Olive Pomace Amendment in Mediterranean Conditions: Effect on Soil and Humic Acid Properties and Wheat (*Triticum turgidum* L.) Yield

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The effects of the addition of either crude or exhausted olive pomace at two rates (10 and 20 t ha^{-1}) on soil and soil humic acid (HA) properties and durum wheat (*Triticum turgidum* L.) yield were investigated in open-field Mediterranean conditions. Soil amendment with olive pomaces produced a significant increase of total organic, total extractable, humified and nonhumified C forms, and available K contents. With respect to control soil HA, humic-like acids isolated from crude and exhausted olive pomaces were characterized by larger phenolic OH group contents, smaller carboxyl group contents, a prevalent aliphatic character, extended molecular heterogeneity, and smaller aromatic polycondensation and humification degrees. In general, application of olive pomaces to soil produced a number of modifications in soil HAs, including the increase of O and acidic functional group contents, C/N ratio, and aliphaticity and the decrease of C/H ratio and N and C contents. Wheat grain yield increased significantly as an effect of olive pomace amendment. In particular, the increases were related to kernel weight, kernel number per square meter, and soil organic matter content. Possibly, the enhanced amount of soil organic matter in olive-pomace-amended soils relieved wheat of drought stress from anthesis to maturity by promoting a good soil structure, thereby reducing water loss by evaporation.

KEYWORDS: Crude olive pomace; exhausted olive pomace; humic acids; Mediterranean agro-ecosystem; soil amendment; *Triticum turgidum* L.; wheat

INTRODUCTION

Olive oil mill extraction is a widely diffused food industry in the Mediterranean region. The byproducts obtained from the traditional three-phase centrifugation process of the olive paste are the olive oil mill wastewater and a solid residue consisting mainly of olive skin, pulp, and pit fragments, which is named olive pomace. The residue obtained from the first olive pressing by traditional and continuous machines is known as "crude" olive pomace (CP), whereas the residue obtained after the extraction of the small amount of oil still present in CP by water steam and/or organic solvents is named "exhausted" olive pomace (EP). Both materials contain a valuable amount of organic matter and plant nutrients (*1, 2*).

Durum wheat is a widely diffused crop in Mediterranean countries, and its yield generally suffers from water limitation in the dryland production systems typically used in this area. In particular, most rainfall occurs during autumn and winter, and water deficit occurs in spring. This rainfall regime typically

results in moderate stress at wheat anthesis and in severe stress during grain filling (*3−5*).

Furthermore, most agricultural soils in the Mediterranean region contain small amounts of organic matter (*6−8*), which is a further limiting factor for wheat growth and production. In particular, soil organic matter, and especially its humified fractions such as humic acids (HAs), constitute not only an important source of nutrients but are also a key factor in promoting a good soil structure that improves water retention (*6, 9−12*). Recycling of CP not further processed and/or EP as soil amendment represents a promising agricultural practice for increasing soil organic matter content, thus improving crop yields in Mediterranean agro-ecosystems. This practice can also help solve environmental hazards and economic costs related to the disposal of olive pomace wastes.

The objectives of this work were to evaluate the effects of CP and EP amendment at different rates on (a) some relevant soil chemical properties; (b) the molecular, structural, and functional characteristics of amended soil HAs; and (c) the yield of durum wheat grown in Mediterranean conditions.

MATERIALS AND METHODS

Olive Pomaces, Soils, and Field Experiment. Samples of CP and EP were collected from an olive oil mill plant located in the Bari

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Olive Pomace Amendment in Mediterranean Conditions

J. Agric. Food Chem., Vol. 53, No. 17, 2005 **6731**

province in southern Italy, which employed a three-phase decanter centrifugation for oil separation. The field experiment was conducted in Spinazzola (Bari province) on a sandy loam soil (sand, 670 g kg^{-1}; silt, 160 g kg^{-1}; clay, 170 g kg^{-1}) classified as a Typic Haploxerept (*13*) or Haplic Calcaric Regosol (*14*). The site is characterized by a thermo-Mediterranean climate (*15*) with mild winters and hot dry summers. The average annual rainfall is 490 mm, which occurs mostly in the autumn and winter, and the average annual temperature is 15 °C.

The experimental design includes nonirrigated plots (2 m × 2 m) arranged randomly, either unamended (S) or amended with CP and EP at rates of 10 and 20 t ha^{-1} on a dry weight basis (CP10, CP20 and EP10, EP20, respectively). Each treatment was performed in four replicates. Olive pomace was applied in late March 2003 and immediately incorporated into soil at a depth of 0−20 cm with a disk harrow. Five surface soil subsamples (Ap horizon, 0−20 cm depth) were collected randomly from each plot in late July 2003 using a soil probe of 3 cm diameter. A soil sample was then obtained for each plot by mixing equal amounts of the five corresponding soil subsamples. In early November 2003, durum wheat (*Triticum turgidum* L.) was planted in rows 15 cm wide at a seeding rate of 250 kg ha^{-1} at a density of 450 plants m^{-2}. During the growing season weeds were controlled by applying the herbicides clodinafop-propargyl [prop-2-ynyl(*R*)-2-[4-(5-chloro-3-fluoropyridin-2-yloxy)phenoxy] propionate] and tribenuron-methyl [2-[4-methoxy-6-methyl-1,3,5-triazin-2-yl(methyl)carbamoylsulfamoyl]-benzoic acid)]at rates of 250 and 15 g ha^{-1}, respectively. Soon after the wheat physiological maturity was reached (mid-July 2004), the entire above-ground wheat biomass was collected from each plot.

