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Distribution of humic substances: effects of long-term application of vermicompost

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Abstract The results indicated that long-term fertilization with vermicompost presented a positive correlation for fulvic acid more than humic acid. Fulvic acid as a part of humic substances can predict metal existence and its bioavailability in soil. In depth of 0-20cm, content of fulvic acid about 6.72 to 9.87%, higher than 20-40 cm about 3.78 to 6.84%. In vermicompost utilization, application 25 Ton ha^{-1} was not significantly differed from 5 Ton ha^{-1}. Meanwhile, organic carbon started ranged 3.29 to 5.32% and 2.59 to 3.64% on soil depth 0-20cm and 20-40cm, respectively. Carbon stock at 0-20cm ranged about 53.02 to 88.29% and 34.02 to 64.30% at 20-40cm. Carbon, it consisted of total organic carbon (carbon stock) and lignin that caused a primary effect on humification processed. A positive correlation between level dose of vermicompost and soil depth for soil pH, fulvic acid, organic carbon, total nitrogen, carbon stock, and lignin showed that organic fertilizer can give a positive impact to remain soil health and quality.

Keywords: Carbon, C-stock, Humification, Soil depth

Introduction

Long-term fertilization, especially for organic fertilizer, not only modifies the structure of humic acid, but also influences the content and stability of organic carbon (Zhang *et al*., 2019). Almost all scientists have investigated the relationship of organic matter on soil organic carbon group, as well as humic substances. They reported significance effect organic matter with altering soil nitrogen (N) cycling in farmlands. Moreover, soil organic carbon (SOC) affects nutrients availability, the emission of greenhouse gases and detoxifying harmful substances in soil. Humic substances represent the stable part of SOC, accounting for between 50 to 80% of organically bound carbon in soil (Gerke, 2022; Yue *et al*., 2023). Sun *et al*. (2021) explained that SOC fractions showed a different trend for seasonal variations, but for the same fraction in the topsoil (0-20 cm) and subsoil (20-40 cm), as well as that in rhizosphere and bulk soil, they had the same trend of change. In addition,

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the humic substances are the main soil fertility indices influencing crop productivity (Xiao *et al*., 2020).

Humic acids constitute the largest organic carbon fraction at the upper soil layer, representing 12-34% of the total SOM (Ben Mahmoud *et al*., 2024). Humic acid-based products have been used in crop production in recent years to ensure the sustainability of agriculture production (Ampong *et al*., 2022). Humic acid effects on soils and crops depend on the humic substances source (Gollenbeek and van der Weide, 2020; Rose *et al*., 2014). The source of humic acid applied depends on various factors such as nutritional composition, mode of production, functional group composition, and intended purpose (Ampong *et al*., 2022; Rose *et al*., 2014). As a results from (Watanabe *et al*., 2001), the proportion of fulvic acid fraction in the subsoil ranged between 21% and 50%, while that of humic acids was less than 8%. There was no relation between the proportion of humic acids of the fulvic acid fraction and the type of land use.

Vermicompost is a nutrient-rich biological fertilizer with a variety of microorganisms that are thought to be significant in increasing the growth and yield of various field crops (Mohite *et al*., 2024). In fact, vermicompost-treated soil has better aeration, porosity, bulk density and water retention in physically properties. Chemically, utilizing vermicompost can improve pH, electrical conductivity, and organic matter content (Lim *et al*., 2015). Furthermore, (Amante, 2024) showed that vermicompost contains considerable amounts of humic acid, such as humic acid 17-36% and fulvic acid 13-30% of the total concentration of organic matter. These substances play a vital role in the carbon cycle and soil mineralization, and act as sources of nutrients. Besides that, vermicompost presents numerous hydrophilic groups (-OH, -COOH, and -SH, among others), a high surface area and vast porosity, thus demonstrating its remarkable adsorptive potential (Landgraf *et al*., 1998; Pereira *et al*., 2014; Pereira and Arruda, 2003). Moreover, (Sharma and Garg, 2017) showed that humic acid content in vermicompost was found to be 28% higher than normal compost, because earthworms fragment the soil more rapidly, hence finer particles react and retain humic acid in a high amount. Vermicomposting accelerates the humification of organic matter and increased it by 0-60% as compared to the natural composting process. This research aimed to investigate the humic substances in two layers of soil after using vermicompost for seven years.

