



ISSN : 2350-0743

www.ijramr.com



International Journal of Recent Advances in Multidisciplinary Research

Vol. 06, Issue 06, pp.5023-5028, June, 2019

## RESEARCH ARTICLE

### COMPARATIVE CHARACTERIZATION OF HUMIC SUBSTANCES EXTRACTED FROM PEAT SOILS OF GOPALGONJ AND KHULNA REGIONS OF BANGLADESH

\*<sup>1</sup>Mohammad Moniruzzaman, <sup>1</sup>Badhan Saha, <sup>1</sup>Afroza Parvin, <sup>1</sup>Priyanka Dey suchi,  
<sup>1</sup>Md. Kamal Hossain and <sup>2</sup>Sirajul Hoque

<sup>1</sup>Soil and environment section, BCSIR Laboratories, Dhaka, Bangladesh Council of Scientific and Industrial Research, Bangladesh

<sup>2</sup>Department of soil, water and environment, University of Dhaka, Bangladesh

#### ARTICLE INFO

##### Article History:

Received 13<sup>th</sup> March, 2019

Received in revised form

09<sup>th</sup> April, 2019

Accepted 18<sup>th</sup> May, 2019

Published online 30<sup>th</sup> June, 2019

##### Keywords:

Humic substances, Extraction, Characterization.

##### \*Corresponding author:

Mohammad Moniruzzaman

#### ABSTRACT

Humic substances act as a chelating agent for metal ion extraction. Soluble humic substances (HS) might be an alternative to use instead of synthetic chemicals such as mineral acid or EDTA. These natural extractants are less expensive, biodegradable, environment friendly, less destructive to soil structure and also improve soil properties. Two peat soils were collected one from Khulna University campus and another from Gopalganj basin. Humic substances were extracted from peat soils. The characterization of humic substances was done by determining various parameters to evaluate their maturity. The quantifications of functional groups of humic substances were also performed. The complexing capacities of humic substances with metal ions depend on its maturity, molecular dimension and presence of functional groups. The optical density, E4:E6 ratio and coagulation thresholds of humic substances of Khulna region indicated more maturity due to intense humification process than that of HS in Gopalganj region. Consequently the functional groups including carboxylic (-COOH), phenolic (-OH) and aliphatic (-OH) of humic substances of Khulna region were relatively higher (from FTIR spectroscopy and potentiometric titration) which increased its metal holding capacity over that of Gopalganj region. Furthermore, the yield of humic and fulvic acids from mature peat of Khulna region were 2.48 and 2.29 folds higher than that of Gopalganj peat, respectively. These characteristics may be attributable to the climatic conditions of the peatland of two regions. Therefore the humic substances of Khulna region were selected for further investigation.

#### INTRODUCTION

Humic substances as part of humus are compounds arising from the physical, chemical and microbiological transformation (humification) of biomolecules. Humic substances consist of a complex mixture of organic substances covering a very wide range in molecular size mainly formed during microbial degradation of dead organic materials (Stevenson and Cole, 1999). Humic substances (HS) found in soils, peats, sediments and natural waters come from the chemical and biological degradation of plants and animals residues in the environment and the synthesis activity of microorganisms (McDonald *et al.*, 2004). In general, humic substances from peat can be considered biogenic organic substances, poly-electrolyte with properties that are similar to bio-colloids, such as dark coloration, heterogeneous nature, high molecular weight, many different molecular sizes (poly-dispersity), refractory, partially biodegradable and with a complex structure (McDonald *et al.*, 2004; Muscolo *et al.*, 2013; Wagner *et al.*, 2016). Humic substances (HS) are composed of humic acid, fulvic acid, and humins, all of which have a different solubility in alkaline and acid solutions (Benedetti *et al.*, 1996; Evangelou *et al.*, 2004). The properties and structure of HS may vary substantially, depending on the characteristics of the water or soil, of the compounds of origin, maturation of the HS and specific conditions of extraction (Fong and Mohamed, 2007; Muscolo *et al.*, 2013; Garcia *et al.*,

2016). These fractions vary in molecular size and functional group content. Humic acid are generally larger in size than fulvic acid in HS isolated from peat (Fong and Mohamed, 2007). However, due to the complexity of HS and different chemical and environmental factors, it is difficult to accurately determine the structure and molecular size of these substances (Rigobello *et al.*, 2017). Humic substances have a complex structure with multiple functional groups that can interact with metal ions in several ways. One way is through electrostatic interactions between negatively charged humic substances and positively charged metal-ions (Evangelou and Marsi, 2001). This research work compares the characteristics of humic substances isolated from peat soils of different regions using different analytical techniques.

