Humic Matter of Compost: Determination of Humic Spectroscopic Ratio (E₄/E₆)

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ABSTRACT

This study aims to determine the quality of compost produced by monitoring the evolution of different humic fractions (humin, total humic substances, humic and fulvic acids) generated during the composting. This is achieved by determining the organic carbon content, humic spectroscopic UV-visible ratio (E_4/E_6), humification parameter estimation and the number of humic fraction. The compost used is produced from a mixture of olive pomace and cattle manure composted for a 5 months at different proportions 50:50, 66:33, 75:25, 80:20.During composting, different fractions of humic substances undergo a decrease in carbon and E_4/E_6 ratio is the lowest in humic acids and the highest in fulvic acids. The results confirm the relative maturity of the final product after 150 days of composting but do not reach the expected humic quality. The fulvic acids predominate on the olive pomace control, while humic acids are dominant on composted mixtures. The estimated number of humic fraction can provide information on the agronomic use of composted mixtures. Composted mixtures can be used on soils at pH 5.5-6.5 whatever the content of the soil organic matter. Olive pomace composted can be used on soils at pH between 6.6 and 8, even higher than 8 and whatever the organic matter content of the soil.

Key words: Cattle manure, composting, fulvic acid, humic acid, humic spectroscopic ratio E_4/E_6, olive pomace, number of humic fraction

Introduction

Organic matter has a very important agronomic interest. Recently, a great effort has been made to the recycling of organic waste, encouraging them to replace the expensive chemical fertilizers. The substantial quantities of humus in these wastes may have great value for maintaining soil health. Soil application of organic wastes and residues of any nature requires, however, that these materials be previously subjected to appropriate treatments.

Composting of the olive mill by-products is a useful method for producing a stabilized product that can be used as a source of plants nutrients and soil conditioner and is a promising solution (Ait Baddi *et al.*, 2001). However, the evolution of the stabilization of the organic matter during the composting has a primordial importance for the control of the efficiency of this process. In this sense, several physical, chemical and biological parameters have been proposed for the evaluation of the compost stability and maturity (Ouatmane *et al.*, 2000; Droussi *et al*., 2006).

Compost maturity has been investigated via the humification characteristics. The monitoring of the humification of organic matter during composting is used to determine the state of maturity of the compost. The quality of compost is related to the proportion of stable humus which is formed after decomposition of organic matter (Robin, 1997; Domeizel *et al*., 2004). Many authors have proposed indices of maturity based on the monitoring of humic substances, humic acids and fulvic acids (Sugahara and Inoko, 1981; Lopez *et al.,* 2002).

Among the indices of maturity proposed, those most representative of the evolution of the maturity of the compost were presented, in particular those indices measuring the evolution of humic acids (HA) compared to fulvic fraction (FF) or fulvic acids (FA). Deiana *et al.,* (1990), Inbar *et al.,* (1991), Ouatmane *et al.,* (2000), Tomati *et al*., (2000), proposed the use of the humification index, the humification rate and the degree of humification and suggested monitoring the molecular weight of the humic substances as an index of stability. These parameters may be good indicators of the progression of the humification processes but do not provide sufficient data concerning the composition of the humic substances.

The objective of the present work was to study the state of humification of the organic matter, according to the age of composting. Determining the degree of product maturity and tracking changes undergone by humic substances will be made by using various physico-chemicals (rate of carbon and ash) and spectroscopic (E_4/E_6 ratio) parameters.

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Material and Methods

The study was focused on olive pomace and cattle manure, composted individually as controls, or composted as mixtures with different proportions by weight (olive pomace/cattle manure: 50:50, 66:33, 75:25, 80:20). These composted mixtures were used and studied in a previous paper (Haddad *et al.*; 2012). The humic substances were extracted from these different mixtures of olive pomace and cattle manure at various stages of composting (1, 8, 80 and 150 days). During the composting process and at the end of the process the humic parameters of the obtained compost were evaluated. Differentiation of humic fractions of compost is one of the means used to determine the quality of the compost.

All samples were passed through a 20 mm sieve and dried at 105°C. For each sample, three replicates were made up and the results presented averages of the latter. Analyzes were performed on different humic fractions (humin, humic acids, fulvic acids) obtained after alkaline extraction of organic matter composts. Thus, various chemical techniques were applied to analyze humic acid fraction extracted from compost at different stages of composting:

Extraction of the humic substances

The extraction of humic substances from compost was performed based on the method of Duchaufour and Jacquin (1966), according to Schnitzer (1982), Dabin, (1971).This method has been used by other authors for the extraction of humic substances from compost formed from olive pomace, straw, olive mill wastewater (Ait Baddi *et al.,* 2003).

For the analysis of the humic substances, humic acids and fulvic acids were determined by extracting compost with 0.1 mol.L^{-1} (0.1 N) NaOH in a ratio of 1/10 (compost/solution) (Kallianou, 2000). It has shown that solution of sodium hydroxide (0.1 mol.L^{-1}) extracts more organic matter than more concentrated solution (Levesque and Schnitzer, 1966; Pansu and Gautheyrou, 2003). A dried, ground sample of compost were dissolved in a solution of NaOH 0.1 N. The solution was stirred for 2 hours and centrifuged for 25 min at 5000 rpm.min^{-1}. This operation was repeated several times until the supernatants obtained were clear or uncolored. The supernatants were collected and filtered on Whatman paper N°42. A solution of total humic matter (THM) was obtained (Domeizel *et al.,* 2004; Ait Baddi *et al.,* 2001; Amir *et al.,* 2003). An aliquot of the (THM) serve to the determination of carbon, and another aliquot will be used for UV-visible spectroscopic determination (E$_4$/E$_6$), while the rest of (THM) supernatant is used for separation of humic acids (HA) and fulvic acids (FA). The insoluble fractions represent humin (HU) for which the organic carbon was determined by calcination method.

Separation of humic and fulvic acids

The humic (HA) and fulvic acids (FA) were fractionated by acidification the rest of (THM) with a sulphuric acid solution (6N). The separation of humic and fulvic acids depend on their different solubility according to pH acid. The filtered solutions or the pooled supernatants were precipitated by acid treatment with 6 N H$_2$SO$_4$ (from pH 12.0 to pH 2.0) for 24 h at 4°C.

The precipitated humic acids, insoluble fraction, were separated from fulvic acids, soluble fraction with non phenolic compounds, by centrifugation for 30 minutes at 6000 rpm.min^{-1}. The precipitated humic acids were solubilized by NaOH solution 0.1mol L^{-1} (Barriuso *et al.,* 1985). An aliquot of HA was used to determine the carbon by calcination at 550°C, and another one for the spectroscopic determination E$_4$/E$_6$. The supernatant, which is the soluble fulvic fractions (FF=fulvic acids +non-humified fractions) was isolated and assayed like the total humic substances, but fulvic acids were estimated as the difference between total humic matter and humic acids (FA = THM - HA). An aliquot of "FF" was used to determine the carbon by calcination at 550°C, and another one for the spectroscopic determination (E$_4$/E$_6$).

Weight fractionation of humic matter

The following procedure modified, for the fractionation of humic matter, is based on that used by Petrik' laboratories (*Quality Compost. CompoStar-DP Addendum, 2002. www.petrik.com /public/learning/ app_3products/ compstar/index2.htm*), which determine the number of humic fraction (NHF). This NHF will give an idea about the quality of the compost and its agronomic use.

400 mL of distilled water are added to 100 g of composted material dried at 60-70°C, while increasing the pH to 12.0 with NaOH 0.1 N. This solution is stirred for 2 hours. The solution was decanted and then the solid phase (Humin) is separated from the supernatant. Then, the supernatant of the first humic matter extraction is weighed; it is the total weight of the total humic matter supernatant [TW$_{THMS}$]. From this total supernatant, three fractions were collected:

- A first fraction of 100g was removed and dried at 60-70°C and weighed, it is the weight of the total extractable humic matter [W_{TEHM}].
- A second fraction of 100 g was taken and the pH was adjusted to 6.0 with a solution of sulfuric acid 6N.
- A third fraction of 100g was collected and the pH was adjusted to 2.0 with a solution of sulfuric acid 6N.

The last two fractions were left to stand for 24 hours at a temperature of 4°C. After, the solid phase in each fraction is separated from the liquid phase by centrifugation (30 minutes at 6000 rpm.min^{-1}). The liquid phase was removed and the solid phase was collected, dried in an oven at 60-70°C and weighed.

