



Ultraviolet-visible and Infrared Spectroscopic Studies of Soil Humic Acids

Minakhi Kachari¹, Punam Belwar¹, Krishna Dutta¹, Annapurna Sarmah¹,
Partha P. Saikia^{1*} and Mrinal K. Baruah¹

¹Department of Chemistry, NNS College, Titabar-785630, Assam, India.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MK, PB, KD and AS collected samples and performed experiments; and authors PPS and MKB designed, analyzed and interpreted data, and wrote the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Structural characterization of humic acids extracted from surface, below 15 cm depth and 30 cm depth tea garden soils have been studied here. Instrumental analysis of the organic substances were carried out using UV-Visible and IR spectroscopies. The results reveal low level of humification of the humic acids occurring in the soil samples and low condensation of these molecules. The predominant aliphatic characters of the humic acid molecules are evident from this study. The occurrence of hydrogen bonded oxygen containing groups, e.g., hydroxyl, carboxyl, etc., have been observed. The presence of nitrogen containing organic compounds as well as unleachable (in acid and alkali treatments) metal-bonded oxygen and nitrogen species are also indicated. The results obtained in this study would be quite helpful for structural understanding of soil organic matters.

Keywords: Humic acid; tea garden soil; UV-visible; infra-red.

*Corresponding author: E-mail: ppsaiikia@yahoo.co.in;

1. INTRODUCTION

Humic acid is a heterogeneous organic substance and, therefore, its most fundamental characteristics is its chemical composition. Like all natural organic compounds, humic acid has aliphatic and aromatic networks with common elements such as carbon, hydrogen, nitrogen, sulphur and oxygen; the content of sulphur is quite less. The structure of humic acid is very complex because it is a mixture of different compounds and hence termed as heterogeneous substance. Some potential sites of the humic acid are mainly oxygen containing functional groups: carboxylic, hydroxyl (alcoholic and phenolic), quinone, keto, etc., groups [1]. Initially, Microbial degradation of dead plant matter results in humic substances. During the decomposition, the carbohydrates tend to disappear rapidly and the lignins, which are phenolic compounds, are accumulated. The transformation of lignin into humic matters may be divided into three stages: (a) formation of oxyquinone, (b) rupturing of the rings resulting in very reactive keto acids, which (c) by subsequent reaction yield condensed ring systems [2]. In a common practice, humic substances in soils and sediments can be divided into three main fractions: humic acids, fulvic acids, and humin. However, the precise properties and structure of a given soil humic acid depend on the source and, also the process and condition of extraction.

Various workers have studied organic matters from different sources and they characterized these matters by using UV-visible, IR, XRD, etc [3,4,5]. In order to understand the nature and type of humic acids, it is important to characterize the substance. In this paper, efforts will be made to determine some structural

parameters of humic acids extracted from soil by UV and IR spectroscopy.

2. MATERIALS AND METHODS

2.1 Soil Samples

Soil samples were collected from the surface, below 15 cm and below 30 cm depth. Soil of a tea garden under Titabar sub-division (Latitude: 26°35'10"N; Longitude: 94°9'35"E), Jorhat district, Assam (Fig. 1). The samples were dried in air. These were ground, sieved and then stored in polythene bags.

2.2 Extraction of Humic Acid

The method of extraction of humic acid has been given elsewhere [6]. Briefly, 100 g air dried soil sample was treated with 0.1M 300 ml NaOH solution with occasional stirring and then kept overnight at room temperature. The soluble humic material was collected and then acidified with 6MHCl until stable pH 1.0 was attained. The material was kept overnight for complete precipitation. The separation of precipitate and washings (with distilled water) were done with a centrifuge. The humic acid was dried in air and then kept in a dessicator.

2.3 Analysis of Humic Acid

The proximate and ultimate analyses of humic acids - moisture, ash, carbon, hydrogen, nitrogen, sulphur and oxygen (by difference) were done to understand the nature of the humic acids at different depths by ASTM D2974-14. Carbon, hydrogen and nitrogen were analysed using a CHN analyser.

