



Strategies to produce commercial liquid organic fertilisers from “alperujo” composts



Germán Tortosa^{a,*}, José Antonio Alburquerque^b, Eulogio J. Bedmar^a, Ghita Ait-Baddi^c, Juan Cegarra^b

^a Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín (EEZ), Agencia CSIC, E-419, 18080 Granada, Spain

^b Department of Soil and Water Conservation and Organic Waste Management, Centro de Edafología y Biología Aplicada del Segura, (CEBAS), Agencia CSIC, E-164, 30100 Murcia, Spain

^c Multidisciplinary Faculty of Taroudant, University Ibn Zohr, Agadir, Hay El Mohammadi (Lastah), BP:271, 83000 Taroudant, Morocco

ARTICLE INFO

Article history:

Received 15 May 2014

Received in revised form

27 June 2014

Accepted 30 June 2014

Available online 10 July 2014

Keywords:

Commercial organic fertilisers

Humic-like substances

“alperujo” composts

Organic carbon extraction

Extraction efficiency

ABSTRACT

Composting is considered an economically-friendly procedure for producing commercial solid organic amendments and fertilisers from the two-phase olive mill waste (called “alperujo”: AL), main by-product of the Spanish olive oil industry. AL composts are characterized by a noticeable organic matter content, mainly of lignocellulosic nature, which determines their humic properties. In this study, we have assayed several extraction conditions in order to release commercial liquid organic fertilisers from AL composts. The following conditions were tested: extraction time, extraction temperature, heat (70 °C) time applied, extraction ratio, extractant agent and alkali concentration. Their effects on organic fraction (total organic, polyphenol- and carbohydrate-like carbons), nutrient concentration and extraction efficiency were evaluated. In general terms, an increase in the extraction time and the combined use of alkali and heat increased significantly the amount of organic carbon solubilised from the compost, affecting the nature of the alkali-soluble organic matter and even showing a chemical degradation of the humic fraction in some cases. The extraction ratio modified the concentration of the organic and inorganic fraction in the extracts, and also their polyphenol and carbohydrate content. The use of a 24 h extraction with 1 M KOH (1:4 or 1:5, extraction ratios) and heat (4 h at 70 °C) allowed us to extract the required amount of C and K from AL compost, being necessary an external source of N and P to complete the fertiliser formulations according to the current Spanish legislation for *Organo-mineral Fertiliser* production (RD 506/2013, 2013).

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Composting is considered a low cost technology for transforming organic wastes and by-products into quality materials that can be used as soil amendments and/or fertilisers. A clear example is found in the Spanish olive oil industry, which generates a large amount of a solid by-product called “alperujo” (AL) (Alburquerque *et al.*, 2004). Composting has been shown as an effective treatment for adding value to AL (Muktadirul Bari Chowdhury *et al.*, 2013) through the production of commercial solid organic amendments and fertilisers (Tortosa *et al.*, 2012). Also, AL composts have shown a good performance in agricultural experiments (Alburquerque *et al.*, 2006, 2007; Fernández-Hernández *et al.*, 2013;

Altieri *et al.*, 2014; Nair *et al.*, 2014). Therefore, the quality of the AL composts has been recognised by the Spanish authorities, including a category for AL compost as an organic amendment in the Spanish legislation for fertiliser production (RD 506/2013, 2013). Such composts are characterized by a high content of organic matter due to the lignocellulosic nature of AL, which forms the basis of their humic properties (García-Gómez *et al.*, 2005; Alburquerque *et al.*, 2009a; Serraniá *et al.*, 2013). Moreover, the composting process is associated with a high degradation of the main components of the organic matter (lignin, cellulose, hemicellulose and proteins); delivering simple carbohydrates and polyphenols which have been proposed in the literature as relevant precursors for the humification process (Sánchez-Monedero *et al.*, 1999; García-Gómez *et al.*, 2005).

The humic substances are considered the most important fraction of organic matter in agricultural soils (Stevenson, 1994). This fact is related with the improvement of physico-chemical and

* Corresponding author.

E-mail address: german.tortosa@eez.csic.es (G. Tortosa).

biological soil properties and also, with positive effects on plant physiology, nutrient uptake and root development (Trevisan et al., 2010). In addition to these facts, composts can be also used for the extraction of soluble humic substances with potential to be used in agriculture, nowadays becoming more commonly found in commercially available liquid fertilisers (Valdrighi et al., 1996; Bidegain et al., 2000; Eyheraguibel et al., 2008). Ait Baddi et al. (2013) and Masmoudi et al. (2013) characterized the humic fraction from AL composts showing that the alkali-extracted humic-like substances presented characteristics close to those of standard humic materials. Moreover, Caravaca et al. (2006) and Kohler et al. (2008) have shown positive effects of the alkali-soluble organic matter application from AL composts on some important legumes crops (*Retama sphaerocarpa* L. and *Medicago sativa* L.).

In Tortosa et al. (2012), we underlined the importance of producing high quality composts in establishing a fertiliser market, which could promote an efficient recycling strategy for AL. Also, we demonstrated the technical feasibility for producing commercial solid organic amendments and fertilisers from AL composts according to the Spanish legislation for fertiliser production (RD 506/2013, 2013). This article constituted a continuation to the former and focuses on the technical feasibility of obtaining liquid organic fertilisers from AL composts, in order to diversify the range of commercial products obtained from AL in either solid (compost) and liquid (*Organo-mineral Fertiliser*) forms. For this, we have tested different extraction conditions in order to optimize the solubilisation of both organic carbon and nutrients with particular emphasis on the humic-like component and its lignocellulosic fraction, especially important due to its positive effects on plant growth.

2. Material and methods

2.1. Composting performance and compost characterisation

A total of seven AL composts were used in the present study and they were obtained in previous experiments where AL was co-composted with animal manures (cow, poultry and sheep manure) using the mechanical turning strategy. Complete details of the composting performance (bulking agent selection, mixing ratio, etc.), process evolution and compost characterisation can be consulted in Cegarra et al. (2006) and Tortosa et al. (2012). The agrochemical characterisation of the AL composts is presented in Table 1.

The AL composts showed common typical characteristics such as alkaline pH (round 8.5), moderate salinity (2.05 ± 0.48 dS m^{-1} of electrical conductivity) and a high organic matter content (close to 70%, especially of lignocellulosic nature: 407.4 ± 44.7 , 179.9 ± 18.4 and 158.3 ± 69.8 g kg^{-1} of lignin, cellulose and hemicellulose contents, respectively). In addition, they presented a high total organic carbon (T_{OC} : 355.8 ± 85.9 g kg^{-1}), a total nitrogen content close to 20.3 ± 1.5 g kg^{-1} (predominantly organic) and a T_{OC}/T_N ratio ranged from 13.5 to 21.0, typical of AL composts (Muktadirul Bari Chowdhury et al., 2013). The water-soluble organic fraction showed in general a low content according to their water-soluble organic carbon (WSC), polyphenols (WSPH) and carbohydrates (WSCH) values (16.8 ± 5.7 , 2.1 ± 0.8 and 3.2 ± 2.4 g kg^{-1} , respectively). Macro and micronutrient contents were noticeable, especially K, Ca and Fe, those ranged between 13.9 ± 10.1 , 40.0 ± 26.5 and 6.0 ± 3.5 g kg^{-1} of average, respectively. In general terms, the AL composts showed germination index (GI) values higher than 70% and a low heavy metal content that confirmed their non-phytotoxic characteristics. Finally, agrochemical characteristics of AL composts used were agreed with those reviewed by Muktadirul Bari Chowdhury et al. (2013).

