

## Chemical Differentiation of Domestic Sewage Sludge and Cattle Manure Stabilized by Microbioreactors: Study by Pyrolysis Coupled to Gas Chromatography Coupled to Mass Spectroscopy

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The aim of the present study was to evaluate the chemical alterations in substrates of vermicomposts from domestic sewage sludge and from cattle manure, besides its quality as an organic fertilizer. Elemental and infrared analysis and UV-Vis spectroscopy were used for their characterizations. Chemical characteristics determined in the vermicomposts indicate that they can be used as organic fertilizers, mainly with regard to organic matter content, pH, C/N ratio, and nitrogen and phosphorus levels. The main constituents of the humic substances extracted from the vermicomposts were obtained by pyrolysis coupled to gas chromatography-mass spectrometry (GC/MS), showing that these compounds are quite similar, despite being produced by domestic sewage sludges or by cattle manure.

**Keywords:** Vermicompost, humic substances, FTIR, UV-Vis, pyrolysis

### Introduction

Many composts obtained from cattle manures, domestic sewage sludges or household wastes, as well as other organic wastes, can be applied in agriculture soils, after reaching its chemical stability. Besides, this practical can be a really great tool for an adequate residue discharge and represents a management strategy that could counteract depletion of organic matter in soils. The addition of organic compost to soils requires that it presents an adequate degree of maturity, which implies stable organic matter content and the absence of phytotoxic pathogenic compounds for plants or animals.<sup>1,2</sup>

Sludge is a labile putrescible material which must undergo a heterotrophic decomposition process and be subject to humification before becoming stabilized substances. An understanding of the principles underlying these processes may give raise to better management plans for the application of the sludge to soil.<sup>3,4</sup>

A method of a biological treatment of organic waste, which involves *Eisenia foetida* earthworm, has been gaining more scientific interest, due to its speed and efficiency.<sup>1,4-6</sup> Vermicomposting process requires a relative low cost technology for the processing or treatment of organic wastes. Earthworms feed on organic matter and utilize only

a small amount for their body synthesis and excrete a large part of the consumed materials in a partially digested form called coprolites.<sup>1</sup>

The earthworms act as microbioreactors leading to various several unit operations that conduce the stabilization of the recent organic matter, transforming it in a more refractory material. Firstly the earthworms swallow the stuff, which is constituted from organic matter plus particulate material, moistening it with their salivary secretions and leading it to the gizzard. The gizzard, which acts as a stone mill, promotes the breakdown of the material. Then, the so crushed material goes into the intestine where it undergoes an enzymatic attack due to the presence of a large content of microorganisms.<sup>3</sup>

Food quality influences not only the size of earthworm populations but also their growth and reproduction rates. It has been reported for some vermicomposting earthworm species that different food diets can affect their growth, reproduction or both,<sup>7,8</sup> and moreover the impact of earthworms on the mineralization and leaching of nitrogen depends on carbon availability.<sup>9</sup>

The presence of humic acids (HA) in earthworm-composted materials has been widely demonstrated based on the vermicompost fraction isolated by the conventional alkaline extraction/acid precipitation method.<sup>8</sup>

It is well known that the quality of composts depends on several factors such as: type of substrate (organic

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residues), aeration, humidity, pH, temperature, and the earthworm species used for vermicomposting.<sup>10</sup> Therefore, it is necessary to evaluate specific characteristics, as composition and abundance of microorganisms, organic C, total N, P and K content, HA and enzymatic activities, in order to evaluate the dynamics of vermicomposting.<sup>10,11</sup>

It has been suggested that the vermicomposting process itself is an effective alternative to eliminate application for sanitising composts from human pathogens, being an excellent alternative to stabilize organic compounds such as sewage sludge.<sup>12</sup> Previous studies have shown that the vermicomposted sample can reach safe levels of pathogens removal, besides the beneficial soil microbes that the earthworms leave behind.<sup>13-15</sup>

Humic substances are formed through chemical and biological transformation and metabolic activity of microorganisms and consist of humic acids, fulvic acids and humin.<sup>16</sup> Chemical and physical characteristics of humic acids and fulvic acids extracted from different locations are dependent on many factors, such as the merits of the original material. In nature, the process of humification is slow and gradual and it can take thousands of years to occur, leading to humic substances with different structural characteristics.<sup>17</sup> The process of composting through the metabolism of the earthworms occurs in a short period of time, leading the recent organic matter to a state of stabilization similar to the one that occurs in the environment if compared to humic substances originated from peat or soil.<sup>18,19</sup>

Pyrolysis coupled to gas chromatography-mass spectrometry (GC/MS) has been used for prediction and identification of the main chemical constituents of the humic acids extracted from several materials such as soils, peat, vermicompost, etc.<sup>20</sup> This process results in a set of small molecular species, which are related to the composition of the original sample, as attested by Marinari *et al.*<sup>21,22</sup>

These small molecules are used to qualitatively identify the unique structure of macromolecules, through adequate and proper standardization, and to provide quantitative information on its composition.<sup>23</sup> As the thermal degradation of macromolecules often produces complex mixtures of smaller molecules, it is necessary to use other related techniques such as chromatography to promote the separation of the species produced.<sup>24</sup> When this association or coupling also includes mass spectrometry, the species produced can be identified.

