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Humus and Humic  
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# Meet the editor



Dr. Abdelhadi Makan has a Ph.D. in Waste Management and Environmental Sciences. He has professional experience in environmental science, chemistry, and waste management. Through all his academic and professional publications, he has fully practiced both qualitative and quantitative research methods. Additionally, he is the founder and CEO of ENQUAS Consulting, an investigation and consultancy office practicing in environmental, quality, and safety domains. Currently, Dr. Makan is a full-time professor at the National School of Applied Sciences (ENSAH), Abdelmalek Es-saadi University, Morocco. In his spare time, he acts as an editorial board member, reviewer, and proofreader for several reputed scientific journals.





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# Preface

The complexity of humic substances and their remarkable properties in agricultural and other applications have attracted and continue to attract the attention of researchers, bringing over the years new knowledge on their structure and physicochemical and biological properties. However, studies have produced controversial results because of the difficulty in identifying a precise relationship between the structure and the activity of these substances. This book discusses recent advances and applications of humus and humic substances in agriculture, industry, and the environment.

During heavy rains, thunderstorms, and summer monsoons, induced runoff results in surface water enrichment with humic and fulvic acids. These soil-generated acids negatively affect the water supply by producing disinfection byproducts during chlorination and serving as sorbents to bind hydrophobic organic contaminants. Chapter 1 investigates the chlorination and phenanthrene sorption for humic and fulvic acids extracted from different soils. It also analyzes and compares their characteristics using  $^{13}\text{C}$ -NMR elemental analysis, spectroscopic analysis, and size exclusion chromatography.

The use of humic substances as growth promoters for farm animals is an emerging practice that deserves more attention. These substances can be administered to the animal through drinking water or feed. Chapter 2 reviews their mechanisms of action inside the body in terms of digestive mucosa protection, antioxidant properties, immune response and inflammation, digestive microbiota, and metabolism. In addition, it addresses the productive response in animals supplemented with humic substances.

Chapter 3 explores the dynamics and function of humus forms in the case of tropical forests as well as factors influencing their development and transformation. Moreover, it reviews new approaches for qualitative and quantitative characterization of humus in tropical environments and debates future challenges for its analysis.

Humic substances are essential organic compounds found in topsoil and aquatic environments. These degraded materials represent a rich source of nutrients for agriculture and crop productivity. In this context, Chapter 4 examines the properties of humic substances and their effect on soil quality and plant health. Finally, Chapter 5 discusses their contribution to improving the agriculture and livestock sector in the African Great Lakes region.

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## Chapter 1

# Humic Acids and Fulvic Acids: Characteristics, Sorption of Hydrophobic Organic Contaminants, and Formation of Disinfection by-Products during Chlorination

*Hang Vo-Minh Nguyen, Jin Hur and Hyun-Sang Shin*

### Abstract

Humic and fulvic acids, which can be extracted from soils, are abundant in surface water because of their high discharges from runoff during torrential rainfall, storm events, and summer monsoon. Both humic and fulvic acids adversely affect water supply as they produce disinfection by-products (DBPs) during chlorination and serve as the sorbent for the binding of hydrophobic organic contaminants. In the present study, we conducted chlorination and phenanthrene sorption for humic and fulvic acids that were extracted from nine soil samples. We also analyzed and compared their characteristics by using elemental  $^{13}\text{C}$  NMR analysis, spectroscopy analysis, and size exclusion chromatography. Our results showed that the changes in their structural characteristic, their DBP formation, and phenanthrene sorption behavior differed critically between humic and fulvic acids. For chlorinated humic acids, high SUVA, low molecular weight, low N/C, and low O groups of aromatic C were associated with high trihalomethane (THM) formation. In comparison, low O groups of aliphatic C in fulvic acids were associated with both oxidation and incorporation in terms of THM formation. Humic acids exhibited higher sorption ability than fulvic acids due to their higher  $MW_w$ , SUVA, and %THLF. These findings provide key information for monitoring water quality in rivers and lakes.

**Keywords:** humic acids, fulvic acids, oxidation reaction, incorporation reaction, trihalomethane, sorption isotherm, hydrophobic organic contaminants

### 1. Introduction

Humic substances are principal compounds that account for 80–90% of soil organic matter [1]. Owing to their complexes of bioactive substances, humic substances can control the stability and ecosystem in soil [2]. Humic substances

are organic macromolecules with multiple properties and contain a wide variety of structural functional groups [3], arising from physical, chemical, and microbiological processes [4]. In aquatic system, humic substances account for 40–60% of natural organic matter [5]; thus, they can have significant impact on water quality. In natural water resources, humic substances are formed from the degradation of plants, animal residues, and soil surface runoff [6]. In the events of torrential rainfall, storm events, and summer monsoon season, humic substances are abundantly formed from upstream land use and soil surface runoff [7–9]. Thus, it is essential to study the humic substances extracted from upland soils for water quality management.

Humic substances are mainly divided into humic acids, fulvic acids, and humin [10]. Humic acids are soluble in water only at  $\text{pH} > 2$ , fulvic acids are soluble in water in all pH conditions, whereas humin is insoluble in water [11]. Thus, because of their solubility, humic and fulvic acids play indispensable roles in dissolving organic matter in aquatic system. Humic and fulvic acids majorly comprise carboxylic, phenolic, carbonyl, hydroxyl, amine, amide, and aliphatic moieties [12]. In surface water, fulvic acids account for the majority of humic substances, whereas humic acids account for only 10% of humic substances [6]. For the disinfection of water for drinking purpose, humic and fulvic acids present in water can react with disinfectant chemicals (i.e., chlorine and ozone) to form disinfection by-products (DBPs) such as trihalomethanes (THMs), haloacetic acids (HAAs), halo ketones, and haloacetonitriles [13, 14]. Among these four DBPs, THMs and HAAs are the two most abundant halogenated DBPs [15, 16]. DBPs are considered to be dangerous to human health because of the presence of potential carcinogens [17] that particularly cause urinary bladder cancer [18, 19]. Humic and fulvic acids are the primary sorbent, which can impact the fate, mobility, and bioavailability of hydrophobic contaminants, especially the presence of polycyclic aromatic hydrocarbons (PAHs) in water system [20, 21]. PAHs contain more than two benzene rings [22] and are the most persistent and toxic organic micropollutants in surface water. Low concentrations of these hydrocarbons can have adverse effects on human health and aquatic systems because they contain carcinogenic, mutagenic compounds and potent immune suppressants [23, 24]. PAHs can be formed from biological process, industrial wastes, petroleum spills, incomplete combustion from nature sources (forest and brush fires), and/or human combustion sources (engine emissions) [24]. Recently, PAHs from urban runoff were reported to be a serious contaminant in rivers and lakes [9, 25, 26]. In addition, PAHs have been widely detected in surface water and drinking water at higher concentrations compared with other persistent organic pollutants [27, 28]. Of the 16 PAHs monitored by the US Environment Protection Agency, phenanthrene (PHE) was reported to be the most abundant PAH in surface water. Similar to humic and fulvic acids, PAHs can produce chlorinated PAHs during chlorination process for drinking water treatment. This is because PAHs contain an electronic-rich system that can be readily attacked electrophilically by hypochlorous acid [29]. The hypobromous acid might also be formed in the presence of  $\text{Br}^-$  during chlorination because  $\text{Br}^-$  ion is ubiquitous in both surface water and chlorine solution [30]. The hypobromous acid reacts with PAHs to form brominated PAHs. In comparison with PAHs, the chlorinated PAHs and brominated PAHs exhibit AhR activity, DNA damaging effects, and mutagenicity, and thus, they present a larger threat to human health [31, 32]. Hence, it is essential to investigate the characteristics of humic and fulvic acids and their PAHs sorption behavior in order to control the formation of DBPs from humic and fulvic acids as well as the formation of halogenated PAHs.

