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Mitigation of Olive Mill Wastewater Toxicity

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The toxicity of olive mill wastewaters (OMW) is commonly attributed to monomeric phenols. OMW were treated in an aerated, stirred reactor containing agricultural soil, where the oxidative polymerization of phenols took place. In 24 h, OMW monomeric phenols decreased by >90%. This resulted in a corresponding reduction in phytotoxicity, as measured by germination tests with tomato and English cress seeds, and in microbial toxicity, as measured by lag phase duration in *Bacillus cereus* batch growth. Soil germination capability after irrigation with OMW was assessed in long-term pot experiments. The relative germination percentage 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, OMW.

KEYWORDS: Olive mill wastewaters (OMW); dephenolization; phytotoxicity; microtoxicity

INTRODUCTION

Most of the world's olive oil production is localized in the Mediterranean basin. Over 2.5 million tons of olive oil is produced yearly in this region in a limited time span (typically in autumn and part of the winter).

Two milling techniques are used, namely, a three-phase systems that produce oil, olive mill wastewaters (OMW), and olive husk (i.e., the solid residue) and a two-phase system that yields oil and a highly hydrated husk. The former, traditional technique is widespread in the south bank, as well as in many small-scale mills in the north bank. The latter technique is usually adopted in large-scale facilities in Spain as well as in Italy. Throughout the present paper, the term OMW refers to only the liquid residue from three-phase mills.

Approximately 30 million cubic meters of OMW per year (*1*) are released and must be disposed of. OMW cannot be disposed of directly in sewage systems because of the high COD (50000−200000 mg/L) and polyphenol content. The deliberate or accidental introduction of large amounts of OMW into urban sewage treatment plants might be catastrophic because of their highly toxic effect on microbial growth (*2*). The anaerobic digestion in dedicated reactors also requires the preemptive removal of toxic polyphenols (*3*, *4*).

Cost-effectiveness of OMW treatment is crucial, because of the relatively low added-value of olive oil and because its production is distributed into small, low-technology units. As a consequence, the direct spreading of OMW onto the soil is the most common disposal technique. Overall OMW amounts released per year and hectare are regulated by local laws. The spreading of OMW onto the agricultural soil has become an accepted practice in Italy according to law n.574/96. The law fixes upper limits to the cubic meters of OMW that can be spread per year per hectare of agricultural field. The procedure relies upon the natural attenuation of polyphenols in soil. On the other hand, OMW contain organic matter (sugars, proteins), mineral salts, and nutrients of potential interest for fertigation, which would be dissipated were OMW merely regarded as a waste.

OMW spreading, however, is by no means a response to irrigation or fertigation requirements. Indeed, (i) it is carried out in the rainy season; (ii) the huge amounts of water involved might exceed by far the actual irrigation needs, even the more so if previous dilution must be performed; and (iii) spreading OMW onto soil might pollute groundwater and surface waters, thus disseminating the problem over an even larger area (*5*).

OMW are toxic toward plants and microorganisms, including soil microflora (*6*−*8*). Even though the high salt content and the relatively low pH of OMW might be phytotoxic and have negative effects on soil biological properties (*9*, *10*), it is common opinion that OMW toxicity is essentially due to monomeric phenols (*11*, *12*). Severe phytotoxic effects may occur on higher plants mainly during germination and seedling development, due to the enhancing action of phenolic compounds on seed dormancy (*7*). Antibacterial activity by phenolics has been demonstrated, as well (*8*, *13*, *14*). Therefore, most proposed detoxification treatments are focused on monomeric phenol removal (*15*, *16*). The effectiveness of any detoxification

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treatment is usually evaluated by measuring the reduction in the content of monomeric phenols, upon treatment.

In previous papers (*17*, *18*), we proposed an extremely simple and low-technology technique for the dephenolization of a model system simulating OMW. As shown in refs *17* and *18*, when solutions of model OMW monomeric phenols are put into contact with a water suspension of agricultural soil within an aerated, stirred reactor, a fast decay in phenol concentration ensues. The treatment promotes phenol oxidation via a corresponding reduction of soil manganese and iron oxides. The reduced metals give rise to the immediate formation of metal−phenol complexes that break down in the presence of oxygen. This yields radicals that endorse the polymerization of monomeric phenols. That polymerization actually takes place is indicated by total organic carbon (TOC) measurements performed on samples drawn from the soil reactor, before and after filtration on 0.2 μm filters. Obviously, the contribution of soil to TOC was taken into account. Incidentally, it was demonstrated (*17*) that the observed decay in the concentration of monomeric phenols cannot be attributed to the adsorption on soil.