Pomace and Soil Analyses. Prior to analyses, pomace and soil samples were air-dried, crushed, and passed through a 2 mm sieve. The principal chemical properties of the samples were determined according to conventional methods (*16*). In particular, (a) dry matter was measured after the sample had been heated overnight at 105 °C; (b) ash content was measured by burning the sample overnight at 550 °C; (c) the pH was determined at a ratio of pomace to water of 1:10 and a ratio of soil to water of 1:2.5; (d) electrical conductivity (EC) was measured on water extracts obtained at a ratio of pomace to water of 1:10 and a ratio of soil to water of 1:5; (e) total organic C (TOC) was determined on 0.5 mm ground sample by dichromate oxidation followed by titration with ferrous ammonium sulfate; (f) total N content was obtained according to the Kjeldahl method; (g) total P and K contents in olive pomaces were determined on nitric and perchloric acid digests of 0.5 mm ground samples by inductively coupled plasma−atomic emission spectrometry; (h) available P content of soils was determined according to the Olsen method; and (i) available K content of soils was measured in 1 M BaCl$_2$ soil extracts using a ratio of soil to extractant of 1:10. The fractions of total extractable C (TEC) and humified C (HA + FA) were isolated from soils according to the procedure described in Ciavatta et al. (*17*). Briefly, 100 mL of a solution of 0.1 M Na$_4$P$_2$O$_7$ and 0.1 M NaOH was added to 2 g of 0.5 mm ground soil sample, and the mixture was shaken for 48 h at 338 K. The supernatant solution was then separated from the residue by centrifugation and filtration. Twenty-five milliliters of the supernatant (TEC fraction) was acidified to pH ∼1 with 50% H$_2$SO$_4$ to allow the precipitation of the HA fraction. The fulvic acid (FA) fraction remaining in solution was purified by passing on a column filled with polyvinylpyrrolidone and then added to the HA fraction. The combined HA and FA fractions were transferred into a 50 mL flask and brought to volume with 0.5 M NaOH. The contents of TEC and (HA + FA)-C were determined by dichromate oxidation followed by titration with ferrous ammonium sulfate. Nonhumified (NH) C content was calculated by difference: NH = TEC − (HA + FA). The degree of humification (DH) was calculated as DH% = 100 × (HA + FA)/TEC, the humification rate (HR) as HR% = 100 × (HA + FA)/TOC, and the humification index (HI) as HI = NH/(HA + FA) (*17, 18*).

Isolation of Humic Acids. The humic-like acids (HLAs) and HAs were isolated according to a conventional procedure (*19*), respectively, from the pomaces and composite soil samples obtained by mixing equal weights of the four corresponding replicates of each treatment. Briefly, a solution of 0.1 M Na$_4$P$_2$O$_7$ and 0.1 M NaOH was added to each air-dried, 2 mm sieved sample using a ratio of extractant to sample of 10:1. The mixture was shaken mechanically in a N$_2$ gas atmosphere

for 24 h at room temperature (293 ± 2 K). The supernatant solution was then separated from the residue by centrifugation at 9600*g* for 30 min. The extraction procedure was repeated three times on the residue that was finally discarded. The combined alkaline supernatants were acidified with 6 M HCl to pH ∼1, allowed to stand for 24 h in a refrigerator, and then centrifuged at 30400*g* for 20 min. The HLA and HA precipitates were purified by repeating three times the following steps: (a) dissolution in a minimal volume of the alkaline extractant; (b) centrifugation as above; (c) removal of the residue; (d) acidification of the recovered alkaline supernatant with 6 M HCl to pH ∼1; (e) standing of the suspension for 24 h at room temperature; and (f) final centrifugation as above. The centrifuged HLAs and HAs were recovered with distilled water and then dialyzed against distilled water using a membrane having a molecular weight cutoff of 6000−8000 Da until the dialysis water gave a negative Cl$^-$ ion test with AgNO$_3$. Finally, the dialyzed HLAs and HAs were freeze-dried and stored at room temperature in plastic vials placed in a desiccator containing P$_2$O$_5$.

Humic Acid Analyses. The moisture content of HLAs and HAs was measured by heating overnight at 105 °C and the ash content by burning overnight at 550 °C. The C, H, N, and S contents of HLAs and HAs were determined in triplicate using a Fisons Instruments (Crawley, U.K.) elemental analyzer model EA 1108. Oxygen content was calculated by difference: O% = 100 − (C + H + N + S)%. Total acidity was determined according to the Ba(OH)$_2$ method and carboxyl group content according to the Ca(CH$_3$COO)$_2$ method, and phenolic hydroxyl group content was calculated by difference (*20*).

The Fourier transform infrared (FT IR) spectra of HLAs and HAs were recorded over the range from 4000 to 400 cm^{-1} on pellets obtained by pressing under reduced pressure a mixture of 1 mg of HA and 400 mg of dried KBr, spectrometry grade. A Nicolet (Madison, WI) 5PC FT IR spectrophotometer operating with a peak resolution of 2 cm^{-1} and Omnic 1.2 software were used to obtain the spectra.