#### MATERIALS AND METHODS

**Source of humic substances:** Peat-basin soils occupy perennially wet basin or valley sites which are mainly deeply flooded in the rainy season. They occur extensively in the Gopalganj-Khulna bills (Rahman, 2005). Gopalganj-Khulna Beel (2,247 sq km) region occupies extensive low-lying areas between the Ganges River Floodplain and the Ganges tidal floodplain (Figure 1). Soils of the area are grey, and dark grey, acidic, heavy clays overlay peat or muck at 25 - 100 cm. General soil types include mainly peat and Non-calcareous Dark Grey Floodplain soils (Banglapedia).

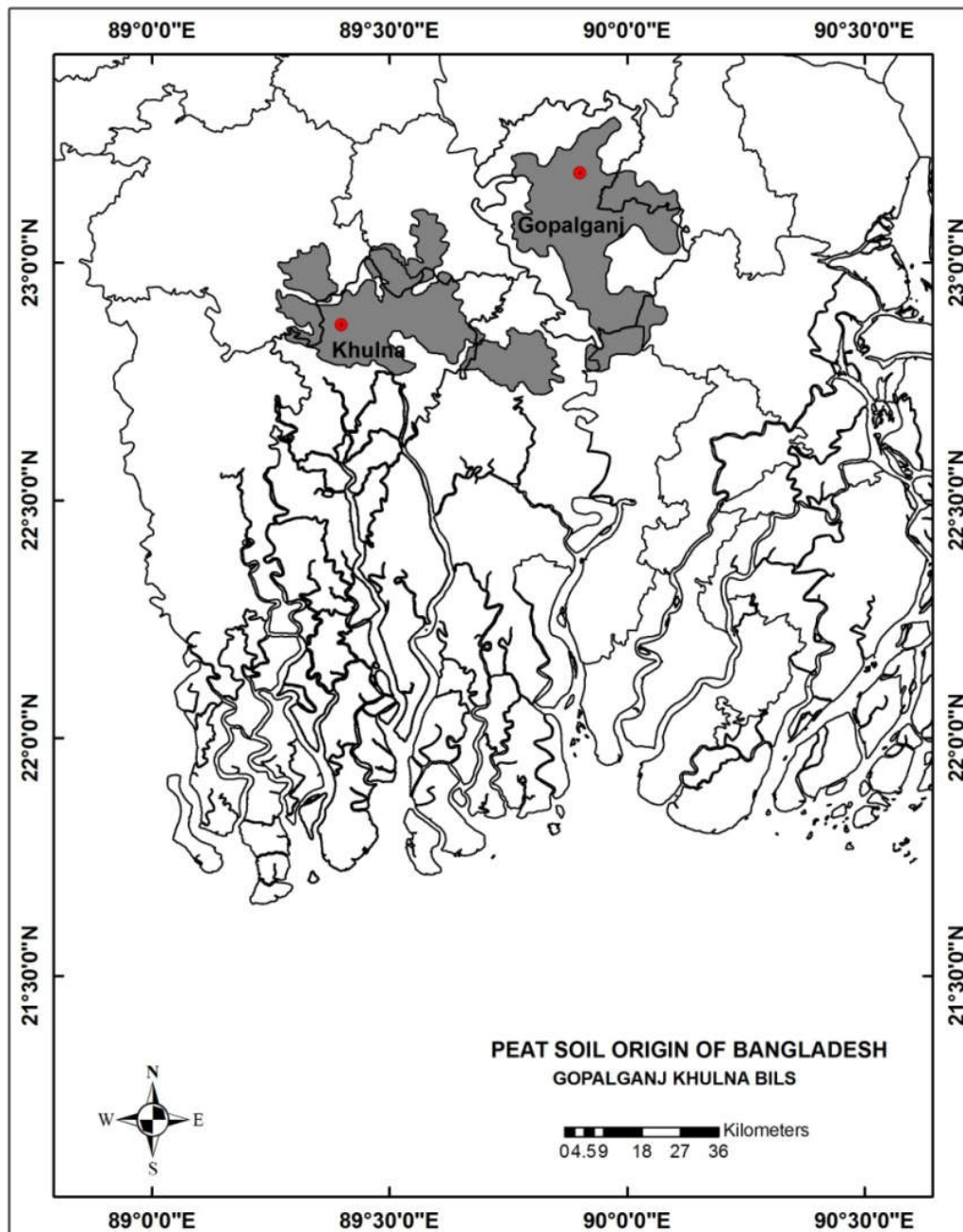


Figure 1. Peat soil distribution in Khulna and Gopalganj district

**Collection of peat samples:** Peat soils were collected from Khulna University campus ( $89^{\circ}23'52.8''\text{E}$  &  $22^{\circ}52'8.4''\text{N}$ ) and Gopalganj basin ( $89^{\circ}54'3.6''\text{E}$  &  $23^{\circ}11'20.4''\text{N}$ ). The GPS-GIS based location map of the sampling site is shown in the Figure 1. The soils of Khulna and Gopalganj regions are belonging to the Harta and Satla soil series, respectively. General features are presented in Table 1 and general description of the Harta and Satla soil series is given below.

