

Research Article

Humic acid characterization in soil from various land uses in Tutur District, Pasuruan Regency of East Java

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Abstract: Changes in land use will impact on the characteristics of humic acid in the soil due to biotic and abiotic factors. The study aimed to characterize humic acid in soil from various land uses in Tutur District, Pasuruan Regency of East Java. Soil sampling was carried out on six land uses, namely mixed garden, coffee plantation, apple orchard, vegetable garden, pine forest, and conservation forest. Analysis of soil samples included soil chemical characteristics (pH, redox, organic-C, and total-N) and characterization of humic acid included colour ratio of E4/E6, total acidity, carboxyl groups, and phenolic groups. The results showed that the land use of coffee plantation provided the best soil fertility indicated by the contents of humic acid, total-N, organic-C in the soil that were higher than that of other land-uses. The highest humification index was obtained for coffee plantation land use with the measurement of the E4/E6 colour ratio of 4.56 index value. The best characteristics based on the total value of acidity, the -COOH group and the phenolic -OH group were observed in the coffee plantation. This was supported by the characteristic of humic acid through the humification index or E4/E6 colour ratio, as well as the low phenolic -OH value which is a characteristic of fulvic acid.

Keywords: *characterization, humic acid, land use, organic-C, total-N*

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Introduction

The diversity of land management affects the unstable and stable soil organic matter. Humic is included in the category of stable soil organic matter (Dębska et al., 2012; Tavares and Nahas, 2014). The naturally stable soil organic matter is formed from the process of degradation of organic matter in the form of plant remains (litter, or remnants of dead twigs) with the help of microbes and biochemical activity (Tavares and Nahas, 2014). Humus is an organic material with high potential and has not been widely developed to improve the quality and productivity of the land. Topsoil is the last product of soil organic matter which has an essential role in maintaining soil sustainability. The presence of topsoil is composed of organic material components such as carbon (C), hydrogen (H), and oxygen (O).

The availability of topsoil is obtained from the overhaul of fallen plant litter and undergoes biochemical decomposition (Susanti and Halwany, 2007). Litter is an organic material that supplies soil organic material from plant parts, both leaves, twigs or roots of plants that die. Organic material will be decomposed to form a simpler material that is widely known as hummus (Suwahyono, 2011). The role of humic compounds contained in topsoil makes the material very important in the soil. Humic compounds have a role in increasing land productivity because humus plays a role in regulating soil nutrient supply (Tan, 2014). Humic substance deficiency will have an impact on decreasing land productivity and its impact on yields and yield quality. Diverse land use has its potential in the supply of soil organic matter. Supplier of soil organic matter, namely diverse vegetation resulting

in varying levels of organic matter decomposition. Land use will provide a different supply of organic carbon. Obtained organic carbon yield in mixed garden by 0.94% and industrial plantations with teak commodities by 0.98% (Edwin, 2016). Whereas other land uses, namely for forest, obtained an organic carbon value of 3.07% and for a field of organic carbon value of 1.03%.

Humus is the primary substance in the formation of humic compounds derived from soil organic matter. The topsoil component consists mainly of organic acids, one of which is humic acid. The fraction of soil humic compounds is divided into humic acid, fulvic acid, and humin compounds, which are insoluble and moist parts (Tan, 2014). The structure of humic acid is mostly composed of phenol groups, carboxylates which are bound to aromatic rings and quinones, which are bridged by nitrogen and oxygen. So it can be concluded that humic acid is an aromatic and aliphatic compound that binds the -COOH, -OH phenolate, -OH alcoholic groups, and allows quinone groups (Yuliyati and Natanael, 2016). Another characteristic of humic compounds is the colour ratio of E4/E6. This ratio is measured by using spectrophotometry to find the wavelength ratio absorption. The colour or absorbance ratio of the humic acid solution at wavelengths of 465 and 665 nm is the goal in the characterization of humic acid. The E4/E6 ratio provided information about the concentration of humic material derived from various soil humic extracts (Khan and Schnitzer, 1972). This E4/E6 ratio is also believed to be a characteristic of the condensation level of the aromatic carbon group (Kononova, 1966). The different characteristics of the humic substance in each land use will also affect soil fertility conditions, for example, the effect on nutrient sorption and cation exchange capacity. This study aimed to characterize humic acid in soil from various land uses in Tutur District, Pasuruan Regency of East Java.