Fluorescence spectra in the emission, excitation, and synchronous-scan modes were obtained on aqueous solutions of each HLA and HA at a concentration of 100 mg L^{-1} after overnight equilibration at room temperature and adjustment to pH 8 with 0.05 M NaOH (*21*). Spectra were recorded using a Perkin-Elmer (PE, Norwalk, CT) LS-5 luminescence spectrophotometer equipped with a PE Data Station 3600 for data generation and processing by PE Computerized Luminescence Spectroscopy (PECLS) software. Emission and excitation slits were set at a 5 nm bandwidth, and a scan speed of 120 nm min^{-1} was selected for both monochromators. Emission spectra were recorded over the range from 380 to 550 nm at a constant excitation wavelength of 360 nm. Excitation spectra were obtained over a scan range of 300−500 nm by measuring the emission radiation at a fixed wavelength of 520 nm. Synchronous-scan excitation spectra were measured by scanning simultaneously both the excitation (varied from 300 to 550 nm) and the emission wavelengths while maintaining a constant, optimized wavelength difference $\Delta\lambda = \lambda_{exc} - \lambda_{em} = 18$ nm (*21*). The overall relative fluorescence intensity (RFI) was expressed in arbitrary units as the unitless reciprocal to the gain used to normalize each emission spectrum (*21*).

Wheat Yield Analyses. The entire wheat plant samples collected were weighed, and then the grain was separated from the straw and also weighed. Moisture contents of the grain and straw were determined by heating for 24 h at 105 °C. Total wheat plant and grain yields were calculated on a moisture-free basis, whereas straw yield was calculated as the difference between total and grain yields. The harvest index was calculated as the percentage amount of grain in the total above-ground biomass. The number of spikes per square meter was determined by dividing the number of spikes contained in all plants in each plot by the plot area. The average kernel weight was calculated as the average of the weights of 1000 kernels taken randomly. The number of kernels per square meter was determined by dividing the grain yield by the average kernel weight. The number of kernels per spike was calculated as the average number of kernels in 20 spikes selected randomly.

Statistical Analysis. Analytical data were subjected to statistical analyses of variance (ANOVA). Mean separations were determined using the Fischer's protected least significant difference test at *P* = 0.05. Simple regression analyses were performed to find possible correlations of the grain yield with any other wheat parameter and

6732 *J. Agric. Food Chem.,* Vol. 53, No. 17, 2005

Brunetti et al.

Table 1. Main Chemical Properties of Olive Pomaces (± Standard Errors of Three Laboratory Replicates)[a]

property	crude pomace	exhausted pomace
dry matter (g kg^{-1})	340 ± 1	780 ± 1
ash (g kg^{-1})	50 ± 4	48 ± 1
pH	5.7 ± 0.1	5.9 ± 0.1
electrical conductivity (dS m^{-1})	3.1 ± 0.1	2.9 ± 0.1
total organic C (g kg^{-1})	450 ± 3	411 ± 2
total N (g kg^{-1})	10.5 ± 0.3	10.8 ± 0.4
total P (g kg^{-1})	0.50 ± 0.04	0.35 ± 0.06
total K (g kg^{-1})	17.1 ± 0.3	15.9 ± 0.2
C/N ratio	43	38

[a] All data except dry matter are expressed on a dry matter basis.

Table 2. Some Chemical Properties of Unamended Soil (S) and Soils Amended with either Crude Pomace or Exhausted Pomace at Rates of 10 and 20 t ha^{-1} (CP10, CP20 and EP10, EP20, Respectively) and Correlation Coefficients (*r*) of the Wheat Grain Yield with These Properties[a]

soil	pH	EC (dS m^{-1})	TOC (g kg^{-1})	total N (g kg^{-1})	available P (mg kg^{-1})	available K (mg kg^{-1})	C/N ratio
S	8.0 a	0.19 a	10.3 d	1.0 a	33 a	186 d	10.0 b
CP10	8.0 a	0.21 a	11.5 c	1.1 a	34 a	253 b	10.8 ab
CP20	7.9 a	0.22 a	12.4 ab	1.1 a	32 a	297 a	11.5 a
EP10	8.0 a	0.19 a	11.7 bc	1.1 a	31 a	220 c	10.6 b
EP20	8.0 a	0.19 a	13.0 a	1.1 a	31 a	268 b	11.5 a
r	−0.197	0.159	0.976**	0.892*	−0.585	0.818	0.913*

[a] Values in the same column followed by the same letter are not significantly different (*P* = 0.05). *, *P* < 0.05; **, *P* < 0.01. EC, electrical conductivity. TOC, total organic C.

analytical data of unamended and amended soils and their HA fractions. The SPSS 11.5.1. program for Windows (SPSS, Inc., 2002, Chicago, IL) was used for statistical analysis.

RESULTS AND DISCUSSION

Olive Pomaces and Soils. The chemical properties of CP and EP (**Table 1**) generally fall within the ranges commonly reported in the literature for these materials (*22−26*). With respect to the EP, the CP has a much smaller dry matter content, similar ash and total N contents, pH, and electrical conductivity (EC), slightly larger TOC content and C/N ratio, and larger contents of total P and total K.

Some chemical characteristics of the unamended soil and soils amended with either CP or EP at rates of 10 and 20 t ha^{-1} are listed in **Table 2**. The control soil features a moderately alkaline pH, small EC, TOC content, and C/N ratio, and levels of total N, available P, and available K adequate for wheat growth (*27*). The pomace-amended soils have pH, EC, and contents of total N and available P similar to those of the control soil, but larger contents of TOC and available K and slightly larger C/N ratios.