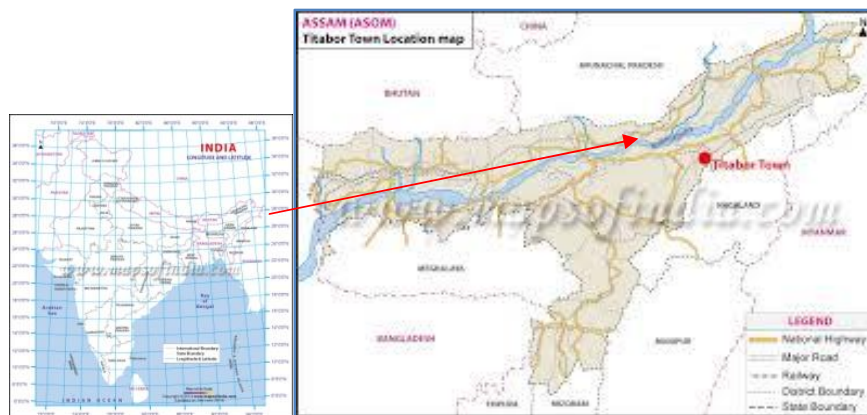


Fig. 1. Geographical location of soil samples

2.4 Measurement of Optical Density

0.01 g dry humic acid was taken in a 50 ml beaker and to it, 0.1M, 5 ml NaOH solution was added. The beaker was kept on a water bath for half an hour to dissolve dry humic acid almost completely. Then the volume of the solution was made to 50 ml by adding more distilled water. The whole solution was warmed at 60-70°C on the water bath. As some fine particles were visible in the solution, these particles were filtered out using Whatman 41 filter paper. The clear solution was taken for the measurement of absorption in the ultraviolet as well as visible regions. Optical density in the range 200-700 nm were recorded in a digital UV-visible spectrophotometer.

2.5 Infrared Study

Infrared spectra of the humic acid samples were recorded in KBr pellets using Perkin-Elmer Model Spectrophotometer in the frequency range 4000 – 400 cm^{-1} .

3. RESULTS AND DISCUSSION

3.1 Elemental Analysis of Humic Acids

The analysis of moisture, ash, carbon, hydrogen, nitrogen, sulphur and oxygen are given in Table 1. The value of oxygen content was calculated by difference. It is observed that the moisture content in humic acids gradually decrease from the surface to 30 cm depth soil. The ash contents in the humic acids differ much.

3.2 UV-Vis Spectroscopic Data Analysis

In the spectra obtained in the UV-Vis region for humic acids from surface soil, 15 cm depth soil and 30 cm depth soil of tea garden, a decrease on the absorption intensity with the increase of the wave length have been observed. These spectra are shown in Fig. 2. According to Stevenson and Schnitzer [7], this may be correlated with the fact that the humic acids possess chromophores which absorb in the whole analyzed region. Another interesting observation is that a minor shoulder is noticed around 265 nm for humic acid from surface soil which is assigned for possessing of aromatic structure in the organic substance. Moreover, some workers reported that the shoulder is due to the structural conjugation of quinone and ketone and the shoulder appears in the region 250-270 nm [8].

For analyzing the UV-Vis. Spectra of humic acids, most of the workers have considered absorptions at 250, 270, 280, 365, 400, 465, 472, 600, 664, and 665 nm wave length [8,9]. Two important ratios $E_{250/365}$ (known as E_2/E_3), $E_{465/665}$ (known as E_4/E_6) are largely used for characterization of humic acid. Other ratios are also considered, these include $E_{270/400}$, $E_{472/664}$, $E_{280/472}$ and $E_{280/664}$. Another term $\Delta\log K$ value has large application because it gives information regarding the optical properties of humic acid. $\log K$ is defined as the difference among the logarithms of the absorbance at 400nm ($\log E_{400}$) and at 600 nm ($\log E_{600}$) [9]. The absorbances at different wave lengths have attributed different significance on the nature of the humic acid. The absorbances at different wave lengths are given in Table 2.

