Evaluation of Vermicompost Maturity Using Scanning Electron Microscopy and Paper Chromatography Analysis

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · APRIL 2014

Impact Factor: 2.91 · DOI: 10.1021/jf4054403

5 AUTHORS, INCLUDING:

D. Senthil Kumar
Kandaswami Kandars college, Paramathi- Vel…
24 PUBLICATIONS **37** CITATIONS
SEE PROFILE

Satheesh Kumar Palanisamy
Università degli Studi di Messina
30 PUBLICATIONS **131** CITATIONS
SEE PROFILE

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Letter

pubs.acs.org/JAFC

Evaluation of Vermicompost Maturity Using Scanning Electron Microscopy and Paper Chromatography Analysis

ABSTRACT: Vermicompost was produced from flower waste inoculated with biofertilizers using the earthworm *Eisenia fetida*. Principal component analysis (PCA) and cluster analysis (CA) were carried out on the basis of physicochemical parameters of vermicomposted samples. From the results of the PCA and CA, it was possible to classify two different groups of vermicompost samples in the following categories: E2 and E5; and E1, E3, E4, and control. Scanning electron microscopy and biodynamic circular paper chromatography analysis were used to investigate the changes in surface morphology and functional groups in the control and vermicompost products. SEM analysis of E1−E5 shows more fragment and pores than the control. Chromatographic analysis of vermicompost indicated the mature condition of the compost materials.

KEYWORDS: *compost maturity, vermicompost, SEM, paper chromotography*

INTRODUCTION

Flower waste is one of the major recyclable materials, existing more during the festival seasons in and around Paramathi-Velur, Namakkal District. It contains low molecular weight proteins, which are not often used for constructive applications. Vermicomposting is defined as a low-cost technology using earthworms as a versatile natural bioreactor for effective recycling of organic waste into nutritious compost production.[1] Chemical fertilizers play an important role in meeting nutrient requirements of the crop, but continuous use of these on lands will have deleterious effects on the physicochemical and biological properties of soil, which in turn is reflected in yield.[2] Thus, there is an urgent need to reduce the use of chemical fertilizers and in turn increase the usage of organic manures, which are known to improve the physicochemical properties of soil and supply available forms of nutrients to the plants.[3] The vermicomposting product of flower waste can be used as a fertilizer because it has a source of minerals and nitrogen for microbial populations, which can be beneficial to plant growth.[4] Recent surveys show that vermicomposting of flower waste is rare. The aim of the present study was to characterize and classify the vermicomposted biofertilizer enriched flower waste samples. Recently we have reported the physicochemical properties of vermicomposted samples;[5] those data are used for principal component analysis (PCA) and cluster analysis (CA) to determine the nature of vermicomposted samples. However, there are few reports in the literature in which biodynamic circular paper chromatography (BPC) and scanning electron microscopy (SEM) analysis are used to describe the vermicomposted samples.[6,7] These results prompted us to determine changes of surface morphology and functional groups during the vermicomposting of flower waste into a nutrient-rich product. In this study, PCA, CA, SEM, and BPC methods are used to characterize the vermicomposted samples. Herein, the results of these studies are discussed in detail.

MATERIALS AND METHODS

The preparation method of compost (C) and vermicompost (E1−E5) samples and their physicochemical properties are reported.[5] The paper chromatography of control and vermicompost samples was performed by following reported procedures.[6,8] Five grams of each sample was ground into a fine paste and individually placed into 125 mL Erlenmeyer flasks each containing 50 mL of 1% NaOH solution, mixed thoroughly by twirling the flask, and kept for 6 h. Five milliliters of sample extract was used for analysis. The retention factor (R_f value), color, pattern, and shape of the vermicompost chromatograms were analyzed. The final control and dried vermicompost samples were sputtered with gold for the clear visibility of the image, and surface morphology was recorded using a JEOL JM-5600 electron microscope at ×2000 magnification.[7,9] Minerals were analyzed by using a UNICAM 939 atomic absorption spectrophotometer (AAS). Student t test, descriptive statistical analysis, such as kurtosis and skewness, were performed on IBM-SPSS statistical software program (version 20 for Windows 8, SPSS, Chicago, IL, USA). PCA and CA were analyzed by using PAST (statistical version 3). Additionally, multivariate statistical methods require variables to confirm the normal distribution; the normality of the distribution of each variable was set by analyzing kurtosis and skewness statistical tests before multivariate statistical analysis was conducted.[10] The original data revealed values of kurtosis and skewness ranging from −2.0 to 1.55 and from −1.068 to 0.79, respectively, indicating that all of the data were in normal distribution.