From the second fraction, at pH 6, the humic high molecular weight [HHMW] was determined; from the third fraction, at pH 2, the sum of the humic medium and high molecular weight is determined [H (M + H)MW].

Other molecular weights are calculated using the following formulas:

Humic medium molecular weight, HMMW = [H (M + H) MW] - [HHMW]

Fulvic low molecular weight, FLMW = [W_{TEHM}]- [H (M + H)MW]

Total humic percentage, THP = [W_{TEHM}] * [TW_{THMS}] / 100

Molecular weights will be arranged in the following sequence [HHMW, HMMW, FLMW] giving for the highest weight the number '1', the second will be the number '2' and the lowest take the number '3'. In this sequence, the molecular weight with the number '1' will play the most important role. This sequence is defined as the number of humic fraction or "NHF".

Humification parameters

Various "empirical" humification parameters, such as the humification index (HI), the humification degree (HD) and the rate of humification (HR) have been calculated and proposed to quickly evaluate the evolution of the stability of the organic matter during and after the maturation period.

The indices of maturity were presented as those measuring the evolution of humic acids (HA) compared to fulvic fractions (FF) or fulvic acids (FA). The various indices determined are:

Humification index (HI) = Carbon of fulvic acid / Carbon of humic acid ratio = "C_{FA}/C_{HA}", or Carbon of humic acid / Carbon of fulvic acid ratio = "C_{HA}/C_{FA}" (Sugahara and Inoko, 1981; Estrada *et al.,* 1987; Jimenez and Garcia, 1992).

Index of polymerization (IP) = Carbon of fulvic fractions / Carbon of total humic matter = "C_{FF} / C_{THM}";

Humification rate (HR%) = (Carbon of total humic matter / Total organic carbon)* 100 = (C_{THM} / TOC)*100 (Morel *et al.,* 1979) ;

Humification Degree (HD%) = (Carbon of total humic matter / Carbon of Humin)* 100 = (C_{THM} / C_{HU}) *100 (Sequi et al., 1986);

where the C_{THM}, C_{HA}, C_{FA}, C_{FF}, C_{HU} and TOC are respectively the dissolved organic carbon in the humic substances, in the humic acids, in the fulvic acids, in the fulvic fractions, in the hunin, and the total organic carbon in the solid sample of compost.

Quantification fractions humic and ultraviolet-visible spectroscopy (E₄/E₆ Ratio)

The measurements of moisture, ash (Chen *et al.,* 1977; Lu *et al.,* 2000), organic carbon content (Nelson and Sommers, 1982; Mustin 1987) and E₄/E₆ ratio (Chen et al., 1977; Schnitzer, 1982) were taken in the solutions obtained from the extractions of total humic substances (THM), humic acids (HA), fulvic acids fractions (FF).

To characterize the humic material, the optical absorbance ratio at 465 and 665 nm (E₄/E₆ or Welt ratio) of alkali-soluble fractions was determined. Humic material is dissolved in a solution of sodium bicarbonate. Approximately 10 mg samples of humic acids dried at 60°C, dissolved in 25 ml 0.05 mol NaHCO₃.L[-1] (0.025M=0.05N) (Mangrich *et al.,* 2000). For total humic matter, 0.5 ml was dissolved and 5 ml for fulvic acid fraction. Zero is established with the solution 0.05 mol (NaHCO₃).L[-1] pure. The absorbances were measured at the wavelengths 465 and 665 nm by UV-visible spectroscopy.

Ultraviolet spectroscopy, already used to quantify and to evaluate the quality of humic substances (Sugahara and Inoko, 1981; Prudent *et al.*, 1995; Watanabe *et al.,* 2001; De Guardia *et al.,* 2002), was used here in order to propose an index of maturity.

Statistical analysis

The statistical interpretation was conducted by analysis of variance studied (ANOVA) according to the procedure of general linear model (GLM procedure) statistical program SAS (Statistical Analysis System, 2004). Comparison of the treatment means is established by calculating the least significant difference (LSD) at the 5%.

Results and Discussion

Characterization of humic substances

In general, the humic substances have a brown color, darker, reflecting their chemical properties (Stevenson, 1982).

Evolution of the carbon of humic matter in mixtures composted

The rate of carbon in humin remains slightly stable until 80 days, and then decreases markedly in all treatments. Indeed, this decrease is more pronounced in the case of manure at the beginning of composting (Figure 1). This trend follows the evolution of the total carbon in the compost described in a study realized previously by Haddad *et al.,* (2012). In the control of the olive pomace, the carbon humin content is the highest, but is the lowest in the manure control, between these two controls groups are the contents of the other treatments. At the beginning of composting, all treatments showed no significant differences between them and the controls. These differences become more significant with the progress of composting especially between the lot of manure and other treatments. The decrease of carbon in the total humic matter, which is faster than in humin, occurs at the beginning of composting to grow slightly after the period of 80 days. However, Carbon contents of THM are as in humin (Figure 2). Regarding the carbon of THM, at the beginning of the process the differences are significant only between the manure controls on the one hand and olive pomace control and the mixture (66:33) on the other. Then, these differences are located only between the lot of manure and other treatments.

The decrease of carbon of HA is between day 8 and day 80 and then becomes stable from this period (Figure 3). The decreased of carbon in the compost of residues of olives and straw with increasing composting is noted by Ait Baddi *et al.,* (2003). By contrast, in another studies, an increase in the concentration of humic acids was observed during composting (Domeizel *et al.,* 2004). Effectively, Senesi and Brunetti, (1996), have reported, for humic acids extracted from other types of wastes (sludge, vermicompost), that with increasing composting time and compost maturity, there is loss of aliphatic groups as more stable humic acids are formed. Carbon contents of HA show fluctuations between the different treatments. At the beginning of the composting, significant differences exist between the manure lot on one hand, and the olive pomace and the mixture (50:50) on the other hand. These differences decrease with time, to become insignificant between different treatments. Carbon contents of HA are lower than those obtained in sludge composts (9 months of composting) (Kallianou, 2000; Amir, 2005), in compost of residues of olives and straw (12 months of composting) (Ait Baddi *et al.,* 2003), in vermicompost of sheep, goats, cattle and poultry for a period of 3 months (Mangrich *et al.,* 2000).

The decrease of carbon of FA, calculated as the difference between carbon of THM and carbon of HA ($C_{FA} = C_{THM} - C_{HA}$), is very clear between day 1 and day 8. Then, the content starts to increase significantly to 150 day (Figure 4). By contrast, the curve of the evolution of carbon determined on the liquid fraction of fulvic acids (FF) follows the same shape decreased as that of THM in all treatments (Figure 5). But, in another study, the concentration of fulvic acids (FA) decreased gradually during the composting process (Ko *et al.,* 2008). Generally, fresh and raw composts contain low levels of HA and higher levels of FA compared to mature compost (Chefetz *et al.,* 1996), but this trend don't occurred in this study. Unlike other fractions, the carbon content of FA showed no significant difference between treatments. However, differences in the carbon of FF are significant between the two controls of olive pomace and manure, at the beginning of composting, but over time these differences become insignificant.

Evolution of the rate of ash mixtures composted

The rate of ash in humin remains slightly stable until 80th day, and then increases sharply in all treatments. In fact, this increase is more pronounced in the case of the manure control at the beginning of composting. In the control of olive pomace, ash content of humin is the lowest, but it's the highest in the control manure, between them the other treatments are situated (Figure 6). This growth, faster in the THM than in humin, occurs at the beginning of composting and then decreased slightly after the period of 80 days (Figure7). At the beginning of composting, only mixed treatments (66:33-75:25) and olive pomace control had showed significant differences with the control of manure, regarding the rate of ash in humin. These differences become more significant with the progress of composting, especially between the lot of manure and other treatments. Regarding the ash content of THM, the differences are significant, at the beginning of the process, only between manure treatment on the one hand and those of the olive pomace and the mixture (66:33) on the other hand. Over time, these differences are significant only between the lot of manure and other treatments. The manure control has the highest rate of ash.

