Valorization of the olive sector effluents as potential fertilizers and their impact on biological, physical and chemical properties of the soil

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The olive mill wastewater (OMW), mill effluent, is accumulated from one year to another, and poses a real environmental problem due to its high polluting power. Current researches in several Mediterranean centers have shown the interest of the soil application of OMW, looking for solving the problem of the disposal of such waste and the nutrition of perennial crops such as olive tree. This is for the sake of preserving the environment and improving the agricultural products quality. The OMW, when applied in adequate quantity and mode, can be used as a fertilizer and improves the nutritional status the olive groves. It contains appreciable amounts of nutrients such as potassium, necessary for the development of the fruit, values of nitrogen, phosphorus, and magnesium of worthy consideration. Their levels of inorganic substances are favourite to the development of microflora soil, improving its physical and chemical properties with respect to its ability to retain water and mineral salts.

Key words: OMW, soil application, environment preservation, products quality, adequate quantity and mode.

INTRODUCTION

The olive oil world production is estimated to 2 million tons per year. After Spain, Italy and Greece, Tunisia is the fourth producer of olive oil. The olive oil culture with its mills is an integral heritage to which many Tunisians are greatly attached to. But, it is clear that this ancient and traditional activity, in Tunisia triturating the olives into oil (traditional and modern oil mills activity), must now comply with the new laws and regulations concerned with pollution control and environmental protection.

The increase in production and the introduction of modern techniques of oil extraction (continuous process) during the last decade have led to a rapid accumulation of wastewater "OMW" from olive mills. These effluents come from 50% of the fruit and the rest from the used water needed to oil extraction. The Mediterranean production of wastewater was in average about 10-16 million m³/year, with 2 to 3 million m³/year for Spain (Cabrera et al., 1996), 2 x 10⁶ m³/year for Italy. In Tunisia, this production was 0.550 million m³/year during the period 1990/2000 (Ben Rouina and Tamallah, 2000).

The physico-chemical characteristics of the effluents deriving from olive oil mills vary significantly depending on the oil extraction technique, the eventual dilution with olive wash water and installations (Tamburino et al., 1999).

The composition, the most frequently found, is: water (88-97%), organic compounds (2-10%), inorganic compound (1-2%), pH (5-5.5) COD (chemical oxygen demand, 60-80 g/l) and BOD5 (biochemical oxygen demand, 22-55 g/l) (Almirante and Di Renzo, 1993).

The high organic content of effluents from oil mills, the presence of inhibitory substances, the seasonal nature of the oil elaboration, territorial fragmentation and dispersion of oil mills pose significant technical and economic difficulties for the flow of OMW, in relation also with all the interest which is attached more and more to the protection of environmental resources. The characteristics of current techniques for wastewater treatment, as well as experience gained in managing impose to seek alternative methods of management to permit to:
i. Contain the costs of the effluent management,
ii. Restore the natural cycles of organic matter and nutrients,
iii. Prevent pollution of water flumes, and
iv. Valorize the elements richness of effluent in the agricultural angle.

Starting with an ecological approach, some researchers believe that the effluent flow in the reconstruction of natural cycles and reuse of biomass, including the spreading of the mill effluent on the agricultural soils, is the preferred method, the simplest and the most effective solution.

This soil application represents one of the possible uses, which is currently considered very prudently.

These researches focus either on a possible pollution phenomena, or to discourage the spreading of OMW on the soil. This result is related to the high content of phenolic substances (Della Monica et al., 1979; Fiestas Ros Ursinos et al., 1981; Potenz et al., 1985), the immobilization of nitrogen in the soil, the abnormal values of salinity (Fiestas Ros Ursinos et al., 1981) and pH (Albi Romero and Fiestas Ros Ursinos, 1960; Della Monica et al., 1979; Fiestas Ros Ursinos et al., 1981) and, finally, the harmful actions identified in cultures (Fiestas Ros Ursinos et al., 1981; Potenz et al., 1985; Proietti et al., 1988). Moreover, other researches valorize water vegetation because of its potassium, phosphorus and organic matter contents, by spraying OMW directly on soil (Levi-Minzi et al., 1992; Braham, 1993; Cabrera et al., 1996; De Monpezat and Denis, 1999; De Monpezat et al., 2000) or indirectly after composting (Tomati et al., 1996).