**Table 1. General features of Harta and Satla soil series with soil classification system**

Soil Series	Location	USDA Soil Taxonomy	General soil types
Harta Series	Khulna University	Typic Haplofibrists	Peat Basin
Satla Series	Gopalganj Basin	Typic Haplohemists	Peat Basin

**Harta series** includes seasonally flooded, poorly to very poorly drained, strongly gleyed which below a depth of 10-20 inches have a buried organic layer of 35 inches or more. Topsoil varies from clay to mucky clay in texture. Those soils

usually remain waterlogged for 8 - 10 months in 6 - 10 feet deep water. They have two phases: Poorly drained; and very poorly drained (Rahman, 2005).

**Satla series** includes seasonally to almost perennially flooded, poorly to very poorly drained, very dark grey or black to very dark greyish brown, organic soils below the topsoil. These soils are developed in organic material derived from decomposed swamp grasses and reeds in the peat basin areas occupying low-lying basin depressions of Khulna-Gopalganj Bill areas. They have two phases poorly drained and very poorly drained (Rahman, 2005). Collected samples were kept in paper bags with appropriate label and the bags were tied securely with tag. The samples were carefully brought to the laboratory of BCSIR for detail analysis.

**Preparation of peat samples:** Peat soils were spread on separate sheet of thick coarse brown paper and dried in air. The air dried sample was oven dried and ground with mortar and

pestle. The ground sample was passed through a 0.5 mm sieve. The sieved samples were mixed thoroughly for making composite samples and preserved.

#### Extraction and quantification of humic substances:

Extraction of humic acid and fulvic acid was done according to new standard method of IHSS (International Humic Substances Society) (Lamar *et al.*, 2014).

**Characterization of humic substances:** The humic and fulvic acids were characterized by various chemical techniques.

**Optical density and E4/E6 Ratio:** The optical density of humic substances and E4/E6 ratio were determined as described by Chen *et al.* (1977). The absorbance was measured at 465 and 665 nm after dissolving 2 mg of freeze-dried humic substances in 25 ml of  $\text{NaHCO}_3$  (0.025M).

**Elemental Analysis:** The C, H, N and S contents of HAs and FAs were determined by using a CHNS analyzer (Perkin-Elmer 2400, USA). Total ash content was determined by the combustion method. The contents of Fe, and Al were determined using Atomic Absorption Spectrophotometer. The oxygen content was calculated by subtracting the C, H, N, S, and ash contents from the total weight.  $\text{SiO}_2$  content was calculated by subtracting the  $\text{Fe}_2\text{O}_3$  and  $\text{Al}_2\text{O}_3$  contents from the total ash content. (Huang *et al.*, 2006).

#### Quantification of functional groups of humic substances

##### Fourier-Transform Infrared (FTIR) Spectroscopy:

Functional groups of humic and fulvic acid were determined by FTIR (Perkin Elmer 1600, USA) spectrophotometer using Infrared spectra from 4000 to 400  $\text{cm}^{-1}$  range, recorded at a rate of 16 nm/s (Huang *et al.*, 2006).

**Potentiometric Titration:** Total acidity was determined by the titration method (Tsutsuki and Kuwatsuka, 1978a). Carboxyl group content was determined by the calcium acetate method of Blom *et al.* (1957). Total hydroxyl group content was determined by the acetylation method of DeWalt and Glenn (1952), modified by Tsutsuki and Kuwatsuka (1978a). Phenolic hydroxyl group content was estimated by subtracting the carboxyl group content from the total acidity. Alcoholic hydroxyl group content was estimated by subtracting the phenolic hydroxyl group content from the total hydroxyl group content. Carbonyl group content was determined by the oximation method of Schnitzer and Riffaldi (1972).

## RESULTS AND DISCUSSION

**Characterization of humic substances:** Humic substances are heterogeneous, high-molecular-weight organic substances that are composed of humic acid, fulvic acid and humins, all of which have a different solubility in alkaline and acid solutions (Zhang *et al.*, 2013). The properties of humic substances such as optical density, E4:E6 ratio, coagulation threshold and functional groups were analyzed. These properties were very important to determine the maturity, molecular condensation, hydrophobic nature as well as complex forming capacity of HS with metal ions.