The increase of ash content of humic acids is between 8th day and 80th day and stabilizes from this period (Figure 8). The ash content showed significant differences between the control manure on one side and the other treatments on the other side especially at 8th day. These differences diminish with time, and become insignificant between the various treatments. In the study realized by Ait Baddi *et al.,* (2003), on the composting mixtures of olive pomace and olive mill wastewater with straw, they noticed that the ash content of HA does not vary during composting and the level of ash remained very similar throughout composting. Note however that the amount of ash depends on the extraction and purification conditions (Bruckert *et al.,* 1993). The ash content of HA are higher than those obtained in sludge composts (Kallianou, 2000; Amir, 2005), residues of olives and straw composted (Ait Baddi *et al.,* 2003) and in vermicompost of sheep, goats, cattle and poultry (Mangrich *et al.,* 2000).
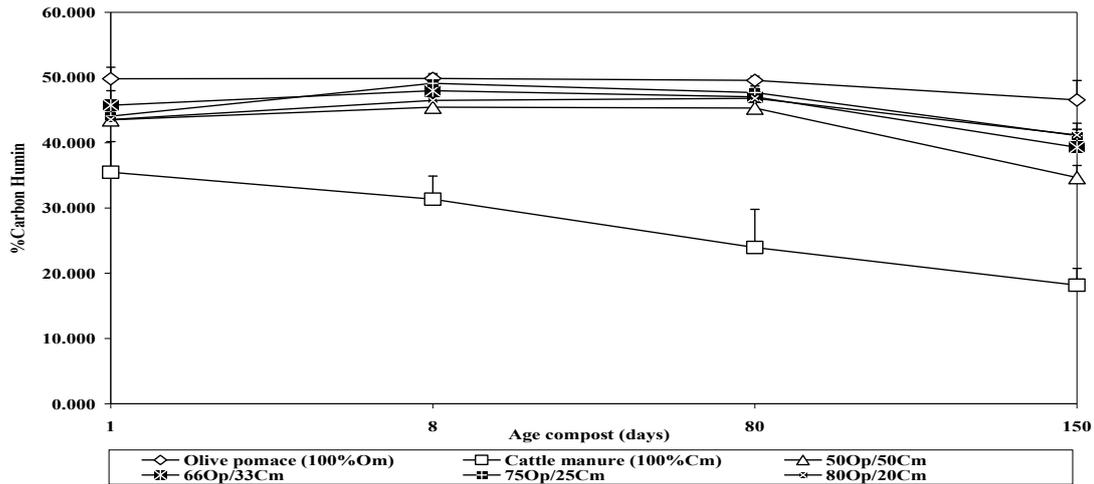

Fig. 1: Evolution of carbon content of Humin-H-(C_H % DM) during composting of mixtures of olive pomace and cattle manure. *Errors bars represent standard errors.*
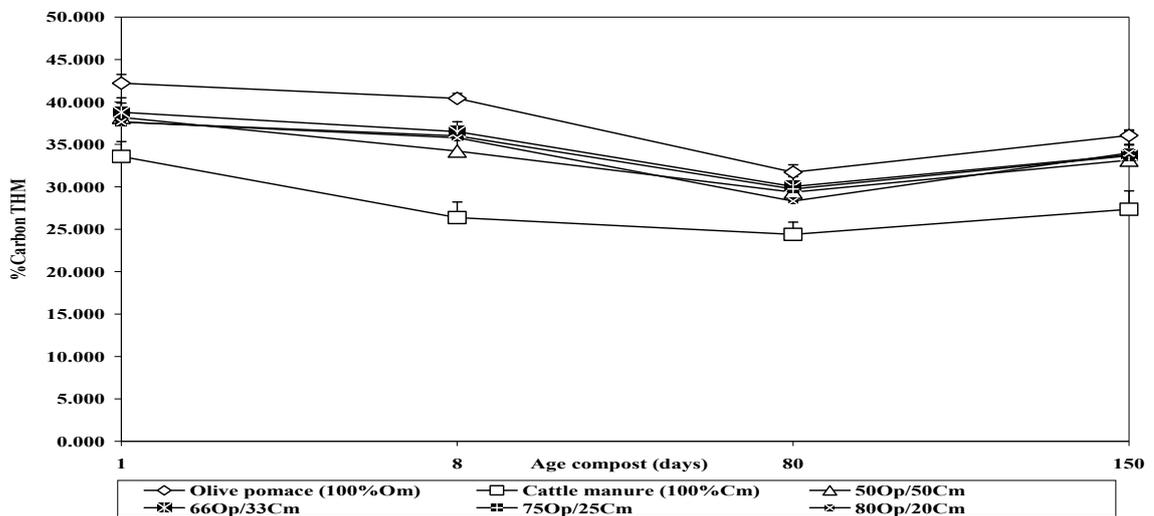

Fig. 2: Evolution of carbon content of Total Humic Matter- THM- (C_{THM} % DM) during composting of mixtures of olive pomace and cattle manure. *Errors bars represent standard errors.*
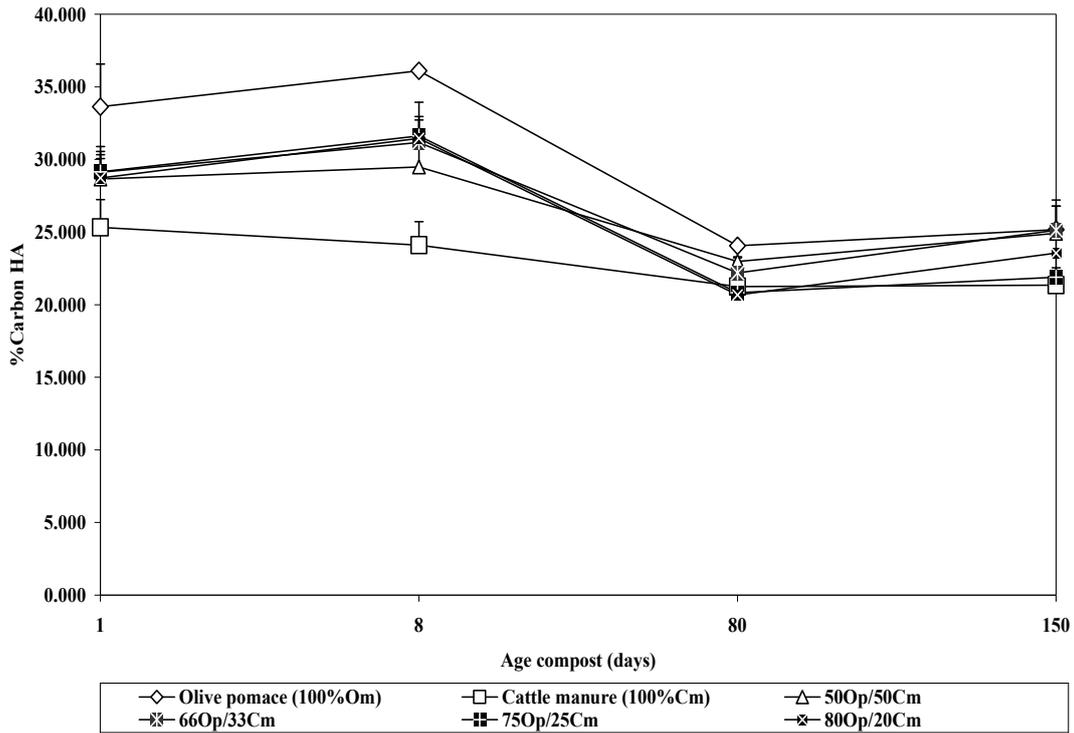
Fig. 3: Evolution of carbon content of Humic Acid -HA- (C_{HA} % DM) during composting of mixtures of olive pomace and cattle manure. *Errors bars represent standard errors.*
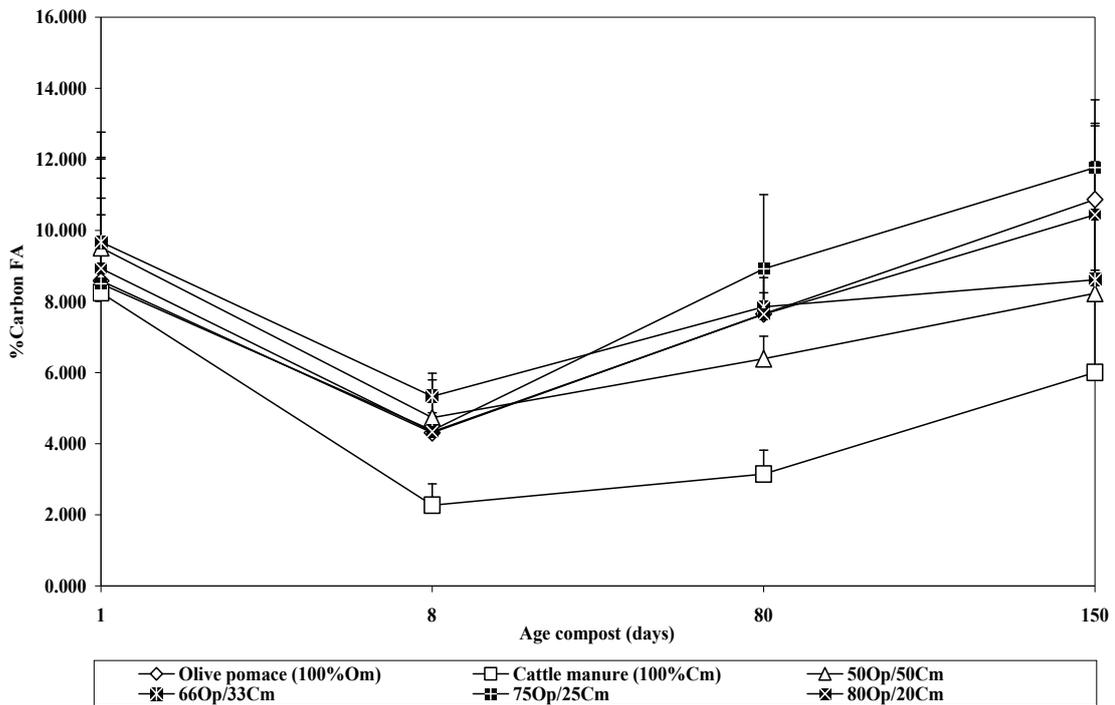