The significant increase of available K in amended soils may be directly related to the large contents of this nutrient in olive pomaces. However, no significant increase of N is measured in amended soils with respect to the control soil, despite the relatively large amount of N in olive pomaces. This result suggests a partial N loss, possibly by N mineralization and subsequent NH$_3$ volatilization, which can be ascribed to the moderately alkaline soil pH and/or nitrification and NO$_3^-$ leaching (*28, 29*).

Despite the slightly larger TOC content of CP as compared to EP, the slightly smaller TOC increase measured in the CP-amended soils suggests the occurrence of more extended

Table 3. Total Extractable C (TEC), Humified C (HA + FA), and Nonhumified C (NH) Contents and Degree of Humification (DH), Humification Rate (HR), and Humification Index (HI) of Unamended Soil (S) and Soils Amended with either Crude Pomace or Exhausted Pomace at Rates of 10 or 20 t ha^{-1} (CP10, CP20 and EP10, EP20, Respectively) and Correlation Coefficients (*r*) of the Wheat Grain Yield with Each Variable[a]

soil	TEC (g kg^{-1})	HA + FA	NH	DH (%)	HR	HI
S	7.9 d	7.0 d	0.9 c	89 a	68 a	0.13 b
CP10	8.8 c	7.3 c	1.5 b	83 b	63 b	0.21 a
CP20	9.6 b	7.8 b	1.9 ab	81 b	63 b	0.24 a
EP10	9.1 c	7.5 b	1.5 b	83 b	64 b	0.20 a
EP20	10.2 a	8.3 a	1.9 a	81 b	64 b	0.23 a
r	0.967**	0.901*	0.985**	−0.964**	−0.844	0.964**

[a] Values in the same column followed by the same letter are not significantly different (*P* = 0.05). *, *P* < 0.05; **, *P* < 0.01.

Table 4. Elemental Composition (± Standard Errors of Three Laboratory Replicates, Moisture- and Ashfree) and Atomic Ratios of Humic-like Acids (HLAs) Isolated from Crude Pomace (CP) and Exhausted Pomace (EP) and Humic Acids (HAs) from Unamended Soil (S) and Soils Amended with either CP or EP at Rates of 10 or 20 t ha^{-1} (CP10, CP20 and EP10, EP20, Respectively) and Correlation Coefficients (*r*) of the Grain Yield with Each Property of Soil HAs

origin of HLA and HA sample	g kg^{-1}					atomic ratio		
	C	H	N	S	O	C/N	C/H	O/C
S	591 ± 7	47 ± 1	46 ± 1	2 ± 1	315 ± 9	15.1	1.1	0.4
CP	616 ± 5	80 ± 1	17 ± 1	1 ± 0	286 ± 6	41.5	0.6	0.3
CP10	563 ± 2	47 ± 2	44 ± 0	3 ± 0	343 ± 5	15.0	1.0	0.5
CP20	581 ± 4	62 ± 0	38 ± 1	2 ± 0	317 ± 3	17.8	0.8	0.4
EP	574 ± 8	68 ± 1	21 ± 0	2 ± 1	337 ± 6	32.0	0.7	0.4
EP10	553 ± 4	50 ± 0	42 ± 0	3 ± 0	352 ± 5	15.5	0.9	0.5
EP20	558 ± 4	50 ± 1	40 ± 0	3 ± 1	350 ± 2	16.3	0.9	0.5
r	−0.677	0.493	−0.831	0.423	0.562	0.578	−0.720	0.598

mineralization processes, that is, larger priming effect, in these soils than in the EP-amended ones. The larger presence of easily biodegradable compounds in CP than in EP may feasibly result in a larger increase of soil microbial activity due to a greater availability of energy sources, thus leading to a larger enhancement in soil organic C decomposition. Furthermore, the slight increase of C/N ratio in amended soils would suggest a slight decrease of organic matter stability, especially at the larger amendment rate.

In comparison to the control soil, olive pomace amended soils have larger contents of TEC, (HA + FA)-C, and NH-C (**Table 3**), especially at the highest amendment rate and for the EP amendment (except for the NH content). Furthermore, both amendments cause a significant decrease of DH and HR and an increase of HI (**Table 3**). These results suggest that amendment with olive pomace, especially with CP, leads to a slight decrease of native soil organic matter stability by increasing the nonhumified organic matter fraction more than the humic fractions.

