.) and olive (*Olea europea* L.) oil mill wates as potential natural pesticides. *Current Topics in Phytochemistry* **3**, 167-177

Andreozzi R, Canterino M, Di Somma I, Lo Giudice R, Marotta R, Pinto G, Pollio A (2008) Effect of combined physico-chemical processes on the phytotoxicity of olive mill wastewaters. *Water Research* **42**, 1684-1692

Aqeel AM, Hameed KM (2007) Implementation of olive mill by products in agriculture. *World Journal of Agricultural Sciences* **3**, 380-385

Aranda E, Sanpedro I, Ocampo JA, García-Romera I (2006) Phenolic removal of olive-mill dry residues by laccase activity of white-rot fungi and its impact on tomato plant growth. *International Biodeterioration and Biodegradation* **58**, 176-179

Arvanitoyannis IS, Kassaveti A (2007) Current and potential uses of composted olive oil waste. *International Journal of Food Science and Technology* **42**, 281-295

Arvanitoyannis IS, Kassaveti A, Stefanatos S (2007) Olive oil waste treatment: A comparative and critical presentation of methods, advantages and disadvantages. *Critical Reviews in Food Science and Nutrition* **47**, 187-229

Asfi M, Ouzounidou G, Sperdouli I, Moustakas M (2006) Effects of olive mill wastewater fertirrigation on growth and photosynthetic characteristics of spinach (*Spinacea oleracea* L.). *15th Congress of Federation of European Societies of Plant Biology*, 17-21 July, Lyon, France, p 187 (Abstract)

Assas N, Ayed L, Marouani L, Hamdi M (2002) Decolorization of fresh and stored-black olive mill wastewaters by *Geotrichum candidum*. *Process Biochemistry* **38**, 361-365

Asses N, Ayed L, Bouallagui H, Rejeb IB, Gargouri M, Hamdi M (2009) Use of *Geotrichum candidum* for olive mill wastewater treatment in submerged and static culture. *Bioresource Technology* **100**, 2182-2188

Baddi GA, Alburquerque JA, Gonzalvez J, Cegarra J, Hadi M (2004) Chemical and spectroscopic analyses of organic matter transformations during composting of olive mill wastes. *International Biodeterioration and Biodegradation* **54**, 39-44

Baeta-Hall L, Ceu Saagua MC, Lourdes Bartolomeu M, Anselmo AM, Fernanda Rosa M (2005) Bio-degradation of olive oil husks in composting aerated piles. *Bioresource Technology* **96**, 69-78

Baldrian P, Zervakis GI, Merhautová V, Ntougias S, Ehaliotis C, Nerud F (2006) The use of hydroxyl radicals-generating systems for the treatment of olive mill wastewaters (OMW). *Folia Microbiologica* **51**, 337-341

Balis C (1994) Enrichment of olive oil mill wastes through microbiological processing. *Proceedings of the VII International Congress on "L'approccio integrato della moderna biologia: uomo, territorio, ambiente"*, 22-25 September 1994, Vieste, Italy, pp 99-115

Balis C, Chatzipavlidis J, Flouri F (1996) Olive mill wastes as substrate for nitrogen fixation. *International Biodeterioration and Biodegradation* **38**, 169-178

Belaqziz M, Lakhal EK, Mbouobda HD, El Hadrami I (2008) Land spreading of olive mill wastewater: effect on maize (*Zea mays*) crop. *Journal of Agronomy* **7**, 297-305

Ben Hamman O, de la Rubia T, Martínez J (1999) Decolorization of olive oil mill wastewaters by *Phanerochaete flavido-alba*. *Environmental Toxicology and Chemistry* **18**, 2410-2415

Ben Rouina B (1994) Repercussions agronomiques de l'epandage des margines comme fertilisant. *International Conference on Land and Water Resources Management in the Mediterranean region II*, Bari, 4-8 September 1994, Istituto Agronomico Mediterraneo, Bari, pp 583-594

Ben Rouina B, Taamallah H, Ammar E (1999) Vegetation water used as a fertilizer on young olive plants. *Acta Horticulturae* **474**, 353-355

Ben Sassi A, Ouazzani N, Walker GM, Ibnsouda S, El Mzibri M, Boussaid A (2008) Detoxification of olive mill wastewaters by Moroccan yeast isolates. *Biodegradation* **19**, 337-346

Benitez J, Beltran-Heredia J, Torregrosa J, Acero JL, Cercas V (1997) Aerobic degradation of olive mill wastewaters. *Applied Microbiology and Biotechnology* **47**, 185-188

Benitez E, Sainz H, Nogales R (2005) Hydrolytic enzyme activities of extracted humic substances during the vermicomposting of a lignocellulosic olive waste. *Bioresource Technology* **96**, 785-790

Blánquez P, Caminal G, Sarrá M, Vicent MT, Gabarrell X (2002) Olive oil mill waste waters decoloration and detoxification in a bioreactor by the white rot fungus *Phanerochaete flavido-alba*. *Biotechnological Progress* **18**, 660-662

Bonari E, Macchia M, Angelini LG, Ceccarini L (1993) The waste water from olive oil extraction: their influence on the germinative characteristics of some cultivated and weed species. *Agricoltura Mediterranea* **123**, 273-280

Borrero C, Trillas I, Avilés M (2009) Carnation Fusarium wilt suppression in four composts. *European Journal of Plant Pathology* **123**, 425-433

Brunetti G, Plaza C, Senesi N (2005) Olive pomace amendment in Mediterranean conditions: effect on soil and humic acid properties and wheat (*Triticum turgidum* L.) yield. *Journal of Agricultural and Food Chemistry* **53**, 6730-6736