Material and methods

Soil sampling and analysis

This research was conducted in an experimental field were in Selupu Rejang District, Rejang Lebong, Bengkulu Province, Indonesia in November 2023. This field has been a closed agriculture system since 2017 with vermicompost as a main organic fertilizer that was used until now. Soil has collected from 2 depth, 0-20 cm and 20-40 cm, with ground drill (mentioned type or series) as much as 1 kg for each treatment. Each block consists of 3 points of drill and then composite it and just 500 g was taken as soil sample. Total were 30-unit samples that were prepared for analysis.

Analysis of physics and chemical properties was conducted in Soil Science Laboratory, University of Bengkulu. The physical properties like bulk density, and chemical properties for instance organic carbon, total nitrogen, soil pH, C/N ratio, humic and fulvic acids, cellulose, hemicellulose, lignin, and carbon stock.

Extraction and fractionation of humic acids and fulvic acids fractions

Total humic and fulvic acids. Soils were extracted with 0,1 N Natrium pyrophosphate + Natrium hydroxide for 60 minutes and allowed to stand overnight. The day after that, extract filtered by filter paper to separate humin and humic substances, such as humic acid and fulvic acid. Filtrate was pipetted as 5 ml (5 gr) into 100 ml volumetric flask. Adding 5 ml 1 mol L^{-1} $K_2Cr_2O_7$ and mixed them. Adding 7,5 ml 96-97% H_2SO_4 and diluted with 50 ml distilled water. After stand overnight, the humic acid and fulvic acid absorbance were measured by spectrophotometry with 561 nm wavelength (used glucose standard 0-250 g kg^{-1}).

Humic acid. Pipetted 5 ml filtrate into 50 ml volumetric flask and added 1 ml 5 mol L^{-1} H_2SO_4. After overnight, filtered with filter paper and rinsed extract that not passed by filter paper with 20 ml 0.01 mol L^{-1} H_2SO_4. Rinsed again with 0.1 mol L^{-1} NaOH (70-80^{o}C) slowly. Pipetted 5 ml extract into 100 ml volumetric flask. Adding 5 ml 1 mol L^{-1} $K_2Cr_2O_7$ and mixed them. Adding 7,5 ml 96-97% H_2SO_4 and diluted with 50 ml distilled water. After stand overnight, the humic acid and fulvic acid absorbance were measured by spectrophotometry with 561 nm wavelength (used glucose standard 0-250 g kg^{-1}).

The humic acid and fulvic acid content were calculated as, (BSIP, 2023)

$$\text{Humic acid and fulvic acid content (\%)} = (\text{C-humic and fulvic acid}) \times 1.79 \quad \text{(a)}$$

$$\text{Humic acid content (\%)} = \text{C-humic content} \times 1.79 \quad \text{(b)}$$

$$\text{Fulvic acid content (\%)} = (a) - (b)$$

Extraction of cellulose, hemicellulose, and lignin

One gr dried sample (a) diluted with 150 ml H_2O or alcohol-benzene and refluxed with 100^{o}C in water bath during an hour. After that, filtered extract with filter paper and rinsed with 300 ml hot water. The residue dried in oven until a constant weight and weighted it (b). Added 150 ml 1 mol L^{-1} H_2SO_4 and then refluxed in water bath for an hour at 100°C. The extract was filtered and rinsed with 300 ml H_2O and dried until constant weigh, after that weighted it again (c). Dried residue was added with 100 ml H_2SO_4 72% and soaked at room temperature for 4 hours. Furthermore, added 150 ml 1 mol L^{-1} H_2SO_4 and refluxed again at 100°C in water bath for an hour. Residue filtered and rinsed with 400 ml H_2SO_4. Residue was heated at 105°C until got constant weight and weighted (d). Finally, residue was ash and weight (e). Lignin and cellulose were calculated (Chesson, 1981) as,

$$\text{Cellulose content (\%)} = (c\text{-}d)/a \times 100\%$$
$$\text{Lignin content (\%)} = (d\text{-}e)/a \times 100\%$$

