Engineering and Technology Journal e-ISSN: 2456-3358
Volume 09 Issue 06 June-2024, Page No.- 4179-4185
DOI: 10.47191/etj/v9i06.01, I.F. – 8.227

Characterization of Humic Acid Using E4/E6 Ratio in Various Land Uses in Jatiarjo Village, Prigen District, Pasuruan

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ABSTRACT: Different land uses can influence the characteristics of humic acid in the soil. The study aimed to characterize humic acid in soil from various land uses in Jatiarjo Village, Prigen Distict, Pasuruan Regency of East Java. Soil sampling was carried out on three land uses, namely mixed gardens, bush lands, and dry lands. Sampling was carried out using the purposive random sampling method, where seven points were taken in each land use. Analysis of soil samples included soil chemical characteristics (pH, organic-C, and CEC) and characterization of humic acid using E4/E6 ratio. The results showed that the land use of bush land provided the best soil fertility indicated by the content of humic acid and C-organic in the soil that were higher than that of other land uses. All land uses in Jatiarjo Village, Pasuruan have advanced humification levels, as evidenced by low E4/E6 ratio values (<5).

KEYWORDS: Carbon Organic; Soil Acidity; Decomposition; Humification ; Bush Land

I. INTRODUCTION

Different land uses, such as agricultural land, plantations, forests, or residential areas, can influence the characteristics of humic acid in the soil. This is due to differences in vegetation types, land management practices, and human activities occurring in each of these land uses. Humic acid, as the main component of humic substances, plays a significant role in determining soil fertility, water retention, and nutrient availability for plant growth. Therefore, characterizing humic acid using spectrophotometric methods (E4/E6 Ratio) across various land uses can provide important information regarding the degree of humification and the quality of soil organic matter. The E4/E6 Ratio (absorbance at wavelengths of 465 nm and 665 nm) is a commonly used method to evaluate the degree of humification and the degree of aromatic condensation of humic acid. This method is based on the principle that aromatic and aliphatic compounds in humic acid have different absorbances at certain wavelengths within the ultraviolet-visible (UV-Vis) spectrum (Aiken et al., 1986; Chen et al., 1977; Stevenson, 1994; Tan, 2014).

This research aims to characterize humic acid from various land uses using the spectrophotometric method (E4/E6 Ratio) and analyze the influence of land use on the properties and quality of humic acid. By characterizing humic acid, it is expected to obtain useful information to evaluate the quality of soil organic matter and assist in developing more effective and sustainable land management strategies.

II. RESEARCH METHODS

A. *Study Area*

This research was conducted from July to December 2023. Soil sampling took place in Jatiarjo Village, Prigen District, Pasuruan, on three Land Use Units (LUUs), namely mixed gardens, bush lands, and dry lands. Sampling was carried out using the purposive random sampling method, where seven points were taken in each LUU. The analysis of soil chemical properties was performed at the Land Resources Laboratory, Faculty of Agriculture, UPN "Veteran" East Java.

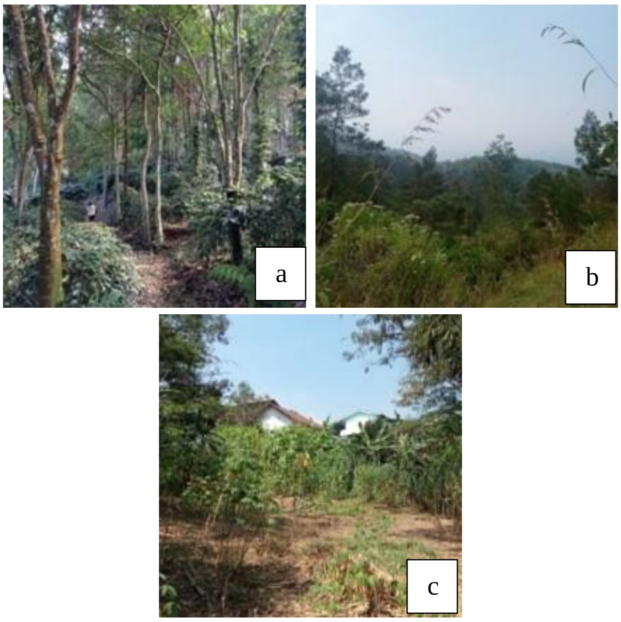

Figure 1. Existing Conditions in 3 Land Use Units (LUUs) in Jatiarjo Village, Pasuruan. a) Mixed Crops; b) Bush Land; c) Dry Land