Many methods to identify the structure of humic and fulvic acids have been reported in the literature. Among them,  $^{13}\text{C}$  NMR is the most common method to identify functional groups and molecular structures such as aromatic and aliphatic C groups. In contrast, elemental analysis, which is a faster method than the  $^{13}\text{C}$  NMR method, reflects the atomic ratios that relate to aromatic C such as H/C and the derived sources of humic substance such as N/C [33]. The humic and fulvic acids can also be compared on the basis of molecular weight (MW) [34]. In addition, spectroscopic techniques such as UV–visible (Vis) absorbance and fluorescence are widely employed because of their simplicity, rapid process, and non-requirement of pretreatment of samples. Specific UV absorbance (SUVA) at 254 nm is an indicator for aromatic C, while their ratio  $E_4/E_6$ ,  $S_{275-295}$ , and  $S_{350-400}$  confirmed its humification and aromaticity [35]. Moreover, the sources of humic substance and its relationship with MW distribution humic substance can be determined using fluorescence properties and their index [36]. Although many studies have examined the characteristics of humic substances and their binding with PAHs, there is still a lack of sufficient information on the characteristics of humic and fulvic acids and their differences, formation of their DPBs, and binding behavior of their PAHs.

In this present study, humic and fulvic acids were extracted from soils and characterized by using  $^{13}\text{C}$  NMR, elemental analysis, MW, UV, and fluorescence methods. The study also reported the different formation of THMs and HAAs and the PHE behavior of humic and fulvic acids due to their different characteristics.

## 2. Study sites and characterization methods

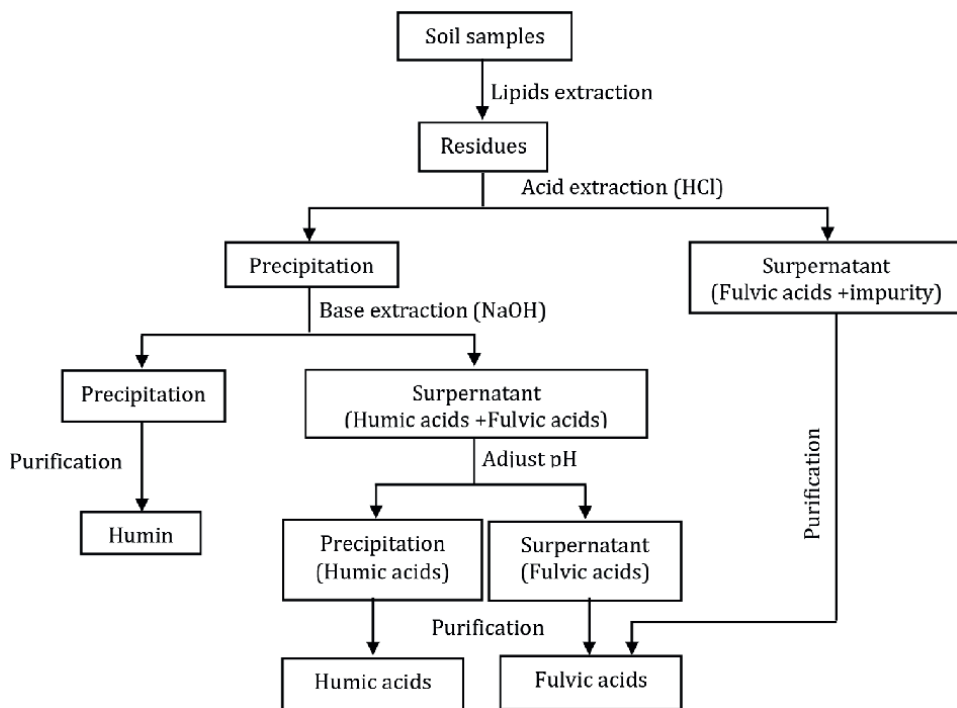
### 2.1 Soil sampling sites and extraction method

For this study, soil samples were collected from six different locations in Korea, and a minimum of 20 km distance was maintained between the sampling sites. Four of these samples were representative of granite soils and were named as Gori (KR), Wolseong (WS), Uljin (UJ), and Yeonggwang (YK). Two soil samples were collected from the foot of Mt. Seorak (Goseong (KS)) and from Mt. Hallan (Jeju Island, volcanic ash soil) (Halla (HL)). Three soil samples, namely Elliott Silt Loam Soil (Cat No. 1BS102M), Canadian peat moss soil sample (sphagnum peat moss), and Aldrich HA (Sigma-Aldrich, CAS no. 1415-93-6), were purchased and named as IHSS, Peat, and AL, respectively.

Humic and fulvic acids were extracted from six sampling soils (KR, WS, UJ, YK, KS, and HL) and Peat, according to the IHSS method [37] and ISO 12782-4:2012 [38]. The extracted fulvic acids were purified using XAD resin concentration and then passed through a Dowex-50X8(H+) column. The AL sample was purified using an acid–base precipitation method [10]. **Figure 1** presents the extraction and purification process of humic and fulvic acids.

### 2.2 Characterization methods

The UV–Vis absorbance of humic and fulvic acids in the 200–800 nm range was investigated using a UV–Vis spectrometer (Shimadzu, UV-1601PC). To measure the dissolves organic matter (DOC), samples were prepared at pH 7.0 and then filtered using a 0.45- $\mu\text{m}$  membrane filter (cellulose acetate, Advantec). The ratio of UV absorbance at 254 nm to the DOC concentrations of the samples was calculated to determine the SUVA values. The UV–Vis absorbance ratio at 465 and 665 nm ( $E_4/E_6$ ) and



**Figure 1.** The extraction and purification process of humic and fulvic acids.

spectral slope were applied to characterize the humic material as well as the aromaticity. The spectra slope was calculated using log-transform linear regression at intervals of 275–295 nm ( $S_{275-295}$ ) and 350–400 nm ( $S_{350-400}$ ). These two narrow bands were chosen for spectral slope calculation because they present the greatest variations from a variety of sources (i.e., marsh, riverine, estuarine, coastal, and open ocean).

