

<https://doi.org/10.37501/soilsa/189544>

# Humic acid structural characteristics in soils under different land uses in Ilesa, Nigeria

Olalekan Oluwatoyosi Salami<sup>1\*</sup>, Olusegun Olufemi Awotoye<sup>2</sup>, Olushola Olajumoke Adesanwo<sup>3</sup>, Samuel Idowu Adebola<sup>2</sup>

<sup>1</sup>Department of Environmental Management and Toxicology, Faculty of Pure and Applied Sciences, Kwara State University, Malete, Nigeria

<sup>2</sup>Institute of Ecology and Environmental Studies, Obafemi Awolowo University, Ile-Ife, Nigeria

<sup>3</sup>Department of Soil Science and Land Resources Management, Obafemi Awolowo University, Ile-Ife, Nigeria

\* Corresponding author: Olalekan Salami, lekansalamio@gmail.com, ORCID id: <https://orcid.org/0000-0002-0304-8195>

## Abstract

Received: 2023-12-01  
Accepted: 2024-06-01  
Published online: 2024-06-01  
Associated editor: Bartłomiej Głina

## Keywords:

Humic acid  
Land use types  
UV-Vis  
FTIR  
Agroecosystem

Numerous studies have examined the spectroscopic and elemental properties of soil humic acids (HA) from different soil orders, but little is known about how land usage affects the features of humic acids from Nigerian alfisol. The aim of the study is to ascertain how land use affects HA properties, which have a significant impact on the quality of organic matter. It was predicted that HA characteristics are dependent on the agricultural use of the soil and its years of existence. The study was conducted on six different types of land uses in Nigeria: a maize farm (MF), a citrus grove (CT), an oil palm plantation (OP), an abandoned alley crop farm (AC), an agroforestry site (AG), and an area with solely natural vegetation (NC). Soil HAs were analysed by Fourier transform infrared (FTIR) and UV-Vis spectroscopies. The percent humic acid yield of 100 g of soil was highest at the uncultivated site. The HA samples appeared to be highly aromatic, condensed, and hydrophobic for all land use types, according to FTIR spectroscopy. The spectra also showed a low E4/E6 absorbance coefficient, indicating a tendency for increased condensation of aromatic structures, higher molecular weight, and greater humification. No significant effect due to land use was observed between the two techniques. However, further in-depth investigation is required on the impacts of factors such as particle size and the potential of HAs from these soils.

## 1. Introduction

Land use types and modifications can affect the chemical composition of soil organic matter (SOM), which is typically dominated by plant and animal biopolymer residues, microbial biomass, and humic compounds (Awotoye et al., 2013; Zhang et al., 2019). Studies have suggested that SOM chemistry is influenced by typical vegetation cover of a particular location (Labaz et al., 2012), presence and activities of earthworms (Filley et al., 2008), tillage practices (Sleutel et al., 2007), fertilizer use (Marinari et al., 2007), and other environmental factors (Ganjegunte et al., 2005), including clay mineralogy (Buurman et al., 2005). Understanding the nature and quality of SOM, which may be helpful in soil management procedures such as carbon management, requires knowledge of the structural and chemical properties of humic acids (HAs).

HAs are intricate colloidal supramolecular combinations that are produced naturally when biological materials that make up a significant portion of humus undergo transformation (Chung et al., 2005). They are insoluble under severely acidic conditions (pH < 2). They have a wide spectrum of chemically related compounds

that contain carboxyl and phenolate groups and occasionally behave functionally as a di- or tribasic acid (Stevenson, 1994). The parent rock, origin and age of deposition and humification of the degraded organic material, soil texture, prevalent environmental and climatic conditions, and hydrological regime all influence the functional group, elemental composition, and characteristics of humic acids (Kechaikina et al., 2011; Gorbov and Bezuglova, 2014; de Melo et al., 2016). HAs in the soil improve soil buffering capacity, support the storage and release of essential nutrients, which enhances plant growth, yield and nutrient uptake (Calvo et al., 2014), root growth and functions (Kholdi et al., 2015), and play an important role in the complexation of heavy metals and organic compounds and the treatment of water (Tang et al., 2014).