So, the aim of this research is to evaluate the effects of spreading the field with mill vegetation water on the olive tree nutrition and several important soil properties.

OVERVIEW ON THE OLIVE MILL WASTEWATER (OMW)

Definition

The OMW is a liquid effluent from the oil mills, obtained after crushing olives. This liquid is an acid residue containing many nutrients, but with real contaminant power, resulting mainly into high polyphenol content.

De Monpezat (1993) considers the OMW as a nitrogen fertilizer, organic, of plant origin, fermentable, non-composted, rich in potash, with a minimum content of phosphorus and magnesium, ensuring a good balance of the soil.

Compositional characteristics of olive mill effluent

OMW is a saline and organic residue; it has variable compositions and concentrations, depending in part on the olive variety, the place of its culture, its degree of ripening and also its mixer with the used water in the phases of extraction and separation of the oil.

OMW is viscous, reddish-brown to black colored, depending on the state of the phenolic compounds degradation and also olives from which it comes; it is characterized by an unpleasant acidic odor. The black polymer, which gives the characteristic color of the OMW, is mainly composed of fatty acids included within a matrix of phenolic origin (Saiz-Jimenez et al., 1986).

The organic fraction of OMW (Table 1) includes sugars, tannins, polyphenols, pectins and lipids (Fiestas Ros Ursinos, 1981). In addition to cellulose and pectins of olive pulp, which constitute the total suspended solids, several other simple sugars such: raffinose, mannose, sucrose, glucose, arabinose and xylose are contained in these effluents (Salvemini, 1985).

Beside sugars, a brown pigment corresponding to the catecholmelaninic compound is found in OMW, in a greater amount (Ranalli et al., 2003). The hydrolysis of such compound permits obtaining also glucose, galactose, rhamnose, and arabinose. Organic acids contained in the OMW are essentially fumaric, glyceric, lactic and malonic acids (Fiestas Ursinos Ros, 1981).

Proteins (from 0.5 to 7.5 g/l) represent the majority of the nitrogen fraction, all amino acids were identified in the oil factory effluents and the main ones are aspartic acid, glutamic acid, proline and glycine (Salvemini, 1985; Ranalli et al., 2003).

Simple phenolic acids, the most representative of these effluents, are synergetic acids, such as p-hydroxyphenylacetic, the vanilic, the veratic, the caffeic, the protocatechuic, the p-coumaric, and the cinnamic (Balice and Cera, 1984). Identified phenolic alcohols include the 4-hydroxyphenyalcool and 3,4-dihydroxyphenylethanol. Tannin levels range from 8 to 16 g/l (Balice and Cera, 1984). OMW contain also anthocyanins.

According to Ranalli et al. (2003), the high content of free phenols of OMW is the origin of the antimicrobial action of these effluents. Indeed, the drupe contains an antimicrobial substance, corresponding to the oleuropein, a bitter glucoside of olives, of phenolic nature, variable at alkaline pH, which action is enhanced in the presence of sodium chloride, and having moreover a power hypotensive activity.

The vegetation water antimicrobial activity was attributed also to phenolic acids. It is not excluded that free fatty acids, similarly, have a negative effect in addition to that exerted by phenols. The olive effluent contains also very small amounts of waxy and resinous, as well as vitamins and hormones, oleanic and mastic acids.

Organic matter is found mainly in solution and to a lesser extent in suspension (16.5 g/l) or emulsion (oil).

From suspended solids, the rate is also very high in absolute values, it is the settleable fraction rich in plant material colloidal which prevails; in addition, there are
significant amounts of inorganic salts of which the largest fraction is soluble (phosphate, sulfate, chloride), while the residual fraction (20%) is insoluble (carbonate, silicate). The mineral fraction of the effluent (Table 2), which represents 1.5% and 0.4% of dry matter, for OMW obtained by classic and continued regimes, contains essentially potassium, sodium, calcium and phosphorus, which are the most abundant minerals (Fiestas Ros Di Ursinos, 1986).