Table 1

Agrochemical characteristics of the AL composts used (ALC1 from Cegarra et al., 2006; ALC2-7 from Tortosa et al., 2012).

Parameters ^a	AL composts						
	ALC1	ALC2	ALC3	ALC4	ALC5	ALC6	ALC7
pH ^b	8.99	8.30	7.97	8.02	8.88	8.52	8.35
EC ^b (dS m^{-1})	2.96	1.70	1.84	2.44	1.69	1.70	2.03
OM (g kg^{-1})	912.2	745.6	760.3	746.0	561.2	572.1	588.2
Lignin (g kg^{-1})	399.2	428.2	467.4	457.2	350.6	375.0	374.1
Cellulose (g kg^{-1})	208.0	176.6	182.8	178.6	146.1	179.2	188.1
Hemicellulose (g kg^{-1})	291.4	208.7	152.2	129.1	131.0	92.0	104.0
T_{OC} (g kg^{-1})	484.9	396.6	403.1	393.2	251.9	272.3	288.4
T_N (g kg^{-1})	23.1	20.2	20.6	21.1	18.6	19.2	19.1
T_{OC}/T_N	21.0	19.5	19.5	18.7	13.5	14.2	15.0
WSCH (g kg^{-1})	8.1	3.2	2.0	4.2	1.5	2.2	1.2
WSPH (g kg^{-1})	2.9	2.0	2.1	3.4	2.1	1.1	1.3
WSC (g kg^{-1})	27.0	19.4	13.5	19.3	16.4	10.6	11.7
P (g kg^{-1})	1.5	2.0	1.9	2.2	1.8	1.8	2.0
K (g kg^{-1})	36.2	11.0	7.9	6.5	12.8	12.7	10.2
Ca (g kg^{-1})	9.4	29.4	20.1	18.7	65.0	72.4	65.2
Mg (g kg^{-1})	1.9	5.7	4.9	4.9	12.7	10.6	10.3
Na (g kg^{-1})	4.1	8.3	6.0	8.2	7.6	6.2	6.2
S (g kg^{-1})	–	2.5	3.6	4.6	5.2	5.4	5.4
Fe (g kg^{-1})	0.5	3.9	9.0	4.8	6.5	11.2	6.0
Cu (mg kg^{-1})	33	32	22	27	16	14	19
Mn (mg kg^{-1})	44	183	193	145	167	199	131
Zn (mg kg^{-1})	50	92	54	39	41	55	49
Pb (mg kg^{-1})	20	8	30	37	11	17	24
Cr (mg kg^{-1})	12	14	12	13	24	22	28
Ni (mg kg^{-1})	10	21	29	35	50	42	80
Cd (mg kg^{-1})	nd	nd	2	7	nd	1	2
P_{HA} (%)	75.3	63.2	63.5	72.0	78.7	63.8	61.9
GI	79	71	94	77	81	70	78

Note: Data was shown as the mean value of four replicates with less than 5% of relative error.

^a Data based on dry weight.

^b Water extract 1:10. EC: electrical conductivity. OM: total organic matter. T_{OC} : total organic carbon. T_N : total nitrogen. WSCH: water-soluble carbohydrates. WSPH: water-soluble phenols. WSC: water-soluble organic carbon. HR: $(ASC/T_{OC}) \times 100$, P_{HA} : $(ASC_{HA}/ASC) \times 100$, ASC: carbon extracted with NaOH 0.1 M, ASC_{HA} : carbon of acid humic-like substances. GI: Germination index by Zucchini Test. nd: not detected and –: not determined.

2.2. Extraction conditions and humic-like fractionation

The current protocol for the preparation of organic fertilisers is based on the procedures proposed by the International Humic Substances Society (IHSS, <http://www.ihss.gatech.edu/>), which are essentially based on an extraction with a strong alkali (NaOH or KOH) under controlled conditions. These procedures were initially developed for comparative studies on the chemical properties of the solubilised organic matter, and its humic fraction (humic and fulvic acids). From a commercial point of view, the extraction efficiency (percentage of organic carbon solubilised with respect to the initial content in the compost), and especially the richness in organic carbon of the obtained extract (expressed by concentration, g L^{-1}), must be optimised to fulfil the legal requirements.

In the present study, both criteria were optimized by modifying several factors such as extractant concentration, extraction time, temperature and ratio (the relationship between the solid organic material and the liquid extractant). From an agricultural point of view, KOH was used instead of NaOH as alkali extractant since K is essential for plant nutrition and Na can affect negatively both soil properties and plant growth. In order to compare the extractant effectiveness, two concentrations (0.1 and 1 M) of KOH were tested. A representative AL compost was used (Cegarra et al., 2006) and the following extraction conditions were tested:

- **Extraction time:** 2, 4, 8, 16, 24, 36 and 48 h were assayed using 25 °C and a 1:20 extraction ratio.
- **Extraction ratio (grams of compost:ml of extractant):** A total of five extraction ratios were assayed: 1:4, 1:5, 1:10, 1:20 and 1:40 using 25 °C during 24 h of extraction.
- **Heat (70 °C) time applied (hours) in a 24 h extraction:** During an extraction of 24 h at 25 °C and extraction ratio of 1:20, increasing heat (from 25 °C to 70 °C) times (0, 1, 2, 4, 8, 12, 16, 20 and 24 h) were applied. For instance, 0 h meant an extraction of 24 h at 25 °C, 12 h meant 12 h at 25 °C plus 12 h at 70 °C, and 24 h meant 24 h at 70 °C, respectively.

Compost used was freeze-dried and ground to less than 0.5 mm and the extractions were performed by constant shaking in sealed bottles (400 mL high-density polyethylene bottles with a height/base ratio of 3, previously acid-washed) in a water bath (Memmert® waterbath, 22 L of capacity with a horizontal shaking system and thermostatic and temporal control system). In order to achieve a proper organic matter solubilisation, the extraction bottles were filled to 70% of their capacity. For each extraction procedure, a total of three extractions ($n = 3$) were made.

Once extraction finished, the soluble organic matter was centrifuged for 20 min at 23 500 g and the supernatant carefully removed. Thus, the supernatant was fractionated into two fractions, humic-like acids (HA) and fulvic fraction (FF) according to the methodology described by [Albuquerque et al. \(2009a\)](#) with some modifications. Briefly, HA was obtained by adding concentrated HCl to the supernatant until it reached pH 2, when HA precipitated during 24 h at 4 °C. After that, the organic fraction was centrifuged as mentioned before and the supernatant was collected (FF). After that, the nutrient and organic carbon contents in the resulting extracts were analysed.

2.3. Chemical characterisation of the organic extracts potentially used as commercial fertilisers

Three extractant procedures (H₂O, KOH 1 M at 25 °C and KOH 1 M at 70 °C) at both 1:3 and 1:20 during an extraction of 24 h were tested. For each extraction procedure, a total of seven extractions ($n = 7$) were made (one per each compost) per duplicate. All composts used are described in [Tortosa et al. \(2012\)](#) and [Cegarra et al. \(2006\)](#). Also, composts used were freeze-dried and ground to less than 0.5 mm and their humic fraction (HA) in the alkaline extracts were obtained as previously described.