RESULTS AND DISCUSSION

The pH of vermicompost E1−E5 and control (C) samples falls in the range of 6.8−7.2 and was significant at $p < 0.01$; decreasing the pH toward neutrality is the energetic process of retaining N to promote nutrient availability to the plant.[11] TN content in C was 1.9%, which increased to 2.29% at the end of the process (Figure 1). A higher value of TN was observed in E5 and E2 followed by E4, E3, and E1, which were significant at $p < 0.001$. The excretory products of earthworm increase the N level in the substrate,[11] and the addition of N-fixing bacteria to the compost medium enhances total nitrogen content.[12] TOC levels were observed high (36%), which was decreased noticeably during the vermicomposting period, reaching a value of 25% by the 80th day, thus indicating the occurrence of extended mineralization. The results of TOC were significant at the $p < 0.001$ level. The decline observed for the C/N ratio from an initial value of 15% in the control to the end of the composting period (11.5%) in E5 indicates advanced TOC decomposition and the achievement of an appropriate degree of TOC stabilization. P, K, Ca, and Mg concentrations in all treatments

Received: December 26, 2013
Revised: March 10, 2014
Accepted: March 17, 2014
Published: March 17, 2014

dx.doi.org/10.1021/jf4054403 | *J. Agric. Food Chem.* 2014, 62, 2738−2741

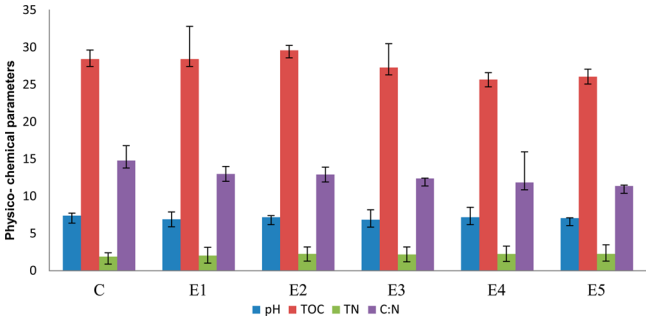

Figure 1. Physicochemical parameters of vermicompost (C, E1–E5).

(E1–E5) were increased significantly compared to control (C); data are presented in Table 1. The higher value of P was recorded at E3, which may be partially due to the earthworm's gut phosphatase and more P discharged by the added phosphobacteria in the substrate. The maximum K level was observed in E5 followed by E2, E3, E4, and E1. A higher value of Ca was observed in E1 and a similar increment of Mg level in E5. The increased level of nutrients in vermicompost was similar to that in the literature.[11,12] The transition metal concentration was decreased in vermicompost samples relative to compost (C) except Zn concentration in E3, which was slightly increased. The decrease in the metal concentration is due to the accumulation of metals (Mn, Zn, and Cu) on the earthworm body.[13] The results of the PCA are given in (Figure 2. Four principal components accounting for 99% of the total variation are retained on the basis of the eigenvalue greater-than-one rule. The first two principal components explain 80.82 and 13.90% of the variance, respectively. The third and fourth principal components are considerably less important, explaining only 3.24 and 1.82% of the variance, respectively. Accordingly, we consider only the first two components, which account for a large proportion of the variation in the data (94% of the variance). It is possible to observe that control and vermicompost treatments are separated into two different groups on the basis of their characteristics. PC1 separates the composts studied in two different groups: group 1A (C, E3) was characterized by high TOC, C:N ratio, and phosphorus; group 1B (E1, E4) was characterized by the lowest value of pH. PC2 included E2 and E5, and it is possible to separate composts that have higher TN contents (2.29%) and reduced level of C:N ratio, 11.39%, respectively. CA was used to detect similar groups to characterize the control and vermicompost samples (Figure 3); it is found that two different groups were ordinated separately from all of the treatments, which confirm PCA.