Land use types contribute to the qualitative and quantitative change of the humic pool of soils explored in other climes. Kotzé et al. (2016) observed decreased soil total SOM and humic substances in cultivated lands due to intensive decomposition. Oktaba et al. (2018) found variations in humic acid levels among different land use types within urbanized plots in Poland. Similarly, Cui et al. (2023) identified differences in humic composition across various agricultural land use types in China. Bielińska et

al. (2018) noted significant distinctions in humic substance composition attributed to anthropogenic influence between urban, suburban, and rural soils. Additionally, studies by Zalba et al. (2016), Banach-Szott et al. (2021), and Bekier et al. (2023) highlighted the changes and modifications of humic acid properties by various land uses. However, there are currently no qualitative investigations on humic acids obtained from various agricultural land use types in Nigeria alfisol. Previous studies on humic acids in Nigeria were carried out on inceptisol and ultisol (Piccolo et al., 2005; Jamala and Oke 2013). Fagbenro and Agboola (1993) checked the effect of humic acid on the growth of teak seedlings on an alfisol, while Adekunle et al., 2007 characterised humic acids associated with some heavy metals. The country already suffers from hunger and food insecurity as a result of low food production, according to Nigeria's Adaptation Communication (ADCOM, 2021). As a result, sustainable agriculture through enhanced soil management methods, increased planting of native vegetation cover, and promotion of greening activities are being implemented as part of soil preservation and climate-smart mitigation and adaptation plans.

In the current study, chemical and spectroscopical techniques were used to assess the composition and structural characteristics of humic acid in soils under various land uses. The aim was to describe the effects of land use on the structural features of humic acid in the soils which may serve as a guide to establishing SOM management, particularly in the humid tropical zone of Africa. Despite differences in years of establishment, it was predicted that changes in humic acid quality would be more noticeable in sites that had been subject to intensive cultivation as opposed to less-disturbed areas.

## 2. Materials and Methods

### 2.1. The study area description

The soils under investigation are farm plots in Leventis Farms, which occupy about 236 hectares of fertile farmland in Ilesa (SW Nigeria) at elevations ranging from 395 to 419 m a.s.l.

The farm is located approximately between latitudes 7°40' N and 4°45' E. The soil of Ilesa belongs to the basement complex of the southwest Nigeria classification, the Okemesi series (Smyth and Montgomery, 1962), and the alfisol order (Ajiboye et al., 2019). The granitic and gneissic basement rocks are overlain by thick weathered materials that underlay the area because of the typical rock types, which include schist and amphibolite complex, granite-gneiss, quartz, and quartz-schist (Akinlalu et al., 2017). With a mean annual temperature of about 28°C and a mean annual rainfall of more than 1600 mm, the region is characterized by the typical dry and wet seasons typical of the lowland rainforest region of south-west Nigeria and is influenced by the Tropical Maritime (mT) and Tropical Continental (cT) air masses (Oyedele et al., 2009).

### 2.2. Analyses of soil physical and chemical properties

Soil samples were collected from five agricultural land use types (Fig. 1) which included a maize farm (MF), a citrus grove (CT), an oil palm plantation (OP), an alley crop farm (AC), agroforestry (AG) and uncultivated soil under natural vegetation (NC). The description and year of establishment for each site are presented in Table 1. For each land use type, three plots of 10 m × 10 m were delineated based on observable field characteristics such as topography and vegetation types. In each plot, three replicates soil samples were collected with a soil auger at 0–15 cm soil depth using the simple random method. Each sample was a bulk of three subsamples collected within the same zone within the plot. The samples were air-dried, crushed with a porcelain mortar and pestle, and sieved through a 2 mm mesh sieve to determine the physical and chemical properties. Soil pH was measured using a WalkLAB TI9000 pH meter in a 1:10 soil water suspension. Soil organic carbon was determined by using the chromic acid oxidation method (Sato et al., 2014) and total nitrogen was determined using microKjeldahl procedure. Particle size distribution was determined by hydrometer method. Available phosphorus was determined using Bray-1 method (Beegle, 2005). In this method, soil samples are first extracted with a solution containing a mixture of Bray's solution (containing ammo-