Among the microorganisms contained in the effluent of olive mill, there is a predominance of yeasts and gram-negative bacteria (Ramos Cormenzana, 1996), there is also schizomycetes, actinomycetes and fungi having at most a lipolytic activity. However, in most cases, there are no micro-organisms, so that the effluent does not pose a problem to the point of considering health and hygiene. Fiestas Ros de Ursino and Borja-Padilla (1996) note that the number of microorganisms is of the order of $10^5$ per ml of OMW and indicate that the bacteria are resistant to hydrolytic polyphenols; the acetogenic bacteria are sensitive to caffeic acid, concentration greater than 0.25 g/l; methanogenic bacteria are sensitive to acids, caffeic cumaric and ferrlic concentration greater than 0.12 g/l; some fungi have an ability to degrade polyphenols, in descending order were: *Trichoderma viridea*, *Botrytis cinerea*, *phomaglomerate*, *Phanerochoete chryosphorum*, *Aspergillus versicolor*, *Aspergillus Niger*, *Cladosporum sphaerspermum*, *Penicillium brericompectum*, *Penicillium hordei* and *Penicillium frecututans* (Fiestas Ros De Borja-Padilla and Ursinos, 1996).

There is a directive proposed by the WHO (World Health Organization), which limits the concentrations of pathogenic microorganisms in wastewater designed for irrigation. This directive limits the concentration of 1000 fecal coliforms per 100 ml in wastewater for the irrigation of products and raw consumption. The OMW are not affected by the pathogens under normal packaging.

**Characterization of the OMW in relation to its polluting power**

The potential pollutant effluent from the implementation of olive quintal is equal to 45 inhabitants; 2 liters of OMW cause pollution equal to 3 people in one day.

On the other hand, if we compare the characteristics of the olive mill wastewater with others from most food industries, we observe that the concentration of organic substance in the OMW is usually 10 times greater. This strong concentration, representing in some cases up to 100 kg/m³, lets us consider the OMW as residual water with stronger polluting power among those from food processing industries.
Table 3. Characteristics of the OMW in relation to their polluting power (Balice et al., 1984, Martin et al., 1991; Berndt et al., 1996).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Discontinued process</th>
<th>Continued process</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.5 – 5</td>
<td>4.7 – 5.2</td>
</tr>
<tr>
<td>COD (g/l)</td>
<td>120 – 130</td>
<td>45 – 60</td>
</tr>
<tr>
<td>BOD5 (g/l)</td>
<td>90 – 100</td>
<td>35 – 48</td>
</tr>
<tr>
<td>Suspended solid (g/l)</td>
<td>1-2</td>
<td>6-9</td>
</tr>
<tr>
<td>Dry matter (g/l)</td>
<td>100-120</td>
<td>50-60</td>
</tr>
<tr>
<td>Inorganic solids (g/l)</td>
<td>12-15</td>
<td>6-7</td>
</tr>
<tr>
<td>Volatile solids (g/l)</td>
<td>88-105</td>
<td>44-55</td>
</tr>
<tr>
<td>Brown pigment melanin catechol (g/l)</td>
<td>35.0</td>
<td>22.5</td>
</tr>
<tr>
<td>Fat (g/l)</td>
<td>0.5 – 1</td>
<td>3 - 10</td>
</tr>
</tbody>
</table>

Table 4. OMW heavy metal Contents (Berndt et al, 1996).

<table>
<thead>
<tr>
<th>Elements</th>
<th>Composition in PPm/dry matter</th>
<th>Supply to sol en Kg/ha/an</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OMW Authorized limited by the UE</td>
<td>50m³ OMW Authorized limited by the UE</td>
</tr>
<tr>
<td>Cadmium</td>
<td>&lt;0.1 40 0.0015 0.15</td>
<td></td>
</tr>
<tr>
<td>Chlorure</td>
<td>10 2.000 0.135 4.5</td>
<td></td>
</tr>
<tr>
<td>Cuivre</td>
<td>68 2.000 0.062 12</td>
<td></td>
</tr>
<tr>
<td>Mercure</td>
<td>&lt;0.01 20 0.00015 1</td>
<td></td>
</tr>
<tr>
<td>Nickel</td>
<td>8 400 0.108 3</td>
<td></td>
</tr>
<tr>
<td>Plomb</td>
<td>&lt;1 1.600 0.013 15</td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>212 6.000 0.212 30</td>
<td></td>
</tr>
</tbody>
</table>

Anyway, the composition and pollution indices values of the effluent are largely dependent on the techniques and the systems used for the extraction of oil from the first material (Table 3; Ranalli et al., 2003).

Heavy metal toxicity of OMW: The heavy metals, elements in low concentration, are normally essential to crop development but can be phytotoxic beyond a certain threshold. From the perspective of ecotoxicity, heavy metals are bio-accumulative elements, presenting risks for soil microflora, for plant growth with reduced yield and quality, and finally for the food chain and the human health.