SEM was used to study the morphological results of the final control and vermicompost samples as shown in Figure 4. In the control sample the cellulose and protein groups were tightly bound with the lignin as an assortment (Figure 4C). Comparison of the micrographs of vermicompost samples E1–E5 suggests that the surface area of compost samples is higher than that of the control. Within the vermicompost samples, the highest proportion of the surface was recorded in

Table 1. AAS Data for Control and Vermicompost Samples

	concentration determined (%)					concentration determined (ppm)			
sample	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
C	0.89	1.16	0.77	0.59	0.18	1947	211	128	60
E1	0.96	1.19	1.02	0.61	0.1	1907	187	52	41
E2	0.96	1.74	0.88	0.68	0.17	1901	183	119	3.1
E3	1	1.6	0.8	0.67	0.18	1887	209	136	53
E4	0.94	1.27	0.82	0.62	0.07	1874	192	90.33	44.3
E5	0.93	1.82	0.93	0.73	0.13	1817	167	98	16
t test[a]	0.004*	0.044**	0.039**	0.029**	0.153	0.012**	0.026**	0.159	0.038**

[a](*) $p < 0.05$ and (**) $p < 0.01$ level of significance in *t* test.

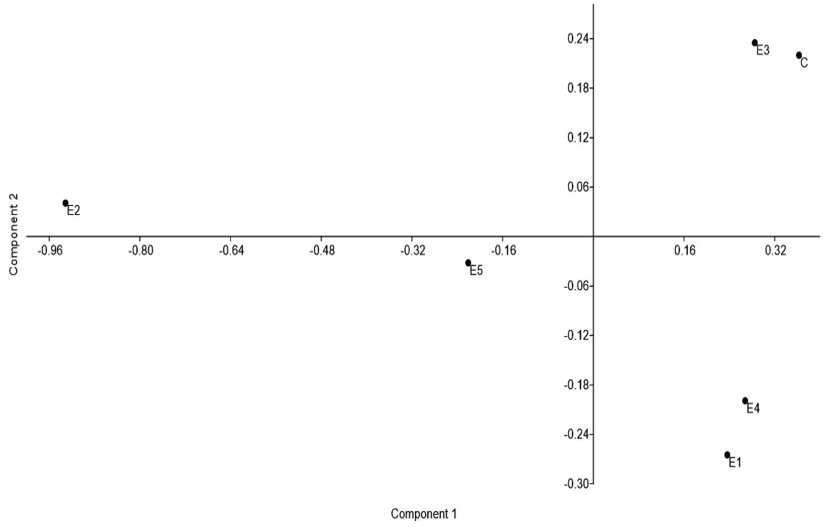

Figure 2. Principal component analysis for control and vermicomposting treatments.

dx.doi.org/10.1021/jf4054403 | J. Agric. Food Chem. 2014, 62, 2738–2741

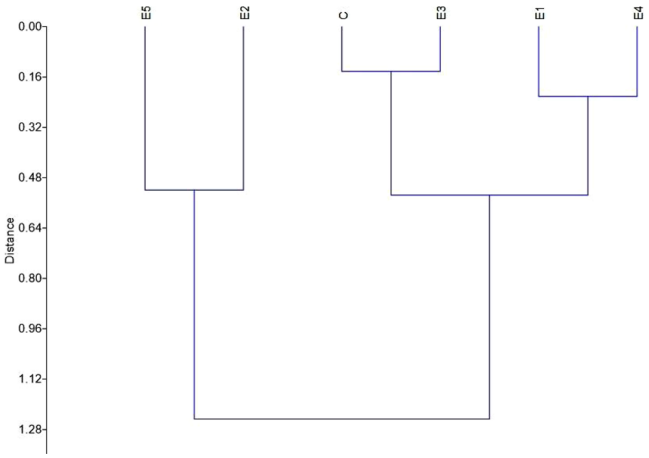

Figure 3. Cluster analysis for control and vermicomposting treatments.