Fig. 1. Different agricultural land use types used for the study. A. Maize farm, B. Citrus grove, C. Oil palm plantation, D. Alley crop farm E. Agroforestry, F. Natural vegetation

**Table 1**  
Characteristic of the land use types used for this study

Land Use Type	GPS Coordinates	Elevation (m)	Years of cultivation	General Description
MF	N 07. 61780° E 004. 77124°	401	30	Usually cultivated with maize and cassava; sometimes intercropped with cowpea/groundnut.
CT	N 07. 61962° E 004. 77570°	400	22	Currently producing <i>Citrus sp.</i> comprising of a mixture of orange species.
OP	N 07. 61448° E 004. 77622°	399	53	Large expanse of oil palm plantation. Still producing scantily. Low management observed. Overgrown with shrubs including <i>Cromolaena odorata</i>
AC	N 07. 61908° E 004. 77739°	406	7	Formerly used for maize farming with <i>Gliricidia sepium</i> now overgrown with the tree.
AG	N 07. 61757° E 004. 77633°	416	30	A form of forest farming consists of exotic trees, cocoyam and an apiary. Example of the trees are <i>Triplochiton sclerozylon</i> , <i>Adansonia digitata</i> , <i>Cola nitida</i> , <i>Irvingia wombulu</i> , <i>Hildergardia barterii</i> , <i>Artocarpusaltilis</i> , <i>Hevea brasiliensis</i> , <i>Tectona grandis</i> .
NC	N 07. 62232° E 004. 77224°	398	23	Tickets of bushes and trees.

MF – maize farm, CT – citrus grove, OP – oil palm plantation, AC – alley crop farm, AG –agroforestry, NC – soil under natural vegetation

nium fluoride and hydrochloric acid). After the extraction, the solution is filtered, and the filtrate is analysed for phosphorus content using colorimetric methods.

### 2.3. Humic acids extraction

Humic acids were extracted from the topsoil (0 – 15 cm). Humic acids were extracted with 0.1 M NaOH using a soil: solvent ratio of 1:5 using the procedure of Schnitzer (1982). Briefly, 20 g of air-dried soil was shaken for 12 hours at room temperature in 100 ml of 0.1 M NaOH, and later left overnight at room temperature. After repeated extraction with the same concentration and quantity of solvent and centrifugation, the HAs extracts were separated from the supernatant by acidification with 0.1 M HCl at a pH of 2.0. The HA precipitates were separated by centrifugation and dialysed in cellophane bags. The powdered humic acids were obtained after freeze drying, and kept in a vacuum desiccator. Extraction yields of humic acids were calculated as the percentage weight recovered from the 100-gram original soil sample used for extraction. The extracted humic acid elemental compositions were determined using a Perkin Elmer 2400 elemental analyzer. Elemental ratios (C/N, H/C and O/C) were reported based on atomic weight percentages.

### 2.4. Fourier transform infrared spectroscopy

A Shimadzu-FTIR-8400S spectrophotometer was used to obtain the FTIR spectra for each of the humic acid samples. The KBr pellets were obtained by applying a pressure of 10,000 kg cm<sup>-2</sup> for 2 minutes to a mixture of 1 mg HA and 100 mg KBr, spectroscopy grade. The spectra for the samples were acquired between 400–4000 cm<sup>-1</sup> measured at 4 cm<sup>-1</sup> resolution, and 45 scans were averaged to reduce noise.