A greater understanding of the chemical structure of humic fraction of organic matter is of extreme importance, since it attributes much of the fertilizing potential of the material to be added in the soil, moreover, understanding the

structural differences between the humic fractions arising from different organic wastes is extremely important for better comprehension of the vermicomposting process and the improvement of its use.

To evaluate the chemical characteristics of the vermicomposts obtained from cattle manure and from domestic sewage sludge, we used pyrolysis coupled to GC/MS, to identification of the major chemical constituents of the humic acids, to understand how the initial material interferes on the final product. In addition, it was evaluated the use of the final product (vermicompost) as an organic fertilizer, turning unwanted waste into a raw material, that is, giving it a sustainable ending.<sup>25</sup>

## Experimental

### Site and experiment description

The sludge used in the experiment was collected in the treatment plant of São Carlos/SP/Brazil. The cattle manure was from a farm located in the region of São Carlos/SP/Brazil. The experiments were conducted in boxes (70 cm height, 70 cm width and 70 cm deep). So, the vermicomposting consisted of these two kinds of substrates: cattle manure and domestic sewage sludge. Moisture was maintained to about 48-53%. Four hundred earthworms (*Eisenia foetida*) approximately the same size (6-7 cm) were added in each box. Homogenized sample mixtures were vermicomposting for 90 days. The experimental boxes were kept under shade and covered with straw to avoid direct sunlight. Earthworms were removed manually at the end of the experiment.

### Characterization of the vermicomposts

The chemical characterization of the vermicomposts were based on the following parameters: pH in CaCl<sub>2</sub>; total Kjeldahl nitrogen (TKN), Hach® method 399; phosphorus, Hach® method 480; total organic carbon (TOC) via Shimadzu® TOC-V<sub>CPH</sub>; cation exchange capacity (CEC), through the occupation of active sites to exchange with hydrogen ions in solution 1 mol L<sup>-1</sup> of glacial acetic acid; organic matter content (OM) and humidity (U), gravimetrically and concentration of viable eggs of helminths, using the method of Yanko.<sup>26</sup>

### Extraction and purification of the humic and fulvic acids from vermicomposts

The extraction and purification of humic acids (HA) and fulvic acids (FA) were performed according to the conventional methodology suggested by the international

humic substances society (IHSS).<sup>27</sup> The HA ash content was determined by burning up the samples in a crucible of platinum, at 560 °C for 4 hours. The levels of ash were below 5%, indicating an acceptable degree of purification for subsequent use of samples for elemental analysis, which is directly affected by the degree of purification of samples, since the oxygen content is obtained by difference with respect to carbon, hydrogen, nitrogen and sulfur.

#### Characterization of the humic and fulvic acids from vermicomposts

Elemental analysis were performed in a Carbo Erba instruments® EA 1110 CHNS-O. The oxygen content was obtained by difference in an ash-free basis.<sup>27</sup> The ratio between the absorbance at 465 and 665 nm,  $E_4/E_6$  ratio, was measured in a 0.025 mol L<sup>-1</sup> sodium bicarbonate solution. Approximately 5.00 mg of HA and FA were dissolved in 50.00 mL of NaOH solution pH 8.40. A spectrometer for UV-Vis Hitachi U3501® with a quartz cuvette of 1 cm optical path was used.<sup>28</sup>

The infrared spectra were obtained in tablets of KBr, with about 0.50 mg of sample to 200.00 mg of KBr. The spectra were analyzed in a Bomem MB-102® spectrophotometer with Fourier transform. Making up 32 scans from 4000 to 400 cm<sup>-1</sup> with resolution of 4 cm<sup>-1</sup>.<sup>19</sup>

All measurements were performed in triplicate and the values expressed as the mean ± standard deviation (SD). The statistic treatment of Student for a confidence level of 95% was done.