Factors influencing the retention of metals by soil are texture, pH and amount of organic matter. In general, the solubility of ions increases when the pH decreases.

In summary, when we irrigated with wastewater, heavily loaded with heavy metals, the risks of accumulation and transfer are amplified on acid and sandy soils. OMW contains very low quantity of heavy metals (Table 4) and regular supply of 50m³/ha/an provides 30 to 100 times less of heavy metals than the limits allowed by the EU standards for the environment (Fiestas Ros Ursinos et al., 1996).

Bicarbonate of olive mill wastewater: The bicarbonate, even at a very low concentration, poses a problem especially in the case of fruit crops or nurseries with sprinkler irrigation, in a period of very low humidity and high evaporation. Under these conditions, it forms a white deposit on the leaves, which are not eliminated by the following watering, these deposits interfere with commercial quality. There is no toxicity, but as the water deposited on the leaves evaporates (partly or completely) during the rotation of the sprinkler, salts concentrate and CO₂ is released in the atmosphere. When these phenomena are sufficiently marked, the less soluble elements in the water, such as CaCO₃, deposit on the leaves.

Fiestas Ros Ursinos et al. (1996), views the low bicarbonate levels of OMW and the precautions, generally taken, to avoid splashing the plant aerial parts with this water, bicarbonate constitutes not a particular disadvantage.

Presence of substance of phenolic nature and fat emulsion: The formation of free phenols is due mostly to the enzymatic hydrolysis of glycosides and esters of olive pulp in the oil elaboration process (Baldi et al., 1995). Phenolic glycosides from the pulp of olives and anthocyanins are present in significant amounts in fresh or newly produced water, the total phenols volume is equal to about 3-4g/l in centrifuged water, this value is doubled in pressurized water.

The antimicrobial power of the vegetation water must be attached to the action of any components that it
contains, very high in absolute values; it is the free phenols plus the brown pigment or the catecholmalaninic compound. This flavonoid is a phenolic polymer that is only found in olives. It is formed during crushing of the fruits from orthodiphenol, of which the pulp is rich, under the action of phenol oxidases (inactive enzyme in whole drupes) which determines firstly the quinonisation, then the polymerization.

One of the most serious dangers to be feared of is however, the potential phytotoxicity of OMW leave residue that accumulate in the soil, because of their high organic content and the presence of substances of phenolic nature, exerting antimicrobial activity.

Fat emulsion may also occur, in case of accumulation, some superficial soil impermeability, reducing and limiting gas exchange with the atmosphere, and consequently the renewal of the air in the cavities of the active layer, with certainly no positive impact on the activity of the aerobic microflora and respiration of the root system (Proietti et al., 1988). A very interesting objectives of pretreatment effluent with a strain of Streptococcus durans, capable of producing high amounts of H₂O₂, able to attack the pigment, the major component of the residue that OMW let in the soil (Jiménez et al., 1986).

Studies, achieved by Garcia et al. (2000), showed that the brown pigment added in solid medium, can also be attacked by certain fungal species as such the fungus SC 26 of Phanerochaete cryosporium strain, which also seems to induce the enzymatic depolymerization of the pigment added in the liquid culture medium, with production of low molecular weight phenols, less resistant to bacterial attack, followed by decolorization of the vegetation water (Perez et al., 1987).

**Characteristics of olive mill wastewater versus its fertilizing power**

The early work concerned with the use of OMW go as far as to the late 50’s when Albi and Fiestas Ros Ursinos, (1960) reported the enrichment of the soil with organic and inorganic materials and the improvement of crop yield after its supply.

Based on a survey of some Spanish provinces, Fiestas Ros Ursinos (1981) found an important practice of the spreading of OMW on soil planted with olive trees (with inputs from 100-200L by tree and the possible addition of lime), the author also relates other culture essays (Corn and Wheat) realized on land treated with OMW which showed slight increases in yields.

The experiments conducted in Spain (Mariost, 1979) showed that the application of 100 m³/ha of OMW on the floor may be a nitrogen fertilizer (50-60 kg nitrogen in organic form) and phosphate (70 -200 kg phosphate as P₂O₅), with a high supply of potassium (350-1100 kg as K₂O).