The basic points of the discussion that follows are (i) to analyze the applicability of the technique to a real OMW system, (ii) to evaluate the results through a comprehensive analysis of both microbial toxicity and phytotoxicity, and (iii) to simulate the medium-term effect of treated OMW spreading onto a soil in terms of its germination capability.

By the way, the phenomena discussed in the present paper mimic, at least to some extent, those occurring in superficial, aerated layers of an agricultural soil when OMW are spread. It should be noted, however, that the time scale of the percolation phenomena is probably shorter than that required to bring about a substantial conversion of the phenolics.

MATERIALS AND METHODS

Analytical Methods and Characteristics of OMW. OMW were from a three-phase olive mill in Puglia (southern Italy). Prior to any further analytical procedure, in order to get rid of the small, residual solid fraction still present, OMW samples were centrifuged at 10000 rpm for 15 min and subsequently filtered (Sartorius cellulose acetate, 0.2 μm). The relevant properties of the OMW were determined by using standard methods (*19*), and each sample was analyzed in triplicate.

The pH and electrical conductivity (dS m^{-1}) of OMW samples were measured using a pH-meter (Hanna Instruments, Hi 9017 electrode CW711) and conductivimeter (Hanna Instruments, Hi 8733), respectively. Cations and anions were determined by atomic absorption spectrometry (Perkin-Elmer Analyst 700), ion chromatography (Dionex DX-120), and a CD20 conductivity detector combined with autosuppression. The total sugar and protein contents were measured according to anthrone (*20*) and biuret (*21*) methods, respectively.

Measurements of the total phenolic content of OMW were performed by both the Folin method (*22*) and HPLC analyses. All analytical determinations were carried out (i) on whole OMW, (ii) on the ethyl acetate extractable fraction (extract), and (iii) on the ethyl acetate nonextractable fraction (exhausted).

Fifty milliliters of OMW was funnel extracted with ethyl acetate (4 × 50 mL), and the organic extracts were combined, dried over sodium sulfate, and evaporated under reduced pressure. The number of extractions (four) was based on the results obtained from thin layer chromatography (TLC).

In Folin method determinations, overall phenol content was evaluated by using the Folin−Ciocalteu reagent (*22*). OMW were centrifuged, filtered at 0.2 μm cutoff, and diluted by 1:10 ratio. Then 0.02 mL of diluted OMW sample was added to 0.83 mL of distilled water together with 0.05 mL of Folin−Ciocalteu reagent (Sigma-Aldrich). After 3 min, reaction was blocked by the addition of 0.10 mL of 6% NaOH (w/v). After 1 h, optical density at 725 nm was measured. The method was calibrated with catechol solutions of known concentration (as suggested in ref *23*). The results were expressed in terms of phenolic concentration (grams per liter).

HPLC analyses were carried out on samples filtered with low phenol absorption cellulose acetate membranes (0.2 μm cutoff, Sartorius, Göttingen, Germany). Use was made of an Agilent HP1100 instrument equipped with a diode array detector and a Zorbax Eclipse XDB-C18 column, 25 cm by 4.6 mm i.d. with a 5 μm particle size.

An efficient gradient of distilled water containing 0.1% v/v of H_3PO_4 (solvent A) and a mix of acetonitrile (70%) and distilled water (solvent B) was used, according to the following elution program: isocratic elution with 85% (A) and 15% (B) for 5 min; gradient to 50% (A) and 50% (B) in 35 min; gradient to 100% (B) in 10 min; gradient to 85% (A) and 15% (B) in 10 min; isocratic elution 85% (A) and 15% (B) for 5 min. The phenolic compounds were identified on the basis of their retention times and their spectra in comparison with standards.

OMW Treatment in Soil Slurries. All reaction experiments were carried out at 20 °C in stirred soil−slurry reactors, as described in Colarieti et al. (*17*, *24*), under continuous air sparging. Two hundred and forty grams of dry soil was added per liter of raw OMW (noncentrifuged and nonfiltered). An agricultural soil from Castelvolturno (Caserta, Italy) was used throughout.

