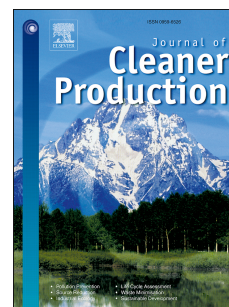


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**Agri-food sludge management using different co-composting strategies: study of the added value of the composts obtained.**

A.B. Morales<sup>1</sup>, M.A. Bustamante<sup>2</sup>, F.C. Marhuenda-Egea<sup>3</sup>, R. Moral<sup>2</sup>, M. Ros<sup>1</sup>, J.A. Pascual<sup>1</sup>

<sup>1</sup>Department of Soil and Water Conservation and Organic Waste Management, Centro de Edafología y Biología Aplicada del Segura, CSIC, PO Box 164, 30100 Murcia, Spain

<sup>2</sup>Department of Agrochemistry and Environment, Miguel Hernandez University, EPS-Orihuela, ctra. Beniel Km 3.2, 03312-Orihuela (Alicante), Spain.

<sup>3</sup>Department of Agrochemistry and Biochemistry. University of Alicante, Apartado 99, E-03080 Alicante, Spain.

**Abstract**

The growth of the agri-food industry has resulted in a strong increase in its sludge generation. This type of waste is often produced in high amounts, concentrated in certain areas, and shows characteristics similar to those of municipal sewage sludge (except for the absence of heavy metals). Composting has been widely studied as a viable alternative for the treatment and recycling of municipal sewage sludge, but little information is currently available concerning the composting of agri-food sludge. Thus, the aim of this work was to increase our knowledge of how the vegetable-derived sludge produced by the agri-food sector can be managed using different co-composting strategies. The work was concerned mainly with the characteristics of the bulking agents used (type and particle size) and their effects on the development of the process and on the potential added value of the composts obtained. For this, four composting piles were prepared using agri-food sludge and two bulking agents (vine shoot prunings and pepper plant pruning waste) at two particle sizes ( $< 1$  cm and  $< 3$  cm). The evolution of the process and the final properties of the composts obtained were studied using classical analytical methods together with advanced instrumental methods (thermal analysis and excitation–emission matrix (EEM) fluorescence spectroscopy). In addition, their physical characteristics and potential suppression of phytopathogens (*Fusarium oxysporum* f. sp. *melonis*) were determined. The results showed differences according to the type and size of bulking agent, the mixtures with vine shoot prunings having a higher biodegradability, which was confirmed by thermogravimetry and EEM fluorescence spectroscopy. Melon plants grown in a mixture which included compost produced using vine pruning waste had a greater shoot weight than those grown on peat alone, while the mixture including compost derived from pepper pruning waste gave a shoot weight similar to that of peat-grown plants. Furthermore, the composts elaborated

using vine shoot prunings had added value with respect to their use as growing media components for seedling production of melon, due to their suppression of *F. oxysporum* f. sp. *melonis*. Therefore, this study has shown that the nature of the bulking agent used is of major importance not only for the development of the composting process, but also for the final properties and potential added value of the end-products obtained.

**Keywords:** agri-food waste, compost, bulking agent, thermogravimetric analysis, fluorescence analysis, phytopathogen suppression.

## 1. Introduction

The food and agricultural industry constitutes the main activity of the European manufacturing sector, representing 14.6% of its output (more than 1,048,000 million €), with a growth of around 3.1% in relation to the previous year, during the period 2013-2014 (MAGRAMA, 2015). The production processes of this industry involves high water consumption, generating effluents with a high organic load due to the contribution of plant remains and soluble compounds derived from the raw materials (proteins, carbohydrates, phenolic compounds, etc.), as well as to the presence of oils and fats remaining after their extraction.

In recent years, the industries of the agri-food sector, involved in the processing of fruits and vegetables for the production of juices and processed vegetables, have significantly improved the quality of their processing procedures and have installed treatment systems to treat their wastewaters, according to the Directive 91/271/CEE of May 21 (DOCE, 1991). Biological treatment is the process most commonly used to treat wastewaters rich in nutrients (Najafpour et al., 2006) and with high organic content (Christensen et al., 2009), such as those of the food-processing industries. However, this

treatment generates a new organic residue, known as agri-food sludge. This type of sludge is usually found in areas of concentrated production; for example, in the Region of Murcia (11,000 km<sup>2</sup>), where 15,000 tonnes were produced in the campaign 2011/2012, with a predicted increase to 40,000 tons by the year 2020 (AGROWASTE, 2014).

The characteristics of this waste are similar to those of urban wastewater produced at sewage treatment plants. However, agri-food sludge also shows a significant difference, since it does not contain heavy metals (Li et al., 2013) or other potentially toxic constituents, since it is generated during the processing of wastewater whose pollutant load is mainly constituted by organic matter (OM) of plant origin. Thus, this sludge has great potential for agricultural uses, due to its high contents of nutrients, such as nitrogen and phosphorus, and OM (Singh and Agrawal, 2008). Currently, the management of this sludge has an associated cost based on the “elimination of the problem” by authorised management agents. In Spain, the management of these wastes is governed by the Law 22/2011, relating to residues and polluted soils (BOE, 2011), which prioritises recycling and other ways of adding value such as energy extraction, thereby avoiding deposition in landfill sites. In addition, under the proposed Plan of the Circular Economy of the European Commission, in 2025 it is intended that the entry of recyclable residues in landfill sites will be prohibited; so, options such as direct application to soil and composting represent viable alternatives for the management of these wastes.

Composting can constitute a suitable method for the recycling of this type of waste, since the compost obtained is a useful organic amendment and/or organic substrate that can be reincorporated into the economic system, helping to solve the disposal problem and reducing emissions of greenhouse gases (Banegas et al., 2007; Himanen and

Hänninen, 2011; Lim et al., 2016). Composting is a biological treatment in which aerobic thermophilic and mesophilic microorganisms use OM as substrate, the main products of this process being fully-mineralised materials (carbon dioxide ( $\text{CO}_2$ ), water and ammonium ( $\text{NH}_4^+$ )) and stabilised OM (mostly humic substances), free of pathogens and phytotoxins, which can be safely used in agriculture (Bustamante et al., 2008a). Diverse biodegradable organic materials can be composted, such as sewage sludge, pruning wastes, animal manures and slurries, forest management residues and remains from the food and agricultural industry. In this sense, the greatest number of studies have been developed using biodegradable organic materials that are generated in large amounts and whose handling or elimination are problematic, such as municipal sewage sludge (Ingelmo et al. 2012). However, not enough information is currently available regarding the composting of sludge from the agri-food industry. Like sewage sludge, agri-food sludge shows several characteristics that require the addition of a bulking agent for its composting, to optimise the properties of the composting mixture - such as the air space, moisture content, carbon to nitrogen (C/N) ratio, particle density, pH and mechanical structure - and thus enhance the decomposition rate. In this sense, ligno-cellulosic by-products, such as wood chips and sawdust, have been commonly used as bulking agents (Pasda et al., 2005; Li et al., 2013).

The composting of biodegradable solid wastes is deemed useful since its end product (compost) can be used as a nutrient-rich organic fertiliser or for land application (Wu et al., 2014). However, the destination and/or use of the compost will depend on its characteristics, which will be determined to a great extent by the raw materials used and the composting process carried out. In particular, additional value can be provided to agri-food sludge when it is composted appropriately with other organic residues generated in the agri-food industry to produce composts suitable for use in seedling

production (Himanen and Hänninen, 2011). Alternatives to chemical fertilisers, such as compost and vermicompost, are becoming increasingly important in the agricultural sector because the use of chemical fertilisers on the soil over a long period of time may affect its ability to sustain healthy plant growth and crop production (Lim et al., 2015a). In addition, the biopesticidal effect of such composts also might help to reduce the use of chemical fungicides; in contrast, peat is hardly ever suppressive against pathogens (Bonanomi et al., 2010). Hence, compost represents an increasingly-attractive alternative to peat in sustainable agricultural production. Peat is the plant growth substrate used most in seedbeds and nurseries. However, the increasing demand for peat as a growing medium in horticulture and its rising cost have intensified the search for alternative high-quality and low-cost substrates. Because of this, different materials, such as residual biomasses and, especially, organic wastes, mostly after proper composting, have been studied as peat substitutes in potting media (Ceglie et al., 2015). Therefore, the main aims of this work were: 1) to evaluate different co-composting strategies for the management of the sludge generated by the vegetable processing industry, considering two main variables: the type of bulking agent and its particle size; 2) to study the characteristics of the composts obtained using chemical, thermogravimetric and spectroscopic techniques and to evaluate their potential suppressive capacity against *Fusarium oxysporum*, with a view to their use as substrates or substrate components in seedling production.

## 2. Material and methods

Four different composting mixtures were tested, using agri-food sludge and two bulking agents prepared at two particle sizes ( $< 1$  cm and  $< 3$  cm), to select the best bulking agent and to assure the quality of the final product (Bustamante et al., 2013).

## 2.1 Composting procedure

The bulking agents used in the mixtures were vine shoot prunings (VP), obtained from a vineyard of Petit Verdot 3 years Rootstock SO4 (*Vitis vinifera* L.), planted in 2007 and located in Fuente Alamo (Albacete, Spain), and pepper plant pruning waste (PP), collected from a commercial greenhouse of bell pepper (*Capsicum annuum* L.) located in Almeria (Spain). The agri-food sludge (SSA) was obtained from a treatment plant of the “Sociedad de Depuracion Virgen de los Dolores S.L.” (Murcia, Spain), which mainly treats wastewater derived from the processing of artichokes and peppers, by means of sequential biological reactors (SBR). The SSA showed some characteristics similar to those of municipal sewage sludge (Singh and Agrawal, 2008), such as high salinity (EC = 15.1 dS/m), slightly alkaline pH (pH = 7.6); notable concentrations of total organic C (30.3%) and total organic matter (OM = 53.4%); a high content of total nitrogen (3.82%); a low carbon to nitrogen ratio (C/N = 7.93) and high contents of phosphorus and potassium (%P = 0.86 and %K = 0.24). However, the SSA also showed low concentrations of heavy metals (Cu: 59.0 mg/kg; Cr: 54.6 mg/kg; Cd: 0.18 mg/kg; Ni: 20.8 mg/kg; Pb: 8.72 mg/kg; Zn: 155 mg/kg; Hg: 0.03 mg/kg), compared to municipal sewage sludge, mainly due to the different characteristics of the type and origin of the wastewater to be purified (Banegas et al., 2007). The bulking agents had the following characteristics: pH 5.56, EC 2.0 dS/m, total organic carbon 48.0%, OM 92.1%, total nitrogen 0.63%, 0.11% P and 0.53% K for VP; pH 9.10, EC 12.9 dS/m, total organic carbon 39.0%, OM 61.0%, total nitrogen 1.91%, 0.35% P and 1.70% K for PP.

The composting mixtures were prepared using the maximum-possible quantity of SSA (between 50 and 60% on a fresh weight basis), while maintaining an initial C/N ratio



appropriate for a correct composting process, in the range of 18-24 (Bernal et al., 2009), and a suitable moisture content (60%) (Lim et al., 2016). A suitable moisture content optimises the microbial activity, leads to a rise in temperature and curtails the time required for compost maturation (Iqbal et al., 2015). Table 1 shows the percentages of the different ingredients present in the composting mixtures, as well as their main chemical properties. Almond shell powder was added to piles 3 and 4 to raise the C/N ratio, while maintaining SSA as the main component, since the PP waste had a high nitrogen content.

