

Characterization of Potential Liquid Organic Fertilizers Obtained by Alkaline Extraction from Two-Phase Olive-Mill Waste Composts

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Two potential liquid fertilizers were produced from two mature “alperujo” composts by using an alkaline extraction (1M KOH at 70°C). The characteristics of the resulting humic-like substances were compared to those extracted according to the international humic substance society protocol by using elemental analysis, FTIR and fluorescence spectroscopy in both excitation-emission matrix and synchronous modes. The results obtained confirmed that the hot alkaline extraction dissolved more polymerized compounds, probably including lignin degradation by-products and/or newly formed aromatic materials. On the other hand, based on fluorescence excitation-emission matrix results, different $\Delta\lambda$ (18, 66, and 100 nm) were selected to provide more information about humic substances in synchronous mode. The results showed that the extracted humic-like substances presented characteristics close to those of standard humic materials. As a consequence, the alkaline extraction of “alperujo” compost with 1M KOH at 70°C can be used to produce liquid organic fertilizers, thus diversifying the marketable products obtained from “alperujo,” the most abundant by-product of the Spanish olive-oil extraction industry. © 2012 American

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INTRODUCTION

Composting is a cheap technology which speeds up the natural biodegradation of organic matter by providing a controlled environment for a great diversity of microorganisms that lead to the mineralization of a large part of the raw

organic matter and the synthesis of new organic compounds via humification processes. As a consequence, it can be considered as a humification technology [1]. The compost obtained has the advantage of containing a large proportion of humic-like substances (HS) which play a major role in soil fertility. These substances are ubiquitous in the environment, occurring in composts, soils, sediments, and waters and may be isolated in abundance from coal-like lignite and leonardite. Previous studies [2, 3] highlighted their use in fertigation as liquid organic fertilizers in modern intensive agriculture. Nowadays, there is considerable evidence about the pollutant impact of mineral fertilizers on the environment, due to their massive application and the fact that crops use them inefficiently. Therefore, an increasing attention has been paid to organic fertilizers obtained from wastes that might provide plant nutrients more properly and help growers maximize crop yields. This fact has positive effects on important issues related to climate change, sustainable agriculture, waste management, and soil protection and resource conservation. Furthermore, fertigation satisfies the requirements of many plant species through their different stages of development and avoids nutrient losses, thus enhancing crop yield and reducing water evaporation and irrigation frequency [4].

Both olive cultivation and olive oil extraction industry have a great economic and social importance in the Mediterranean countries, being Spain the main producer worldwide of olive oil. The industrial activity associated to olive oil sector generates large quantities of organic by-products in many Mediterranean countries, being a solid lignocellulosic material known as olive husk or “alperujo” (AL) one of the most-abundant agro-industrial wastes generated nowadays in Spain with about 5 million tons produced in the last season. AL may be recycled by composting for agricultural uses [5–9]. AL compost has a high organic matter content, partially

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humified, and it can be used to produce liquid organic fertilizers based on the extraction of its humic-like fraction. But little information is available about it.

Therefore, the aim of this work was to evaluate the feasibility and potential use of AL compost to produce liquid organic fertilizers (LOFs) containing humic-like substances by using alkaline extraction. The obtained LOFs were characterized by elemental and spectroscopic (FTIR) analyses and by fluorescence spectroscopy in both excitation-emission matrix (3D-EEM) and synchronous modes. Comparison of these results with the HS characteristics obtained by the international humic substance society (IHSS) protocol from the same materials and with those of Suwannee River humic and fulvic acids were also performed.

MATERIAL AND METHODS

Compost Preparation

The AL composts were obtained in a previous experiment where four trapezoidal piles 1.5 m high and 4×6 m² base, each weighing 10 tons, were composted using the mechanical turning strategy. The piles were made by mixing 91% of the wet lignocellulosic solid olive-mill by-product (“alperujo,” AL) + 9% (on wet weight basis) of fresh horse manure (HM) as bulking agent. Two of the piles received 4,450 L of a fatty-proteinaceous animal waste (AW) (64% AL/6% HM/30% AW on fresh weight basis), which led to the end-compost CO-AW, while the two other piles did not receive AW (CO). AW was applied in five sequential additions to evaluate the use of cocomposting with AL as disposal method for AW. AW was rich in readily available N and had a high content of Na and K salts, while AL mainly contained lignocellulose and generally lower levels of nutrients than AW or HM. The bulking agent selection, mixing ratio, and turning schedules were based on the experience gained from previous AL composting experiments [9]. Complete details of the composting process evolution and the quality of the composts obtained were previously reported by Alburquerque *et al.* [10].

Alkaline Extraction Procedure and Humic Substance Fractionation from AL Compost

The LOFs extracted from milled (<3 mm) mature CO and CO-AW composts by caustic potash (COK and CO-AWK) were obtained by constant shaking of sealed bottles containing the composts and 1M KOH (1:3 w/v) for 24 h in a water bath at 70°C. This extraction condition is based on previous experiments where the most suitable methods (extractant type, extraction time, and temperature) for LOFs extraction from AL compost were evaluated [11, 12]. Potassium hydroxide was used instead of sodium hydroxide due to the important role played by K in the plant metabolic processes and several enzymatic reactions, and to avoid the detrimental effects of sodium on soil [13]. After centrifugation at 23,000g for 30 min, the alkaline extracts were carefully removed, dialyzed using a Spectra Por membrane (MW CO 7000 Da) to eliminate excess salts, and freeze dried. Using the IHSS protocol, extracts were also isolated under N₂ flux with 0.1M NaOH (1:10 w/v) at room temperature (<http://www.ihss.gatech.edu/>) from the same composts (CONa and CO-AWNa). Moreover, 50 mL of the four alkaline extracts were carefully adjusted to pH 2.0 by adding 3M H₂SO₄. The precipitate (humic-like acids, HA) was allowed to coagulate for 24 h at 4°C, and separated from the supernatant by centrifugation (23,000g for 30 min) (fulvic fraction, FF).