2.4. Analytical methods

The following parameters were determined in the alkaline extracts obtained in Section 2.2: the total alkali-extracted organic carbon (ASC) and that in the FF (ASC_{FF}) were determined using an automatic carbon analyser for liquid samples (TOC-V CSN + TNM-1 Analyser, Shimadzu); carbon in humic-like acids (ASC_{HA}) was calculated by subtracting ASC_{FF} from ASC (ASC: ASC_{HA} + ASC_{FF}). The lignocellulosic fraction presented in the alkaline extracts affected by extraction procedures assayed was evaluated according to alkali-soluble polyphenols and carbohydrates concentration. Polyphenols and carbohydrates were measured spectroscopically according to [García-Gómez et al. \(2005\)](#) in the alkaline extracts. The analyses were based on Folin-Ciocalteu ($\lambda = 725$ nm) for the former and sulphuric acid-antrone ($\lambda = 625$ nm) chemical reactions for the latter. Caffeic acid and D-glucose were used as the standards, respectively, and the results were expressed as polyphenols and carbohydrates-like carbon content (ASCP and ASCC, respectively) referred to both standards in order to calculate their percentage respect to ASC ([Table 2](#)).

Table 2

Perceptual contribution (%) of alkali-soluble polyphenol (ASCP) and carbohydrates (ASCC) to alkali-soluble organic carbon (ASC) extracted and its humic acid fractions (HA) at different extraction procedures.

Extraction time (hours)	0.1 M KOH			1 M KOH		
	ASCP	ASCC	ASC	ASCP	ASCC	ASC
2	13.0b	15.0e	100.0	7.1 ab	18.1d	100.0
4	12.3b	14.3d	100.0	7.5b	17.0 ab	100.0
8	13.2b	15.3e	100.0	7.3.b	18.0e	100.0
16	12.6b	13.0c	100.0	6.1a	15.8a	100.0
24	10.4 ab	12.0c	100.0	6.6a	15.9a	100.0
36	8.8a	10.9b	100.0	7.9b	16.9 ab	100.0
48	7.9a	8.5a	100.0	10.6c	17.9c	100.0
	ASCP _{HA}	ASCC _{HA}	ASC _{HA}	ASCP _{HA}	ASCC _{HA}	ASC _{HA}
2	21.7d	18.8d	100.0	9.5b	17.3c	100.0
4	19.0c	16.4c	100.0	9.5b	18.5c	100.0
8	19.9c	18.5d	100.0	8.9 ab	17.2c	100.0
16	18.0c	15.2c	100.0	7.0a	14.9a	100.0
24	13.4b	12.4b	100.0	7.8a	14.7a	100.0
36	10.5 ab	11.7a	100.0	9.8b	15.9a	100.0
48	9.2a	11.8a	100.0	12.8c	16.3b	100.0
Extraction ratio	0.1 M KOH			1 M KOH		
	ASCP	ASCC	ASC	ASCP	ASCC	ASC
1:40	9.1c	15.6c	100.0	6.8b	16.2c	100.0
1:20	9.7c	12.0c	100.0	6.6b	15.7c	100.0
1:10	5.9b	8.2b	100.0	4.9a	11.8b	100.0
1:5	3.4a	5.2a	100.0	5.1a	7.5a	100.0
1:4	3.6a	4.6a	100.0	4.7a	6.8a	100.0
	ASCP _{HA}	ASCC _{HA}	ASC _{HA}	ASCP _{HA}	ASCC _{HA}	ASC _{HA}
1:40	10.5c	18.8c	100.0	7.5b	15.0c	100.0
1:20	12.2c	12.6c	100.0	7.8b	14.5c	100.0
1:10	6.2b	6.9b	100.0	5.5a	11.8b	100.0
1:5	2.7a	5.2a	100.0	5.6a	5.1a	100.0
1:4	3.3a	5.1a	100.0	4.9a	4.7a	100.0
Heat (70 °C) time applied (hours)	0.1 M KOH			1 M KOH		
	ASCP	ASCC	ASC	ASCP	ASCC	ASC
0	10.6a	11.1b	100.0	6.6a	16.0b	100.0
4	12.7b	10.4 ab	100.0	10.3c	9.2a	100.0
12	13.0b	10.6 ab	100.0	9.7b	8.6a	100.0
16	12.9b	9.8a	100.0	10.1c	8.6a	100.0
24	13.4c	9.8a	100.0	10.7c	9.2a	100.0
	ASCP _{HA}	ASCC _{HA}	ASC _{HA}	ASCP _{HA}	ASCC _{HA}	ASC _{HA}
0	12.8a	11.7b	100.0	7.2a	14.1c	100.0
4	15.1b	9.7a	100.0	11.8b	7.8a	100.0
12	15.6b	10.1a	100.0	11.5b	8.0a	100.0
16	14.8b	9.0a	100.0	12.3bc	8.1a	100.0
24	15.6b	9.4a	100.0	13.7c	9.5b	100.0

Note: same lower-case letters in columns within the same parameters means no statistical differences according to Tukey's test at $P < 0.05$.

The freeze-dried liquid samples of the water/alkaline extracts and their humic fraction obtained in 2.3 section were characterized for macro and micronutrients (P, K, Ca, Mg, Na, S, Fe, Cu, Mn, Zn) carried out by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Varian ICP 720-ES) after microwave digestion at the Instrumental Technical Services of the "Estación Experimental del Zaidín" (CSIC, <http://www.eez.csic.es>) and Ionomics Laboratory of "Centro de Edafología y Biología Aplicada del Segura" (CSIC, <http://www.cebas.csic.es>). Finally, soluble organic carbon and nitrogen were analysed using a TOC-V CSN + TNM-1 Analyser, Shimadzu liquid analyser after 0.45 µm filtration.

2.5. Statistical analysis

For each extraction procedure, a descriptive statistical analysis (mean, absolute and relative errors) was carried out. Also, we

performed some inferential statistical analyses such as the analysis of variance (ANOVA) within procedures (extraction time and ratio, heat time applied and extraction agents and its concentrations), assuming a normal distribution and homoscedasticity of the data. Finally, as a *post-hoc* analysis, the Tukey's test ($p < 0.05$) was used.

3. Results and discussion

3.1. Effects of extraction time (hours) on organic carbon solubilisation

The total amount of organic carbon extracted (ASC) and that corresponding to its HA fraction (ASC_{HA}) are presented in Fig. 1. Both showed a linear increase from 2 to 48 h with 0.1 M KOH, resulting in a maximum concentration of 6.1 and 4.4 g L⁻¹ after 48 h, respectively. A similar behaviour occurred using 1 M KOH until 24 h, and after that, a slight slowdown of the ASC was shown, indicating a saturation of the alkali extraction capacity. In general, the use of 1 M KOH doubled the amount of organic carbon extracted in ASC for all the time periods studied, especially due to the ASC_{HA} increases. The extraction efficiency showed a similar behaviour to organic carbon concentration (Fig. 2) for ASC and ASC_{HA} . Similar results were reported by Roletto and Ottino (1984) using poplar and spruce bark composts. They found a more relevant effect of KOH concentration on extraction efficiency, obtaining an efficiency close to 40% for 1 M and lower for 0.1 M KOH (30%) in a 48 h extraction.