The mixtures produced were placed in 350-L thermo-composters made of high-density polyethylene (HDPE) and with a lateral system of natural ventilation to guarantee aerobic conditions; in each thermo-composter were installed probes for automatic monitoring of the temperature. The piles were turned when the temperature in the mixtures decreased, to provide aeration and to improve the homogeneity of the materials, thus favouring the composting process. Each pile was turned 2-3 times depending on the development of the temperature; piles 1 and 2 were turned twice (at 10 and 31 days) and piles 3 and 4 were turned three times (at 5, 14 and 33 days). The moisture content was maintained within the range 40-70%, as determined in samples collected periodically. According to previous composting studies, very-low moisture values ( $< 40\%$ ) can cause early dehydration within the pile and inhibit the microbial activity (Bertoldi et al., 1983), while high moisture contents ( $> 70\%$ ) may produce anaerobic conditions due to waterlogging (Tiquia et al., 1998). The bio-oxidative phase of composting was considered finished when the temperature was close to the ambient and re-heating after turning did not occur. Then, the composts were left to mature over a period of one month, approximately.

Composting samples were collected at the beginning of the process, during the thermophilic phase, at the end of the bio-oxidative phase and at the end of maturation. Samples were taken manually at three sites of the pile, from the whole profile (top to bottom). Composite, representative samples were obtained after mixing and homogenising thoroughly the three sub-samples. Each representative sample was divided into three fractions in the laboratory: one was dried in a drying-oven at 105 °C for 24 h to determine the moisture content, the second was immediately kept in refrigeration (4 °C) for determination of the microbial groups and the third was air-dried and ground to less than 0.5 mm for the rest of the analytical determinations.

## *2.2 Chemical determinations in the compost samples*

In the raw materials and composting samples, pH and electrical conductivity (EC) were determined in a 1:10 (w/v) aqueous extract; the moisture was measured in accordance with the standard method CEN13039 (European Committee for Standardization, 1999). The OM was assessed by determining the loss-on ignition at 550 °C for 24 h, in accordance with the standard method CEN13039. Total nitrogen (TN) and total organic carbon (TOC) were determined by dry combustion at 950 °C using an elemental analyser (Truspec CN, Leco, St. Joseph, Mich., USA). Phosphorus (P) was assessed colorimetrically as molybdovanadate phosphoric acid, and potassium (K) by flame photometry (Jenway PFP7 Flame Photometer, Jenway Ltd., Felsted, Dunmow, Essex, UK). Heavy metals (Cu, Zn, Cd, Cr, Pb, Ni, Hg) were analysed, after digestion with a nitric acid-perchloric acid mixture (HNO<sub>3</sub>/HClO<sub>4</sub>), by inductively coupled plasma-mass spectrophotometry (ICP-MS PQ-ExCell, VG-Thermo Elemental, Winsford, Cheshire, UK). Water-soluble organic carbon (WSC) was extracted with deionised water (1:20, w/v) and measured in an automatic carbon analyser (TOC-VCSN, Shimadzu

Corporation, Kyoto, Japan). Losses of OM due to OM mineralisation were calculated from the initial ( $X_1$ ) and final ( $X_2$ ) ash contents according to the equation (1) of Paredes et al. (2000):

$$(1) \text{ OM loss (\%)} = 100 - 100 \frac{[X_1 (100 - X_2)]}{[X_2 (100 - X_1)]}$$

In addition, in the final composts, the contents of Na, Ca, Mg, Fe, Mn and B were determined by ICP-MS, after  $\text{HNO}_3/\text{HClO}_4$  digestion. The presence of pesticides in the raw materials was determined using an Agilent Technology 6890 gas chromatograph (AT, Palo Alto, CA, USA) equipped with an HP5973 mass selective detector. All the analyses were made in triplicate. Finally, the phytotoxicity of the compost was evaluated as the germination index, which was determined using seeds of *Lepidium sativum* L. (Zucconi et al., 1981). The physical properties, including total pore space, bulk density, shrinkage, air capacity and total water holding capacity, were determined in the mature composts, according to the methods used by Bustamante et al. (2008b).

### 2.3 Microbial determinations and analysis of enzymatic activities

The microbiological groups studied were *Salmonella* (pre-enrichment in buffered peptone water, 24 h at 37 °C, followed by Salmonella Xpress warm incubation, 24 h at 41.5 °C, VIDAS test), *Listeria monocytogenes* (Fraser warm incubation, 24 h at 30 °C, VIDAS test), faecal Coliforms (*Escherichia coli* (*E. coli*)) (TBX incubation, 24 h at 44 °C), faecal Streptococcus (KAA incubation, 24-48 h at 37 °C) and *Clostridium perfringens* (TSC incubation, 24 h at 44 °C). The results for *E. coli*, faecal coliforms and *C. perfringens* were expressed as the number of colony-forming units per gram of fresh compost (CFU/g compost) and for *Salmonella* and *Listeria* as presence or absence.

The  $\beta$ -glucosidase activity was determined according to the method of Tabatabai (1982). This method is based on the colorimetric estimation of the p-nitrophenol (PNP)

released by the hydrolysis of p-nitrophenyl-b-D-glucopyranoside (PNG) at 37 °C for 1 h. The dehydrogenase activity was determined according to the method of Trevors et al. (1982), as modified by Garcia et al. (1993). This method is based on the measurement of the iodonitrotetrazolium formazan (INTF) formed when the material is incubated with 2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride (INT) for 20 h at 20 °C, in darkness, since the latter acts as an electron acceptor in the absence of a buffer, being reduced to its corresponding formazan salt, which is red in colour.

#### 2.4. Fluorescence analysis

For the fluorescence analysis, compost samples were dried at 65 °C. One gram of each sample was extracted with 20 ml of deionised water by shaking for 24 h at room temperature. The extracts were filtered through Whatman No. 2 paper. Then, 1 ml of each sample was centrifuged at 15000 rpm for 10 min in a SIGMA 2K15 centrifuge (rotor Nr. 12143, Osterode, Germany). Fluorescence spectra were obtained with an FP-6500 spectrofluorometer (JASCO, Japan). The excitation source was a 150-W xenon lamp. Contour maps of EEM spectra were obtained for water extracts of the composts. One hundred microlitres of the sample, filtered and centrifuged, were diluted in 2 ml of 20 mM phosphate buffer pH 8.0 (Marhuenda-Egea et al., 2007). The emission (Em) wavelength range was fixed from 300 to 550 nm in 2 nm steps, whereas the excitation (Ex) wavelength was increased from 220 to 400 nm in 5-nm steps. The slit widths were 5 nm and the compost extract was irradiated in a 1-cm-path-length, fused silica cell (Hellma). The UV–visible spectra of the diluted samples were acquired (Shimadzu UV-160 spectrophotometer, 200–800 nm, 1-cm quartz cuvette, Japan) and the absorbance was always lower than 0.1 at 254 nm, to reduce the absorbance of the solution and eliminate potential inner filter effects (Mobed et al., 1996). The fluorescence intensity

was normalised to a quinine sulphate standard and expressed as quinine sulphate equivalent (QSE) in parts per billion (ppb) (Coble et al., 1993). The 45° ridge line of high fluorescence intensities in the upper left corner of the EEMs results from first-order Rayleigh scattering of the incident light from the excitation grating. Similarly, the 22.5° ridge line of high intensity on the right side is associated with second-order Rayleigh light scattering, where excited light is emitted at an emission wavelength twice that of the excitation wavelength. A new “excision, interpolation” technique was used to eliminate Rayleigh and Raman scattering peaks (Zepp et al., 2004). Windows-based software, Sigma-Plot 2001 (SPSS Inc.), was used to plot the contour maps. The increment of the contour lines was 5. In order to quantify the relative changes in the EEM spectra and for comparison of spectra, the percent fluorescence response ( $P_{i,n}$ ) was calculated, using the integration method proposed by Chen et al. (2003).

### 2.5 Thermal analysis

To carry out the thermogravimetric determinations, the compost samples were dried in an air stream, ground in an agate mill and sieved through a 0-125  $\mu$ m mesh. Thermal analyses were performed with a TGA/SDTA851e/LF/1600 instrument (Mettler Toledo, Barcelona, Spain). All the samples were combusted with a mixing stream of oxygen/helium (20/80%), a gas flow of 100 ml min<sup>-1</sup> within a temperature range of 25 to 800 °C, a heating rate of 20 °C min<sup>-1</sup> and a sample weight of about 15 mg. The parameter R1, an important parameter obtained with this technique, was calculated considering the overall loss of mass due to the loss of aliphatic materials (at 430 to 600 °C) and carbohydrate molecules (at 200 to 430 °C) during the combustion process.

### 2.6 In vivo assay of the potential suppressive capacity of the composts obtained

Different growing media were elaborated using 1:1 (w/w) mixtures of the respective composts obtained (C1, C2, C3 and C4) and black peat (T-C1, T-C2, T-C3 and T-C4), for the cultivation of melon (*Cucumis melo* L. cv. Giotto) seedlings. Pure black peat was used as control (Peat). Seeds of melon were sown in the different growing media tested as treatments, with a covering of vermiculite, in polystyrene trays, one seed per well (5 cm in diameter). The seeds were left to germinate in a growth chamber at  $28 \pm 1$  °C and 90-95% relative humidity. After germination, the seedlings were transferred to the same growing medium, in separate trays (three replicates per treatment). Then, the trays were distributed randomly on rails in a polyethylene-covered greenhouse with natural daylight conditions. Once the first true leaf had appeared (15 days after sowing), six replicates (10 plants each) were inoculated with 2 ml of a suspension of conidia of the pathogen *Fusarium oxysporum* f. sp. *melonis* (FOM), to obtain a final concentration of  $3.5 \cdot 10^5$  CFU g<sup>-1</sup> of FOM. In addition, as a control treatment and to evaluate any possible effect on the substrate, another 10 plants (for each of the 6 replicates for each growing medium) were watered with 2 ml of deionised water.

The pathogen FOM was isolated from infected muskmelon plants from a greenhouse nursery. Conidia were recovered as described by Blaya et al. (2013). The trays were moistened periodically by manual watering, following the normal nursery routine. Finally, the seedlings were harvested 40 days after being transplanted, for measurement of the fresh weight of their aerial parts. Also, the seedlings inoculated with FOM were weighed and the suppressive effect of the composts was evaluated. The effect of *F. oxysporum* was estimated as the percentage fresh weight loss of the melon plants grown on a medium (treatment) infected with *F. oxysporum* with respect to the same medium without inoculation of the phytopathogen (López-Mondejar et al., 2012).

## 2.7 Statistical analysis

The data for the loss of OM during the composting process were fitted to a kinetic function by the Marquardt-Levenberg algorithm, using the Sigmaplot 11.0 computer program. A first-order kinetic model (2) was used for OM degradation during composting (Bernal et al., 1996):

$$(2) \text{ OM losses (\%)} = A (1 - e^{-kt})$$

A is the maximum degradation of OM (% C),  $k$  the rate constant ( $d^{-1}$ ) and  $t$  the composting time (days). The root mean square (RMS) and significance values (F-values) were calculated to compare the fittings of different functions and the statistical significance of curve fitting.