Fig. 4: Evolution of carbon content of Fulvic Acid -FA- (C_{FA} % DM) during composting of mixtures of olive pomace and cattle manure. *Errors bars represent standard errors.*
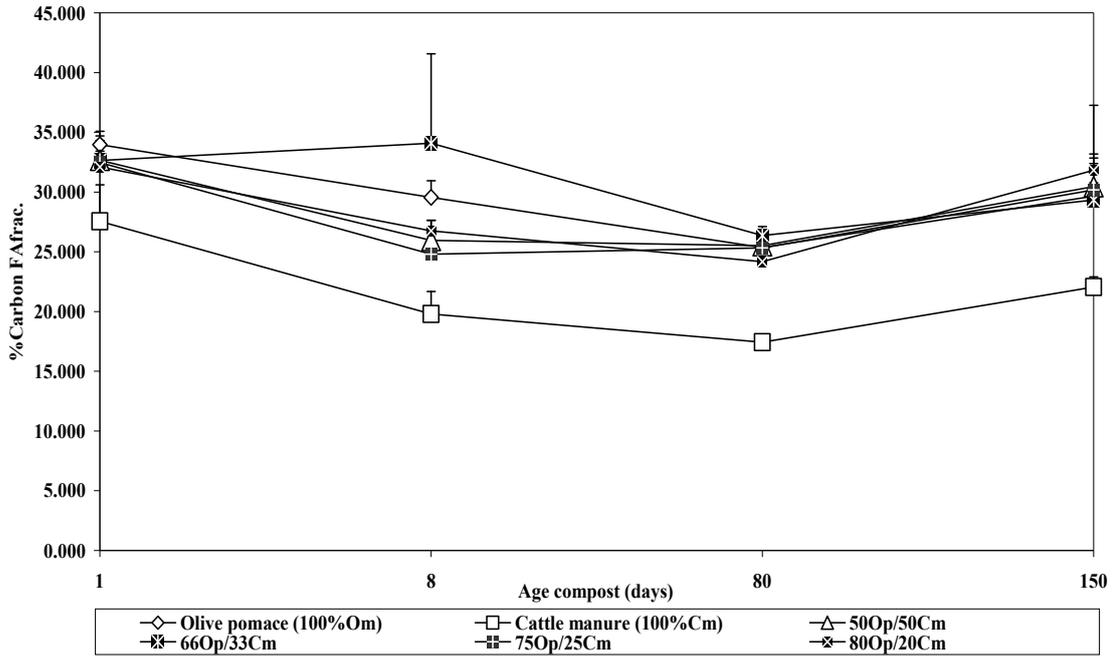
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Fig. 5: Evolution of carbon content of Fulvic Fractions –FF- (C_{FF} % DM) during composting of mixtures of olive pomace and cattle manure. *Errors bars represent standard errors.*
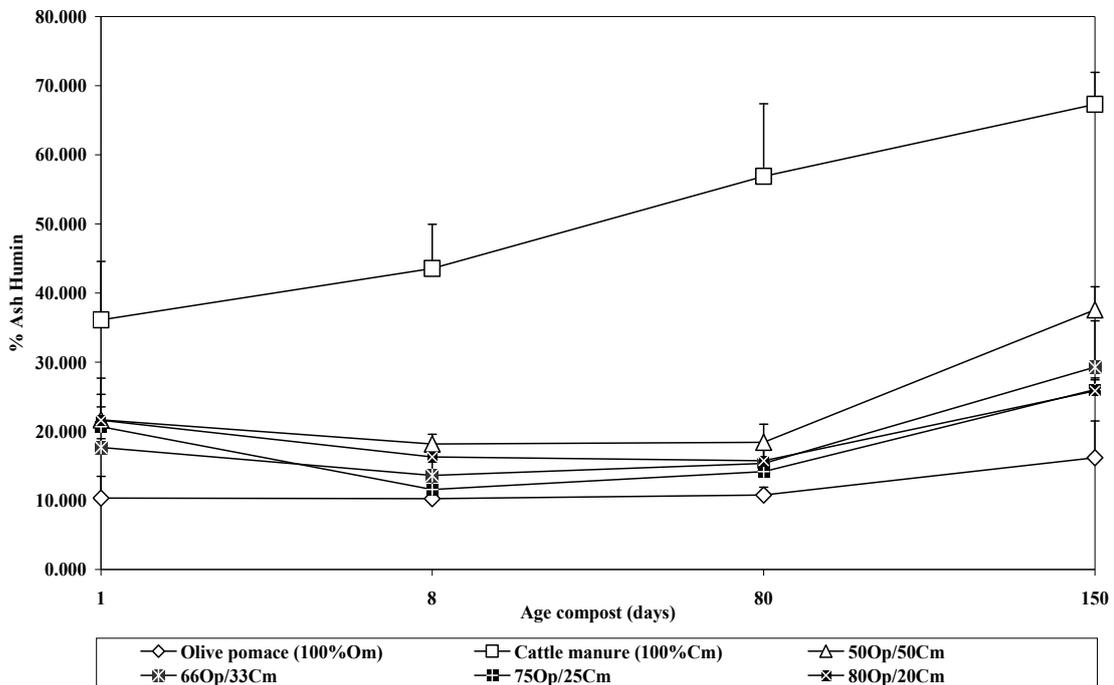