It is known that alkaline treatments of lignocellulosic materials can alter chemical bounds of lignin monomers by hydrolysing uronic and acetic esters (Xiao et al., 2001), and also lignin-hemicellulose complexes, releasing small phenolic molecules such as *p*-coumaric and ferulic acids (Spencer and Akin, 1980). On the other hand, Sjöström (1991) and Coelho et al. (1988) demonstrated the positive effect of alkaline treatments on carbohydrates solubilization and found that some carbohydrates could be linked to soil HA through ester or amino bound neutral amino sugars, being an important component of this fraction. In ASC and ASC_{HA} , the contribution of phenolic-like and carbohydrate-like carbon with respect to ASC (Table 2), remained constant with time for 1 M KOH, but not with 0.1 M KOH, that showed a slight decrease with time. In general, the effect of KOH concentration produced an increase in both phenolic-like and carbohydrate-like carbon around 2.5 times in all extracting times studied (data not shown).

According to these results, the extraction time can optimise the efficiency of the process, especially for ASC concentration (g L⁻¹) under low alkaline concentration when non-saturated extraction conditions are carried out. By using 1 M KOH at 25 °C, a saturation condition was reached after 24 h.

3.2. Effects of extraction ratio on organic carbon solubilisation

As expected, ASC (g L⁻¹) was linear and positively related to extraction ratio (Fig. 1). Thus, the highest values of its concentration were obtained using 1:4 as extraction ratio; 20.1 and 42.3 g L⁻¹ with 0.1 and 1 M KOH, respectively. As was noticed before, the principal component of the ASC was always the HA fraction. In general terms, the KOH concentration produced an increase nearly double in ASC concentration, for all extraction ratio ranges studied. ASC and ASC_{HA} concentrations were much higher at larger values of extraction ratio (as was expected), similar results that Roletto and Ottino (1984) found using two lignocellulosic waste composts, 0.5 M NaOH and extraction ratio values of 1:10 and 1:100. On the contrary, the efficiency (%) was only slightly affected by the extraction ratio, showing a decrease along the extraction ratio

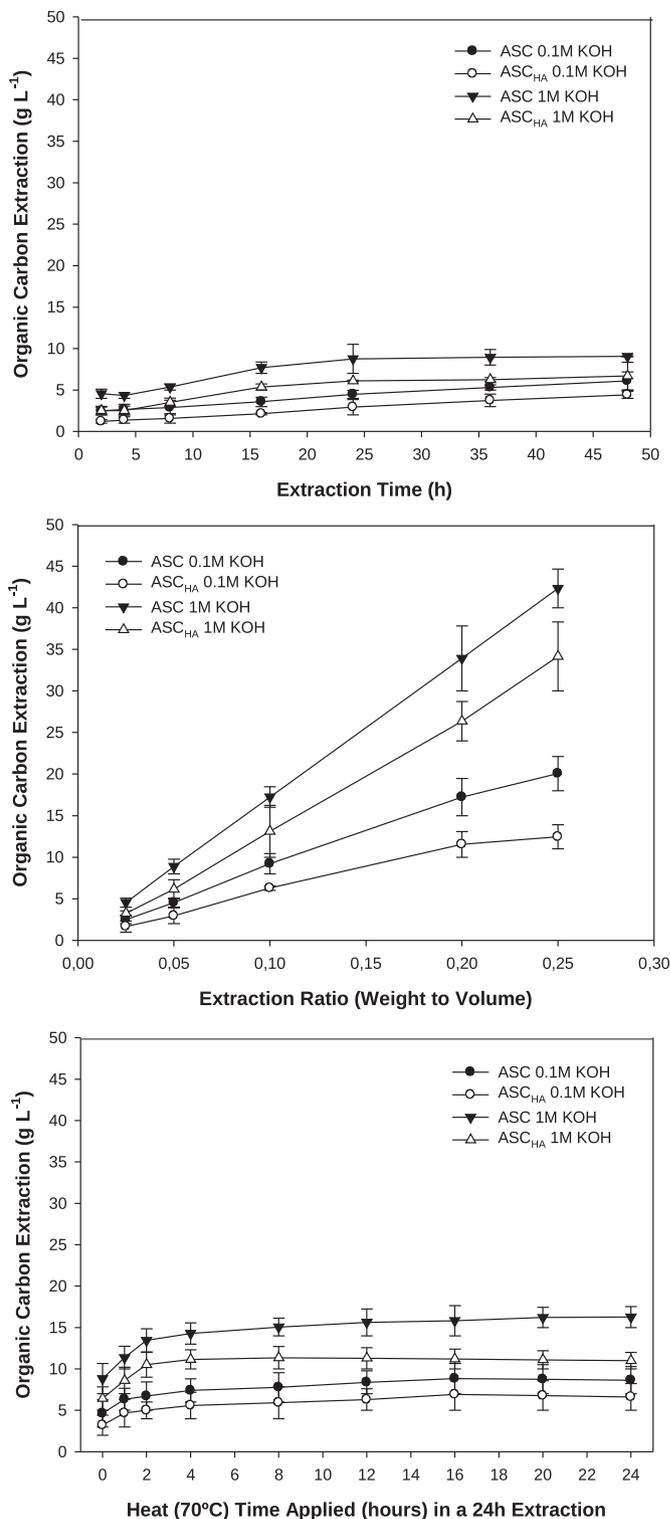


Fig. 1. Effects of extraction time, extraction ratio and heat (70 °C) time applied (hours) in a 24 h extraction in the organic carbon extraction (g L⁻¹) and its humic acid fraction (HA) using 0.1 and 1 M KOH as extractant agents. Extraction ratio is represented as the quotient between weight (g) and volume (ml) (1:4 = 0.25; 1:5 = 0.20; 1:10 = 0.10; 1:20 = 0.05; 1:40 = 0.025).

assayed (Fig. 2). This effect was previously noted by Roletto and Ottino (1984) in the humic carbon efficiency obtained with NaOH and Na₄P₂O₇ as extraction agents using 1:10 and 1:100 extraction ratios during several extraction times (3, 6, 12 and 24 h).

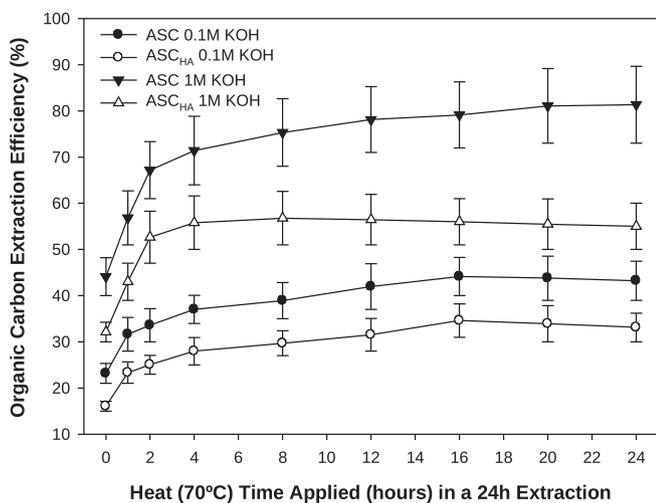
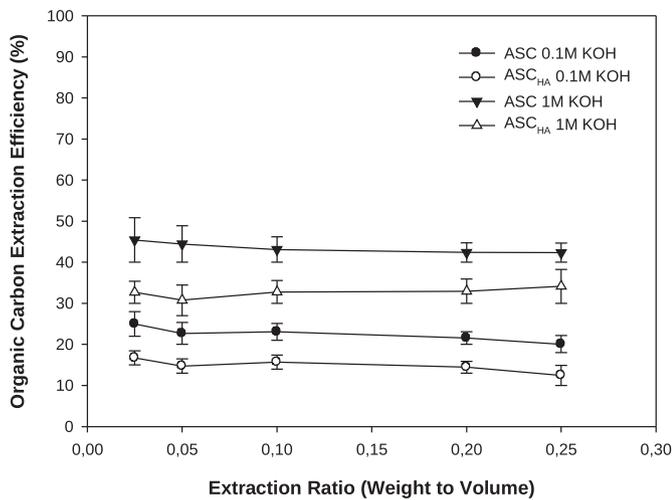
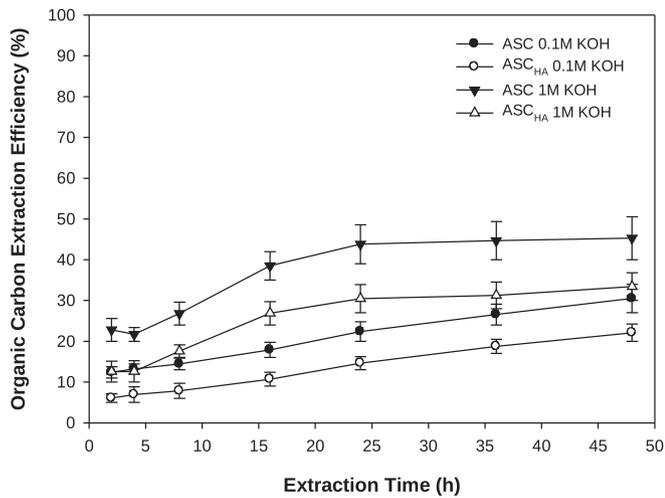