A fluorescence spectrometer (Perkin Elmer LS50B) was used to obtain synchronous fluorescence spectra. It is known that fluorescence intensity can alternate with measurement time depending on external conditions such as humidity. Thus, the measured fluorescence intensities were normalized as units of quinine sulfate (QSE) equivalents based on the fluorescence of a diluted series of quinine sulfate dehydrate in 0.05 M sulfuric acid at an excitation/emission wavelength of 350/450 nm. Both the excitation and emission slits were fixed at 10. The difference between the emission wavelength and the excitation wavelength ( $\Delta\lambda$ ) was fixed at 30 nm and then measured from 250 to 600 nm to determine synchronous fluorescence spectrum. The relative fluorescence regions were classified into four groups: protein-like (%PLF) fluorescence, fulvic-like (%FLF) fluorescence, humic-like (%HLF) fluorescence, and terrestrial humic-like (%THLF) fluorescence. These groups of fluorescence regions corresponded to the relative percentage of fluorescence intensity at wavelengths of 250–300, 300–380, 380–420, and 420–600 nm, respectively.

The apparent weight-average molecular weight ( $MW_w$ ) values were determined using size exclusion chromatography. The polydispersity of samples with the relative precision of  $MW_w$  and  $MW_n$  were less than 5% and 7%, respectively. The elemental composition ratios of humic and fulvic acids (C/H, N/C, and (N + O)/C) were determined using CHNS-932 and VTF-900 (LECO Co.).



The cross-polarization magic-angle spinning method was used to determine carbon structure via  $^{13}\text{C}$  NMR spectroscopy (Bruker Avance II, 500 MHz). The spectrum was measured at 300 K with a 90-pulse width of 4.5 s, 1.5 ms contact time, 3 ms pulse delay time, and 6.0 kHz spinning speed. A qualitatively good signal-to-noise ratio was obtained by using a total of  $3 \times 10^4$  scan signal free induction decays and a line broadening function of 40 Hz. Then, the C functional groups were determined by integrating the area of the spectra in the chemical shift area: 0–50 ppm (alkyl C), 50–110 ppm (O-alkyl C), 110–145 ppm (C,H-aryl), 145–165 ppm (O-aryl phenol), and 165–190 ppm (carboxyl).

### 2.3 Chlorination of humic and fulvic acids and THMs/HAAs measurement

Humic and fulvic acids extracted from six sampling soils (KR, WS, UJ, YK, KS, and HL), Peat, AL, and IHSS were diluted to 1 mg C/L. Then, 1 ml phosphate buffer was added to 50 ml of diluted humic and fulvic acids to adjust their pH value to  $7.0 \pm 0.2$ . Then, the humic and fulvic acids were incubated for 2–3 h before chlorination. Chlorination of the humic and fulvic acids was conducted using the Aldrich's sodium hypochlorite solution (available chlorine >4%) ( $\text{NaOCl}$ ). The glassware required for the experiment was washed with acetone and then baked at  $400^\circ\text{C}$  for 1 h to remove any remaining organic matter. A constant dose of 5 mg  $\text{Cl}_2/\text{L}$  was added to each sample for chlorination. The final solutions were sealed and stored in the dark at  $25^\circ\text{C}$  for 24 h. Then, a 10% sodium sulfite solution was injected into the solutions to suppress the formation of additional by-products by residual chlorine.

THMs and HAAs were analyzed using USEPA Method 551.1 and Method 552.3. A micro-electron capture detector (Agilent 6890 GC-ECD) was used to conduct gas chromatography of the liquid–liquid extracts in order to quantify the different THMs and HAAs. This was followed by diazomethane derivatization. Four species of THM, i.e., such as chloroform (CF), dichlorobromomethane (DCBM), dibromochloromethane (DBCM), and bromoform, were measured. HAAs were analyzed using the Drinking Water Quality Process Test Method (ES 05552.2.). Three substances, i.e., dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), and dibromoacetic acid (DBAA), were used in this analysis.

### 2.4 PHE adsorption experiment and analysis

PHE (purity >97%) was purchased from Aldrich and used without further purification. The stock solution (1.0 mg/L) was prepared by dissolving an excess in methanol to make a saturated solution. This solution was filtered through a  $0.45\text{-}\mu\text{m}$  cellulose acetate membrane filter (Advantec). In this filter, the sorption of PHE is negligible. The solution was sterilized by adding 5 mM  $\text{CaCl}_2$  and 0.01 mM  $\text{HgCl}_2$ , and its pH was adjusted to 6 by adding either 0.1 M NaOH or 0.1 M HCl. Then, 100, 90, 80, 70, 60, 40, and 30% stock solution was used with 20 mg of humin added in a 10-mL glass vial in order to perform the PHE adsorption experiments. The headspace was kept minimal to reduce the solute vapor loss and minimize the effect of surface adsorption. In addition, separately manufactured vial caps were used. The experiment was conducted using a rotator (at 30 rpm). Based on the preliminary tests for apparent equilibrium, the reaction time was set to 5 d. After the reaction, a centrifuge (5000 rpm, 15 min) was used to separate the supernatant and precipitate from each sample. The HPLC (YoungLin, UV730D) was used to measure PHE concentration in the supernatant. The mobile phase for HPLC was prepared using acetonitrile and

ultrapure water (80:20 v/v) with a flow rate of 1.8 mL/min. A C18 4.6 × 150 mm reverse-phase column (Supelcosil LC-18DB) was used to perform separation analysis using a UV detector (at 254 nm).

