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Understanding the Vermicompost Process in Sewage Sludge: A Humic Fraction Study

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Abstract The objective of this research was the characterization of the humic substances isolated from domestic sewage sludge (DSS) *in natura* and after three months of vermicomposting, treated with *Eisenia foetida*, in order to help the understanding about the main differences between the maturation degree of both organic residues. Elemental analysis, infrared and UV-Vis spectroscopy were used for the characterization of the humic substances. By comparing both humic substances, those from DSS *in natura* presents a great potential as fertilizer, whereas the humic substances from DSS vermicompost produced in three months shown a satisfactory percentage of humic acids, thus being able to be incorporated into the soil as a conditioner or fertilizer and thereby offering an adequate environmental destiny for this residue, transforming an unwanted material into a valuable product. It is notable to mention that the number of viable helminth ova is reduced during the process of vermicomposting. It has shown that vermicomposting process is a powerful tool to increase the stability of this residue.

Keywords Fulvic acids, Humic acids, Vermicomposting, Domestic sewage sludge

1. Introduction

Domestic sewage sludge (DSS) is an organic residue generated during the treatment of wastewater stations in the Wastewater Treatment Plant [1-3]. Among many processes of final disposal of DSS (after being stabilized), it is worth to consider the agriculture recycling of this residue due to the adequacy for agricultural and environmental health [4, 5]. Among of several sources of organic matter applied to the agriculture, the use of vermicompost has been considered as a great alternative to sustainability, therefore it is presented as a material rich in macronutrients, consequently, it can be used as fertilizer or conditioner of soils [6, 7].

Vermicomposting is the process of transformation of recent organic matter in stabilized organic matter through the action of earthworms along to the flora that lives inside their digestive dealings [8-11]. In the gut of earthworms, the not digested organic matter along to those organic matter that was not assimilated are expelled together with earth particles in the form of an organic compound rich in nutrients, easily assimilated by plants, which receives the name of coprolites [6, 5, 12].

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Humic substances are formed through chemical and biological degradation of organic matter and metabolic activity of microorganisms and consist of humic acids, fulvic acids and humin [13]. The physical and chemical 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 may take thousands of years to occur, leading to humic substances with different structural characteristics [14, 15].

The process of composting through the metabolism of the earthworms occurs in a short period of time - about 3 months -, leading the recent organic matter to a state of stabilization similar to what occurs in the environment if compared to humic substances originated from peat or soil, thanks to several enzymes that live inside the earthworms and are able to accelerate the humification process [16, 17].

This research had as main objective the comparison between the domestic sewage sludge *in natura* and the humic substances generated after 3 months from the same domestic sewage sludge vermicomposted to be used as fertilizers or soil conditioners. For sample characterization, techniques such as elemental analysis and spectroscopy in UV/Vis and infrared regions were used.

The physicochemical characterization of the DSS *in natura* and after 3 months of vermicomposting was done in order to assess the fertilizing potential of the residue. In addition, one sample of humic acids from peat was submitted to the same analysis in order to compare the humification degree of the DSS in natura and of the vermicomposted DSS.

The quantification of the number of viable helminth ova during the vermicomposting process was done in order to evaluate the sanitary conditions of the material. For its determination, the Yanko method was used [18-20].

2. Materials and Methods

Samples Preparation

Samples were collected in the Wastewater Treatment Plant of São Carlos city, SP, Brazil. The samples were from DSS previously dried at 50℃, with about 40% moisture, without being stabilized by any process, the sludge was collected *in natura*. For the vermicomposting process approximately 60 kg of the same material were used.

Preparation of the vermicomposts

To prepare the vermicomposts, boxes of 0.70 m length, 0.70 m wide and 0.70 m height were constructed. These boxes were filled up with DSS, and then approximately 1,000 worms of the specie *Eisenia foetida* per m² were inoculated in controlled conditions of humidity and temperature for the adaptation of the earthworms [21].

After the inoculation of the earthworms, the material was composted for three months. At the end of the vermicomposting process, the vermicomposts so obtained were collected and divided into samples of 100.00 g, and submitted to extraction and purification of humic acids (HA) and fulvic acids (FA). For the samples of DSS *in natura* the FA and HA were immediately extracted and purified after the collection of the material at the Wastewater Treatment Plant.