#### Pyrolysis analysis on GC/MS

To carry out the pyrolysis it was used a pyrolyser microfurnace at an internal temperature of 610 °C and an interface temperature of 400 °C. A sample of 0.50 mg of humic acids was weighed into a platinum crucible and introduced into the pyrolyser frontier lab. PY 2020IS. A fused silica capillary column of 30 m long, 0.25 mm I.D. with Rtx-5MS stationary phase, film thickness 0.25 µm were used. Helium was used as a carrier gas. The gas chromatographic (GC) conditions were as follow: pressure of 100.00 kPa, split 100:1, heating ramp starting at 40 °C for 1 min and increased 6 °C *per* minutes until a temperature of 250 °C (resulting in an analysis time of 60 min), detector temperature 200 °C, detection starting at 0.10 min and ending at 60 min, with He as carrier gas. The pyrolyzer was connected to a 2010 gas chromatograph with a GCMS-QP2010 plus, series C7050044 quadrupole mass spectrometer (Shimadzu corp) operated in electron impact ionization (EI) mode. Compounds were monitoring

at 45-300 *m/z*, identified based on their mass spectra, GC retention times, and comparison with library mass spectra using two libraries of mass spectra standard computer: NIST05 and NIST05s.

## Results and Discussion

### Vermicompost characterization

The chemical characteristics of the vermicomposts from sewage sludge (SSV) and from cattle manures (CMV) were analyzed and evaluated. The data are presented on the Table 1. The moisture content of the SSV reached (48.19 ± 0.33)% and of the CMV reached (53.36 ± 2.97)%, both are close to the maximum limit of 50% established by brazilian legislation for composted solid wastes used as fertilizer.<sup>29</sup>

The average OM content for SSV and for CMV ranged from (48.19 ± 0.33)% to (27.46 ± 1.09)%, respectively. Brazilian legislation establishes an organic matter content of a minimum of 40% for fertilizers. Therefore SSV attends these needs.<sup>29</sup> The C content of SSV and CMV were (25.26 ± 0.79)% and (11.31 ± 2.34)%, respectively. Data revealed a significant difference between both vermicomposts. The SSV had the lowest C/N ratio value, due to its N content being the highest. However in general the final content of nitrogen in vermicomposting is dependent on the initial nitrogen present in the waste and the extent of its decomposition.<sup>30</sup> According to Suthar,<sup>31</sup> rapid mineralization as well as decomposition of organic matter, in vermicomposting process, lowers the C/N ratio. Carbon/nitrogen ratio of substrate material reflects the organic waste mineralization and stabilization during the process of composting or vermicomposting. The loss of carbon as carbon dioxide through microbial respiration and simultaneous addition of nitrogen by worms in the form of mucus and nitrogenous excretory material lowered the C/N ratio of the substrate. C/N ratio below 20 is an indicative of acceptable maturity.

The pH provides valuable information about the decomposition stage of organic matter during the humification process. The raw organic matter is naturally acidic. Values of vermicompost pH close to neutrality indicate that biological stabilization of the material has occurred.<sup>32</sup>

The CMV was slightly alkaline. This property helps to neutralize the deleterious effects of heavy metals possible present in the environment and ameliorate the soils because, in general, brazilian soils are acid, leading to the need of using limestone to correct soil pH.<sup>33</sup> Considering these soil characteristic, the addition of CMV could decrease or

**Table 1.** Chemical characteristics of the vermicomposts prepared from domestic sewage sludges (SSV) and from cattle manure (CMV)

Parameter	SSV <sup>a</sup>	CMV <sup>a</sup>	Legislation <sup>29</sup>
Moisture / %	48.19 ± 0.33	53.36 ± 2.97	50.00 (max.) <sup>b</sup>
pH	4.98 ± 0.02	8.43 ± 0.03	6.00 (min.) <sup>c</sup>
HA / %	0.93 ± 0.08	2.60 ± 0.22	–
FA / %	0.21 ± 0.01	0.68 ± 0.05	<sup>d</sup>
CEC / (cmol kg <sup>-1</sup> )	13.49 ± 0.34	24.64 ± 0.22	–
OM / %	48.19 ± 0.33	27.46 ± 1.09	40.00 (min.)
Total N / %	2.41 ± 0.95	1.12 ± 0.18	1.00 (min.)
Total C / %	25.26 ± 0.79	11.31 ± 2.34	15.00 (min.)
C/N ratio	9.88 ± 0.24	11.69 ± 0.54	12.00 (min.)
Total P / (mg kg <sup>-1</sup> )	77.96 ± 0.66	7.89 ± 0.38	–

<sup>a</sup>Means of three replicates ± standard deviation; <sup>b</sup>max. = maximum; <sup>c</sup>min. = minimum; <sup>d</sup>it is not defined on Brazilian legislation.