Humic acid has aromatic networks and the extent of degree of condensation is related to the humification process. E_4/E_6 ratio signifies these behaviors of the organic substance. Various researchers [7,9] reported that E_4/E_6 ratio is related to the aromaticity and to the degree of condensation of the chain of aromatic carbons of the humic acid and could be used as humification index. Progressive condensation is indicated by a decrease in the E_4/E_6 ratio (15). In our study, the values of the E_4/E_6 ratios for humic acid extracted from surface soil, 15 cm depth soil and 30 cm depth soil are in the range 5.607-5.881 (Table 2). Our results are in excellent agreement with Kononova [10] that the value of E_4/E_6 ratio for soil humic acid is reported to be about 5.0. This value for lignite humic acid is 3.8 [11] revealing that the humic acids, under study, are less condensed than lignite humic acid. Moreover, high ratios reveal presence of large quantities of aliphatic structures and low quantities of condensed aromatic structures [12] According to Uyguner et al. [13], this ratio is inversely related to the degree of aromaticity, particle size, molecular weight and acidity. Typically E_4/E_6 ratio is larger for non-humified material by the presence of proteins and carbohydrates which increase the absorbtivity at the UV region of the spectrum [14].

The E_4/E_6 ratios for the humic acids, under study, have almost similar values (Table 2) suggesting that the degree of condensed aromatic system, molecular weights, particle size and acidity of these organic compounds, although extracted from different soil samples, do not differ much.

The coefficient $E_{270/400}$ characterizes the degradation of phenolic/quinoid core of humic

acid to simpler carboxylic aromatic compounds (Uyguner and Kbolet, 2005). In our study, the $E_{270/400}$ value is highest in the humic acid from surface soil and lowest that from the 15 cm depth soil (Table 2).

The $E_{280/472}$ reflects the proportion between lignins and other materials at the beginning of humification. The $E_{280/664}$ denotes the relation between non-humified and strongly humified material. Low ratio reflects a high degree of aromatic condensation and indicate a higher level of organic material humification [15]. Quite high values have been obtained for the humic acids, under study (Table 2). High values

attribute a low degree of aromatic condensation which further suggest a lower level of humification of these organic substances.

The $E_{472/664}$ ratio is used to indicate the degree of condensation and polymerization of aromatic constituents. Low ratio reflects a high degree of aromatic condensation and indicate a higher level of organic material humification [15]. For the humic acids, under study, reasonably high values of $E_{472/664}$ ratio have been found (Table 2), which reveal a low degree of aromatic condensation and further confirms the lower level of humification of these organic compounds.

Table 1. Proximate and ultimate analysis of humic acids

Humic acid from	Proximate analysis				Ultimate analysis*				
	Moisture (%)	Ash (%)	Volatile matter (%)	Fixed carbon (%)	%C	%H	%N	%S	%O
Surface soil	7.53	3.61	34.64	54.22	15.99	2.62	1.59	0.61	79.19
15 cm depth	6.62	12.58	35.43	45.37	23.49	2.62	2.24	0.63	71.02
30 cm depth	5.54	6.84	39.74	47.88	21.68	2.67	1.91	0.58	73.16

*Moisture and ash free basis

Table 2. UV-Vis absorbances at various wave lengths and their ratios for humic acids from tea garden soils

Humic acid from	A_{280}	$E_{270/400}$	$E_{280/472}$	$E_{280/664}$	$E_{472/664}$	E_2/E_3	E_4/E_6	$\Delta \log K$
Surface soil	1.455	3.897	3.769	42.794	11.353	3.0	5.727	0.841
15 cm depth soil	3.0	2.924	2.988	35.294	11.812	1.875	5.881	0.844
30 cm depth soil	2.603	3.769	3.625	41.984	11.581	2.619	5.607	0.839

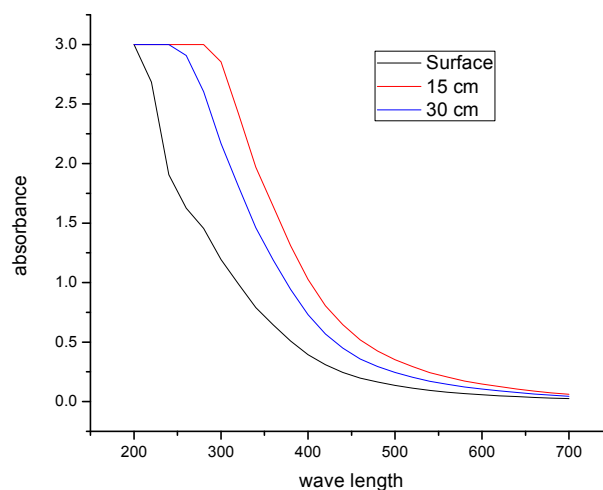


Fig. 2. UV-Vis spectra of humic acids extracted from surface soil, 15 cm depth soil and 30 cm depth soil of tea garden