Fig. 2. Effects of extraction time, extraction ratio and heat (70 °C) time applied (hours) in a 24 h extraction in the organic carbon extraction efficiency (%) and its humic acid fraction (HA) using 0.1 and 1 M KOH as extractant agents. Extraction ratio is represented as the quotient between weight (g) and volume (ml) (1:4 = 0.25; 1:5 = 0.20; 1:10 = 0.10; 1:20 = 0.05; 1:40 = 0.025).

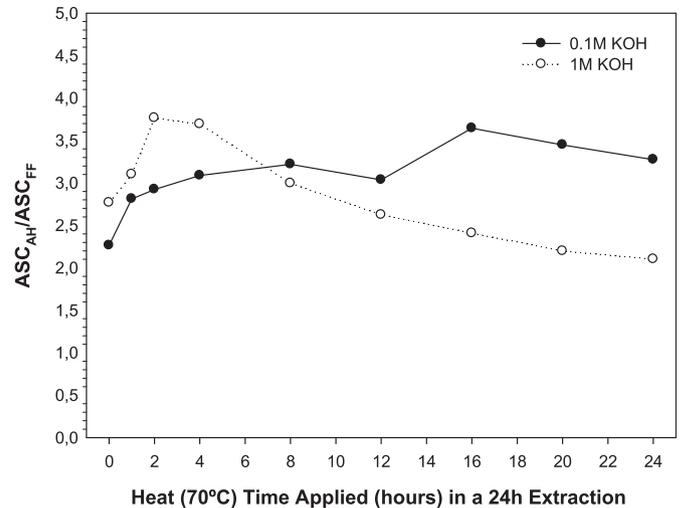


Fig. 3. Effects of heat (70 °C) time applied (hours) in a 24 h extraction in the Polymerization Index (ASC_{AH}/ASC_{FF}).

Also, similar results were obtained for polyphenol and carbohydrate-like carbon solubilisation, increasing their concentrations in ASC and ASC_{HA} with extraction ratio values (data not shown). Their perceptual contribution presented the opposite behaviour to ASC concentration, being more important at slower extraction ratio values, especially for the carbohydrate-like carbon (Table 2). The greater percentual contribution of polyphenols and carbohydrates were obtained at low ratios (less concentrated extracts). These results suggested that the extraction ratio was a key factor for modifying the lignocellulosic nature of the ASC (more polyphenols and carbohydrates contribution under low extraction ratios and vice versa).

According to our results, the extraction ratio can optimise the ASC concentration (g L⁻¹), using either 0.1 or 1 M KOH, without significantly affecting the extraction efficiency. Thus, the more low extraction ratios are used, the more concentrated organic carbon (ASC) will be obtained.

3.3. Effects of heat (70 °C) time applied (hours) in a 24 h extraction on organic carbon solubilisation

In general, heat time applied produced an important increase in both organic carbon concentration (g L⁻¹) and extraction efficiency (%) in ASC obtained, especially notable after only 2 h at 70 °C (Figs. 1 and 2). Maximum values were recorded after 24 h at 70 °C giving 8.6 g L⁻¹ and 16.3 g L⁻¹ for ASC concentration, and 43.2 and 81.3% for its extraction efficiency with 0.1 and 1 M KOH, respectively. For both ASCs, the main fraction was HA as ASC_{HA} data revealed (Figs. 1 and 2) and a degradation process of this fraction was registered in the extraction with 1 M KOH as was shown in the polymerization index (ASC_{AH}/ASC_{FF}) (Fig. 3). According to this data, the important decrease of this index after 8 h at 70 °C occurred due to the HA degradation and consequently, an increase in its FF fraction. This alkaline degradation of HA with temperature extraction was also obtained by several authors as Yamamoto *et al.* (1989), Cegarra *et al.* (1994) and Ouattmane *et al.* (2000), using several organic materials such as Andisol soil, peat and composts. Temperature could improve chemical degradation and solubilisation of lignin and polysaccharides from lignocellulosic agricultural wastes as was noted by Sjöström (1991). In our case, only a quantitative increase of polyphenols-like and carbohydrates-like carbon was produced (data not shown) with temperature and its time applied, not

Table 3
Nutrient concentration (mg L⁻¹) and nutrient extraction efficiency (%) in water and alkali extracts (1 M KOH) and its humic acid fractions (HA) at different extraction procedures.

Nutrient	Extraction ratio	Extractant							
		H ₂ O		KOH 1 M (25 °C)			KOH 1 M (70 °C)		
		(mg L ⁻¹)	(%)	(mg L ⁻¹)	(%)	HA ^a (%)	(mg L ⁻¹)	(%)	HA ^a (%)
C	1:3	5940aA	5.5	37 642aB	35.1	78.2	62 906aC	58.7	92.2
	1:20	1234bA	7.6	7234bB	44.9	80.6	7989bC	48.1	94.1
N	1:3	603aA	9.2	3702aB	56.2	77.7	6191aC	94.0	94.2
	1:20	235bA	24.1	400bB	40.5	69.2	517bC	52.3	88.1
K	1:3	878aA	26.4	35 772aB	—	—	36 098aB	—	—
	1:20	256bA	52.0	23 338bB	—	—	23 549bB	—	—
P	1:3	67aA	10.2	155aB	7.9	73.3	210aB	11.1	61.3
	1:20	7bA	6.6	25bB	3.2	65.5	43bC	7.9	59.2
Ca	1:3	457aA	3.7	1754aB	14.2	41.6	2285aC	18.5	33.0
	1:20	77bA	3.6	110bB	26.9	62.4	156bC	27.0	41.2
Mg	1:3	128aA	5.2	185aB	12.1	26.7	262aC	21.1	31.1
	1:20	20bA	4.5	12bA	4.5	21.9	30bA	7.5	29.1
Na	1:3	157aA	6.8	332aB	14.2	28.1	430aC	18.5	23.6
	1:20	19bA	5.4	94bB	26.9	33.2	94bB	27.0	36.4
Fe	1:3	50aA	2.0	316aB	12.1	74.4	514aC	21.1	83.6
	1:20	1bA	0.3	19bB	4.5	60.6	29bC	7.5	76.0
Cu	1:3	1aA	16.9	4aA	57.7	97.1	5aA	77.5	92.2
	1:20	0aA	3.8	0aA	40.0	95.0	1aA	49.1	89.1
Mn	1:3	2aA	3.1	7aA	12.6	30.3	10aA	17.7	42.3
	1:20	0aA	0.7	0aA	5.5	25.4	1aA	9.1	38.0
Zn	1:3	1aA	8.6	7aA	42.4	56.6	12aA	70.8	78.2
	1:20	0aA	4.6	1aA	36.7	52.7	1aA	53.9	75.1

Note: Data was shown as the mean value of seven replicates with less than 5% of relative error. Same lower-case letters in columns within the same nutrient and same capital letters in files within the same extraction ratio means no statistical differences according to Tukey's test at $P < 0.05$.