even avoid the limestone addition. The shift in pH domestic sewage sludges for higher pH values after vermicomposting could be due to microbial decomposition during the process of vermicomposting<sup>31</sup> and, in addition, due to the production of CO<sub>2</sub> and organic acids by microbial decomposition, during the bioconversion process.<sup>30</sup> Similarly, it was pointed out that a shift in pH is related to the mineralization of the nitrogen and phosphorous into nitrites/nitrates and orthophosphates and bioconversion of the organic material into intermediate species of the organic acids.<sup>34</sup>

The cation exchange capacity of wastes ranged from 13.49 (domestic sewage sludge) to 24.64 (cattle manure) cmolc kg<sup>-1</sup>, reflecting certain variation in the ability of these organic materials in adsorb cations. This attribute also reflects on the differences into the degree of maturation of the vermicompost.

It is worth to note that the earthworms are able to eliminate the helminth ova. The domestic sewage sludge presents 10.00 ova *per* 4 g of dry basis. After the vermicomposting this value fell down to < 0.25 ova *per* 4 g.

According to the Brazilian legislation, until 1.00 ova *per* 4 g is allowed. That means the residue so produced could be applied to agriculture, at least in this sanitary aspect.

#### Humic and fulvic acids characterization

Table 2 show the results of the composition analysis of the humic and fulvic acid extracts. It is well known that alkali solution dissolves protoplasmic and structural components from fresh and incompletely humified plant residue<sup>34,35</sup> with the formation of humus-like substances.

The humic acid fraction generally increases during vermicomposting, demonstrating that the humification of the organic matter takes place. Although in the humic acids obtained from SSV (SSV-HA) this tendency was not so clear.

The HA content present in both vermicomposts are relatively high, indicating the dominant contributors to these humic substances, especially in CMV in which the raw materials are from vegetables (Table 2).

**Table 2.** Chemical characteristics of the humic and fulvic acids obtained from the vermicomposts prepared from domestic sewage sludges and from cattle manure

Parameter	SSV-HA <sup>a</sup>	CMV-HA <sup>b</sup>	SSV-FA <sup>c</sup>	CMV-FA <sup>d</sup>
HA / %	0.93 ± 0.08	2.60 ± 0.22	–	–
FA / %	–	–	0.21 ± 0.01	0.68 ± 0.05
E <sub>4</sub> /E <sub>6</sub> ratio	3.26 ± 0.22	6.35 ± 1.28	16.18 ± 0.24	13.01 ± 0.14
C / %	48.83 ± 0.83	50.32 ± 0.11	24.47 ± 0.022	27.57 ± 0.22
H / %	7.51 ± 0.03	6.10 ± 0.04	4.37 ± 0.09	8.04 ± 0.15
N / %	8.14 ± 0.08	3.86 ± 0.01	2.09 ± 0.03	2.41 ± 0.02
S / %	1.16 ± 0.07	1.07 ± 0.05	0.92 ± 0.06	0.90 ± 0.02
O / %	34.36 ± 0.12	38.65 ± 0.10	68.15 ± 0.32	61.08 ± 0.40

<sup>a</sup>SSV-HA: humic acids from sewage sludge; <sup>b</sup>CMV-HA: humic acids from cattle manure vermicompost; <sup>c</sup>SSV-FA: fulvic acids from sewage sludge;

<sup>d</sup>CMV-FA: fulvic acids from cattle manure vermicompost.

According to previous investigations, the absorptivity of humic materials increases with rising molecular weight percentage of C, degree of condensation and ratio of aromatic C, to aliphatic C, whereas the absorptivity of fulvic acids from various sources are fairly similar.<sup>36</sup>

The comparison between the samples studied showed that the percentage of organic C was slightly higher for humic acids obtained from CMV (CMV-HA):  $(50.32 \pm 0.11)\%$ , and slightly lowest for the SSV-HA:  $(48.83 \pm 0.83)\%$ .

The ratio of optical densities or absorbances of aqueous humic and fulvic acids solutions at 465 and 665 nm ( $E_4/E_6$ ) is widely used by scientists for the characterization of these materials. The decline of the  $E_4/E_6$  rate is directly related with the increase in molecular weight and condensation of aromatic carbons, and it is inversely related to aromaticity.<sup>27</sup>

Samples of CMV-HA showed values for the  $E_4/E_6$  rate higher than the values obtained for SSV-HA, which suggests less condensation of the molecule and higher proportion of aliphatic structures, Table 2. According to Chen *et al.*,<sup>37</sup> low  $E_4/E_6$  values would reflect relatively large particle size and higher molecular weight. In addition,  $E_4/E_6$  is also related to an increase in the oxygen content of the humic substances.<sup>38</sup> The highest  $E_4/E_6$  ratios reached by FA in comparison to HA point to the presence of small molecular species, with relatively high O-containing functional groups. These values are in concordance with the results presented here for (CMV-HA) sample.