Della Monica et al. (1979), and Pentez et al. (1985) noted that OMW initially determine significant changes of chemical, physico-chemical and biological nature; thereafter microbial activities transform and degrade organic matter, the organic nitrogen is mineralized and the soil is enriched in salts (especially potassium), which results in a beneficial fertilizing effect. All the nitrogen is present in organic form. Inorganic nitrogen (nitrate and ammoniac) is usually present only in trace amounts. Organic nitrogen, potassium, phosphorus is retained in the first ten centimeters of the soil with good absorbency. Provision of nitrate nitrogen by OMW is done progressively by slow mineralization and the water supply is negligible, since it corresponds to an irrigation of 10 mm in maximum (De Monpezat et al., 1992).

Compared to conventional olive trees fertilization, a contribution of 10m³/ha of OMW corresponds to fertilization (De Monpezat et al., 1992):

- i. Normal in magnesium,
- ii. High in phosphorus,
- iii. Very high in potassium,
- iv. Variable in nitrogen but generally very high.

These enrichments (Table 5) justify the interest of the agricultural valorization of OMW that can be either a basal fertilizer, or a maintenance fertilizer and can be made:

- i. By soil application, either directly or after storage, and/or
- ii. Composting with a carbon support and the spreading of the compost in the field.

**THE MAIN EFFECTS OF OMW ON THE SOIL**

**Effects of application of OMW on physical soil properties**

**Effects on aggregation and structural stability**

The OMW, in addition to its value as a fertilizer (Fiestas Ros Ursinos et al., 1981; Fiestas Ros Ursinos, 1986), may be used, in particular, in olive trees irrigation (Marsilio et al., 1990; Berndt et al., 1996) and have hydrophobic properties and binders. These properties promote its use as a natural conditioner of the top of the soil. According Mellouli (1996), OMW, incorporated into the soil, improve the structure and consolidate aggregates. In fact, the formed aggregates gained appreciable bonds between the particles and resist thus to the breakdown (binding nature of OMW). On the other hand, the aggregates acquire a hydrophobic property without being totally impermeable (hydrophobic character of OMW). This allows the reduction of imbibition rate of
aggregates in a reduction of the compression of the air. The OMW, through their hydrophobic powers and binder, promotes the formation of a much more efficient than the natural one, which its porosity and its structure act as a barrier against evaporation, this fact would have a positive impact on the water conservation for germination and development of cultures. As well, an improvement of hydrodynamic properties is resulted, with an increase of the total porosity, of the pore drainage and the hydraulic conductivity (Mellouli, 1996). This can be compared to the results obtained by Gabriels et al. (1972) in sandy soil, treated with bituminous emulsions, demonstrating the effectiveness of mulch, consisting of aggregates, having a thickness less than 5.35 mm, and allowing for better soil stability and a substantial reduction in evaporation.

In addition to the hydrophobic properties of OMW and binders, it is noted that the improvement of the stability of the soil treated with OMW could also be due to the polymers that it contains. Saiz-Jimenez et al. (1986) have identified mono and polysaccharides, polyphenols, proteins, lipids and metals in the form of salts. The architecture of these polymers could enable them to organize the edifices of soil without altering the exchange capacity (De Monpezat, 1994).

### Effects of application of OMW on chemical soil properties

#### Effect on organic matter

The amount of organic matter varies in OMW from 5 to 10%, it is a fermentable material in the soil, undergoing a decomposition phase, when OMW is neutralized by the soil solution and its temperature exceeds 10 °C to allow sufficient activity of the microbial flora of the soil. Incorporated into the soil in adequate doses, the OMW provides a significant improvement in its chemical composition, and the content of soil organic matter, initially very low (less than 0.2%), is found upper than the unit after five years of repeated spraying (Ben Rouina et al., 2000).