Analytical Methods

The following parameters were determined in the alkaline extracts obtained: the total alkali-extracted organic carbon (TEC) and that in the FF (FFC) were determined using an automatic carbon analyzer for liquid samples (TOC-V Ana-

lyzer, Shimadzu); carbon in humic-like acids (HAC) was calculated by subtracting FFC from TEC. The percentage of humic-like acids (PHA) defined as “(HAC/TEC) × 100,” the humification index (HI) as “(HAC/TOC) × 100,” the humification ratio (HR) as “(TEC/TOC) × 100” and the degree of polymerization as “HAC/FFC,” were calculated from the above carbon values [14, 15].

The freeze-dried samples were characterized for C, H, S, and N using an ANA 1500 Carlo Erba model 1106 microanalyzer, and O was calculated as the difference from 100% (ash-free dry weight). P and K were determined by Inductively Coupled Plasma spectrometry (ICP-OES Iris Intrepid II XDL, Thermo Scientific) after HNO₃/HClO₄ digestion of freeze-dried samples. The ratio between absorbance at 465 and 665 nm (E4/E6 ratio) was determined from the optical densities according to Chen *et al.* [16].

A 2-mg portion of each sample was ground up with 300 mg KBr and compressed under vacuum for 10 min. The pellets obtained were analyzed with a Perkin-Elmer 16 FPC spectrophotometer from 4000 to 400 cm⁻¹ [17].

A 20-μL volume of alkaline extracts was filtered at 0.45 μm and diluted in 2 mL of buffer solution (0.01M NaH₂PO₄, pH 6.8). Fluorimetric analyses were performed at room temperature (20°C ± 2°C) on a SAFAS model SP2000 computer-controlled spectrofluorimeter (SAFAS, flx, version 6.7.0.6 Monaco). Fluorescence spectra were recorded in a 2 mL quartz cuvette. Three-dimensional (3D) fluorescence and excitation-emission matrix (3D-EEM) mode was generated at excitation and emission slit widths of 5 nm. Fluorescence EEMs were collected for excitation wavelengths from 250 to 600 nm with sequential 10 nm increments of excitation wavelength. These spectra were recorded under photomultiplier voltage of 700 V and scan time of 0.04 s. After analysis of the 3D-EEM of each sample, different Ex/Em intervals were selected for synchronous scanning: Δλ18, Δλ66, and Δλ100 nm. The synchronous-scan excitation was measured by simultaneously scanning both the excitation (varied from 200 to 600 nm) and the emission wavelengths while keeping in every scan a constant wavelength ($\Delta\lambda = \lambda_{em} - \lambda_{exc} = 18$ nm; $\Delta\lambda = \lambda_{em} - \lambda_{exc} = 66$ and $\Delta\lambda = \lambda_{em} - \lambda_{exc} = 100$). No corrections for fluctuation of instrumental factors or for scattering effects were applied to experimental spectra. However, corrections by subtracting the blank were made and the absorbance of samples in the UV-visible was always lower than 0.1 at 254 nm to eliminate potential inner filter effects [18]. A comparative discussion on the spectra is acceptable, at least on a qualitative basis, since all were recorded on the same instrument, using the same experimental conditions. For all fluorescence analyses, 5 nm slit widths were maintained on excitation and emission monochromators.

Statistical Analyses

Statistical analyses of data were performed using the SPSS (Statistical Package for Social Sciences) 11.5 program for Windows. The data were subjected to ANOVA and differences between means were determined using Tukey's test ($P < 0.05$).

RESULTS AND DISCUSSION

Characterization of the Alkaline Extracts Obtained from AL Compost

The HS extracted from mature AL composts with KOH 1M at 70°C (COK and CO-AWK) showed a higher humification degree compared to those extracted with NaOH 0.1M at ambient temperature (Table 1), suggesting the predominance of more polymerized compounds and probably including lignin degradation by-products or newly formed materials generated by the hot 1M KOH. The use of heat and a higher alkaline extractant concentration increased the yield of alkali-soluble organic matter extracted from AL composts. Romaris-

Hortas *et al.* [19] employed microwave energy at four levels, reaching temperatures of 75, 100, 125, and 150°C, to assist the alkaline extraction of humic and fulvic acids from marine sediments. They reported an increase of the extraction yields and shorter extraction times for both humic and fulvic acids as temperature increased, meaning that HS alkaline extraction is enhanced at high temperatures. Also, similar findings were reported by Eyheraguibel *et al.* [20] who indicated that alkali HS extracted from homogeneous poplar sawdust resulted from lignin and hemicellulose degradation and presented similar characteristics to HS extracted from soil or compost.

To obtain further information about the humification degree of the LOFs studied (CO-AWK, COK, CO-AWNa, and CONa), the E4/E6 ratio was calculated (Table 1). The E4/E6 ratio provides useful information about the degree of condensation of the aromatic rings. Senesi *et al.* [21] explained that the E4/E6 absorbance ratio of the humic substances estimates the degree of humification and/or the molecular size. According to these authors, a higher ratio indicates a lower degree of aromatic condensation and the presence of relatively large proportions of aliphatic structures. In agreement with the above, the lowest E4/E6 found in CO-AWK (Table 1) suggested a higher degree of aromatic condensation in the CO-AWK compared to the others.