**Optical Density:** Optical density of humic substances decreased with increasing wavelength. Among two different sources of humic and fulvic acids, optical density of humic substances showed considerable variation. The optical densities of humic and fulvic acids extracted from peat from

Gopalganj region were lower than that of Khulna region (Figure 2 and 3). During comparative studies of the chemical structure and the optical properties of humic and fulvic acids of different origins it was found that younger humic substances, in a chemical sense, have a lower optical density than more mature humic substances (Kononova, 1966). This indicated that humic substances extracted from peat of Gopalganj region were younger than that of Khulna region.

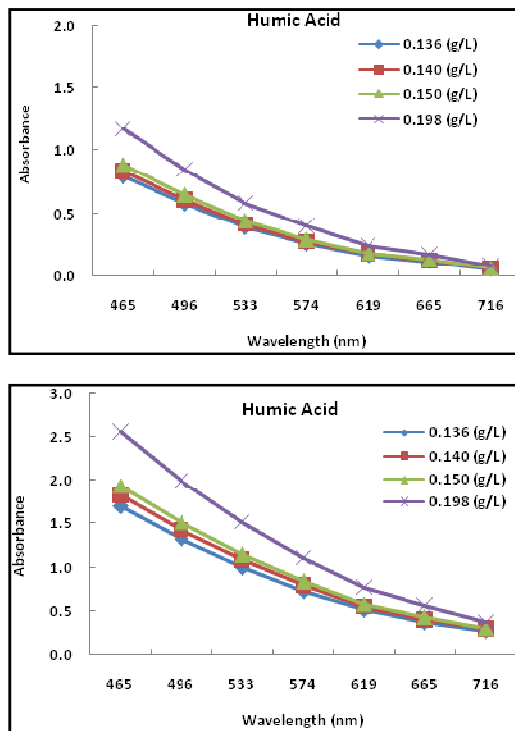


Figure 2. Optical density of humic acid extracted from peat collected from Gopalganj and Khulna region at different carbon concentration

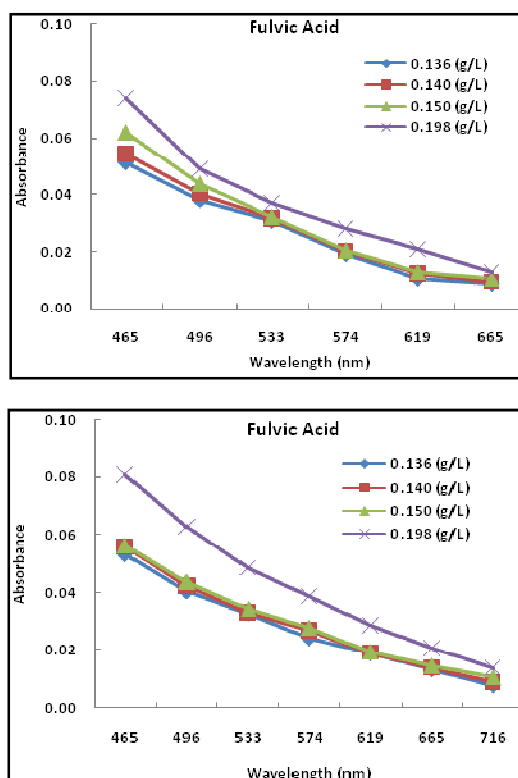


Figure 3. Optical density of fulvic acid extracted from peat collected from Gopalganj and Khulna region at different carbon concentration

**E4/E6 Ratio:** The optical parameter E4/E6 ratio expresses molecular condensation. Scheffer (1954) showed that the ratio of the extinction E at the wavelengths 465 nm and 665 nm (the so-called E4/E6 ratio) is independent of the concentration of carbon in solution and by reflecting the steepness of the spectrophotometric curve, is a characteristic of humic substances. The mean E4:E6 ratio value was high (HA 5.8 and FA 6.94) in peat of Gopalganj region followed by Khulna region (HA 3.99 and FA 4.57) (Table 2). According to Chen *et al.* (1977), the E4/E6 ratio is a parameter inversely related to the molecular dimension. Chanyasak *et al.*, (1982) reported that E4/E6 ratio has been immensely useful as an assessment index, which usually decreases during humification. This index is widely used as an indicator for evaluating the maturity of humic substances.

As shown in Table 2, the E4/E6 ratio of both HA and FA of Khulna region decreased with increasing humification process and higher molecular weight compound was formed by increased condensation and aromatization processes which produced more polycondensed humic substances during humification. So, in respect of E4/E6 ratio, it can be concluded that humic substances of Khulna region were more mature than that of Gopalganj region. Kononova (1966) found that the E4/E6 ratio  $\geq 5.0$  indicates a reduced hydrophobic structure while that  $\leq 5.0$  suggests an increased hydrophobic structure. So, according to E4/E6 ratio humic substances of Khulna region showed increased hydrophobic and HS of Gopalganj region indicated reduced hydrophobic structure.