B. Soil Chemical Analysis

Analysis of soil acidity

Soil pH analysis is conducted using the conductometric method. A soil sample weighing 10 grams is placed into a shaking bottle, then 20 ml of ion-free water (H_2O) solution is added. Subsequently, the sample is shaken using an electric shaker for 30 minutes. The pH suspension measurement is performed using a pH meter.

Analysis of soil organic carbon

Soil organic carbon analysis is conducted using the Walkey and Black method. A soil sample passing through a 0.5 mm sieve, weighing 0.25 grams, is placed into a 100 ml volumetric flask, then 5 ml of $K_2Cr_2O_7$ and 10 ml of concentrated sulfuric acid are added, and then homogenized. After 1 hour, distilled water is added up to the mark and homogenized, then left to reach room temperature. Absorbance is measured using a spectrophotometer with a wavelength of 560 nm.

Analysis of Soil Cation Exchange Capacity (CEC)

Soil Cation Exchange Capacity (CEC) analysis is conducted using the colorimetric method. A soil sample passing through a 2 mm sieve is weighed at 1 gram and then mixed with 20 ml of Ammonium Acetate before being shaken with an electric shaker for 10 minutes. After shaking, the extract is filtered using filter paper to separate soil particles from the filtrate. The next step involves rinsing with 50% Alcohol three times to remove soluble cations. Then, percolation is performed using 10% NaCl at 20 ml. After the percolation stage, the filtrate is diluted with distilled water at a ratio of 1:9. Once homogenized, 2 ml of the filtrate is transferred to another reaction tube to which 5 ml of tartrate buffer, 5 ml of Na-phenol, and 5 ml of 5% NaOCl are added, then homogenized and used for absorbance reading. Absorbance reading is done using a spectrophotometer at a wavelength of 660 nm (Eviati *et al.*, 2023).

Humic Acid Extraction

A soil sample weighing 10 g is extracted with 100 ml of 0.5 N NaOH solution (1:10). The sample is then shaken for 24 hours and cooled for 16 hours with occasional shaking. The next step involves separating non-humic substances from humic compounds. Separation is done using Whatman 41 filter paper to obtain humic compounds. The substance is then added to 6 N HCl until the pH of the solution reaches 2. The addition of 6 N HCl forms two layers. The solution is separated again using Whatman 41 filter paper. The obtained precipitate is rinsed with CO_2-free distilled water to remove residual chlorine in the humic acid. It is then placed in an oven at 105ºC to determine the percentage of humic acid and at 60ºC for humic acid characterization (Seran, 2011).

Characteristic of Humic Acid (using E4/E6 Ratio)

Characteristic of Humic Acid (using E4/E6 Ratio) is determined using the spectrophotometric method (Chen *et al.*, 1977). A humic acid sample weighing 0.02 grams is dissolved in 10 ml of 0.05 N $NaHCO_3$, then absorbance is measured using a UV-Vis spectrophotometer at wavelengths of 465 nm and 665 nm.

C. Data Analysis

The obtained data is tabulated and analyzed using Microsoft Excel and SPSS software. Data analysis includes descriptive analysis, ANOVA, HSD test (Tukey), and regression analysis. The resulting data is then presented in the form of graphs and tables.