Carbon stock analysis

Soil carbon stock was calculated according to BSNI (2011),

$$Ct = Kd \times \rho \times \%\ \text{organic carbon}$$

Where Ct was soil carbon stock (g cm^{-2}), Kd was soil depth (cm), ρ was bulk density (g cm^{-3}), % organic carbon was found by carbon content in soil through laboratory analysed.

Results

Seven years organic fertilization, typically vermicompost, affected numbers of humic substances and soil properties. The utilization vermicompost with different levels of dose showed too early humification as forming humus. Humic acid and fulvic acid content represented humification process and correlated with other forms of carbon, for instance organic carbon and stock carbon. Lignin, cellulose, and hemicellulose can be part of determining decomposition. Environmental variables such as soil acidic (pH), nitrogen, and bulk density of soil can support how humification would ensue.

Interaction level dose of vermicompost and soil depth

The level dose of vermicompost and soil depth showed have significant effects with soil pH, fulvic acid content, organic carbon, total nitrogen, carbon stock, and lignin (Table 2; Figure 1). Both treatments did not have significant effect (P-value > 0.005) on humic acid content, C/N ratio, bulk density, cellulose, and hemicellulose content (Table 2). However, neither level dose of vermicompost nor soil depth showed any interaction effect in this study.

Table 1. Soil properties and humic substances content after long-term fertilization

Level dose of vermicompost	pH	Hum (%)	Ful (%)	Org-C (%)	C/N	N-tot (%)	Bulk-d (g cm^{-3})	C-Stc (%)	Cell (%)	Hemi (%)	Lig (%)
---------- *0-20 cm* ----------											
5-ton ha^{-1}	5.59	2.46	9.26	3.29	9.18	0.36	0.81	53.02	13.27	20.43	32.23
10-ton ha^{-1}	5.77	2.09	7.94	3.53	8.07	0.43	0.75	56.20	9.50	16.20	30.20
15ton ha^{-1}	5.82	2.62	6.72	4.15	10.14	0.41	0.92	76.30	10.03	15.27	34.20
20-ton ha^{-1}	5.83	2.60	7.30	4.89	10.74	0.46	0.82	79.48	10.43	16.23	36.63
25-ton ha^{-1}	5.87	2.60	9.87	5.32	10.37	0.51	0.84	88.29	11.85	18.30	37.79
---------- *20-40 cm* ----------											
5-ton ha^{-1}	5.34	2.49	6.84	2.59	10.08	0.26	0.70	34.02	11.63	15.83	26.67
10-ton ha^{-1}	5.66	2.29	3.78	3.00	9.85	0.30	0.87	51.68	11.57	15.87	28.00
15ton ha^{-1}	5.59	2.41	4.06	3.22	9.13	0.35	0.83	53.06	8.50	18.77	26.67
20-ton ha^{-1}	5.67	2.57	5.77	3.36	9.28	0.36	0.96	64.30	12.50	16.03	34.33
25-ton ha^{-1}	5.77	2.27	5.25	3.64	8.89	0.41	0.83	60.26	10.67	17.30	33.20

Hum = Humat acid; Ful = Fulvic acid; Org-C = Organic Carbon; N-tot = Nitrogen Total; C-stc = Carbon Stock; Bulk-d = Bulk density; Cell = Cellulose; Hemi = Hemicellulose; Lig = Lignin

Table 2. Analysis of variance from dose level of vermicompost, two depths of soil, their interaction, and coefficient of variance on soil humic substances after long-term fertilization