One-way analysis of variance (ANOVA) and the least significant difference (LSD) test at  $P < 0.05$  were used to assess the significance of differences among the values of each parameter studied during composting. The Tukey test was used to test the statistical significance of differences among the composts with respect to the seedling growth assay in trays. All statistical tests were conducted using the SPSS 22.0 software package.

## 3. Results and discussion

The development of the composting process can be assessed using different parameters, the temperature profile and the evolution of the OM fraction being two of the main aspects that indicate the correct progress of the process (Bustamante et al., 2008a; Bernal et al., 2009).

### 3.1 Evolution of the temperature during the composting process

According to previous studies, temperature is one of the main controlling factors in the composting process (Banegas et al., 2007). High temperatures are essential for the

destruction of pathogenic organisms, and decomposition is more rapid in the thermophilic temperature range (Chen et al., 2014). The evolution of the temperature was similar in all the piles (Fig. 1). The thermophilic phase (above 40 °C) was reached in the first five days in all piles, reflecting the rapid initiation of the process, and was maintained for approximately two weeks. This behaviour was also observed in studies of the co-composting of sewage sludge using different bulking agents (Doublet et al., 2011; Himanen and Hänninen, 2011). Piles 1 and 4 showed the highest temperature values (> 70 °C), while pile 3 displayed thermophilic conditions during a longer period of time than the other piles, which reflects the effect of the bulking agent used and its particle size in the mixture. This effect was also observed by Bustamante et al. (2013) during the co-composting of the solid fraction of pig slurry anaerobic digestate with different bulking agents, for which the mixture with vine shoot prunings also showed the longest thermophilic phase. On the other hand, pile 2, elaborated using VP (particle size < 3 cm), showed the lowest temperature values, probably due to the higher influence of the particle size in this material, with an important lignocellulosic nature, which could have inhibited the microbial attack, slowing down the degradation of the mixture during composting (Bustamante et al., 2012).

In general, after the first turnings, a rise in the temperature values was observed in all the piles, as reported also by Bustamante et al. (2013). In general, after the last turning, the temperature started to decrease, approaching the ambient temperature, probably due to the depletion of readily-biodegradable components (Varma and Kalamdhad, 2015).

### *3.2 Evolution of the organic matter fraction and study of the compost quality using classical analytical determinations*



The initial concentration of OM in piles 1 and 2 was higher than in piles 3 and 4 (Table 2) due to the greater average OM content of VP (92.1%) compared to PP (61.0%). The OM contents decreased throughout the composting process in all the mixtures, due to the mineralisation processes (Banegas et al., 2007), the percentages of decrease from the initial values being 10.74%, 16.94%, 13.22% and 18.65% in piles 1-4, respectively. These values are similar to or higher than those reported by different authors. Himanen and Hänninen (2011) observed a decrease in the OM of 8% during composting of aerobic sludge; Banegas et al. (2007) observed a decrease of 12.7% during composting of sewage sludge with sawdust; and Molla et al. (2004) found a decrease of 13.78% when they composted sewage sludge with rice straw as bulking agent.

The OM losses in all piles were significant during the bio-oxidative phase of composting (Fig. 2), corresponding to the maximum microbial activity; the pile with the smallest particle size of PP (pile 3) showed the lowest mineralisation rate in the early stage. The OM degradation profile during composting, according to the OM losses, followed a first-order kinetic equation in all piles. Curve fitting of the experimental data gave the following parameter values, all the equations being significant at  $P < 0.001$ :

Pile 1:  $A = 33.41$  (1.90),  $k = 0.2002$  (0.0543),  $RMS = 11.97$ ,  $F = 75.23$ ,  $SEE = 3.46$ ;

Pile 2:  $A = 55.52$  (4.31),  $k = 0.0676$  (0.014),  $RMS = 22.69$ ,  $F = 102.40$ ,  $SEE = 4.76$ ;

Pile 3:  $A = 31.76$  (3.73),  $k = 0.0278$  (0.0069),  $RMS = 3.62$ ,  $F = 191.70$ ,  $SEE = 1.90$ ;

Pile 4:  $A = 35.44$  (2.28),  $k = 0.0709$  (0.0168),  $RMS = 11.14$ ,  $F = 88.21$ ,  $SEE = 3.34$ ,

where RMS is the residual mean square and SEE is the standard error of the estimate.

The  $A$  and  $k$  values obtained were close to the ranges found by Banegas et al. (2007), during co-composting of aerobic and anaerobic sewage sludge with sawdust, and differed from those obtained in composting experiments using other organic wastes (Bernal et al., 1996; Bustamante et al., 2008a, 2012). Pile 1 showed a higher rate

constant ( $k$ ), indicating that the degradation of the mixture was faster than in the other piles, probably due to the higher proportion of SSA in this mixture. A great proportion of the decrease in OM can be attributed to the mineralisation of the OM fraction of SSA, since the OM of the bulking agents is a more-structured fraction less susceptible to microorganism attack (Banegas et al., 2007; Bustamante et al., 2012).

The C/N ratio has been widely mentioned as an index of compost stability and maturity (Chowdhury et al., 2014; Li et al., 2013), with values below 20 being suggested as indicative of mature compost (Bernal et al., 2009). The C/N ratios of this study are presented in Table 3. The values of this parameter decreased with time in all the piles and all the mature composts showed values between 10 and 16, indicating an acceptable degree of maturation. According to Lim et al. (2014), a final C/N ratio of approximately 10-15 is often taken as an indication of humic material formation and the enhanced stability of treated organic wastes. Piles 3 and 4 showed, from the beginning, C/N ratios below 20, which demonstrates, as underlined in numerous previous studies, that this parameter should be used not as an absolute value with regard to the state of maturity (Bustamante et al., 2013) but rather as a parameter for monitoring the process, since it shows a decline in all cases (Bustamante et al., 2008a). The water-soluble C (WSC) decreased throughout the composting process, principally during the bio-oxidative phase (Table 2), as a consequence of the degradation of simple, water-soluble organic compounds. The WSC values of the mixtures after composting ranged from 0.58 to 0.83%, being similar to the values reported in other co-composting assays (Banegas et al., 2007; Bustamante et al., 2012, 2013) and close to the limiting values suggested by different authors in order to consider a compost sufficiently mature: <1% or <1.7% (Bernal et al., 2009).

The OM concentration, the C/N ratio and the WSC declined in all the piles during composting, whereas the N concentration increased (Table 2) due to a concentration effect caused by the strong degradation of the labile organic-C compounds, which reduced the weight of the composting mass. Although the N concentration can also decrease, due to ammonia volatilisation and/or nitrate leaching, the loss of OM was greater than that of ammonia. At the end of the composting process, the increase in the N concentration was greatest in piles 1 and 2, probably because these piles showed less volatilisation than piles 3 and 4, since the temperature was lower (Ekling et al., 2007), although another aspect to consider is the bulking agent used (Bustamante et al., 2013). This increase in the N contents was more notable in the bio-oxidative phase, similar to the results reported by Bustamante et al. (2012) during co-composting of the solid fraction of anaerobic digestates with vine shoot prunings. At the end of the composting process, the piles had similar levels of N (2.3-2.4 %).

The germination index (GI) was another parameter evaluated to determine the maturity of the composts obtained (Zucconi et al., 1981). All the composts exhibited values above 60% (Table 3); so, they can be considered mature and free of phytotoxicity. Also, during composting, the initial materials are degraded through a variety of biological and biochemical processes in which enzymes play an essential role; hence, the activities of dehydrogenase and  $\beta$ -glucosidase have been used widely as indicators of compost maturity (Tiquia, 2005). In this study, dehydrogenase activities of 69.84, 77.37, 111.04 and 108.69  $\mu\text{mol INTF g}^{-1} \text{ h}^{-1}$  and  $\beta$ -glucosidase activities of 16.09, 12.85, 46.22 and 48.96  $\mu\text{g PNF g}^{-1} \text{ h}^{-1}$  were found for composts 1, 2, 3 and 4, respectively, the lowest values occurring in the composts derived from vine shoot prunings (1 and 2). This demonstrates the greater degree of stabilisation of these composts, in comparison with those elaborated using the remains of pepper plants, owing to their greater

biodegradability and hence more-rapid stabilisation. Both of the enzymatic activities were relatively low when compared with previous work (Tiquia, 2005; Barrena et al., 2008), in which it was found that these activities decreased during composting due to the loss of easily-biodegradable substrates.

### *3.3 Evolution of the organic matter fraction and study of the compost quality using thermal and spectroscopic approaches*

The degree of maturity was also measured by thermogravimetry (TG), differential thermogravimetry (DTG) and differential thermal analysis (DTA), which have been reported as useful tools for the structural and chemical assessment of natural OM (Smidt and Lechner, 2005, Marhuenda-Egea et al., 2007). These thermal methods are based on programmed heating of the samples in a controlled atmosphere. Different components in the sample, which undergo transformations at different temperatures, produce a graph whose shape reflects the chemical composition and structure of the sample. These thermal degradation techniques have been used to assess compost stability and maturity (Otero et al., 2002; Mondini et al., 2003). Thermal analysis has the advantage that it is simple, fast and reproducible and can be performed on the whole sample without requiring pre-treatments (Marhuenda-Egea et al., 2007).

The profiles of TG, DTG and DTA indicate important characteristics of the OM. The profiles of the four piles were similar (Fig. 3), but significant differences were found between the initial and final compost samples. The loss of mass before 100 °C is mainly due to the loss of the residual water in the samples (Lim and Wu, 2015). The loss of mass at other temperatures corresponds to different types of compound. Carbohydrates, such as cellulose, are degraded between 200 and 400 °C; the piles were rich in these compounds, as was expected due to their plant origin. Between 400 and 600 °C, the loss

of mass corresponds to aliphatic material. In all the piles, the R1 ratio increased during composting, revealing a high sensitivity of this parameter to the chemical changes induced by the bio-transformation of organic materials (Torres-Climent et al., 2015). The R1 values for the initial samples of piles 1, 2, 3 and 4 were 0.158, 0.156, 0.262 and 0.224, respectively. For the final samples, the R1 values for piles 1, 2, 3 and 4 were 0.177, 0.180, 0.342 and 0.358, respectively. This indicates transformation of the OM, corresponding to a loss of the more-degradable material and an increase in the aliphatic compounds, and thus an increase in R1, giving a final material that was mature and stable.

In the DTA profiles, the percentage of the energy released is shown in relation to the temperature (Fig. 4), thus showing the energy that would be available to the microorganisms during the composting process (Otero et al., 2002). The first exothermic peak, in the range 200-430 °C, was characteristic of the cellulosic component, and the second exothermic peak, in the range 430-600 °C, was characteristic of more-recalcitrant molecules. Thus, the energy released below 500 °C in piles 1 and 2, elaborated with VP, was around 96% at both the beginning and end of the composting process, while for piles 3 and 4, elaborated with PP, the initial values were around 97%, but these decreased to 88%. The results show that VP contained more energy available to microorganisms than did PP waste, thus explaining the greater biodegradability of VP.