Materials and Methods

Study area

A field survey took place from November 2018 to March 2019. Soil sampling was carried out at mixed garden in Ngembal Village, coffee plantations in Tutur, Sumberpitu, and Kalipucang Villages, apple orchard in Andonosari Village, pine forest, vegetable garden in Wonosari Village, and conservation forest in Kayukebek Village of Tutur District, Pasuruan Regency. Sampling was made at a depth of 0-20 cm. Each land use was taken as much as five times as a repetition of land. Soil analysis was conducted in the Laboratory of Land Resources at the Faculty of Agriculture, UPN "Veteran", East

Java. The analysis covered soil chemistry and characterization of humic acid.

Soil chemical analysis

Soil chemical analyses were conducted following standard methods of "Petunjuk Teknis Analisis Kimia Tanah, Air, Tanaman dan Pupuk" (Page et al., 1982; Eviati and Sulaeman, 2009). Soil chemical analysis included actual pH, redox potential, total-N, and organic-C.

Humic acid extraction

Extraction of humic compounds (humic acid and fulvic acid) was carried out using the modified method of Stevenson (1994). A 10 g soil sample was extracted using a 0.5 mL NaOH solution of 100 mL (1:10). The sample was then shaken for up to 24 hours and cooled down for 16 hours with occasional shaking. The next stage was centrifuging the sample with a speed of 1,500 rpm. Separation of the substance was made using Whatman 41 filter paper to obtain the humic compound. The substance was then added to HCl 6 N until the pH of the solution reached 2. The addition of HCl 6 N formed two layers. The solution was separated again with Whatman 41 filter paper. The precipitate obtained was rinsed using CO₂-free distilled water to remove the residual chlorine in humic acid. It was then put in an oven with a temperature of 105°C for determining the percentage of humic acid and 60°C for the characterization of humic acid (Seran, 2011).

Characteristic of humic acid

Analysis of humic acid characteristics was conducted following the standard method of humic acid characterization by functional group (Schnitzer and Gupta, 1965). The analysis of humic acid characteristics included water analysis of total acidity, carboxyl group, and phenolic group.

Analysis of total acidity

Total acidity is the measure of the total concentration of carboxylic acid, and phenolic hydroxyl groups present in a compound. 20 mL of 0.2 N BaSO₄ solution was added to 50 mg of humic acid and nitrogen was bubbled through it. The mixture was refluxed for twenty-four hours, the suspension was filtered off, the residue was washed with distilled water, and the filtrate was titrated potentiometrically at pH 8.4 with standard 0.5 N HCl solution. A blank titration was performed simultaneously.

Analysis of carboxyl group

A solution of 10 mL of 1 N (CH₃COO)₂Ca in 40 mL of water was added to 50 mg humic acid. The mixture was refluxed for twenty-four hours, filtered, the precipitate was washed, followed by the potentiometric titration of the filtrate at pH 9.8 with

0.1 N NaOH solution. A blank titration was performed simultaneously.

Analysis of phenolic groups

The concentration of phenolic hydroxyl groups was determined by subtracting the concentration of carboxylic acid groups expressed in milliequivalent per gram of humic acid from the total acidity.

Analysis of the E4/E6 spectrophotometry ratio

Optical densities of 0.1 g/L of a solution of humic acid in 0.01 N NaOH were measured for wavelengths 465 nm and 665 nm using a spectrophotometer, and the ratio of the two optical densities was determined (Chen et al., 1977).

Results and Discussion

Soil pH

The conditions in the study area indicate that there are differences in the chemical properties of the soil in each land use. The results showed that coffee plantation had a low pH of 4.82 and the highest pine forest of pH 5.46 (Table 1). The overall average acidity of the soil into the very acidic and slightly acidic category based explains that the pH range of 4.6-5.0 is in the very acidic category and the pH range is 5.1-5.5 included in the category rather acid (McCauley et al., 2017). This can be attributed to the soil organic matter content as one source of soil acidity. The acidity level of six land-uses showed no significant difference at the Anova test of 5% level. Soil pH indirectly affects the decomposition of soil organic matter. The effect of soil pH in decomposition is on the presence of soil microbes because microbes cannot reproduce under conditions of too low or high pH (Sayara et al., 2010). Renovation of organic matter is possible to occur more quickly under slightly acidic soil conditions because when pH conditions are low the availability of nutrients for microbes is sufficient so that its activity increases (Mrozik et al., 2003). Results of soil pH measurements showed that the land use of coffee garden had the lowest pH value. These conditions allow the overhaul of soil organic matter to occur more quickly.

