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Olive pulp and its effluents suitability for soil amendment

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Abstract

Olive pulp (OP) and its effluents produced after digestion processes were characterised and their suitability as soil amendment materials were investigated. Results showed that OP and its effluent for hydrogen (EH_2) and methane production (ECH_4) contain high amount of organic matter, remarkable concentration of nutrients and negligible content of heavy metals. Decreasing concentrations of low molecular weight phenols (monomeric phenols) and increasing amount of humic-like materials were found passing from OP to EH_2 and ECH_4. The effects on both wheat seed germination and seedlings growth were also investigated. Addition of OP at the highest doses delayed both seed germination and seedling growth. These effects decreased when the OP and its effluents were incorporated into the soil. On the contrary an enhancement of seedlings growth was detected by addition of EH_2 and ECH_4. Enhancement effects also were found out by addition of lower OP concentrations. The phytotoxic effects decreased when the products were incorporated into the soil.

Keywords: Olive pulp; Phenolic compounds; Physicochemical characterisation; Seed germination; Wheat seedling growth

1. Introduction

The olive oil industry is extremely important from an environmental sustainability point of view because of the significant amount of wastes produced that lead to economic, technical and organisational constraints in the adoption of an environmentally sustainable disposal [1–3].The technology for olive oil extraction has progressed significantly since the beginning of the 1970, when the three-phase centrifugation system appeared. It separates the oil from an aqueous phase (olive mill wastewater) and a solid phase (olive husk) in a continuous process. The main inconvenience of this system is a large production of wastewaters characterised by a heavy pollutant load [2–5]. Numerous attempts have been made in order to reduce their environmental impact, such as treating them physically, chemically and biologically or applying them directly to soils; nevertheless the disposal of wastewaters continues to be a very difficult process [4].

In the 1990, the olive oil industry has adopted a new continuous centrifugation system with a two-phase decanter, which separates the oil from a solid humid by-product called olive pulp (OP), constituted by variable quantities of olive pulp, stones, residual oil and vegetative waters [6,9]. Even though the direct application of OP onto land is an inexpensive way of disposing and recycling nutrients and organic matter, it might be a source of pollution and could have an unfavourable environment impact. Many studies have reported the toxic effects of oil by-products on plants and soil microbial activity, because of their content of monomeric phenolics, fatty acids and mineral salts [5–12].

An interesting alternative for a sustainable disposal of OP is its use in energy recovery. It is a good potential energy source because of its high polysaccharide content and can produce ethanol, hydrogen and methane through aerobic and anaerobic digestion [13]. However, only when the by-products remaining at the end of the digestion processes are eliminated might their use in agriculture as amendments be feasible.

The aim of this research, within the UE BIOTROLL[1] Project, was the chemical characterisation of olive pulp (OP) and the effluents arising from its anaerobic digestions for the production of hydrogen (EH_2) and methane (ECH_4) and to assess their effect on the germination and the early stages of wheat

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development, so as to examine whether they can be re-used in agriculture.

2. Materials and methods

The olive pulp (OP) was collected from a two-phase decanter mill and was submitted to two different fermentation processes resulting in one effluent from hydrogen production (EH_2) and one from methane production (ECH_4) [12]. Urea and disodium phosphate were added during the biotransformation processes to make up for N and P deficiencies. Detailed information on the two digestion processes has been reported by Gavala et al. [13].

Varying amounts of fresh samples of OP, EH_2 and ECH_4 were: (i) freeze-dried to perform the chemical analyses; (ii) stored at 4 °C for direct use as a fresh material; (iii) frozen at −20 °C for further analysis.

2.1. Analytical methods

In order to chemically characterise OP, EH_2 and ECH_4, the following parameters were determined: dry matter (d.m.) content, ash content, pH, electrical conductivity (EC), total lipid content, total content of phenolic compounds. The analyses of the most important low molecular weight phenols (LMWP), organic carbon (TOC), extracted organic carbon (TEC), humic-like and fulvic-like acids (HLA + FLA), nitrogen (N), ammonium and nitrates and the most representative macro and micronutrients were also performed. The d.m. content, ash content, pH and EC were determined directly using the fresh materials, while the other analyses were carried out on the freeze-dried products.