Treatment	pH	Hum (%)	Ful (%)	Org-C (%)	C/N	N-tot (%)	Bulk-d (g cm^{-3})	C-Stc (%)	Cell (%)	Hemi (%)	Lig (%)
---------- ***P-value*** ----------											
Dose level of vermicompost	.0003***	.9371ns	.020*	.005**	.884ns	.0001***	.545ns	.0002***	.789ns	.728ns	.0015**
Depth of soil	.0004***	.8267ns	.000***	.0004***	.698ns	.000***	.874ns	.0001***	.793ns	.663ns	.0008**
Interaction	.692ns	.9845ns	.3256ns	.501ns	.383ns	.607ns	.397ns	.349ns	.800ns	.355ns	.513ns
CV (%)	1.89	34.35	20.94	18.11	18.21	10.24	16.34	16.24	42.29	19.12	9.40

Hum = Humat acid; Ful = Fulvic acid; Org-C = Organic Carbon; N-tot = Nitrogen Total; C-stc = Carbon Stock; Bulk-d = Bulk dentsity; Cell = Cellulose; Hemi = Hemicellulose; Lig = Lignin; CV = Coefficient of Variance; * $P<.005$; ** $P<.001$; *** $P<.0001$

Contents of humic acid and fulvic acid fractions

Application of vermicompost, at 5 levels of dose, promoted the formation of humic and fulvic acid, mainly on their interaction on different depths of soil (Table 2). The humic acid content represented an insignificant effect, but at 0-20cm and 20-40cm have 2.46 to 2.60% and 2.27 to 2.57%, respectively (Table 1). On the other hand, fulvic acid content has a positive correlation with vermicompost and soil depth (Figure 1b), where at two depths have about 6.72 to 9.87% and 3.78 to 6.84% (Table 1). As result from Machado *et al*., (2020) stated that fulvic acid is a substance that is more soluble and tends to be degraded, rapidly. This condition caused by lignin substrate content in soil, as occurred in this study that vermicompost tended more lignin in soil (Table 2; Figure 1e).

Soil carbon stock

Soil carbon stock was a belowground response to land use change and less obvious than aboveground (Hairiah *et al*., 2020). Improvement level dose of vermicompost can result in the increase of soil carbon stock, where the application of 25-ton ha^{-1} vermicompost can produce soil carbon stock 74.27% (Figure 1). Soil depth can present a different amount of carbon stock, there are 70.65% and 52.66% respectively (Table 4; Figure 1).

The application of vermicompost on fulvic acid content, identically showed significantly different at 5-ton ha^{-1} application, the highest value is 8.04% but not significantly different with 25 Ton ha^{-1}, as much as 7.56% (Table 4). This result might be predicted by nitrogen released that caused by utilization of vermicompost, which is this organic fertilizer has the numbers of nitrogen as its characteristic can give nitrogen supply into soil (Table 3).

Table 3. The properties of vermicompost and its comparison with national standards

Vermicompost properties	Value	National standards
Organic carbon (%)	19.71	9.8-32
Total nitrogen (%)	1.65	0.40
C/N ratio	11.92	10
pH	6.8-7.9	6.8-7.49
Water content (%)	55	50

Note: National standard used SNI 19-7030-2004; value adopted from the research study that conducted by Riwandi *et al.* (2022)