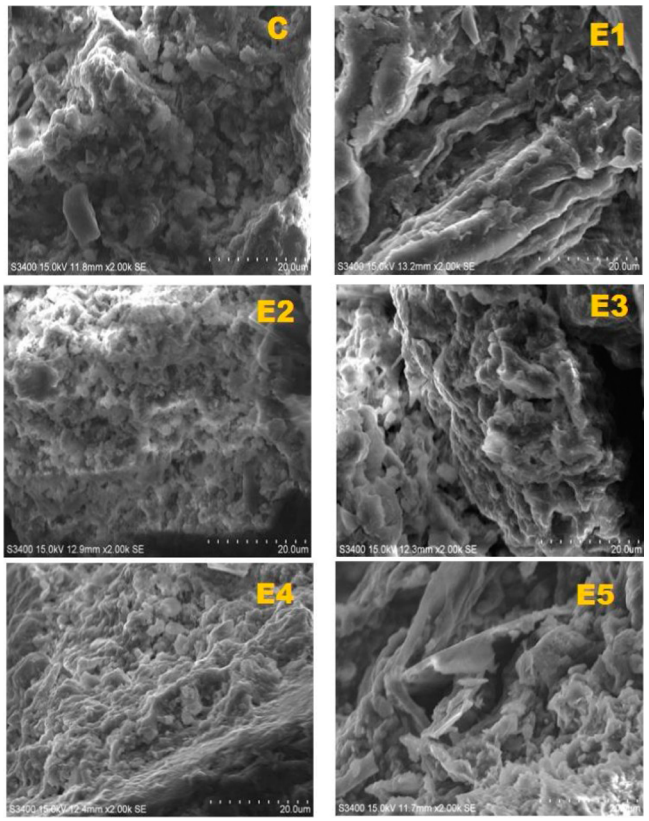

Figure 4. Micrographs by SEM of flower waste vermicompost after 80 days in the following forms: (C) control compost; (E1) vermicompost; (E2) vermicompost + *Azospirillum*; (E3) vermicompost + Phosphobacterium; (E4) vermicompost + blue-green algae; (E5) vermicompost + *Rhizobium*.

E2 followed by E4 and E5. It is observed that the particle size of the vermicompost was smaller than that of control compost. The surface area of vermicompost samples showed single particles packed together to form aggregates. Such aggregation is responsible for the uncertainty about a real surface area of vermicompost because the internal area was not fully accessible. Moreover, different morphological features occurring within vermicompost might be due to their different chemical structures.[5] In addition, in the final vermicompost product (Figure 4, E1–E5), the protein and lignin matrix is disaggregated by earthworms and microbes. The presence of earthworms and microbes shows more numerous surface irregularities that confirm the final compost has attained compost maturity.[7] These images indicate a surface region with an irregular morphology as well as a high number of pores and can corroborate the high CEC value. Similar results were reported for their vermicompost samples.[7,9]

In the past the focus of the application of the chromatography method was on the soil and compost assessment. In contrast to the assessment of soils and composts, there are hardly any methodical works on the evaluation of chromatography pictures of foods.[6,14] The chromatogram of the initial substrate shows three clear zones: (a) an absence of color in the outer zone, which reflects the lack of colloidal substances; (b) a faint brown color indicates the availability of less organic matter; (c) the inner small violet zone indicates the quantity of available minerals, which is clearly observed in E1.[6,8,14] These zones were entirely different from the final substrate in the present study. The final day of samples showed the inner zone with violet radiation confirmed the concentration of the mineralization was increased at the end of the composting process (Figure 5). E2 shows a high level of mineralization process