The three materials were dried in an oven at 105 °C for 24 h, to a constant weight, for the d.m. determination and at 600 °C for 24 h for the ash content analysis. The pH and EC were measured in a water extract, 3:50 (w/v) and 1:10 (w/v), respectively. The total lipid content was determined using the Soxhlet method with petroleum ether as extractant [14]. Total content of phenolic substances, expressed as gallic acid, was determined by means of the Folin Ciocalteau method using water + methanol (1:1) in a ratio 1:20 (w/v) as extracting solution for the colorimetric determination [15,16]. Low molecular weight phenols (LMWP) were characterised by HPLC after extraction at pH 7 with a buffer solution of 0.03 M EDTA–Na_2 + 0.06 M KH_2PO_4 + 0.04 M $Na_2HPO_4 \cdot 2H_2O$ at 120 °C for 1 h and acidification with 10 M HCl. The phenolic compounds identified were: oleuropein (the most important phenol in olive fruits and leaves, but also found in a lower quantity in olive oil residues) and the following LMWP (<350 Da): hydroxytyrosol, tyrosol, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, gallic acid and vanillin; catechol was not separated due to its high photolability. The analyses were performed at 280 nm and 25 °C, with a variable UV–vis detector. Separations were achieved on a Phenomenex C18 reversed-phase column (150 mm × 4.6 mm, 5 mm). A gradient of water:acetic acid (100:1, v/v) as solvent A and methanol:acetonitrile:acetic acid (95:5:1, v/v/v) as solvent B at a constant flow rate of 1.5 mL min^{-1} as mobile phase was used, according to the following elution program: isocratic elution with 5%B for 2 min, followed by a gradient to 25%B over 5 min, with a further stepwise gradient to 40%B at 8 min, 50%B at 10 min and 100%B at 5 min, held for 5 min and returned to initial conditions over 5 min [17,27]. The quantification of compounds was performed by external standard calibration. The TOC was determined with the Springer-Klee method using 1/3 M $K_2Cr_2O_7$ as oxidant [18]. The TEC and the HLA + FLA were extracted in NaOH + $Na_4P_2O_7$ 0.1 M; a polyvinylpyrolidone column was used to separate the humic-like substances from the non-humic substances, 1/3 M $K_2Cr_2O_7$ was used as oxidant [19,20]. The total nitrogen content was determined using an elemental analyser. Ammonium and nitrate were measured after extraction with hydrochloric acid (1:1) + water and steam distillation with MgO and Devarda alloy [21]. Total phosphorus was measured colorimetrically according to the Murphy–Riley method [22]. The other macro and micronutrients were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) after digestion with nitric acid 65%.

All results are means of tests performed in triplicate and have been reported with their standard deviation.

2.2. Germination and seedling growth

To study the effects of OP, EH_2 and ECH_4 on wheat (*Triticum durum* L., cultivar Duilio), two different experiments were carried out: a paper study and a soil study. The three by-products were applied at different doses, usually utilized for wheat fertilisation, in order to assess if there were any positive or negative or no effects on seed germination and seedling growth of wheat, with or without soil as growth medium. Both studies were held in a 100 mm × 10 mm Petri dishes, in a growth chamber settled at 21 °C from 6 to 20 h and at 15 °C from 20 to 6 h, with a photoperiod of 14 h/10 h light/dark cycle. The procedure based on the rules for seed testing [23] was used.

2.2.1. Paper study

The experiment was carried out using a germination paper disk as growth medium. The three fresh by-products (OP–EH_2–ECH_4), adjusted to the same water content, were spread on the germination paper at doses of 60, 120 and 180 kg N ha^{-1} (standard N doses for wheat fertilisation) and at 70 tonnes ha^{-1} (corresponding to: 420, 273 and 175 kg N ha^{-1} for OP–EH_2–ECH_4, respectively) and 80 m^3 ha^{-1} (corresponding to: 559, 329 and 210 kg N ha^{-1} for OP–EH_2–ECH_4, respectively) of materials, and were compared to a no-nitrogen control (C = 0 kg N ha^{-1}). The latter two doses, expressed like tonnes or m^3 ha^{-1} of material, correspond to Italian legal limits for the application of sludges from agro-industrial origin (Law no. 99/1992) to which OP was compared and for olive mill wastewaters (Law no. 574/1996) to which EH_2 and ECH_4 were compared, respectively. The agronomic doses of 60, 120 and 180 kg N ha^{-1} correspond to the addition of 9.4, 18.8 and 28.2 tonnes ha^{-1} of fresh OP, of 15.4, 30.8 and 46.2 tonnes ha^{-1} of fresh EH_2 and of 24, 48 and 72 tonnes ha^{-1} of fresh ECH_4, respectively. Before use, all the materials were shaken to avoid sedimentation and ensure homogeneity. Distilled water was used

as a control (C). Soon after spreading the three by-products on germination paper, 20 kernels of durum wheat were placed in each Petri dish. The dishes were initially kept in the dark for 4 days and subsequently the photoperiod was adjusted as previously reported. They were watered with deionised water when necessary. The number of germinated seeds was counted daily until the end of the germination process and then calculated as a percentage of seeds sown. The criterion to establish germination was the emergence of a primary root and a coleoptile the same length as the seed [23]. The seedlings were grown for 10 days after which the following parameters were recorded: shoot height, root and shoot dry biomass (at 105 °C for 24 h) and number of abnormal seeds, i.e. those seeds characterised by seedlings with weak development or deformed structure [23].