A modified Freundlich adsorption isotherm Eq. (1) was used to analyze the adsorption results. The Freundlich equation is related to multi-layer and heterogeneous adsorption and is, thus, commonly applied to organic matter and hydrophobic pollutant adsorption [39]. The Freundlich adsorption constant ( $K_{FOC}$ ) and isotherm linearity constant ( $n$ ) were derived from the slope and y-intercept, respectively, as per Eq. (1):

$$S_{OC} = K_{FOC} \times (C_e / C_{scl})^n \quad (1)$$

where  $S_{OC}$  is the concentration of the PHE adsorbed on the humin ( $\mu\text{g}/\text{kg C}$ ),  $C_e$  is the freely dissolved PHE concentration ( $\mu\text{g}/\text{L}$ ),  $C_{scl}$  is the supercooled solubility of PHE at 25°C (5970  $\mu\text{g}/\text{L}$ ) in supercooled aqueous solution, and  $K_{FOC}$  ( $\mu\text{g}/\text{kg C}$ ) and  $n$  are the Freundlich adsorption model parameters (adsorption isotherm linearity increases as  $n$  increases). The single-point sorption is as follows:

$$K_{OC} = \frac{S_{OC}}{C_i} = K_{FOC} \times C_i^{n-1} / C_{scl}^n \quad (2)$$

From Freundlich sorption coefficient, the Gibbs energy change ( $\Delta G$ ) can be calculated as follows [40]:

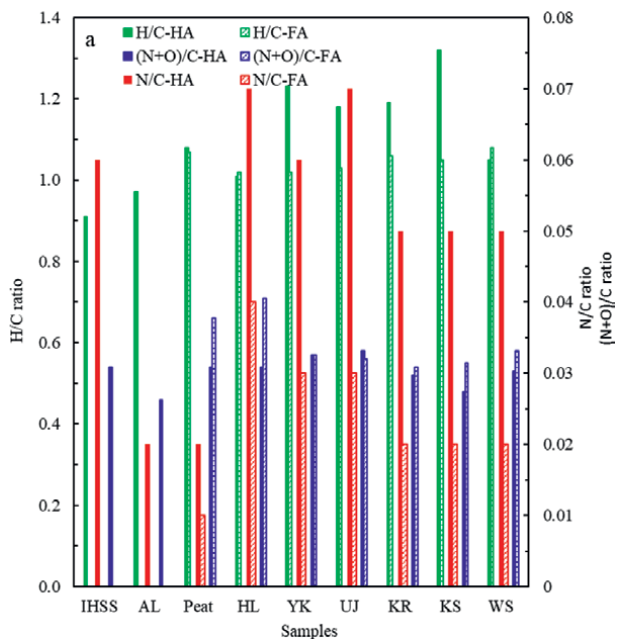
$$\Delta G^\circ = -RT \ln K \quad (3)$$

where  $T$  is the absolute temperature in kelvins,  $R$  is the gas constant (8.314  $\text{J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ ), and  $K$  is the Freundlich adsorption coefficient ( $K_{OC}$ ). The  $K$  value is recalculated as a dimensionless coefficient by multiplying it by 55.5 (number of moles of water per liter of solution) to correct the  $\Delta G^\circ$  values [41]:

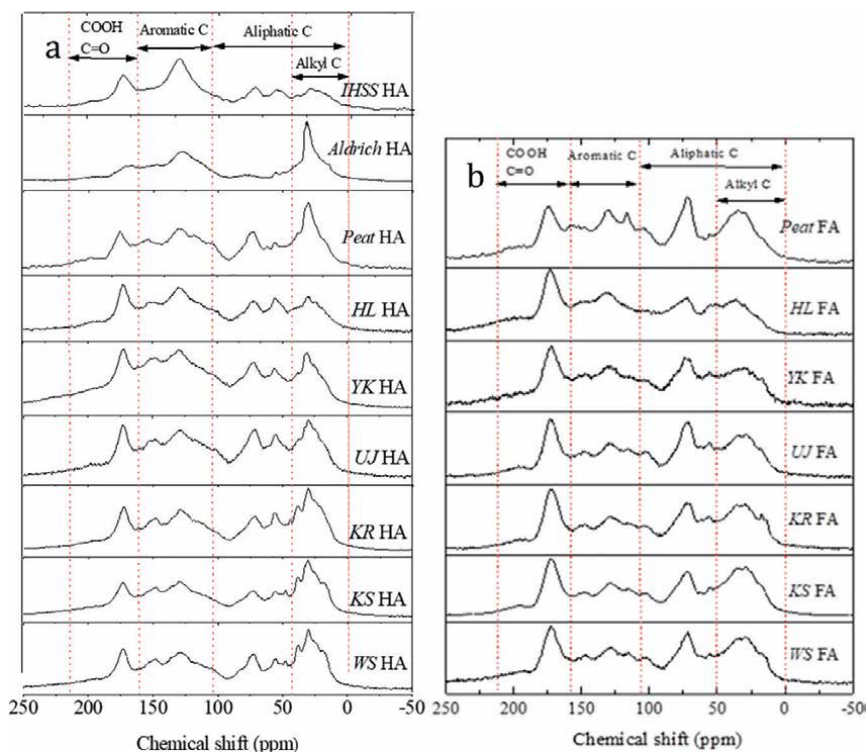
$$\Delta G^\circ = -RT \ln(55.5K) \quad (4)$$

### 3. Characteristics of humic and fulvic acids

The humic acids exhibited higher values of elemental composition of H/C and N/C ratios compared with those of fulvic acids for almost samples (**Figure 2**). Fulvic acids exhibited higher values of (N + O)/C and, thus, presented higher polarity than those values for humic acids (**Figure 2**). Similar to previous research works, aromatic C (110–165 ppm) in humic acids was higher than that in fulvic acids, whereas the aliphatic C (0–110 ppm) exhibited the opposite trend (**Figure 3**). In addition, only O-alkyl C proportions in fulvic acids presented higher values compared with those in humic acids for all soil samples, except for Peat and HL soil. In contrast, O-alkyl C, C,H-aryl C, and O-aryl C exhibited the opposite trend. Moreover, the carboxyl and carbonyl groups (165–210 ppm) in fulvic acids were higher compared with the values in humic acids for all soil samples. Higher aromatic C compounds (C,H-aryl and O-aryl phenol) in humic acids indicated higher amount of lignin and polyphenol from various plants [42]. In addition, the presence of higher O-alkyl and carboxyl groups such as peptides and organic acids indicated higher solubility of humic acids