III. RESULT AND DISCUSSION

IV. Land Uses	Parameters		
	Actual pH	SOC (%)	CEC (m.e/100 g)
Mixed Cropping	**5,72 a**	7,91 b	**37,09 b**
Bush Land	5,68 a	**8,94 b**	32,33 b
Dry Land	6,06 a	2,42 a	25,59 a
HSD 5%	**ns**	*	*

*Note : SOC (Soil Organic Carbon), CEC (Cation Exchange Capacity). * (significantly different), ns (not significantly different). Numbers followed by the same letters show no significant difference in the Honestly Significant Difference (HSD) test level of 5%.*

Soil acidity is one of the crucial factors influencing the properties and characteristics of humic acid in soil. Humic acid is a complex organic compound formed from the decomposition of organic matter and is a primary component of soil humus (Stevenson, 1994).

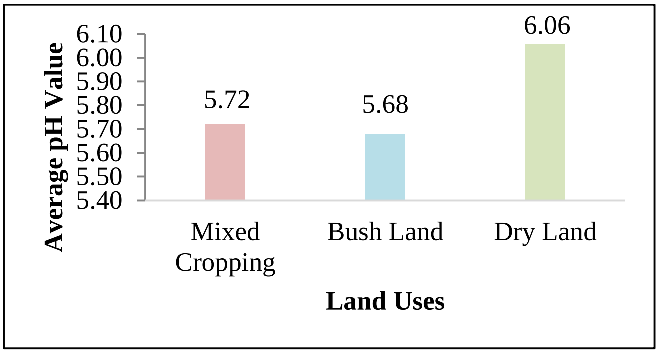

Figure 2. Soil acidity in several land uses in Jatiarjo Village

Soil pH analysis in the research area (Figure 2) indicates differences in soil chemical properties among land uses. The average soil acidity across all land uses falls within the slightly acidic category according to Eviati et al. (2023) which defines the pH range of 5.6 - 6.0 as slightly acidic. This may be related to the soil organic matter content as one of the sources of soil acidity.

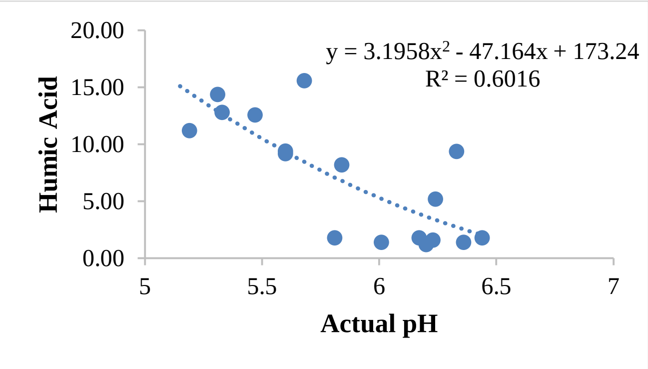

Figure 3. Relationship between actual pH and humic acid

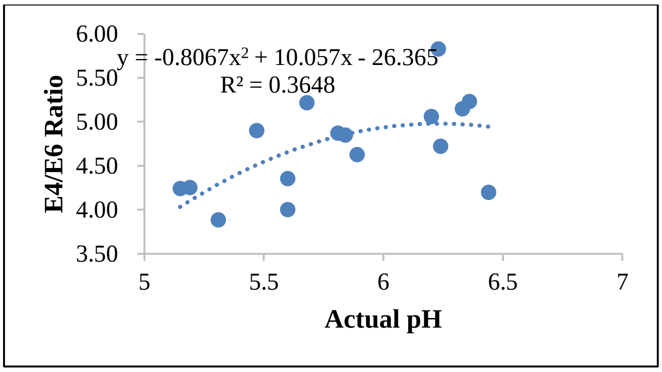

Figure 4. Relationship between actual pH and E4/E6 ratio

Humification process (formation of humic acid) is the final stage of decomposition. The influence of soil pH on decomposition lies in its effect on the presence of soil microbes because microbes cannot proliferate in excessively low or high pH conditions (Sayara et al., 2011). Organic matter decomposition may occur more rapidly in slightly acidic soil conditions because at low pH, microbial nutrient availability is sufficient, leading to increased microbial activity (Mkrozik et al., 2003).