**Coagulation Threshold:** Requirement of time as well as CaCl<sub>2</sub> concentrations for coagulation of humic and fulvic acids were investigated by Kononova (1966). Data present in Table 3 showed the coagulation threshold values obtained with humic and fulvic acids from different regions. These coagulation results indicated that peat of Khulna region has more condensed molecular structure of HA and FA than Gopalganj region.

**Elemental compositions:** Results of elemental compositions of HA and FA are presented in Table 4. Decreasing carbon content may be explained by more intense decomposition of organic and decreasing H content indicated substitution or fusion of aliphatic chains to form aromatic groups' carbon in HA of Khulna region. A relative increase of nitrogen in HA could be due to incorporation of N in HA through condensation of lignin with proteins (Stevenson, 1994) or due to complexation of N with lignin (Hsu and Lo, 1999). Peat of both regions showed the lower C/O ratio in FA than HA indicated the presence of more O-containing group in FA such as carboxylic-ketonic (C=O, COO-) and phenolic-alcoholic (OH). Moreover the HA and FA of Khulna region showed the lower C/O ratio in indicated more O-containing group (C=O, COO, OH) than that of Gopalganj region.

**Yield of humic substances:** The increased yields of humic substances (HS) were observed in peat of Khulna region possibly due to higher humification process compare to that of Gopanganj peat (Table 5). In general, younger peat contains lower levels of HS as compared to mature one (Chefetz *et al.*, 1981; Inbar *et al.*, 1990). This trend was also observed in the present study. The yields of humic and fulvic acids from mature peat of Khulna region were 2.48 and 2.29 folds higher than that of Gopalganj peat, respectively (Table 5).

**Table 2. The E<sub>4</sub>/E<sub>6</sub> ratio of Humic and fulvic acids of peat collected from different regions**

Carbon Concentration (g/L)	Gopalganj		Khulna	
	Humic Acid	Fulvic Acid	Humic Acid	Fulvic Acid
0.136	5.818	6.991	4.077	4.589
0.140	5.798	6.900	4.043	4.569
0.150	5.804	6.890	3.944	4.537
0.196	5.789	6.981	3.937	4.578

**Table 3. Coagulation threshold of humic and fulvic acids of peat collected from different regions**

Time required for coagulation	Gopalganj		Khulna	
	Humic Acid	Fulvic Acid	Humic Acid	Fulvic Acid
	Concentration of Calcium chloride (meq/L)			
1 hour	9.0	9.5	8.0	8.5
4 hours	8.0	8.0	6.5	7.0
6 hours	7.0	8.0	6.0	7.0
24 hours	6.0	7.5	5.5	6.0

**Table 4. Elemental composition (%) of humic and fulvic acids from peat of different regions**

Sources	HS	C	H	N	S	O	C/H	C/O
Gopalganj Peat Soil	HA	48.4	5.6	3.7	0.51	41.7	8.64	1.16
	FA	43.2	5.8	2.2	0.81	48.0	7.45	0.90
Khulna Peat Soil	HA	47.3	5.3	4.1	0.31	43.0	8.92	1.10
	FA	41.5	5.4	3.9	0.65	48.6	7.69	0.85

HS = Humic Substances, HA = Humic Acid FA = Fulvic Acid

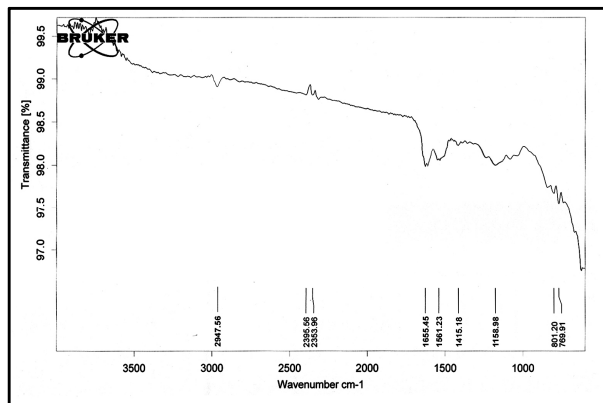
**Table 5. Yields of humic acid and fulvic acid from peat of Gopalganj and Khulna region**

Sources	Humic Substances	Yield (%)
Peat of Gopalganj region (Satla soil series)	Humic acid	3.14
	Fulvic acid	2.09
Peat of Khulna region (Harta soil series)	Humic acid	7.81
	Fulvic acid	4.78