2.2.2. Soil study

The second experiment was carried out in addition to the paper study, to evaluate whether the effects exerted by the three by-products on germination and seedling growth change as a consequence of their interactions with soil. The test was performed in the same condition of the paper study (Section 2.2.1), but in this case 50 g of soil was used in each Petri dish as growth medium. The soil was a top layer of Typic Ustipsamment soil [24], sampled at a depth of 0–40 cm from an area of Southern Italy characterised by large-scale olive oil production. Before use, the soil was air-dried and crushed to pass through a 2 mm sieve [25]. The analyses were carried out according to the chemical methods of soil analysis [25] and the main physical and chemical characteristics of the soil are reported in Table 1. At the beginning of the experiment the OP–EH_2–ECH_4, adjusted to the same water content, were mixed with the soil at agronomic doses of 60, 120 and 180 kg N ha^{-1}, that were compared to a no-nitrogen control (C = 0 kg N ha^{-1}). Distilled water was used as a control. Some days before the experiment started, the soil was moistened with deionised water up to its water holding capacity (20%, p/p) to stabilise microbial activity. Twenty kernels of durum wheat per Petri dish were placed on the soil at 0 (T0), 15 (T15), 30 (T30) and 60 (T60) days after soil treatment with the three olive oil by-products, in order to assess whether there were any differences in phytotoxicity over time as a consequence of their degradation. The experiment was carried out in the same way as the paper study. The germination percentage was measured daily until the end of the germination process. After 10 days, the seedlings were removed from their substrate and were rinsed with water to remove soil particles. The shoot height, root and shoot dry biomass and number of abnormal seedlings were then determined.

Both experiments were performed in triplicate using a completely randomised design. Results were submitted to a one-way ANOVA, means were reported with their standard deviations and compared by using the Student's–Newman–Keuls test for multiple comparisons.

Table 1
Physical and chemical properties of the Typic Ustipsamment soil [24]

Parameter	
Texture	
Sand (%)	80
Silt (%)	7
Clay (%)	13
pH (pH-unit)	7.60
Electrical conductivity, EC (dS m^{-1})	0.17
Total nitrogen, TNK (mg kg^{-1})	600
Available P (mg kg^{-1})	12.3
Exchangeable K (mg kg^{-1})	112
Total organic carbon, TOC (%)	0.48
Organic matter, OM (%)	0.79
Total extracted organic carbon, TEC (%)	0.31
Humic-like substances, HLA + FLA (%)	0.24
Humification rate, HR[a] (%)	50

[a] HR = (HLA + FLA)/TOC × 100.

3. Results and discussion

3.1. Chemical characterisation

The main chemical characteristics of the three by-products are shown in Tables 2 and 3. The analyses performed on the freeze-dried products have been expressed both for the dry matter and the fresh material.

OP has the highest dry matter content, whereas ECH_4 and EH_2 show a significant reduction, respectively 9.8% and 4.6%, both because OP was diluted to promote fermentation processes and probably because part of the organic matter was used by fermentation agents as a substrate (Table 2). The ash content shows a similar trend; however, in this case, there are no differences between the two effluents (Table 2). OP and EH_2 have acidic pH while ECH_4 is moderately alkaline, probably, as a consequence of the addition of urea during the anaerobic digestions (Table 2). The EC is higher in OP and ECH_4, whereas EH_2 shows a lower value (Table 2).

For the remaining chemical parameters determined, the three by-products were compared on a dry matter basis. Lipid content is higher in OP and EH_2 than in ECH_4 (Table 2). Total phenolic compounds, TEC, HLA + FLA show an increase in digested materials, whereas TOC quantities are similar in the three by-products (Table 2). The trend observed suggests that microorganisms, during the biological processes for hydrogen and methane production, might have used a more readily available source of C (e.g. lipids, carbohydrate and polysaccharides) as a preferential substrate probably transforming part of it into a more recalcitrant form of C, such as phenolic and humic-like substances. This hypothesis is also supported by the apparent humification rate (AHR) that increases from 23% in OP to 37% in EH_2 and 53% in ECH_4, and by the total phenolic compounds (13.7% in OP, 25.7% in EH_2 and 32.6% in ECH_4): both parameters show the progressive polymerization of the materials (Table 2).