The value of $\Delta\log K$ coefficient can be used for describing degree of humification of organic matter. On the basis of $\Delta\log K$ coefficient, humic acids can be divided into three types [16]:

- Humic acids of higher degree of humification for which the values of $\Delta\log K$ coefficient is up to 0.6;
- Those humic acids which have values between 0.6-0.8, and
- humic acids have $\Delta\log K$ values within range 0.8-1.1 [16]. High values of $\Delta\log K$ and their minor variation (0.839-0.844) observed in the humic acids (Table 2) suggest that these organic matters did not undergo high degree of humification and the nature of the molecules are almost similar.

3.3 Infrared Spectra Analysis

In an infrared spectrum, a number of bands have been found which give some information regarding nature of bonding between two atoms. In a heterogeneous organic compound like humic acid, the molecule has large number of different functional groups and linkages. Infrared spectrum will give some information about the structural features of the humic acid.

Infrared spectra of humic acids extracted from surface soil, 15 cm depth soil and 30 cm depth soil of a tea garden are presented in Fig. 3. Each spectrum shows some distinct bands and an attempt will be made here to interpret these bands; these are discussed below.

3.3.1 3350-3450 cm^{-1} region

In the Fig. 3, a broad band has been found in this region for all the humic acids. Precisely, this band for humic acid from surface soil occurs at 3435.8 cm^{-1} , that from 15 cm depth soil at 3422.5 cm^{-1} and that from 30 cm depth soil at 3421.4 cm^{-1} . The band occurs in this region is usually attributed to O-H stretching vibration. In the undissociated state or free state, OH groups absorbs near 3600 cm^{-1} . When there is hydrogen bonding in the association of molecules, the frequency of the O-H stretching vibration is considerably reduced. According to Silverstein et al. [17], this broad band is assignable to O-H stretching vibration of the polymeric association of the molecules. Thus the bands occurring in the $3421.4\text{-}3425.8 \text{ cm}^{-1}$ region in these humic acids, under study, can be attributed to hydrogen bonded OH groups occurring in these organic substances.

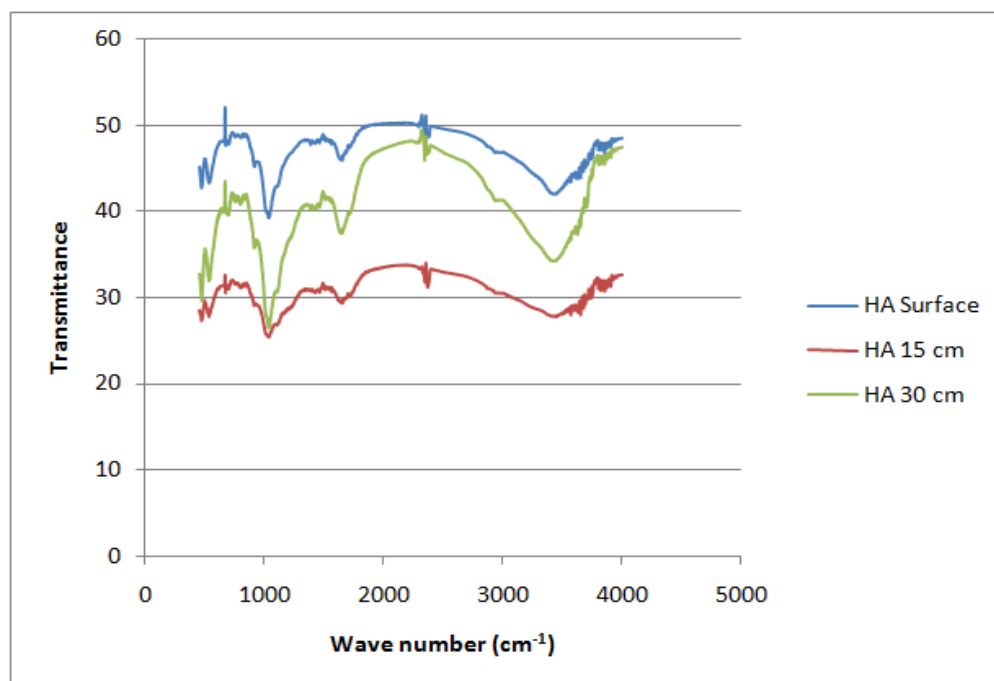


Fig. 3. Infrared spectra of humic acids from surface soil, 15 cm depth soil and 30 cm depth soil of tea garden