Lower soil pH (acidic) results in humic acid from forest soil having higher aromaticity and polymerization degree compared to humic acid from agricultural and plantation soil. However, at higher soil pH (basic), such differences become less significant or even disappear (Silva et al., 2018).

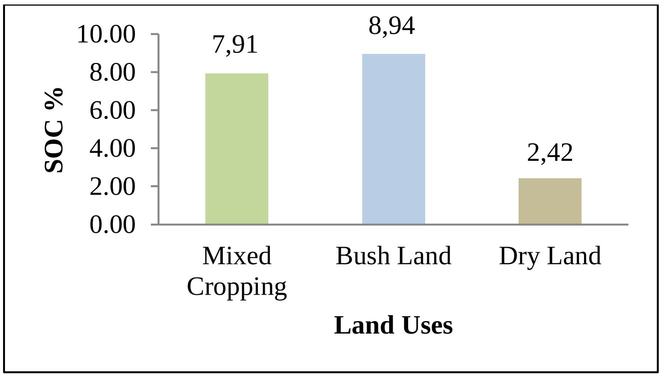

Figure 3. Average of Soil Organic Carbon (SOC) in Various Land Uses

Soil organic carbon (SOC) content reflects the amount of organic matter, including humic substances, present in it. The higher the SOC content, the more humic substances are contained in the soil. Humic substances contribute to soil structure improvement, increasing Cation Exchange Capacity (CEC) (Figure 4), and providing nutrients to plants (Stevenson, 1994; Tan, 2014).

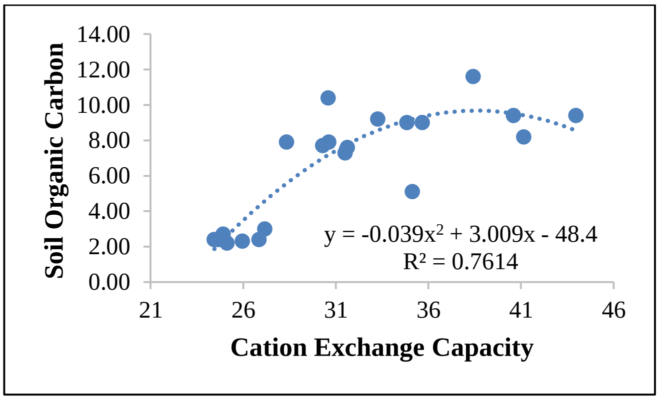

Figure 4. Relationship Between CEC and SOC

The highest organic C content is obtained in bush land at 8.94%. This may be due to the dominance of shrub vegetation in bush land, which is a type of plant with high polyphenol content, resulting in high humic content in the decomposition end product. This is consistent with Rashed et al.'s (2022) statement that some shrub species have high total polyphenol content, ranging from 25.9 to 104.6 mg/g dry weight. Polyphenols are compounds produced by plants and can contribute to humic substance formation through the humification process. Polyphenols, as one of the main sources of organic matter, can undergo humification processes in soil. These compounds are present in plant tissues and can enter the soil through the decomposition of plant residues (Kögel-Knabner, 2002). Polyphenols can react with other compounds such as proteins, carbohydrates, and nucleic acids during the humification process, forming more complex and stable structures in humic substances (Tan, 2014). In mixed cropping areas, vegetation is predominantly composed of coffee, sengon, and pine trees. According to Ramalakshmi et al. (2008), coffee beans contain a considerable amount of polyphenolic compounds, especially chlorogenic acid and its derivatives. Robusta coffee contains a polyphenol content ranging from 7.4 to 10.1% dry weight, while Arabica coffee beans have a polyphenol content ranging from 5.1 to 7.9% dry weight. Pine wood and pine bark contain various polyphenolic compounds such as condensed tannins and lignin. The polyphenol content in Pinus radiata wood ranges from 0.2 to 2.4% dry weight, while Pinus radiata bark contains a polyphenol content ranging from 12.4 to 16.7% dry weight (Scalbert et al., 1986). Sengon trees (Paraserianthes falcataria) contain several polyphenolic compounds such as flavonoids, tannins, and phenolic acids. Sengon leaves have a polyphenol content ranging from 1.4 to 2.2% dry weight