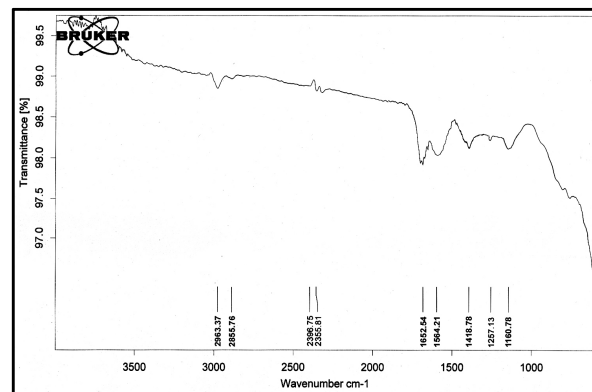
**FTIR Spectroscopy:** From FTIR spectra of humic acid of Gopalganj (GHA): one small peaks at 2963 cm<sup>-1</sup> (aliphatic C-H), a sharp peak of unsaturated C=C at 1655 cm<sup>-1</sup> (aromatic C=C, COO-, C=O), a sharp peak at 1561 cm<sup>-1</sup> (amide II bonds), a peak at 1415 cm<sup>-1</sup> (phenolic OH deformation and C-O stretch), a sharp peak at 1158 cm<sup>-1</sup> (symmetric bonding of aliphatic CH<sub>2</sub>, OH, or C-O stretch of various groups), and a peak at 801 cm<sup>-1</sup> (aromatic CH out of plane bending) were observed (Figure 4). The main absorbance bands in humic acid spectra of Khulna region were two small peaks at 2963 and 2855 cm<sup>-1</sup> (aliphatic C-H), a sharp peak of unsaturated C=C at 1652 cm<sup>-1</sup> (aromatic C=C, COO-, C=O), a sharp peak at 1564 cm<sup>-1</sup> (amide II bonds), a peak at 1418 cm<sup>-1</sup> (phenolic OH deformation and C-O stretch), a shoulder at 1257 cm<sup>-1</sup> (aromatic C, C-O stretch), a sharp peak at 1160 cm<sup>-1</sup> (symmetric bonding of aliphatic CH<sub>2</sub>, OH, or C-O stretch of various groups), a peak in the 1100-1000 cm<sup>-1</sup> range (C-O stretch of polysaccharide, Si-O stretch) observed (Figure 4). Inbar *et al.* (1990) found similar peak in FTIR spectra of humic acid. The band assigned to aliphatic C-H stretch occurred at 2950 cm<sup>-1</sup> was lower and the band 2850 cm<sup>-1</sup> was absent in GHA. Peaks in the polysaccharide region at 1160 cm<sup>-1</sup> decreased, while the 1420 cm<sup>-1</sup> peak became sharper in KHA, as compared to a HA spectrum of Gopalganj peat, which indicated less -OCH<sub>3</sub> and -OH polysaccharide groups in HA of Gopalganj peat (Figure 4). The relative height of the aromatic region at 1650 cm<sup>-1</sup> and 1250 cm<sup>-1</sup> rose as the humification process proceeded from GHA to KHA which

Table 6. Functional groups of humic and fulvic acids in peat soils of Gopalganj and Khulna regions

Sources	Humic Substances	Total Acidity (meq g <sup>-1</sup> )	Carboxyl (meq g <sup>-1</sup> )	COOH	Phenolic <sup>a</sup> (meq g <sup>-1</sup> )	OH	Aliphatic <sup>b</sup> OH (meq g <sup>-1</sup> )	Total (meq g <sup>-1</sup> )	OH	Carbonyl (meq g <sup>-1</sup> )
Gopalganj Peat Soil (Satla)	Humic acid	5.05	3.12		1.93		2.58	4.51		3.12
	Fulvic acid	5.96	4.64		1.32		2.12	3.44		4.16
Khulna Peat Soil (Harta)	Humic acid	6.28	4.07		2.21		2.97	5.18		3.75
	Fulvic acid	7.42	5.41		2.01		2.34	4.35		4.76

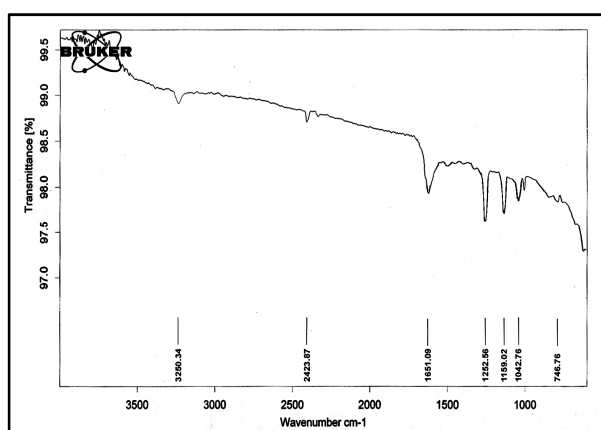


(A)