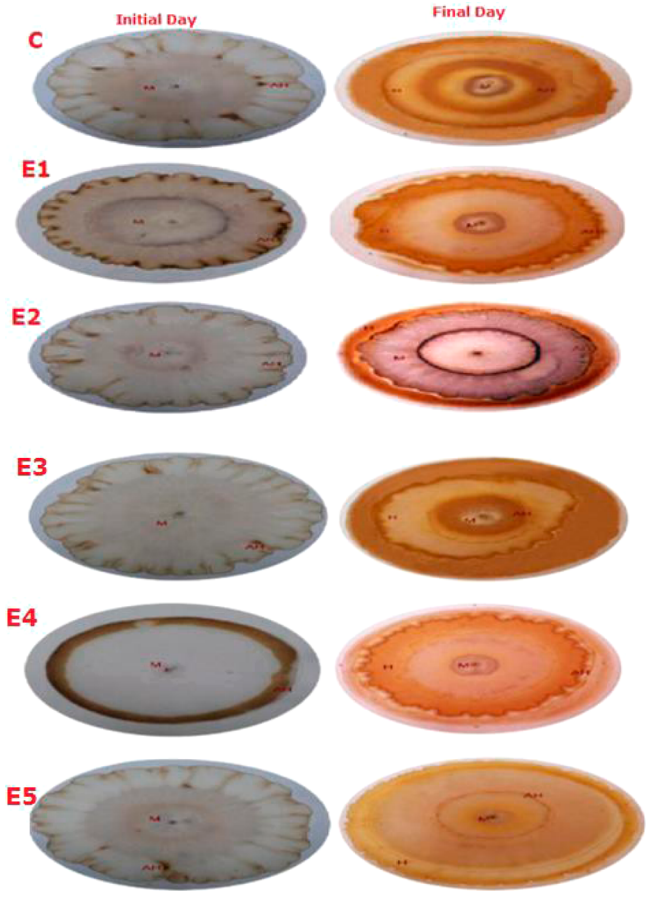

Figure 5. Biodynamic paper chromatography of flower waste vermicompost after 80 days in the following forms: (C) control compost; (E1) vermicompost; (E2) vermicompost + *Azospirillum*; (E3) vermicompost + Phosphobacterium; (E4) vermicompost + blue-green algae; (E5) vermicompost + *Rhizobium*.

followed by E5, whereas in the remaining samples the mineralization process was not clearly visible. All of the final samples showed an evenly distributed brown color in the outer zone that indicates the presence of humus substances in the final

dx.doi.org/10.1021/jf4054403 | J. Agric. Food Chem. 2014, 62, 2738–2741

samples. The existence of humus in all samples around neutral pH values is a sign of maturity of the vermicompost samples. Similar chromatography results of vermicompost samples are reported in the literature.[8,14] The R_f values of different color bands/patterns of all samples are given in Table S1 (see the Supporting Information). In this study, a circular chromatographic method has been used for the purpose of determining differences in the formation of humus in the compost and biofertilizer-enriched vermicompost samples that cannot be determined by chemical analysis.[7]

Vermicomposting led to the drop of pH, decrease in TOC and C:N ratio, and increase in nitrogen content compared to the control sample. The reduction of the C/N ratio was reported from an initial value of 15% in C and to the end of the composting (11.5%) in E5, which indicates organic carbon decomposition in the final vermicompost. However, by the employment of statistical analysis, PCA and CA, it was possible to classify and characterize the vermicompost samples. BPC and SEM techniques proved useful tools to monitor the maturity of vermicompost during the composting process. Critical investigation of our study results indicates that the epigeic earthworm *E. fetida* was capable of decomposing flower waste into nutrient-enriched products and can play a significant role in solid waste management.