To obtain more information on the phenolic content of the three by-products, the most important LMWP (monomeric phenols), which correspond to the most toxic phenolic fraction for plants and soil microorganisms [8,11,26–28] were identified. The LMWP content varies in the three materials (Fig. 1). OP has the highest content of total LMWP: 2.37 g kg^{-1} d.m., whereas EH_2 and ECH_4 contain respectively 1.22 and 0.96 g kg^{-1} d.m.

Table 2
Chemical characteristics of olive pulp (OP) and effluents derived from its anaerobic digestions for the production of hydrogen (EH_2) and methane (ECH_4)

Parameters	OP		EH_2		ECH_4	
	f.m.[a]	d.m.[a]	f.m.	d.m.	f.m.	d.m.
Dry matter (%)	28.4 ± 0.67[b]		9.8 ± 0.09		4.6 ± 0.77	
Density (kg dm^{-1})	1.092 ± 0.10		1.054 ± 0.07		1.048 ± 0.06	
Ash (%)	1.50 ± 0.10		0.55 ± 0.07		0.60 ± 0.00	
pH	5.39 ± 0.01		5.12 ± 0.02		8.05 ± 0.06	
Electrical conductivity (dS m^{-1})	1.27 ± 0.06		0.58 ± 0.00		1.13 ± 0.03	
Total lipids (g kg^{-1})	55	194 ± 7.1	24	249 ± 6.9	4.0	81 ± 6.6
Phenolic compounds (g kg^{-1})	3.9	13.7 ± 0.39	2.5	25.7 ± 0.92	1.5	32.6 ± 2.48
TOC (C %)	16.6	58.4 ± 0.53	6.0	61.8 ± 0.57	2.6	56.3 ± 3.66
TEC (C %)	5.9	20.6 ± 1.10	3.7	37.5 ± 0.23	1.8	39.9 ± 0.45
HLA + FLA (C %)	3.8	13.4 ± 0.29	2.2	22.3 ± 0.64	1.4	29.8 ± 0.53
[c]AHR (%)	23	23	37	37	53	53
Total nitrogen (g kg^{-1})	6.4	22.4 ± 0.8	3.9	40 ± 1.8	2.5	54 ± 1.2
NH_4-N (mg kg^{-1})	15	51 ± 4	81	823 ± 58	479	10442 ± 1555
NO_3-N (mg kg^{-1})	6.8	24 ± 3	8.0	82 ± 4	1.2	27 ± 1
Carbon/nitrogen (C/N) ratio		24.4		13.5		8.7
Total P_2O_5 (g kg^{-1})	0.97	3.4 ± 0.07	1.05	10.7 ± 0.15	1.18	25.8 ± 0.45
K_2O (g kg^{-1})	6.0	20 ± 0.5	40	41 ± 0.1	3.0	60 ± 0.3
SO_3 (g kg^{-1})	1.03	3.64 ± 0.18	0.55	5.57 ± 0.20	0.41	9.03 ± 0.95

[a] f.m. = fresh material; d.m. = dry matter.
[b] ±S.D.
[c] Apparent humification rate, AHR (%) = (HLA + FLA)/TOC × 100.

(Fig. 1, sum of LMWP). Hydroxytyrosol was the most detected compound and its content decreases from OP through EH_2 to ECH_4. Ferulic acid shows a similar trend, even though the value detected is lower than hydroxytyrosol (−53%). Tyrosol was found only in OP and ECH_4 (−72% than hydroxytyrosol) while it was practically negligible in EH_2, probably as a consequence of the biological fermentations involved. The quantity of the remaining LMWP is low, less than 0.10 g kg^{-1} of d.m.; *p*-cumaric acid was the only LMWP that remained undetected. It is important to note that the OP is the material poorest in total phenolic compounds (Table 2) and richest in LMWP (Fig. 1). The consequence is that OP could have phytotoxic effects on higher plants mainly during germination and seedling development, due to the enhancing action of phenolic compounds on seed dormancy [11]. These findings supported the hypothesis that both biological processes (EH_2 and ECH_4) contribute to the microbial polymerization of LMPW towards phenolic macromolecules (humic-like materials) (Table 2).

Total N content (Table 2) that is predominantly in the organic form increases with OP digestion. A very large quantity of ammonium was measured in the digested effluents (Table 2) due to the addition of urea at the beginning of the fermentation process as a source of N for the microorganisms. Nitrate content is negligible in all three products (Table 2). The C/N ratio is higher in OP (24.4) and decreases in the digested samples, with values of 13.5 in EH_2 and 8.5 in ECH_4 (Table 2); this suggests that the

Table 3
Total macro and micronutrients and heavy metal content in the three olive oil by-products (mg kg^{-1})