The C, H and O contents are in the range commonly reported for humic acids.<sup>27</sup> Elemental composition of both humic acids is comparable although SSV-HA contains higher N species.<sup>39</sup>

The atomic ratios of H/C, O/C and C/N are often used to identify humic substances from different sources, to monitor structural changes of humic substances in different environments, and to elucidate structural formulae for humic substances.<sup>40</sup>

Results of elemental analysis allowed estimating H/C, O/C, C/N atomic ratios for humic acids (Table 3), giving additional information about the humification processes in the samples. Atomic ratios have been preferably used to establish the degree of condensation, diagenetic

transformations, as well as environmental conditions under which they were created.<sup>27</sup>

H/C atomic ratio is often associated with the degree of condensation or aromaticity and turn with the degree of humification, with larger values indicates greater amounts of aliphatic groups, typical of less humified materials. O/C and C/N atomic ratios, respectively, indicate the content of oxygenated groups and the degree of the incorporation of N into the humic structure. In general, more humified samples exhibit larger values of O/C and C/N.<sup>27</sup>

The value of H/C ratio is an indicative (in general) of the degree of maturity in humic substances.<sup>41,42</sup> Even indirectly, lower H/C reflects the existence of more condensed aromatic rings or substituted ring structures. Atomic H/C ratios show a value of  $1.46 \pm 0.01$  for CMV-HA. This ratio suggests a degree of aromaticity for CMV-HA higher than for SSV-HA ( $1.85 \pm 0.01$ ).

The O/C ratio is considered an indicator of carbohydrate and carboxylic contents of humic substances and it can be used to compare HA and FA. The O/C ratio is also representative of the degree of humification. A decrease of this ratio commonly suggests an increase in the aromatic condensation. By comparing the O/C ratio of HA and FA from different origins, a progressive decrease in aromaticity suggests that the maturity of the humic substances is higher. The O/C and O/H ratios are indicative of oxidation and follow parallel trends. The average for both ratios was higher for the FA fraction than for the HA fraction because of the higher overall level of oxidation in the FA fraction.<sup>27</sup> CMV-HA presents more N-containing groups ( $15.22 \pm 0.04$ , compared to  $6.99 \pm 0.08$  for SSV-HA), indicating a higher maturity reached during the composting of cattle manure.

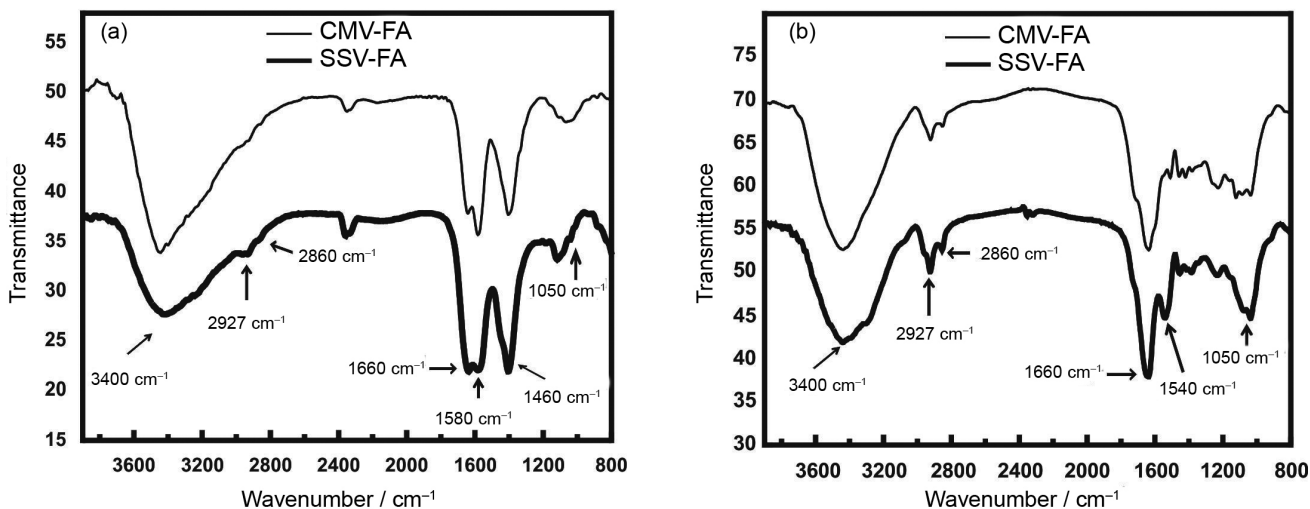
In order to evaluate differences in the structure of all materials, Fourier transformer infrared (FTIR) spectroscopy was used, whereas this technique is a qualitative tool for monitoring chemical groups and bands of humic substances.<sup>43,44</sup> The FTIR spectra of the SSV-HA and CMV-HA are shown in Figure 1.

