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CHARACTERIZATION AND EVALUATION OF VERMICOMPOSTING MATERIALS

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Abstract

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The agricultural sector and growing food production needs rely on chemical fertilizers. This initially had positive effects on production, but over the last decade, the negative impacts of overuse have resulted in low crop productivity, increased pest and disease infestation, soil degradation, and consequent adverse effects on environmental parameters. As one of the possibilities in organic farming, we found vermicomposting as the most sustainable practice. For the assembly of vermicomposting, mature cattle manure was utilized in three different combinations with organic material and earthworm inoculations (the household waste, the grass clippings, and mixture). The experiment was set up by random block design in three replications. Analyses of qualitative parameters of the final product showed a pH reaction from neutral to slightly alkaline. The nitrogen compound varied between 2.7% and 2.9% and the organic carbon varied between 45.59% and 47.41%, showing a C/N ratio of 16.7–16.67. The potassium content varied between 1.1% and 1.2% K_2O, and the phosphorus content varied from 0.3% to 0.5%. The experiment gave quite good results of vermicomposting of household waste with manure, showing a neutral pH reaction, optimal content of ash and carbon with almost similar and good NPK contents and increased C/N ratio of 16.7, and with satisfying levels of trace elements and the heavy metal content under the limits. This combination of household waste and manure also showed best characterization of humic and fulvic acids in vermicompost, ranging from 1505 to 1778 cm^{-1} and from 729 to 998 cm^{-1}, respectively. These results indicate that vermicomposting can increase the quality of the final product.

Key words: vermicomposting, earthworms, recycling, FT-IR analysis, organic waste.

Introduction

Vermicomposting is a mesophilic process (Edwards, Burrows, 1988) that involves the joint action of earthworms (active at 10–32 °C) and mesophilic microbes (Benitez et al., 1999) for the conversion of organic wastes into a valuable product called vermicompost. Sinha et al. (2010) stated that earthworms restore and improve soil fertility and significantly boost crop productivity. It has been found that over the last 20 years, 75% of the agricultural sector relies on chemical fertilizers in agricultural production. This initially had positive effects on production, but over the last decade, the negative impacts of overuse have resulted in low crop productivity, increased pest and disease infestation, soil degradation, and consequent adverse effects on environmental parameters. As a result, researchers and farmers in many nations have begun to use organic agriculture, creating particular markets for organic products through the use of biopesticides, biocontrol agents, and various organic matter sources. This can be also supported by the increased use of solid organic waste produced at different levels, which mainly includes 46% of organic waste globally, incorporated into soil and water causing various pollutants. Such organic waste is recycled by processes like vermicomposting, which may produce organic fertilizers rich in nutrients that are an enriched source of beneficial microbes. Increased public interest and environmental problems associated with the utilization of manure are directing farmers toward alternative solutions, and composting enables considerable reduction in environmental problems associated with the employment of assorted manure fertilizers. One of the simplest explanations for vermicomposting is that it is a process that uses earthworms to transform organic residues into a secondary product named vermicompost that can be used as a fertilizer for crop production (Dominguez, 2004). Vermicompost improves the physical, chemical, and biological properties of the soil and also contributes to organic enrichment (Chauhan, Singh, 2013). Vermicomposting is the biotechnological method of composting by using different species of earthworms to boost waste conversion mechanism and achieve a better product (Devi, Prakash, 2015). According to Ndegwa and Thompson (2001), vermicompost is an excellent homogenous product that