Contour EEM fluorescence spectra of water-soluble organic matter (WSOM) from the compost piles at the different stages of the composting process are shown in Fig. 5. The spectra indicate the presence of different fluorophores, each characterised by an Ex/Em wavelength pair. These fluorophores contributed to the final contour EEM spectrum. Parallel Factor Analysis (PARAFAC) was performed in order to detect the fluorophores

or components. These components can be associated with different biomolecules, such as humic and fulvic substances or peptides (Ohno and Bro, 2006). For the WSOM from the composting samples, PARAFAC models with three components were calculated. The excitation and emission spectral loadings for the three components are displayed in Figure 5. The first and second PARAFAC components were associated with humic ( $\lambda_{\text{ex}}$  ~260 nm and ~355 nm,  $\lambda_{\text{em}}$  ~448 nm) and fulvic ( $\lambda_{\text{ex}}$  ~240 nm and ~320 nm,  $\lambda_{\text{em}}$  ~398 nm) substances (Leenheer and Croué, 2003; Sierra et al., 2005; Ohno and Bro, 2006). The third PARAFAC component was associated with peptides containing residues of tryptophan and tyrosine ( $\lambda_{\text{ex}}$  ~225 nm and ~280 nm,  $\lambda_{\text{em}}$  ~360 nm) (Leenheer and Croué, 2003; Yamashita and Tanoue, 2003). The intensity of the fluorescence peaks decreased during composting for all piles. This could indicate a decrease in the WSOM, the fraction of the OM that is most available to the microorganisms.

### *3.3 Properties and potential added value of the composts obtained*

Compost quality can be evaluated with a variety of techniques, at a chemical and/or biological level, to study the two main aspects considered prior to agricultural use: its stability and maturity (Bustamante et al., 2008a). In addition, several agricultural sectors, such as the soilless crop production sector, demand composts with added-value properties that justify the associated production cost of the compost and also satisfy the necessities of these specific agricultural activities, such as the suppressive capacity against phytopathogenic microorganisms (Bustamante et al., 2012). For this, different methods (analytical, instrumental and biological) have been used to evaluate the properties of the composts obtained, as well as their potential added value.

#### *3.3.1 Physico-chemical, chemical and microbiological properties*

The main physico-chemical, chemical and microbiological properties of the final composts are shown in Table 3. At the end of the composting process, composts 1 and 2 showed final values of pH close to neutrality and within the range (6.0-8.5) suggested for the agricultural use of compost (Hogg et al., 2002), but higher than those established (5.2–6.3) for their use as growing media (Bustamante et al., 2008b). In addition, the EC values of composts 1 and 2 were lower than those of composts 3 and 4, the values of the latter two exceeding  $3.5 \text{ dS m}^{-1}$ , the limit indicated by Lemaire et al. (1985) for vigorous seedling growth in a growing medium. This result is due to the characteristics of PP, with a higher salt content than VP ( $12.9 \text{ dS m}^{-1}$  for PP and  $2.0 \text{ dS m}^{-1}$  for VP), as was also observed in a previous experiment using both materials as bulking agents (Bustamante et al., 2013). The compost pH and EC are parameters of great importance because they affect the availability of nutrients, the germination of seeds and the growth of the plants. Moreover, the EC reflects the salinity of an organic amendment: a high salt concentration may cause phytotoxicity (Shak et al., 2014).

Apart from N, potassium (K) and phosphorus (P) in fertilisers are widely studied because K improves the water use efficiency of plants and enhances crop growth, while P determines the agronomic potential for crop production (Lim et al., 2015b). The concentrations of the macronutrients (NPK) were high and the differing particle sizes did not produce significant differences. These values are close to those reported by other authors: slightly higher than those obtained in co-composting experiments with municipal sewage sludge (Doublet et al., 2011; Himanen and Hänninen, 2011) and lower than those of other studies of the co-composting of other organic wastes (Bustamante et al., 2008a, 2012, 2013). The micronutrient concentrations varied among the four composts, the concentrations of calcium and iron being highest in the piles prepared using VP as bulking agent (probably due to the higher proportion of SSA used

in these mixtures), which could be significant in the biological control of some plant diseases.

The concentrations of heavy metals were much lower than those found previously in municipal sewage sludge, principally due to the fact that the latter arises from the treatment of heavily-polluted flows from industry (Smith, 2009). As has been mentioned previously, the raw materials used in these composts are of vegetable origin, intended for human consumption; thus, their heavy metal levels are minimal. The concentrations in the four composts were below the limits established for compost by the Spanish legislation and the European guidelines (BOE, 2013; European Commission, 2001).

In addition, according to this legislation and the European guidelines, the levels of human pathogenic microorganisms in the composts were below the maximum limit for the use of composts as fertilisers. Table 3 shows the absence of *Salmonella* and concentrations of *E. coli* below 1000 CFU g<sup>-1</sup>. The levels of other pathogens, such as *Listeria monocytogenes*, faecal *Streptococci* or *Clostridium*, indicate the quality of these products (Banegas et al., 2007; Bustamante et al., 2008c). The composts showed an absence of *Listeria monocytogenes* and the faecal *Streptococci* were below the limit of 5000 CFU g<sup>-1</sup> (fresh weight) recommended by Strauch (1987) for compost sanitation. The presence of *Clostridium* in piles 1 and 3, whereas it was below the detection limit in piles 2 and 4, can be explained by the use in these piles of a bulking agent of lesser diameter, which probably led to the existence of anaerobic microniches that allowed the bacteria to survive, in spite of the turnings of the piles (Pourcher et al., 2005). Another potential negative aspect is the presence of the remains of chemical pesticides since both the sludge and (of greater importance) the bulking agents are of agricultural origin



and had been treated with chemical pesticides. However, pesticides were not detected in the sludge, bulking agents or composts (data not shown).

### 3.3.2 *Physical properties*

Table 4 shows the main physical properties of the composts obtained, in comparison to the values recommended for an ideal substrate for the cultivation of plants in pots (Abad et al., 2001). In general, all the composts displayed values of the physical properties within the range of reference values for an “ideal” substrate (Abad et al., 2001), except for the total water holding capacity (TWHC) in composts 1 and 2. In addition, these two composts also showed the highest values of air capacity, favouring their use as substrates in spite of their lower water retention capacities (Bustamante et al., 2012); the latter can be overcome by using more-frequent irrigation with lesser quantities of water. In relation to this, composts 1 and 2 also showed the highest values of total pore space, indicating the presence in the final compost of particles of greater size - which result in higher porosity and, therefore, higher aeration capacity. Bustamante et al. (2008b) reported, in a study of the use of winery-distillery composts as growing media components, that higher values of air capacity could be due to a greater proportion of particles of size >1 mm.

### 3.3.3 *Potential capacity for suppression of F. oxysporum: in vivo assay*

An *in vivo* assay was performed with melon plants to study the potential suppressive capacity of the composts obtained. For this, the composts were used as growing media, with peat as the control, for infected and non-infected plants. Forty days after transplanting, the fresh weight of the aerial parts (shoots) of the non-infected plants grown with compost was similar to or greater than the values obtained for the plants

grown in peat (Figure 7A). Composts 1 and 2 produced a greater fresh weight than composts 3 and 4, possibly due to the negative effect of the EC of the latter two (Lemaire et al., 1985).

Figure 7B shows the fresh weight of the aerial parts of the melon plants in the presence of the pathogen *Fusarium oxysporum* f. sp. *melonis* (FOM). These results were used to evaluate the potential added value of the composts, concerning their suppressive effect. The growing media (treatments) prepared with composts 1 and 2 produced a lesser reduction of fresh weight than the peat control, indicating their suppressive effect. This did not happen with composts 3 and 4, which showed a decrease in the fresh weight similar to that observed with peat. This capacity for suppression of FOM was also reported by Bustamante et al. (2012) in an *in vitro* assay using composts elaborated using vine shoot prunings as bulking agent. Therefore, the importance of the co-composting agent is clear, not only in the composting process, but also for the potential added value of the compost obtained, which in this case is related to its suppressive effect.

#### 4. Conclusions

The results of this study have provided more information concerning the best strategy for the management by composting of the sludge generated by the vegetable-processing sector. They show that co-composting of this waste using bulking agents, especially vine shoot pruning waste, constitutes a feasible and sustainable strategy for its management, thus avoiding the current environmental and economic costs associated with its disposal. Therefore, this study has confirmed the importance of the bulking agent, not only for the development of the composting process, but also for the final properties and potential added value of the end-products obtained. Regarding the latter

aspect, a soilless organic substrate with suppressive capacity against the phytopathogen *Fusarium oxysporum* f. sp. *melonis* was obtained by using vine prunings as bulking agent instead of pepper pruning waste. Also, the applicability of advanced instrumental techniques, such as thermogravimetry and EEM fluorescence spectroscopy, to assess the rate of OM stabilisation and the recalcitrant C in compost samples has been demonstrated. This will help to predict the agronomic behaviour of compost. Industrial scaling-up of the best mixtures identified here should be carried out, to verify the added value of the end-products obtained.

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## 6. References

- Abad, M., Noguera, P., Burés, S., 2001. National inventory of organic wastes for use as growing media for ornamental potted plant production: case study in Spain. *Bioresour. Technol.* 77, 197-200.
- AGROWASTE, 2014. Results of the Project AGROWASTE LIFE10/ENV/ES/469. Available in: [www.agrowaste.eu](http://www.agrowaste.eu)
- Banegas, V., Moreno, J.L., Moreno, J.I., García, C., León, G., Hernández, T., 2007. Composting anaerobic and aerobic sewage sludges using two proportions of sawdust. *Waste Manage.* 27, 1317-1327.

- Barrena, R., Vázquez, F., Sánchez, A., 2008. Dehydrogenase activity as a method for monitoring the composting process. *Bioresour. Technol.* 99, 905-908.
- Bernal, M.P., Albuquerque, J.A., Moral, R., 2009. Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour. Technol.* 100, 5444–5453.
- Bernal, M.P., Navarro, A.F., Roig, A., Cegarra, J., García, D., 1996. Carbon and nitrogen transformation during composting of sweet sorghum bagasse. *Biol. Fertil. Soils* 22, 141-148.
- Bertoldi, M. de, Vallini, G., Pera, A., 1983. The biology of composting. *Waste Manage. Res.* 1: 157-176.
- Blaya, J., López-Mondejar, R., Lloret, E., Pascual, J.A., Ros, M., 2013. Changes induced by *Trichoderma harzianum* in suppressive compost controlling *Fusarium* wilt. *Pest. Biochem. Physiol.* 107, 112-119.
- BOE, 2011. Ley 22/2011, de 28 de julio, de residuos y suelos contaminados. *Boletín Oficial del Estado* 181, 85650-85705.
- BOE, 2013. Real Decreto 506/2013 sobre productos fertilizantes. *Boletín Oficial del Estado* 164, 51119-51207.
- Bonanomi, G., Antignani, V., Capodilupo, M., Scala, F., 2010. Identifying the characteristics of organic soil amendments that suppress soil-borne plant diseases. *Soil Biol. Biochem.* 42, 136–144.
- Bustamante, M.A., Albuquerque, J.A., Restrepo, A.P., de la Fuente, C., Paredes, C., Moral, R., Bernal, M.P., 2012. Co-composting of the solid fraction of anaerobic digestates, to obtain added-value materials for use in agriculture. *Biomass Bioenergy* 43, 26-35.