Fig. 6: Evolution of ash content of Humin-H-(Ash_H % DM) during composting of mixtures of olive pomace and cattle manure. *Errors bars represent standard errors.*
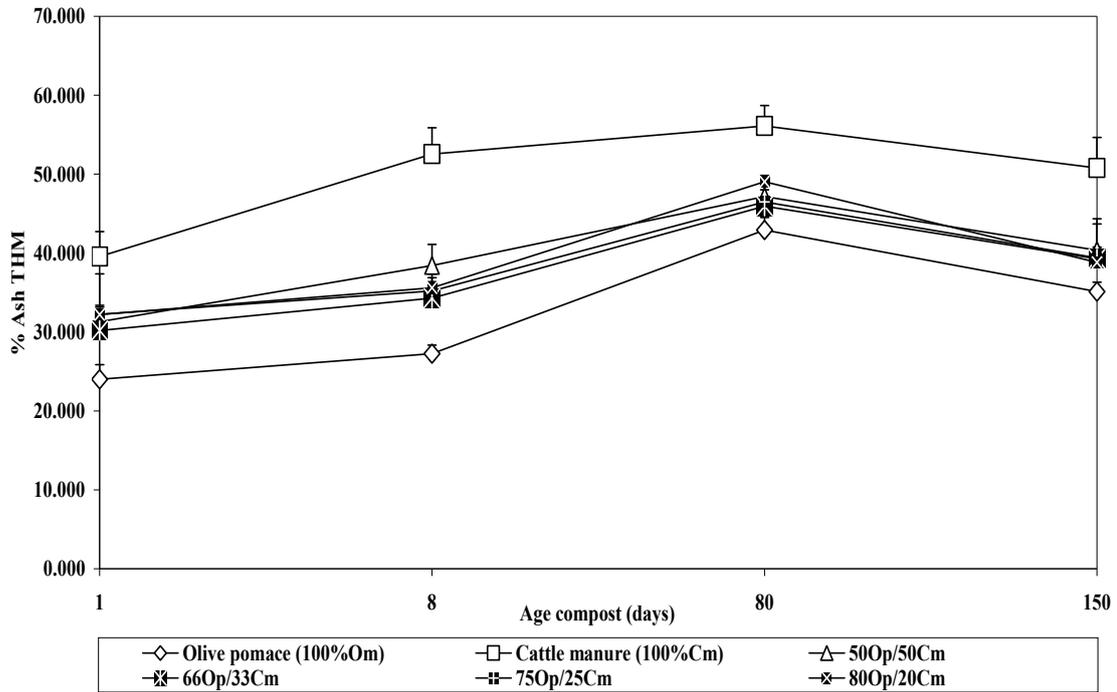
Fig. 7: Evolution of ash content of Total Humic Matter- THM- (Ash$_{THM}$ % DM) during composting of mixtures of olive pomace and cattle manure. *Errors bars represent standard errors.*
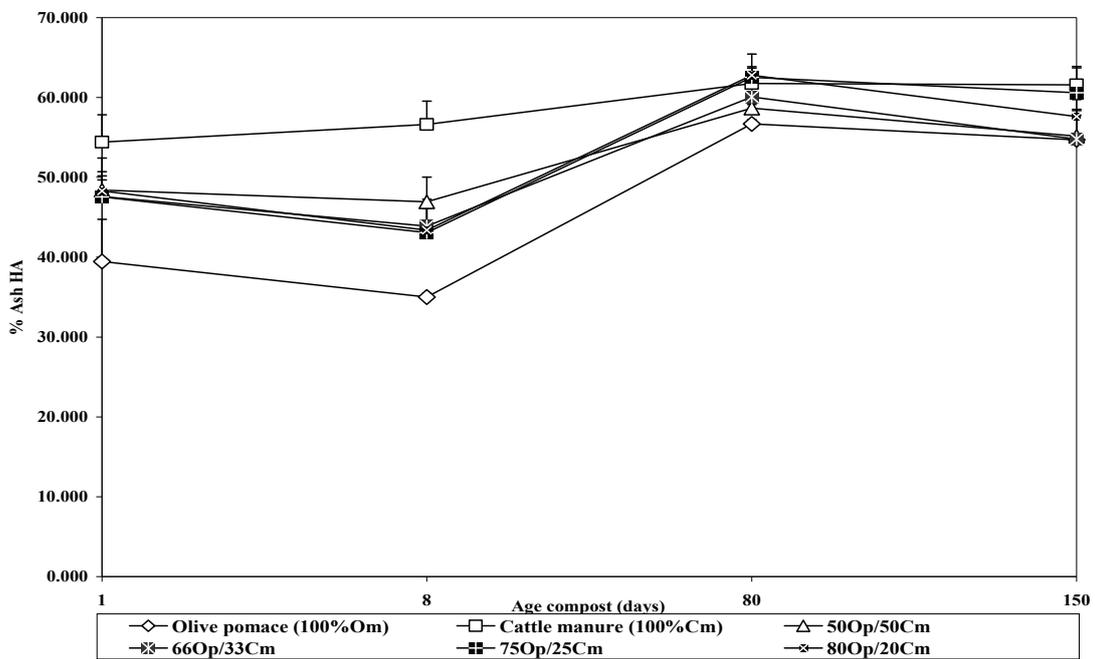

Fig. 8: Evolution of ash content of Humic Acid -HA- (Ash$_{HA}$ % DM) during composting of mixtures of olive pomace and cattle manure. *Errors bars represent standard errors.*
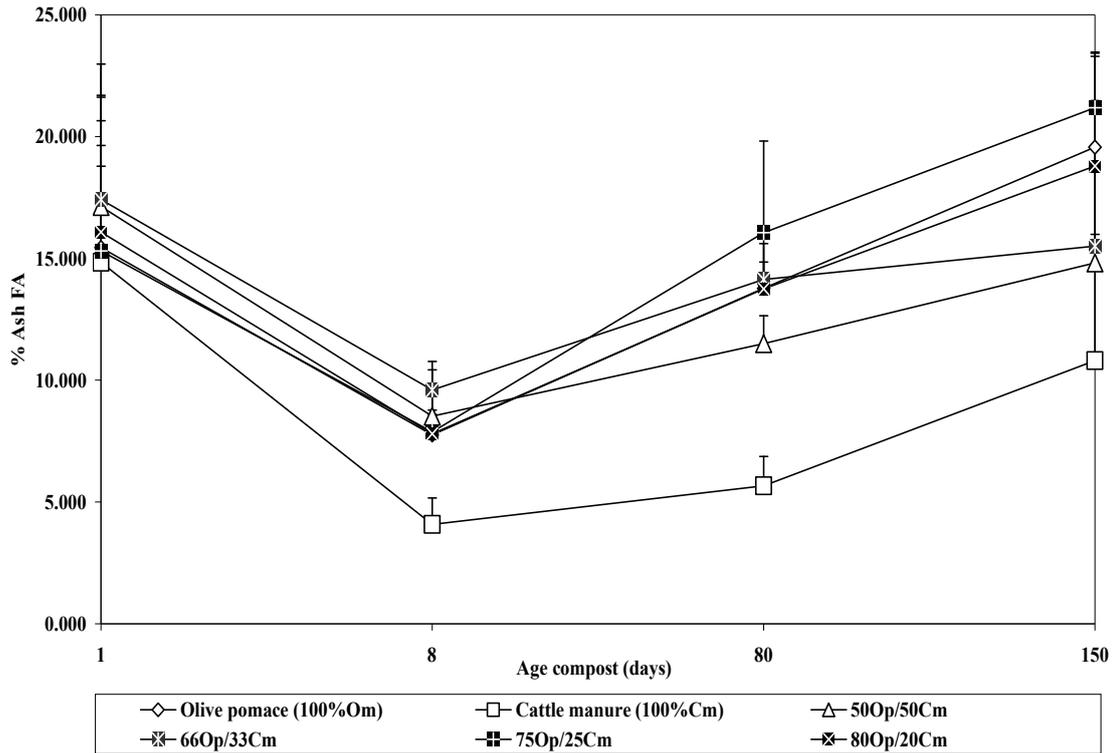
Fig. 9: Evolution of ash content of Fulvic Acid -FA- (Ash$_{FA}$ % DM) during composting of mixtures of olive pomace and cattle manure. *Errors bars represent standard errors.*
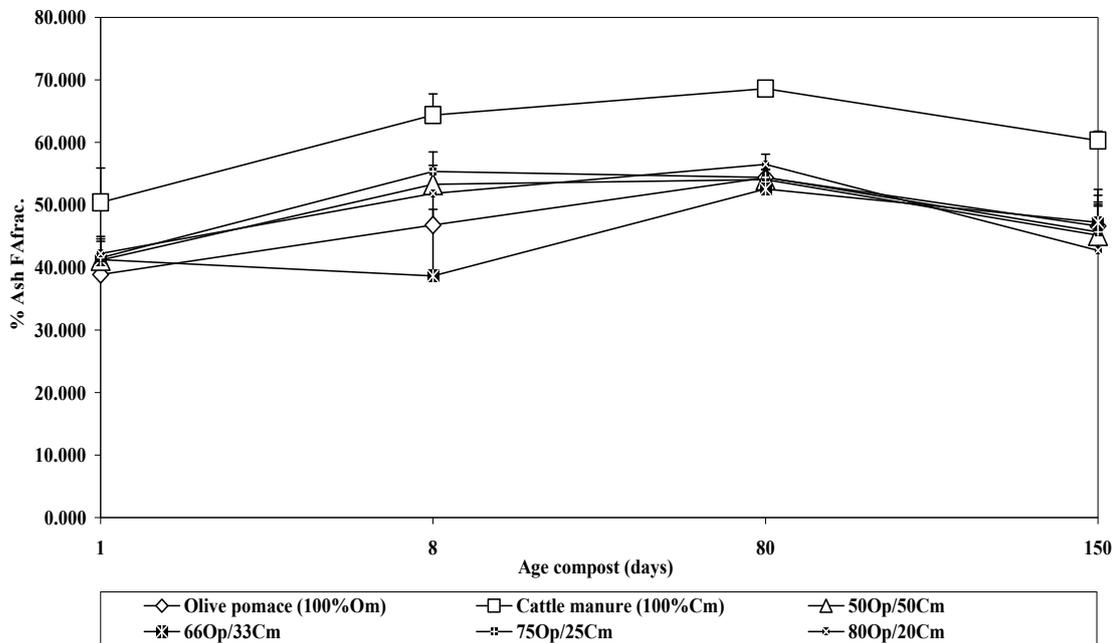