Humic Acids: Elemental and Functional Group Composition. The elemental composition and atomic ratios of HLAs isolated from CP and EP and the HAs from control and amended soils are shown in **Table 4**. The CP-HLA has larger or much larger C and H contents and C/N ratio and smaller or much smaller O, N, and S contents and C/H and O/C ratios than S-HA. In contrast, EP-HLA has smaller C and N contents and C/H ratio, similar S content and O/C ratio, and larger H and O contents and C/N ratio than S-HA. With respect to S-HA, the
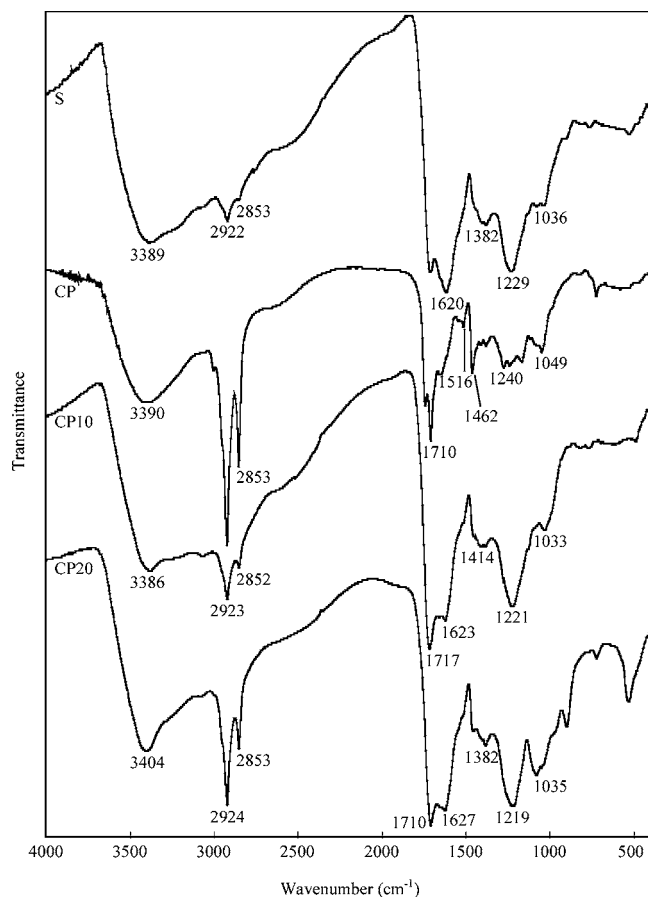
Olive Pomace Amendment in Mediterranean Conditions

J. Agric. Food Chem., Vol. 53, No. 17, 2005 **6733**

Figure 1. FT IR spectra of humic-like acids isolated from crude olive pomace (CP) and humic acids from unamended soil (S) and soils amended with either 10 or 20 t ha^{-1} of CP (CP10 and CP20, respectively).
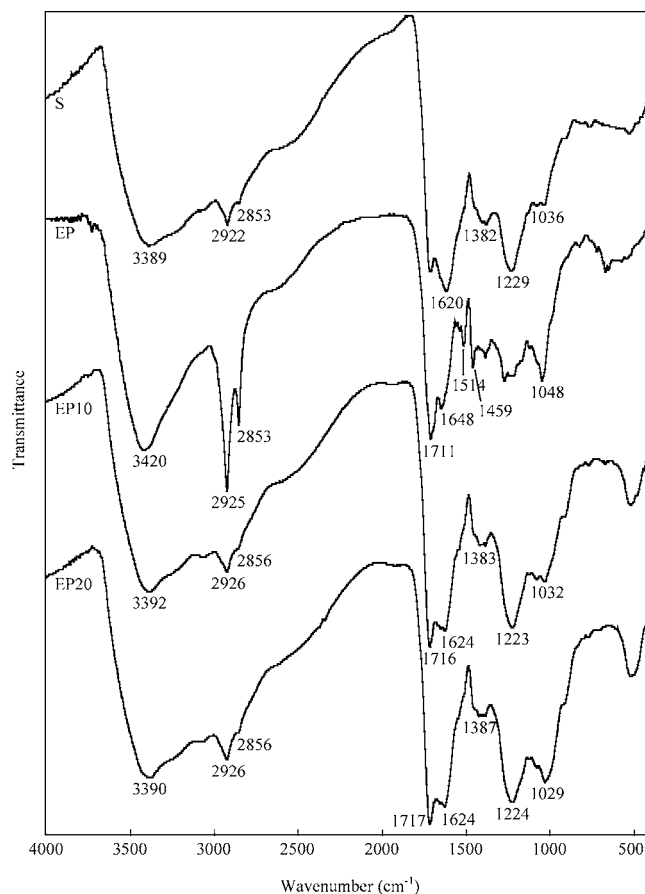
Table 5. Acidic Functional Group Contents (Moisture- and Ashfree) and Relative Fluorescence Intensity (RFI) of Humic-like Acids (HLAs) Isolated from Crude Pomace (CP) and Exhausted Pomace (EP), Humic Acids (HAs) from Unamended Soil (S) and Soils Amended with either CP or EP at Rates of 10 or 20 t ha^{-1} (CP10, CP20 and EP10, EP20, Respectively), and Correlation Coefficients (*r*) of the Grain Yield with Each Property of Soil HAs[a]

origin of HLA and HA sample	total acidity (mmol g^{-1})	COOH (mmol g^{-1})	phenolic OH (mmol g^{-1})	RFI (arbitrary units)
S	4.7	4.3	0.4	3.0
CP	5.9	2.2	3.7	3.2
CP10	6.0	4.6	1.4	3.5
CP20	7.1	4.5	2.6	3.6
EP	5.9	2.0	3.9	3.6
EP10	6.6	5.3	1.3	4.1
EP20	6.5	5.3	1.2	4.1
r	0.910*	0.685	0.648	0.865

[a] *, $P < 0.05$.

HAs from CP- and EP-amended soils have similar or slightly larger H, O, and S contents and C/N and O/C ratios and slightly smaller C and N contents and C/H ratios.

The acidic functional group compositions of CP-HLA and EP-HLA are similar to one another but differ markedly from that of S-HA (**Table 5**). In particular, total acidity and especially phenolic OH group contents of CP-HLA and EP-HLA are larger and carboxyl group contents are smaller than those of S-HA. The HAs isolated from amended soils show greater total acidity and phenolic OH and carboxyl group contents than S-HA.