- Bustamante, M.A., Moral, R., Paredes, C., Vargas-García, M.C., Suárez-Estrella, F., Moreno, J., 2008c. Evolution of the pathogen content during co-composting of winery and distillery wastes. *Bioresour. Technol.* 99, 7299–7306.
- Bustamante, M.A., Paredes, C., Marhuenda-Egea, F.C., Pérez-Espinosa, A., Bernal, M.P., Moral, R., 2008a. Co-composting of distillery wastes with animal manures: Carbon and nitrogen transformations in the evaluation of compost stability. *Chemosphere* 72, 551-557.
- Bustamante, M.A., Paredes, C., Moral, R., Agullo, E., Perez-Murcia, M.D., Abad, M., 2008b. Composts from distillery wastes as peat substitutes for transplant production, *Resour. Conserv. Recycl.* 52, 792–799.
- Bustamante, M.A., Restrepo, A.P., Albuquerque, J.A., Pérez-Murcia, M.D., Paredes, C., Moral, R., Bernal, M.P., 2013. Recycling of anaerobic digestates by composting: effect of the bulking agent used. *J. Clean. Prod.* 47, 61-69.
- Ceglie, F.G., Bustamante, M.A., Ben Amara, M., Tittarelli, F., 2015. The challenge of peat substitution in organic seedling production: optimization of growing media formulation through mixture design and response surface analysis. *Plos One* 10(6):e0128600.
- Chen, Y., Yu, F., Liang, S., Wang, Z., Liu, Z., Xiong Y., 2014. Utilization of solar energy in sewage sludge composting: Fertilizer effect and application. *Waste Manage.* 34, 2014–2021.
- Chen, Y.N., 2003. Nuclear magnetic resonance, infra-red and pyrolysis: application of spectroscopic methodologies to maturity determination of composts. *Compos. Sci. Util.* 11, 152-168.

- Chowdhury, A.K.M.M.B., Michailides, M.K., Akratos, C.S., Tekerlekopoulou, A.G., Pavlou, S., Vayenas, D.V., 2014. Composting of three phase olive mill solid waste using different bulking agents. *Int. Biodeterior. Biodegrad.* 91, 66-73.
- Christensen, A., Gurol, M.D., Garoma, T., 2009. Treatment of persistent organic compounds by integrated advanced oxidation processes and sequential batch reactor. *Water Research* 43, 3910–3921.
- DOCE, 1991. COUNCIL DIRECTIVE of 21 May 1991 concerning urban waste water treatment (91/271/EEC). *Diario Oficial de la Unión Europea* 135, 40-52.
- Doublet, J., Francou, C., Poitrenaud, M., Houot, S., 2011. Influence of bulking agents on organic matter evolution during sewage sludge composting; consequences on compost organic matter stability and N availability. *Bioresour. Technol.* 102, 1298–1307.
- Eklind, Y., Sundberg, C., Smars, S., Steger, K., Sundh, I., Kirchmann, H., Jonsson, H., 2007. Carbon turnover and ammonia emissions during composting of biowaste at different temperatures. *J. Environ. Qual.* 36, 1512-1520.
- European Commission (EC), 2001. Working document on biological treatment of biowaste. 2<sup>nd</sup> draft. Brussels; DG ENV.A.2./LM/biowaste/2<sup>nd</sup> draft.
- European Committee for Standardization, 1999. Soil Improvers and Growing Media – Determination of Organic Matter and Ash. Standard CEN EN 13039:1999 E. European Committee for Standardization, Brussels.
- Garcia, C., Hernández, T., Costa, F., Ceccanti, C., Ganni, A., 1993. Hydrolases in the organic matter fractions of sewage sludge: changes with composting. *Bioresour. Technol.* 45, 47-52.

- Himanen, M., Hänninen, K., 2011. Composting of bio-waste, aerobic and anaerobic sludges – Effect of feedstock on the process and quality of compost. *Bioresour. Technol.* 102, 2842-2852.
- Hogg, D., Favoino, E., Centemero, M., Caimi, V., Amlinger, F., Devliegher, W., Brinton, W., Antler, S., 2002. Comparison of Compost Standards within the EU, North America and Australia. The Waste and Resources Programme (WRAP), Oxon.
- Ingelmo, F., Molina, M.J., Soriano, M.D., Gallardo, A., Lapeña, L., 2012. Influence of organic matter transformations on the bioavailability of heavy metals in a sludge based compost. *J. Environ. Manage.* 95, S104-S109.
- Iqbal, M.K., Nadeem, A., Sherazi, F., Khan, R.A., 2015. Optimization of process parameters for kitchen waste composting by response surface methodology. *Int. J. Environ. Sci. Technol.* 12 (5), 1759-1768.
- Leenheer, J.A., Croué, J.P., 2003. Characterizing dissolved aquatic organic matter. *Environ. Sci. Technol.* 37, 18A-25A.
- Lemaire, F., Dartigues, A., Rivière, L. M., 1985. Properties of substrate made with spent mushroom compost. *Acta Hort.* 172, 13-29.
- Li, X., Xing, M., Yang, J., Zhao, L., Dai, X., 2013. Organic matter humification in vermifiltration process for domestic sewage sludge treatment by excitation–emission matrix fluorescence and Fourier transform infrared spectroscopy. *J. Hazard. Mater.* 261, 491-499.
- Li, Z., Lu, H., Ren, L., He, L., 2013. Experimental and modelling approaches for food waste composting: A review. *Chemosphere* 93, 1247–1257.
- Lim, P.N., Wu, T.Y., Clarke, C., Daud, N.N.N., 2015b. A potential bioconversion of empty fruit bunches into organic fertilizer using *Eudrilus eugeniae*. *Int. J. Environ. Sci. Technol.* 12 (8), 2533-2544.

- Lim, S.L., Lee, L.H., Wu, T.Y., 2016. Sustainability of using composting and vermicomposting technologies for organic solid waste biotransformation: recent overview, greenhouse gases emissions and economic analysis. *J. Clean. Prod.* 111, 262-278.
- Lim, S.L., Wu, T.Y., 2015. Determination of maturity in the vermicompost produced from palm oil mill effluent using spectroscopy, structural characterization and thermogravimetric analysis. *Ecol. Eng.* 84, 515-519.
- Lim, S.L., Wu, T.Y., Clarke, C., 2014. Treatment and biotransformation of highly polluted agro-industrial wastewater from a palm oil mill into vermicompost using earthworms. *J. Agric. Food Chem.* 62 (3), 691-698.
- Lim, S.L., Wu, T.Y., Lim, P.N., Shak, K.P.Y., 2015a. The use of vermicompost in organic farming: Overview, effects on soil and economics. *J. Sci. Food Agric.* 95 (6), 1143-1156.
- López-Mondéjar, R., Blaya, J., Obiol, M., Ros, M., Pascual, J.A., 2012. Evaluation of the effect of chitin-rich residues on the chitinolytic activity of *Trichoderma harzianum*: *In vitro* and greenhouse nursery experiments. *Pest. Biochem. Physiol.* 103, 1-8.
- MAGRAMA, 2015. Informe Industria Alimentaria 2013-2014 del Ministerio de Agricultura, Alimentación y Medio Ambiente de España. Available in: <http://www.magrama.gob.es/es/alimentacion/temas/industria-agroalimentaria/informacion-economica-sobre-la-industria-agroalimentaria/>
- Marhuenda-Egea, F.C., Martínez-Sabater, E., Jordá, J., Sánchez-Sánchez, A., Moral, R., Bustamante, M.A., Paredes, C., Pérez-Murcia, M.D., 2007. Evaluation of the aerobic composting process of winery and distillery residues by thermal methods. *Thermochimica Acta* 454, 135-143.



- Mobed, J.J., Hemmingsen, S.L., Autry, J. L., McGown, L.B., 1996. Fluorescence characterization of IHSS humic substances: Total luminescence spectra with absorbance correction. *Environ. Sci. Technol.* 30, 3061-3065.
- Molla, A.H., Fakhru'l-Razi, A., Alam, Md.Z., 2004. Evaluation of solid state bioconversion of domestic wastewater sludge as a promising environmental-friendly disposal technique. *Water Res.* 38, 4143–4152.
- Mondini, C., Dell' Abate, M.T., Leita, L., Benedetti, A., 2003. An integrated chemical, thermal and microbiological approach to compost stability evaluation. *J. Environ. Qual.* 32, 2379–2386.
- Najafpour, G.D., Zinatizadeh, A.A.L., Lee, L.K., 2006. Performance of a three-stage aerobic RBC reactor in food canning wastewater treatment. *Biochem. Eng. J.* 30, 297-302.
- Ohno, T., Bro, R., 2006. Dissolved organic matter characterization using multiway spectral decomposition of fluorescence landscapes. *Soil Sci. Soc. Am. J.* 70, 2028-2037.
- Otero, M., Calvo, L.F., Estrada, B., García, A.I., Morán, A., 2002. Thermogravimetry as a technique for establishing the stabilization progress of sludge from wastewater treatments plants. *Thermochim. Acta* 389, 121–132.
- Paredes, C., Roig, A., Bernal, M.P., Sánchez-Monedero, M.A., Cegarra, J., 2000. Evolution of organic matter and nitrogen during co-composting of olive mill wastewater with solid organic wastes. *Biol. Fert. Soils* 32, 222-227.
- Pasda, N., Limtong, P., Oliver, R., Montange, D., Panichsakpatana, S., 2005. Influence of bulking agents and microbial activator on thermophilic aerobic transformation of sewage sludge. *Environ. Technol.* 26 (10), 1127-1136.
- Pourcher, A.M., Morand, P., Picard-Bonnaud, F., Billaudel, S., Monpoeho, S., Federighi, M., Ferré, V., Moguedet, G., 2005. Decrease of enteric microorganisms from

rural sewage sludge during their composting in straw mixture. *J. Appl. Microbiol.* 99, 528–539.

Shak, K.P.Y., Wu, T.Y., Lim, S.L., Lee, C.A., 2014. Sustainable reuse of rice residues as feedstocks in vermicomposting for organic fertilizer production. *Environ.Sci. Pollut. Res.* 21 (2), 1349-1359.

Sierra, M.M.B., Giovanela, M., Parlarite, E., Soriano-Sierra, E.J., 2005. Fluorescence fingerprint of fulvic and humic acids from varied origins as viewed by single-scan and excitation/emission matrix techniques. *Chemosphere* 58, 715-733.

Singh, R.P., Agrawal, M., 2008. Potential benefits and risks of land application of sewage sludge. *Waste Manage.* 28 (2), 347–358.

Smidt, E., Lechner, P., 2005. Study on the degradation and stabilization of organic matter in waste by means of thermal analyses. *Thermochim. Acta* 438/1-2, 22-28.

Smith S.R., 2009. A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. *Environ. Int.* 35, 142-156.

Strauch, D., 1987. Microbiological specifications of disinfected compost. In: de Bertoldi, M., Ferranti, M.P., L'Hermite, P., Zucconi, F. (Eds.), *Compost: Production, Quality and Use*. Elsevier, London, pp. 210– 229.

Tabatabai, M.A., 1982. Soil enzymes. p. 903-948. In: A.L. Page (Ed.), *Methods of soil analysis. Part 2. Chemical and microbiological properties*. American Society of Agronomy, Madison.

Tiquia, S.M., 2005. Microbiological parameters as indicators of compost maturity. *J. Appl. Microbiol.* 99, 816-828.

Tiquia, S.M., Tam, N.F.Y., Hodgkiss, I.J., 1998. Changes in chemical properties during composting of spent pig litter at different moisture contents. *Agric. Ecosyst. Environ.* 67, 79-89.