Cabrera F, Lopez R, Martínez-Bordiu A, Dupuy de Lome E, Murillo JM (1996) Land treatment of olive oil mill wastewater. *International Biodeterioration and Biodegradation* **38**, 215-225

Canet R, Pomares F, Cabot B, Chaves C, Ferrer E, Ribó M, Albiach MR (2008) Composting olive mill pomace and other residues from rural southeastern Spain. *Waste Management* **28**, 2585-2592

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

Capasso R, Evidente A, Schivo L, Orru G, Marcialis MA, Cristinzio G (1995) Antibacterial polyphenols from olive oil mill waste waters. *Journal of Applied Bacteriology* **79**, 393-398

Casa R, D'Annibale A, 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

Cayuela ML, Bernal MP, Roig A (2004) Composting olive mill waste and sheep manure for orchard use. *Compost Science and Utilization* **12**, 130-136

Cayuela ML, Sánchez-Monedero MA, Molina J, Roig A (2005) Compost production from olive oil processing. *BioCycle* **46**, 64-65

Cayuela ML, Sánchez-Monedero MA, Roig A (2006) Evaluation of two different aeration systems for composting two-phase olive mill wastes. *Process Biochemistry* **41**, 616-623

Cayuela ML, Millner PD, Meyer SL, Roig A (2008) Potential of olive mill waste and compost as biobased pesticides against weeds, fungi, and nematodes. *Science of the Total Environment* **399**, 11-18

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

Cereti CF, Rossini F, Federici F, Quaratino D, Vassilev N, Fenice M (2004) Reuse of microbially treated olive mill wastewater as fertilizer for wheat (*Triticum durum* Desf.). *Bioresource Technol* **91**, 135-140

Clijsters H, Cuypers A, Vangronsveld J (1999) Physiological responses to heavy metals in higher plants; defence against oxidative stress. *Zeitschrift für Naturforschung* **45**, 730-734

Codounis M, Katsaboxakis K, Papanicolaou D (1983) Progress in the extraction and purification of anthocyanin pigment from the effluents of olive-oil extracting plants. *Lebensmittel-Wissenschaft und -Technologie* **7**, 567-572

Colarieti ML, Toscano G, Greco G Jr. (2006) Toxicity attenuation of olive mill wastewater in soil slurries. *Environmental Chemistry Letters* **4**, 115-118

Cummins I, Brazier-Hicks M, Stobiecki M, Franski R, Edwards R (2006) Selective disruption of wheat secondary metabolism by herbicide safeners. *Phytochemistry* **67**, 1722-1730

D'Annibale A, Casa R, Pieruccetti F, Ricci M, Marabottini R (2004a) *Lentinula edodes* removes phenols from olive-mill wastewater: impact on durum wheat (*Triticum durum* desf.) germinability. *Chemosphere* **54**, 887-894

D'Annibale A, Ricci M, Quaratino D, Federici F, Fenice M (2004b) *Panus tigrinus* efficiently removes phenols, color and organic load from olive-mill wastewater. *Research in Microbiology* **155**, 596-603

D'Annibale A, Crestini C, Vinciguerra V, Giovannozzi Sermanni G (1998) The biodegradation of recalcitrant effluents from an olive mill by a white-rot fungus. *Journal of Biotechnology* **61**, 209-218

Dhouib A, Aloui F, Hamad N, Sayadi S (2005) Complete detoxification of olive mill wastewater by integrated treatment using the white rot fungus *Phanerochaete chrysosporium* followed by anaerobic digestion and ultrafiltration. *Biotechnology* **4**, 153-162

Dhouib A, Ellouz M, Aloui F, Sayadi S (2006) Effect of bioaugmentation of activated sludge with white-rot fungi on olive mill wastewater detoxification. *Letters in Applied Microbiology* **42**, 405-411

Di Gioia D, Barberio C, Spagnesi S, Marchetti L, Fava F (2002) Characterization of four olive-mill-wastewater indigenous bacterial strains capable of aerobically degrading hydroxylated and methoxylated monocyclic aromatic compounds. *Archives in Microbiology* **178**, 208-217

Di Gioia D, Fava F, Bertin L, Marchetti L (2001) Biodegradation of synthetic and naturally occurring mixtures of mono-cyclic aromatic compounds present in olive mill wastewaters by two aerobic bacteria. *Applied Microbiology and Biotechnoogy* **55**, 619-626

Di Giovacchino L, Basti C, Costantini ML, Ferrante ML, Surrichio G (2001) Effects of olive oil mill waste water spreading on soil cultivated with maize and grapevine. *Agricultura Mediterranea* **131**, 33-41

Dias AA, Bezerra RM, Pereira AN (2004) Activity and elution profile of laccase during biological decolorization and dephenolization of olive mill wastewater. *Bioresource Technology* **92**, 7-13

Douds DD, Johnson CR, Koch KE (1988) Carbon cost of the fungal symbiot relative to net leaf P accumulation in a split-root VA mycorrhizal symbiosis. *Plant Physiology* **86**, 491-496

Ehaliotis C, Papadopoulou K, Kotsou M, Mari I, Balis C (1999) Adaptation and population dynamics of *Azotobacter vinelandii* during aerobic biological treatment of olive-mill wastewater. *FEMS Microbiology Ecology* **30**, 301-311

Ehaliotis C, Zervakis G, Anoliefo O, Papadopoulou K, Kardimaki A (2003) The capacity of agricultural soils to auto-regulate bioremediation of olive-mill waste water. In: Sasek V, Glaser JA, Baveye P (Eds) *The Utilization of Bioremediation to Reduce Soil Contamination: Problems and Solutions*, Kluwer Academic Publishers, Dordrecht, pp 353-358

Ehaliotis C, Zervakis GI, Karavitis P (2005) Residues and by-products of olive-oil mills for root-zone heating and plant nutrition in organic vegetable production. *Scientia Horticulturae* **106**, 293-308