De Monpezat and Denis (1999) noted that spreading 333 m³/ha of OMW, in an adult olive tree orchard, grown traditionally, corresponds to an increase in the rate of organic matter more than 1%, and a month after the

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units kg/ha</th>
<th>Supply per ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic matter OM</td>
<td>OM</td>
<td>400 to 1800</td>
</tr>
<tr>
<td>Total nitrogen N</td>
<td>N</td>
<td>50 to 200</td>
</tr>
<tr>
<td>Phosphorus P₂O₅</td>
<td>P₂O₅</td>
<td>65 to 200</td>
</tr>
<tr>
<td>Potash K₂O</td>
<td>K₂O</td>
<td>350 to 1100</td>
</tr>
<tr>
<td>Magnesium MgO</td>
<td>MgO</td>
<td>15 to 150</td>
</tr>
<tr>
<td>Calcium CaCo</td>
<td>CaCo</td>
<td>15 to 100</td>
</tr>
</tbody>
</table>

*Table 5. Mean supply of nutrients on the basis of an application of 100m³/ha/year of OMW (De Monpezat et al, 1992).*

Effects on water soil characteristics

Studies concern the effects of OMW application on the physical characteristics of the soil were conducted by Pagliai (1996). The author noted an increase in the porosity of the field and a reduced tendency to the formation of crusts in the surface that limit the volume of infiltration.

Experiments, done in Tunisia by Mellouli (1996), showed that the hydrophobicity of stable aggregates of mulch, established by the OMW, induced in the beginning of the rain, a preferential flow at the macro pores, while moisturizing slowly the aggregates, but at some time (an hour later), when the mulch reached its holding capacity, the flow is slowed and infiltration becomes more important, it results, consequently, in a higher humidity than in the soil without OMW.

Effect on the reaction pH of soil

The normal pH range for irrigation water is 6.5 to 8.4. Some water irrigation, with a pH outside this range, may still be satisfactory, but we must expect nutrition or toxicity problems (Berndt et al., 1996). While, being acids, OMW modify only slightly the soil pH, probably due to the buffering capacity of the soil itself (Levi-Minzi et al., 1992).

Ben Rouina and Tamallah (2000) noted that, after an application of 200m³ of OMW on a sandy soil, soil pH remained unchanged (8.4 in startup tests and after 6 consecutive years of application), this could result following the high buffering capacity of soils in southern Tunisia, rich in active calcite and the intense ammonification by microflora, resulting in a significant increase in the rate of organic matter in the presence of favorable conditions (moisture and soil temperature).

The OMW still has a very low pH, often close to 5, and this acidity can be easily neutralized by the addition of lime in effluents and these fees can be avoided if the soil is limestone, which is often the case in Mediterranean regions.
applying, the C/N ratio is doubled. The obtained enrichment is sustainable, since five years later; it still 2.25% of organic matters in the soil against 1.8% in the non-enriched soil.

Studies by Ben Rouina and Tamallah (2000) showed that after the first application, the rate of organic matter, initially very low, is at least tripled for different treatments: its respective values increased from 0.10 to 0.28% with adose of 50m³, 0.10 to 0.31% with the dose of 100m³ and 0.05 to 0.56% with the dose of 200m³. This indicates an improvement in the organic matter soil content, inducing a series of improvements for the fertility, the water holding capacity, the micro-organisms activity, the growth and the fructification of the trees. After application of OMW, the organic carbon, the available phosphorus, the potassium, the calcium and the magnesium increased in the soil, but decreased during the recovery phase following the infiltration of mobile components, the mineralization of the organic material and the available phosphorus fixation (Cabrera et al., 1996).

Effects on the mobility of minerals and trace content elements: The experiments conducted in Spain (Maristó, 1979) showed that the application of 100 m³/ha of OMW on the soil may correspond to a nitrogen fertilization as 50-60kg of nitrogen in organic form and phosphate fertilization as 70-200 kg of phosphate in P₂O₅ form, in addition to a high supply of potassium (350-1100 kg as K₂O).

The tests realized by the Canal of Provence Company have shown that the potassium content in the product remained in an exchangeable situation in the soil, so available to the plants. With a massive 333m³ of OMW supply, the amount of the exchangeable potassium in the soil was multiplied by 3 and 5 years later, when the level of potassium in the soil is back to normal (there was no blocking between the elements fertilizers). Similarly, these large doses have made significant quantity of magnesium, have increased the rate of exchangeable magnesium, and multiply by 10 the rate of available phosphorus. While, the total phosphorus rate did not change due to the low OMW phosphorus content (De Monpezat, 1993).

According to Levi-Minzi et al., (1992), the contribution of OMW in a calcareous soil enhanced the liberation of soluble inorganic cations, in consequence this action has an enrichment of the first soil centimeters, and in descending amounts, by potassium, phosphorus and nitrogen.

