Green innovative biodegradation of polyphenols in oil mill wastewaters to produce first class soil conditioner

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Olive oil production worldwide

* Olive oil is a key ingredient of the Mediterranean diet and its consumption is rapidly increasing worldwide.

* According to the International Olive Oil Council:
  
  **Olive oil production:**
  
  - **70%** since 1987
  - **2,861,500 tons** for the 2009/2010 period
  - **75%** comes from Mediterranean Region

Serious environmental problems:

High amounts of by-products

- 3–phase systems
  - olive pomace (OP)
  - olive mill wastewater (OMWW)
  - two-phase olive mill waste (TPOMW)

- 2–phase systems

✓ Total amount of OMW~ 10 million m³/year
Main characteristics of OMWW:
- High chemical oxygen demand (COD) concentration (45–220 mg/L)
- Low pH (4–5),
- High suspended solids concentration (up to 50 g/L)
- Other recalcitrant organic compounds, water-soluble phenols and polyphenols originating from the olives

Typical OMWW composition by weight:
- 83–94% water,
- 4–16% organic compounds
  - sugars, polyphenols, polyalcohols, pectins, and lipids, nitrogenous compounds, organic acids, carotenoids, tannins
- 0.4–2.5% mineral salts
  - chlorides, sulphates and phosphates, potassium, calcium, iron, magnesium, sodium, copper.
Usual treatment and disposal practice followed in Greece – environmental impacts:

- Neutralization with lime and disposal in evaporation ponds/lagoons.
  - Overflow and affect neighbouring systems
  - Polyphenols and other organic compounds → high COD → low Dissolved Oxygen → induction of anaerobic conditions → odor nuisance
- Direct disposal into soil, sea or rivers.
  - Oil compounds → increased soil hydrophobicity and decrease water retention and infiltration rate
  - Polyphenols → bactericide and phytotoxic properties cause alterations in N cycle, changes in soil microbial activity as well as contamination of surface- and groundwater.
  - High phosphorus contents → eutrophication
  - Lipids form an impenetrable film, blocks out sunlight and oxygen → hypoxia
## Advantages

<table>
<thead>
<tr>
<th>Advantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduction of organic pollutants</td>
</tr>
<tr>
<td>Integrated olive oil mill wastes management</td>
</tr>
<tr>
<td>End-product is a neutralised compost material</td>
</tr>
</tbody>
</table>

## Disadvantages

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Time consuming process (8-12 months)</td>
</tr>
<tr>
<td>Need for large land area</td>
</tr>
<tr>
<td>Product is often not the expected due to dependence on many parameters.</td>
</tr>
</tbody>
</table>

## Compost

- organic matter source
- increases soil fertility and the cation exchange capacity
- improves soil water capacity
- favors microbial activity in the soil
- helps in the breakdown of pesticides and other organic substances
- acting sedative in the development of soil-borne pathogens
- reduces the bioavailability of heavy metals
## Materials

- Olive mill waste waters from 3-phase mill
  - They may be replaced by 2-phase mill wastes
- All plant materials that remains in olive mills before olive oil extraction.
  - They may be replaced by other green residues.
- Biocatalyst

## Method:

- Mixture of OMWW and crushed plant residues, 50:50
- Addition of biocatalyst
- Stacking of the composting mixture to piles
- Monitoring of physicochemical parameters
- Aeration of the mixture
- Wetting whenever moisture < 50%
- Biostabilization for 2 months
Biocatalyst

Innovative solid substrate based on a special organic rock, mineral origin, inoculated with soil microorganisms laboratory cultivated.