Parameters	OP		EH_2		ECH_4	
	f.m.[a]	d.m.[a]	f.m.	d.m.	f.m.	d.m.
Calcium (CaO)	1588	5590 ± 125[b]	727	7425 ± 58	593	12911 ± 95
Magnesium (MgO)	347	1220 ± 36	262	2675 ± 14	152	3317 ± 19
Sodium (Na_2O)	60	214 ± 15	910	9300 ± 110	950	20710 ± 360
Aluminum (Al)	27.5	97 ± 8	10.5	108 ± 0.5	64	1392 ± 10
Cadmium (Cd)	–[c]	–[c]	–[c]	–[c]	0.023	0.5 ± 0.0
Cobalt (Co)	–[c]	<1	–[c]	<1	0.09	2.0 ± 0.0
Chromium (Cr)	–[c]	<0.5	0.15	1.5 ± 0.0	0.14	3.0 ± 0.0
Copper (Cu)	4.3	15 ± 2	2.75	28.1 ± 0.2	2.1	45.7 ± 0.4
Iron (Fe)	123	433 ± 32	66.3	677 ± 2.6	60.5	1317 ± 12
Manganese (Mn)	8.2	29 ± 4	2.9	30.0 ± 0.1	2.3	49.2 ± 0.4
Nickel (Ni)	<2	5 ± 1	0.7	7.0 ± 0.0	0.5	10.5 ± 0.1
Lead (Pb)	<2	4 ± 1	0.5	5.4 ± 0.1	0.2	4.7 ± 0.4
Zinc (Zn)	6	21 ± 3	6.6	67 ± 0.1	4.5	97 ± 0.5

[a] f.m. = fresh material; d.m. = dry matter.
[b] ±S.D.
[c] Detection limit.

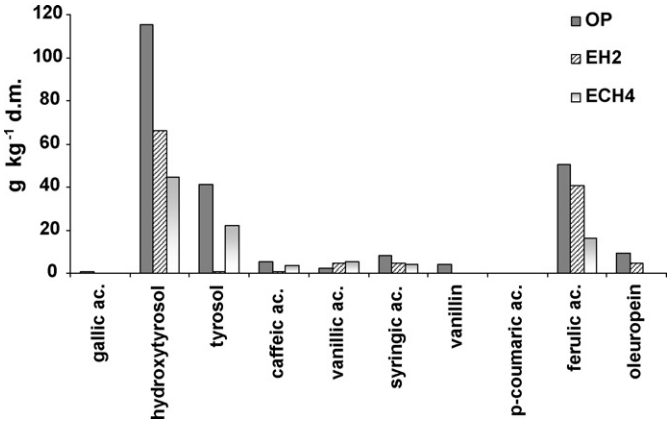

Fig. 1. Concentration of the various free phenolic compounds.

two digested materials, and in particular ECH_4, might release in soil inorganic N more easily than OP. The concentration of phosphorus (Table 2) increases markedly in the digested samples, as a consequence of the addition of phosphates as nutrients for microorganisms during the microbiological transformations. The content of potassium and sulphur follows a similar trend to that observed for phosphorus, even though the values are lower (Table 2).

The content nutrient (Ca and Mg) can be considered negligible for crop fertilisation, while the concentration of sodium increases in the effluents (Table 3) as a consequence of the addition of Na_2HPO_4 salt before the digestions. Among the micronutrients, only the amount of iron, present in bioavailable forms (organic matter Fe-complexes) could be of agronomic interest. The amount of heavy metals, including the micronutrients copper and zinc, is negligible (Table 3).

3.2. Germination and seedling growth

3.2.1. Paper study

The effects of the three by-products at the various doses both on seedling germination and number of abnormal is shown in Fig. 2. At the beginning of the germination process, 2 days after sowing, the percentage of germinated seedlings decreased markedly compared to the control with OP application at doses

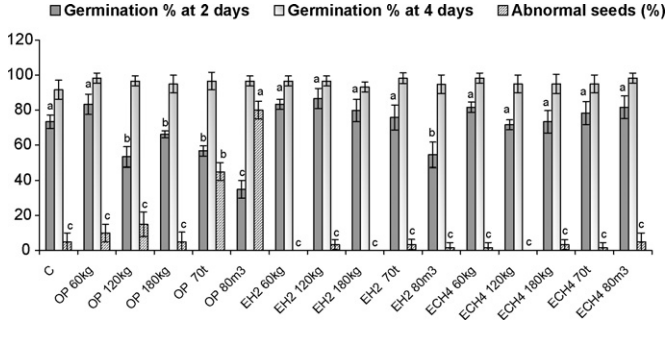

Fig. 2. Paper study: effects of olive pulp (OP) and effluents from hydrogen (EH_2) and methane production (ECH_4) applied at different N-doses on the germination (%) and on the number of abnormal seeds (%). Histograms of the same shape with the same letter or without letter do not differ per $p < 0.05$ (test SNK).