Redox potential

Reduction and oxidation conditions are determinants of oxygen availability in the soil. Redox reactions play a role in many activities in the soil, including the overhaul of organic matter, microbial activity, and soil respiration (Stepniewski et al., 1994). Redox potential is the value of the reaction conditions of oxidation and reduction occurring in the soil. Redox values in the entire study area ranged from 99 to 133 mV that was in the category of medium-low

reduction (Table 1). The overhaul of soil organic matter is influenced by microbial activity, where oxygen is one of the elements needed. When the soil experiences aerobic or oxidation conditions, the overhaul of soil organic carbon becomes CO₂ faster due to oxygen availability for microbes (Nawaz et al., 2014). The determination of the redox value criteria is based on the description of that 100-300 is a low reduction (Schüring et al., 2001). This happens because Fe has a reduced condition of Fe³⁺ to Fe²⁺. Moderate and low reduction values mean that the soil sometimes experiences water saturation but not for too long. This condition can be related to the condition of the soil moisture regime, namely the humidity which bumpy means that there is a long wet condition, but there are days when the soil is dry (Staff, 2014). This medium-low reduction condition supports the formation of more stable soil organic carbon. Humate that is formed in the soil is a compound formed from organic and inorganic compounds and microbes in remodelling. Humic can form in oxygen-deficient soil conditions or wet soil conditions (Keller et al., 2009).

Soil organic carbon

The role of soil organic carbon is essential in the formation of topsoil, where topsoil is a stable organic material (Lehmann and Kleber, 2015). Several factors influence the condition of soil organic carbon in the soil. The organic carbon content of the soil is described as a bucket with an output (Siringoringo, 2013). Inputs are in the form of plant residues and other additional organic materials. The storage is in the form of organic carbon which slowly settles. The output is the conversion of CO₂ into the air through the decomposition of organic matter (Lubna and Sembiring, 2013). The coffee plantation can provide a high supply of plant litter, so the results of this study showed that coffee plantation had the highest organic carbon content of 3.21% and mixed plantation had the lowest soil organic carbon content of 1.20% (Table 1).

The decrease in organic-C content in the soil can be caused by changes in management and land use so that the availability of stable carbon will be reduced (Ermadani et al., 2018). The organic-C content will undoubtedly determine the amount of humic acid that will form in the soil. The relationship between organic-C content and humic acid is shown by the linearity graph (Figure 1) where the increased organic-C content will create a high humic acid content. Loss of soil organic carbon will reduce humic acid content because carbon which is stable will also be reduced. Humification is very closely related to the decomposition, mineralization, and stabilization of soil organic carbon (Zech et al., 1997). The coffee plantation is very supportive of the formation of humic acid because the existing

vegetation and microclimate in the system support the decomposition and mineralization processes of stable carbon. The land use of coffee plantation showed the highest organic-C content (sig. 0.05), so it also affected the content of humic acid formation. The content of humic acid in the coffee plantation

(1.86%) was the highest amongst the other land uses (Table 2). This is closely related to the overhaul of organic material. Management of organic-C in the soil, especially in tropical climate regions, is difficult due to the rapid rate of organic decomposition (Ermadani et al., 2018).

Table 1. Soil chemical properties in several land uses.

Land Usage	pH	Redox (mV)	Organic-C (%)	Total-N (%)
Mixed Plantation (MP)	4.98	123	1.20	0.30
Coffee Plantation (CP)	4.82	126	3.21	0.59
Apple Plantation (AP)	5.37	105	2.72	0.57
Vegetable Garden (VG)	5.28	99	1.89	0.41
Pine Forest (PF)	5.46	133	2.06	0.37
Conservation Forest (CF)	5.02	118	2.96	0.44
LSD 5%	ns	*	**	**

Note: ns = not significantly different, * = significantly different, ** = very significantly different.

Addition of organic matter in the form of cow dung (40 kg/tree) and the return of plant litter to the soil provide additional stable organic carbon. This is consistent with land management research according to (Ermadani et al., 2018) where the addition of organic material in the form of litter plants can supply humic acid (AH) of 0.56%.

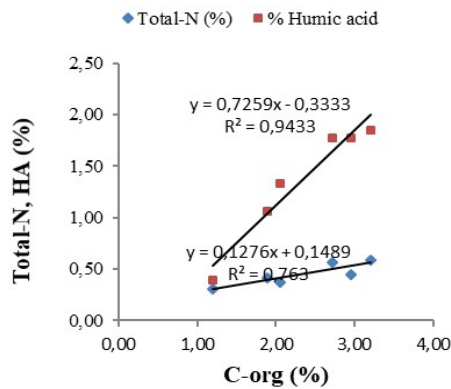


Figure 1. Relationship of organic-C with humic acid and total-N.