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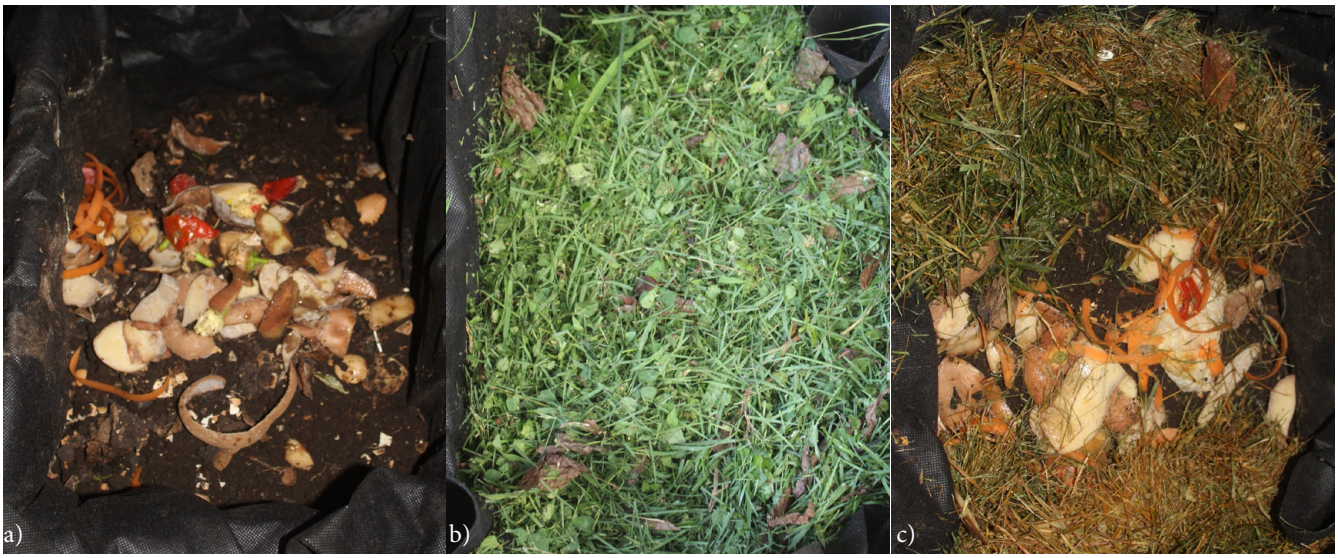

Fig. 1. Organic waste mixtures for vermicomposting (a – household waste; b – grass clippings; c – mixture).

has desirable esthetics, has minimal contamination levels, and tends to hold more nutrients over a longer period without adversely impacting the environment. The nature of humic substances in vermicompost is important in terms of predicting its efficiency during application. Fourier transform infrared (FT-IR) spectroscopy analysis is one of the most promising tools for characterizing heterogeneous organic matter by providing comprehensive information on the composition, properties, and behaviors of the samples (Cuetos et al., 2010). FT-IR spectroscopy provides structural and compositional information about the functional groups present in the samples of organic substrates. Thus, this method helps to validate the decomposition products of substrates that contain a variety of functional groups, such as polypeptides, polysaccharides, lignin, and aliphatic, aromatic, carboxylic, or phenolic groups. The fulvic- and humic-like substances are resistant to microbial degradation, and these more humified materials generally have a higher chemical stability, increasing the residence time of organic matter in the environment and, consequently, improving soil structure and fertility (Santos et al., 2010). In the present research, vermicompost maturity and lignocellulosic organic substances predominant in different types of vermicompost were characterized based on the presence of functional groups by FT-IR spectroscopy. The aim of this study is to provide a characterization and quality of vermicompost enriched with the nutrients and microorganisms utilized from mixed biowaste using earthworms.

Material and methods

For the assembly of vermicompost, partly mature manure was utilized in three different combinations with organic material and earthworms. Mature manure was used as an initial substrate for composting with California earthworms (*Eisenia foetida).* The experiment was organized in line with a random block design in three replications. The initial mature manure was placed in three combinations with household waste and grass clippings, while the controls were a combination of the previous two combinations. Moistening (if required) was performed to maintain the moisture level at 40–50%. Composting material was made in plastic airtight containers with a 40 L capacity and dimensions of 36x35x31 cm (LxWxH). Dark waterproof textiles with small perforations was put to the bottom and sidewalls. Each individual box received an addition of epigeic California earthworms (10 worms per kg of material) along with an initial quantity of 5 kg of manure for each treatment. In each box, 1 kg of the mentioned organic materials and the same amount of fabric were added to the surface at weekly intervals. Vermicompost was mixed, finely sieved, sampled, and analyzed after 6 months. The mixtures of treatments used are shown in Figure 1.