3.3.2 2800-3100 cm⁻¹ region

The absorption characteristics for C-H stretching vibration observed in the infrared spectra of organic substances are occurring as small but broad bands. The bands occurring at 2926 and 2853 cm⁻¹ are due to asymmetrical and symmetrical stretching vibrations of C-H of methylene groups, respectively [17]. Another important band appears at frequencies slightly higher than 3000 cm⁻¹ which may be assigned to aromatic C-H stretching vibration.

The infrared spectra, in Fig. 3, show that the humic acid from 15 cm depth soil records a minor band occurring at 3034.7 cm⁻¹; this band is not found in the spectra of other two humic acids. The band at 3034.7 cm⁻¹ in the infrared spectrum of the humic acid from 15 cm depth soil attributes C-H stretching vibration of the aromatic compounds. This result indicates that this humic acid has comparatively higher aromatic content than that of other humic acids extracted from surface soil and 30 cm depth soil. The presence of highest percentage of carbon content in this sample (Table 1) supports our view. But it is also known that absence of a pronounced band at frequency slightly higher than 3000 cm⁻¹ may be due to extensive substitution of the aromatic ring or masking from the broad band due to O-H stretching. There is a strong likelihood that absence of the band at near 3034.7 cm⁻¹ for humic acids from surface and 30 cm depth soils may be attributed to extensive substitution of aromatic ring present in the organic compounds.

The occurrence of aliphatic content in humic acid may be examined with the help of infrared spectroscopy. Absorption due to aliphatic C-H stretching vibration varies considerably, being strong in some preparations but not in others. In the spectra of humic acids, under study. Bands of intense absorption at near 2926 and 2853 cm⁻¹ have been obtained. The bands at 2930.6 and 2867.1 cm⁻¹ for humic acid from surface soil; that from 15 cm depth soil shows bands at 2936.2 and 2868.7 cm⁻¹; and that from 30 cm depth soil, bands at 2931.8 and 2870.3 cm⁻¹ are observed. These two weak absorption bands at near 2926 and 2853 cm⁻¹ attribute to asymmetric and symmetric stretching vibration of C-H aliphatic, respectively.

3.3.3 1600-1800 cm⁻¹ region

A characteristic band occurring in the infrared spectrum of an organic compound at slightly

above 1700 cm⁻¹, commonly at 1720 cm⁻¹. is generally attributed to the stretching vibration of C=O of carboxylic acids, aldehydes and ketones. In the spectra of humic acids, under study, absorption bands occur in the range 1719.4-1721.6 cm⁻¹ (Fig. 3). The bands are very weak. Hydrogen bonding and resonance can weaken the C=O bond. It is known that internal hydrogen bonding reduces the frequency of the C=O stretching to a greater degree than does intermolecular hydrogen bonding [17]. The band at 1720 cm⁻¹ often shifts to a slightly higher frequency when humic substances are methylated. This can be attributed to the fact that the C=O of esters generally absorb at higher frequencies than the C=O of the corresponding acids. The frequency of absorption for both acid and ester forms in humic substances are lower than normal which have been explained by the occurrence of conjugated C=O groups and/or aromatic acids.

Another band of quite moderate intensity occurring around 1650 cm⁻¹ has been observed in all the humic acids extracted from tea garden soils of varying depths; 1637.6, 1650.7 and 1650.8 cm⁻¹ for humic acids from surface, 15 cm depth and 30 cm depth soils, respectively. The C=C stretching mode of unconjugated olefins usually shows moderate to weak absorption at 1667-1640 cm⁻¹ [17]. Monosubstituted olefins, i.e., vinyl groups, absorb near 1640 cm⁻¹ with moderate intensity. Disubstituted *trans*-olefins, tri-, and tetra-alkyl substituted olefins absorb at or near 1670 cm⁻¹, disubstituted *cis*-olefins and vinylidene olefins absorb near 1650 cm⁻¹ (Silverstein et al). The occurrence of moderate intensity bands at and near 1650 cm⁻¹ in all the humic acids, under study, may be attributed to C=C stretching vibrations of substituted olefins.