### 2.5. UV-visible spectroscopy

The UV-Vis absorption spectra of the humic acid samples were recorded on a Shimadzu UV-1650PC UV-Vis spectrophotometer in a 1 cm quartz cell. Measurements were made between 200 and 700 nm after dissolving 5.0 mg of each dry HA sample in 5 ml of 0.1 M NaOH (pH 12.0), followed by dilution (1:10) with 0.01 M NaOH. The solution was thoroughly shaken and then left overnight to allow enough time for the humic acid to dissolve. A solution of 0.01 M NaOH was used as a blank. The  $E_2/E_3$  (at absorbances of 254 and 365 nm),  $E_4/E_6$  (at absorbances of 465 and 665 nm), and  $A_{254}/A_{436}$  (at absorbances of 254 and 436 nm), ratios were also calculated.

## 3. Results and Discussion

The physical and chemical properties of the soil at each of the six sites are shown in Table 2. All soil samples contained a high percentage of sand fraction (500–592 g kg<sup>-1</sup>), and most of the soil texture was sandy clay loam. The soil pH ranged from 4.44 to 5.23, indicating that it was acidic. The organic carbon content ranged from 1.03 to 1.48 g kg<sup>-1</sup> in the following order: oil palm plantation > agroforestry > natural vegetation > alley crop farm > citrus grove > maize farm. Oil palm plantations had the highest phosphorus concentrations (18.3 mg kg<sup>-1</sup>) and alley crop farms had the highest soil nitrogen content (0.37 g kg<sup>-1</sup>). The humic acid yield from soil ranged from 1.02% to 2.45%, with the highest yield found in soil under natural vegetation. A previous study found that land areas with similar land-use patterns exhibit similar soil chemical characteristics (Tian et al., 2017). Our investigation revealed that vegetated regions showed superiority over cultivated or barren land. These findings underscore the

**Table 2**  
Physical and chemical characteristics of the soil and extraction yields of humic acid

Soil Property	MF	CT	OP	AC	AG	NC
<b>pH</b>	4.50 ± 0.11	4.72 ± 0.02	5.23 ± 0.18	4.53 ± 0.09	5.22 ± 0.18	4.44 ± 0.13
OC (g kg <sup>-1</sup> )	1.03 ± 0.17	1.10 ± 0.14	1.48 ± 0.01	1.31 ± 0.05	1.46 ± 0.01	1.34 ± 0.05
P (mg kg <sup>-1</sup> )	4.41 ± 1.82	5.36 ± 1.46	18.93 ± 4.06	6.32 ± 1.07	12.09 ± 1.28	6.57 ± 0.97
N (g kg <sup>-1</sup> )	0.31 ± 0.02	0.33 ± 0.02	0.32 ± 0.01	0.37 ± 0.02	0.25 ± 0.03	0.29 ± 0.01
<b>% Humic Acid Yield</b>	1.2 ± 0.09	1.02 ± 0.16	1.02 ± 0.16	1.05 ± 0.15	1.76 ± 0.14	2.45 ± 0.41
<b>Clay</b> (g kg <sup>-1</sup> )	28.2 ± 7.21	27.4 ± 10.41	29.2 ± 3.14	31.8 ± 7.44	25.2 ± 12.36	38.0 ± 13.14
<b>Silt</b> (g kg <sup>-1</sup> )	16.4 ± 0.91	13.4 ± 3.23	20.4 ± 5.37	17.0 ± 1.50	20.6 ± 6.21	12.0 ± 8.81
<b>Sand</b> (g kg <sup>-1</sup> )	55.4 ± 1.35	59.2 ± 16.94	50.4 ± 19.09	51.2 ± 15.80	54.2 ± 3.51	50.0 ± 20.54
<b>Texture classification (USDA)</b>	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay

MF – maize farm, CT – citrus grove, OP – oil palm plantation, AC – alley crop farm, AG – agroforestry, NC – soil under natural vegetation, OC – Organic carbon, P – Available Phosphorus, N – Total Nitrogen. Data are expressed as mean ± standard deviation

potential advantages of ensuring land cover and rehabilitating vegetation to enhance soil nutrient conditions.