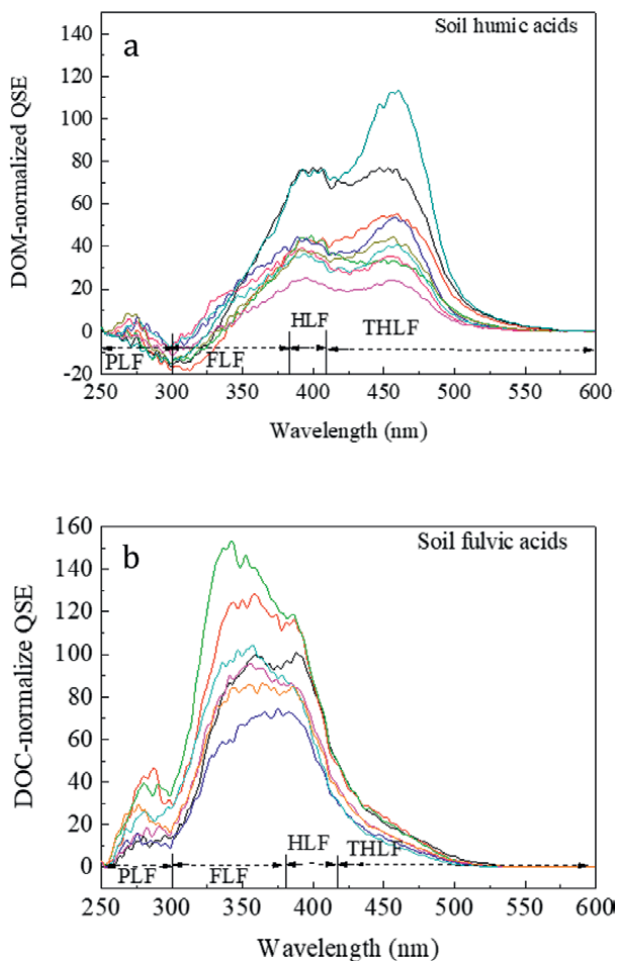
**Figure 2.**  
 Atomic ratios of humic acids (HA) and fulvic acids (FA) extracted from soils.



**Figure 3.**  
<sup>13</sup>C NMR spectra of (a) humic acids (HA); and (b) fulvic acids (FA) extracted from soils.

compared with fulvic acids [43]. Fulvic acids contain high carboxyl groups, and hence, the appearance of both COOH and  $\text{-HC=CH-}$  structure in these acids might affect the result of H/C [44]. Thus, fulvic acids exhibited lower H/C ratios as compared to the values of these ratios in humic acids. The  $\text{MW}_w$  values of humic acids ranged from 2545 to 4411 Da and were higher than the values of fulvic acids (from 1751 to 2584 Da). In comparison with fulvic acid, humic acids presented higher polydispersity index ( $\text{MW}_w/\text{MW}_n$ ), revealing a larger distribution of MW for humic acids. Thus, humic acids extracted from soils revealed higher H/C (affected by COOH and  $\text{-HC=CH-}$  structure), N/C,  $\text{MW}_w$ , and  $\text{MW}_w/\text{MW}_n$  and lower polarity and O-alkyl C compared with fulvic acids. In particular, fulvic acids contained higher aliphatic C owing to their extremely higher values of O-alkyl C.

SUVA,  $E_4/E_6$ ,  $S_{275-295}$ , and  $S_{350-400}$  were employed to identify the difference in spectroscopic spectra between humic and fulvic acids. Humic acids presented higher SUVA values than those of fulvic acids, whereas  $E_4/E_6$  exhibited the opposite trend. The negative relationship between  $E_4/E_6$  and the degree of condensation



**Figure 4.** Fluorescence spectra of (a) humic acids<sup>\*</sup>; and (b) fulvic acids extracted from soils. <sup>\*</sup>Figure 4a was presented in research [20].

of the aromatic carbon network and/or the MW has facilitated the increased usage of the  $E_4/E_6$  ratio in the identification of humification and aromaticity of soil organic matter [35, 45, 46]. The lower values of  $E_4/E_6$  for humic acids are associated with the higher values of SUVA, aromatic carbon (110–165 ppm), and  $MW_w$ . Of all soil samples,  $S_{350-400}$  presented higher values of SUVA, aromatic carbon (110–165 ppm), and  $MW_w$  for fulvic acids than for humic acids, whereas the soil sample  $S_{275-295}$  did not present any trend. Thus, spectra slope at longer wavelengths could be used as an effective index to distinguish the dissolved organic matter between humic acids and fulvic acids.

**Figure 4** shows the synchronous fluorescence spectra of soil humic acids and fulvic acids. As shown in this figure, soil humic acids presented higher peaks at THLF regions, whereas fulvic acids exhibited lower peaks at FLF and HLF regions. With respect to fluorescence relative distribution, compared with fulvic acids, humic acids presented lower %FLF and %HLF, but higher %THLF values. The humification index (HIX) also presented higher values for humic acids than for fulvic acids. Based on these findings, it can be said that soil humic acids were more condensed with polymerized humic-like structure (higher SUVA, aromatic C,  $MW_w$  and %THLF), whereas fulvic acids contained high levels of carbonyl and quinone, aliphatic groups, and oxygen functional groups related to fulvic- and humic-like fluorescence materials (higher  $(N + O)/C$ ,  $E_4/E_6$ ,  $S_{350-400}$ , %FLF, %HLF O-alkyl, and carboxyl groups). These specific different molecular characteristics between humic and fulvic acids are important as they can aid in investigating the structural changes, generation of DBPs under chlorination, and PHE sorption behavior.

## **4. DBPs formation and the structural changes of humic and fulvic acids extracted from soils**

### **4.1 DBPs formation of humic and fulvic acids extracted from soils**

For the comparison of DBPs formation between humic and fulvic acids extracted from soils, the concentrations of THM, HAA, and their species ( $\mu\text{g/L}$ ) were normalized to DOC ( $\text{mg/L}$ ) and named as specific THM/HAA formation potential (STHMFP/SHAAFP) and CF, DCBM, DBCM, DCAA, TCAA, and DBAA. It was found that the formation of STHMFP and SHAAFP significantly differed between humic acids and fulvic acids. Humic acids exhibited higher STHMFP values that ranged from 141.9 to 194.6  $\mu\text{g/mg}$  compared with the lower values in fulvic acids (98.7 to 100.6  $\mu\text{g/mg}$ ). In both humic acids and fulvic acids, chloroform was the most dominant compound of STHMFP, accounting for over 95% of STHMFP. Similar to the trend of the values of STHMFP, SHAAFP values for humic acids were found to be 5.5 times higher (ranging from 259.0 to 390.0  $\mu\text{g/mg}$ ) compared with the SHAAFP values for fulvic acids (ranging from 19.8 to 54.9  $\mu\text{g/mg}$ ). TCAA was the most abundant (83%) SHAAFP species in humic acids, whereas it accounted for only 17% in fulvic acids. In humic acids, SHAAFP (presenting as TCAA) presented higher values than STHMFP (presenting as CF). In contrast, STHMFP presented much higher values than SHAAFP for fulvic acids. During chlorination, humic molecules having higher aromatic content, such as humic acids, first reacted with chlorine to form TCAA and then form CF. This is the reason why TCAA production was higher than CF production [47]. In addition, fulvic acids contained alkyl, carbohydrate, and carboxyl groups that were less capable of producing CF and TCAA under chlorination than the

aromatic carbon components [48]. Thus, the difference in DPB formation between humic and fulvic acids might be explained by the differences in their molecular structure.