In Colarieti et al. (*17*) it was shown that soil nature and composition are quite immaterial, as far as the efficacy of the treatment is concerned, provided iron and manganese oxides are present. Nonetheless, an extensive characterization of the soil was carried out. The chemical analyses were performed on air-dried and sieved (<2 mm) soil samples by standard methods (*25*). The soil is a clay soil (clay, 48%; sand, 20%; silt, 32%) with alkaline reaction (pH 8.2) and a high content of organic carbon (27.3 g kg^{-1}), a great available phosphate level (55 mg kg^{-1}), and low quantities of available cations (Ca, K, Na, etc.). It can be classified as Vertic Xerofluvent according to USDA Soil Taxonomy (*26*). The soil had high levels of both extractable Mn (473 mg kg^{-1}) and Fe (192 mg kg^{-1}) and therefore appears to be particularly suitable for the treatment.

The reactor consisted of a Pyrex-glass vessel (overall volume = 220 mL) equipped with a two-blade impeller driven by a small electric motor (220 rpm). Ports were present for suspension sampling and for continuous gas sparging and venting. Gas flow rates were measured by a bubble flow meter. Under the adopted experimental conditions, the rate of oxygen gas−liquid mass transfer was sufficient to ensure near-equilibrium values for dissolved oxygen concentration, even in the presence of oxygen-consuming reactions (*17*, *24*).

Two milliliters of slurry samples was drawn periodically. Prior to any further determination, the samples were centrifuged at 10000 rpm for 15 min. The supernatant was then filtered with the 0.2 μm cutoff cellulose acetate membranes.

Reference runs in the absence of soil were performed, as well.

Typical overall treatment times lasted 24 h. A medium-small olive mill produces 1 m^3 of OMW per day. Therefore, this is the associated reactor size.

Microtoxicity Tests. At a preliminary stage, halo zone tests, according to Baer et al. (*27*), were performed on *Bacillus megaterium* in a Stainer medium recommended for recovery and enumeration of soil bacteria.

The agar medium was pre-inoculated at 50 °C with 0.5 mL of a *B. megaterium* overnight culture. Each Petri dish contained three or four steel cylinders, 1 cm in diameter and 1 cm in length. Upon medium solidification, 100 mL of either treated or untreated OMW was added in each well. The dishes were incubated in darkness at 4 °C for 4 h and then at 28 °C for 24 h. The antibacterial activity was determined as bacterial growth inhibition area.

In all further experiments, the microbial toxicity was determined according to the following experimental procedure. Briefly, the technique consists of the incubation of 2.5 mL of growth medium (50 g L^{-1} peptone, 30 g L^{-1} meat extract) added with 22 mL of diluted OMW samples (treated and untreated, respectively). The samples were inoculated with 0.5 mL of a diluted overnight *Bacillus cereus* (6E/2) preculture (optical density at 600 nm OD_{600nm} = 1.2) at 37 °C, directly drawn from the agar plate.

Table 1. Composition of OMW

chemical	concn, g L^{-1}
phenols	4.50
proteins	1.97
sugars	17.1

Samples were periodically withdrawn and their OD_{600nm} values measured. Reference runs were carried out with distilled water in place of OMW.

Analysis of the growth curve yields two parameters: the lag time (h) and the specific rate (h^{-1}) in the exponential phase.

Germination Tests. Germination tests were carried out in triplicate on *Lycopersicon esculentum* (tomato) and *Lepidium sativum* (English cress) seeds. Usually, 20 seeds were incubated for 5−7 days (tomato) or 3 days (cress) at 25 ± 1 °C in the dark over Whatman no. 1 paper filters in 90 mm Petri dishes. Paper filters were wet with 5 mL of OMW samples. Control tests were performed with paper filters wet with distilled water. A primary root >2 mm was considered as the end germination point.

The relative germination percentage (RGP) was calculated for each treatment as $RGP = 100(G_s/G_c)$, where G_s and G_c are the numbers of roots germinated in the sample and control, respectively. The germination index (GI) was also calculated as $GI = 100(G_s/G_c)(L_s/L_c)$, where L_s and L_c are the mean root length in the sample and in the control, respectively.