The elemental composition of humic acids is an important chemical property. Table 3 presents the elemental analysis (in weight %) of the humic acid samples analysed, revealing no statistically significant differences. The percentages of carbon and nitrogen were grouped around 57% and 3%, respectively. The percentage composition of each element (C, H, O, and N) from the various land uses was distributed within the range of generally accepted percentages in soil humic acids (Yonebayashi and Hattori, 1988; Lodhi, et al., 2013). According to these results, it can be deduced that the HAs are aromatic and comparable in terms of carbon species distribution. The C/N ratio (20.5–28.2) observed in this study was highest in the alley crop farm compared with the other land uses, while the H/C ratio was higher in the HA from agroforestry soils. Kumada (1987) proposed that the H/C ratio is an appropriate metric for defining humic acid humification since it reflects the progress of humification. When these results are compared to humic acids from soils from other natural environments, it becomes clear that all humic acids studied, except for soils from agroforests, are mature or more humified (low H/C, i.e., H/C < 1), and their O/C ratios are consist-

ent with those found in terrestrial environments (Lodygin et al., 2017).

The UV-visible spectra of the soil humic acid are shown in Fig. 2. All spectra showed a sharp peak in the region between 210 and 240 nm with a slight hump, characteristic of humic substances. This hump is attributed to the absorption of radiation by the double bonds C=C, C=O and N=N of the aromatic or unsaturated components of humic substances (Domeizel et al., 2004). Beyond this region, the spectra followed a featureless, monotonic exponential decrease in absorption with increasing wavelengths from 300–700 nm. The oil palm plantation HA had a lower absorbance than the other land use types in the spectra range. The absorption intensities of humic acids from agroforestry and uncultivated sites are comparable, but much lower than those from alley crop farm and citrus grove. The variation in hump found at different land use types could be linked to variations in aromatic compound concentrations, which are symptomatic of differences in the humification process. This could imply that the aromaticity and molecular weight of humic acids, which are known to increase as the humification process matures, increased more in citrus grove HA than in other land uses.

**Table 3**  
Elemental composition and ratio of soil HA from different land use.

Land Use Types	Elemental composition (%)				Atomic ratio		
	C	H	O	N	C/N	O/C	H/C
MF	56.3 ± 0.11	4.24 ± 0.09	36.3 ± 0.14	3.18 ± 0.06	20.7 ± 0.61	0.48 ± 0.00	0.90 ± 0.02
CT	56.3 ± 0.10	4.24 ± 0.09	36.3 ± 0.14	3.17 ± 0.05	20.7 ± 0.59	0.48 ± 0.00	0.90 ± 0.02
OP	57.4 ± 0.32	4.28 ± 0.07	35.2 ± 0.29	3.13 ± 0.04	21.4 ± 0.31	0.46 ± 0.01	0.89 ± 0.02
AC	57.14 ± 0.22	4.24 ± 0.09	36.3 ± 0.14	2.36 ± 0.27	28.2 ± 2.47	0.48 ± 0.00	0.89 ± 0.02
AG	55.4 ± 0.50	5.25 ± 0.32	36.3 ± 0.14	3.15 ± 0.04	20.5 ± 0.67	0.49 ± 0.01	1.14 ± 0.08
NC	57.0 ± 0.17	4.47 ± 0.01	35.1 ± 0.37	3.25 ± 0.08	20.5 ± 0.68	0.46 ± 0.01	0.94 ± 0.00

MF – maize farm, CT – citrus grove, OP – oil palm plantation, AC – alley crop farm, AG – agroforestry, NC – soil under natural vegetation. Data are expressed as mean ± standard deviation.

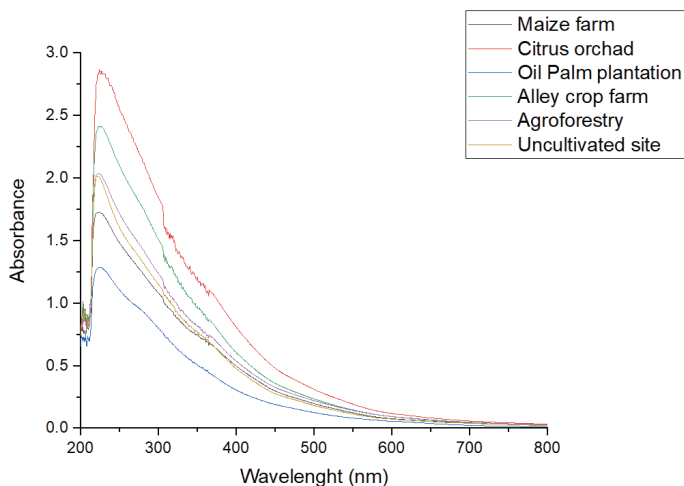


Fig. 2. The UV-visible spectra of soil humic acid isolated from different soil types