D. Senthil Kumar[*,†]
P. Satheesh Kumar[§]
N. M. Rajendran[#]
V. Uthaya Kumar[⊥]
G. Anbuganapathi[†]

[†]Department of Zoology, Kandaswami Kandar's College, Paramathi-Velur, 638 182 Tamil Nadu, India
[§]Department of Biological and Environmental Sciences, University of Messina, Messina 98166, Italy
[#]Department of Chemistry, Pondicherry University, Puducherry 605 014, India
[⊥]Department of Zoology, Bharathiyar University, Coimbatore, Tamil Nadu, India

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(D.S.K.) E-mail: drskumar1968@gmail.com.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Hand, P.; Hayes, W. A.; Frankland, J. C.; Satchell, J. E. The vermicomposting of cow slurry. *Pedobiologia* **1988**, *31*, 199−209.
(2) Sarkar, R. K.; Karmakar, S.; Chakraborty, A. Response of summer green gram (*Phaseolus radiatus*) to nitrogen, phosphorus application and bacterial inoculation. *Indian J. Agron.* **1997**, *38* (4), 578−581.
(3) Ramanathan, B.; Alagesan, P. Evaluation of millicompost versus vermicompost. *Curr. Sci.* **2012**, *103* (2), 140−143.
(4) Raju, A. A.; Rose, C.; Muralidhara Rao, N. Enzymatic hydrolysis of tannery fleshings using chicken intestine proteases. *Anim. Feed Sci. Technol.* **1997**, *66* (1), 139−147.
(5) Kumar, D. S.; Kumar, P. S.; Rajendran, N. M.; Anbuganapathi, G. Compost maturity assessment using physicochemical, solid-state spectroscopy, and plant bioassay analysis. *J. Agric. Food Chem.* **2013**, *61*, 11326−11331.
(6) Pfeiffer, E. *Chromatography Applied to Quality Testing*; Biodynamic Literature: Wyoning, RI, USA, 1984; 44 pp.
(7) Ravindran, B.; Dinesh, S. L.; John Kennedy, L.; Sekaran, G. Vermicomposting of solid waste generated from leather industries using epigeic earthworm *Eisenia fetida*. *Appl. Biochem. Biotechnol.* **2008**, *151*, 480−488.
(8) Perumal, K.; Vatsala, T. M. *Utilization of Local Alternative Materials in Cow Horn Manure (BD 500) Preparations: A Case Study on Biodynamic Vegetable Cultivation*; Murugappa Chettiar Research Centre: Tharamani, Chennai, India. 2002; 26 pp.
(9) Pereira, M. G.; Arruda, M. A. Z. Vermicompost as a natural absorbent material. Characterization and potentialities for cadmium absorption. *J. Braz. Chem. Soc.* **2003**, *14* (1), 39−47.
(10) Satheeshkumar, P.; Khan, A. B. Identification of mangrove water quality by multivariate statistical analysis methods in Pondicherry coast, India. *Environ. Monit. Assess.* **2012**, *184* (6), 3761−3774.
(11) Suthar, S.; Singh, S. Vermicomposting of domestic waste by using two epigeic earthworms (*Perionyx excavatus* and *Perionyx sansibaricus*). *Int. J. Environ. Sci. Technol.* **2008**, *5* (1), 99−106.
(12) Kaushik, P.; Garg, V. K. Dynamics of biological and chemical parameters during vermicomposting of solid textile mill sludge mixed with dung and agricultural residues. *Bioresour. Technol.* **2004**, *94*, 203−209.
(13) Kumar, D. S. Study on biofertilizer enriched flower waste vermicompost by using *Eisenia fetida* and its application on agriculture and aquaculture. Ph.D. thesis, Periyar University, Salem, Tamil Nadu, India, 2013; 152 pp.
(14) Ismail, S. A. Status of composting in India − an Indian earthworm's eye view. In *Proceedings Indo-US Workshop on Vermitechnology in Human Welfare*; Edwards, C. A., Jeyaraj, and Jeyaraj, I., Eds.; Coimbatore, India, 2007; pp 110−124.