Figure 2. FT IR spectra of humic-like acids isolated from exhausted olive pomace (EP) and humic acids from unamended soil (S) and soils amended with either 10 or 20 t ha^{-1} of EP (EP10 and EP20, respectively).

These results indicate a difference between S-HA and pomace-HLAs and between CP-HLA and EP-HLA, which are clearly due to their different origin and genesis. Residues and byproducts of the olive mill agroindustry are characterized by large polyphenol contents (*24*, *30−32*), which may explain the larger phenolic OH group contents measured in pomace-HLA with respect to soil HAs. Pomace amendment affects slightly the elemental composition but increases the content of acidic functionalities of soil HA, probably as a result of the partial incorporation of pomace-HLAs into soil HA. Furthermore, pomace applications might have favored microbial oxidation reactions leading to the formation of additional acidic oxygenated functional groups in amended soil HAs.

Humic Acids: FT IR Spectra. The FT IR spectra of CP-HLA and EP-HLA differ markedly from the spectrum of S-HA (**Figures 1** and **2**). The main features of these spectra and their corresponding assignments, according to Bellamy (*33*), MacCarthy and Rice (*34*), and Stevenson (*9*), are the following: (a) a common, intense broad band at about 3400 cm^{-1} usually attributed to O−H stretching and, secondarily, to N−H stretching of various functional groups; (b) two sharp bands at about 2925 and 2855 cm^{-1} due to aliphatic C−H group stretching, the relative intensity of which varies in the order CP-HLA > EP-HLA > S-HA; (c) an absorption at about 1710 cm^{-1} due to C=O stretching of various carbonyl groups, which is much more intense in CP-HLA and EP-HLA than in S-HA; (d) a broad band in the region between 1660 and 1600 cm^{-1}, which is generally considered to be an envelope of unresolved absorptions mainly due to aromatic C=C, C=O stretching of amide groups (amide I band), quinonic C=O, and/or C=O of H-bonded
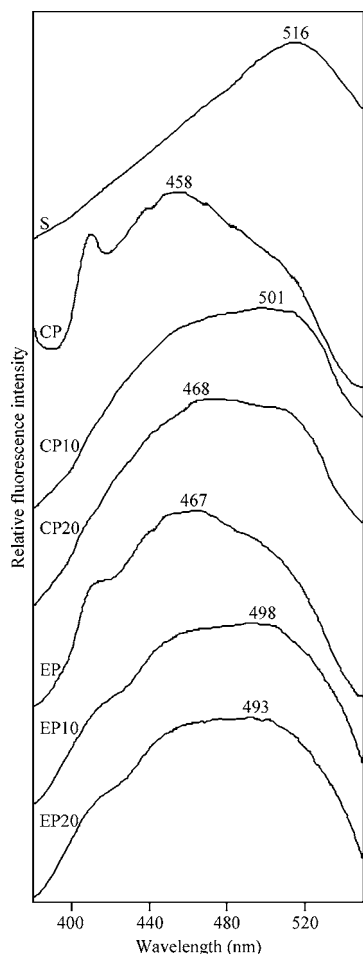
6734 *J. Agric. Food Chem.,* Vol. 53, No. 17, 2005

Brunetti et al.

Figure 3. Fluorescence emission spectra of humic-like acids isolated from crude olive pomace (CP) and exhausted olive pomace (EP) and humic acids from unamended soil (S) and soils amended with either CP or EP at rates of 10 or 20 m³ ha⁻¹ (CP10, CP20 and EP10, EP20, respectively).

Figure 4. Fluorescence excitation spectra of humic-like acids isolated from crude olive pomace (CP) and exhausted olive pomace (EP) and humic acids from unamended soil (S) and soils amended with either CP or EP at rates of 10 or 20 m³ ha⁻¹ (CP10, CP20 and EP10, EP20, respectively).

conjugated ketones, which is less intense in CP-HLA and EP-HLA than in S-HA; (e) a band of medium intensity at about 1540 cm⁻¹ preferentially ascribed to N—H deformation and C═N stretching of amides (amide II band), which is evident only for CP-HLA and EP-HLA; (f) a medium-intensity band at about 1460 cm⁻¹ attributed to aliphatic C—H, which is very sharp in CP-HLA and EP-HLA, but a weak shoulder in S-HA; (g) a common band of medium intensity centered at about 1380 cm⁻¹ possibly due to C—H deformation of CH₂ and CH₃ groups and/or to antisymmetric stretching of COO⁻ groups; (h) a broad band of medium-strong intensity centered between 1270 and 1220 cm⁻¹ and generally ascribed to C—O stretching and O—H deformation of carboxyl groups and C—O stretching of aryl ethers, the intensity of which is much larger in S-HA; and (i) an absorption at about 1040 cm⁻¹, generally attributed to C—O stretching of polysaccharides or polysaccharide-like substances, which is more intense in CP-HLA and EP-HLA than in S-HA.

The FT IR spectra of HAs isolated from amended soils are more similar to the spectrum of S-HA than to the spectra of CP-HLA and EP-HLA (**Figures 1** and **2**). However, the absorptions ascribed to aliphatic structures, carboxyl groups, and polysaccharides are relatively more intense in the FT IR spectra of amended soil HAs than in the spectrum of S-HA and tend to increase with the amendment rate, especially in the spectra of HAs from CP-amended soils.