An average weight sample was collected for qualitative analysis that included the standard chemical parameters as substitutes pH reaction (in 1 M KCl) potentiometrically in accordance with BAS ISO 10390:2009 v/v 1:5), and active pH reaction in water. Nitrogen contents in vermicomposting mass were determined by the Kjeldahl method (BAS ISO 11261: 2010). Determination of potassium and phosphorus was performed by wet incineration and sample analysis on a phosphorus spectrophotometer and a potassium flame photometer, respectively. Values are expressed as P_2O_5 and K_2O in percentage. Total carbon was calculated from the results obtained by the determination of organic matter. The C/N ratio was calculated using the information of total carbon in dry matter and total nitrogen. The dry matter content was obtained by drying the sample at 105 °C to constant mass. A digital portable conductivity meter (HACH semsyion5) was used to measure salinity, total dissolved salts (TDS), and electrical conductivity in a 1:2 (w/v) ratio. Volatile solid content was determined by loss ignition method (dry mass basis) at 550 °C for 1 h. The total organic carbon content was calculated from volatile solids (Mohee et al., 2008). Extraction of elements soluble in aqua regia (Ca, Mg, Zn, Cu, Cd, Fe, Pb, and Na) was done according to ISO 11466:2000 and their determination was done by the method of flame and graphite atomic absorption spectrometry (ISO 11047:2000) on the device Perkin Elmer FIAS 800.

Table 1. Proposed attributions for wavelengths (cm^{-1}) of FT-IR spectra.

Fulvic acid (FA) Visible features of the spectra			Humic acid (HA) Visible features of the spectra		
A	2935	Asymmetric CH stretch of –CH2–	A	2935	Asymmetric CH stretch of –CH2–
	2850	Symmetric CH stretch of –CH2–, a weak shoulder, barely visible in some spectra		2850	Symmetric CH stretch of –CH2–, more prominent than in FA
B	1720	A weak shoulder, only a distinct peak in FK5	B	1720	Weakly visible next to the 1650 peak
C	1600–1650	Aromatic C, carbonyl, and/or COO–	C	1600-1650	Aromatic C, carbonyl, and/or COO–
D	1415	C–O stretch	D	1520	Aromatic C=C stretch, not visible in FA
E	1225	Shoulder, C stretch, OH deformation of –COOH	E	1450	CH deformation and bending
F	1080	Stretching C–OH of aliphatic	F	1420	C–O stretch
				1225	Shoulder, C stretch, OH deformation of –COOH

Spectra of humic and fulvic acids were obtained using diffuse reflectance infrared spectroscopy (FT-IR) on Nicolet iS10 with diffuse reflectance accessory. The samples were scanned as 64 scans, with a 4 cm^{-1} resolution, in the range of 4000–400 cm^{-1}. The background was scanned against a golden plate standard, and postprocessing in the form of atmospheric correction along with automatic smoothing was performed. Peak intensities were obtained using the TQ Analyst software. Through this method, we calculated the aromaticity index (AIr), which reflects C–C double bond "density" in a molecule and which can be calculated solely from the exact molecular masses of single organic compounds (Koch, Dittmar, 2006). In the present study, the spectra's different absorption peaks/patterns and values showed the presence or absence of specific functional groups in the samples. In numerous FT-IR studies (Kumar et al., 2015), relevant peaks attributing to specific functional groups and/or compounds were ascertained, and these peaks were used as a guide for interpretation (Table 1). For FT-IR analysis, the mid-IR spectrum (400–4000 cm^{-1}) divided into four regions (the single bond region 2500–4000 cm^{-1}, the triple bond region 2000–2500 cm^{-1}, the double bond region 1500–2000 cm^{-1}, and the fingerprint region 600–1500 cm^{-1}) is the most widely used for organic sample analysis. Banding at around 2935 cm^{-1} is attributable to aliphatic C–H–, C–H_2–, and C–H_3– group stretching, while the broad band between 1660 and 1600 cm^{-1} is usually attributed to aromatic C=C and C=O stretching of amide groups (amide I band) and quinonic C=O and/or C=O of H-bonded conjugated ketones (Xue et al., 2009).