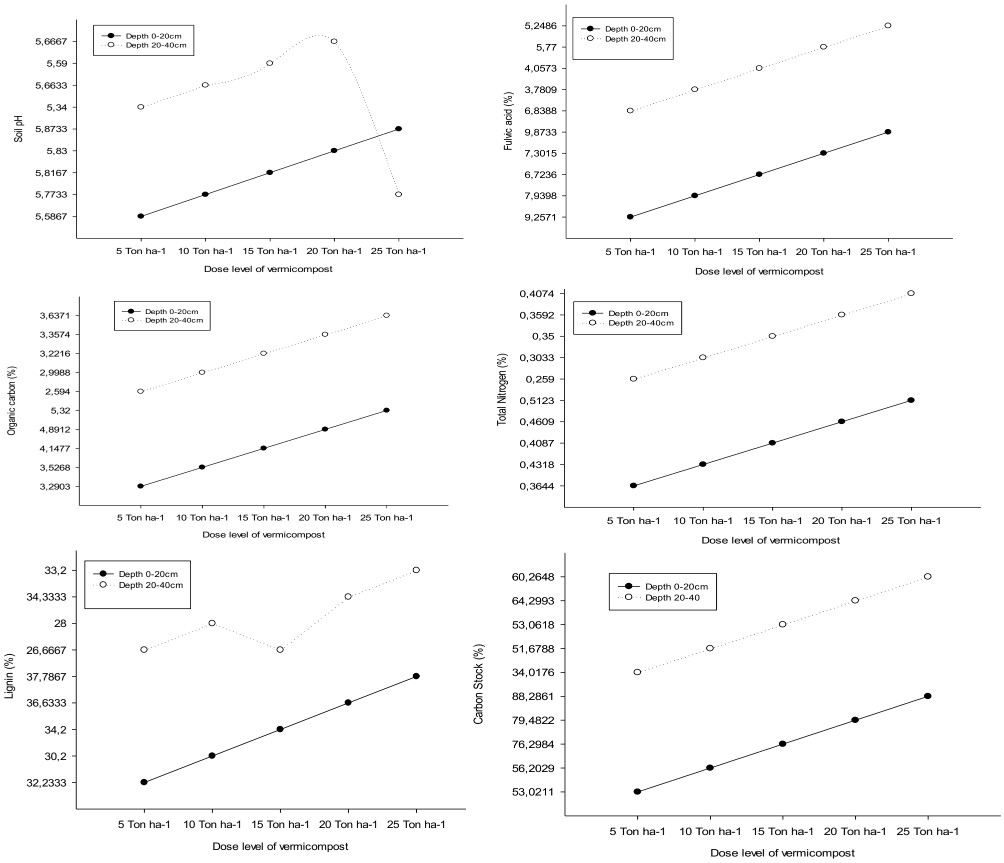

Figure 1. Distribution of (a) soil pH, (b) fulvic acid, (c) organic carbon, (d) total nitrogen, (e) lignin, and (f) carbon stock on two depth of soil (0-20 cm and 20-40 cm)

Table 4. Relationship number of soil properties after long-term application of vermicompost

Treatment	pH	Fulvat acid	Organic-C	Total N	Carbon stock	Lignin
---Dose level of vermicompost---						
5 Ton ha^{-1}	5.46^{b}	8.04^{a}	2.94^{b}	0.31^{c}	43.52^{c}	29.10^{b}
10 Ton ha^{-1}	5.71^{a}	5.85^{b}	3.26^{b}	0.36^{b}	53.94^{bc}	29.45^{b}
15 Ton ha^{-1}	5.70^{a}	5.39^{b}	3.68^{ab}	0.37^{b}	64.48^{ab}	30.43^{b}
20 Ton ha^{-1}	5.74^{a}	6.53^{ab}	4.12^{a}	0.41^{b}	71.89^{a}	35.48^{a}
25 Ton ha^{-1}	5.82^{a}	7.56^{a}	4.48^{a}	0.46^{a}	74.27^{a}	35.49^{a}
---Soil depth---						
Depth 0-20 cm	5.77^{a}	8.21^{a}	4.23^{a}	0.43^{a}	70.65^{a}	34.21^{a}
Depth 20-40 cm	5.60^{b}	5.13^{b}	3.16^{b}	0.33^{b}	52.66^{b}	29.77^{b}

Note: Different lowercase letter indicates significant differences among different levels of vermicompost dosage and soil depth (*$p < 0.05$*)