(Fidrianny et al., 2015). In dry land use, vegetation is dominated by cassava plants. Cassava (Manihot esculenta) also contains a number of polyphenolic compounds, although its content is relatively lower compared to plants such as coffee or pine. In a study conducted by Medoua et al. (2007), the total polyphenol content in cassava leaves ranged from 27.8 to 57.2 mg/g dry weight, depending on the cultivar and location of growth. A study by Ayoola et al. (2011) found that cassava tuber peel extract contained a total polyphenol content of 5.4 mg/g dry weight.

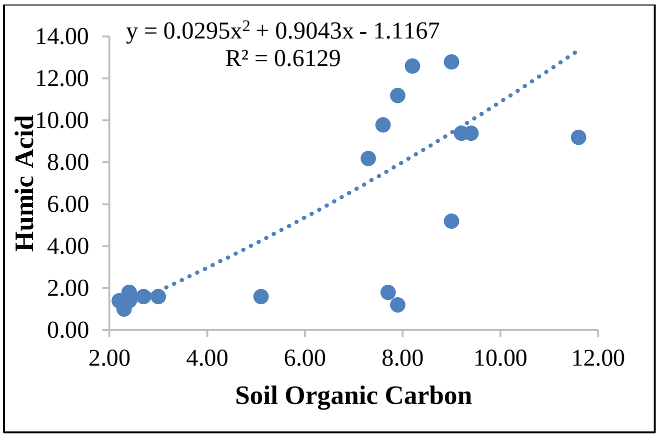

Figure 5. Relationship Between SOC and Humic Acid

In Figure 5, soil organic carbon content reflects the amount of organic matter, including humic substances. The higher the organic carbon content, the higher the percentage of humic substances in the soil. A low E4/E6 ratio indicates a high degree of aromaticity and polymerization of humic substances, usually associated with more stable and condensed humic substances (Chen et al., 1977; Stevenson, 1994; Tan, 2003; Zbytniewski & Buszewski, 2005).