Before the inoculation of worms on composting mixture, the manure utilized in the trial was analyzed.

The results were reported as the mean of three replicates with standard error (SE). Analysis of variance (ANOVA) and Tukey's test were used to test the effects of different chemical properties of the vermicompost products formed due to the earthworm activities. The probability level used for statistical significance was $p < 0.05$ for all measured parameters. For statistical processing, the data's GenStat 7 software (Laws Agricultural Trust, Rothamsted Experimental Station) was used.

Results and discussion

Results of initial cattle manure used in the trial indicated alkaline pH reaction (8.3) and lower nitrogen and higher carbon content compared to vermicompost treatment that resulted in a C/N ratio of 25.57. Potassium content was low (0.5% K_2O), similar to the phosphorus content (0.8% P_2O_5). Results of this trial with vermicomposting for basic parameters are shown in Table 2.

The collected data indicate that using organic material together with cattle manure showed a pH reaction from neutral to slightly alkaline. In this trial, cow's manure was used as the basis for vermicompost treatments, which showed lower pH compared to the initial one, probably due to the presence of various vegetables and fruit home waste and, of course, earthworms' activity. This is in line with Gandhi et al. (1997) who reported that the earthworm activity reduced not only the pH, but also the C/N ratio in manure. According to Dickerson (2001), the recommended pH for vermicomposting is around 6–7, as in lower pH, the bacterial activity decreases, and the worms do not like it, and therefore will migrate or probably die. Brinton (2000) suggested a pH 6.5-8.4 threshold for the A-class range of vermicomposting. According to organic amendments for pH range, a pH of 6.0–8.5 is suggested in several legislations (e.g., Italy, Belgium, Spain) to ensure compatibility with most plants (Hogg et al., 2002). We can confirm that in our research, all three vermicomposting mixtures have pH neutral, accept alkaline active pH in mixture of cattle manure and grass clippings probably due to the grass component, but anyway all the values fit in the suggested range. A larger population of earthworms can significantly contribute to the aeration of the compost mass, which prevents a drastic drop in the pH value, while aerobic condition affects ammonium consumption and prevents pH increment (Rostami, 2011). The nitrogen compound in the three treatments presented varied between 2.7, 2.8, and 2.9% N, while the total carbon content varied between 45.59, 46.39, and 47.41%, showing a C/N ratio of 16.7, 16.29, and 16.67, respectively. C/N compounds in vermicomposting mixtures decreased up to 34%–36% from the initial cattle manure. Significant decrease in C/N ratio compared to the initial cattle manure was found in vermicompost with grass clippings, probably due to the uniform mass that can be digested by the earthworms easily. A C/N ratio less than 20 confirms organic waste mineralization, which indicates compost maturity; however, a C/N ratio less than 12 is also preferred for agricultural purpose (Shak et al., 2014). Rostami (2011) stated that vermicompost process will progress properly by starting the process with a C/N ratio around 25–30 and it will decrease during the process.

Table 2. Chemical characteristics of vermicompost.