To further investigate the HAs, the spectra were subjected to a comparison of absorption coefficients at different wavelengths: 465 nm and 665 nm ( $E_4/E_6$ ), 254 nm and 365 nm ( $E_2/E_3$ ), and 254 nm and 436 nm ( $E_2/E_4$ ). The results, shown in Table 4, fall within the same classification ranges and suggest a similar degree of humification among the different soils, with humic acid from agroforestry soil having the lowest  $E_4/E_6$  ratio. A low  $E_4/E_6$  ratio ( $\sim 3.0$ ) indicated a higher aromatic character, while a high ratio ( $\sim 8$ ) indicated a higher aliphatic character (Lodhi, et al., 2013). These values, obtained from our study, confirmed the presence of extensive aromatic conjugation, a high degree of polycondensation, and a high molecular weight in the humic acid samples. The  $E_2/E_3$  ratio, which measures aromaticity, humification degree, and molecular weight of organic matter, yielded similar values in this study (2.16–2.44) compared to the reference soil HA value (2.30) established by the International Humic Substances Society (Takács and Füleky, 2010). This similarity indicated comparable levels of humification, condensation of aromatic rings, and molecular weight (McDonald et al., 2004). The source of organic materials was identified using the ratio  $A_{254}/A_{436}$  ( $E_2/E_4$ ). According to Battin (1998), values between 4 and 11 indicated that organic matter came primarily from terrestrial sources, which was consistent with the results of the present study (4.18–4.99). Although this ratio is frequently used

for aquatic humic substances, Abbt-Braun and Frimmel (1999) found that humic acid with a lower ratio derived from terrestrial sources had a higher aromaticity and hydrophobicity.

The IR spectra of the HAs (Fig. 3) showed similar patterns to our pilot study (Salami et al., 2023), with broad peaks around the 3300–3400  $\text{cm}^{-1}$  region, which were assigned to the presence of O-H stretching and N-H functional groups. A peak centered at 1635  $\text{cm}^{-1}$  resulting from the H-bonded C=O stretching of amide I band, conjugated ketones and aromatic C=C stretching was very strong and sharp in the IR spectra of all the HAs. A band at 2883–2949  $\text{cm}^{-1}$  (aliphatic C-H stretching) occurred with weak intensity in the spectra from HA samples from citrus grove, maize farm, alley crop farm and oil palm plantation. The low strength of these bands could indicate that the humic acid molecules have fewer aliphatic carbon chains or that the aliphatic groups have suffered some degree of oxidation or chemical change, resulting in lower absorption signals. This could be due to the degree of decomposition, microbial activity, or environmental factors that influence the synthesis and stabilization of humic compounds in soil.

Bands at 1710–1870  $\text{cm}^{-1}$  attributed to C=O of  $\text{CO}_2\text{H}$  were present in the humic acid spectra but absent in the HA of citrus grove. Carboxylic acids are important functional groups in humic substances, originating from the partial decomposition and transformation of organic matter in soils. These groups contribute to the acidic nature of humic substances and play significant roles in various soil processes, such as nutrient retention, metal complexation, and soil pH buffering. A band at 1541  $\text{cm}^{-1}$  (aromatic C=C, NH deformation of amide II) was strong in the spectra of maize and citrus farms indicating the prevalence of aromatic and nitrogen-containing compounds in these soils. Small peaks resulting from O-H deformation and C-O stretching of phenol and aliphatic C-H were present in the IR spectra of HA from agroforestry and oil palm plantations at 1456  $\text{cm}^{-1}$  as well as at the 1365–1396  $\text{cm}^{-1}$  region for all the land use types.