* Patent 2004010018 (2004) Owner Dr Dinos Chassapis Ass. Professor University of Athens

Typical analysis:
- Microorganism population (Bacteria, mycetes, actinomycetes,) $2 \times 10^9$ c.f.u./g
- Humic substances 30% (dry basis)
- Mineral content 38% (dry basis)

* Accelerates 5 times the biochemical reactions in the compost.
* Enhance the bio-oxidative phase of composting

necessary microorganisms for the decomposition of polyphenols, carbohydrates, lipids and other organic substances

* Operating at wide ranges of pH
* Suitable for Mediterranean climate conditions.
* Active even in extreme environments

Humic acids
### Physicochemical parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OMWW</th>
<th>Initial mixture</th>
<th>Soil conditioner (60 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>90.3</td>
<td>68.1</td>
<td>48.9</td>
</tr>
<tr>
<td>Electrical conductivity (mS/cm)</td>
<td>41</td>
<td>1.92</td>
<td>1.8</td>
</tr>
<tr>
<td>pH</td>
<td>5.48</td>
<td>5.7</td>
<td>7.3</td>
</tr>
<tr>
<td>Bulk density (g/ml)</td>
<td>0.98</td>
<td>0.33</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Electrical conductivity for initial mixture and soil conditioner has measured in ratio 1:5 in water and pH in 1:10.
## Changes in some critical parameters during composting (dry weight basis)

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<th>OMWW</th>
<th>Initial mixture</th>
<th>Soil conditioner (60 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash (% w/w)</td>
<td>7.3</td>
<td>14.0</td>
<td>21.9</td>
</tr>
<tr>
<td>Organic matter (% w/w)</td>
<td>92.7</td>
<td>86.0</td>
<td>78.1</td>
</tr>
<tr>
<td>Total organic carbon (% w/w)</td>
<td>53.8</td>
<td>49.9</td>
<td>45.3</td>
</tr>
<tr>
<td>Total Kjeldahl nitrogen (% w/w)</td>
<td>1.7</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>C/N</td>
<td>31.6</td>
<td>38.4</td>
<td>34.8</td>
</tr>
<tr>
<td>Humic acids (% w/w)</td>
<td>n.d</td>
<td>5.8</td>
<td>8.0</td>
</tr>
<tr>
<td>Total phenols mg/kg</td>
<td>374.3</td>
<td>80.3</td>
<td>32.3</td>
</tr>
</tbody>
</table>

10% reduction

91.4% reduction of polyphenols
## Evaluation of OMW soil conditioner as Soil Media

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean value of produced Soil Conditioner</th>
<th>Soil Substrates /media (Optimum values)</th>
<th>Soil Substrates of sowing /nurseries (Optimum values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total organic content (TOC) % w/w</td>
<td>78.1</td>
<td>-</td>
<td>80.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH</td>
<td>7.3</td>
<td>5 – 7.5</td>
<td>5.5-7</td>
</tr>
<tr>
<td>Electrical conductivity (EC) dS m&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>1.8</td>
<td>≤3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>≤0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total N % w/w</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>40</td>
<td></td>
<td>&lt;500&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zn (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>123</td>
<td></td>
<td>&lt;1500&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cd (μg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.20</td>
<td></td>
<td>&lt;5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cr (μg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.10</td>
<td></td>
<td>&lt;200&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ni (μg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>28</td>
<td></td>
<td>&lt;100&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pb (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.05</td>
<td></td>
<td>&lt;1000&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Soil Medium for growing plants | Produced OMW soil conditioner | Optimum Soil Substrate
--- | --- | ---
EAW (vol %) + WBC (vol%) | 49.8-60.0 | 55-65
AS (vol%) | 15.6-30.8 | 20–30
TPS (vol%) | 73.5-80.7 | 85
Bulk density g. L⁻¹ | 440-500 | 400

EAW: easily available water, AS: air space, WBC: water buffering capacity and TPS: total pore space
## Comparison of composts produced from OMWW in Greece