- Torres-Climent, A., Gomis, P., Martín-Mata, J., Bustamante, M.A., Marhuenda-Egea, F.C., Pérez-Murcia, M.D., Pérez-Espinosa, A., Paredes, C., Moral R., 2015. Chemical, thermal and spectroscopic methods to assess biodegradation of winery-distillery wastes during composting. *Plos One* 10(9): e0138925.
- Trevors, J.T., 1986. Bacterial growth and activity as indicators of toxicity. *Toxicity Testing Using Microorganisms*. Eds.: G. Bitton and B.J. Dutka. CRC Press. Boca Ratón. Florida.
- Varma, V.S., Kalamdhad, A.S., 2015. Evolution of chemical and biological characterization during thermophilic composting of vegetable waste using rotary drum composter. *Int. J. Environ. Sci. Technol.* 12 (6), 2015-2024.
- Wu, T.Y., Lim, S.L., Lim, P.N., Shak, K.P.Y., 2014. Biotransformation of biodegradable solid wastes into organic fertilizers using composting or/and vermicomposting. *Chem. Eng. Trans.* 39, 1579-1584.
- Yamashita, Y., Tanoue, E., 2003. Chemical characterization of protein-like fluorophores in DOM in relation to aromatic acids. *Mar. Chem.* 82, 255-271.
- Zepp, R.G., Sheldon, W.M., Moran, M.A., 2004. Dissolved organic fluorophores in southeastern U.S. coastal waters: correction method for eliminating Rayleigh and Raman scattering peaks in excitation-emission matrices. *Mar. Chem.* 89, 15-36.
- Zucconi, F., Pera, A., Forte, M., Bertoldi, M., 1981. Evaluating toxicity of immature compost. *BioCycle* 22, 54-57.

**Figure captions**

**Figure 1.** Temperature profiles of the composting piles during the process. Pile 1: 58% SSA + 42% PV1; Pile 2: 57% SSA + 43% PV2; Pile 3: 50% SSA + 33% PP1 + 17% almond shell powder; Pile 4: 52% SSA + 34% PP3 + 14% almond shell powder. SSA: agri-food sludge; PV1: vine shoot pruning waste with particle size < 1cm; PV3: vine shoot pruning waste with particle size < 3cm; PP1: pepper plant pruning waste with 1 cm particle size; PP3: pepper plant pruning waste with 3 cm particle size.

**Figure 2.** Organic matter losses during the composting of piles 1-4. Pile 1: 58% SSA + 42% PV1; Pile 2: 57% SSA + 43% PV2; Pile 3: 50% SSA + 33% PP1 + 17% almond shell powder; Pile 4: 52% SSA + 34% PP3 + 14% almond shell powder. Symbols are the experimental data (n=3) and lines represent the curve-fitting. SSA: agri-food sludge; PV1: vine shoot pruning waste with particle size < 1cm; PV3: vine shoot pruning waste with particle size < 3cm; PP1: pepper plant pruning waste with 1 cm particle size; PP3: pepper plant pruning waste with 3 cm particle size.

**Figure 3.** Curves of thermogravimetry-TG (dashed line) and of derivative thermogravimetry-DTG (continuous line) of initial (above) and final (below) compost samples from pile 4 [52% SSA + 34% PP3 + 14% almond shell powder]. SSA: agri-food sludge; PP3: pepper plant pruning waste with 3 cm particle size.

**Figure 4.** Curves of differential thermal analysis-DTA (continuous line) and percentage of energy release (dashed line) of initial (above) and final (below) compost samples from pile 4 [52% SSA + 34% PP3 + 14% almond shell powder]. SSA: agri-food sludge; PP3: pepper plant pruning waste with 3 cm particle size.

**Figure 5.** Spectra of excitation–emission matrix (EEM) fluorescence of pile 1 [58% SSA + 42% PV1] and pile 4 [52% SSA + 34% PP3 + 14% almond shell powder]. The increase in level curves was 20 for all the figures. SSA: agri-food sludge; PV1: vine shoot pruning waste with particle size < 1cm; PP3: pepper plant pruning waste with 3 cm particle size.

**Figure 6.** The three excitation–emission matrix (EEM) PARAFAC components.

**Figure 7.** Fresh shoot weight (grams) of melon seedlings grown in the different media (treatments), 40 days after transplanting. (A) without inoculation of the pathogen and (B) with inoculation of *F. oxysporum* (FOM). Error bars represent the standard errors. For each compost, values with the same letter do not differ significantly according to Turkey's post hoc test ( $P < 0.05$ ).

Table 1. Proportions (fresh weight basis) and main characteristics (dry weight basis) of the raw materials used in the composting piles.

	<i>Initial matrix composition (f.w.)</i>					<i>General chemical properties (d.w.)</i>				
	SSA (%)	PV1 (%)	PV3 (%)	PP1 (%)	PP3 (%)	Almond shell powder (%)	TOC/TN	TN (%)	P (%)	K (%)
Pile 1	58	42					22	1.61	0.31	0.43
Pile 2	57		43				22	1.56	0.29	0.43
Pile 3	50			33		17	18	2.01	0.44	1.19
Pile 4	52				34	14	18	1.94	0.45	1.18

SSA: agri-food sludge; PV1: vine shoot pruning waste with particle size < 1cm; PV3: vine shoot pruning waste with particle size < 3cm;

PP1: pepper plant pruning waste with particle size of 1 cm; PP3: pepper plant pruning waste with particle size of 3 cm.

TOC: total organic C; TN: total nitrogen; f.w.: fresh weight basis; d.w.: dry weight basis.

Table 2. Evolution of the organic matter (OM), TOC/TN ratio, water-soluble organic C (WSC) and total N (TN) during composting (dry weight basis).

Composting phase <sup>a</sup>	OM (%)	TOC/TN	WSC (%)	TN (%)
<i>Pile 1: 58% SSA + 42% PV1</i>				
I (0)	75.83 ± 0.04	23.75 ± 0.21	1.49 ± 0.54	1.61 ± 0.01
T (10)	70.69 ± 0.27	18.42 ± 0.14	1.54 ± 0.34	1.96 ± 0.01
E (37)	68.17 ± 0.32	15.55 ± 0.14	0.81 ± 0.28	2.41 ± 0.01
M (78)	67.68 ± 0.02	16.06 ± 0.04	0.58 ± 0.17	2.35 ± 0.01
<i>LSD</i>	<i>0.7</i>	<i>0.49</i>	<i>1.17</i>	<i>0.04</i>
<i>Pile 2: 57% SSA + 43% PV2</i>				
I (0)	83.17 ± 0.07	23.40 ± 0.27	1.62 ± 0.48	1.56 ± 0.02
T (10)	79.84 ± 0.11	20.01 ± 0.23	1.27 ± 0.18	1.73 ± 0.02
E (37)	70.49 ± 0.06	17.01 ± 0.09	0.93 ± 0.23	2.27 ± 0.01
M (78)	69.08 ± 0.03	16.05 ± 0.06	0.83 ± 0.14	2.36 ± 0.01
<i>LSD</i>	<i>0.23</i>	<i>0.56</i>	<i>0.94</i>	<i>0.05</i>
<i>Pile 3: 50% SSA + 33% PP1 + 17% almond shell powder</i>				
I (0)	59.07 ± 0.26	17.97 ± 0.08	1.37 ± 0.37	2.01 ± 0.01
T (10)	55.59 ± 0.12	15.64 ± 0.13	0.96 ± 0.29	1.89 ± 0.01
E (37)	51.00 ± 0.10	11.98 ± 0.21	0.82 ± 0.21	2.43 ± 0.02
M (78)	46.39 ± 0.34	10.80 ± 0.07	0.82 ± 0.60	2.36 ± 0.00
<i>LSD</i>	<i>0.55</i>	<i>0.43</i>	<i>1.29</i>	<i>0.04</i>
<i>Pile 4: 52% SSA + 34% PP3 + 14% almond shell powder</i>				
I (0)	61.10 ± 0.17	18.30 ± 0.03	1.31 ± 0.26	1.94 ± 0.00
T (10)	55.80 ± 0.31	16.65 ± 0.23	0.99 ± 0.50	1.70 ± 0.03
E (37)	50.19 ± 0.57	12.05 ± 0.06	0.90 ± 0.55	2.39 ± 0.00
M (78)	49.71 ± 0.34	11.48 ± 0.09	0.79 ± 0.27	2.27 ± 0.01
<i>LSD</i>	<i>1.23</i>	<i>0.39</i>	<i>1.36</i>	<i>0.06</i>

<sup>a</sup> Days in brackets. I: initial phase of composting; T: thermophilic phase of composting; E: end of the bio-oxidative phase; M: maturity phase. TOC: total organic carbon. Values reported as mean ± standard error. SSA: agri-food sludge; PV1: vine shoot pruning waste with particle size < 1cm; PV3: vine shoot pruning waste with particle size < 3cm; PP1: pepper plant pruning waste with 1 cm particle size; PP3: pepper plant pruning waste with 3 cm particle size.

Table 3. Final properties of the mature composts obtained.

	Compost 1	Compost 2	Compost 3	Compost 4
<i>Physico-chemical, chemical and biological properties</i>				
pH	7.94	8.22	8.97	8.74
EC (dS/m)	3.52	3.18	5.55	5.57
TN (%)	2.35	2.36	2.36	2.27
P (%)	0.48	0.46	0.64	0.64
K (%)	0.61	0.63	2.06	1.83
Ca (%)	10.6	12.5	8.64	8.08
Mg (%)	0.86	0.91	0.87	0.86
Na (%)	0.25	0.24	0.20	0.21
Fe (%)	0.14	0.16	0.27	0.26
Cu (mg/kg)	32.7	33.2	42.7	40.9
Mn (mg/kg)	30.0	49.4	112	70.0
Zn (mg/kg)	161	169	171	153
B (mg/kg)	65.6	67.2	82.0	72.4
Cd (mg/kg)	0.10	0.07	0.24	0.13
Cr (mg/kg)	33.1	25.5	18.6	49.8
Pb (mg/kg)	5.25	3.28	12.4	7.84
Ni (mg/kg)	13.0	11.1	8.83	27.2
Hg (mg/kg)	0.02	0.02	0.04	0.06
G.I. (%)	70.1	75.5	71.2	79.3
<i>Microbial pathogen groups</i>				
<i>Salmonella</i>	ND	ND	ND	ND
<i>Listeria</i>	ND	ND	ND	ND
<i>Clostridium</i>	$9.00 \cdot 10^1$	<10	$5.10 \cdot 10^2$	$1.10 \cdot 10^2$
<i>Streptococcus faecalis</i>	$1.40 \cdot 10^2$	$7.00 \cdot 10^1$	<10	<10
<i>Escherichia coli</i>	<10	$4.00 \cdot 10^1$	<10	<10

Data referred to microbial groups are expressed as CFU/g compost, the rest of

parameters are determined on a dry weight basis. EC: electrical conductivity; G.I.:

germination index; ND: not detected in 25 g compost; for other abbreviations, see

Tables 1 and 2.