Analytical Methods

The chemical characterization of the domestic sewage sludge and of the vermicompost from domestic sewage sludge were based on the following parameters: pH in $CaCl_2$; a microwave furnace was utilized to prepare the samples for determination of both nitrogen Kjeldahl total (NKT) and phosphorus by Hach® method 399 and Hach® method 480, respectively; organic carbon (TOC) via Shimadzu® TOC-V OHC; cation exchange capacity (CEC), through the occupation of active sites to exchange with hydrogen ions in solution 1 mol L^{-1} of glacial acetic acid; organic matter content (OM) and humidity (U); and concentration of viable helminth ova, using the method of Yanko. The samples were collected each 15 days, up to 90 days, and compared to the DSS *in natura*.

Extraction, purification and characterization of HA and FA

The extraction and purification of HA and FA were performed according to the conventional methodology suggested by the International Humic Substances Society (IHSS) [22]. The HA ash content was determined by burning up the samples in a crucible of platinum, at 550℃ for 4 hours. The levels of ash were below 5%, indicating an acceptable degree of purification for subsequent use of the samples for elemental analysis. 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 [23]. The ratio between the absorbance at 465 and 665 nm, E4/E6 ratio, was measured in $NaHCO_3$ 0.025 mol L^{-1} solution. Approximately 5.00 mg of HA and FA were dissolved in 50.00 mL of solution with pH 8.40. A spectrometer for UV/Visible Hitachi U3501® with a quartz cuvette of 1 cm optical path was used. 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^{-1} with resolution of 4 cm^{-1}. Measurements were made in triplicate, and the error for a confidence interval of 95% calculated by the Equation 1:

$$E = \pm (t x \sigma)/\sqrt{n} \quad (1)$$

where, E = error, t = coefficient Student t tabulated, σ = standard deviation, n = 3 determinations.

3. Results and Discussion

Characterization of the Domestic Sewage Sludge and of its Vermicompost

For understanding which of the two materials possess a greater fertilizer potential, the physicochemical characterization of the DSS *in natura* and of the obtained vermicompost from the DSS was performed (Table 1). For comparison purposes two peats are also presented, once peat usually presents a higher fertilizing potential.

The vermicompost from the sewage sludge showed up a slender increase in the pH value, reflecting the earthworm action. According to Bidone [24], from the earthworms esophagus the calcific glands secrete calcium carbonate, controlling its content in the animal organism. The CO_2 produced through breathing is eliminated with the excess of absorbed calcium from the soil, making up $CaCO_3$, which is released outside along with the non-digested particles such as excrements.

Table 1. Physicochemical characterization of the DSS and of the obtained vermicompost from the DSS in comparison to peat [28]

Parameters	DSS	Vermicompost	Peat [28]
pH	4.45 (0.03)	5.01 (0.01)	3.52 (0.02)
CEC ($cmol_c\ kg^{-1}$)	24.54 (0.54)	17.07 (0.40)	22.50 (1.58)
OM (% weight)	57.25 (0.20)	49.23 (0.67)	65.85 (0.00)
U (% in weight)	47.90 (0.65)	47.00 (0.33)	27.56 (0.00)
TKN (mg g^{-1})	2.44 (0.03)	3.37 (0.04)	12.76 (0.00)
P (mg g^{-1})	1.12 (0.09)	1.18 (0.07)	-
TOC (% in mass)	31.00 (2.33)	21.32 (0.44)	34.90 (0.1)
Viable helmints ova 4.00 g (in a dry basis)	10.00	< 0.25	-

Mean (error) for n = 3

The vermicompost showed a lower value of TOC and CEC than the sewage sludge *in natura* and the peat. Sinha et al. [4] and Xing et al. [25] observed that substrates with high OM content can present loses of CO_2 (between 20 and 43% of TOC) up to the end of the vermicomposting. These results are in agreement with the literature that reported meaningful reduces in TOC after the inoculation of earthworms in different kinds of organic substrates [26,27]. The values of CEC are reduced due to the degradation of some carboxylic groups, that are able to contribute to the CEC, during the degradation process due to the earthworms action.