Table 4
Total amount of low molecular weight phenols (LMWP) (monomeric phenols) added with the three olive oil by-products

Dose	LMWP added (kg ha^{-1})		
	OP	EH_2	ECH_4
60 kg N ha^{-1}	5.9	1.8	1.1
120 kg N ha^{-1}	11.9	3.7	2.1
180 kg N ha^{-1}	17.8	5.5	3.2
70 tonnes ha^{-1}	47.1	8.4	3.1
80 m^3 ha^{-1}	58.8	10.1	3.7

higher than 60 kg N ha^{-1} and with EH_2 at 80 m^3 ha^{-1} ($p = 0.01$). At the end of germination, 4 days after sowing, no phytotoxic effects were found with any of the treatments. These results demonstrate that OP is able to enhances the seed dormancy [11] when the total amount of LMWP added was around 8 kg ha^{-1} (Table 4). OP had a negative effect not only on the germination energy, but also on the number of seeds characterised by an abnormal growth of seedlings. In particular, with the application of 70 tonnes ha^{-1} and 80 m^3 ha^{-1} of product, corresponding to 47.1 and 58.8 kg ha^{-1} of total LMWP added, the number of abnormal seeds increased remarkably, compared to the control ($p = 0.01$).

The effects of the treatments on seedling growth are reported in Fig. 3. OP at 70 tonnes ha^{-1} and 80 m^3 ha^{-1} doses had a negative effect on plant development: the shoot height and shoot weight decreased compared to the control by 55% and 53%, respectively, and the root weight decreased by 37%. No differences from the control were observed with the other doses ($p = 0.01$). On the contrary, with ECH_4, for all doses and EH_2, at 120 and 180 kg N ha^{-1}, even a significant increase in seedling growth was observed compared to the control ($p = 0.01$).

The toxicity of OP can be related to its greater content of LMWP (Fig. 1, Table 4) that are characterised by the highest toxic effects on plants and soil microorganisms [5,11]. Phenols delay, but not completely inhibit the germination [11,29], whereas they seem to affect seedling growth more than germination. The shoot and root parameters were markedly reduced at both doses 70 tonnes ha^{-1} and 80 m^3 ha^{-1} and this reduction can only be attributed to the higher LMWP added (47.1 and 58.8 kg ha^{-1}, respectively). The maximum quantity of LMWP

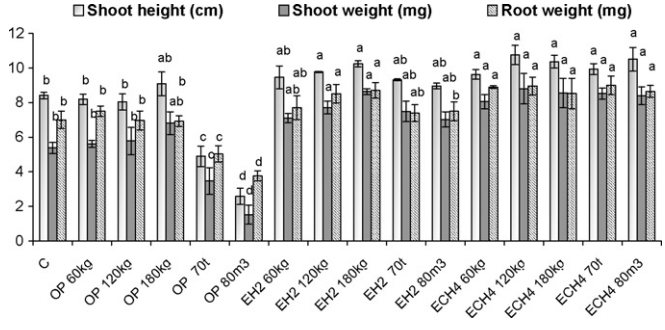

Fig. 3. Paper study: effects of olive pulp (OP) and effluents from hydrogen (EH_2) and methane production (ECH_4) applied at different N-doses on the shoot and root dry biomass of the single seedling (mg) and on the shoot height (cm).

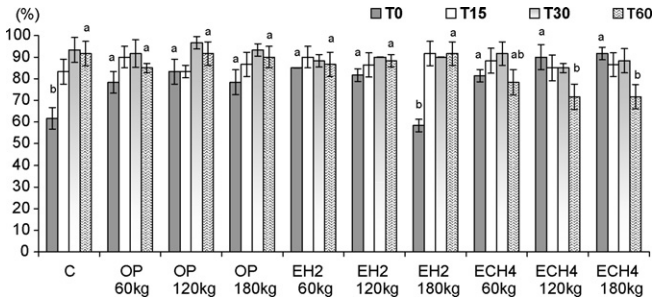

Fig. 4. Soil study: effects of olive pulp (OP) and effluents from hydrogen (EH_2) and methane production (ECH_4) applied at different N-doses and at different times of wheat sowing on germination at 3 days (%).

added with ECH_4 and EH_2 (3.7 and 10.1 kg ha^{-1}, respectively) is less than the amount added with OP at 120 kg N ha^{-1}.

3.2.2. Soil study

The effects of the three products applied at different doses varied in function of wheat sowing time. At the beginning of the germination process (Fig. 4), with sowing at T0, a significant increase in germination energy (+35% compared to the control) was found with all treatments with the exception of EH_2 at 180 kg N ha^{-1} that does not differ from the control ($p = 0.01$). With sowing at T15 and T30 no differences among treatments were witnessed, whereas with sowing at T60 a decrease in germination energy (−19% compared to the control) was observed with the application of ECH_4 ($p = 0.01$), probably as a consequence of phytotoxic compounds released by the degradation of the product. At the end of the germination process no significant differences among treatments were observed at the different wheat sowing times, hence the products did not exert any negative effects on germination.