Fig. 10: Evolution of ash content of Fulvic Fraction –FF- (Ash$_{FF}$ % DM) during composting of mixtures of olive pomace and cattle manure. *Errors bars represent standard errors.*

As for fulvic acids (FA) calculated by difference, the decreasing rate of the ashes of FA is very clear between day 1 and day 8. The content starts to increase significantly to day 150 (Figure 9). As against, for the fraction of fulvic acids (FF), the curve evolution of ashes followed an increasing shape to 80th day then decrease after this period (Figure 10). Unlike other fractions, the ash content of FA showed no significant difference between treatments. As against, at the beginning of composting differences in the ashes of FF are significant between the two controls of olive pomace and manure. Over time these differences become insignificant.

Evolution of the report 'Welt' (E_4/E_6) in mixtures composted

The results, shown in Figures 11, 12 and 13, show the evolution of variable spectroscopic E_4/E_6 ratio measured in the different fractions of humic substances extracted from different treatments at different times of composting. However, a decrease in absorbance is noticed in the first phase of composting to 80th day, and then a renewed increase is recorded until 150th day of composting.

These results are in agreement with those obtained by Amir (2005) and Amir *et al.*, (2003). These variations can be explained by a phenomenon of mineralization in the first phase followed by a resynthesis of humic molecules complex in the maturation phase of composting as suggested by Senesi *et al.,* (1996). However, this increase in the ratio E_4/E_6, is due, perhaps, to a high proportion of carboxylic functions (Ait Baddi *et al.,* 2003).

A slight increase in the ratio E_4/E_6 during composting involves a reduction of the molecular weight of humic structures probably by elimination of aliphatic compounds, an increase in oxygen content as suggested by Chen *et al.,* (1977). According to Riffaldi *et al.,* (1983), the decrease in the E4/E6 ratio may be ascribed to either an increase in the molecular weight of the excluded fraction, or to a decrease in the total acidity of this fraction. E_4/E_6 ratio of HA plus FA and HA decreased during composting, and one FA was increase (Li *et al.,* 2001).
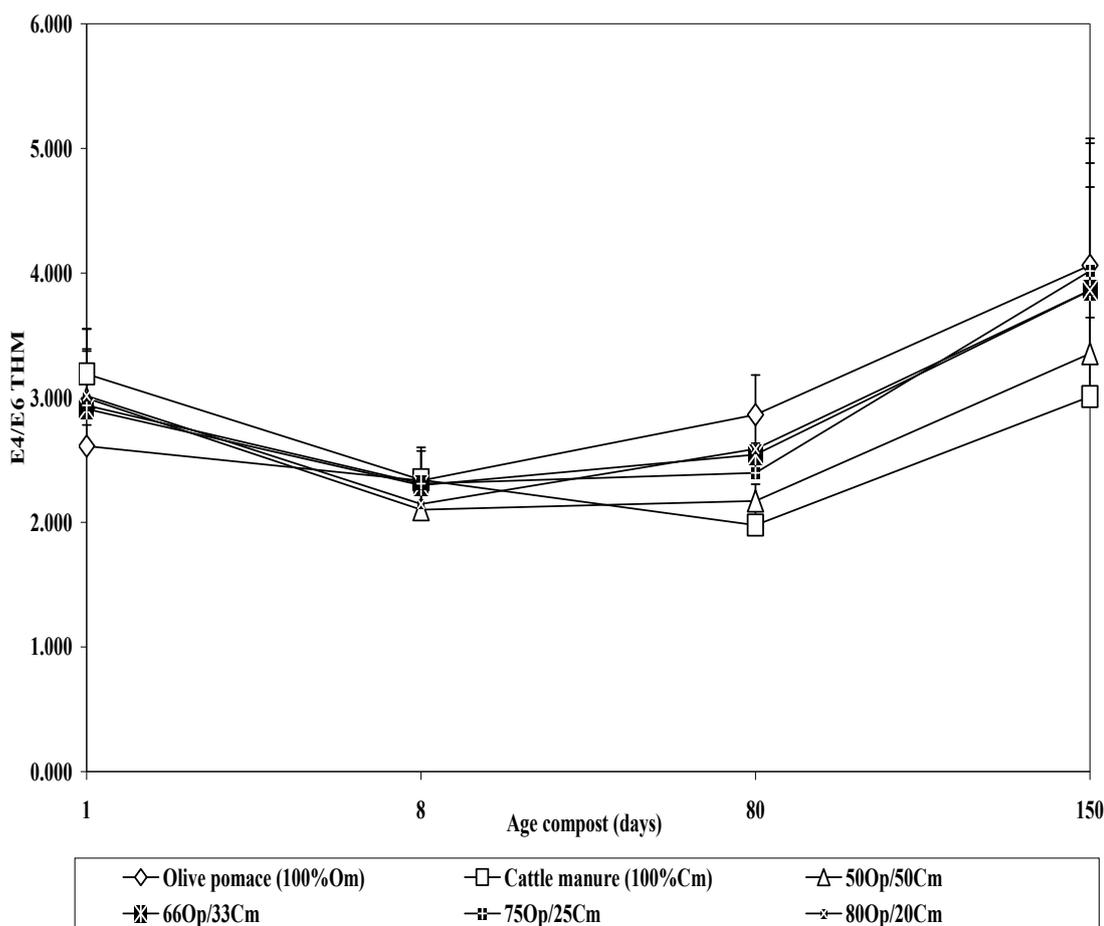