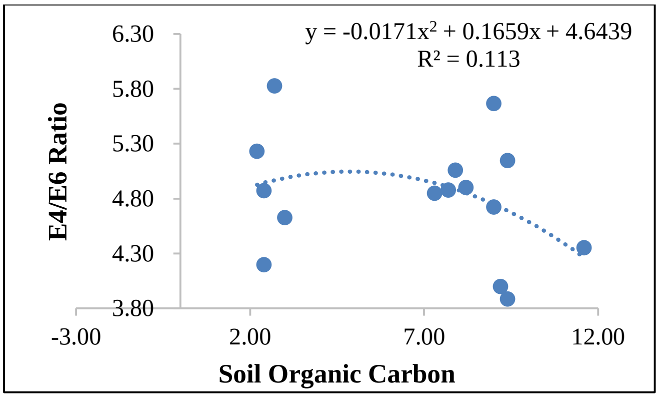

Figure 6. Relationship Between SOC and E4/E6 Ratio

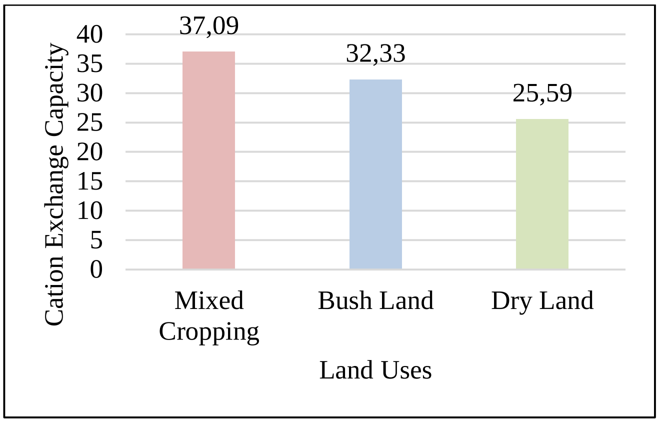

Figure 7. Average of CEC in Various Land Uses

In general, the humic substance content in soil significantly contributes to soil CEC through the negative charge held by humic substances. However, the correlation between CEC and humic substances may vary depending on land use types, management practices, and other environmental factors. Humic substances, which are the end products of organic matter decomposition, have negative charges that significantly contribute to soil CEC (Brady & Weil, 2008). This is due to the presence of functional groups such as carboxyl (-COOH) and phenolic (-OH) groups that can release protons and provide a negative charge on the surface of humic particles (Tan, 2014). This negative charge allows humic substances to bind and exchange important cations such as Ca2+, Mg2+, K+, dan Na+, thereby increasing soil CEC. The relationship between humic acid and CEC (Figure 8) shows a positive correlation. This is in line with Ren et al.'s (2018) research, which indicates a strong positive correlation between soil CEC and humic substance content in various land uses such as forests, grasslands, and agricultural land.

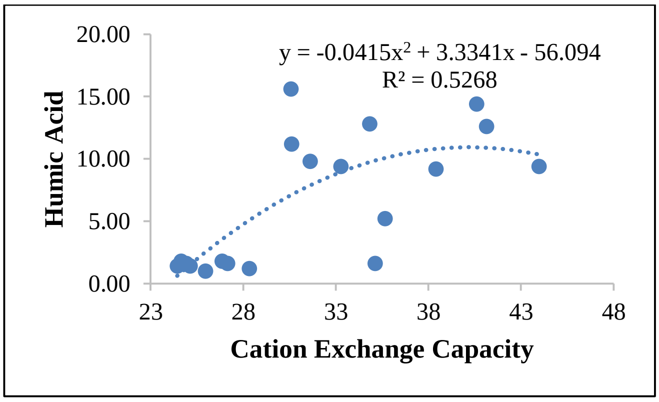

Figure 8. Relationship Between CEC and Humic Acid

Soils with higher humic substance content tend to have higher CEC as well. Harada et al. (2021) also found that CEC increases with the increasing degree of organic matter humification in soil. More humified organic matter contains more negatively charged functional groups, thereby contributing to increased soil CEC. Therefore, it can be concluded that there is a strong positive correlation between soil CEC and humic substance content, especially in soils with high organic matter content and advanced humification levels. Humic substances significantly contribute to soil CEC through their negative charges, which can bind and exchange important cations for plants. The E4/E6 ratio (absorbance at wavelengths of 465 nm and 665 nm) is used to evaluate the aromaticity and polymerization degree of humic acid. A low E4/E6 ratio indicates high aromaticity and polymerization degree of humic substances, usually associated with more stable and condensed humic substances (Chen et al., 1977; Martins et al., 2011).

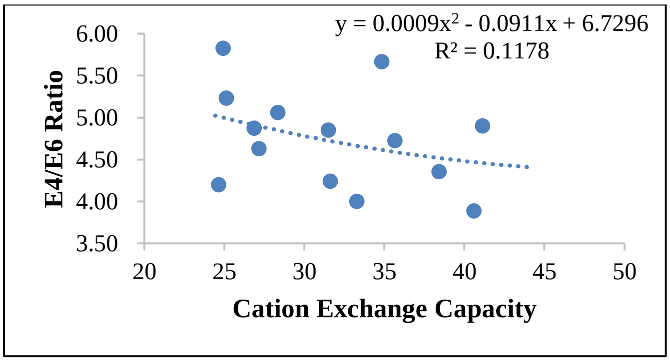

Figure 9. Relationship Between CEC and E4/E6 Ratio

Table 2. Humic substances of some land uses

Land Uses	Humic Acid	Fulvic Acid	Humin
		%	
Mixed Cropping	**0,09 b**	22,24 a	**69,03 a**
Bush Land	**0,09 b**	**24,53 a**	66,73 a
Dry Land	0,01 a	17,63 a	80,86 b
HSD 5%	*	ns	*