The shoot dry weight response to the application of the materials is shown in Fig. 5. When the wheat was sown at T0 and at T30, a significant increase in shoot weight, compared to the control, was observed with ECH_4 at the highest doses ($p = 0.05$). With sowing at T15 no negative effects were observed, whereas when the wheat was sown at T60, a significant increase, compared to the control, was found with ECH_4 at all doses (+18%) and with EH_2 at 180 kg N ha^{-1} (+19%) ($p = 0.01$). Shoot height does not vary in function of the different treatments applied. Additionally, when the wheat was sown at T0 a significant

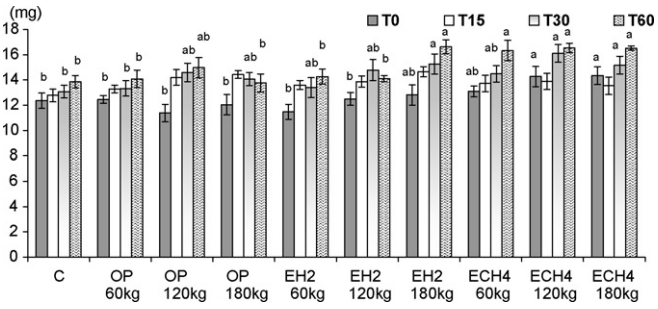

Fig. 5. Soil study: effects of olive pulp (OP) and effluents from hydrogen (EH_2) and methane production (ECH_4) applied at different N-doses and at different times of wheat sowing on shoot dry weight (mg).

increase of the root dry weight, compared to the control, was observed with EH_2 (+37%) and ECH_4 (+35%) at doses of 120 and 180 kg N ha^{-1} ($p = 0.01$); no negative effects were found at the other sowing times.

Germination and seedling development were not negatively affected by the application of the three by-products, on the contrary, a stimulatory effect (biostimulant effect) on plant growth was detected with the two effluents.

4. Conclusions

The possibility of using OP, EH_2 and ECH_4 as soil amendment seems to be very promising. They have shown chemical characteristics compatible with a potential use in agriculture, due to the amount of organic carbon, nutrients and a negligible concentration of heavy metals. Comparing OP to its effluents EH_2 and ECH_4 an increase in the AHR was observed. From the agronomic point of view the increase of AHR can be considered positive, because the addition of stable organic biomasses improve the soil's chemical, physical and biological properties.

Considering the effects on wheat, only OP at highest doses of 70 tonnes ha^{-1} and 80 m^3 ha^{-1} (never used for wheat) delayed, but not inhibited, the germination and negatively affected seedling growth. With EH_2 and ECH_4, on the contrary, a biostimulant effect, with a significant increase in plant growth, was detected. Therefore, at N doses normally used for wheat, the three products did not exert any relevant phytotoxicity on seed germination and seedling growth; moreover, the phytotoxic effects decreased when the products were incorporated into the soil.

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References

[1] A.C. Caputo, F. Scacchia, M. Pelagagge, Disposal of by-products in olive oil industry: waste to energy solutions, Appl. Therm. Eng. 23 (2003) 197–214.
[2] D. Mantzavinos, N. Kalogerakis, Treatment of olive mill effluents. Part I: organic matter degradation by chemical and biological processes—an overview, Environ. Int. 31 (2005) 289–295.
[3] R. Sarika, D. Mantzavinos, N. Kalogerakis, Treatment of olive mill effluents. Part II: complete removal of solids by direct flocculation with polyelectrolytes, Environ. Int. 31 (2005) 297–304.
[4] M. Beccari, M. Majone, C. Riccardi, F. Savarese, L. Torrisi, Integrated treatment of olive oil mill effluents: effect of chemical and physical pretreatment on anaerobic treatability, Water Sci. Technol. 40 (1999) 347–355.
[5] M.D. Gonzales, E. Moreno, J. Quevedo Sarmiento, A. Ramos Cormenzana, Studies on antibacterial activity of waste waters from olive oil mills inhibitory activity of phenolic and fatty acids, Chemosphere 20 (1990) 423–432.
[6] E. Bonari, M. Macchia, L.G. Angelici, L. Ceccarini, The waste waters from olive oil extraction: their influence on the germinative characteristics of some cultivated and weed species, Agric. Med. 123 (1993) 273–280.
[7] R. Cardelli, C. Benitez, Changes of chemical properties in two soils amended with moist olive residues, Agric. Med. 128 (1998) 171–177.