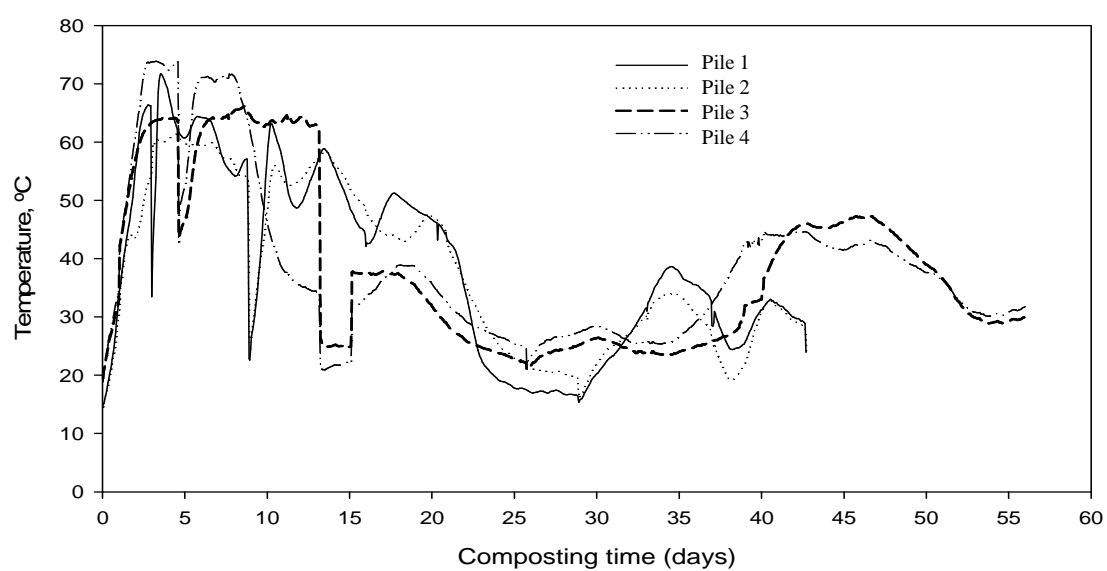


Table 4. Physical properties of the composts obtained.

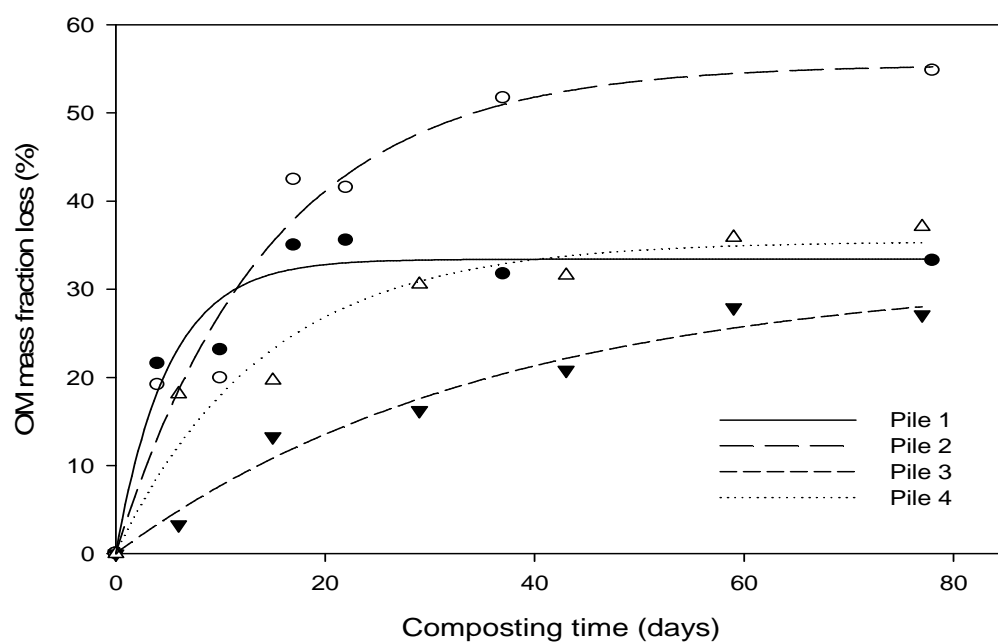
	I.S. <sup>1</sup>	Compost 1	Compost 2	Compost 3	Compost 4
BD (g/cm <sup>3</sup> )	≤ 0.40	0.23b ± 0.00	0.21c ± 0.00	0.26a ± 0.00	0.26a ± 0.00
TPS (% vol)	> 85	87.54b ± 0.05	87.85a ± 0.07	86.44d ± 0.01	86.91c ± 0.10
Shrinkage (% vol)	< 30	17.18b ± 0.08	16.22b ± 0.35	23.39a ± 1.48	23.36a ± 0.44
AC (% vol)	20-30	36.00b ± 0.52	48.70a ± 0.08	23.60c ± 0.21	22.50c ± 0.29
TWHC (mL/L)	550-800	516c ± 4.65	392d ± 1.40	629b ± 2.29	644a ± 3.91

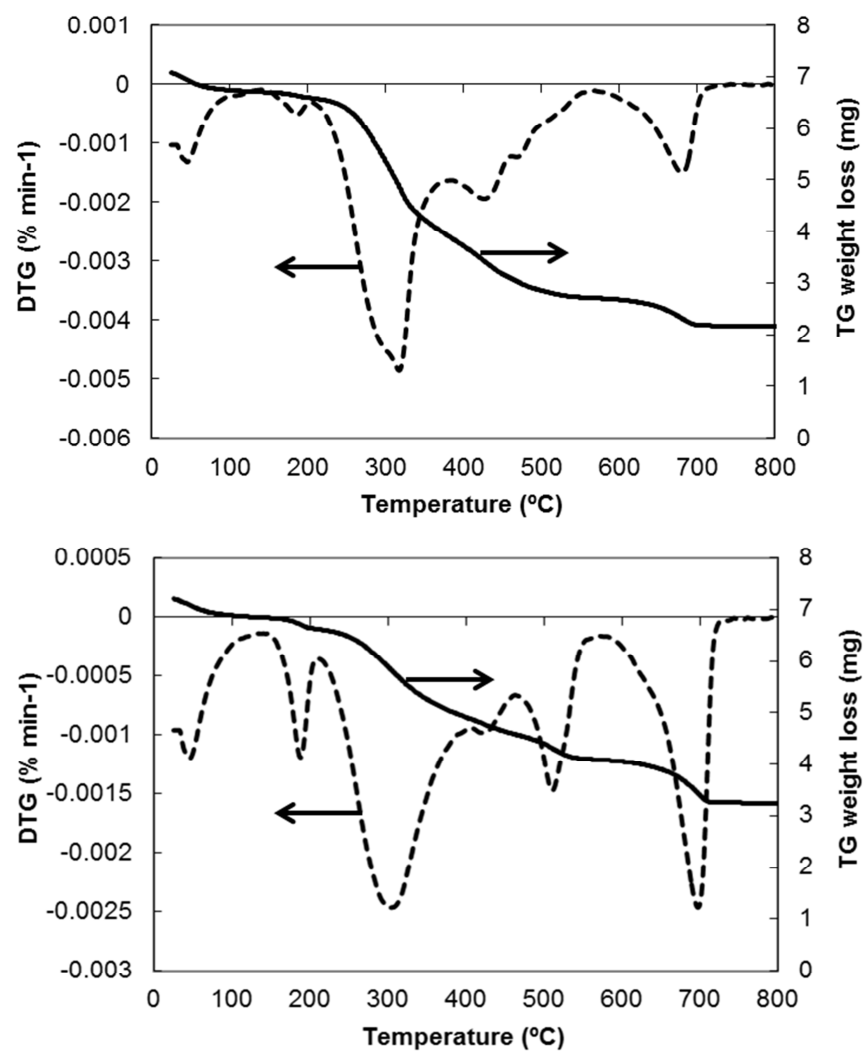
<sup>1</sup> Ideal substrate according to Abad et al. (2001). BD: Bulk density; TPS: Total pore space; AC: air capacity; TWHC: Total water holding capacity.

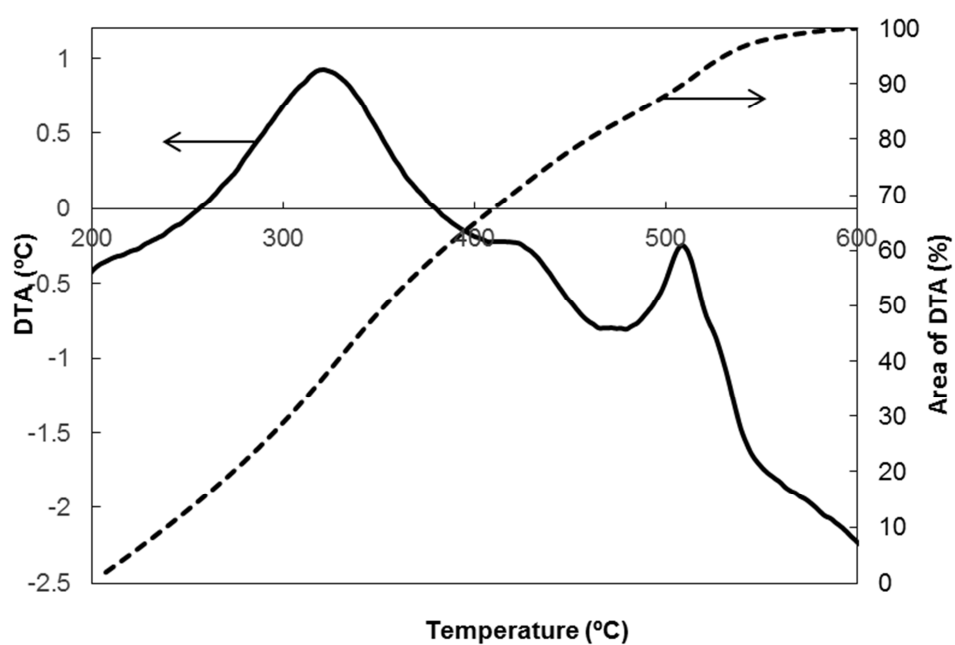
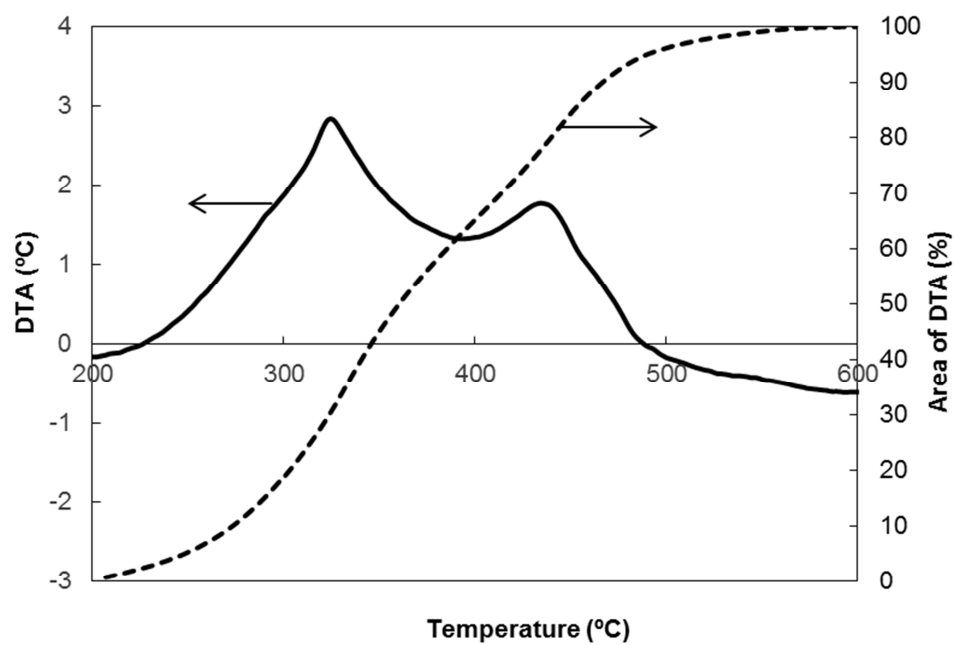
Mean values ± standard error. In columns, values followed by the same letter are not statistically different according to the Tukey-b test at  $P < 0.05$ .