Conjugation with a C=C bond results in delocalization of the pi electrons of unsaturated groups. Delocalization of the pi electrons of the C=O group reduces the double bond character of the C to O bond, causing absorption at lower wave numbers (or longer wavelengths). Conjugation with an olefinic or phenyl group causes absorption in the 1685-1666 cm⁻¹ region. Additional conjugation may cause a slight further reduction in frequency [17].

3.3.4 1300-1500 cm⁻¹ region

Aromatic C-C ring stretching is observed in this region; generally, a weak band at 1497 cm⁻¹ and comparatively a higher absorption at near 1460

cm^{-1} appear in the spectrum. On the other hand, a band occurs at near 1430 cm^{-1} for asymmetrical vibration of C-H bonds of bending methyl and methylene groups. The symmetrical C-H bending vibrations of methyl groups occur near 1380 cm^{-1} .

Infrared spectrum of humic acid extracted from the surface soil shows three bands in this region: a weak band at 1497.4 cm^{-1} , and two medium intensity bands at 1450 and 1384.8 cm^{-1} . In case of humic acid extracted from 15 cm depth soil, only two bands at 1498 and 1459 cm^{-1} appeared in the spectrum. On the other hand, three bands at 1498.4 , 1459 and 1429.5 cm^{-1} are obtained in the spectrum of humic acid extracted from 30 cm depth soil.

All the three humic acids show band at $1497.4 - 1498.4 \text{ cm}^{-1}$ region and 1459 cm^{-1} . The bands can definitely be attributed to aromatic C-C ring stretching, thus confirms the presence of aromatic network in the organic substances. Moreover, asymmetrical bending vibration of C-H bonds of methyl group at 1429.5 cm^{-1} for the humic acid extracted from 30 cm depth soil and symmetrical C-H bending vibration of methyl groups at 1384.8 cm^{-1} in the humic acid from surface soil suggest the occurrence of aliphatic networks in these humic acids.

3.3.5 900-1100 cm^{-1} region

A band appears in the region $800-950 \text{ cm}^{-1}$ region attributed to asymmetrical ring stretching in which the C-C bond is stretching during contraction of the C-O bonds. Literature survey reveals that the band in the region $1000-1100 \text{ cm}^{-1}$ is due to C-O stretching vibration of polysaccharides [18,19] and some others reported that the band occurs in this region is due to Si-O stretching vibration [20,21]. While studying the humic acid from various soil samples and river sediment sample, it has been reported that the band at near 1050 cm^{-1} is due to C-O stretch of polysaccharides [22]. Moreover, the characteristic behavior of ether linkages is observed in this region by the stretching vibration of the C-O-C bonds. The vibration involving oxygen atom in ether results in greater dipole moment changes showing more intense bands for ether [17]. Some minor variation of the position of the bands occur. The C-O-C group in a six membered ring absorbs at the same frequency as in acyclic ether. As the ring becomes smaller, the asymmetrical C-O-C stretching vibration moves progressively to the

lower wave numbers; the symmetrical stretching moves to the higher wave numbers.

The spectra of humic acid extracted from soils of different depths show three distinct bands in this region: a weak band at 1105.4 cm^{-1} , a sharp band with quite high absorption at 1032 cm^{-1} and a medium absorption band at 912.1 cm^{-1} for the humic acid from surface soil, for humic acid from 15 cm depth soil, the bands occur at 1101.3 , 1032.9 and 912.8 cm^{-1} and for humic acid from 30 cm depth soil, the bands appear at 1101.3 , 1031.5 and 915 cm^{-1} . Appearance of the band at 1101 cm^{-1} may be attributed to the vibrations of the ether groups. The bands at near 1032 cm^{-1} may be due to the contribution of polysaccharides but the contribution of Si-O stretching vibration cannot be completely ruled out because all the humic, under study, contain considerable amount of ash. Further the band at near 912 cm^{-1} may be assigned to the asymmetric ring stretching of C-C bond.