[8] R. Capasso, G. Cristinzio, A. Evidente, F. Scognamiglio, Isolation, spectroscopy and selective phytotoxic effects of polyphenols from vegetable waste waters, Phytochemistry 31 (1992) 4125–4128.
[9] J.A. Alburquerque, J. Gonzalvez, D. Garcìa, J. Cegarra, Agrochemical characterisation of alperujo, a solid by-product of the two-phase centrifugation method for olive oil extraction, Bioresour. Technol. 91 (2004) 195–200.
[10] R. Casa, A. D'Annibale, F. Pieruccetti, S.R. Stazi, G. Giovannozzi-Sermanni, B. Lo Cascio, Reduction of the phenolic components in olive-mill wastewater by an enzymatic treatment and its impact on durum wheat (*Triticum durum*) germinability, Chemosphere 50 (2003) 959–966.
[11] M.G. Krogmeier, J.M. Bremner, Effects of phenolic acids on seed germination and seedling growth in soil, Biol. Fert. Soils 8 (1989) 116–122.
[12] A. Saviozzi, R. Levi Minzi, R. Cardelli, A. Biasci, R. Riffaldi, Suitability of moist olive pomace as soil amendment, Water Air Soil Poll. 128 (2001) 13–22.
[13] H.N. Gavala, I.V. Skiadas, B.K. Ahring, G. Lyberatos, Potential for biohydrogen and methane production from olive pulp, Water Sci. Technol. 52 (1/2) (2005) 209–215.
[14] AOAC, Official Methods of Analysis, 16th ed, Association of Official Analytical Chemists, Arlington, VA, 1995.
[15] J.D. Box, Investigation of the Folin Ciocalteau phenol reagent for the determination of polyphenolic substances in natural waters, Water Res. 17 (1983) 511–525.
[16] D. Ryan, H. Lawrence, P.D. Prenzler, M. Antolovich, K. Robards, Recovery of phenolic compounds from olea europaea, Anal. Chim. Acta 445 (2001) 67–77.
[17] L. Lesage-Meessen, D. Navarro, S. Maunier, J.C. Sigoillot, J. Lorquin, M. Delattre, J.C. Simon, M. Asther, M. Labat, Simple phenolic content in olive oil residues as a function of extraction systems, Food Chem. 75 (2001) 501–507.
[18] C. Ciavatta, L. Vittori Antisari, P. Sequi, Determination of organic carbon in soils and fertilizers, Commun. Soil Sci. Plant Anal. 20 (1989) 759–773.
[19] C. Ciavatta, M. Govi, L. Vittori Antisari, P. Sequi, Determination of organic carbon in aqueous extracts of soils and fertilizers, Commun. Soil Sci. Plant Anal. 22 (1991) 795–807.
[20] C. Ciavatta, M. Govi, Use of insoluble polyvinylpyrrolidone and isoelectric focusing in the study of humic substances in soils and organic wastes: a review, J. Chromatogr. 643 (1993) 261–270.
[21] AOAC, Official Methods of Analysis, 15th ed, Association of Official Analytical Chemists, Arlington, VA, 1990.
[22] J.G. Murphy, J. Riley, A modified single solution method for the determination of phosphate in natural waters, Anal. Chim. Acta 27 (1962) 31–36.
[23] International Rules for Seed Testing, Amendments of Rules of the International Seed Testing Association, ISTA, 2003.
[24] USDA, Soil Taxonomy, 6th ed, Keys to Soil Taxonomy, 1998.
[25] SSSA, in: D.L. Sparks (Ed.), Methods of Soil Analysis, Chemical Methods, Part 3, Soil Science Society of America, Madison, WI, 1996, Book Series no. 5.
[26] G. Aliotta, A. Fiorentino, A. Oliva, F. Temessi, Olive oil mill wastewater: Isolation of polyphenols and their phytotoxicity in vitro, Allelopathy J. 9 (2000) 9–17.
[27] M. Della Greca, P. Monaco, G. Pinto, A. Pollio, L. Previtera, F. Temessi, Phytotoxicity of low molecular weight phenols from olive mill waste waters, Bull. Environ. Contam. Toxicol. 67 (2001) 352–359.
[28] A. Fiorentino, A. Gentili, M. Isidori, P. Monaco, A. Nardelli, A. Parrella, F. Temessi, Environmental effects caused by olive mill wastewaters: toxicity comparison of low molecular weight phenol components, J. Agric. Food Chem. 51 (2003) 1005–1009.
[29] R.D. Williams, R.E. Hoagland, The effects of naturally ocurring phenolic compounds on seed germination, Weed Sci. 30 (1982) 206–212.