Elemental Analysis

The elemental analysis of LOFs (COK, CO-AWK, CONa, and CO-AWNa) showed that the contents of carbon, hydro-

Table 1. Evaluation of humification indices and UV-visible ratio (E4/E6).

Samples	HR (%)	HI (%)	PHA (%)	HAC/FFC	E4/E6
CO-AWK	33.7b	27.9b	82.9a	4.8a	4.5c
COK	35.1a	29.3a	83.7a	5.1a	6.4b
CO-AWNa	14.7c	11.8c	80.3ab	4.1ab	7.8ab
CONa	9.5d	7.2d	75.3b	3.1b	9.4a
Significance	***	***	*	*	**

*, **, and ***: Significant at $P < 0.05$, 0.01 , and 0.001 , respectively. For each parameter, values followed by the same letter are not statistically different according to Tukey's test at the 5% probability level.

HR: humification ratio defined as $(\text{TEC}/\text{TOC}) \times 100$, HI: humification index defined as $(\text{HAC}/\text{TOC}) \times 100$, and PHA: percentage of humic-like acids defined as $(\text{HAC}/\text{TEC}) \times 100$. TEC: total alkali-extracted organic carbon, HAC: humic-like acid carbon, FFC: fulvic-like acid carbon, and TOC: total organic carbon.

gen, and nitrogen were lower in CO-AWK than in CO-AWNa (Table 2) and a similar trend was observed when COK and CONa were compared. HS extracted by 1M KOH from AL compost where AW was added (CO-AWK) showed the highest N/C atomic ratio, which can be due to the high nitrogen content of AW. Santin *et al.* [22] attributed high N/C values to the immobilization of nitrogen during humification due to the incorporation of microbial nitrogen-rich compounds into humic substances or to the stabilization of nitrogen rich-compounds by different processes. H/C ratio was clearly higher in alkaline extracts from CO-AWK and COK (underwent to strong/severe extraction conditions). Furthermore, the O/C ratio was higher in the case of HS extracted by 1M KOH, which suggested an increase in O-containing functional groups (carboxylic-ketonic and phenolic-alcoholic) in these samples (more oxidized molecules in the HA structure), probably due to the greater alkalinity of the extractant and the high temperature employed. Therefore, the highest severity of the extraction conditions with heat should favor the release of substances with a humic-like nature, leading to oxidation and condensation reactions of incompletely-humified materials.

Comparison of the elemental composition of CO-AWK, COK, CO-AWNa, and CONa with that of humic acids from soils, coals or peats [23, 24] showed lower O/C and higher H/C and N/C ratios for CO-AWK, COK, CO-AWNa, and CONa (Table 2). This reflects the much younger form of the HA from AL compost. However, the elemental composition of the HA extracted from AL compost was similar to the HAS from agro-industrial waste composts ([21], Table 2), suggesting that the 1M KOH extraction at 70°C led to the production of reproducible and stable humic products.

HS extracted by 1M KOH from AL composts (COK and CO-AWK) were both black liquids presenting higher total organic carbon and nitrogen contents in comparison with HS extracted according to the IHSS protocol using 0.1M NaOH at room temperature under N_2 flux (CONa and CO-AWNa, Table 3).

Table 3. Nutrient content of the alkaline extracts obtained from AL composts.

	CO-AWK	COK	CO-AWNa	CONa	Spanish legislation [26]
TOC ($g L^{-1}$)	43.9	45.8	5.8	3.7	≥ 40
TN ($g L^{-1}$)	6.1	5.7	1.4	1.0	≥ 20
P_2O_5 ($g L^{-1}$)	0.4	0.5	0.1	0.1	≥ 20
K_2O ($g L^{-1}$)	56.6	49.4	7.0	4.9	≥ 20

TOC: total organic carbon and TN: total nitrogen.

Table 2. Elemental composition (%) and atomic ratios ($\times 100$) on an ash-free dry weight basis.

Samples	C	N	H	O	H/C	O/C	N/C
CO-AWK	53.8	6.1	6.3	33.8	141	47	10
COK	57.4	5.8	6.7	30.1	141	39	9
CO-AWNa	60.0	6.6	6.7	26.6	135	33	9
CONa	73.4	7.5	8.2	10.9	134	11	9
Soil humic acids*	53.4–59.9	1.9–3.9	3.4–4.8	30.3–39.3	73–102	39–55	3–6
Lignite and Leonardite humic acids**	52.3–60.7	0.8–1.6	3.7–4.7	34.5–43.3	62–103	43–62	1–2
Compost humic acids†	51.5–62.5	2.4–4.8	4.4–9.5	23.7–41.6	100–167	30–60	3–7

*Schnitzer and Preston [23].

**García [24].

†Senesi *et al.* [21].

Rocha *et al.* [25] reported that the extraction of HS from soils, using alkaline solvents comprised the ionization of the acid groups and solvation of the conjugated bases in aqueous medium. These authors explained that the counter-ions Na^+ and K^+ , responsible for the interaction and solvation of the negative charges dissociated from the humic macromolecules, having a different behavior in aqueous medium, resulting in the increase of HS yields when KOH is used.