As the humic acids have considerable amount of nitrogen, therefore, the presence of aliphatic C-N bond is not unlikely. The aliphatic C-N stretching vibration occurs in the range $1020-1250 \text{ cm}^{-1}$, the variation of the position of the band depends on substitution while the aromatic C-N stretching vibration occurs in the range $1180-1380 \text{ cm}^{-1}$ with two bands. As there is no band in this region in all the humic acids, therefore, occurrence of aromatic C-N compounds in these organic substances are very poorly indicated. The bands at 1100 and 1032 cm^{-1} may also be possible by the contribution of aliphatic C-N bonds. The occurrence of aliphatic C-N compounds in the humic acids is quite possible from proteins because proteins in soil are obtained from decomposed vegetations.

3.3.6 400-700 cm^{-1} region

A mono-substituted benzene exhibits two strong bands between $690-710 \text{ cm}^{-1}$ and between $730-770 \text{ cm}^{-1}$ [20]. Ortho-substituted benzene shows only a strong band between $735-770 \text{ cm}^{-1}$; meta-substituted benzene also exhibits two bands but at different positions and the intensity of one band is stronger than the other (one band at $680-725 \text{ cm}^{-1}$ and other band at $750-810 \text{ cm}^{-1}$) while para-substituted benzene gives a single strong band at higher frequency (between $790-850 \text{ cm}^{-1}$) than ortho-substituted benzene [23].

Infrared spectrum of humic acid from surface soil shows bands at 829.5 and 695.8 cm^{-1} . The

spectrum of humic acids from 15 cm depth soil shows bands at 798.3 and 693.6 cm^{-1} and that of humic acid from 30 cm depth soil shows bands at 785.7 and 696.5 cm^{-1} . Since meta-substituted benzene gives two bands in the regions 750-810 cm^{-1} and 680-725 cm^{-1} , thus strongly suggest that all the humic acids, studied here, have meta-substituted benzene compounds mainly. However, the contribution of ortho-substituted and para-substituted benzene compounds cannot be completely ruled out.

It has been shown in the elemental analysis above that the humic acids have very low percentage of sulphur. Sulphur may occur in natural organic matter as C-S and S-S bonds. The stretching vibration assigned to C-S bond shows band in the region 600-700 cm^{-1} . But the S-S stretching vibration is weak and is occurring in the range 400-500 cm^{-1} . The bands in the region 400-800 cm^{-1} may not be attributed to either C-S or S-S stretching vibration because the sulphur contents in the humic acids extracted from different soils are less than 1%.

The region from 600-1400 cm^{-1} is generally called finger print region. This is because the patterns of absorption of the infrared radiation in this region are unique to any particular compound, just as a person's finger prints are unique.

The band occurring around 540 cm^{-1} is generally assigned to M-O stretching vibration while the M-N stretching vibration occurs at a lower wave number than the M-O stretching vibration. The M-N stretching vibration commonly appears near 480 cm^{-1} .

In our study, all the humic acids have quite high percentages of oxygen content. Oxygen content is mainly contributed by the oxygen containing functional groups present in the organic substances. Thus there is a strong likelihood of the formation of metal-humic acid complexes where occurrence of M-O bonds are quite obvious. The sharp band at 553.9 cm^{-1} for humic acid from surface soil, at 531.6 cm^{-1} for humic acid from 15 cm depth soil and at 538.2 cm^{-1} for humic acid from 30 cm^{-1} depth soil can be attributed to stretching vibration of M-O bonds.

The presence of nitrogen containing compounds in humic acids have been discussed above. The nitrogen functional groups are excellent ligands which can bind strongly with metal forming chelate type of compounds. Thus M-N bonds are

present in the organic substances. It is important that chelate complexes containing M-O and M-N bonds could not be cleaved by the treatment of alkali and acid, used for the preparation of humic acids from soil. As all the humic acids show bands almost at same position, i.e., at 468 cm^{-1} , from surface soil, at 468.5 cm^{-1} from 15 cm depth soil and at 471.1 cm^{-1} from 30 cm depth soil, these bands may be correlated by the contribution of M-N bonds occurring in these humic acids.

4. CONCLUSION

In conclusion, it may be said that humic acids from different depth tea garden soils are undergoing less humification process resulting in less condensed networks where aliphatic molecules dominate. Hydrogen bonded hydroxyl groups, carboxyl carbonyl, substituted olefins, presence of polysaccharides, nitrogen species, etc., have been detected in these humic acids. Further, chelate-type of complexes containing M-O and M-N have also been indicated.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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