A strong band at 1097  $\text{cm}^{-1}$  or a shoulder at 1008  $\text{cm}^{-1}$  was assigned to the C-O stretching of polysaccharide or polysaccharide-like substances. The sharp peak at 1033  $\text{cm}^{-1}$  is attributed to Si-O vibrations of clay impurities (Olk et al., 1999) and indicates the presence of clay material that was probably not completely removed during the purification process. The spectra showed that the HA from the soil is much transformed

Table 4

The UV-vis absorbance parameters of soil humic acid samples under different land use

Land Use Type	$E_2/E_3$	$E_4/E_6$	$E_2/E_4$
Maize farm	2.16 ± 0.15	5.20 ± 0.00	4.18 ± 0.48
Citrus grove	2.32 ± 0.02	5.63 ± 0.43	4.50 ± 0.15
Oil palm plantation	2.44 ± 0.14	5.03 ± 0.19	4.99 ± 0.34
Alley crop farm	2.37 ± 0.07	5.58 ± 0.38	4.93 ± 0.28
Agroforest	2.26 ± 0.05	4.71 ± 0.49	4.45 ± 0.20
Natural vegetation	2.28 ± 0.03	5.04 ± 0.20	4.84 ± 0.19

Data are expressed as mean ± standard deviation.

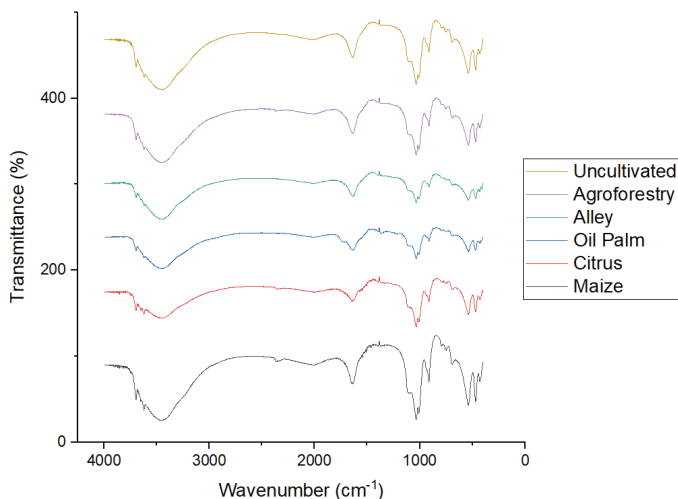


Fig. 3. The FTIR spectra of soil HA studied at different soil types

and have a condensed structure that produces broad and weak bands. Similar findings have been reported in soils from South Africa, Botswana, and Tanzania (Almendros et al., 2003; Almendros et al., 2005). This could be due to the conventional tropical weather conditions and a possible consequence of previous fires. This research provided details on the many functional group types that were present in these soils as well as the typical properties of humic acids, one of which was a strongly aromatic character.

#### 4. Conclusion

In this study, soil humic acids were characterized using two spectroscopic techniques: UV-VIS and FTIR. Depending on the agricultural management type, the humic acid characteristics of the soil slightly differed. The soil in agroforests has the highest level of humification. Our prediction that isolated humic acids from less disturbed soils would have more aromatic compounds in their molecules and higher fluorescence intensity proved valid. The results of this study support prior findings that changing the OM status through agriculture led to the loss of its protective characteristics by usually reducing the hydrophobicity acquired under forest and increasing the relative concentration of oxidation products. It was further demonstrated that all examined samples of soil humic acids had identical primary peak positions. The uniformity of the C/N values among the different humic fractions, which indicates the refractory nature of these materials, suggests that they can withstand microbial degradation using low-disturbance management techniques.

#### Acknowledgements

We would like to thank the management of the Leventis Foundation Nigeria Agricultural Training School Farm in Ilesa, Nigeria, for allowing in collecting soil samples at several areas for this study.

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