El Hadrami A, Belaqziz M, El Hassni M, Hanifi S, Abbad A, Capasso R, Gianfreda L, El Hadrami I (2004) Physico-chemical characterization and effects of olive oil mill wastewaters fertirrigation on the growth of some Mediterranean crops. *Journal of Agronomy* **3**, 247-254

El Hassani FZ, Zinedine A, Bendriss Amraoui M, Errchidi F, Mdaghri Alaoui S, Aissam H, Merzouki M, Benlemlih M (2009a) Characterization of the harmful effect of olive mill wastewater on spearmint. *Journal of Hazardous Materials* **170**, 779-785

El Hassani FZ, Bendriss Amraoui M, Zinedine A, Aissam H, Mdaghri Alaoui S, Merzouki M, Benlemlih M (2009b) Changes in leaf phenols and other physiological parameters of peppermint in response to olive mill wastewater application. *International Journal of Agriculture and Biology* **11**, 413-418

Elliot LF, Stevenson FJ (1977) *Soil for Management of Organic Wastes and Waste Waters*, Soil Science Society of America, Madison, WI, 650 pp

Ergül FE, Sargın S, Öngen G, Vardar Sukan F (2009) Dephenolisation of olive mill wastewater using adapted *Trametes versicolor*. *International Biodeterioration and Biodegradation* **63**, 1-6

Erhart E, Burian K, Hartl W, Stich K (1999) Suppression of *Pythium ultimum* by biowaste composts in relation to compost microbial biomass, activity and content of phenolic compounds. *Journal of Phytopathology* **147**, 299-305

Ettayebi K, Errachidi F, Jama L, Tahri-Jouti MA, Sendide K, Ettayebi M (2003) Biodegradation of polyphenols with immobilized *Candida tropicalis* under metabolic induction. *FEMS Microbiology Letters* **223**, 215-219

Estaun V, Calvet C, Pages M, Grases JM (1985) Chemical determination of fatty acid, organic acid and phenols during olive marc composting process. *Acta Horticulturae* **172**, 263-270

Fadil K, Chahlaoui A, Ouahbi A, Zaid A, Borja R (2003) Aerobic biodegradation and detoxification of wastewaters from the olive oil industry. *International Biodeterioration and Biodegradation* **51**, 37-41

Fiestas Ros de Urcinos JA (1986) Vegetation water used as fertilizer. In: FAO-UNDP (Ed) *Proceedings of the International Symposium on Olive By-products Valorization*, Seville, pp 321-330

Fiestas Ros de Urcinos JA, Borja Padilla R (1992) Use and treatment of olive mill wastewater: current situation and prospects in Spain. *Grasas y Aceites* **43**, 101-106

Filidei S, Masciandaro G, Ceccanti B (2003) Anaerobic digestion of olive oil mill effluents: evaluation of wastewater organic load and phytotoxicity reduction. *Water, Air and Soil Pollution* **145**, 79-94

Fiorelli F, Pasetti L, Galli E (1996) Fertility-promoting metabolites produced by *Azotobacter vinelandii* grown on olive-mill wastewaters. *International Biodeterioration and Biodegradation* **38**, 165-167

Fiorentino A, Gentili A, Isidori M, Lavorgna M, Parrella A, Temussi F (2004) Olive oil mill wastewater treatment using a chemical and biological approach. *Journal of Agricultural and Food Chemistry* **52**, 5151-5154

Fischler M, Wortmann CS, Feil B (1999) Crotalaria (*C. orchroleuca* G. Don) as a green manure in maize-bean cropping systems in Uganda. *Field Crop Research* **61**, 97-107

Fountoulakis MS, Dokianakis SN, Kornaros ME, Aggelis GG, Lyberatos G (2002) Removal of phenolics in olive mill wastewaters using the white-rot fungus *Pleurotus ostreatus*. *Water Research* **36**, 4735-4744

Fuller WH, Warrick AW (1985) *Soils in Waste Treatment and Utilization*, CRC Press, Boca Raton, FL, 240 pp

Gallego SM, Benavides MP, Tomaro ML (1996) Effect of heavy metal ion excess on sunflower leaves: Evidence for involvement of oxidative stress. *Plant Science* **121**, 151-159

Galli E, Pasetti L, Fiorelli F, Tomati U (1997) Olive-mill wastewater composting: microbiological aspects. *Waste Management and Research* **15**, 323-330

Gavalda D, Sheiner JD, Revela JC, Merlina G, Kaemmerer M, Pinelli E, Guiresse M (2005) Agronomic and environmental impacts of a single application of heat-dried sludge on an alfisol. *Science of the Total Environment* **343**, 97-109

García García I, Jimeńez Penã PR, Bonilla Venceslada J, Martín Martín A, Martiń Santos MA, Ramos Gomez E (2000) Removal of phenol compounds from olive mill wastewater using *Phanerochaete chrysosporium*, *Aspergillus niger*, *Aspergillus terreus* and *Geotrichum candidum. Process Biochemistry* **35**, 751-758

García-Gómez A, Bernal MP, Roig A (2002) Growth of ornamental plants in two composts prepared from agroindustrial wastes. *Bioresource Technology* **83**, 81-87

Gharsallah N, Labat M, Aloui F, Sayadi S (1999) The effect of *Phanerochaete chrysosporium* pretreatment of olive mill waste waters on anaerobic digestion. *Resources, Conservation and Recycling* **27**, 187-192

Giannoutsou EP, Meintanis C, Karagouni AD (2004) Identification of yeast strains isolated from a two-phase decanter system olive oil waste and investigation of their ability for its fermentation. *Bioresource Technology* **93**, 301-306