Because of the cation deficit of OMW in aqueous solution, and the high content of potassium, and after metabolism of nitric nitrogen, there is a chemical effect on the soil solution; this causes the mobilization of phosphate and sulfate anions which are added to the chloride anions contained in the OMW (Berndt et al., 1996).

Effects of the application of OMW on biological soil activity

A decrease in the number of microorganisms of the genus Bacillus was identified as a result of the flow of the effluent into the soil. According to the test results, with doses of OMW up to 800 m³/ha, realized on olive tree grown in pots or orchard, Proietti et al. (1988), found no change in photosynthetic activity, transpiration, stomata conductance, chlorophyll, carbohydrate and specific leaf weight. After 14 months of treatment, the authors noted that the microbial load of the land is unchanged.

According to Ben Rouina et al. (2000), an intense microbial life is settled into the soil after application of OMW, the respirometric activity (mg of CO₂ x 24 hours) shows a significant improvement comparatively to the soil control. The identification of this Flore shows that it is composed of mesophilic bacteria, including two strains of nitrogen-fixing, cellulolytic and pectinolytic bacteria, yeasts and molds.

The mineralization of carbon soil is linked to the presence of a microbial population with a relationship between the size and the mineralizing activity. Incorporated into the soil, the organic charge of OMW is mineralized more or less quickly depending on the factors and soil conditions (texture, soil structure, nitrogen availability, pH, salinity ... and climate (temperature, humidity).

In the presence of OMW, no alteration in chemical and microbiological compositions was found. However, a proliferation of microbial population, particularly that of nitrogen fixing, and then stimulation of the respiratory activity of the microflora in the soil were established (Flourish et al, 1990; Marsilio et al, 1990) and exerted secretions (Flourish et al., 1990). On the other hand, Mekki et al. (2002) show that after in vitro incubation of soils, treated with OMW, the respiration, reflecting the intensity of carbon mineralization, is inversely proportional to the organic matter provided by the OMW, this is due to the report C/N mainly, which increases significantly during the incorporation of the OMW in the soil. Hatzipavlides et al. (1986) reported that the soil microflora, enhanced by OMW, becomes capable of decomposing phytotoxic elements such as polyphenols. De Monpezat (1994) and Berndt et al. (1996) have explained this by the fact that polyphenols are destroyed during microbiological processes in the soil, the different carbon chains that support the soil microflora have an architecture which allows it to organize with buildings humic soil without altering the properties of exchange.

Following the application of OMW in the soil, intense microbial activity is enhanced, this activity shows a significant improvement compared to the initial soil. The responsible flora is composed of coliform bacteria (cellulolytic and pectinolytic), typically responsible for the degradation of organic matter, denitrifying bacteria and nitrogen-fixing, yeasts and molds (Hamdi, 1993; Mekki et
Effect of application of OMW on nitrification

The studies of De Monpezat and Denis (1999), done in Aix-en-Provence (France), on clay-limestone soil, receiving a single contribution in the winter of 50 m³ of OMW, showed that during the two years following the application, there was no enrichment of the under soil in nitrate. Levels in the control and in the treated under soils are between 4 and 20 ppm. One month after the application, the C/N doubled. After 5 years, there were 2.25% of organic matter in the enriched soil against 1.8% in the non-enriched soil; during this period, the C/N decreased from 14.39 to 10.67 and returned to the normal. During the year of the application, there were two peaks of nitrification in the soil enriched with OMW, first in April and May, and then in September, with nitrate contents varying between 45 and 58 ppm.

The optimal conditions for nitrification are a soil temperature of above 12 °C and humidity of 20%, corresponding to a normal microbiological activity (De Monpezat, 1993). After a follow-up, during one year, of the liberation of nitrates with an input of 50 m³/ha of OMW to the soil, De Monpezat (1993) notes that OMW liberate, in the top 30 cm of the soil, a nitrate surplus, equivalent to 60 units of nitrogen in April-May and September-October, when the soil temperature exceed 12 °C without training nitrates in depth.

EFFECT OF APPLICATION OF OMW PRODUCTION

The experiments conducted in Spain (Mariost 1979) on soil devoted to vegetable crops, with inputs up to 200 m³/ha of OMW, 10 days before transplanting, have provided significant increases in production. Other tests carried out in the greenhouse and in pots, by growing a forage grass in soil treated with doses between 200 and 800 m³/ha, showed for the grass planted at a periodic-distance longer than 45 days after the application of OMW, increases in production from 120 to 145% compared to the control. However, negative effects were noted with sowing, done immediately or after 15 days of the application of OMW on the soil. This highlights temporary phytotoxic effects on crops if the application does not occur enough time before (at least three weeks according to Mariost) the beginning of the vegetative activity.