The FTIR spectra of the humic acids showed rather similar adsorption bands, since many similar structural and functional groups coexist in humic acids, absorption band at  $3400\text{-}3440\text{ cm}^{-1}$  assigned to (OH) from bound

**Table 3.** Atomic ratios for humic and fulvic acids

Parameter	SSV-HA <sup>a</sup>	CMV-HA <sup>b</sup>	SSV-FA <sup>c</sup>	CMV-FA <sup>d</sup>
H/C	$1.85 \pm 0.01$	$1.46 \pm 0.01$	$2.14 \pm 0.02$	$3.50 \pm 0.04$
O/C	$0.53 \pm 0.00$	$0.58 \pm 0.00$	$2.09 \pm 0.03$	$1.66 \pm 0.02$
C/N	$6.99 \pm 0.08$	$15.22 \pm 0.04$	$13.66 \pm 0.14$	$13.34 \pm 0.05$

<sup>a</sup>SSV-HA: humic acids from SSV; <sup>b</sup>CMV-HA: humic acids from CMV; <sup>c</sup>SSV-FA: fulvic acids from SSV; <sup>d</sup>CMV-FA: fulvic acids from CMV.



**Figure 1.** Infrared spectra of CMV-FA and SSV-FA (a), and of CMV-HA and SSV-HA (b).

water and phenolic and carboxylic groups, symmetrical and anti-symmetrical stretching assigned to C–H of aliphatic or cyclic groups at 2950 cm<sup>-1</sup>, a strong peak at 1640–1650 cm<sup>-1</sup> assigned to conjugated C=C and stretching of COO<sup>-</sup> groups, and a broad adsorption band at 1220–1230 cm<sup>-1</sup> assigned to C–O stretching and OH bending from carboxylic acids, alcohols, esters and ethers.<sup>45,46</sup>

A broader adsorption band at 1035 cm<sup>-1</sup> suggests stretching of groups C–O. The broad band between 1550–1650 cm<sup>-1</sup> resulted from the overlap of several vibrational modes of HA, and is assigned to the asymmetric COO<sup>-</sup> stretch of carboxyl groups located at different environments in the HA. In this region we also find aromatic C=C stretch, and the presence of various functionalities containing C=O, like quinones, ketones, ethers and proteins.

The SSV-HA spectra indicate some aromatic units (1538 cm<sup>-1</sup> stretch) and a defined lignin pattern additional band at 1460 cm<sup>-1</sup>. Absorption bands at 1234 cm<sup>-1</sup> of SSV-HA was attributed to phenol deformations and absorption due to the C–O stretching mode of polysaccharides exhibited bands between 1170 and 1000 cm<sup>-1</sup>. The main differences between FTIR spectra from HA were found in the region between 1508 and 1034 cm<sup>-1</sup>.

The broad band at 1400 cm<sup>-1</sup> could be due to the absorption of some vibrational modes such as symmetric C–H bending of terminal methyl, isopropyl, and tertiary butyl groups, to aromatic C–C stretch, and to carboxylate symmetric stretching.

The FTIR spectra show the difference between the humic substances from CMV and the humic substances from SSV. The spectra of CMV-FA and SSV-FA present the same bands but with different intensities, pointed that the results for both samples are similar. Nevertheless

spectra of CMV-HA and SSV-HA also differ regarding to the intensities of the bands.

Results suggest that the chemical constitution of the humic fractions studied are basically similar, despite slightly different. In this way, to better understand the structure of these compounds, pyrolysis technique was used coupled to GC/MS to fragment identification.