Pot Experiments. Pot experiments were performed according to the method of Piotrowska et al. (*28*). Briefly, the soil (100 g) was placed in plastic pots and supplemented with 36 mL of untreated OMW. This amount corresponds to a field rate of 80 m^3 ha^{-1}, that is, to the maximum allowed amount of OMW to be spread onto agricultural soil according to the Italian legislation (law n.574, 1996). Thus, a moisture content of ≈50% of the WHC was reached. Control runs were carried out on soil supplemented with 36 mL of distilled water. Samples were incubated under controlled conditions of humidity and temperature, in a climatic chamber at 25 °C, in the dark, and periodically analyzed. Each sample was replicated four times. At any given incubation time, germination tests were performed on *L. esculentum* (tomato) as described above with 8 g of control soil and soil plus untreated OMW. In the paper by Piotrowska et al. (*28*), germination experiments were performed in triplicate and only RGP values were determined. For comparison purposes, in the present paper the same procedure was applied to treated OMW.

RESULTS AND DISCUSSION

OMW Treatment in Soil Slurries. *Physicochemical Properties.* The basic physicochemical properties of OMW can be summarized as follows: pH 4.9; electrical conductivity, 11.6 dS m^{-1}; Na, 0.045 g L^{-1}; K, 3.5 g L^{-1}; Ca, 0.03 g L^{-1}; Mg, 0.03 g L^{-1}.

Table 1 reports the composition (grams per liter) of whole OMW in terms of "phenols", proteins, and sugars.

With regard to the determination of OMW phenolic content, it was performed according to the Folin method (*22*). This is an almost universal technique, adopted in most papers dealing with OMW dephenolization (*23*). It should be noted that the overall phenolic concentration is expressed, as usual, in terms of grams of a pure, reference phenol per liter of OMW, independently of the actual OMW composition. In our case, the photometric Folin reading has been worked out adopting the extinction coefficient pertaining to catechol as suggested in Catalano et al. (*23*).

Table 2 reports the distribution of the Folin reading between the extract (containing prevalently monomeric phenols) and the exausted fraction of OMW (containing polymeric phenols and other phenolic compounds such as phenyl glucosides) in terms of catechol concentration. The corresponding data obtained on OMW samples treated with soil for 24 h are reported, as well.

Table 2. Folin Reading of Treated and Untreated Whole OMW and Their Extract and Exhausted Fractions

	Folin reading, g L^{-1}		
sample	untreated	treated	reduction, %
whole OMW	4.50	2.10	53
extract	0.90	0.10	89
exausted	3.30	1.80	45

Table 3. Major Monomeric Phenols of Untreated and Treated OMW As Determined by HPLC Analysis

		untreated OMW		treated OMW		
phenol	retention time, min	area	concn, g L^{-1}	area	concn, g L^{-1}	reduction, %
hydroxytyrosol	11.9	11028	0.649	88.6	5.1×10^{-3}	99
3,4-dihydroxy-benzoic acid	12.8	817	0.011	557	7.0×0^{-3}	32
tyrosol	17.8	1907	0.086	662	0.030	65
caffeic acid	21.8	2670	0.023	272	2.0×10^{-3}	90
coumaric acid	28.6	999	7×10^{-3}	328	2.0×10^{-3}	67
total		17421	0.776	1908	0.045	93

The results are internally consistent, because the sum of the Folin readings of both the extract and the exausted is in good agreement with the results on the whole OMW samples.

As to the measurement of the actual OMW phenolic content, it should be noted that the best part of the Folin reading is localized in the exhausted fraction. Therefore, it cannot be due to monomeric, extractable phenols. Even when more exhaustive extraction procedures were used (data not shown), the Folin reading of the extract did not reach >30% of that measured with the whole OMW.

To explain this result, one could speculate on a possible interference by proteins that are present at 1.97 g L^{-1} and do supply a Folin reading. This cannot be entirely the case because measurements on albumin solutions at this concentration yield a Folin reading of 0.073 g L^{-1}.

Capasso (*29*) and Capasso et al. (*30*) demonstrated that polymeric phenols and phenyl glucosides are contained in OMW at high concentration. Both contribute to the Folin reading, are localized in the exhausted fraction, and could provide, at least partly, reason for the results reported in **Table 2**.

A detailed analysis by HPLC on the extracts of both whole and treated OMW was carried out. The results are reported in **Table 3**.

Pure component tests enabled the identification of the major monomeric phenol components (i.e., those individually contributing >4% to the overall chromatogram area) according to the retention time (**Table 3**). Individual concentrations were calculated on the basis of the extinction coefficient measured with pure component solutions.

The HPLC results on extracts are in excellent agreement with those of **Table 2** (total phenol amount = 0.776 g L^{-1} against 0.900 g L^{-1}).