The TKN value was higher for the vermicompost than for DSS in natura, showing up the influence of the earthworms in the mineralization of the organic matter. A meaningful variation for the phosphorus value after the vermicomposting was not observed.

Characterization of HA and FA

Table 2 shows the physicochemical characteristics of HA from the DSS *in natura* and from the vermicomposted DSS. Table 3 shows the physicochemical characteristics of FA from the DSS *in natura* compared to the FA from vermicompost produced from the DSS composted for 3 months. In Table 2 is also presented a HA obtained from peat for comparison purposes [28]. Peat is formed by the low decomposition of plant and animal materials found in wetlands such as lakes, lowland rivers, coastal plains and mazurian [23]. Peat *in natura* has 90%, or more, of water in its composition, when it is dry with air this content can be reduced to 40%. According to the International Peat Society, peat is classified according to its decomposition level [29]. Comparing vermicompost with the peat, vermicompost is a very recent material that has a lesser stabilized chemical state.

Observing Tables 2 and 3 it is noticed a decrease in the HA content after the vermicomposting process, and an increase in the FA content. Therefore it suggests a continuous process; the HA from DSS is a labile material (if compared to a HA from DSS after vermicomposting), which can be transformed into FA. This fact is probably a result of a selective action of the microorganisms present in the sewage sludge.

In this work it was observed an increase on the FA content after the vermicomposting, which is in non-agreement with the literature. According to some authors it was observed that the FA content decreases after the vermicomposting [30]. The increase on the FA content here observed is an indicative of a continuous process and of the importance of the original material. Here it was compared the humic substances obtained from the same material but with different degrees of ageing: a fresh material versus a vermicomposted one.

The percentage in weight values of C, H, N, S, O and the atomic ratios H/C, O/C, C/N and (O + N)/C for HA and FA are presented in the Tables 2 and 3, respectively. The obtained ratios H/C for the HA and FA samples from the DSS vermicomposted were lower than the HA and FA samples from the DSS *in natura*, showing up a larger proportion of condensed structures in the HA and FA from the vermicomposted samples than in the fresh samples. The same happens to the O/C and (O+N)/C ratios showing up a lower quantity of oxygenated groups for the samples of vermicomposted HA and these results are similar to HA from peat.

Table 2. Physicochemical characteristics of HA from the DSS *in natura* and of the vermicomposted DSS comparing to the literature values

Parameters	Sewage sludge HA	Vermicompost HA	Peat HA [28]
Ash content m/m	3.12 (0.43)	2.39 (0.50)	1.00 (0.03)
EQ* w/w	2.37 (0.19)	1.16 (0.10)	8.60 (0.05)
EQ/OM w/w**	4.15 (0.33)	2.41 (0.21)	16.51 (0.96)
% C	39.00 (0.68)	49.02 (0.11)	55.82 (0.06)
% N	4.01 (0.15)	8.85 (0.05)	3.11 (0.04)
% H	6.31 (0.05)	8.01(0.02)	4.45 (0.09)
% S	2.31 (0.08)	1.14 (0.07)	nd
% O	48.19 (0.51)	32.98 (0.00)	36.63 (0.09)
C/N	11.87 (0.56)	7.69 (0.16)	20.96 (0.27)
H/C	1.94 (0.06)	1.65 (0.03)	0.96 (0.02)
O/C	0.94 (0.02)	0.42 (0.02)	0.49 (0.00)
(O + N)/C	0.97 (0.02)	0.56 (0.09)	0.54 (0.00)
Ratio E_4/E_6	1.65 (0.00)	3.26 (0.22)	3.77 (0.02)
Δ Log K	0.26 (0.00)	0.63 (0.02)	0.58 (0.02)
Rf	100.79 (0.02)	45.67 (1.62)	67.68 (1.61)
H	0.97 (0.1)	1.04 (0.1)	---

Mean (error) for n = 3; EQ* = Extracted Quantity of HA;
EQ/OM** = Extracted Quantity of HA normalized by organic matter content;
nd = not determined; H = index of hydrophobicity