#### **4.2 Changes in spectroscopic characteristics and TOC and their relationship after chlorination**

After chlorination, both humic and fulvic acids exhibited lower SUVA and TOC values. TOC removal presented higher values for fulvic acids. This demonstrated the presence of more organic carbon components in fulvic acids that can be easily mineralized to CO<sub>2</sub> during chlorination as compared to humic acids. During chlorination, 50–80% of chlorine oxidated humic substances into CO<sub>2</sub>, whereas only 5–10% of chlorine participated in the incorporation reaction to form DBPs [49]. SUVA presented higher reduction values for humic acids than for fulvic acids. This indicated that high aromatic C (presented by high SUVA values) were transformed into lower ones (lower SUVA) by splitting the aromatic rings and decomposing the unsaturated carbon rather than converting it into CO<sub>2</sub>. After chlorination, humic and fulvic acids showed different changes in terms of fluorescence relative distribution. Moreover, after chlorination, only %THLF values decreased for humic acids, whereas %FLF and %THLF reduced for fulvic acids. The values of  $\Delta\%FLF$ ,  $\Delta\%HLF$ , and  $\Delta\%THLF$  were calculated based on the differences in each relative distribution before and after chlorination ( $FL_{\text{before}} - FL_{\text{after}}$ ). These values were used to further examine the mechanisms of chlorination for humic and fulvic acids.

The relationship between the changes in humic/fulvic acid characteristics (TOC removal, SUVA removal,  $\Delta\%FLF$ ,  $\Delta\%HLF$ , and  $\Delta\%THLF$ ) due to chlorination and their original structural characteristics (SUVA, MW, C,H-aryl, and O-alkyl) can clarify the chlorination-induced structural changes in humic and fulvic acid in more depth. SUVA values were found to be strongly positively correlated with SUVA removal for both humic and fulvic acids ( $p < 0.05$ ). Moreover, SUVA values were strongly negatively correlated with TOC removal values for humic acids ( $p < 0.01$ ) and non-significantly correlated with TOC removal values for fulvic acids ( $p > 0.1$ ). The higher SUVA values and aromatic carbon contents of humic acids than those of fulvic acids resulted in the reaction of more aromatic carbon compounds in humic acids with chlorine. This led to the production of higher DBP. In addition, for humic acids, higher MW<sub>w</sub> materials with richer N groups and a higher proportion of O-alkyl C reacted with chlorine to yield higher reductions of TOC and FLF components. In comparison, lower MW<sub>w</sub> materials with a higher percentage of C,H-aryl and O-aryl phenol and fewer N groups were associated with a higher reduction in SUVA and THLF values. In other words, high-MW<sub>w</sub> humic acids with aliphatic properties, high nitrogen content, and a low degree of unsaturation mostly reacted with chlorine via an oxidation reaction. In comparison, low-MW<sub>w</sub> humic acids with high aromatic C and low nitrogen content mainly reacted with chlorine via incorporation.

#### **4.3 Specific relationship between DPBs and humic and fulvic acid characteristics**

The correlations between STHMFP and humic/fulvic acid characteristics determined the effects of the molecular structures of humic/fulvic acids on DBP production. For humic acids, STHMFP was positively correlated with C,H-aryl, O-aryl phenol, and alkyl

C ( $p < 0.05$ ) but negatively correlated with N/C ratio,  $MW_w$ , TOC removal,  $\Delta\%FLF$ , O-alkyl C, and carboxyl ( $p < 0.05$ ). In summary, low-MW aromatic C such as C,H-aryl, O-aryl phenol, and aliphatic C such as alkyl C in humic acids were considered as DBP precursors as they generated STHMFP during the chlorination reaction. On the contrary, high N groups of O-alkyl C in humic acids participated in oxidation reactions during chlorination. For fulvic acids, STHMFP presented a strong positive correlation with alkyl C and negative correlations with C,H-aryl and O-aryl phenol ( $p < 0.05$ ). The chlorination mechanism of fulvic acids shown in our study differed from those reported in previous studies that demonstrated that aromatic C, especially phenol components, were associated with the highest value of STHMFP [50, 51]. The reasons behind these notable findings can be explained as follows. First, unlike those in the humic acids, the aromatic contents in fulvic acids might not play a major role in the generation of STHMFP via incorporation. Second, the presence of N groups combined with aromatic molecules in the fulvic acids resulted in the formation of N-DBPs, and not in the generation of STHMFP and SHAAFP [52]. In previous studies, amino acids such as aspartic acids and asparagine acids in humic substances were also reported to yield N-DBPs (i.e., dichloroacetonitrile and halonitromethanes) during chlorination [16, 53]. In the present study, there was no relationship between the SHAAFP and HAA species and the humic/fulvic acid characteristics. In summary, the low-MW and low-N/C components of aromatic C and alkyl C in the humic acids might form STHMFP via incorporation reactions. In comparison, only low-N/C aliphatic compounds such as alkyl generated STHMFP in the fulvic acids. This detail will be helpful in elucidating the formation of aromatic/aliphatic N-DBPs for humic and fulvic acids in future.  $MW_w$  and  $\Delta\%THLF$  were the appropriate factors for predicting the values of STHMFP in humic acids. In comparison,  $\Delta\%FLF$  and Alkyl C were applied for fulvic acids.

In particular, this study presented a new interpretation of differences between humic/fulvic acids in terms of molecular structure characteristics and chlorine reaction, including oxidation and incorporation reaction, and successfully provided sufficient factors to predict THM generation.

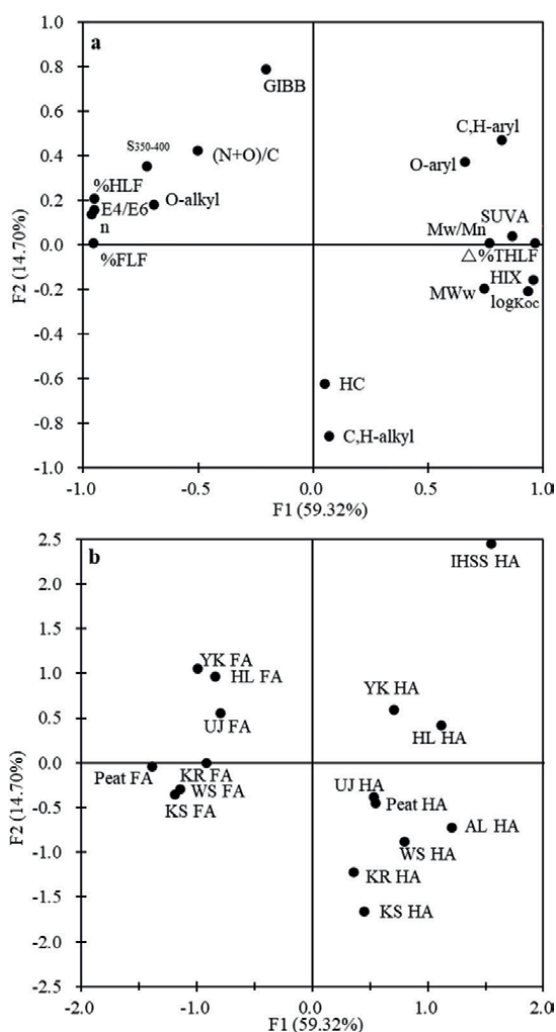
## **5. Phenanthrene adsorption isotherm of humic and fulvic acids**