*Note : * (significantly different), ns (not significantly different). Numbers followed by the same letters show no significant difference in the Honestly Significant Difference (HSD) test level of 5%.*

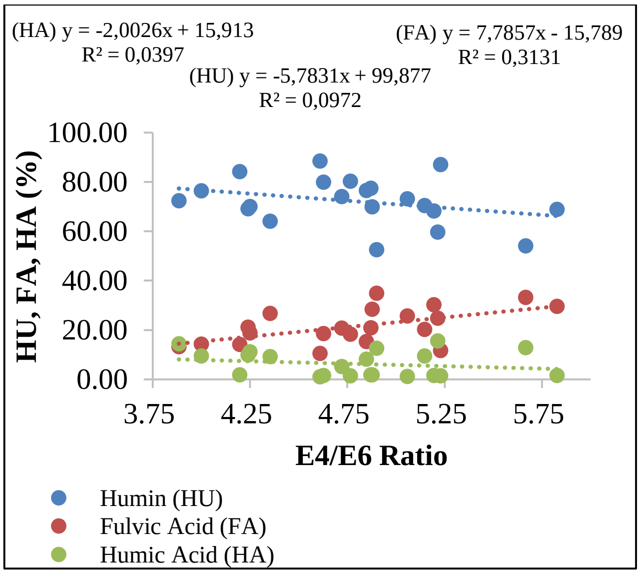

Figure 10. Relationship between humic substances and E4/E6 Ratio

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

Land Uses	E4/E6 Ratio	Status
Mixed Cropping	4,71 a	High
Bush Land	4,77 a	High
Dry Land	4,88 a	High
HSD 5%	**ns**	

Note : ns (not significantly different). Numbers followed by the same letters show no significant difference in the Honestly Significant Difference (HSD) test level of 5%.

The E4/E6 ratio (absorbance at wavelengths of 465 nm and 665 nm) is used to evaluate the aromaticity and polymerization degree of humic acid. A low E4/E6 ratio indicates a high degree of aromaticity and polymerization (Chen et al., 1977). Based on Figure 10, humic acid has the lowest E4/E6 ratio. This is because humic acid has a higher molecular weight, higher aromaticity and polymerization degree, and more functional groups compared to fulvic acid. The humin fraction is a fraction of humic substances that are insoluble in both bases and acids, and have a higher aromaticity and polymerization degree compared to humic acid (Stevenson, 1994; Tan, 2014). A low E4/E6 ratio (usually <4) indicates more humified humic acid, which is more stable and has a higher polymerization degree. A high E4/E6 ratio (>4) indicates less humified humic acid, which is less stable and has a lower polymerization degree (Chen et al., 1977; Zbytniewski & Buszewski, 2005).