One could reasonably assume that the actual concentration of monomeric phenols in untreated OMW is 0.776 g L^{-1}, that is, that localized in the extract, as determined by HPLC.

With regard to the main point of the research, that is, the reduction in monomeric phenol content produced by the treatment, it is apparent that a drastic drop in the overall phenolic content is achieved, as indicated by the 92.8% reduction in the

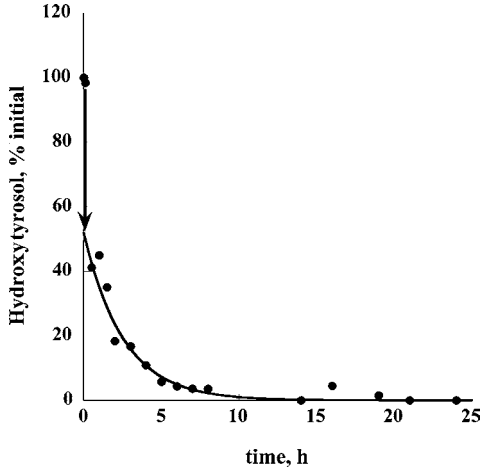

Figure 1. Hydroxytyrosol decay during OMW treatment.

extract when determined by HPLC (89% by Folin). These results, obtained with real, whole OMW, agree with those discussed in Colarieti et al. (*17*, *24*) with reference to model solutions of monomeric phenols.

The different reductions undergone by each individual phenol reported in **Table 3** stem from different reactivities in the oxidative polymerization promoted by soil manganese and iron oxides (*17*, *31*−*33*). As found in ref *18*, *o*-diphenols are rapidly converted in soil slurries by a prevalently abiotic reaction.

In our OMW samples, hydroxytyrosol is the key phenol, because it accounts for approximately two-thirds of the overall chromatogram area. **Figure 1** reports the hydroxytyrosol decay in the course of OMW treatment. All of the features of hydroxytyrosol decay match with those already observed with *o*-diphenols in soil slurries (*17*, *18*, *24*), namely, (i) a sudden, initial drop in concentration, (ii) first-order kinetics, and (iii) almost complete conversion achieved in <5 h. This strongly supports the polymerization mechanism already demonstrated with model *o*-diphenols. A dominating biotic contribution associated with slower reaction has been found for monophenols (*18*). In any case, full removal of monomeric phenols is attained in 24 h.

OMW Toxicity. Experiments were performed by measuring the germination index, according to the technique discussed under Materials and Methods, with both untreated and treated OMW samples at different dilutions, on both tomato and English cress. The results are reported in **Figure 2** in terms of germination index versus the OMW percent amount used in the test. A typical dose−response behavior is followed.

Higher GI values of the treated sample, as compared to those pertaining to the untreated one, are observed at any given sample percentage. This effect holds true for both tomato and cress and shows that the soil treatment is quite effective in reducing OMW phytotoxicity along with the reduction in phenol concentration.

The data of **Figure 2** have been replotted in terms of germination index versus overall major monomeric phenolics concentration (grams of phenols per liter) in the sample. As already stated, according to the HPLC readings of the extract reported in **Table 3**, the phenol concentration of undiluted, untreated OMW is 0.776 g L^{-1}. That of the undiluted, treated samples is 0.045 g L^{-1}.

The results are reported in **Figure 3** for tomato and cress, respectively.

Provided that the phytotoxicity depended only on the overall phenolic content, one would expect that, for either vegetable, the GI should be univocally related to the overall phenol

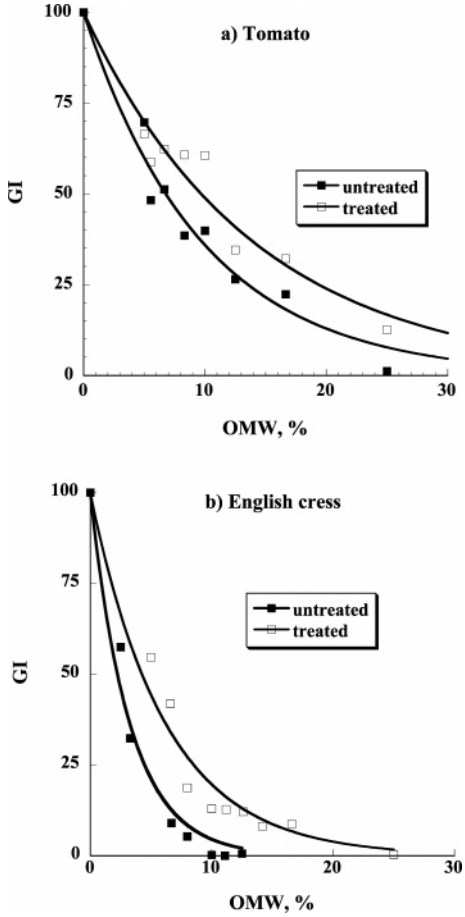

Figure 2. Germination indices (GI) of (**a**) tomato and (**b**) English cress in the presence of untreated and treated OMW at different percentages.