Discussion

Research indicates that vermicompost application does not significantly improve humic acid in surface and sub-surface layer on soils. Utilization of vermicompost with same repetition of level dose during seven years ago clarified not all organic practices could give rise to humic acid as a part of decomposition process in soil, where this experiment pronounced that strongest significancy with carbon organic, carbon stock and nitrogen total. As a land agriculture, many activities are conducted on this site, such as soil tillage, fertilization, harvesting, and other activities that correlation with plant yield, it would influence humic substances, mainly humic acid and fulvic acid. For instance, soil tillage would give a certain impact, can be positive or negative. This is line with Mahmoud *et al*. (2024) stated that humic acid content has invers correlation with depth layer of soil. Humic acid from deep layers has small particle size and the lowest molecular weights. In similar ways, aromatic condensation and humification degrees were increasing with deep layers. Moreover, aridity and organic matter exposure to oxidation by tillage practices accelerated the SOM decomposition and mineralization. For this reason, intensive tillage in arid climate enhances mineralization against humification and reduces content of humic acids. On the other hand, Navarrete *et al*. (2010) stated that any activities on soil can decline humic acids content where fulvic acid might be increased on topsoil.

A positive correlation between organic fertilizers with fulvic acids content showed that decomposition in soils, mainly in surface and under layer, tend to improve soil organic carbon and implied with carbon stock. This is line with Wu *et al*. (2014) that long-term organic fertilization will increase carbon in soil, however the unexpected result exhibits any improvement in concentration of weathered silicon and aluminum and incorporated with fulvic acids. Moreover, there are more than twice as many fulvic acids observed in cultivated land and as many 3.4 to 8.7 times larger under forest (Watanabe *et al*., 2001). On the other hand, Ukalska-Jaruga *et al*. (2018) also stated that fulvic acid had strongest correlation with total organic carbon (approximately reached 22.6%). On his research, fulvic acid derived from degradation and decomposition of molecular-weight hydrophobic and amphiphilic compound of organic material.

The application of vermicompost at 25 Ton ha^{-1} is the optimum level for giving a significant difference on soil pH, fulvic acid content, total organic carbon, total nitrogen, and carbon stock (Table 4). This implied that this level could increase charge of cation or anion, so it tends to rise soil pH and not separate from the role of carbon and nitrogen. This result is lined with research from Setyowati *et al*. (2023) and Kartika *et al*. (2023) stated that range level of application vermicompost for enhancing availability of soil nitrogen and especially for shallot was 15 to 40 Ton ha^{-1}. The significant difference of cation exchange capacity (CEC) in soil after using 15 Ton ha^{-1} reach more than 40 me $100g^{-1}$ and categorized in very high. This variable can be used as a category for soil fertility, crop growth, and soil contamination. Meanwhile, shallots show positive impact with their growth and yield at this level dose.

The improvement of nitrogen as impact from organic supply represents a contradictory consequence. As Liu *et al*. (2021) stated that his result promoted the enhancing availability of phosphor when the excessive nitrogen exists in salt-affected soil by fulvic acid applications. In this case, fulvic acid plays a role in providing the organic anions and binding anion-cations such as Al^{3+}, Fe^{3+}, and Ca^{2+} (Staunton and Leprince, 1996; Nagarajah *et al*., 1970; Perassi and Borgnino, 2014). The increasing nitrogen, correlated with the number of vermicompost applied as long as 7 years, arises from nutrient release as impact from nutrient cycle in soil. Vermicompost has 1.65% total nitrogen (Table 3), and this amount will be accumulated in time-by-time application. This situation is related with study that conducted by Kartika *et al*. (2023) that showed the significant improvement in nitrate after 5 weeks incubation through vermicompost. Results from study that conducted by Zeng *et al*. (2021) stated that the chemical composition of Soil Organic Carbon (SOC) stock was divided into iron-linked humin (HMi), clay-combine humin (HMc), insoluble humin residue (HMr), humic acid (HA) and fulvic acid (FA). The stock of theses chemical components was in the order of HMr (643.95 -269.90 g m^{-2}) > FAs (144.85 – 943.71 g m^{-2}) > HAs (39.67 – 389.55 g m^{-2}) > HMc (24.43 – 142.89 g m^{-2}) > HMi (20.80 – 59.57 g m^{-2}). The stock of these components and their variations generally showed a higher concentration in topsoil, where also obviously affected by soil type, soil depth, and its interaction ($p < 0.01$) with greater contributions from soil dept than soil type, especially for humin.

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