Fig. 11: Evolution of spectroscopy Welt ratio (E_4/E_6) of Total Humic Matter- THM- during composting of mixtures of olive pomace and cattle manure. *Errors bars represent standard errors.*
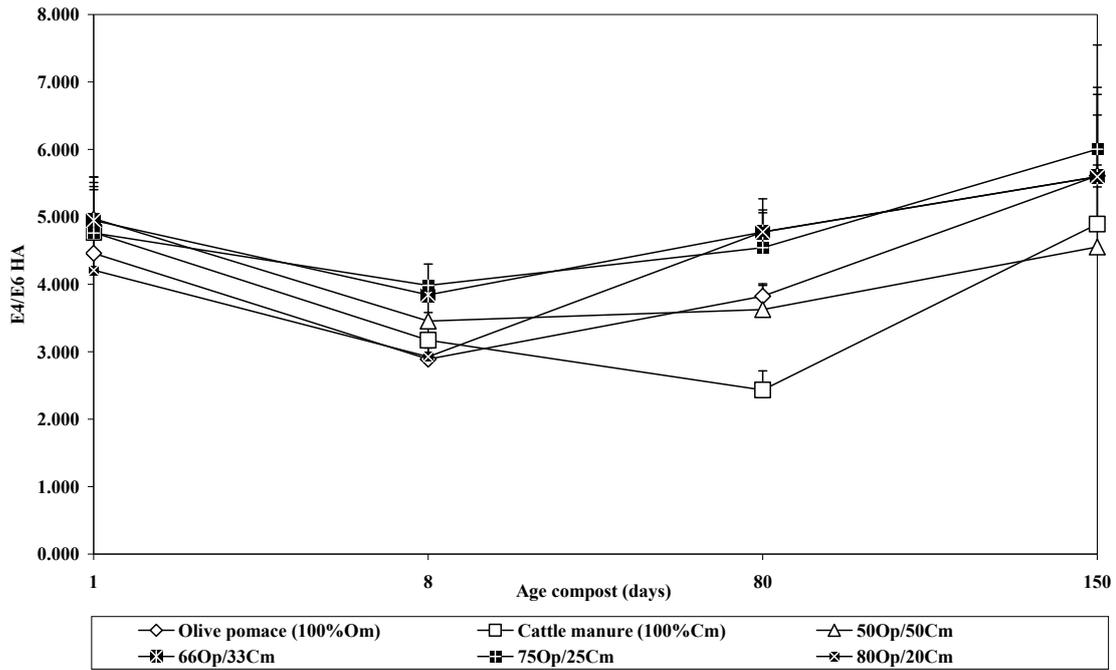
Fig. 12: Evolution of spectroscopy Welt ratio (E_4/E_6) of Humic Acid- HA- during composting of mixtures of olive pomace and cattle manure. *Errors bars represent standard errors.*
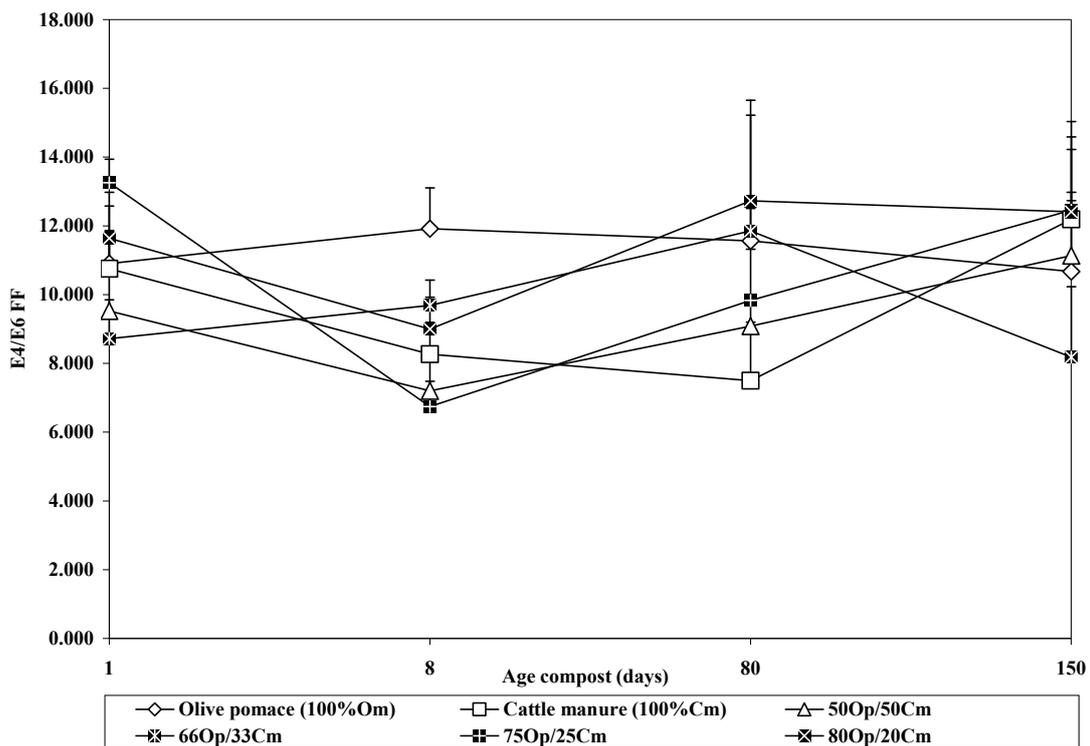
Fig. 13: Evolution of spectroscopy Welt ratio (E_4/E_6) of Fulvic Fraction-FF- during composting of mixtures of olive pomace and cattle manure. *Errors bars represent standard errors.*

At the end of composting, the value of this ratio varies between 3 and 4 for THM and between 4.5 and 6 for the HA. These values reflect a greater heterogeneity of these molecules composing solutions and a more balanced distribution of humic and fulvic fractions. As against, this ratio varies between 7 and 13 solutions for fulvic

fractions. The E_4:E_6 ratios for HA were found generally higher than that for some organic wastes composted in a study realized by Unsal and Ok, (2001). The results suggest that HA are characterized by a low degree of condensation and humification (Senesi and Brunetti, 1996; Unsal and Ok, 2001). For Schnitzer, (1971), E_4/E_6 ratio is generally less than 5 for humic acids and for fulvic acids it is between 6 and 8.5. Other studies have found approximately similar values (5.44 to 5.7) for HA and 8.88 to 9.9 for the FA (Chen *et al.,* 1977). According to these authors, E_4/E_6 is related to the molecular weight of humic substances, it is a good indicator of the size of the molecules present in solution, reliable and easy to measure. E_4/E_6 ratio is used to characterize the different humic samples providing an estimate of the molecular weight (MW) and the composition of the molecules they contain. It varies inversely with the molecular weight of the particles. Particles of high MW (humic acids) have a low ratio ($E_4/E_6 < 5$), those of low MW (fulvic acids) have a high ratio ($5 < E4/E6 < 10$) (Chen et al., 1977). In a study conducted by Eyheraguibel, (2004), and performed on humic extracts of different materials, it has been demonstrated that high E_4/E_6 ratio, on the order of 10, confirms the presence of fulvic acids majority indicating preferential formation of small molecules. As against, the results obtained by Ait Baddi *et al.,* (2003) reported that humic acids extracted from the residues of olive presses have a low molecular weight. This can be explained, according to Garcia *et al.*, (1989), the presence of the oxidation process characteristic of humification.

In general, it is accepted that the E_4/E_6 ratio is an indicator of the intensity of humification (Schnitzer and Khan, 1972; Flaig *et al.,* 1975). The lower the ratio, the compost is mature, more humus particles (not particulate organic matter) are large and complex. Conversely, the higher the ratio, the lower the compost is mature. This report spectroscopic E_4/E_6 of humic acids relatively low (Freppaz *et al.,* 2002), less than 5, indicating good conditions for humification of HA of high molecular weight with a predominance of aromatic molecules in their structure so the presence of humic acids, hence a decomposed, so that a ratio of greater than 5 indicates the presence of fulvic acids (Duval, 1993) and thus a less advanced decomposition and a non-stabilization of humic substances formed in the reactions with the parts of the mineral ground (Bech *et al.,* 2002).

By comparing the characteristics of humic acids of our composts (Carbon - E_4/E_6) with those of the soil taken as reference (mean values of soil humic acids are indicated as follows: C = 56.2% - E_4/E_6 = 5 by Schnitzer, (1972, 1978), these composts are characterized by a lower content of carbon. The result for the ratio E_4/E_6 is in agreement with that obtained by Amir, (2005), for composted sludge but whose carbon contents are higher than those observed in our compost. This can probably be attributed to the richness of our raw materials peptide compounds, as well as the low degree of aromaticity of young humic material (Riffaldi *et al.,* 1983). On the other hand, the E_4/E_6 ratio of HA is comparable to the reference soil. This may be consistent with the suggestion proposed by Riffaldi *et al.,* (1983), and Piccolo *et al.,* (1990), about the production of oxidized humic macromolecular structures in sewage sludge.

Weight Composition of humic substances (humic matter)

Concerning the composition by weight of the different fractions of humic substances, there are no significant differences between treatments at the beginning and at the end of composting (150 days) (Figure 14). All treatments had a slight decrease in the amount of all fractions of humic substances at 150th day except that of the FA. The evolution of the amount of HA is however almost constant. Witter and Lopez-Real (1987) have found that the amount of humic acid extracted from a mixture of straw and sewage sludge did not change during 52 weeks of composting. However, according to Morel *et al.,* (1985), the amount of humus extract is not a good indicator of the biomaturity for all types of composts. It should be added that the extraction of humus varies with the age and maturity of the compost.