concentration independent of the way it has been achieved starting from the original sample, for example, by dilution or by soil treatment. As a consequence, the data for the untreated and treated samples should be correlated by the same curve.

The detoxification achieved by the treatment, however, is by far lower than that associated with the observed reduction in phenol concentration. This implies that toxic components other than monomeric phenols are still present in OMW.

With regard to the microtoxicity, the tests performed with *B. megaterium* by measuring the bacterial growth inhibition zone with treated and untreated OMW, respectively, resulted in a limited reduction of the inhibition zone upon treatment (<27%). In light of the following results, the technique appears to be rather insensitive. Therefore, all further microtoxicity tests were performed according to the growth-curve technique with *B. cereus* described under Materials and Methods. Typical growth curves are reported in **Figure 4**.

It can be seen that the specific growth rates are unaffected by the presence of OMW, whereas a marked increase is produced in the time lag.

It is apparent that OMW microtoxicity is strongly reduced by the soil treatment, as indicated by the data of the treated sample being very close to those of the reference, distilled water run.

Figure 5 reports a summary of the experimental results, in terms of time lag versus percent of OMW for both untreated and treated OMW.

The strong reduction in microtoxicity produced by soil treatment is apparent, because the time lag of the growth curve

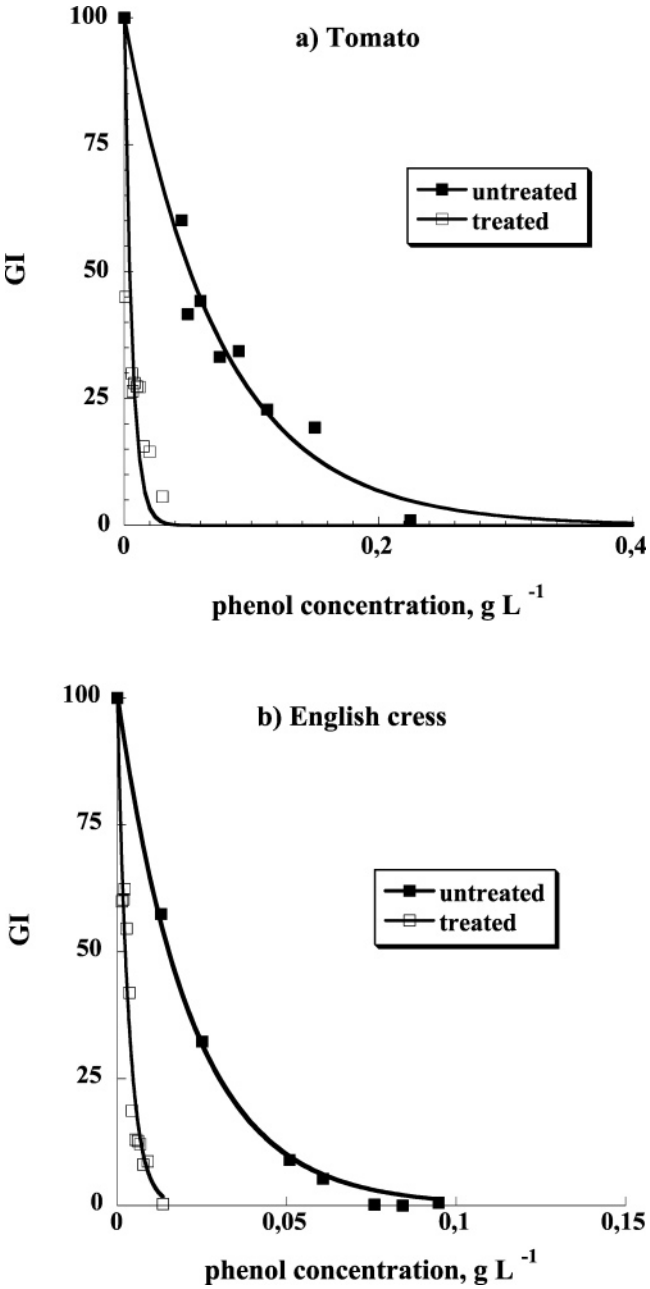

Figure 3. Germination indices (GI) of (**a**) tomato and (**b**) English cress in the presence of untreated and treated OMW as a function of monomeric phenol concentration.

in the presence of treated OMW samples is by far lower that that of untreated OMW at equal dilution.