Table 3. Physicochemical characteristics of FA from the DSS *in natura* and of the vermicomposted DSS

Parameters	Sewage sludge FA	Vermicompost FA
EQ* w/w	0.75 (0.03)	1.31 (0.05)
EQ/OM w/w**	0.21 (0.02)	0.44 (0.04)
% C	7.66 (0.68)	25.00 (0.23)
% N	0.50 (0.01)	2.13 (0.06)
% H	3.87 (0.34)	5.07 (0.08)
% S	1.06 (0.11)	0.88 (0.06)
% O	86.90 (0.42)	66.92 (0.38)
C/N	17.66 (0.42)	13.68 (0.14)
H/C	6.05 (0.40)	2.44 (0.02)
O/C	8.51 (0.09)	2.01 (0.03)
(O + N)/C	8.56 (0.09)	2.08 (0.04)
Ratio E_4/E_6	16.02 (3.32)	13.01 (0.24)
Δ Log K	1.53 (0.07)	1.31 (0.18)
Rf	2.88 (0.50)	1.27 (0.02)
H	0.91 (0.1)	1.13 (0.1)

Mean (error) for n = 3; EQ* = Extracted Quantity of FA;
EQ/OM** = Extracted Quantity of FA normalized by organic matter content;
H = index of hydrophobicity

The C/N ratio reflects the mineralization and stabilization along the vermicomposting process. The HA and FA from vermicomposts showed up a lower value for the C/N ratio when it is compared to DSS suggesting loses of carbon dioxide by the microbial breathing and the secretion addition of the earthworms along the vermicomposting process.

According to Kononova [31], independently on the origin of the humic substances, the UV-Vis spectra are similar because of the presence of π bonds and conjugations that promote the orbital superposition resulting in similar energy absorptions. The parameters Δlog K is defined as the difference between the absorptions in 400 nm and 600 nm [32], and Rf is defined as the absorption value in 600 nm, normalized by the organic carbon mass present in the solution [33]. The parameters E_4/E_6 and Δlog K decrease with the degree of humification while the parameter Rf increases with humification. One must consider, however, that these parameters are strongly affected by the presence of non humic substances and the origin of the raw material [32-34]. It should be noticed that the humic substances extracted from sewage sludge evaluated as well as the humic substances extracted from the vermicomposted material are better understood as humic-like substances.

It can be observed in Table 2 that DSS *in natura* samples showed up values that justifies the higher aromatic degree than the HA vermicomposts values. As E_4/E_6 and Δlog K parameters decrease inversely to the aromatic degree, and the Rf parameter increases with the increase of the aromatic degree. These results are an indicative of the higher aliphatic character of the HA vermicomposted upon it is compared to the DSS *in natura*. However, when it is compared to the peat values, the vermicomposted HA present characteristics similar to the HA from peat, signalizing the humified character of the material.

All the FT-IR spectra (Figure 1) have a large band located between 3700 cm^{-1} to 2800 cm^{-1}, centered around 3400 cm^{-1} attributed to OH stretching from alcohols, phenols or carboxylic acids and N-H, proving the abundant presence of OH groups in the FA and HA. For the vermicomposted HA and FA these bands were stronger [34].

The bands centered at 2920 and 1460 cm^{-1} are related to the aliphatic stretching of the CH, what proves the aliphatic character of the studied HA and FA [35]. The band at 1040 cm^{-1} observed in the HA from the DSS can be attributed to the (CO) stretching of polysaccharide presents in this HA structure. The relation between the absorptions at 2927 cm^{-1} and at 1040 cm^{-1} is related to the hydrophobicity index, named H, (high values mean higher resistance to microorganisms degradation). The small H of the HA from DSS (0.97±0.01 - Table 2) is an indicative of weak resistance to microbial degradation when it is compared to the HA from the vermicompost (1.04±0.01 - Table 2) [36]. The same behavior is observed to fulvic acids: 0.91 ± 0.01 for FA from DSS and 1.13 ± 0.01 for FA from vermicompost (Table 3).