Many researchers have suggested the indices of maturity based on the monitoring of humic substances, HA and FA. However, because the contents of HA and FA normally vary depending on the source of raw materials, it was limited in its use as an indicator of maturity in different composts. Among the indices of humification proposed, Inbar *et al.,* (1990). Jimenez and Garcia, (1992), reported that the C_{HA}/C_{FA} ratio was one of the best parameters related to the degree of compost maturity and the best indicator of maturity and humification. They considered that a value of C_{HA}/C_{FA} ratio higher than 1.6-1.9 indicates the maturity of the compost (city-refuse and sewage sludge composts). In our study, the C_{HA}/C_{FA} ratio of all of the composts taken from the end of the composting process (150 days) was higher (1.86-2.26) indicating that the maturity of compost was satisfied for any agricultural uses (Table 1). Furthermore the ratio of carbon humic acid (C_{HA}) /carbon of fulvic acids fractions (C_{FF}) are close to 1. According to Bech *et al.,* (2002), the ratio of C_{HA} / C_{FA} can be close to 1, and the humic characteristics should be always good, but the AH will have a low molecular weight, similar to the structure of FA (E_4/E_6 ratio close to 6). This may be due to HA with molecules similar in size and structure to those of FA.
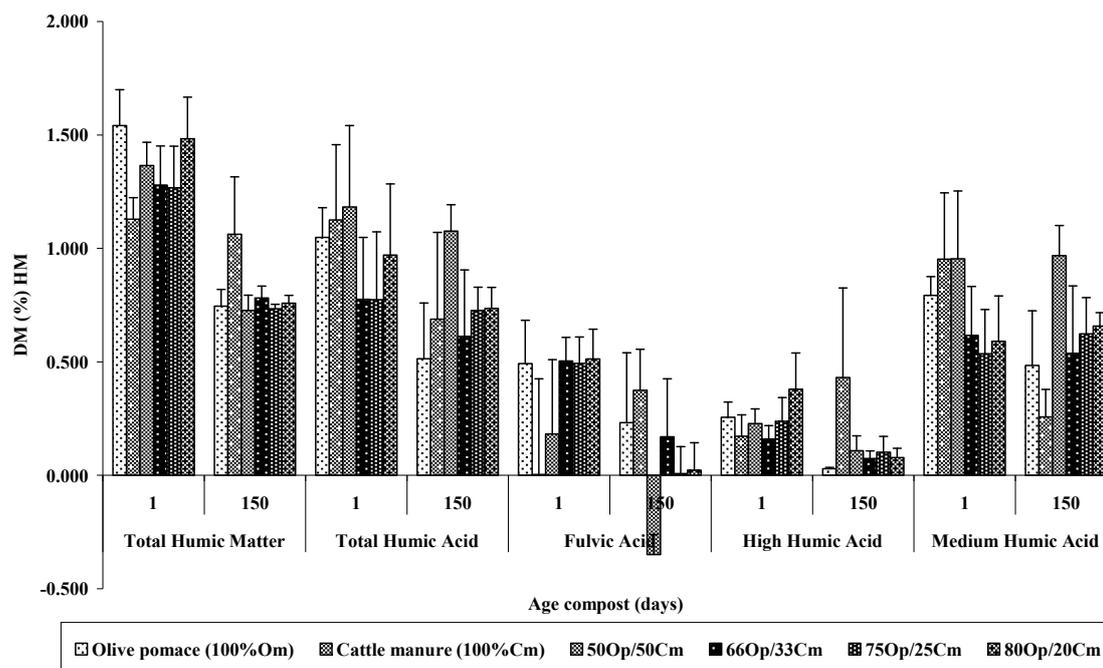
Fig. 14: Percentage of total humic matter, different humic acids, fulvic fraction, expressed as dry weight of composted mixtures of olive pomace and cattle manure

In general, the HA/FA ratio decreased in all treatments, and therefore there is an increase in the amount of FA compared to the HA. This is in contradiction with other studies on different types of waste composted (Feller *et al.,* 1983; Saviozzi *et al.,* 1988; Senesi and Brunetti, 1996). For them, during this process, there is a loss of aliphatic groups, accompanied by a training stable HA with the progression of the duration of composting and maturity. Raj and Antill, (2011), showed that the HA:FA ratio of all the composts increased significantly with composting time mainly due to pronounced decrease in fulvic acid production. However, Bernal *et al.,* (1998), and Veeken *et al.,* (2000), reported that HA:FA ratio should not be considered a good indicator for determining the degree of maturity of compost prepared by using different types of farm wastes.

Table 1: Changes of humic acid / fulvic acid (C_{HA}/C_{FA}) and fulvic acid / humic acid (C_{FA}/C_{HA}) ratios during composting of mixtures of olive pomace and cattle manure.

Treatments	Humification index (HI)							
	(C_{HA}/C_{FA})				(C_{FA}/C_{HA})			
	J1	J8	J80	J150	J1	J8	J80	J150
Olive pomace (100%Op)	3.92	8.38	3.15	2.32	0.255	0.119	0.318	0.432
Cattle manure (100%Cm)	3.07	10.62	6.75	3.56	0.326	0.094	0.148	0.281
50Op/50Cm	3.01	6.23	3.59	3.03	0.332	0.161	0.278	0.330
66Op/33Cm	3.01	5.85	2.83	2.92	0.332	0.171	0.354	0.343
75Op/25Cm	3.43	7.23	2.33	1.86	0.292	0.138	0.428	0.538
80Op/20Cm	3.22	7.26	2.71	2.26	0.311	0.138	0.370	0.443

In all treatments, HD (= C_{THM} / C_{HU}) is 77-95% and HR (C_{THM}/TOC) is 80-96% at the end of composting (150 days) (Table 2). These values are higher than those that are considered as standards (DH > 70% and HR > 35%) for well humified or well-matured organic materials (Ciavatta *et al.,* 2001). However, Raj and Antill, (2011), consider that the humic acid C (C_{HA}) as a percentage of TOC (C_{HA}/TOC x 100) > 30% may be proposed to asses the maturity of composts prepared using a mixture of different farm and agro-industrial wastes. The ratio of carbon of fulvic acids -calculated by difference- to humic acids (C_{FA} / C_{HA}) varies between 0.2 to 0.4, and which is less than 0.5. This indicates a good humification of compost. The rate and degree of humification are highest in manure control compared to other treatments. These values are higher than those obtained by Amirante *et al.,* (2002), for compost (olive pomace 82%, chicken manure 10% and straw 8%). For these authors, the bio-oxidative process of composting the mixture allowed obtaining an organic amendment with a good degree of humification at 87%. For Estrada *et al.,* (1987), the C_{FA} /C_{HA} ratio is proposed to estimate or assess the maturity of humus, but this is very variable between different composts of different origin. The DH varies with the nature of the raw materials, for example, lignin-rich materials tend to yield more humus than lignin-poor materials. At

the same time, the extractability of the humus varies with age or maturity, and is influenced by the presence of clay minerals and metals with which it forms insoluble complexes. The amount of extractable humus is therefore not a good indicator of the biomaturity of all types of composts (Morel *et al.,* 1985). For example, Witter and Lopez-real, (1987), found that the extractable humic acid (HA) content of a straw and sludge mixture did not vary during 52 weeks of composting. The index of polymerization (IP) decreased during the process of composting, which can be explained by the reduction in the representation of the fulvic fraction. Furthermore, for Saviozzi *et al.,*(1988), these dependent parameters were not considered as indicators of compost biomaturity of all types of waste or residues.

Table 2: Maturation indices (IP, HD, HR) calculated by carbon analyses of mixtures of olive pomace and cattle manure after 150 days of composting

Treatments	Index of Polymerization (IP) (C_{FF}/C_{THM})	Humification Degree (HD) (C_{THM}/C_{HU})*100	Humification Rate (HR) (C_{THM}/TOC)*100
Olive pomace (100%Op)	0.359	77.38	80.62
Cattle manure (100%Cm)	0.485	150.5	154.0
50Op/50Cm	0.449	95.58	96.21
66Op/33Cm	0.402	85.86	86.66
75Op/25Cm	0.404	81.92	82.76
80Op/20Cm	0.423	82.49	82.23

By setting the number of humic fractions of different treatments or NFH, the olive pomace control is as type "3 2 1", and the fulvic acids predominate. As against, for mixtures composted, the NFH is type "2 1 3", humic acids are dominant and they are mostly formed by medium molecular weight humic acids. Therefore, according to the differentiation of compost quality established by Petrik laboratories (www.petrik.com 2002) and based on the NFH, our composted mixtures can be used on soils with a pH 5.5-6.5 and whatever the content of the soil organic matter, and they can be used on soils with pH <5.5 but the organic matter content must be more than 4%. As against, the olive pomace composted in our study can be used on soils with pH between 6.6 and 8 and even higher than 8 and whatever the organic matter content of the soil. However, the percentage total humic of all treatments is below 20%, but according to laboratories Petrik, quality compost should have a percentage total humic greater than 20%. Hence, according to this calculated parameter, our composted mixtures do not reach the expected humic quality.

Conclusion

The results indicate that, under our experimental conditions, all mixtures of olive pomace and cattle manure are suitable for composting seen humic parameters evaluated. However, the quantity and quality of humus in the compost product is considered an important indicator of the stability and maturity of organic matter. Humic characterization shows that the duration of 3 months seems sufficient for the production of mature compost which is in contradiction with the C/N ratio determined on the same material in another study by Haddad et al., (2012). This implies the need to continue the composting process for a longer period. Indeed, the duration of composting depends on the source materials used.

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