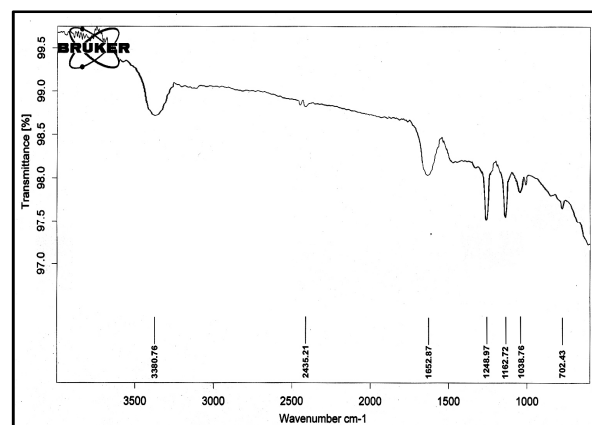


(B)

Figure 4. FTIR Spectrum of Humic acid (HA) of Gopalganj peat soil (A) and Khulna peat soil (B)



(A)



(B)

Figure 5. FTIR Spectrum of Fulvic acid (FA) of Gopalganj peat soil (A) and Khulna peat soil (B)

indicated the humic substances of Khulna region had become more uniform and stable with the attainment of maturity level (Hsu and Lo, 1999). The ratio between the aromatic carbon/aliphatic carbon ( $1650/2850 \text{ cm}^{-1}/\text{cm}^{-1}$ ) increased from GHA to KHA as 0.79 to 1.54 and the aromatic carbon/amide II ratio increased from 0.94 to 1.52, respectively (Fig 4). These changes in FTIR spectra indicate that easily degradable organic matter constituents such as short aliphatic chains, polysaccharides and alcohols are chemically or biologically oxidized, leading to increased aromatic structures of high stability (Gerasimowicz and Bayler, 1985). The FA spectrum was similar to that of HA, as shown in Figure 5. A relatively weaker peak at  $1650 \text{ cm}^{-1}$  as compared to HA was found, indicating that fewer C=C bonds were present in FA and a lower degree of aromaticity. However, a sharp peak occurred at  $1040 \text{ nm}$  regions (C-O stretch of polysaccharide, Si-O stretch), which did not occur in the HA spectra (Fig. 5). This indicated that more O-containing group (C=O, COO-) than that of HA fraction.

**Quantification of functional groups:** The quantifications of functional groups of humic substances were obtained from FTIR spectroscopy and potentiometric titration.

As revealed in the functional group analysis, the total acidity, carboxyl (-COOH), phenolic (-OH), aliphatic (-OH) and carbonyl groups of humic substances were found higher in peat of Khulna region due to intense humification processes than that of Gopalganj peat. Therefore HS of Khulna region have more functional groups compare to HS of Gopalganj region (Table 6).

## Conclusion

The results obtained from present study indicated that, the humic substances obtained from peat soils of Khulna and Gopalganj regions were different in apparent molecular weights, maturity, coagulation capacity and functional groups. The complex forming capacities of humic substances with ions depend on its molecular dimension and presence of functional groups. The optical density, E4:E6 ratio and coagulation thresholds of humic substances of Khulna region indicated more maturity due to intense humification process than that of HS in Gopalganj region. Consequently the functional groups including carboxylic (-COOH), phenolic (-OH) and aliphatic (-OH) and yields of humic substances of Khulna region were relatively higher which increased its ion holding capacity over

that of Gopanganj region. These characteristics may be attributable to the climatic conditions of the peat land of two regions. The preliminary characterization of these native HS may further be confirmed with some advanced analytical techniques such as the solid state NMR, Raman spectroscopy and others. Therefore humic substances of Khulna region were selected for further investigation due to maturity and intense functional groups.