Based on the current Spanish legislation on fertilizers [26], it can be concluded that none of the alkaline extracts obtained from AL compost fulfilled any of the categories for organo-mineral fertilizers, which was mainly due to low N and particularly P solubilized under the conditions studied (Table 3). However, C and K contents in the extracts obtained with 1M KOH and heat met the minimum legal requirements. This makes the obtained alkaline extracts excellent raw materials for preparing commercial organo-mineral liquid fertilizers by mixing them with mineral fertilizers.

FTIR Spectroscopy

FTIR spectroscopy is a qualitative tool for monitoring chemical groups, which is widely used in HS studies. The FTIR spectra of CO-AWK, COK, CO-AWNa, and CONa (Figure 1) were analyzed taking into account earlier studies of HS [17, 21]. This led to the attribution of the main absorption bands presented in Table 4. Comparison of the FTIR spectra of CO-AWK, COK, CO-AWNa, and CONa showed that these spectra

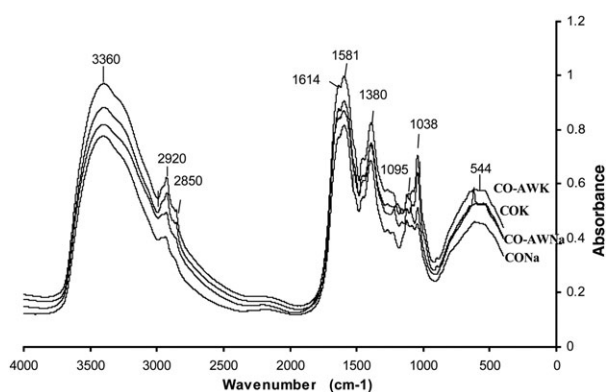


Figure 1. FTIR spectra of CO-AWK, COK, CO-AWNa, and CONa.

Table 4. Main assignments of the FTIR spectral peaks.

Wavelength (cm^{-1})	Assignment
3300–3500	O[–]H vibrations of the hydroxyl groups of phenols, alcohols and carboxyl functions and N[–]H vibrations from amides and amines
2925	Symmetric CH stretching in [–]CH ₃ and [–]CH ₂ [–] of aliphatic chains.
2840	Asymmetric CH stretching in [–]CH ₃ and [–]CH ₂ [–] of aliphatic chains
1654–1640	C=O stretching in quinines, ketonic acids and primary amides.
1580	C=N stretching in secondary amides
1540–1510	Aromatic C=C stretching and/or N[–]H deformation
1460–1440	Aliphatic C[–]H deformation of structures such as fatty acids and waxes occurring in composts.
1400–1380	O[–]H deformation, C=O stretching of phenols, anti-symmetric COO [–] stretching and aliphatic C[–]H deformation
1260–1200	C[–]OH stretching of aromatic groups and C[–]O[–]C stretching of aryl ethers and phenols
1170	Alcohol function vibrations
1080–1030	C[–]O[–]C stretching of carbohydrates
975–775	Out-of-plane bending of aromatic C[–]H

are qualitatively similar, presenting the same overall pattern. The inspection of FTIR spectra of CO-AWK, COK, CO-AWNa, and CONa indicated that the differences between HS for the two extractions are mainly associated to the contents and the characteristics of the oxygenated and amide groups.

The spectra pointed out the high intensity of the 1614 and 1581 cm^{-1} peaks, which can be related to the enrichment in aromatic C=C, quinone, and ketone vibrations and to protein peptide linkages, respectively (Figure 1). The presence of protein groups in CO-AWK and CO-AWNa was due mainly to the high nitrogen content of AW. In addition, the spectra featured a clear shift of the band at 1720 to 1635 cm^{-1} , which was attributed by Fong and Mohamed [27] to an extension of bond conjugation. Furthermore, Senesi *et al.* [21] attributed the high intensity around 1650 and 1385 cm^{-1} to an increase in the abundance of COO[–] groups upon interaction with Fe^{3+} and/or Cu^{2+} ions. Similarly, Chai *et al.* [28] attributed the higher adsorption intensity at 1650 cm^{-1} and at 1720 cm^{-1} , to the high abundance of aromatic groups and to oxygen-rich functional groups, respectively. In addition, the presence of the peak at 1230 cm^{-1} in CO-AWK and COK samples and its absence from the CO-AWNa and CONa samples confirmed the increase in carboxylic and/or phenolic hydroxyl groups. This fact is consistent with the higher O/C atomic ratio and the extent of humification.

Fluorescence Spectroscopy

Three-Dimensional Excitation/Emission Matrix (3D-EEM)

Three-dimensional excitation/emission matrix (3D-EEM) fluorescence spectra provide a powerful spectroscopic tool for determining fluorophore diversity [29]. The 3D-EEM presented a number of distinct peaks that are generally ascribed to humic fluorophores (Figures 2 and 3). The presence of these different fluorophores, is each characterized one by an excitation/emission ($\lambda_{\text{Ex}}/\lambda_{\text{Em}}$) wavelength pair. The 3D-EEM of CO-AWK, COK, CO-AWNa, and CONa showed a broad emission band ($\lambda_{\text{exc}}/\lambda_{\text{em}}$: 280 nm–475 nm/350 nm–550 nm). Coble [29] already noted the presence of this broad emission band ($\lambda_{\text{exc}}/\lambda_{\text{em}}$: 250–400 nm/380–550 nm) as the result of conjugated systems and explained that the emission wavelength was strongly dependent on the degree of conjugation, on substitution and on the presence of heteroatoms.