Greco G Jr., Letizia Coliareti M, Toscano G, Iamarino G, Rao MA, Gianfreda L (2006) Mitigation of olive mill wastewater toxicity. *Journal of Agricultural and Food Chemistry* **54**, 6776-6782

Greco G Jr., Toscano G, Cioffi M, Gianfreda L, Sannino S (1999) Dephenolisation of olive mill waste-waters by olive husk. *Water Research* **33**, 3046-3050

Hachicha S, Cegarra J, Sellamia F, Hachicha R, Drira N, Medhioub K, Ammar E (2009a) Elimination of polyphenols toxicity from olive mill wastewater sludge by its co-composting with sesame bark. *Journal of Hazardous Materials* **161**, 1131-1139

Hachicha R, Hachicha S, Trabelsi I, Woodward S, Mechichi T (2009b) Evolution of the fatty fraction during co-composting of olive oil industry wastes with animal manure: Maturity assessment of the end product. *Chemosphere* **75**, 1382-1386

Hachicha S, Sallemi F, Medhioub K, Hachicha R, Ammar E (2008) Quality assessment of composts prepared with olive mill wastewater and agricultural wastes. *Waste Management* **28**, 2593-2603

Hamdi M, BouHamed H, Ellouz R (1991) Optimization of the fermentation of olive mill waste-waters by *Aspergillus niger*. *Applied Microbiology and Biotechnology* **36**, 285-288

Hamdi M, Ellouz R (1992) Use of *Aspergillus niger* to improve filtration of olive mill waste-waters. *Journal of Chemical Technology and Biotechnology* **53**, 195-200

Hanifi S, El Hadrami I (2008) Olive mill wastewaters fractioned soil application for safe agronomic reuse in date palm (*Phoenix dactylifera* L.) fertilization. *Journal of Agronomy* **7**, 63-69

Hegedus A, Erdei S, Horvath G (2001) Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. *Plant Science* **160**, 1085-1093

Hoitink HAJ, Boehm MJ (1999) Biocontrol within the context of soil microbial communities: A substrate-dependent phenomenon. *Annual Review of Phytopathology* **37**, 427-446

Isidori M, Lavorgna M, Nardelli A, Parrella A (2004) Chemical and toxic evaluation of a biological treatment for olive-oil mill wastewater using commercial microbial formulations. *Applied Microbiology and Biotechnology* **64**, 735-739

IOOC (International Olive Oil Council) (2008) Available online: http://www.internationaloliveoil.org

Jaouani A, Guillen F, Penninckx MJ, Martínez AT, Martínez MJ (2005) Role of *Pycnoporus coccineus* laccase in the degradation of aromatic compounds in olive oil mill wastewater. *Enzyme and Microbial Technology* **36**, 478-486

Jaouani A, Sayadi S, Vanthournhout M, Penninck MJ (2003) Potent fungi for decolorization of olive oil mill wastewaters. *Enzyme and Microbial Technology* **33**, 802-809

Kalmis E, Azbar N, Yildiz H, Kalyoncu F (2008) Feasibility of using olive mill effluent (OME) as a wetting agent during the cultivation of oyster mushroom, *Pleurotus ostreatus*, on wheat straw. *Bioresource Technology* **99**, 164-169

Karpouzas DG, Rousidou K, Papadopoulou KK, Bekris F, Zervakis GI, Singh BK, Ehaliotis C (2009) Effect of continuous olive mill wastewater applications, in the presence and absence of nitrogen fertilization, on the structure of rhizosphere-soil fungal communities. *FEMS Microbiology Ecology* **70**, 56-69

Kavroulakis N, Ntougias S, Zervakis GI, Ehaliotis C, Haralampidis K, Papadopoulou KK (2007) Role of ethylene in the protection of tomato plants against fungal pathogens conferred by an endophytic *Fusarium solani* strain. *Journal of Experimental Botany* **58**, 3853-3864

Kavroulakis N, Papadopoulou KK, Ntougias S, Zervakis GI, Ehaliotis C (2006) Cytological and other aspects of pathogenesis-related gene expression in tomato plants grown on a supressive compost. *Annals of Botany* **98**, 555-564

Kavroulakis N, Ehaliotis C, Ntougias S, Zervakis GI, Papadopoulou K (2005) Local and systemic resistance against fungal pathogens of tomato plants elicited by a compost derived from agricultural residues. *Physiological and Molecular Plant Pathology* **66**, 163-174

Kissi M, Mountadar M, Assobhei O, Gargiulo E, Palmieri G, Giardina P (2001) Roles of two white-rot basidiomycete fungi in decolorisation and detoxification of olive mill waste water. *Applied Microbiology and Biotechnology* **57**, 221-226

Kistner T, Jung V, Olsson E, Nitz G, Heubergre H, Aslanius BW, Schnitzler WH (2004) Effect of olive mill wastewater on young hydroponically grown tomato plants. *Journal of Applied Botany* **78**, 25-31

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

Kotsou M, Mari I, Lasaridi K, Chatzipavlidis I, Balis C, Kyriacou A (2004) The effect of olive oil mill wastewater (OMW) on soil microbial communities and suppressiveness against *Rhizoctonia solani*. *Applied Soil Ecology* **26**, 113-121

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 wastewater. *Bioresource Technology* **96**, 317-322

Levi-Minzi R, Saviozzi A, Riffaldi R, Falzo L (1992) Land application of vegetable water: effects on soil properties. *Olivae* **40**, 20-25

Linares A, Caba JM, Ligero F, de la Rubia T, Martínez J (2003) Detoxification of semisolid olive-mill wastes and pine-chip mixtures using *Phanerochaete flavido-alba*. *Chemosphere* **51**, 887-891