The band at around 1660-1600 cm^{-1} (carboxylate asymmetric stretching) increases in intensity while the band centered at 1450 cm^{-1} decreases, when compared HA and FA from DSS to HA and FA from vermicomposted DSS. This band could be attributed to some vibration modes such as symmetric C-H bending of terminal methyl, isopropyl, and tertiary butyl groups and to aromatic C-C stretching. Then, for HA and FA the band at 1660-1600 cm^{-1} is related to carboxylic groups. For HA from DSS the intense band at 1450 cm^{-1} is reflect of the great presence of aliphatic groups, agreeing to the aliphatic character of the HA.

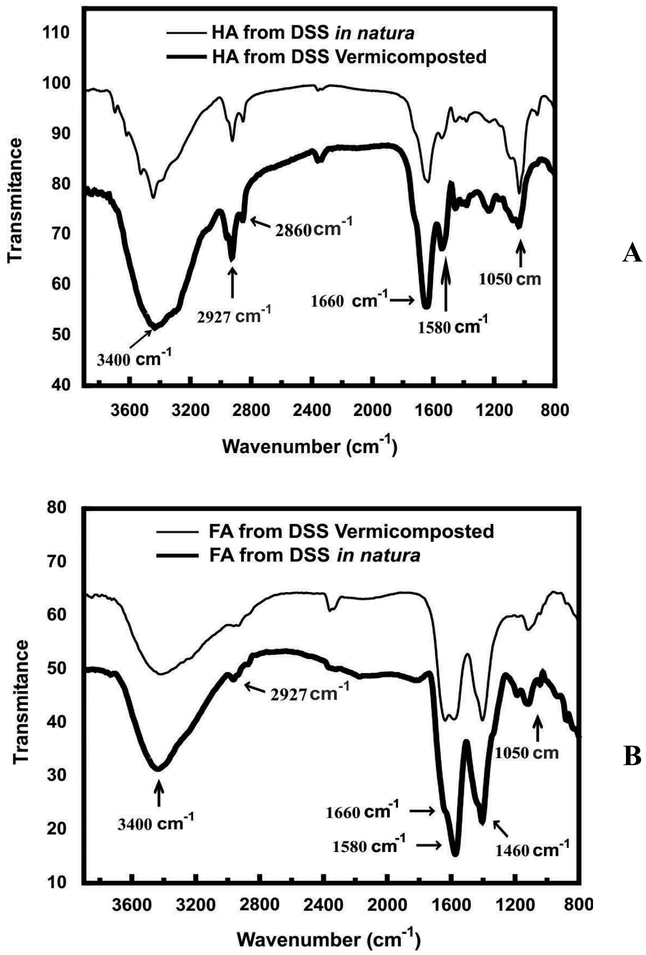

Figure 1. FT-IR spectra of (A) HA from DSS *in natura* and HA from DSS vermicomposted; (B) FA from DSS vermicomposted and FA from DSS *in natura*

Table 4. Variation in the concentration of viable helminth ova along the vermicomposting process (results expressed on a dry basis)

Vermicomposting period (days)						
0	15	30	45	60	75	90
Concentration of viable helminth ova (in 1 g of dry sample)						
10.00	8.00	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25

During the vermicomposting process there is a decrease in the number of viable helminth ova (Table 4). The concentration of viable helminth ova is reduced during the vermicomposting due to the process of feeding the earthworms. They feed on decaying organic matter and they swallow during their feeding the sewage sludge. In the process they end up swallowing food also microorganisms and helminth ova present. When passing through the digestive tract these microorganisms die and part of the viable eggs are destroyed. Another point is that some metals are absorbed and retained in the tissue of earthworms, it may have occurred with the helminth ova, although, toxicological tests should be performed to investigate this proposition.

4. Conclusions

From the FT-IR spectra strong bands at 1450 cm^{-1} for HA and FA extracted from the DSS *in natura* and for the vermicomposted DSS were observed. The bands prove the high degree of aliphatic compounds for the studied samples.

The properties of the analyzed vermicompost were similar to the established values from the Brazilian legislation [37], which determines the criterions for the agriculture use of DSS in the São Paulo state, mainly the concentration of pathogen microorganisms, signalizing that the DSS vermicompost is able to be used as a soil conditioner or fertilizer.

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