Parameters	Initial cattle manure	Cattle manure and domestic waste	Cattle manure and grass clippings	Mixture (control)
pH H_2O	8.3 ± 0.2	7.2 ± 0.14^{ns}	8.3 ± 0.44^{ns}	7.1 ± 0.47^{ns}
pH KCl	7.5 ± 0.1	6.8 ± 0.9^{ns}	7.0 ± 0.37^{ns}	6.7 ± 0.34^{ns}
% N	1.9 ± 0.11	2.7 ± 0.01^{ns}	2.8 ± 0.10^{ns}	2.9 ±0.12^{ns}
% P_2O_5	0.5 ± 0.21	0.3 ± 0.11^{ns}	0.3 ± 0.31^{ns}	0.5 ± 0.15^{ns}
% K_2O	0.8 ± 0.14	1.2 ± 0.08^{ns}	1.1 ± 0.09^{ns}	1.2 ± 0.19^{ns}
% Ash	17.4 ± 0.85	21.58 ± 0.99^{ns}	20.20 ± 1.4^{ns}	18.46 ±1.05^{ns}
% C	48.6 ± 3.6	45.59 ± 2.9^{ns}	46.39 ± 5.3^{ns}	47.41 ± 3.8^{ns}
C/N	25.57 ± 2.2	16.7 ± 1.1^{ns}	16.29 ± 2.1^{ns}	16.67 ± 1.3^{ns}
g/L TDS	2.01 ± 0.63	2.46 ± 0.32^{ns}	2.81 ± 0.83^{ns}	2.56 ± 0.61^{ns}
‰ Salinity	0.5 ± 0.55	2.53 ± 0.32^{ns}	2.90 ± 0.86^{ns}	2.67 ± 0.61^{ns}
mS/cm EC	1.01 ± 0.3	4.70 ± 0.18^{ns}	5.33 ± 0.45^{ns}	4.92 ± 0.79^{ns}
% Ca	1.98 ± 0.15	2.27 ± 0.50^{ns}	2.45 ± 0.14^{ns}	2.26 ± 0.11^{ns}
% Mg	0.50 ± 0.18	0.67 ± 0.11^{a,b}	0.9 ± 0.1^{a}	0.53 ± 0.06^{b}
mg/kg Cd	0.52 ± 0.03	0.60 ± 0.02^{a}	0.53 ± 0.02^{b}	0.53 ± 0.02^{b}
mg/kg Pb	4.46 ± 0.52	11.85 ± 0.81^{ns}	10.47 ± 0.79^{ns}	8.13 ± 2.01^{ns}
mg/kg Cu	38.4 ± 2.34	40.7 ± 4.41^{ns}	43.2 ± 0.51^{ns}	42.8 ± 0.98^{ns}
mg/kg Zn	119.7 ± 6.32	183.0 ± 10.7^{ns}	161.9 ± 4.6^{ns}	171.6 ± 2.99^{ns}
mg/kg Fe	3.19 ± 1.2	5.96 ± 0.88^{a}	4.29 ± 0.49^{a,b}	3.39 ± 0.79^{b}
mg/kg Na	100.7 ± 11.2	586 ± 16.46^{a}	338 ± 17.95^{b}	514 ± 47.66^{a}
HK IAr	-	4.486 ± 0.01^{ns}	4.422 ± 0.09^{ns}	4.366 ± 0.19^{ns}
FK IAr	-	9.37 ± 0.5^{ns}	8.25 ± 1.33^{ns}	8.41 ± 0.66^{ns}
HK 1650	-	6.854 ± 0.1^{c}	7.379 ± 0.2^{b}	7.402 ± 0.1^{a}
HK 2920	-	1.528 ± 0.1^{c}	1.671 ± 0.8^{b}	1.697 ± 0.4^{a}
FK 1650	-	815 ± 0.4^{ns}	682 ± 0.11^{ns}	760 ± 0.15^{ns}
FK 2920	-	871 ± 0.1^{ns}	830 ± 0.1^{ns}	895 ± 0.1^{ns}

Notes: Values of three different vermicomposts are given as mean ± standard error (n = 3); ns – nonsignificant. Means in a row followed by different letters are significantly different at $p < 0.05$ (Tukey's test).