### **5.1 Comparison of phenanthrene adsorption isotherm for humic and fulvic acids**

All the experimental data of humic and fulvic acids fit well with the Freundlich model, presented by the high degree of correlations ( $R^2 > 0.99$ ). Also, for both humic and fulvic acids, all the sorption isotherms were non-linear ( $n < 1$ ). For humic acids, PHE adsorption coefficient ( $K_{OC}$ ) ranged from  $3.7 \times 10^4$  to  $7.0 \times 10^4$  mL/g, while for fulvic acids, it ranged from  $1.4 \times 10^4$  to  $2.2 \times 10^4$  mL/g. The higher sorption affinity of humic acids might be explained by the higher aromatic and condense humic structure. High isotherm linearity ( $n$ ) values were associated with a low degree of natural organic matter maturation and less heterogeneous sorption-site energy distribution [54]. The  $n$  values of humic acids were lower than those of fulvic acids. In humic acids, the hydrophobic nature enhanced more coiled or aggregated structures to provide specific non-ideal binding sites [55]. In addition, high polarity might reduce the sorption affinity [56]. The Gibbs free energy values were negative for both humic and fulvic acids at 20°C, revealing the thermodynamically favorable and spontaneous adsorption process [57].

## 5.2 Correlations between humic and fulvic acid structural characteristics and phenanthrene adsorption isotherm

For humic acids, sorption coefficient ( $\log K_{OC}$ ) was positively related to HIX and negatively correlated with  $S_{350-400}$ . In comparison,  $\log K_{OC}$  of fulvic acids presented positive correlation with %THLF and negative relationship with %FLF and  $E_4/E_6$ . No relationship was observed between  $\log K_{OC}$  and humic/fulvic acid relative carbon distribution. High sorption affinity was found to be positively related to the condense structure with high aromatic C and humification, presented by the high values of HIX and %THLF and low values of %FLF and  $E_4/E_6$ . For humic acids,  $n$  presented positive correlation with H/C, %FLF, and %HLF and negative relationship with SUVA and %THLF. However, no relationship was observed between  $n$  values and their structural characteristics for fulvic acids. The UV spectroscopic and fluorescence characteristics



**Figure 5.** (a) Factor loading plot for selected structural characteristics and PHE sorption of humic and fulvic acids as the first two principal components; and (b) factor score plot for humic and fulvic acids as the first two principal components.



of humic and fulvic acids were primarily related to their sorption ability. In a previous study, PAH sorption ability was reported to be closely related to 3D fluorescence [58].

The principal component analysis was applied for 18 selected parameters in order to interpret the specific PHE binding behavior of humic and fulvic acids. The first two principal components (PCs) explained approximately 59.32% for PC1 and 14.70% for PC2 (**Figure 5**). PC1 was interpreted as a factor associated with the PHE sorption behavior, whereas PC2 presented for C,H-alkyl, H/C, and free energy. Thus,  $\log_{K_{oc}}$  was found to be positively related to  $MW_w$ , SUVA, HIX, %THLF, and  $M_w/M_n$ . Moreover,  $n$  values were positively correlated with %FLF, %HLF,  $E_4/E_6$ , and O-alkyl (**Figure 5a**). From the factor score plot presented in **Figure 5b**, the PHE sorption behavior of humic and fulvic acids was found to be completely different. Humic acids presented higher  $MW_w$ ,  $M_w/M_n$ , SUVA, and %THLF, which were related to sorption affinity ( $\log_{K_{oc}}$ ). In comparison, fulvic acids were related to higher  $n$  and fulvic-/humic-like components, lower degree of condensation of the aromatic carbon, and the MW (higher  $E_4/E_6$  and O-alkyl). In summary, UV and fluorescence characteristics are powerful techniques to determine the PHE sorption behavior of humic and fulvic acids extracted from soils.

## 6. Conclusion

In this chapter, the structure characteristics of humic and fulvic acids extracted from soils were clarified. Soil humic acids were found to be more condensed and have a polymerized humic-like structure (higher SUVA, aromatic C,  $MW_w$ , and %THLF). In comparison, fulvic acids contained high levels of carbonyl and quinone, more aliphatic groups, and more oxygen functional groups related to fulvic- and humic-like fluorescence materials (higher  $(N + O)/C$ ,  $E_4/E_6$ ,  $S_{350-400}$ , %FLF, %HLF O-alkyl, and carboxyl groups). The differences in the molecular characteristics between humic and fulvic acids resulted in the differences in the generation of DBPs under chlorination and the PHE sorption behavior. For chlorination, the low-MW and low-N/C components of aromatic C and alkyl C in the humic acids might form STHMFP via incorporation reactions. However, only low-N/C aliphatic compounds such as alkyl generated STHMFP in the fulvic acids. Humic acids presented higher sorption coefficient ( $\log_{K_{oc}}$ ) because of the presence of higher  $MW_w$ ,  $M_w/M_n$ , SUVA, and %THLF. In comparison, fulvic acids are related to higher  $n$  and fulvic-/humic-like components and lower degree of condensation of the aromatic carbon and the MW. UV and fluorescence characteristics are powerful techniques to indicate the PHE sorption behavior of humic and fulvic acids extracted from soils. Compared with atomic ratio and relative C distribution, the UV and fluorescence characteristics approach provides the key information for water system managers to better predict and mitigate the formation of DBPs in chlorine-treated water and the behavior of hydrophobic organic contaminants in aquatic environment.

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## **Conflict of interest**

The authors declare no conflict of interest.

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## Chapter 2

# Mechanisms of Action of Humic Substances as Growth Promoters in Animals

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and Guillermo Téllez-Isaias*

### Abstract

A review of the latest research on the addition of humic substances (HSs) in the drinking water or feed of farm animals including poultry, pigs, dairy cows and calves, goats, and rabbits was carried out. The results reinforces the promoted protective action of HS on the digestive mucosa, their antioxidant properties, immunomodulatory, and anti-inflammatory attributes, the suggested microbial shift to a probiotic-type bacteria in the gut, including antiparasitic and antifungal effects, as well as their influence on the higher efficient of minerals, proteins, and lipids utilization within the body. The outstanding improvements in health, productivity, and meat and milk quality obtained from animals supplemented with HS are common features in the majority of available research. The main benefits in the gastrointestinal tract stem from the formation of protective barriers on the epithelial mucosa due to its colloidal properties and stimulation of mucin production. HS also promotes the development of probiotic microbiota and positive changes in bacterial fermentation patterns, which results in improved intestinal health and integrity. As a result, a cascade of benefits is derived within the body, reinforcing antioxidant protection responses, immunomodulation, and anti-inflammation mechanisms, as well as improving nutrient utilization efficiency. However, the truly molecular mechanisms of action of HS in the intestine and throughout the body remain unknown.