In **Figure 6** the data of **Figure 5** have been replotted in terms of time lag versus overall phenol concentration according to the same procedure adopted in producing **Figure 3**.

The data of both untreated and treated samples are correlated by the same curve. This suggests that microtoxicity is indeed due to monomeric phenols only and, therefore, that the virtually complete removal of the latter, as achieved by the soil treatment, results in a corresponding complete detoxification.

Pot Experiments. Provided that the results achieved with *B. cereus* could be extended to most other soil microorganisms, the treated OMW could be safely disposed of by spreading them onto soil. Thus, the fertilizing potential of OMW could be entirely exploited with minor, if any, negative effects on the soil fertility.

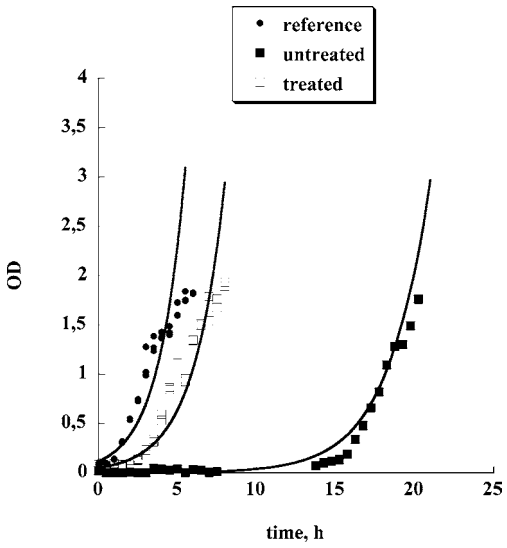

Figure 4. Typical growth curves of *B. cereus* in the presence of untreated and treated OMW.

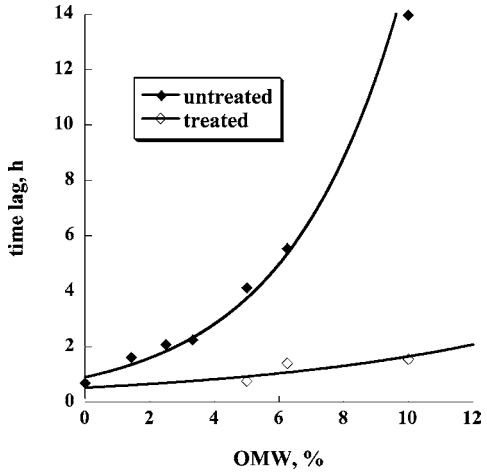

Figure 5. Time lag values (h) of *B. cereus* growth curves in the presence of different percentages of untreated and treated OMW.

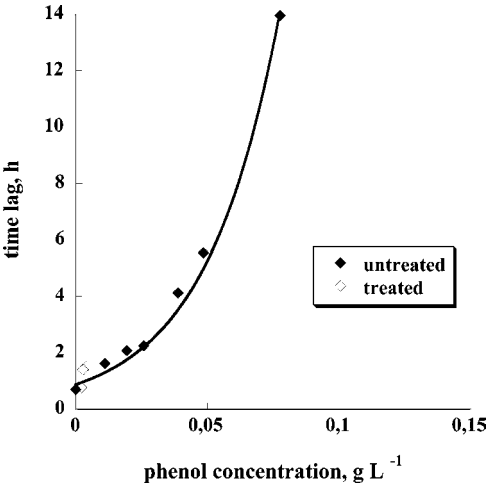

Figure 6. Time lag values (h) of *B. cereus* growth curves in the presence of untreated and treated OMW as a function of monomeric phenol concentration.

The evaluation of the effectiveness of a detoxification process of wastes, however, should not only look at pollutant removal but also monitor whether and how soil biological functions are affected by the detoxified waste.