The test results with doses up to 200 m³/ha of OMW, performed on adult olive tree and realized by Ben Rouina et al. (2000), has resulted in the absence of phytotoxic effects on the trees. After six years of the study, with a moderate supply of 100 m³/ha, the annual production increased from 430.5 kg/tree (740 kg/ha) in the control to 560.1 kg/tree (954 kg/ha), corresponding consequently to an annual increase of 214 kg per hectare and per year.

With its fertilizer effect and its beneficial actions on the water soil retention, the OMW made in reasonable doses (50 and 100m³/ha) allows a significant improvement in the growth of trees, such doses allow the development of fruiting shoots, which seem longer and with more leaves than the control. It cannot be without consequence on the importance of flowering, expressed by the number of clusters and the number of fruit set, and these differences persist until the harvest by obtaining heavier fruits (1.24 g to 100m³ dose/ha against only 1.02 g in the control) and containing more oil (Ben Rouina et al., 2000).

Long-term studies conducted on 10 years aged trees, treated with OMW at doses up to 150m³/ha showed:

i. An increase in the land fertility, coupled with greater availability of phosphate and potash,
ii. A remarkable metabolizing activity (with the COD of the aqueous extract which is reduced to 80%, about 60 days after the treatment),
iii. An absence of phytotoxic defects on plants,
iv. A more vigorous vegetative growth
v. An improved nutritional status of plants,
vi. Significant increases in production (Catalano et al.,

<table>
<thead>
<tr>
<th>Soil control</th>
<th>Yeast; mold (%)</th>
<th>Denitrifying bacteria (%)</th>
<th>Streptomyces (%)</th>
<th>Sporulating bacteria (%)</th>
<th>Coliforms (%)</th>
<th>Pseudomonas (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.10^4 CFU/g of ds</td>
<td>33</td>
<td>31</td>
<td>23</td>
<td>8</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>60.10^4 CFU/g of ds</td>
<td>52</td>
<td>11</td>
<td>32</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>80.10^4 CFU/g of ds</td>
<td>37</td>
<td>16</td>
<td>41</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>57.10^4 CFU/g of ds</td>
<td>32</td>
<td>22</td>
<td>38</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>
It was the same in the case of vineyards treated with OMW at doses up to 150 m³/ha, where increases in the production and in the product quality were noticeable.

**EFFECT OF OMW APPLICATION ON THE GERMINATION PROCESS**

The results obtained by Levi-Minzi *et al.* (1992) indicate that doses of 80 m³/ha of OMW does not have a negative impact either on the germination and the time of lifting of crops such as Corn, the Barley, Wheat and Sunflower, with the necessity, however, of allowing a time interval of 50-60 days between application of OMW and planting. Regarding aspects relating to a possible genotoxicity of OMW, no effect of mutagenesis has been demonstrated for *Vicia faba* in doses reaching about 60m³/ha; once this threshold is exceeded, the occurrence of toxic effects in *Vicia faba* compromised the reliability of the results of genotoxicity tests. In addition, the experiment demonstrated the ability of OMW, at their application on sandy soil, to significantly reduce the genotoxic activity of a strong known mutagenic in plant systems, such as the maleic hydrazide.

Furthermore, inhibition of germination due to the presence of polyphenols, with a variable tolerance depending on the crop, was obtained for OMW in aqueous solution (Perez *et al.*, 1986) and when the seedlings were made into the soil immediately or shortly after application (Fiestas Ros Ursinos *et al.*, 1981; Levi-Minzi *et al.*, 1992). However, for later planting, the toxic effects disappear with time, the germination index and yields became significantly higher in the treated soil even at doses up to 800m³/ha of modern mills wastewater: continuous system (Fiestas Ros Ursinos *et al.*, 1981; Flourish *et al.*, 1990; Levi-Minzi *et al.*, 1992; Berndt *et al.*, 1996).

If the quantities and modes of OMW application were respected to the soil, the hydrophobic properties and binding of OMW, additionally to the secreted substances, OMW could be the source of a creation, in the soil, of structural and hydric conditions favorable for germination and crop development (Mellouli, 1996).

**REFERENCES**


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