Our C/N has decreased, which is inconsistent with the earlier assertion. According to Tchobanoglous et al. (1993), carbon is reduced because the heterotrophic bacteria use organic material as a source of electron and carbon is oxidized to CO_2 and released to the atmosphere. It is probable that the complexity of the mixtures required a longer mineralization process time since the carbon content (C%) was higher in the treatment with domestic waste and grass clipping. According to Boruah et al. (2019), the reduction in organic carbon during vermicomposting is mainly attributed to the respiratory activity of microorganisms and earthworms with a synchronized increase in nitrogen added through the mucus and nitrogenous excretion by the worms. The potassium content varied between 1.1 and 1.2% K_2O, and phosphorus varied within 0.3–0.5% P_2O_5. Slightly different results were obtained by Pukan et al. (2013) using slurry method (nonenriched and enriched) and conventional method. They reported that the total K_2O content varied between 0.69, 0.73, and 0.65%, while the P_2O_5 content varied between 1.1, 1.51, and 0.82%. According to Bhat et al.'s (2013) findings, the final vermicompost covered approximately 2.47% P_2O_5 and 2.37% K_2O. However, domestic waste and its nutritional value, as well as the starting material and favourable conditions that can accelerate earthworm activity, may be to responsible for the diversity in nutrient compounds. Higher potassium content is expected in organic compounds, and we recorded 37–50% higher content of potassium compared to the initial cattle manure. Suhane (2007) asserts that vermicompost has a minimum of four times more nutritive value than cattle manure compost. The experiment gave quite good results of vermicomposting of household waste with cattle manure, showing a neutral pH reaction and also showing the best content of ash and carbon with good NPK contents and with slightly increased C/N ratio of 16.7. It is to be noted that a decline within the C/N ratio to <20 indicates a sophisticated degree of organic matter stabilization and reflects a satisfactory degree of maturity of organic wastes (Senesi, 1989). It is believed that a C/N ratio below 20 is indicative of acceptable maturity, while a ratio of 15 or lower is preferable (Morais, Queda, 2003). As we can see in Table 1, the ash content was increased in vermicomposting materials compared to initial cattle manure. Garg et al. (2008) confirmed that increase in the ash content illustrates faster rate of consuming the wastes by the earthworms and the microbial assimilation is also performing the degradation process rapidly.

In our trial regarding different organic wastes, EC as the most important factor of vermicompost phytotoxicity varied between 4.7 and 5.33 mS/cm. This result is in accordance with Majlessi et al. (2012), who reported a value of 4.9 mS/cm in the final product. Increase in EC is caused by ion discharge. Some

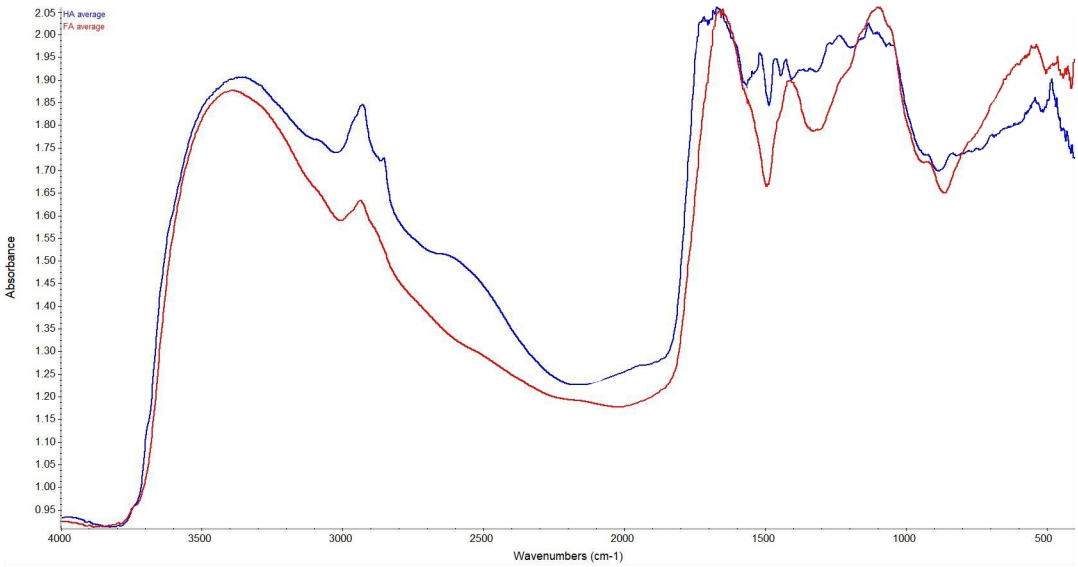

Fig. 2. FT-IR spectral averages of humic and fulvic acids in vermicompost samples.