López-Piñeiro A, Albarrán A, Rato Nunes JM, Barreto C (2008a) Short and medium-term effects of two-phase olive mill waste application on olive grove production and soil properties under semiarid mediterranean conditions. *Bioresource Technology* **99**, 7982-7987

López-Piñeiro A, Fernández J, Albarrán A, Rato Nunes JM, Barreto C (2008b) Effects of de-oiled two-phase olive mill waste on Mediterranean agricultural soils and the wheat crop. *Soil Science Society of America Journal* **72**, 424-430

López-Piñeiro A, Fernández J, Rato Nunes JM, García A (2006) Response of soil and wheat crop to the application of two-phase olive mill waste to Mediterranean agricultural soils. *Soil Science* **171**, 728-736

López-Piñeiro A, Murillo S, Barreto C, Muñoz A, Rato JM, Albarrán A, García A (2007) Changes in organic matter and residual effect of amendment with two-phase olive-mill waste on degraded agricultural soils. *Science of the Total Environment* **378**, 84-89

López-Piñeiro A, Silva C, Nunes JM, Rozas MA, García A (2002) Effects of solid olive mill waste application on soil properties of irrigated olive grove. In: Rubio JL, Morgan RRC, Asins S, Andreu V (Eds) *Man and Soil at the Third Millennium*, Geoforma, pp 117-1123

Manios T (2004) The composting potential of different organic solid wastes, experience from the island of Crete. *Environment International* **29**, 1079-1089

Mantzavinos D, Kalogerakis N (2005) Treatment of olive mill effluents: Part I. Organic matter degradation by chemical and biological processes – an overview. *Environment International* **31**, 289-295

Mari I, Ehaliotis C, Kotsou M, Balis C (2003) Respiration profiles in monitoring the composting of by-products from the olive oil agro-industry. *Bioresource Technology* **87**, 331-336

Martín J, Sampedro I, García-Romera I, García-Garrido JM, Ocampo JA (2002) Arbuscular mycorrhizal colonization and growth of soybean (*Glycine max*) and lettuce (*Lactuca sativa*) and phytotoxic effects of olive mill residues. *Soil Biology and Biochemistry* **34**, 1769-1775

Martínez-Nieto L, Ramos Cormenzana A, García Pareja MP, Garrido Hoyos SE (1992) Biodegradation de compuestos fenolicos del alpechin con *Aspergillus terreus. Grasas y Aceites* **43**, 75-81

Martirani L, Giardina P, Marzullo L, Sannia G (1996) Reduction of phenol content and toxicity in olive oil mill waste waters with the ligninolytic fungus *Pleurotus ostreatus*. *Water Research* **30**, 1914-1918

Mechri B, Echbili A, Issaoui M, Braham M, Elhadj SB, 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

Mechri B, Ben Mariem F, Baham M, Ben Elhadj S, Hammami M (2008) Change in soil properties and the soil microbial community following land spreading of olive mill wastewater affects olive trees key physiological parameters and the abundance of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry* **40**, 252-161

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

Mekki A, Dhoib A, Sayadi S (2007) Polyphenols dynamics and phytotoxicity in a soil amended by olive mill wastewaters. *Journal of Environmental*

Management **84**, 134-140

Mellouli HJ, Hartmann R, Gabriels D, Cornelis WM (1998) The use of olive mill effluents ("margines") as soil conditioner mulch to reduce evaporation losses. *Soil and Tillage Research* **38**, 85-91

Mocquot B, Vangronsveld J, Clijsters H, Mench M (1996) Copper toxicity in young maize (*Zea mays* L.) plants: Effects on growth, mineral and chlorophyll contents and enzyme activities. *Plant and Soil* **182**, 287-300

Montemurro F, Convertini G, Ferri D (2004) Mill wastewater and olive pomace compost as amendments for rye-grass. *Agronomie* **24**, 481-486

Morales F, Abadia A, Abadia J (1998) Photosynthesis, quenching of chlorophyll fluorescence and thermal energy dissipation in iron-deficient sugar beet leaves. *Australian Journal of Plant Physiology* **25**, 403-412

Moreno E, Pérez J, Ramos-Cormenzana A, Martínez J (1987) Antimicrobial effect of wastewater from olive oil extraction plants selecting soil bacteria after incubation with diluted waste. *Microbios* **51**, 169-174

Morillo JA, Aguilera M, Antízar-Ladislao B, Fuentes S, Ramos-Cormenzana A, Russell NJ, Monteoliva-Sánchez M (2008) Molecular microbial and chemical investigation of the bioremediation of two-phase olive mill waste using laboratory-scale bioreactors. *Applied Microbiology and Biotechnology* **79**, 309-317

Morillo JA, Antízar-Ladislao B, Monteoliva-Sánchez M, Ramos-Cormenzana A, Russell NJ (2009) Bioremediation and biovalorisation of olive-mill wastes. *Applied Microbiology and Biotechnology* **82**, 25-39

Nevens F, Reheul D (2003) The application of vegetable, fruit and garden waste (VFG) compost in addition to cattle slurry in silage maize monoculture: Nitrogen availability and use. *European Journal of Agronomy* **19**, 189-203

Niaounakis M, Halvadakis CP (2004) *Olive-mill Waste Management: Literature Review and Patent Survey*, Typothito-George Dardanos, Athens, 498 pp

Nico AI, Jiménez-Díaz RF, Castillo P (2004) Control of root-knot nematodes by composted agro-industrial wastes in potting mixtures. *Crop Protection* **23**, 581-587

Ntougias S, Russell NJ (2001) *Alkalibacterium olivoapovliticus* gen. nov., sp. nov., a new obligately alkaliphilic bacterium isolated from edible-olive washwaters. *International Journal of Systematic and Evolutionary Microbiology* **51**, 1161-1170

