Germination index as a tool to assess phytotoxicity of olive mill solid wastes

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Olive oil production is a relevant industry especially in Mediterranean countries. This activity generates high loads of solid and liquid effluents, whose amounts and composition strongly depend on the extraction procedure applied at the mill. While in Portugal three-phase extraction is predominant, in Spain, for example, the technology is moving towards a less water demanding procedure involving a two-phase system. This modern technique increases the load of olive mill solid waste (OMSW) produced as well as its pollutant content. The management method often involves direct soil application, mainly due to its significant organic matter and nutrient content (namely potassium). However, the high organic load and the presence of toxic substances such as polyphenols may cause land, groundwater and surface water contamination (Caputo *et al*, 2003; Roig *et al*, 2006).

Indeed, OMSW composition is complex and variable (Morillo *et al*, 2009). The occurrence of specific compounds depends on the fruit, climatic conditions, storage time and the processing technique. Among the phenolic compounds, hydrotyrosol, tyrosol, caffeic acid, ferulic acid, vannilic acid and p-coumaric acid are reported as some of the most relevant in the waste composition. In addition, around 98% of the total phenolic content of olive fruit can be found in OMSW (Obied *et al*, 2005).

This study aims to investigate the main compounds responsible for phytotoxicity of OMSW. For that, residues coming from two-phase and three-phase olive oil extraction were collected and characterized regarding their physical, chemical and phytotoxic properties.

OMSW was characterised regarding their moisture, volatile solids (VS), pH, electrical conductivity (EC), total Kjeldahl nitrogen (TKN), total phenolic content (TPh) and chemical oxygen demand in the solid phase (COD). In this study, the germination index (GI) was determined through phytotoxicity assays to assess the potential for inhibition of seed germination, namely using garden cress (*Lepidium sativum*). This parameter was calculated by means of the percentage of germination and the percentage of radicle length in relation to blank tests (Trautmann and Krasny, 1997). In case of a GI higher than 100%, a beneficial effect on seed growth is expected, whilst GI lower than 60% a significant phytotoxic effect is predictable. As an example, Table 1 shows results obtained for OMSW sample characterized in this work and some data from literature.

Table 1. OMSW main physic-chemical characteristics (mean±sd).

Parameter	This work	Fezzani and Cheikh (2008)	Droussi *et al* (2009)	Morillo *et al* (2009)
Moisture (%)	36.8±0.4	-	26.2	62.16
VS (%)	93.7 ± 2.5	97.5 ± 0.5	97.27	90.66
pH	4.8 ± 0.1	-	5.88	5.48
EC(µS/cm)	1365	-	3330	2990
TKN (g/kgTS)	8.5±0.9	12 ± 0.5	7.0	11.99[a)]
TPh (mg GAE/g TS)	0.58±0.06	-	0.35	1.36[b)]
COD (gO_2/kg)	2249 ± 91	1100 ± 5	-	
GI (%)	63.9±11	-	-	

[a)]- as TN; [b)]- as phenols in % (w/w) of total organic matter.

These results show that this waste is characterized by a high organic content and acidic pH, which is in agreement with the literature. In what regards to GI, this waste is moderately phytotoxic since this parameter is slightly higher than 60% (Zucconi *et al*, 1981).

Thus, it would be important to identify the main potential phytotoxic compounds. Thus, several molecules present in OMSW composition were identified based on literature and their individual influence on germination index (GI) was assessed in a systematic manner. The goal was to correlate OMSW phytotoxicity with some key compounds. Fig. 1 shows, as an example, the *Lepidium Sativum* germination index as function of phenol concentration.

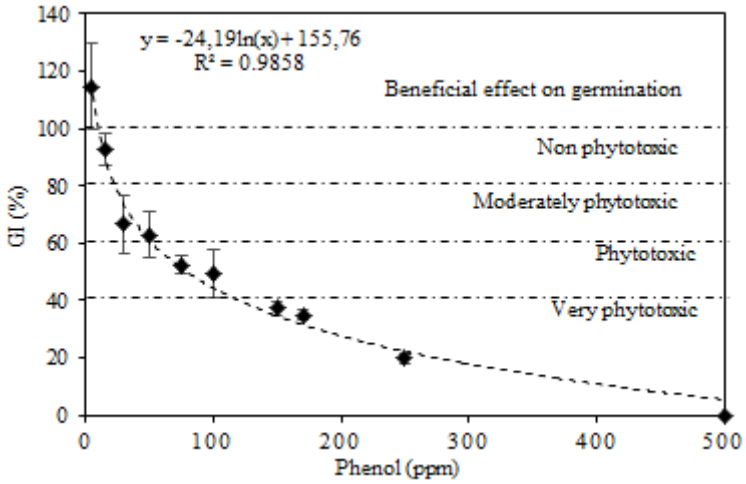

Fig. 1. Effect of phenol concentration in *Lepidium Sativum* GI.

The results revealed that the phytotoxicity of phenol is only significant for concentrations higher than 50 ppm, since in this case GI is lower than 60%. This approach is very useful, once based on the variation of the concentration of individual phenolic compounds, the main substances potentially responsible for OMSW phytotoxicity are identified. In this manner, in the future it will be possible tailoring a specific treatment scheme to reduce the impact of this agro-waste in the soil.

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