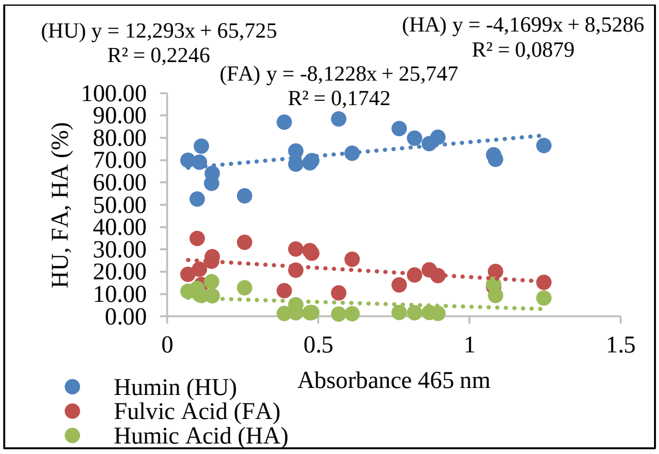

Figure 11. Relationship between absorbance 465 nm and humic substances

In Figure 11, it can be seen that humin has the highest absorbance value, followed by fulvic acid, and the lowest is humic acid. Absorbance at a wavelength of 465 nm (E4) in humic acid is caused by the π→π* electron transition in aromatic structures and auxochrome groups such as carboxyl (-COOH), carbonyl (C=O), and quinone. These aromatic compounds and auxochrome groups absorb UV-Vis radiation in the wavelength range of 465 nm. A higher E4 value indicates a higher content of aromatic compounds and auxochrome groups in humic acid (Chen et al., 1977; Stevenson, 1994). A high E4 value indicates a high content of aromatic compounds in humic acid.

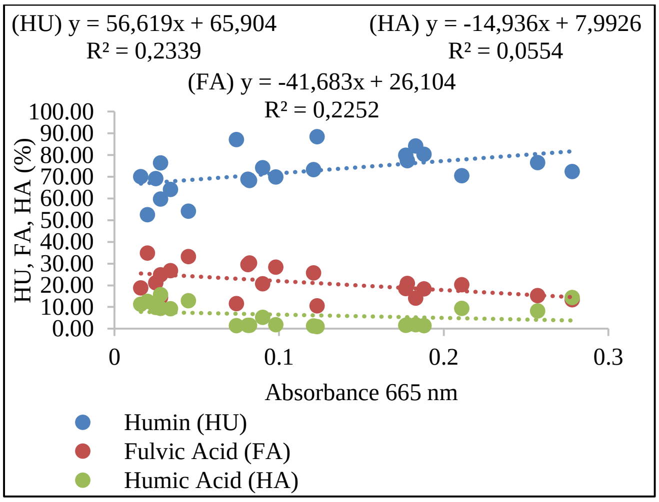

Figure 12. Relationship between absorbance 665 nm and humic substances

(Figure 12) humin has the highest E6 absorbance, followed by fulvic acid, and the lowest absorbance is humic acid. Absorbance at a wavelength of 665 nm (E6) in humic acid is caused by the n→π* electron transition in conjugated carbonyl (C=O) groups and quinone structures. Conjugated carbonyl groups and quinone structures absorb UV-Vis radiation in the wavelength range of 665 nm. A higher E6 value indicates a higher content of conjugated carbonyl groups and quinone structures in humic acid (Chen *et al.*, 1977; Stevenson, 1994).

CONCLUSIONS

The results showed that the land use of bush land provided the best soil fertility indicated by the content of humic acid and C-organic in the soil that were higher than that of other land uses. From the research results, it can be concluded that all Land Use Units (LUUs) in Jatiarjo Village, Pasuruan have advanced humification levels, as evidenced by low E4/E6 ratio values (<5). The lower the E4/E6 ratio, the higher the aromaticity and polymerization degree of humic substances. A low E4/E6 ratio also indicates more stable and condensed humic substances. Supporting parameters such as pH, CEC, and organic C depict the environment and availability of humic substances, while humate percentage and E4/E6 ratio reflect the structural characteristics and stability of humic substances. However, it should be noted that the E4/E6 ratio only provides partial information on the characteristics of humic acid and must be interpreted carefully in the context of sources, environmental conditions, and extraction and analysis techniques used. Combining with other characterization methods such as FTIR spectroscopy, NMR, or fluorescence can provide a more comprehensive picture of the properties and structure of humic acid.

ACKNOWLEDGMENT

We express our gratitude to Allah S.W.T. for the grace and blessings, enabling us to complete this research successfully. We also extend our sincere thanks to our families and friends from Department of Agrotechnology UPN "Veteran" Jawa Timur, who have provided assistance, support, and constructive criticism throughout the preparation of this article.

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