Ntougias S, Kavroulakis N, Zervakis GI, Ehaliotis C, Papadopoulou KK (2008a) Composts deriving from two-phase olive mill wastes (alpeorujo) may be used in potting media and suppress soil-borne pathogens. In: Kalogerakis N, Fava F, Banwart SA (Eds) *Proceedings of the 4th European Bioremediation Conference*, 3-6 September 2008, Technical University of Crete, Chania, Greece, ID 169, pp 1-4

Ntougias S, Papadopoulou K, Zervakis GI, Kavroulakis N, Ehaliotis C (2008b) Suppression of soil-borne pathogens of tomato by composts deriving from agro-industrial wastes abundant in Mediterranean regions. *Biology and Fertility of Soils* **44**, 1081-1090

Ntougias S, Zervakis GI, Fasseas C (2007a) *Olivibacter sitiensis* gen. nov., sp. nov., a novel bacterium isolated from alkaline olive-oil mill wastes in the region of Sitia, Crete. *International Journal of Systematic and Evolutionary Microbiology* **57**, 398-404

Ntougias S, Zervakis GI, Fasseas C (2007b) *Halotalea alkalilenta* gen. nov., sp. nov., a novel osmotolerant and alkalitolerant bacterium from alkaline olive mill wastes, and emended description of the family *Halomonadaceae* Franzmann et al. 1989, emend. Dobson and Franzmann 1996. *International Journal of Systematic and Evolutionary Microbiology* **57**, 1975-1983

Ntougias S, Kavroulakis N, Ehaliotis C, Papadopoulou K, Zervakis GI (2006a) Isolation and screening of indigenous microorganisms capable to detoxify wastes of the olive-oil industry. In: *Proceedings of the 2nd International Seminar on Biotechnology and Quality of Olive Tree Products Around the Mediterranean Basin (OLIVEBIOTEQ 2006)* (Vol II), 5-10 November 2006, Marsala - Mazara del Vallo, Italy, pp 541-544

Ntougias S, Zervakis GI, Ehaliotis C, Kavroulakis N, Papadopoulou KK (2006b) Ecophysiology and molecular phylogeny of bacteria isolated from alkaline two-phase olive mill wastes. *Research in Microbiology* **157**, 376-385

Ntougias S, Ehaliotis C, Zervakis G, Katsaris P, Papadopoulou K (2003) Monitoring the composting process of different agricultural wastes and evaluation of the effects of the final products on plant growth. In: Lekkas TD (Ed) *Proceedings of the 8th International Conference on Environmental Science and Technology*, 8-10 September 2003, University of the Aegean & GlobalNEST, Lemnos, pp 666-673

Olivieri G, Marzocchella A, Salatino P, Giardina P, Cennamo G, Sannia G (2006) Olive mill wastewater remediation by means of *Pleurotus ostreatus*. *Biochemical Engineering Journal* **31**, 180-187

Ouzounidou G, Ilias I (2005) Hormone-induced protection of sunflower photosynthetic apparatus against Cu toxicity. *Biologia Plantarum* **49**, 223-228

Ouzounidou G, Asfi M, Sotirakis N, Papadopoulou P, Gaitis F (2008a) Olive mill wastewater triggered changes in physiology and nutritional quality of tomato (*Lycopersicon esculentum* Mill.) depending on growth substrate. *Journal of Hazardous Materials* **158**, 523-530

Ouzounidou G, Asfi M, Moustakas M (2008b) The impact of olive-mill wastewaters (OMW) on physiology and nutritional value of *Pisum sativum* L. *VI International ISHS Symposium on Mineral Nutrition of Fruit Crops*, 19-23 May, Faro, Portugal, p 84 (Abstract)

Paixao SM, Mendoca E, Picado A, Anselmo AM (1999) Acute toxicity evaluation of olive mill wastewaters: a comparative study of three aquatic organisms. *Environmental Toxicology* **14**, 263-269

Papadimitriou EK, Chatjipavlidis I, Balis C (1997) Application of composting to olive mill wastewater treatment. *Environmental Technology* **18**, 101-107

Papafotiou M, Kargas G, Lytra I (2005) Olive-mill waste compost as a growth medium component for foliage potted plants. *HortScience* **40**, 1746-1750

Papafotiou M, Phsyhalou MK, Chatzipavlidis I, Chronopoulos J (2004) Olive-mill wastes compost as growing medium component for the production of poinsettia. *Scientia Horticulturae* **102**, 167-175

Paredes C, Bernal MP, Cegarra J, Roig A (2002) Bio-degradation of olive mill wastewater sludge by its co-composting with agricultural wastes. *Bioresourse Technology* **85**, 1-8

Paredes C, Bernal MP, Roig A, Cegarra J (2001) Effects of olive mill wastewater addition in composting of agroindustrial and urban wastes. *Biodegradation* **12**, 225-234

Paredes C, Cegarra J, Bernal MP, Roig A (2005) Influence of olive mill wastewater in composting and impact of the compost on a Swiss chard crop and soil properties. *Environment International* **31**, 305-312

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

Paredes C, Roig A, Bernal MP, Sánchez-Monedero MA, Cegarra J (2000) Evolution of organic matter and nitrogen during co-composting of olive mill wastewater with solid organic wastes. *Biology and Fertility of Soils* **32**, 222-227

Paredes MJ, Monteoliva-Sanchez EM, Moreno J, Pérez P, Ramos-Cormenzana A, Martínez J (1986) Effect of waste waters from olive oil extraction plants on the bacterial population of soil. *Chemosphere* **15**, 659-664

Paredes MJ, Moreno E, Ramos-Cormenzana A, Martínez J (1987) Characteristics of soil after pollution with wastewaters from olive oil extraction plants. *Chemosphere* **16**, 1557-1564