FT IR analysis confirms the marked structural and functional differences between S-HA and pomace HLAs in that the latter
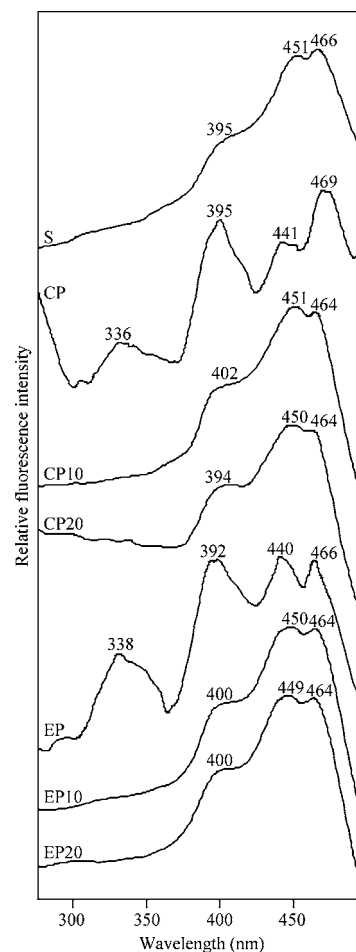
show a prominent aliphatic character and are rich in carbonyl and N-containing groups and polysaccharide components. As an effect, amended soil HAs appear to incorporate, at least partially and increasingly with amended rate, especially for CP-amended soils, the structural and functional moieties in which pomace-HLAs are rich.

Humic Acids: Fluorescence Spectra. The relative fluorescence intensity (RFI) value of EP-HLA is slightly larger than that of CP-HLA, and both are slightly larger than that of S-HA. The RFI values of the HAs isolated from soils amended with either CP or EP are generally greater than those of S-HA, CP-HLA, and EP-HLA (**Table 5**).

The fluorescence emission spectra (**Figure 3**) of all HLA and HA samples feature a typical broad band with the maximum centered at 516 nm for S-HA, at 458 nm for CP-HLA, and at 467 nm for EP-HLA. The amended soil HAs feature the emission maximum at a shorter wavelength than that of S-HA and a broad shoulder that extends to shorter wavelengths.

The fluorescence excitation spectra (**Figure 4**) of CP-HLA and EP-HLA are very different from the spectrum of S-HA and are characterized by four major peaks at short (336 or 338 nm), intermediate (392 or 395 nm), and long (441 or 440 and 469 or 466 nm) wavelengths. The excitation spectrum of S-HA features a prominent band with two peaks (466 and 451 nm) at long wavelength and a faint shoulder at 395 nm. The excitation spectra of amended soil HAs are similar to one another and to
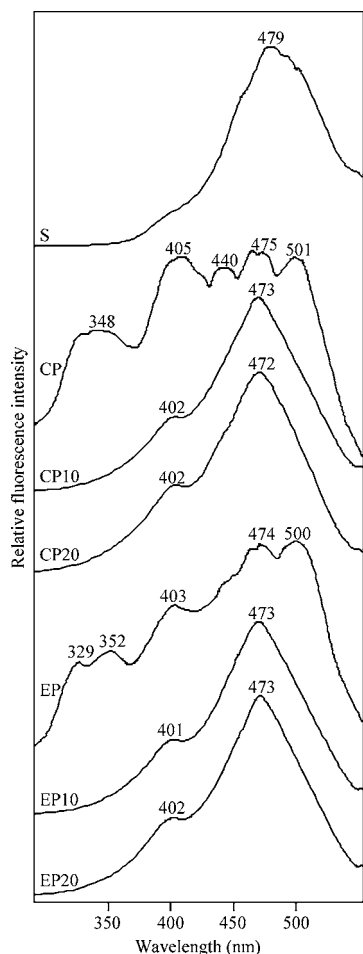
Olive Pomace Amendment in Mediterranean Conditions

J. Agric. Food Chem., Vol. 53, No. 17, 2005 **6735**

Figure 5. Fluorescence synchronous scan spectra of humic-like acids isolated from crude olive pomace (CP) and exhausted olive pomace (EP) and humic acids from unamended soil (S) and soils amended with either CP or EP at rates of 10 or 20 m³ ha⁻¹ (CP10, CP20 and EP10, EP20, respectively).

the spectrum of S-HA. However, with respect to S-HA, amended soil HAs, especially CP20-HA, exhibit a more intense shoulder at intermediate wavelength, and the band at ∼465 nm is slightly less intense than that at ∼450 nm.

The fluorescence synchronous scan spectra (**Figure 5**) of EP-HLA and especially of CP-HLA are also very different from the spectrum of S-HAs. The CP-HLA features four prominent bands of similar relative intensities at intermediate and long wavelengths (405, 440, 475, and 501 nm) and a less intense band at shorter wavelength (348 nm). The synchronous scan spectrum of sample EP-HLA is characterized by two prominent peaks at 500 and 474 nm and a series of less intense peaks at intermediate (403 nm) and short (352 and 329 nm) wavelengths. The S-HA shows a unique peak at long wavelength (479 nm)

accompanied by a shoulder at longer wavelengths. The synchronous scan spectra of amended soil HAs resemble more the spectruum of S-HA than those of CP-HLA and EP-HLA in showing a prominent unique peak at long wavelength (∼473 nm) and a weak shoulder at ∼402 nm.