researchers explained that it happened due to the breakdown of organic substrates, ammonium, phosphate, potassium, nitrate, and calcium (Gong et al., 2019; Yuvaraj et al., 2019; Paul et al., 2020). Mitchell (1997) stated that the EC of vermicompost from cattle manure in his research was 2.5–3.1 mS/cm, while Tiquia and Tam (1998) found in their research on seeds and root elongation of cabbage and spinach that compost with an EC of 3.5–4.7 mS/cm did not reduce germination. Vermicompost-based biological testing on excessive salinity may provide reliable confirmation of phytotoxicity. Salinity can also develop from nitrogen mineralization and production of organic acids. Even these factors indicate the stability of the compost and not its maturity. Iannotti et al. (1994) found that 32% of the analyzed samples showed EC values greater than 4 dS/m (6.1–15 dS/m), probably due to the raw material used (i.e., food waste). The levels of heavy metals like Pb, Cd, Cu, and Zn in vermicompost were found to be below the permissible limit, regardless of the different materials used for vermicomposting. Different materials used in all three treatments in the trial resulted in a calcium content that varied between 2.53 and 2.9%, and this was in accordance with the findings of Suthar (2009) in vermicompost (2.0–2.57% Ca). It was reported by Srimathi et al. (2019) that significant increment of magnesium was found in vermicompost (from 0.37–0.41% to 0.35–0.65%), which is in line with our results. Based on our trial, it is important to emphasize the significantly higher content of magnesium in vermicompost with grass clippings, probably due to the rich chlorophyll content in grass. Magnesium is the central atom of the chlorophyll molecule, with each molecule containing 6.7% magnesium. For the total concentrations of Cd, Fe, and Na, a significant difference was found among the treatments. All three values (Cd, Fe, and Na) were significantly higher in the first treatment with cattle manure and home waste, which probably is due to some residues applied with waste, and these values were lower in the initial material. The heavy metal contents in the final vermicompost were under the US EPA standard limits (US EPA, 1993).

Li et al. (2011) and Busato et al. (2012) have reported that vermicomposting process caused disappearance of easily biodegradable compounds and increase of aromatic compounds, which was confirmed by FT-IR analysis in our trial, where the lowest readings were obtained for home waste and cattle manure. Humic acids showed more diversity in functional groups or they are more prominent than those of fulvic acids. Humic acids also showed greater absorbance, as expected, since they are darker. The index of aromaticity was calculated from the ratio of aromatic (1650 cm^{-1}) to aliphatic (2935 cm^{-1}) peaks, with baselines set between 1853–860 cm^{-1} and 3000–2800 cm^{-1} respectively (Niemeyer et al., 1992; Francioso et al., 1998). Table 1 and Figure 2 show the averages for humic acid (HK) and fulvic acid (FK); smaller the number, greater the aromaticity, and therefore stability of the organic compounds. The peaks at the 2920 cm^{-1} region (aliphatic C–H) were broader and weaker for humic group in all samples of vermicompost (Fig. 3). A narrow and sharp peak at 1620 cm^{-1} is indicative of aromatic moieties and is evident in the fulvic group of samples (Fig. 3).

The results indicated a decreasing trend of aliphatic C–H stretching at 2920 cm^{-1} that confirms lipids and carbohydrate degradation. In general, decrease in aliphatic compounds, N-containing groups, carbohydrates, and enrichment of aromatic components in vermicomposting samples confirmed the organic transformation and maturity. FT-IR analysis confirmed the presence of aliphatic and mostly aromatic compounds due to the stability and maturity of vermicomposting material, which was more prominent in samples of home waste and cattle manure.

Vermicompost has been determined to have the potential to increase the rate of organic matter and the capacity of carbon sequestration based on the applied organic material throughout the treatments.