In the 3D-EEM of CO-AWK, COK, CO-AWNa, and CONa, a less marked first fluorophore showed a maximum at $\lambda_{\text{Ex}}/\lambda_{\text{Em}}$ ~320 nm/350 nm. The emission ($\lambda_{\text{Ex}}/\lambda_{\text{Em}}$: ~280 nm/350 nm) was generally attributed to proteinaceous constitu-

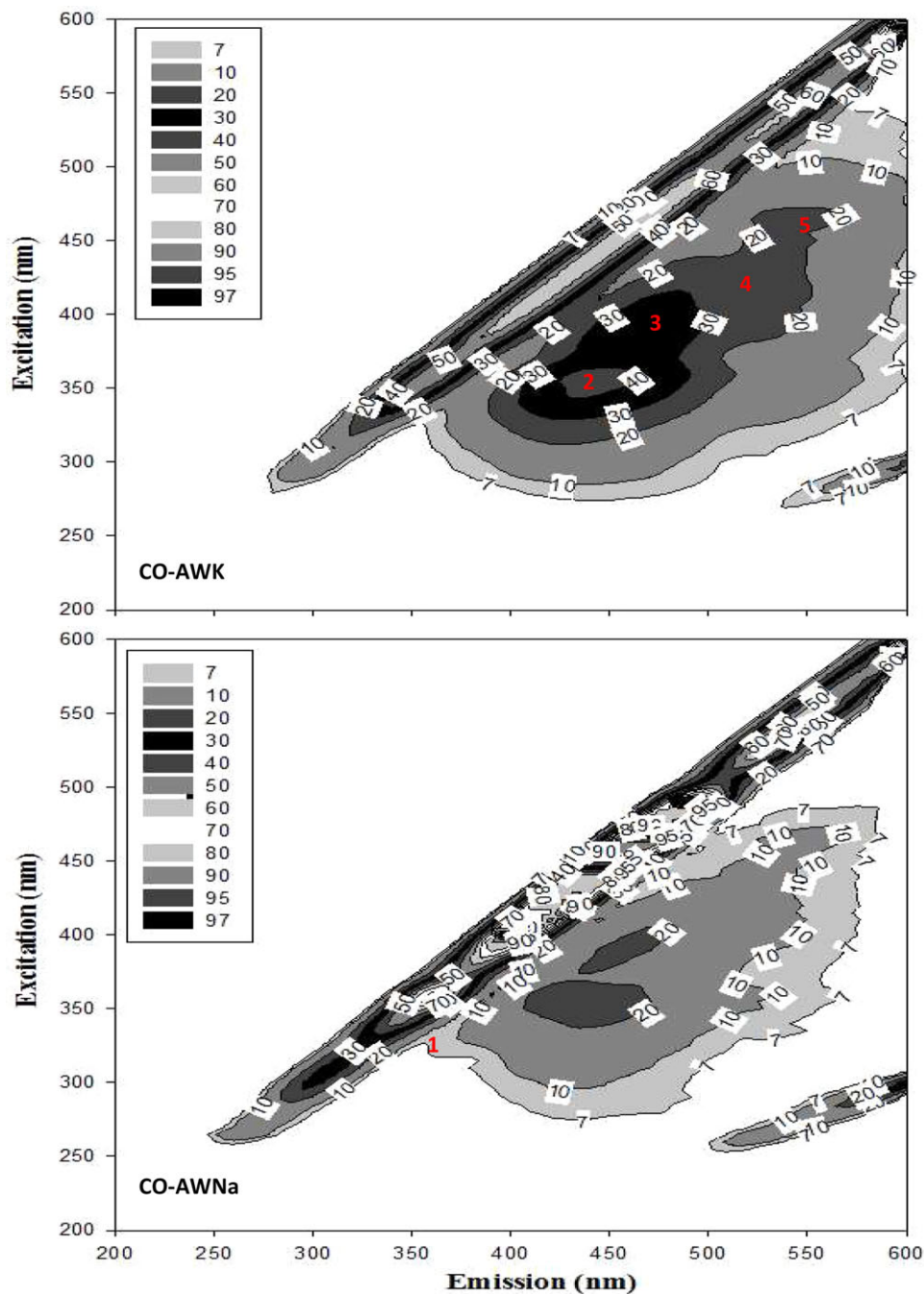


Figure 2. Emission excitation matrix (EEM) of CO-AWK and CO-AWNa. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

ents (tryptophan-like) [29, 30]. This peak can be considered as a distinctive marker for the proteinaceous materials originating from the AW but it was more abundant in CO-AWNa (Figure 2) and CONa (Figure 3), which could be due to the milder alkaline extraction conditions which, unlike 1M KOH, permitted the extraction of proteinaceous structures.

The second and the third fluorophores had maxima at $\lambda_{Ex}/\lambda_{Em} \sim 350/440$ nm and $\sim 375/475$ nm, respectively, which coincide with peaks typical of fulvic acid compounds. The intensity of these two peaks was greatest in CO-AWK, which can be explained by the fact that different molecular components derived from lignin and other degraded plant materials, may contribute to the fluorescence of these peaks and could be incorporated into the humic macromolecules during their

formation [31]. In this respect, Antizar-Ladislao *et al.* [32] reported that an increase of fluorescence intensity may result from an increased molecular complexity associated with aromatic structures and conjugated double bonds. These two peaks were also more intense for CO-AWNa which can be related to the effect of AW addition that could provide additional biodegradable organic matter and active biomass to composting materials.