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Produced Soil Conditioner</th>
<th>Compost from OMWW Greek -1st</th>
<th>Compost from OMWW Greek-2nd</th>
</tr>
</thead>
<tbody>
<tr>
<td>water buffering capacity (%)</td>
<td>147.8</td>
<td>248.7</td>
<td>n.a.</td>
</tr>
<tr>
<td>Humic Acids (%)</td>
<td>8</td>
<td>5.84</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>Electrical conductivity (dS / m)</strong></td>
<td><strong>1.7</strong></td>
<td><strong>2.2</strong></td>
<td><strong>1.1</strong></td>
</tr>
<tr>
<td>pH</td>
<td>7.3</td>
<td>7.7</td>
<td>7.5</td>
</tr>
<tr>
<td>Organic Matter (%)</td>
<td>78.1</td>
<td>74.1</td>
<td>39</td>
</tr>
<tr>
<td><strong>Microrganisms (c.f.u. / g )</strong></td>
<td><strong>23 .10^8</strong></td>
<td><strong>3,6 .10^8</strong></td>
<td>n.a.</td>
</tr>
<tr>
<td>N %</td>
<td>1.3</td>
<td>1.0</td>
<td>1.4</td>
</tr>
<tr>
<td>P (ppm)</td>
<td>n.a.</td>
<td>445</td>
<td>48.7</td>
</tr>
<tr>
<td>K (%)</td>
<td>n.a.</td>
<td>0.7</td>
<td>0.32</td>
</tr>
<tr>
<td>Zn (ppm)</td>
<td>123</td>
<td>49.7</td>
<td>20.1</td>
</tr>
<tr>
<td>Cu (ppm)</td>
<td><strong>40</strong></td>
<td>26.7</td>
<td>6.9</td>
</tr>
<tr>
<td>Ni (ppm)</td>
<td>28</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Cd (ppm)</td>
<td>0.18</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Pb (ppm)</td>
<td>0.05</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Cr (ppm)</td>
<td>0.1</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Hg</td>
<td>Ø</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td><em>Escherichia coli, Salmonella Spp.</em> (Enterobacteriaceae)</td>
<td>Ø</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>
I. Germination rates
Used as a growth substrate 4 mixtures

a- 100 % v/v Perlite
b- 50 % v/v Perlite : 50 % v/v OMW produced soil conditioner
c- 66.66 % v/v Perlite : 33.33 % v/v OMW produced soil conditioner
d- 100 % v/v OMW produced soil conditioner
II. μg Chlorophyll / g fresh plant tissue

Preliminary experiments on lettuce, Lactuca sativa (Asteraceae) seedlings growth under the influence of the produced OMW soil conditioner, based on weight of Chlorophyll / plant tissue

Used as development substrate 4 mixtures

<p>| | | | |</p>
<table>
<thead>
<tr>
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<th></th>
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<tbody>
<tr>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
</tr>
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<td>d- 100 % v/v OMW produced soil conditioner</td>
</tr>
</tbody>
</table>
III. Growth of the underground part of the plants

![Graph showing growth and weight of underground part of seedlings over time.]

- a- 100 % v/v Perlite
- b- 50 % v/v Perlite : 50 % v/v OMW produced soil conditioner
- c- 66.66 % v/v Perlite : 33.33 % v/v OMW produced soil conditioner
- d- 100 % v/v OMW produced soil conditioner
FIELD EXPERIMENTS ON VEGETABLES
During the planting seedlings on the line:
50 plants by adding 250g «produced soil conditioner" in the planting pit,
50 plants by adding 250g other compost from OMWW in planting pit
50 plants by adding 500g «produced soil conditioner"
50 plants by adding 500g other compost from OMWW in planting pit;
50 plants without any soil conditioner (control)

POT EXPERIMENTS ON ORNAMENTALS
Usage in containers filling with roses, geranium, bougainvillea, jasmine as a supportive medium with red soil about 30%, in flower beds of herbaceous sensitive floriculture, palm trees, Benjamin, etc. to improve soil structure.
The above was used instead of classical peat

- Showed no phytotoxicity as soil medium component in vegetable plantations and ornamental plants.
- Logged positive effect on plant growth
- Could replaces common used soil substrates much more expensive

*Experiments performed in the farms of the Union of Agricultural Cooperatives of Rethymnon, Crete*
Proposed method is low cost of investment and operation, converts a toxic waste into a soil conditioner product.

Reduced production time (2 months compared to 12 and 18 months common procedure).

Chania soil conditioner shows positive effect on plant growth and
can replace the more expensive black-humus peat.