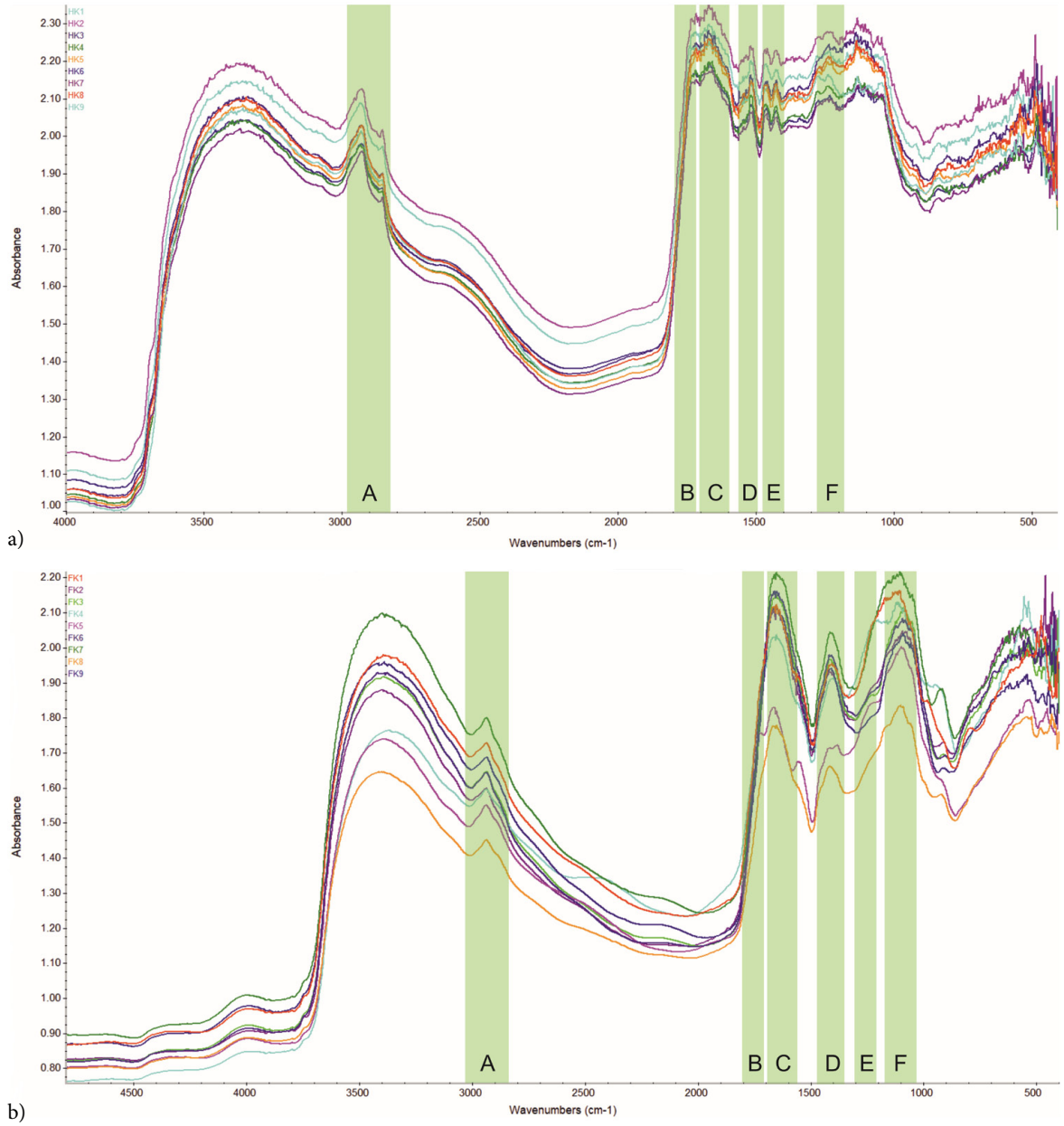

Fig. 3. FT-IR spectroscopy readings of all vermicompost samples for humic (a) and fulvic (b) groups.

Conclusion

An appropriate pH environment and N and C contents, which are reflected in a favorable C/N ratio, create an environment in which earthworms can reproduce, recycle, and efficiently process organic substances and ultimately result in quality vermicompost. Through vermicomposting, the nutrient content could be increased, resulting in good and adequate fertilization manure that could be widely applied. But when it comes to pH and EC of the obtained vermicompost, the most suitable soil for application would be the one with lower pH, especially the combination of cattle manure and grass clippings. The recycling of organic material through vermicomposting could be definitely a positive ecofriendly way of reducing waste and producing organic fertilizer amendments.

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