^a Nutrients in humic acid fraction (HA) are expressed as % of the total nutrient solubilization in the liquid extracts.

affecting their perceptual contribution to ASC and ASC_{AH} obtained with 0.1 and 1 M KOH, respectively (Table 2). This fact could be explained due to the increase registered by ASC that was specifically produced by other organic fractions solubilisation. Several authors have obtained similar results. Xu et al. (2005) obtained an increase in ferulic and *p*-coumaric acids, and related phenolic compounds concentration from sugarcane bagasse. Sun et al. (2001) showed the effect of temperature in the quantitative determination of hydroxycinnamic acids in wheat, rice, rye, and barley straws. Finally, Xu et al. (2006) solubilised 56, 57, 59, 61 and 62% of the initial hemicellulose content in sugarcane bagasse with an extraction of 18 h using 1 M NaOH at 20, 25, 30, 35 and 40 °C, respectively.

As was noted before, high temperature increased the amount of organic carbon extracted and its extraction efficiency, and in some cases, could produce degradation of HA releasing less-complex organic compounds (similar to fulvic fraction). It is important to note that this increase was produced using a high energy condition (70 °C), probably not suitable for large volume extraction systems commercially available due to its important economical cost. For that reason, a few hours of heat applied (2–4 h) could be enough to increase concentration and efficiency significantly. According to Figs. 1, 6.9 and 14.5 g L⁻¹ were obtained with only 4 h using 0.1 and 1 M KOH, respectively, representing 80.2 and 88.9% of the total ASC concentration solubilised.

3.4. Chemical characterisation of the organic extracts

By using distilled water as extractant agent (Table 3), the extracted nutrients showed generally low concentrations (being higher with the 1:3 extraction ratio as expected), giving maximums of 5 940, 603 and 878 mg L⁻¹ for C, N and K, respectively. The rest of the macro and micronutrients presented low extraction efficiencies (only P and Cu values reached around 10% using a 1:3 extraction

ratio), with the exception of N and K. The results obtained were consistent with those described by Gutiérrez-Miceli et al. (2008), Tejada et al. (2008) and Alburquerque et al. (2009b), who characterized leachates derived from cow manure, forage vermicomposts and AL composts, respectively. These extracts can be used in a similar way to “compost tea”, where the biological effects prevailed over the chemical effects (Scheuerell and Mahaffee, 2004; Hargreaves et al., 2009).

With 1 M KOH at 25 °C, a quantitative increase was observed in the concentration of the main nutrients studied, especially C and N (also K was elevated due to the use of the alkali extractant). Regarding the extraction efficiency, C, N, Cu and Zn presented a percentage close to 50%, being too low for nutrients such as P, Fe, Mg and other micronutrients. At 70 °C, a generalized increase was shown in all nutrient concentrations in both extraction ratios assayed, especially in organic carbon than doubled the concentration obtained at 25 °C (62.9 versus 37.6 g L⁻¹ at 70 °C). Regarding other nutrients, their extractive efficiency was higher than that obtained at 25 °C, being in some cases above 75% in the case of N, Cu and Zn. In all cases, ASC and the main nutrients were attached to acid humic-like acid fraction (Table 3).

Finally, the extraction ratio affected the T_{OC}/T_N ratio of the organic liquid extracts, as was shown in Fig. 4. For both alkali extractions, a negative relationship was produced, decreasing this ratio with higher extraction ratio values. Indeed, T_{OC}/T_N ratio decreased from around 19–16 to 10 along 1:20 to 1:3 of extraction ratios. The highest T_{OC}/T_N ratio value was obtained at 25 °C using 1:20 as extraction ratio. These results were consistent with those published by Alburquerque et al. (2009b) and Kohler et al. (2008). In the former, authors obtained alkali-soluble organic matter with T_{OC}/T_N round 8–10 using composts made with AL, horse manure and a fatty-proteinaceous waste with 0.5 M KOH at different temperatures. In the latter, authors obtained an alkali-soluble organic matter from AL composts with T_{OC}/T_N round 20 using 0.1 M KOH at

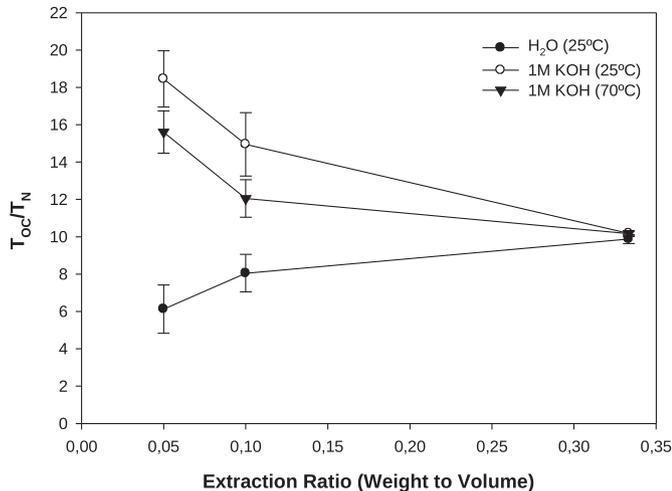


Fig. 4. Effect of extraction ratio in the T_{OC}/T_N ratio of the liquid organic extracts obtained with H₂O, 1 M KOH (25 °C) and 1 M KOH (70 °C). Extraction ratio is represented as the quotient between weight (g) and volume (ml) (1:3 = 0.33; 1:10 = 0.1; 1:20 = 0.05).

1:20 of extraction ratio. It is important to note that T_{OC}/T_N values of round 20–30 in an organic substrate solid or liquid could enhance microbial activity. This fact was shown by Caravaca et al. (2006) in the rizosphere of *Retama sphaerocarpa* (L.) after alkali-soluble organic matter addition, showing an increase in soil microbial activity. This means that it is possible to enhance of specific microbial population activity in agricultural soils which could favour crop development and nutrient metabolism uptake.

3.5. Legal requirements to produce commercial liquid organic fertilisers from AL composts

According to the Spanish legislation for fertiliser production (RD 506/2013, 2013), AL composts could be included in several commercial categories such as *AL Compost*, *Humic Organic Amendment*, *Compost Organic Amendment*, *NPK Organic Fertiliser*, and at least, 19 types of *Organo-mineral Fertilisers*, either as solid or liquid formulations by mixing with other *organic and/or mineral fertilisers* as was demonstrated in a previous study (Tortosa et al., 2012).