Finally, the fourth and the fifth fluorophores located at $\lambda_{Ex}/\lambda_{Em} \sim 425/525$ nm and $\sim 460/540$ nm, respectively corresponded to peaks typical of humic-like acids. Similarly, Chen *et al.* [33] reported that natural organic matter fractions possess unique broad bands, with a maximum at $\sim 435\text{--}450$ nm and a shoulder at ~ 475 nm. Additionally, Provenzano *et al.*

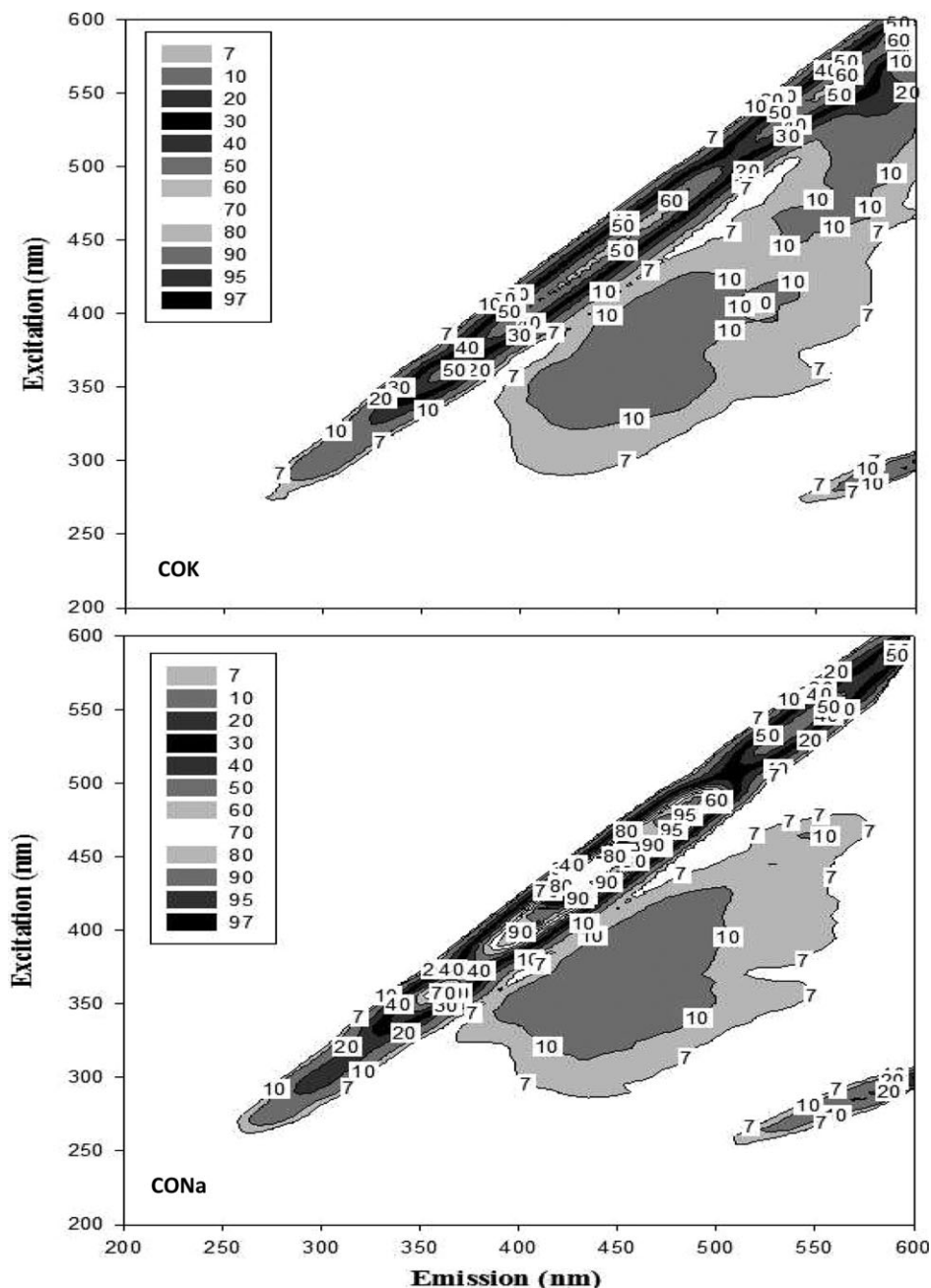


Figure 3. Emission excitation matrix (EEM) of COK and CONa.

[34] reported the occurrence, in the EEM spectrum of soil humic acids, of a peak at Ex/Em 445/525nm and a less intense peak at Ex/Em 370/495nm and other peaks at Ex/Em 450/530 nm and at Ex/Em 450/535 nm in the EEM spectra of peat humic acids and compost humic acids, respectively.

The higher intensity of the humic acid peaks in CO-AWK and COK in comparison with CO-AWNa and CONa can be due to the effect of harsh alkaline extraction at 70°C on the degradation of the lignin fraction and/or newly formed products in the extract of AL compost. The latter trend was confirmed by a shift of emission maxima to longer wavelengths, which was attributed by Chen *et al.* [33] to the increase of molecular size and aromatic content. Moreover, these results are confirmed by the trend of the fluorescence index proposed by Chen *et al.* [33] which is the ratio of emission inten-

sity at 450 nm over 500 nm at 340 nm excitation. The fluorescence index was higher in CO-AWK (1.90) than in COK (1.66), CO-AWNa (1.63), and CONa (1.60). As a consequence, the rise in this index indicated an increase in the structural complexity of the alkali humic-like substances, which therefore showed more accentuated humic characteristics.