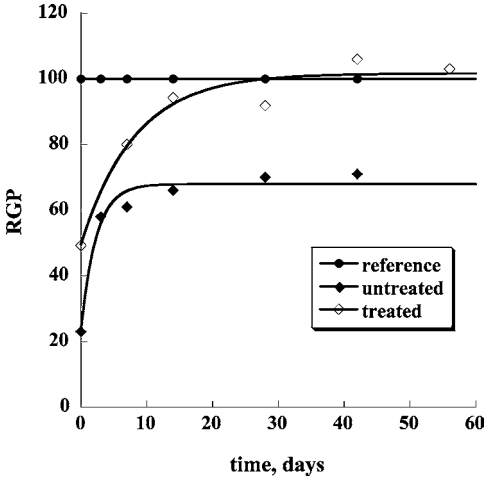

Figure 7. RGP of control and OMW- and treated-OMW-amended soils. The amounts of OMW added correspond to 80 m^3 ha^{-1} OMW.

In a previous paper (*28*), several chemical and biochemical soil properties were examined to demostrate the short-term self-regenerating potential of a soil subjected to OMW spreading. Particular attention was devoted to the soil germination capability in the presence of OMW.

Figure 7 reports the RGP values obtained in the course of pot experiments performed with treated OMW and compared to those already published in Piotrowska et al. (*28*) with water and untreated OMW, respectively.

It should be noted that (i) soil does not entirely recover its germination capability in the time scale of the experiment when OMW are spread at the maximum allowed amounts and (ii) OMW treatment results in a complete long-term recovery and in a relevant increase of RGP values at any time.

The residual higher phytotoxicity of the treated OMW at short incubation times is still indicative that toxic OMW components, that is, other than monomeric phenols, not eliminated by the treatment might be responsible of the lower germination capability of the soil. A possible negative influence of soil EC could also be ruled out, because nonsubstantial changes of their values occurred upon treated and untreated OMW application (data not shown).

In conclusion, the results presented here confirm that a simple treatment, using an agricultural, easily available soil applied to OMW, was very effective in strongly decreasing the OMW monomeric phenols content.

The remarkable decrease of monomeric phenols did not result, however, in a corresponding reduction of toxicity toward germination of both tomato and cress seeds. Although the specific biochemistry of toxic action is still not clear, several organic chemicals including phenols might exhibit a narcosis mode of toxic action, which is mainly due to the non-covalent interaction at membrane levels. As assessed by Wang et al. (*34*), the phytotoxicity of phenols to higher plants such as *Cucumis sativus* was separately modeled in term of narcosis and bioreactivity. Possibly, other components, unaffected by the applied detoxification treatment, are still present in the treated OMW and negatively affect the germination of plant seeds.

Furthermore, possible toxic effects of intermediate byproducts of phenol transformation, that is, dimers, trimers, and low molecular weight polymers, cannot be excluded.

Conversely, a complete abatement of bacterial toxicity was observed, as assessed by *B. cereus* growth tests. As demonstrated by Park et al. (*35*) and Colarieti et al. (*18*), polymerization strongly decreases the antibacterial toxicity of the monomeric, phenolic precursors. Indeed, phenolic compounds are predominantly membrane-active agents. They may damage the cell membrane, causing the release of intracellular components and the intracellular coagulation of cytoplasmic constituents. Cell death or cell growth inhibition may result (*36*). In contrast, polymeric phenolic derivatives have an extremely slow diffusion and most of the damaging phenomena are consistently depressed.

The residual toxicity of the treated OMW toward soil germination capability, shown in the pot experiments at low incubation times compared to untreated OMW (**Figure 7**), also indicates that the removal of monomeric phenols is not sufficient to detoxify the waste completely. Previous investigations (*28*) demonstrated that temporary and permanent changes in several chemical and biochemical soil properties occurred upon OMW addition. It was concluded that the impact of OMW on soil properties was the result of conflicting effects, depending on the relative amounts of beneficial and toxic organic and inorganic compounds present (*28*).

The complete recovery of soil germination capability observed at long incubation times might suggest that the toxic compounds, still present in the treated OMW, could have been removed by adsorption/entrapment phenomena on/in the soil matrix as well as by further soil biotic and abiotic transformations.

ABBREVIATIONS USED

OMW, olive mill wastewaters; GI, germination index; RGP, relative germination percentage; OD, optical density; HPLC, high-performance liquid chromatography; TLC, thin layer chromatography; WHC, water-holding capacity.

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