The legal requirements for liquid *Organo-mineral fertilisers* are: *Liquid N* (N_{TOTAL} : 8%, $N_{ORGANIC}$: 1% and $C_{ORGANIC}$: 5%), *Liquid NPK* (N_{TOTAL} + P_2O_5 + K_2O : 8%, N_{TOTAL} : 2%, $N_{ORGANIC}$: 1%, P_2O_5 : 2%, K_2O : 2% and $C_{ORGANIC}$: 4%), *Liquid NP* (N_{TOTAL} + P_2O_5 : 6%, N : 2%, $N_{ORGANIC}$: 1%, P_2O_5 : 2% and $C_{ORGANIC}$: 4%), *Liquid NK* (N_{TOTAL} + K_2O : 6%, N_{TOTAL} : 2%, $N_{ORGANIC}$: 1%, K_2O : 2% and $C_{ORGANIC}$: 4%) and *Liquid PK* (P_2O_5 + K_2O : 6%, P_2O_5 : 2%, K_2O : 2% and $C_{ORGANIC}$: 4%). According to Table 3, 1 M KOH (25 °C) obtained N_{TOTAL} : 0.37%, $N_{ORGANIC}$: 0.37%, P_2O_5 : 0.04%, K_2O : 4.31% and $C_{ORGANIC}$: 3.76%; while 1 M KOH (70 °C) showed N_{TOTAL} : 0.62%, $N_{ORGANIC}$: 0.62%, P_2O_5 : 0.05%, K_2O : 4.59% and $C_{ORGANIC}$: 6.29%. In both cases using an extraction ratio of 1:3. Therefore, neither alkali extraction procedures assayed could fulfil the legal requirements listed above due to their scarce N_{TOTAL} and P content, nor for K or $C_{ORGANIC}$ concentrations. In order to solve it, an external source of N and P can be added such as concentrated nitric and/or phosphoric acid, that also, it can be used to adjust pH to neutral values of the alkaline organic liquid obtained.

4. Concluding remarks

According to the above results, some conclusions can be listed in order to optimize the solubilisation of organic carbon and nutrients from AL composts:

- Alkali-extractant agent (KOH) concentration significantly affects the extraction performance. The concentration of the alkali-extracted organic carbon (ASC) obtained with 1 M KOH was around twice compared to 0.1 M KOH.
- The extraction time increased the amount of organic carbon extracted and the extraction efficiency, especially using 0.1 M KOH. With 1 M KOH, a saturation condition was reached after 24 h.
- The application of heat (70 °C) significantly affected the nature of humic alkali-soluble organic matter, increasing efficiency and concentration and showing a chemical degradation of the humic-like acid fraction (HA).
- The extraction ratio affected the concentration of both organic, including polyphenols and carbohydrates, and inorganic fractions.

5. Guidelines and economical cost

Based on these conclusions and taking into account the legal requirements for *Organo-mineral Fertilisers* production commented in 3.5 section, some extracting procedures to solubilise humified organic matter could be recommended:

- Extracting agent and its concentration: 1 M KOH gives higher extraction yields and concentrated ASC.
- Extraction time: 24 h.
- Extraction ratio: The higher a ratio is, the more concentrated it will be and the greater the humic nature of organic matter it could have. For this, extraction ratios of 1:4 or 1:5 are recommended.
- Time for applying heat (70 °C): From 2 to 4 h at 70 °C could be enough to notably increase the extraction efficiency and the concentration. As it was noticed before, heat time applied increased ASC concentration but with only 4 h, close to 80% of the ASC solubilised was obtained.
- External source of N and P: Concentrated nitric and/or phosphoric acid or another organic/inorganic source could be used.

In Tortosa et al. (2012) we presented the economical cost for the composting process (2150 € to produce 60 t of AL composts). For the *Organo-mineral Fertilisers* production, several factors have to be taking into account:

- Extraction equipment, which depends of the total volume wished. There are several commercially available like the “compost tea brewers”. Also, it is needed a shaking system working under mechanical or air-pressure injection process. The heating system is the most expensive feature. The total cost for an extraction equipment for producing 1000 L should cost round 600–800 €.
- Composts, water and chemical products. The compost production was presented in Tortosa et al. (2012) and it was 36 € per t of AL compost produced. The chemical products needed (KOH, concentrated nitric and/or phosphoric acid or another organic/inorganic source) and the tap water used could cost round 100 €.
- Filtration system. A supernatant removing system is needed and could cost round 100€.
- Electrical consume. It is directly related to the shaking and heating system applied. Using our recommendations, the electrical cost could be round 50–100 €.

Finally, the *Organo-mineral Fertilisers* production cost from AL composts could be less than 0.4 € per litre obtained using an extraction equipment of 1000 L of capacity. This cost was calculated

without taking into account the initial investment for the extraction and filtration equipments acquisition.

Acknowledgements

Some experiments have been carried out under the framework of the EU contract FAIR5-CT97-3620. The authors thank "Aceites Guadalentín S.A." olive mill for financial and technical support, and D. Francis Lewis for improvement of the written English.