Perez JD, Esteban E, Gomez M, Gallardo-Lara F (1986) Effect of wastewater from olive processing on seed germination and early plant growth of different vegetable species. *Journal of Environmental Science and Health* **21**, 349-357

Perez JD, Gallardo-Lara F, Esteban E (1980) Aspectos a considerar en el empleo del alpechin como fertlizante. I. Evaluacion de su efecto fitotoxico inhibitor dela germinacin de semillas. *Cuadernos de Ciencias Biologicas* **1**, 59-67

Perez-Piqueres A, Edel-Hermann W, Alabouvette C, Steinberg C (2006) Response of soil microbial communities to compost amendments. *Soil Biology and Biochemistry* **38**, 460-470

Piperidou CI, Chaidou CI, Stalikas CD, Soulti K, Pilidis GA, Balis C (2000) Bioremediation of olive oil mill wastewater: chemical alterations induced by *Azotobacter vinelandii*. *Journal of Agricultural and Food Chemistry* **48**, 1941-1948

Prasad KVSK, Pardha Saradhi P, Sharmila P (1999) Concerted action of antioxidant enzymes and curtailed growth and zinc toxicity in *Brassica juncea*. *Environmental and Experimental Botany* **42**, 1-10

Quaratino D, D'Annibale A, Federici F, Cereti CF, Rossini F, Fenice M (2007) Enzyme and fungal treatments and a combination thereof reduce olive mill wastewater phytotoxicity on *Zea mays* L. seeds. *Chemosphere* **66**, 1627-1633

Raimbault M, Mazard D (1980) Culture method to study fungal growth in solid fermentation. *European Journal of Applied Microbiology and Biotechnology* **9**, 199-209

Ramos-Cormenzana A (1986) Physical, chemical, microbiological and biochemical characteristics of vegetation water. In: FAO (Ed) *Symposium on Olive By-Products Valorization*, 5-7 March 1986, Seville, pp 19-40

Ramos-Cormenzana A, Juárez-Jiménez B, Garcia-Pareja MP (1996) Antimicrobial activity of olive mill waste-waters (alpechin) and biotransformed olive oil mill wastewater. *International Biodeterioration and Biodegradation* **38**, 283-290

Raviv M (2008) The use of compost in growing media as suppressive agent against soil-borne diseases. *Acta Horticulturae* **779**, 39-49

Rinaldi M, Rana G, Introna M (2003) Olive mill wastewater spreading in southern Italy: effects on a durum wheat crop. *Field Crops Research* **84**, 319-326

Robles A, Lucas R, De Cienfuegos GA, Gálvez A (2000) Biomass production and detoxification of wastewaters from the olive oil industry by strains of *Penicillium* isolated from wastewater disposal ponds. *Bioresource Technology* **74**, 217-221

Roig A, Cayuela ML, Sánchez-Monedero MA (2004) The use of elemental sulphur as organic alternative to control pH during composting of olive mill wastes. *Chemosphere* **57**, 1099-1105

Roig A, Cayuela ML, Sánchez-Monedero MA (2006) An overview on olive mill wastes and their valorisation methods. *Waste Management* **26**, 960-969

Rouvalis A, Iliopoulou-Georgudaki J, Lyberatos G (2004) Application of two microbiotests for acute toxicity evaluation of olive mill wastewaters. *Fresenius Environmental Bulletin* **13**, 458-464

Ruiz JC, de la Rubia T, Perez J, Martínez Lopez J (2002) Effect of olive oil mill wastewater on extracellular ligninolytic enzymes produced by *Phanerochaete flavido-alba. FEMS Microbiology Letters* **212**, 41-45

Saadi I, Laor Y, Raviv M, Medina S (2007) Land spreading of olive mill wastewater: effects on soil microbial activity and potential phytotoxicity. *Chemosphere* **66**, 75-83

Saiz-Jiménez C, Gómez-Alarcón G (1986) Effects of vegetation water on fungal microflora. In: FAO (Ed) *International Symposium on Olive By-Products Valorization*, 5-7 March 1986, Seville, pp 61-68

Sampedro I, Cajthaml T, Marinari S, Petruccioli M, Grego S, D'Annibale A (2009) Organic matter transformation and detoxification in dry olive mill residue by the saprophytic fungus *Paecilomyces farinosus*. *Process Biochemistry* **44**, 216-225

Sampedro I, Aranda E, Díaz R, García-Sanchez M, Ocampo JA, García-Romera I (2008) Saprobe fungi decreased the sensitivity to the toxic effect of dry olive mill residue on arbuscular mycorrhizal plants. *Chemosphere* **70**, 1383-1389

Sampedro I, D'Annibale A, Ocampo JA, Stazi SR, García-Romera I (2007a) Solid-state cultures of *Fusarium oxysporum* transform aromatic components of olive-mill dry residue and reduce its phytotoxicity. *Bioresource Technology* **98**, 3547-3554

Sampedro I, Marinari S, D'Annibale A, Grego S, Ocampo JA, García-Romera I (2007b) Organic matter evolution and partial detoxification in two-phase olive mill waste colonized by white-rot fungi. *International Biodeterioration and Biodegradation* **60**, 116-125

Sampedro I, D'Annibale A, Ocampo JA, Stazi SR, García-Romera I (2005) Bioconversion of olive-mill dry residue by *Fusarium lateritum* and subsequent impact on its phytotoxicity. *Chemosphere* **60**, 1393-1400

Sampedro I, Aranda E, Martín J, García-Garrido JM, García-Romera I, Ocampo JA (2004a) Saprobic fungi decrease plant toxicity caused by olive mill residues. *Applied Soil Ecology* **26**, 149-156

Sampedro I, Romero C, Ocampo JA, Brenes M, García-Romera I (2004b) Removal of monomeric phenols in dry mill olive residue by saprobic fungi. *Journal of Agricultural and Food Chemistry* **52**, 4487-4492