To confirm these observations and the accordance of the fluorophores in the 3D-EEM of CO-AWK, COK, CO-AWNa, and CONa, bovine serum albumin (BSA), Suwannee river fulvic acids (SRFA), and Suwannee river humic acids (SRHA) were analyzed using the same conditions and the same apparatus. The 3D-EEM of BSA confirmed the presence of one center (~280/350 nm) that coincided with the protein-like fluorophore in the spectra of CO-AWK, COK, CO-AWNa, and CONa. The spectra of SRFA and SRHA confirmed the pres-

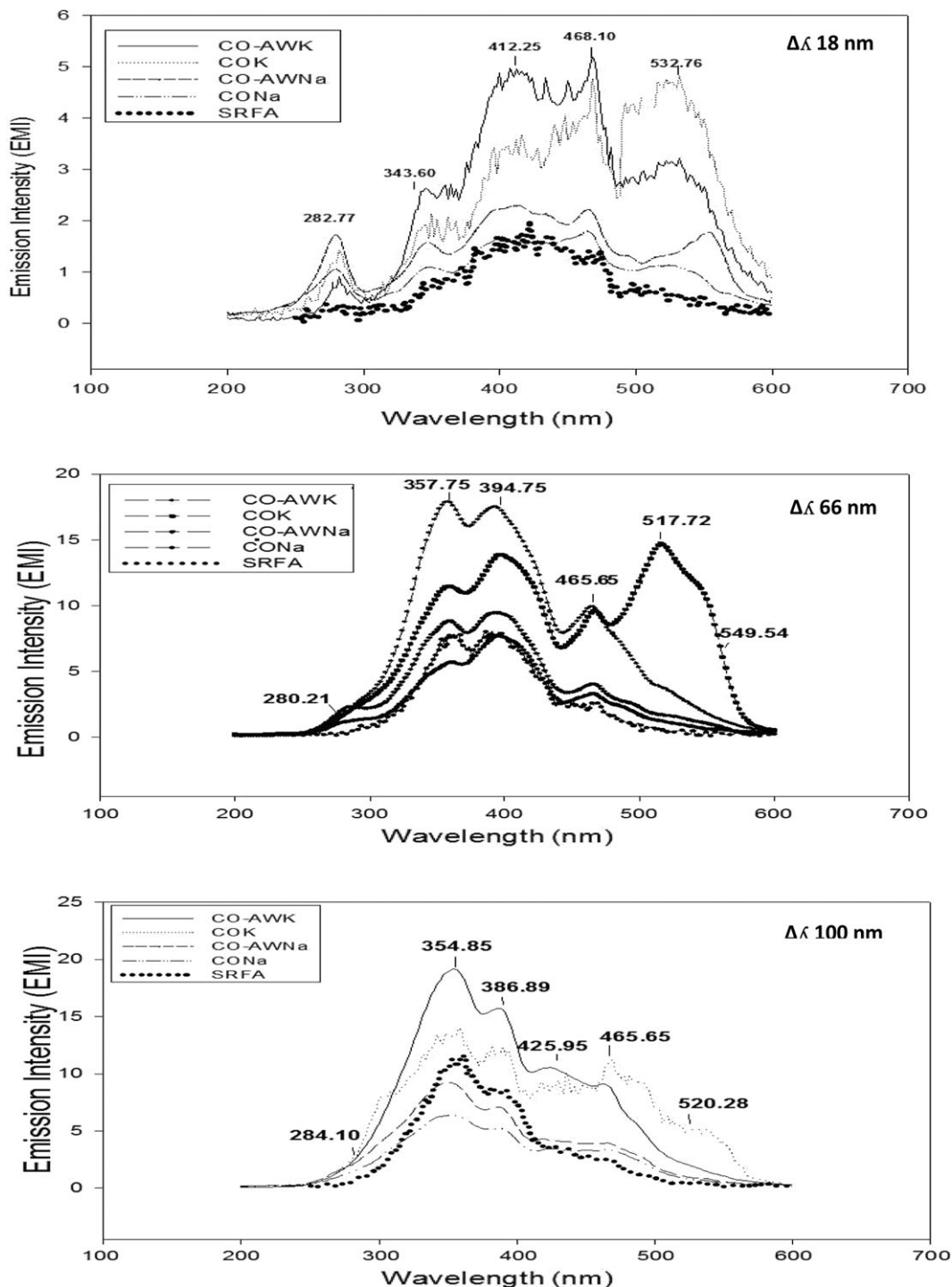


Figure 4. Synchronous fluorescence spectra of CO-AWK, COK, CO-AWNa, and CONa at $\Delta\lambda$ 18, 66, and 100 nm.

ence of two centers ($\sim 350/460$ nm and $\sim 380/475$ nm) that were coincident with fulvic-acid-like fluorophores and two others ($\sim 430/525$ nm and $\sim 450/560$ nm) which coincided with humic-acid-like fluorophores in the 3D-EEM of CO-AWK, COK, CO-AWNa, and CONa.

Synchronous Mode

Synchronous fluorescence has been reported to have several advantages over conventional fluorescence excitation and emission spectroscopy in studying organic matter [31].