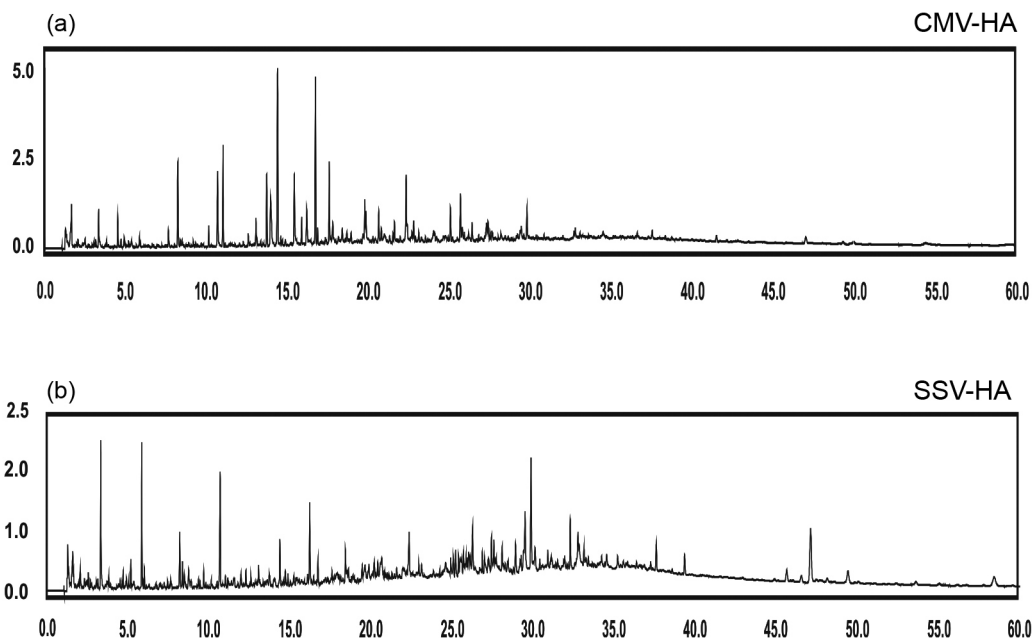
Analytical pyrolysis process results in a set of small molecular species which are related to the composition of the original sample. Such small molecules are used to qualitatively identify the original structure of macromolecules through adequate standards and provide quantitative information of its composition.

In Figure 2 two representative pyrograms of HA are presented.

All pyrograms obtained for the HA and FA studied were very similar, changing only the relative amount of each product (peak area) suggesting that both HA have the same groups, however, in different quantities. In comparison to FA, HA present peaks with longer times, what suggests that HA present higher molecular weight species.

Table 4 shows the main five possible assignments for the pyrolysis products, for each peak pyrogram found in the humic fractions studied as well the percentage of similarity, the fragments of intensity greater than 50%, molecular formulae and presence or not in FA and in HA.

The pyrograms showed, generally, 56 peaks. For assignment of the possible fragments, only peaks of higher intensity and better resolution were evaluated. Here, for each peak chosen, it is reported the compound with the highest percentage of similarity (Table 4). However, the top 5 fragmentation spectra for each peak pyrogram were evaluated (data no shown).



**Figure 2.** Representative pyrograms of HA from cattle manure (a) and from domestic sewage sludge (b).

**Table 4.** Possible assignments for the pyrolysis products, percentage of similarity (%), molecular formula, presence (+) or absence (-) in FA and in HA

Possible assignments	Molecular formulae	Similarity / %	<i>m/z</i> (observed above 50% intensity of the spectrum)	FA	HA
Toluene	C <sub>7</sub> H <sub>8</sub>	95	92; 91	+	+
2-nonenal, (E)	C <sub>9</sub> H <sub>16</sub> O	84	70; 55	+	+
Ethylbenzene	C <sub>8</sub> H <sub>10</sub>	86	91	+	+
Ethylbenzene	C <sub>8</sub> H <sub>10</sub>	86	91	+	+
Exo-norbomyl alcohol	C <sub>7</sub> H <sub>12</sub> O	77	94; 79; 68; 67; 66	+	+
4-(decyloxy)benzaldehyde	C <sub>17</sub> H <sub>30</sub> O <sub>2</sub>	73	123; 122; 121; 57	+	+
3-cyclohexene-1-ethanol, beta, 4-dimethyl	C <sub>10</sub> H <sub>18</sub> O	84	95; 94; 93; 68; 67	+	+
4H-1,4-epoxy-4a,7-methanonaphthalene, 1,5,6,7,8a-hexahydro-, (1.alpha., 4a.alpha., 7.alpha., 7a.beta)-	C <sub>11</sub> H <sub>14</sub> O	75	162; 133; 105; 95; 94; 91; 81; 79; 77	+	+
Bicyclo[3.1.0]hex-3-en-2-one, 4-methyl-1-(methylethyl)	C <sub>10</sub> H <sub>14</sub> O	81	108; 107	+	+
Phenol, 2-methoxy-	C <sub>7</sub> H <sub>8</sub> O <sub>2</sub>	93	124; 109	+	+
Phenol, 4-methyl	C <sub>7</sub> H <sub>8</sub> O	85	108; 107	+	+
1-undecene	C <sub>11</sub> H <sub>22</sub>	78	83; 70; 69; 56; 55	+	+
Azulene, 1,2,3,5,6,7,8,8 <sup>a</sup> -octahydro-1,4-dimethyl -7-(1-methylethenyl)-, [1S-(1.alpha., 7.alpha., 8a.beta.)]	C <sub>15</sub> H <sub>24</sub>	64	108; 107; 105; 93; 91; 81; 79; 55	-	+
6,7-dimethyl-3,5,8, 8a-tetrahydro-1H-2-benzopyran	C <sub>11</sub> H <sub>16</sub> O	79	134; 119; 91	-	+
2,4-cycloheptadien-1-one, 2,6,6-trimethyl	C <sub>10</sub> H <sub>14</sub> O	81	107; 91	-	+
Phenol, 2-methoxy-4-methyl	C <sub>8</sub> H <sub>10</sub> O <sub>2</sub>	90	138; 123	-	+
1-pentadecene	C <sub>15</sub> H <sub>30</sub>	86	97; 83; 70; 69; 57; 56; 55	-	+
Dodecane, 2,6,11-trimethyl	C <sub>15</sub> H <sub>32</sub>	79	71; 57	-	+
Bioalletrin	C <sub>19</sub> H <sub>26</sub> O <sub>3</sub>	67	123; 91	-	+
(7S) – trans-bicyclo[4.3.0]-3-nonen-7-ol	C <sub>9</sub> H <sub>14</sub> O	69	120; 92; 91; 79	-	+
Phenol, 4-ethyl-2-methoxy	C <sub>9</sub> H <sub>12</sub> O <sub>2</sub>	84	152; 137	-	+
1-pentadecanol	C <sub>15</sub> H <sub>32</sub> O	83	97; 83; 70; 69; 57; 56; 55	-	+
Benzene, 2-methoxy-1,3,5-trimethyl-	C <sub>10</sub> H <sub>14</sub> O	70	150; 135	-	+