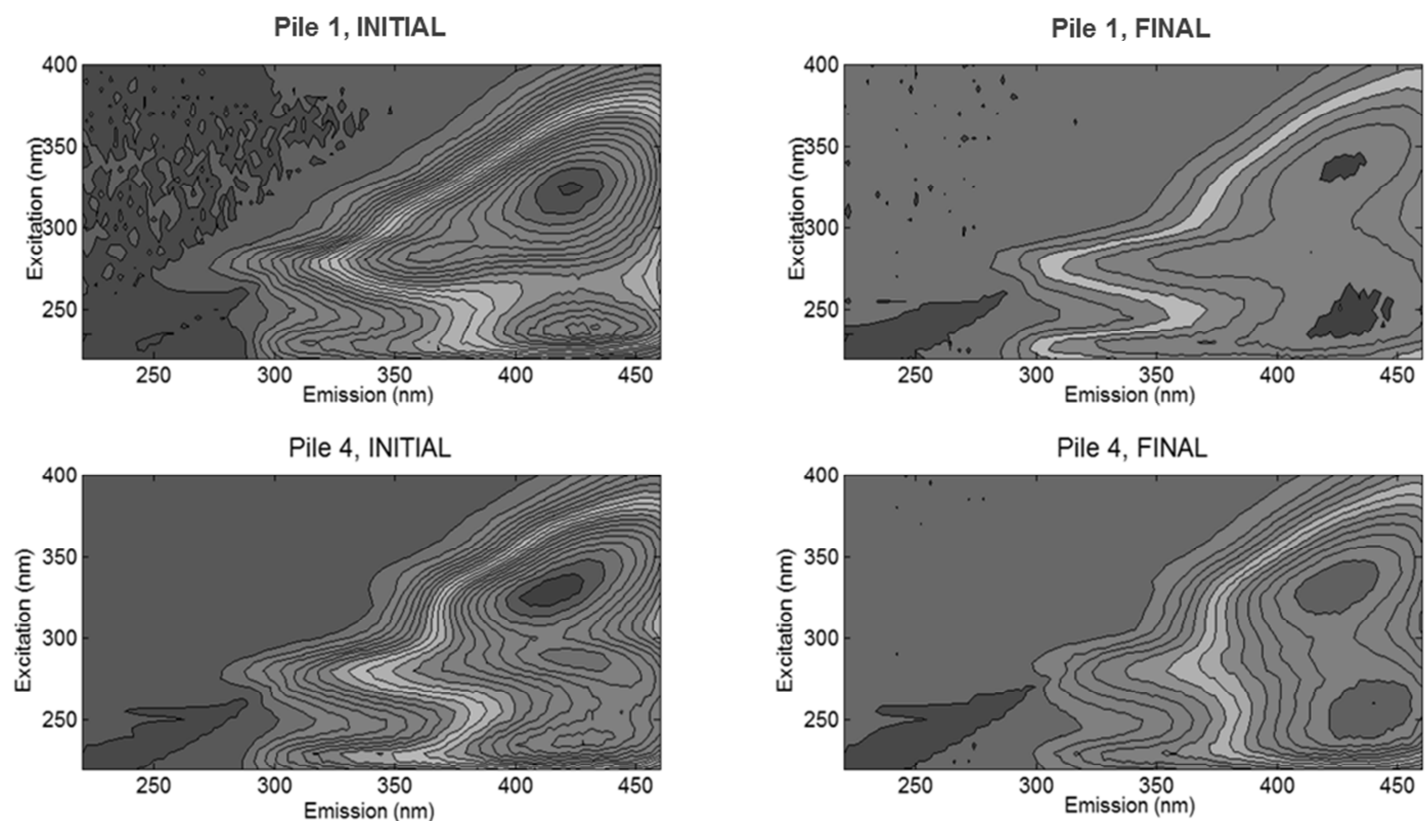


**Figure 1.**

**Figure 2.**

**Figure 3.**

**Figure 4.**

**Figure 5.**

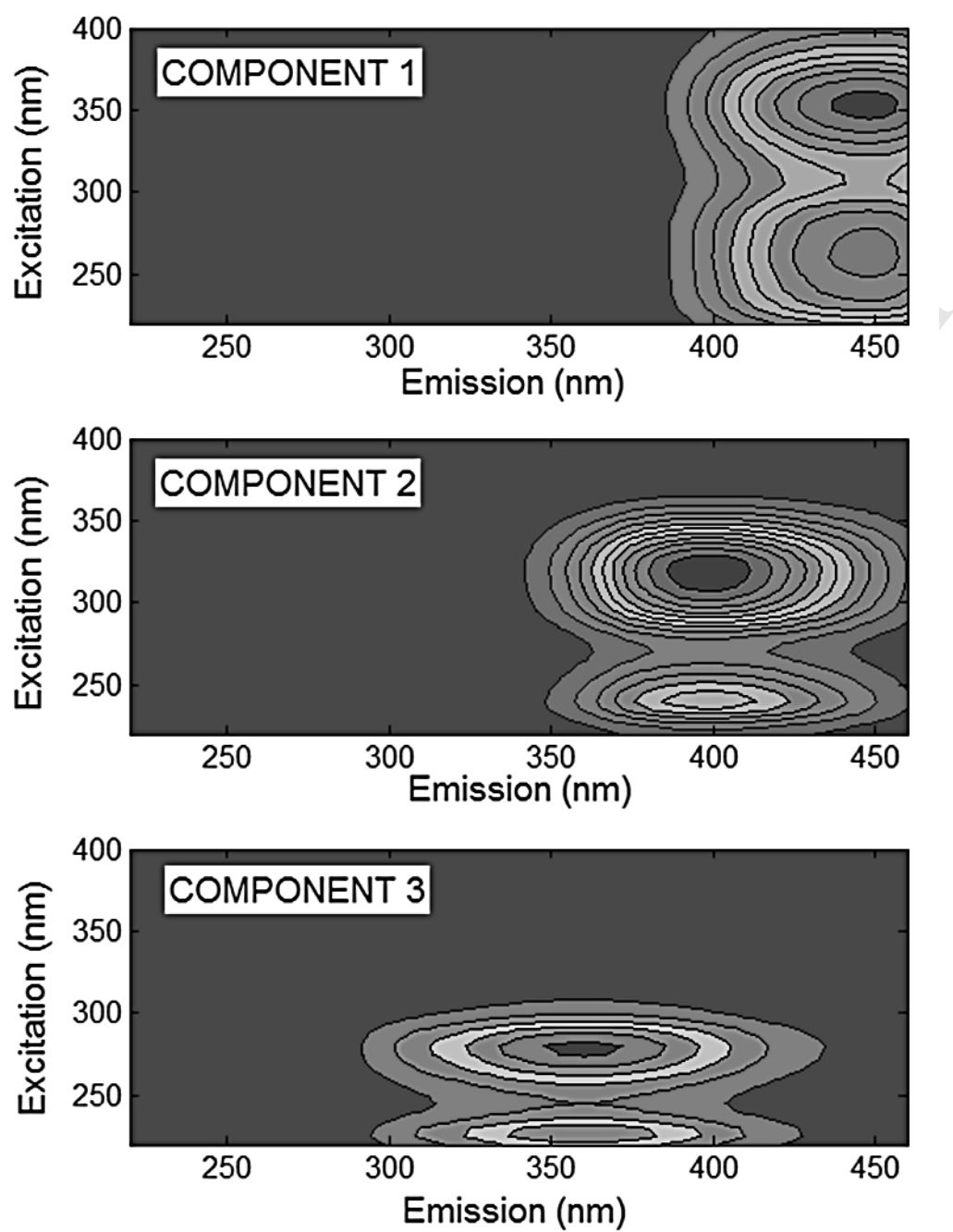
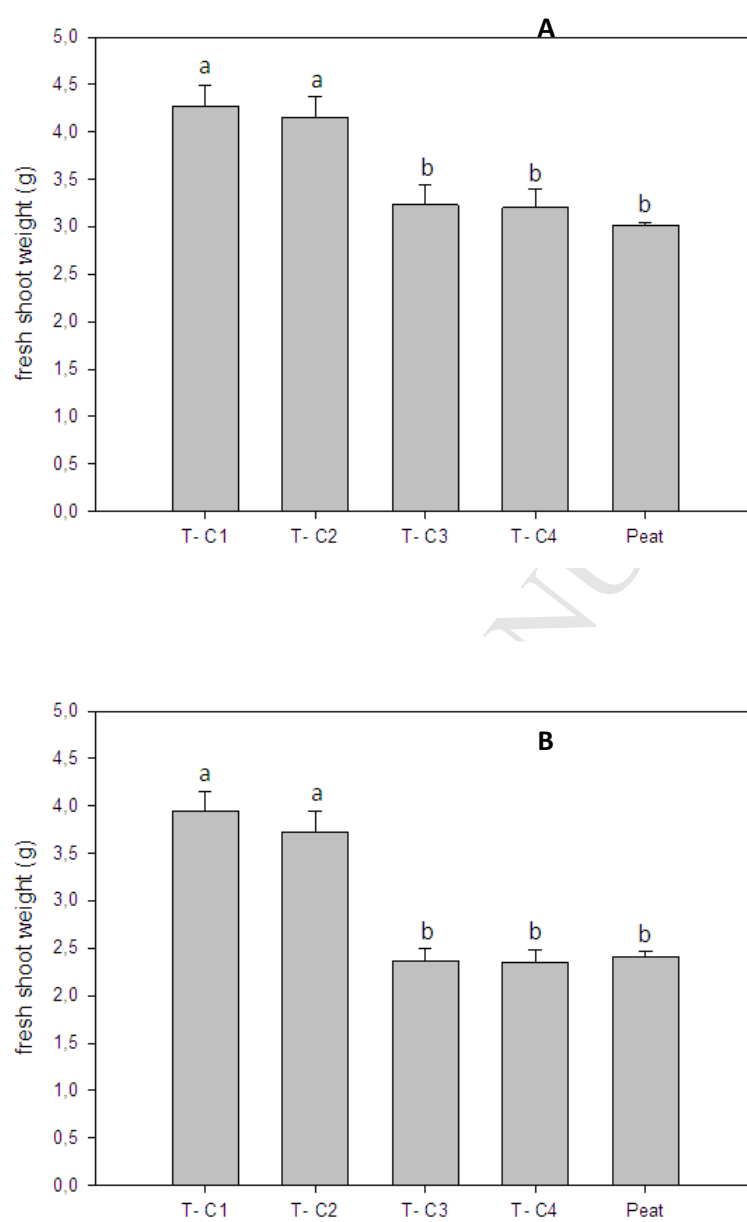


Figure 6.

**Figure 7.**



**Highlights**

Agri-food sludge can be recycled by co-composting with vine or pepper plant pruning.

The composts produced showed a suitable quality to be used as growing media.

Composts with vine pruning showed suppression against *Fusarium oxysporum* f.sp. *melonis*