It consists in simultaneously varying both excitation and emission wavelengths while keeping a constant wavelength interval $\Delta\lambda (= \lambda_{em} - \lambda_{ex})$ between them. Further, the synchronous technique can offer higher sensitivity and allows differentiation of the fluorescence spectra of samples of various origins. It also enables further “hidden” fluorophores to be detected [31]. Note that the principal difficulty involved in this technique, is to find the ideal $\Delta\lambda$ which will produce the most representative spectra of each fluorophore. In this respect, Miano and Senesi [35] investigated the effect of varying $\Delta\lambda$ on the shape of the synchronous-

scan spectra of a series of fulvic and humic acids and observed that a value of $\Delta\lambda = 18$ nm produced the best overall spectral resolution. Other studies [36] reported the effect of using various $\Delta\lambda$ (20, 50, 80, 100, and 150 nm) on the shape of synchronous spectra of fulvic and humic acids of various origins. These authors reported that at $\Delta\lambda = 20$ nm, protein-and/or phenol-like structures can be clearly distinguished in synchronous spectra. However, the protein-like and humic-like structures were clearest at intermediate $\Delta\lambda$ (50 and 80 for example) and at $\Delta\lambda$ 100 and 150 nm the HS were distinguished most.

Taking into account that these correspondences can change from sample to sample depending on the width of the spectral range, the synchronous spectra were obtained at $\Delta\lambda = 18, 66,$ and 100 nm in the present study for CO-AWK, COK, CO-AWNa, and CONa. As shown in Figure 4, spectra from CO-AWK, COK, CO-AWNa, and CONa were very similar at all $\Delta\lambda$. Indeed, the synchronous spectra of CO-AWK, COK, CO-AWNa, and CONa at $\Delta\lambda = 18, 66,$ and 100 nm exhibited all peaks with emission wavelengths ~ 280 nm, ~ 350 nm, ~ 390 nm, ~ 450 nm, and ~ 550 nm.

The attributions of signals at ~ 280 nm and at ~ 350 nm were attributed, to lignin-derived structural moieties and to simple aromatic rings respectively in the molecules [37]. Senesi *et al.* [31] reported that peaks at the lowest λ_{em} (450/430 nm) may be accounted for by fluorescent phenolic groups conjugated to a carbonyl group through the benzene ring. However, maxima observed at intermediate λ_{em} (470/450 nm) may be ascribed either to hydroxycoumarin-like moieties originating from lignin, or to fluorophores of the Schiff-base type, $-N=C-C=N-$ originating from polycondensation of carbonyls with amino groups. Moreover, peaks at ~ 510 nm and at ~ 550 nm are related, according to Senesi *et al.* [31], to the presence of high molecular weight fractions.

The synchronous spectra of Suwannee River fulvic acids analyzed using the same conditions and apparatus (Figure 4) at $\Delta\lambda = 18, 66,$ and 100 nm confirmed the presence of two peaks at ~ 350 nm, ~ 390 nm and a shoulder at ~ 460 nm. The resolution of these peaks in the standard spectra higher, especially for $\Delta\lambda = 66$ nm and $\Delta\lambda = 100$ nm. Additionally, note that the synchronous spectra peaks presented different values at $\Delta\lambda = 18, 66,$ and 100 nm, which could be related to the high degree of conjugation and the presence of abundant aromatic structures [31].

CONCLUSION

The extraction method used in the present work to produce liquid organic fertilizers from two mature "alperujo" composts using 1M KOH at 70°C for 24 h generated black liquids with a high total organic carbon (>40 g L⁻¹) and rich in humic-like substances ($>80\%$ as humic-like acids and a polymerization degree of about 5). The characterization of these compounds by elemental analysis showed lower contents of carbon, hydrogen, and nitrogen, and a higher oxygen content in those extracted with 1M KOH at 70°C (CO-AWK and COK) than in CO-AWNa and CONa (extracted with 0.1 NaOH at ambient temperature). This suggests an increase in O-containing functional groups in CO-AWK and COK samples, probably due to the higher alkalinity of the extractant and the high temperature employed. The use of FTIR and fluorescence spectroscopy confirmed the predominance of more polymerized compounds, including probably lignin degradation by-products or newly formed materials generated by 1M KOH and high temperature (70°C). These results confirmed that the use of 1M KOH at 70°C permitted the extraction of humic-like substances from "alperujo" composts with a high potential to produce commercial organic fertilizers.

ABBREVIATIONS

3D-EEM	three-dimensional fluorescence excitation-emission matrix.
AL	"alperujo" (two-phase solid olive-mill waste).
AW	fatty-proteinaceous animal waste.
BSA	Bovine serum albumin.
CO-AWK	extract obtained from "alperujo"/horse manure/animal waste compost with 1M KOH at 70°C.
CO-AWNa	extract obtained from "alperujo"/horse manure/animal waste compost with 0.1M NaOH at ambient temperature.
COK	extract obtained from "alperujo"/horse manure compost with 1M KOH at 70°C.
CONa	extract obtained from "alperujo"/horse manure compost with 0.1M NaOH at ambient temperature.
FF	fulvic-like acid fraction.
FFC	fulvic-like acid carbon.
HA	humic-like acids.
HAC	humic-like acid carbon.
HI	humification index.
HM	horse manure.
HR	humification ratio.
HS	humic-like substances.
IHSS	international humic substance society.
LOFs	liquid organic fertilizers.
PHA	percentage of humic-like acids.
SRFA	Suwannee river fulvic acids.
SRHA	Suwannee river humic acids.
TEC	total alkali-extracted organic carbon.
TOC	total organic carbon.

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