**Table 4.** (continuation)

Possible assignments	Molecular formulae	Similarity / %	<i>m/z</i> (observed above 50% intensity of the spectrum)	FA	HA
Benzene, 2-methoxy-1,3,5-trimethyl-	C <sub>10</sub> H <sub>14</sub> O	69	150; 135	-	+
2-cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)	C <sub>11</sub> H <sub>16</sub> O	63	164; 149; 135; 122; 110; 93; 91; 79; 77; 67; 55	-	+
7-hexadecenal, (Z)	C <sub>16</sub> H <sub>30</sub> O	72	83; 81; 71; 69; 67; 57; 55	-	+
7-hexadecenal, (Z)-	C <sub>16</sub> H <sub>30</sub> O	70	83; 81; 71; 69; 67; 57; 55	-	+
1-tetradecanol	C <sub>14</sub> H <sub>30</sub> O	87	97; 84; 83; 70; 69; 57; 56; 55	-	+
Octadecane, 1-chloro-	C <sub>18</sub> H <sub>37</sub> Cl	74	71; 57	-	+
1-nonadecene	C <sub>19</sub> H <sub>38</sub>	87	111; 97; 83; 69; 57; 55	-	+
1-nonadecene	C <sub>19</sub> H <sub>38</sub>	84	111; 97; 83; 69; 57; 55	-	+
9-Octadecenoic acid (Z)-, 9-octadecenyl ester, (Z)-	C <sub>36</sub> H <sub>68</sub> O <sub>2</sub>	77	97; 96; 83; 82; 81; 69; 55	-	+
Acetic acid, chloro-, hexadecyl ester	C <sub>18</sub> H <sub>35</sub> ClO <sub>2</sub>	76	97; 83; 70; 69; 57; 56; 55	-	+

As can be seen from Table 4, there is a myriad of pyrolysis products that must be studied and analyzed so that a structural model for FA and HA could be proposed. It is worth to note also that the basic units of humic fractions studied (although presenting different degrees of maturation) are the same.

## Conclusions

The chemical characteristics of the vermicomposts such as, organic matter content, pH, H/C, O/C and C/N ratios and concentration of phosphorus, besides good sanitary health indicate the possible use of such materials as organic fertilizers. Furthermore, the humic acids from the so obtained vermicomposts were also characterized. The FTIR spectra show the difference between the humic substances from CMV and the humic substances from SSV. The spectra present similar bands but with different intensities. The data obtained from pyrolysis bring light the same conclusions: the materials have some similar characteristics but are different structurally.

Pyrolysis coupled to GC/MS was used for identification of the major chemical constituents of the humic and fulvic acids. In addition, the use of the final product (vermicompost) as an organic fertilizer was evaluated, turning undesirable wastes into raw materials with high added values, that is, giving it a sustainable environmentally ending.

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