The large overall fluorescence intensities and the short wavelengths measured for the main fluorescence peaks of samples EP-HLA and CP-HLA suggest the presence of simple structural components of wide molecular heterogeneity and small molecular weight, degree of aromatic polycondensation, level of conjugated chromophores, and humification degree (*21*). On the contrary, the small fluorescence intensities and long wavelengths of major peaks of S-HA may be ascribed to the presence of an extended, linearly condensed aromatic ring network and other unsaturated bond systems capable of a great degree of conjugation in large molecular weight units of great humification degree (*21*). The HAs from amended soils exhibit slight but measurable differences from S-HA, which confirms the partial incorporation of simple and low humified components of pomace HLAs into soil HA. This effect appears to increase with increasing amendment rate, especially when CP is applied.

Wheat Yield. The wheat yield parameters of the unamended soil and soils amended with either CP or EP at rates of 10 and 20 t ha⁻¹ are shown in **Table 6**. Soil application of CP and EP determines a significant increase of total and grain dry matter yields, spike and kernel numbers per square meter, and kernel weight. A small but not significant increase of straw dry matter and harvest index and a decrease of the number of kernels per spike are also apparent. Except for the total dry matter yield, no significant difference in yield parameters is measured between the variously amended soils.

The grain yield is correlated positively with the total yield ($P < 0.05$), the number of spikes per square meter ($P < 0.01$), the number of kernels per square meter ($P < 0.001$), and the kernel weight ($P < 0.001$) and negatively with the number of kernels per spike ($P < 0.01$) (**Table 6**). Thus, the increase of grain yield in soils amended with olive pomaces may be primarily related to the increased number of kernels per square meter and kernel weight and secondarily to the increase of spikes per square meter and decrease of kernels per spike.

The correlation coefficients of the grain yield with the chemical parameters of unamended and amended soils are indicated in the bottom rows of **Tables 2** and **3**. The grain yield is correlated positively and highly significantly ($P < 0.01$) with TOC, TEC, and nonhumified C contents and significantly ($P < 0.05$) with humified C and total N content, but negatively with DH and HI. No significant correlation appears to exist between grain yield and pH, EC, available P and K contents, and HR. Among the elemental and functional compositional data of unamended and amended soil HAs, only total acidity appears to be correlated positively and significantly with the grain yield (bottom rows of **Tables 4** and **5**).

Table 6. Wheat Yield Parameters and Correlation Coefficients (*r*) of the Grain Yield with Each Variable[a]

	dry matter yield (t ha⁻¹)			harvest index (%)	spikes (no./m²)	kernels		
soil	grain	straw	total			no./m²	no./spike	av wt (mg)
S	2.1 b	5.1 a	7.2 c	30 a	416 b	6424 b	15 a	33 b
CP10	2.8 a	5.3 a	8.1 bc	35 a	582 a	7542 ab	13 a	37 a
CP20	3.1 a	6.4 a	9.5 ab	32 a	628 a	7921 a	13 a	38 a
EP10	2.9 a	5.7 a	8.6 abc	34 a	584 a	7843 a	14 a	37 a
EP20	3.3 a	6.9 a	10.1 a	32 a	701 a	8395 a	12 a	39 a
r		0.863	0.942*	0.578	0.990**	0.997***	−0.978**	0.993***

[a] Means followed by the same letter within the same column are not significantly different according to LSD ($P = 0.05$). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Water deficit at wheat anthesis may lead to a decrease of wheat grain yield by reducing the spike number and, consequently, the kernel number per unit area. Furthermore, drought stress from anthesis to maturity, especially if accompanied by high temperatures, reduces the duration and rate of grain filling and, hence, the kernel weight (*3–5, 27, 35*). The results described above thus suggest that the increase of wheat grain yield in soils amended with olive pomaces may be related primarily to the increased content of both humified and nonhumified soil organic matter fractions. Most probably, the enhanced amount of soil organic matter relieves wheat of drought stress from anthesis to maturity by promoting a good soil structure, thereby reducing water loss by evaporation. Furthermore, regression analyses show that grain yield increases with decreasing DH and increasing HI, that is, with decreasing soil organic matter stabilization, which may be attributed to its greater degradability and ease in releasing plant nutrients. Results obtained also suggest that grain yield is affected positively to some extent by the increased total content of acidic functional groups in amended soil HAs. These groups are known to promote the capacity of HA to bind mineral particles together, thus improving soil structure, porosity, and water-holding capacity (*9, 10, 12*). The poor or absence of correlations found between grain yield and pH, EC, N, and available P and K may be due to the little effect of pomace application on these parameters and/or to their adequate initial levels for wheat growth.

Conclusions. Soil amendment with crude and exhausted olive pomaces determines some modification of general soil properties, including increase of TOC, TEC, humified C, nonhumified C, and available K contents. Furthermore, the compositional, structural, and functional chemical characteristics of native soil HA are modified by the partial incorporation of HLA components typical of applied pomaces. In particular, O and acidic functional group contents, C/N ratio, and aliphaticity increase, whereas C/H ratio and N and C contents decrease in pomace-amended soil HAs. These effects are generally more evident in the HAs from CP-amended soils than in the HAs from EP-amended soils and tend to increase with increasing amendment rate.

Olive pomace amendment of soils affects positively durum wheat yields in Mediterranean conditions by increasing the spike density and especially the kernel number per unit area and kernel weight. The enhanced amount of organic matter in pomace-amended soils appears to play a major role in improving wheat performance, possibly by increasing moisture retention in amended soils, thus minimizing the water deficit effects from wheat anthesis to maturity.

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Olive Pomace Amendment in Mediterranean Conditions

J. Agric. Food Chem., Vol. 53, No. 17, 2005 **6737**

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