Sánchez-Monedero MA, Cayuela ML, Mondini C, Serramiá N, Roig A (2008) Potential of olive mill wastes for soil C sequestration. *Waste Management* **28**, 767-773

Sanjust E, Pompei R, Rescigno A, Rinaldi A, Ballero M (1991) Olive milling wastewater as a medium for growth of four *Pleurotus* species. *Applied Biochemisry and Biotechnology* **31**, 223-235

Sasanelli N, Greco P, D'Addabbo T, Coiro MI, Lamberti F (2003) The use of olive mill wastes for the control of root-knot nematodes. *Communications in Agricultural and Applied Biological Sciences* **68**, 135-138

Sayadi S, Allouche N, Jaoua M, Aloui F (2000) Detrimental effects of high molecular-mass polyphenols on olive mill wastewaters biotreatments. *Process Biochemistry* **35**, 725-735

Sayadi S, Ellouz R (1993) Screening of white rot fungi for the treatment of olive mill waste-waters. *Journal of Chemical Technology and Biotechnology* **57**, 141-146

Scioli C, Vollaro L (1997) The use of *Yarrowia lipolytica* to reduce pollution in olive mill wastewaters. *Water Research* **31**, 2520-2524

Segarra G, Casanova E, Borrero C, Avilés M, Trillas I (2007) The suppressive effects of composts used as growth media against *Botrytis cinerea* in cucumber plants. *European Journal of Plant Pathology* **117**, 393-402

Sierra J, Marti E, Montserrat G, Cruaas R, Garau MA (2001) Characterisation and evolution of a soil affected by olive oil mill wastewater disposal. *Science of the Total Environment* **279**, 207-214

Smith SE, Read DJ (1997) *Mycorrhizal Symbiosis*, Academic Press, San Diego, 605 pp

Taccari M, Stringini M, Comitini F, Ciani M (2009) Effect of *Phanerochaete chrysosporium* inoculation during maturation of co-composted agricultural wastes mixed with olive mill wastewater. *Waste Management* **29**, 1615-1621

Takashi Y, Hatano T, Miyamoto K, Okuda T (1994) Antitumor and related activities of ellagitannin oligomers. *Polyphénols Actualités* **11**, 26-29

Tamburino V, Zimbone SM, Quattrone P (1999) Storage and land application of olive-oil wastewater. *Olivae* **76**, 36-45

Tomati U, Di Lena G, Galli E, Grappelli A, Buffone R (1990) Indolacetic acid production from olive wastewaters by *Arthrobacter* spp. *Agrochimica* **34**, 223-228

Tomati U, Galli E (1992) The fertilizer value of waste waters from the olive processing industry. In: Kubat J (Ed) *Humus its Structure and Role in Agriculture and Environment*, Elsevier Science Publishers B.V., Amsterdam, pp 117-126

Tomati U, Galli E, Fiorelli F, Pasetti L (1996) Fertilizers from composting of olive-mill wastewaters. *International Biodeterioration and Biodegradation* **44**, 155-162

Tsioulpas A, Dimou D, Iconomou D, Aggelis G (2002) Phenolic removal in olive oil mill wastewater by strains of *Pleurotus* spp. in respect to their phenol oxidase (laccase) activity. *Bioresource Technology* **84**, 251-257

Vassilev N, Fenice M, Federici F, Azcon R (1997) Olive mill waste water treatment by immobilized cells of *Aspergillus niger* and its enrichment with soluble phosphate. *Process Biochemistry* **32**, 617-620

Vassilev N, Vassileva M, Azcon R, Fenice M, Federici F, Barea JM (1998) Fertilizing effect of microbially treated olive mill wastewater on *Trifolium* plants. *Bioresource Technology* **66**, 133-137

Vlyssides AG, Bouranis DL, Loizidou M, Karvouni G (1996) Study of a demonstration plant for the co-composting of olive-oil processing wastewater and solid residue. *Bioresource Technology* **56**, 187-193

Wahid A, Ghazanfar A (2006) Possible improvement of some secondary metabolites in salt tolerance of sugarcane. *Journal of Plant Physiology* **163**, 723-730

Wang SY, Ballington JR (2007) Free radical scavenging capacity and antioxidant enzyme activity in deerberry (*Vaccinium stamineum* L.). *LWT-Food Science and Technology* **140**, 1352-1361

Wright DP, Scholes JD, Read DJ (1998) Effects of VA mycorrhizal colonization on photosynthesis and biomass production of *Trifolium repens* L. *Plant Cell and Environment* **21**, 209-216

Yangui T, Rhouma A, Triki MA, Gargouri K, Bouzid J (2008) Control of damping-off caused by *Rhizoctonia solani* and *Fusarium solani* using olive mill waste water and some of its indigenous bacterial strains. *Crop Protection* **27**, 189-197

Yesilada O, Sik S, Sam M (1998) Biodegradation of olive oil mill wastewater by *Coriolus versicolor* and *Funalia trogii*: Effects of agitation, initial COD concentration, inoculum size and immobilization. *World Journal of Microbiology and Biotechnology* **14**, 37-42

Yurekli F, Yesilada O, Yurekli M, Topcuoglu SF (1999) Plant growth hormone production from olive oil mill and alcohol factory wastewaters by white rot fungi. *World Journal of Microbiology and Biotechnology* **15**, 503-505

Zervakis G, Yiatras P, Balis C (1996) Edible mushrooms from olive mill wastes. *International Biodeterioration and Biodegradation* **38**, 237-243

Zouari N, Ellouz R (1996) Microbial consortia for the aerobic degradation of aromatic compounds in olive oil mill effluent. *Journal of Industrial Microbiology* **16**, 155-162