References

- Ait Baddi, G., Albuquerque, J.A., Tortosa, G., Winterton, P., Hafidi, M., Cegarra, J., 2013. Characterization of potential liquid organic fertilizers obtained by alkaline extraction from two-phase olive-mill waste composts. *Environ. Prog. Sustain. Energy* 32, 1170–1178.
- Albuquerque, J.A., González, J., García, D., Cegarra, J., 2004. Agrochemical characterisation of "alperujo", a solid by-product of the two-phase centrifugation method for olive oil extraction. *Bioresour. Technol.* 91 (2), 195–200.
- Albuquerque, J.A., González, J., García, D., Cegarra, J., 2006. Composting of a solid olive-mill by-product ('alperujo') and the potential of the resulting compost for cultivating pepper under commercial conditions. *Waste Manag.* 26, 620–626.
- Albuquerque, J.A., González, J., García, D., Cegarra, J., 2007. Effects of a compost made from the solid by-product ('alperujo') of the two-phase centrifugation system for olive oil extraction and cotton gin waste on growth and nutrient content of ryegrass (*Lolium perenne* L.). *Bioresour. Technol.* 98, 940–945.
- Albuquerque, J.A., González, J., Tortosa, G., Baddi, G.A., Cegarra, J., 2009a. Evaluation of 'alperujo' composting based on organic matter degradation, humification and compost quality. *Biodegradation* 20, 257–270.
- Albuquerque, J.A., Bautista-Carrascosa, I., Lidon, A., García-de-la-Fuente, R., Girbent, J., Abad, M., Cegarra, J., 2009b. Co-composting an animal fatty-proteinaceous waste with a solid lignocellulosic by-product from the olive oil industry ("alperujo"). *J. Chem. Technol. Biotechnol.* 84, 918–926.
- Altieri, R., Esposito, A., Baruzzi, G., Nair, T., 2014. Corroboration for the successful application of humified olive mill waste compost in soilless cultivation of strawberry. *Int. Biodeterior. Biodegrad.* 88, 118–124.
- Bidegain, R.A., Kaemmerer, M., Guiesse, M., Hafidi, M., Rey, F., Morard, P., Revel, J.C., 2000. Effects of humic substances from composted or chemically decomposed poplar sawdust on mineral nutrition of ryegrass. *J. Agric. Sci.* 134, 259–267.
- Caravaca, F., Tortosa, G., Carrasco, L., Cegarra, J., Roldán, A., 2006. Interaction between AM fungi and a liquid organic amendment with respect to enhancement of the performance of the leguminous shrub *Retama sphaerocarpa*. *Biol. Fertil. Soils* 43, 30–38.
- Cegarra, J., García, D., Navarro, A., Bernal, M.P., 1994. Effects of heat on the alkali extraction of humic substances from peat. *Commun. Soil. Sci. Plant Anal.* 25, 2685–2695.
- Cegarra, J., Albuquerque, J.A., González, J., Tortosa, G., Chaw, D., 2006. Effects of the forced ventilation on composting of a solid olive-mill by-product ('alperujo') managed by mechanical turning. *Waste Manag.* 26, 1377–1383.
- Coelho, R.R., Linhares, L.F., Martín, J.P., 1988. Sugars in hydrolysates of fungal melanins and soil humic acids. *Plant Soil.* 106, 127–133.
- Eyheraguibel, B., Silvestre, J., Morard, P., 2008. Effects of humic substances derived from organic waste enhancement on the growth and mineral nutrition of maize. *Bioresour. Technol.* 99 (10), 4206–4212.
- Fernández-Hernández, A., García-Ortiz Civantos, C., Roig, A., Sánchez-Monedero, M.A., 2013. Composts prepared with two phase olive mill waste "alperujo" as growing media. In: *International Symposium on Growing Media, Composting and Substrate Analysis*. ISHS Acta Horticulturae 1013, ISBN 978-90-66057-36-4.
- García-Gómez, A., Bernal, M.P., Roig, A., 2005. Organic matter fractions involved in degradation and humification processes during composting. *Compost Sci. Util.* 13, 127–135.
- Gutiérrez-Miceli, F.A., García-Gómez, R.C., Rincón Rosales, R., Abud-Archila, M., Oliva Llavén, M.A., Guillén Cruz, M.J., Dendooven, L., 2008. Formulation of a liquid fertilizer for sorghum (*Sorghum bicolor* (L.) Moench) using vermicompost leachate. *Bioresour. Technol.* 99, 6174–6180.
- Hargreaves, J.C., Sina Adl, M., Warman, P.R., 2009. The effects of municipal solid waste compost and compost tea on mineral element uptake and fruit quality of strawberries. *Compost Sci. Util.* 17 (2), 85–94.
- Kohler, J., Tortosa, G., Cegarra, J., Caravaca, F., Roldán, A., 2008. Impact of DOM from composted "alperujo" on soil structure, AM fungi, microbial activity and growth of *Medicago sativa*. *Waste Manag.* 28, 1423–1431.
- Masmoudi, S., Jarbouia, R., El Fekib, H., Gea, T., Medhiouba, K., Ammar, E., 2013. Characterization of olive mill wastes composts and their humic acids: stability assessment within different particle size fractions. *Environ. Technol.* 34, 787–797.
- Muktadirul Bari Chowdhury, A.K.M., Akrotas, C.S., Vayenas, D.V., Pavlou, S., 2013. Olive mill waste composting: a review. *Int. Biodeterior. Biodegrad.* 85, 108–119.
- Nair, N., Altieri, R., Esposito, A., Saville, K., 2014. Recent studies on preparation of humified compost using olive mill waste for horticultural purposes. In: *International Symposium on Organic Matter Management and Compost Use in Horticulture*. ISHS Acta Horticulturae 1018, ISBN 978-94-62610-04-0.
- Quatmane, A., Provenzano, M.R., Hafidi, M., Senesi, N., 2000. Compost maturity assessment using calorimetry, spectroscopy and chemical analysis. *Compost Sci. Util.* 8, 124–134.
- RD 506/2013, 2013. Real Decreto 506/2013, de 28 de junio, sobre productos fertilizantes. *Boletín Oficial del Estado* 164, 51119. Sec. I. Available in Spanish at: <http://www.boe.es/boe/dias/2013/07/10/pdfs/BOE-A-2013-7540.pdf> (last accessed 22.06.14.).
- Roletto, E., Ottino, P., 1984. Analytical characterization of humic substances from composted lignocellulosic residues. *Agric. Wastes* 11, 181–195.
- Sánchez-Monedero, M.A., Roig, A., Cegarra, J., Bernal, M.P., 1999. Relationships between water-soluble carbohydrate and phenol fractions and the humification indices of different organic wastes during composting. *Bioresour. Technol.* 70, 193–201.
- Scheuerell, S.J., Mahaffee, W.F., 2004. Compost tea as a container medium drench for suppressing seedling damping-off caused by *Pythium ultimum*. *Phytopathology* 94 (11), 1156–1163.
- Serramiá, N., Sánchez-Monedero, M.A., Roig, A., Contin, M., De Nobili, M., 2013. Changes in soil humic pools after soil application of two-phase olive mill waste compost. *Geoderma* 192, 21–30.
- Sjostrom, E., 1991. Carbohydrate degradation products from alkaline treatment of biomass. *Biomass Bioenerg.* 1, 61–64.
- Stevenson, F.J., 1994. *Humus Chemistry: Genesis, Composition, Reactions*, second ed. John Wiley & Sons, Inc, ISBN 978-0-471-59474-1.
- Spencer, R.R., Akin, D.E., 1980. Rumen microbial-degradation of potassium hydroxide-treated coastal bermudagrass leaf blades examined by electron-microscopy. *J. Anim. Sci.* 51, 1189–1196.
- Sun, R.C., Sun, X.F., Zhang, S.-H., 2001. Quantitative determination of hydroxycinnamic acids in wheat, rice, rye, and barley straws, maize stems, oil palm frond fiber, and fast-growing poplar wood. *J. Agric. Food Chem.* 49, 5122–5129.
- Tejada, M., Gonzalez, J.L., Hernandez, M.T., Garcia, C., 2008. Agricultural use of leachates obtained from two different vermicomposting processes. *Bioresour. Technol.* 99, 6228–6232.
- Tortosa, G., Albuquerque, J.A., Ait Baddi, G., Cegarra, J., 2012. The production of commercial organic amendments and fertilisers by composting of two-phase olive mill waste ("alperujo"). *J. Clean. Prod.* 26, 48–55.
- Trevisan, S., Ornella, F., Quaggiotti, S., Serenella, N., 2010. Humic substances biological activity at the plant-soil interface. *Plant Signal. Behav.* 5 (6), 635–643.
- Valdrighi, M.M., Pera, A., Agnolucci, M., Frassinetti, S., Lunardi, D., Vallini, G., 1996. Effects of compost-derived humic acids on vegetable biomass production and microbial growth within a plant (*Cichorium intybus*)-soil system: a comparative study. *Agric. Ecosyst. Environ.* 58, 133–144.
- Xiao, B., Sun, X.F., Sun, R., 2001. Chemical, structural, and thermal characterizations of alkali-soluble lignins and hemicelluloses, and cellulose from maize stems, rye straw, and rice straw. *Polym. Degrad. Stab.* 74, 307–319.
- Xu, F., Sun, R.C., Sun, J.X., Liu, C.F., He, B.H., Fan, J.S., 2005. Determination of cell wall ferulic and *p*-coumaric acids in sugarcane bagasse. *Anal. Chim. Acta* 552, 207–217.
- Xu, F., Sun, J.X., Liu, C.F., Sun, R.C., 2006. Comparative study of alkali- and acidic organic solvent-soluble hemicellulosic polysaccharides from sugarcane bagasse. *Carbohydr. Res.* 341, 253–261.
- Yamamoto, S., Honna, T., Miyata, O., Takata, H., 1989. Alteration of humus characteristics on the extraction by heating with alkaline solvent. *Bull. Fac. Agric. Tamagawa Univ.* 42, 1–6.