Total soil nitrogen

Mineralization of soil organic matter will release some nutrients into the soil. Nutrients produced by the decomposition of organic materials include C, N, P, K and S, which are the most constituent (Zech et al., 1997). The results showed the highest total nitrogen content of coffee plantation was 0.59%, and that of the mixed plantation was 0.30% so that total nitrogen had a range of values of 0.30-0.59% (Table 1). The total nitrogen content in the soil is a constituent of humic acid in the form of amino acids. Humic acid monomer forms with the composition of

$C_{308}H_{335}O_{90}N_5$ (Schulten and Leinweber, 1996). Figure 2 shows that there is a positive relationship, and total nitrogen has the effect of $R^2= 0.7320$, equivalent to 73% in the formation of humic acid. The graph shows a positive relationship which means that any increase in total nitrogen results in an increase in humic acid. The value of total soil nitrogen content in the coffee plantation was higher than other land uses. The total N content of coffee plantation of 0.59% was the highest value compared to other land management. The existence of legume plant vegetation can be suspected as a source of nitrogen supply in the soil. Litter of protective plants, coffee plants, and ground cover crops will be returned to the soil and will contribute nutrients for the production of coffee plants (Evizal et al., 2014). Besides acting as a shade, legume plants are able to provide nitrogen nutrients. Legume plants such as Lamtoro (*Leucaena leucocephala*), Sengon (*Albizia chinensis*), fixation by legume plants will be able to increase N nutrients so as to increase soil fertility cheaply and have no impact on the environment (Prawoto, 2008)

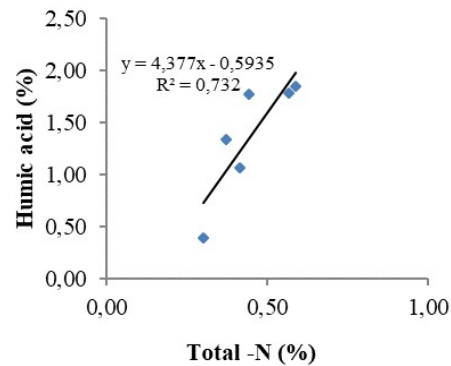


Figure 2. Relationship of total-N with humic acid.

Table 2. Humidity substance of some land usages.

Land Use	Humic acid (%)	Fulvic acid (%)	Humin (%)
Mixed Plantation (MP)	0.39 a	3.79 a	95.82 a
Coffee Plantation (CP)	1.85 b	6.90 a	91.25 a
Apple Plantation (AP)	1.78 b	7.33 a	90.90 a
Vegetable Garden (VG)	1.06 ab	9.50 a	89.44 a
Pine Forest (PF)	1.33 ab	7.86 a	90.81 a
Conservation Forest (CF)	1.77 b	5.57 a	92.65 a

Remarks: Numbers followed by the same letters in the same column indicate no significant difference in the Least Significant Difference (LSD) test level of 5%.

E4 / E6 spectrophotometry

The colour ratio produced by the extraction of humic acid with NaHCO₃ solution can be used as a humification index. The value of the E4/E6 ratio mentioned is also an indicator to determine the maturity of soil organic carbon which is characterized by a mature decomposition activity (Senesi et al., 1996). Assessments using more light absorption methods were developed because they were able to show a more detailed humification index with stages certain. Colour ratio values for some land uses are shown in Table 3, which show the lowest ratio in the coffee plantation and the highest ratio in the use of land for conservation forest. The low value of the E4/E6 colour ratio indicates that condensation and polymerization of high organic carbon have occurred (Seran, 2011). This means that with these conditions, the level of humification becomes high.

Table 3. Value of the E4/E6 ratio (humification index).

No.	Land Use	E4/E6
1	Mixed Plantation (MP)	4.98 ab
2	Coffee Plantation (CP)	4.56 a
3	Apple Plantation (AP)	5.72 ab
4	Vegetable Garden (VG)	4.72 a
5	Pine Forest (PF)	4.91 a
6	Conservation Forest (CF)	6.18 b

Remarks: Numbers followed by the same letters show no significant difference in the LSD test level of 5%.