## REFERENCES

- Benedetti, M.F., Riemsdijk, W.H.V., Koopal, L.K., Kinniburgh, D.G., Gooddy, D.C. and Milne, C.J. 1996. Metal ion binding by natural organic matter: from the model to the field. *Geoch. Cosmo. Acta.*, 60: 2503–2513.
- Blom, L., Edelhausen, L. and D.W.V. Krevelen. 1957: Chemical structure and properties of coal. XVIII. Oxygen groups in coal and related products. *Fuel*. 36: 135-153.
- Chefetz, B., Hatcher, P.G., Hadar, Y. and Chen, Y. 1981. Chemical and biological characterization of organic matter during composting of municipal solid waste. *J. Environ. Qual.*, 25, 776-785.
- Chen, Y., Senesi, N. and Schnitzer, M. 1977. Information provided on humic substances by E4/E6 ratios. *J. Soil Sci. Soc. Am.*, 41: 352-358.
- DeWalt, C.W. and Glenn, R.A. 1952. Analytical acetylation-application to coal hydrogenation products. *Anal. Chern.*, 24: 1789-1795.
- Evangelou, M.W.H., Daghan, H. and Schaeffer, A. 2004. The influence of humic acids on the phytoextraction of cadmium from soil. *Chemosphere*, 57: 207-213.
- Evangelou, V.P. and M. Marsi, 2001. Composition and metal ion complexation behaviour of humic fractions derived from corn tissue. *Plant and Soil.*, 229: 13–24.
- Fong, S. S. and Mohamed, M. 2007. Chemical characterization of humic substances occurring in the peats of Sarawak, Malaysia. *Organic Geochemistry*, v. 38, p. 967-976. <https://doi.org/10.1016/j.orggeochem.2006.12.010>.
- García, A. C., Souza, L. G. A., Pereira, M. G., Castro, R. M., JGarcía-Mina, M. and Zonta, E. 2016. Structure-Property-Function Relationship in Humic Substances to Explain the Biological Activity in Plants. *Scientific Reports*, 6: 20798.
- Hsu, J.H. and Lo, S.L. 1999. Chemical and spectroscopic analysis of organic matter transformations during composting of pig manure. *Environ. Pollut.*, 104, 189–196.
- Huang, G.F., Wu, Q.T., Wong, J.W.C. and Nagar, B.B. 2006. Transformation of organic matter during co-composting of pig manure with sawdust. *Bioresour. Tech.*, 97: 1834-1842.
- Inbar, Y., Chen, Y. and Hadar, Y. 1990. Humic substance formed during the composting of organic matter. *Soil Sci. Soc. Am. J.*, 54: 1316–1323.
- Kononova, M.M. 1966. Soil Organic Matter: Its Nature, Its Role in Soil Formation and in Soil Fertility. Pergamon Press Ltd., Oxford. 45-49.
- Lamar, R.T., Mayhew, D.C. Olk, L. and Bloom, P.R. 2014. A New Standardized Method for Quantification of Humic and Fulvic Acids in Humic Ores and Commercial Products. *Journal of AOAC International*, 97(3): 721-730.
- McDonald, S., Bishop, A. G., Prenzler, P. D. and Robards, K. 2004. Analytical chemistry of freshwater humic substances. *Analytica Chimica Acta*, v. 527, p. 105-124, <https://doi.org/10.1016/j.aca.2004.10.011>.
- Muscolo, A., Sidari, M. and Nardi, S. 2013. Humic substance: Relationship between structure and activity. Deeper information suggests univocal findings. *Journal of Geochemical Exploration*, v. 159, p. 57-63, <https://doi.org/10.1016/j.gexplo.2012.10.012>.
- Rahman., M.R. 2005. Soils of Bangladesh. Soil Resources Development Institute. Dhaka-1215. Bangladesh.
- Rigobello, E. S., Campos, S. X., de Azevedo, E. R., Dantas, A. D. B. and Vieira, E. M. 2017. Comparative characterization of humic substances extracted from freshwater and peat of different apparent molecular sizes. *Ambiente & Água - An Interdisciplinary Journal of Applied Science*, ISSN 1980-993X - doi:10.4136/1980-993X. 775-785.
- Rigobello, E. S., Dantas, A. D. B., Di Bernardo, L. and Vieira, E. M. 2011. Influence of the apparent molecular size of aquatic humic substances on colour removal by coagulation and filtration. *Environmental Technology*, 32: 1767-1777.
- Schnitzer, M. and Riffaldi, R. 1972. The determination of quinone groups in humic substances. *Soil Sci. Soc. Am. Proc.*, 36: 772-777.
- Stevenson, F.J. and Cole, M.A. 1999. Cycles of Soil. Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients. 2nd Edition, John Wiley & Sons, New York.
- Tsutsuki, K. and Kuwatsuka, S. 1978a. Chemical studies on soil humic acids. II. Composition of oxygencontaining functional groups of humic acids. *Soil Sci. Plant Nutr.*, 24, 547-560.
- Wagner M., Schmidt, W., Imhof, L., Grübel, A., Jahn, C. and Georgi, D. 2016. Characterization and quantification of humic substances 2Dfluorescence by usage of extended size exclusion chromatography. *Water Research*, v. 93, p. 98-109, 2016. <https://doi.org/10.1016/j.watres.2016.01.050>.
- Zhang, Y., X. Yang, S. Zhang, Y.Tian, W. Guo, and J. Wang. 2013. The influence of humic acids on the accumulation of lead (Pb) and cadmium (Cd) in tobacco leaves grown in different soils. *Journal of Soil Science and Plant Nutrition*, 13(1): 43-53.

\*\*\*\*\*