The decrease in the value of the E4/E6 ratio also indicates that the dominance of material with high molecular weight, so that it can be argued that humic acid formation has occurred (Jeong et al., 2010). The E4/E6 ratio is determined using the absorbance value or the absorption of light using the spectrophotometric method. The value obtained from the absorption of light between wavelengths of 465 nm and 665 nm determines the ratio for the level of humification. The wavelength of 465 nm is the absorption of light for components associated with the initial humification process, while the

wavelength of 665 nm is used to determine the mature humification component so that a low ratio value indicates the level of humification or the formation of high humic acid (Seran, 2011). Humification index assessment is divided into two levels, namely low and high, where the low index value category is in the 6-8 value range. A high index is indicated by the colour ratio of 3-5 that was obtained from the ratio of light absorption of 465 nm and 665 nm (Tan, 2014). The E4/E6 ratio indicated that the level of humification in the coffee plantation was the highest followed by the vegetable garden, pine forest, mixed garden, apple orchard, and the lowest was conservation forest (Table 3). This result is supported by previous studies with that the colour ratio of E4/E6 in coffee plantation had a range of values of 4.06-4.79 that indicates a high humification index (Putra et al., 2016).

Total acidity, carboxyl groups and phenolic groups

The values of total acidity ranged from 7.61 to 8.36 meq/g, carboxyl groups ranged from 1.88 to 2.17 meq/g, and phenolic groups ranged from 5.67-6.72 meq/g (Table 4). The total acidity and functional groups of humic acid are largely determined by the quality of plant tissue that supplies soil organic carbon. Litter produced by plants has different qualities; this refers to the content of compounds that are easily degraded and difficult to degrade. The content of the -COOH (carboxyl) group is largely determined by the content of lignin compounds in plant litter (Rahmawati, 2011). The breakdown of the aromatic ring inside the lignin compound will form an aromatic ring - COOH. While phenolic -OH values are largely determined by degradation in the later stages after the formation of the -COOH group. The characteristic of humic acid is the lower total acidity value compared to fulvic acid (Rahmawati, 2011). This study showed that the total acidity values were included in the range that had been done by previous researchers that ranging 5.70 to 8.90 meq/g (Schnitzer, 1986). The-COOH values observed in this research also fall into the range of the -COOH values of previous studies. However, the phenolic -OH values were outside the range of that previously reported by other researchers. The Anova

from characteristics of total acidity, carboxyl and phenolic groups of humic acid did not show significantly different results in each land use.

However, when viewed in terms of the contents of the -COOH and -OH phenolic groups, the differences appeared.

Table 4. Total value of acidity and functional groups.

Land Use	Total Acidity (meq/g)	Carboxyl Groups (meq/g)	Phenolic Groups (meq/g)
Mixed Plantation (MP)	8.05 a	1.94 a	6.11 a
Coffee Plantation (CP)	7.61 a	1.94 a	5.67 a
Apple Plantation (AP)	7.95 a	2.09 a	5.87 a
Vegetable Garden (VG)	8.75 a	2.02 a	6.72 a
Pine Forest (PF)	7.63 a	1.88 a	5.75 a
Conservation Forest (CF)	8.36 a	2.17 a	6.19 a
Schnitzer (1986)	5.70-8.90	1.5-5.70	2.70-3.50

Remarks: Numbers followed by the same letters show no significant difference in the LSD test level of 5%.

The carboxyl value determines the molecular weight of humic acid because the constituent of humic acid is derived from the -COOH group which is the result of degradation of lignin compounds (Ogner and Schnitzer, 2006). The carboxyl value in the conservation forest is the highest value at 2.17 meq/g while the lowest in the pine forest at 1.88 meq/g. However, the highest lignin and humic acid compounds produced are in the land use of coffee plantation. This is due to the -OH group value on the conservation forest land use is higher than the coffee plantation. Phenolic-OH group is a dominance of a group derived from fulvic acid, so there is still a dominance of folic acid compared to humic acid (Eshwar et al., 2017). This is also seen in the vegetable garden, although carboxyl values were high, the -OH group was more dominant. So that the humic acid formed was far less than fulvic acid. The coffee plantation consists of land management forms containing conservation, and the quality of soil organic matter is higher than that of other land management. Land management for coffee plants is an example in the management of soil organic matter, where raw/fresh organic material contains leaf litter, dead branches of dead plants produce soil. The high supply of organic matter gives an impact on the high soil organic carbon content, increases the availability of humic substances and produces humic with the best characteristics.

Conclusion

Land use with the highest humification is in a coffee plantation with a value of E4/E6 4.56. Then in a row, the vegetable garden, pine forest, mixed garden, apple orchard, and conservation forest. Characteristics based on the total value of acidity, the -COOH group and the best phenolic -OH group are in the coffee plantation. This is supported by the characteristic of humic acid through the humification

index or E4/E6 colour ratio, as well as the low phenolic -OH value which is a characteristic of fulvic acid. The coffee plantation is the best choice in the humification process due to the supply of soil organic matter through the return of crop residues to the soil that provides stable organic carbon and produces humic substances with the best characteristics.

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