**Keywords:** humic substances, farm animals, mucosal protection layers, microbiota, antioxidant and immune responses, nutrient utilization, performance

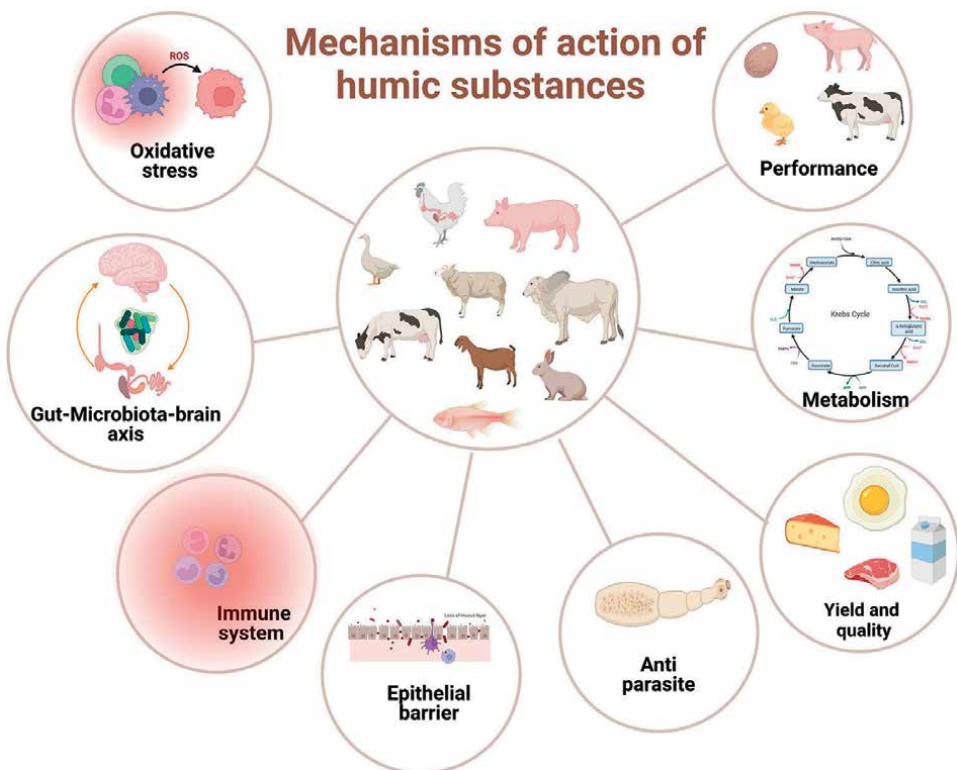
### 1. Introduction

Humic substances (HSs) are organic compounds derived during the humification process of decaying plants and animals and are mainly composed of humic acid (HA) and fulvic acid (FA). HSs have been utilized in humans for ages as nutritional supplements and therapeutic purposes. The first mentions of the therapeutic applications of HS may be found in Sanskrit and ancient Chinese and Roman texts, where they were assigned magical properties [1, 2]. Some of the therapeutic characteristics of HS are mentioned in the Chinese Materia Medica Pharmacology Compendium, which

dates back to the Ming Dynasty in the fifteenth century; their medicinal usage was permitted by the China Drug Administration, and they were known as “the gold of medicine” in China [3]. Another important medical application of HS is its usage in balneotherapy in ancient Babylon, in Lower Mesopotamia and in the Roman Empire [4]. Information about the use of HSs in humans has recently been published, including medical issues, preclinical trial outcomes, and successful clinical therapies [5–7].

HSs are molecules with anti-inflammatory, antioxidant, antiparasitic, antibacterial, and antiviral properties, according to the European Agency for the Evaluation of Medicinal Products’ Committee for the Evaluation of Veterinary Products [5, 8], and the feasibility of using them orally in horses, dogs, pigs, and birds in doses of 500 to 2000 mg/kg of live weight for the treatment of diarrhea, dyspepsia, and acute poisoning was documented; it was also indicated that HSs exert a protective action on the mucosa of the intestine and have antiphlogistic, adsorbent, antitoxic, and antimicrobial properties.

The various structures and functional groups give HS properties such as colloidal, spectral, electrochemical, and ion exchange, which confers significant adsorption capacity [5, 9]. HS has been shown in animals to be able to modulate the harmful effects of a variety of xenobiotics and unwanted compounds that enter the digestive tract through feed and drinking water due to their colloidal properties and propensity to form chelates [10, 11]. HS has also been shown to minimize the accumulation of some heavy metals in tissues, including mercury, cadmium, and zinc in fish [12], lead and cadmium in rats [13], and cadmium, zinc, and lead in chickens [9–11]. The main research findings and some proposed theories about the mechanism of action of



**Figure 1.**  
Main benefits observed in animals provided humic substances.

HS in animals are discussed in the following sections. **Figure 1** summarizes the main benefits observed in animals provided HS.

## 2. Formation of protective layers in the digestive mucosa

Due to their colloidal characteristics and high capacity to form aggregates within solutions, it has been proposed that HSs have the ability to create protective layers on the epithelial mucous membrane of the digestive tract, preventing the penetration of pathogenic bacteria or toxic substances produced by bacteria [8, 14, 15]. The ability of HS to form polymers in a media with a slightly alkaline pH, such as the intestine, is credited with the creation of protective barriers [16]. HS also interacts with biomolecules like collagen, promoting the resistance and maturity of its fibers, resulting in an increase in the intestinal villi's integrity [17].

HSs were found to increase intestinal viscosity, inhibit bacterial translocation from the intestine to the liver, and lower the serum levels of an intestinal permeability marker, fluorescein isothiocyanate dextran (FITCd), in a recent study [15]. HS has also been demonstrated to positively regulate mucine-2 (MUC-2) gene expression in the cecum mucosa [18]. MUC-2 is a main gel-forming mucin that serves as the principal barrier component of mucus layers as well as a storage location for secretory immunoglobulin A (IgA). The number of goblet cells in the villi of the jejunum of broiler chickens fed with HS was similarly found to be increased 24 hours following a diet change challenge [19]. HS may increase the protective mucus layer in the small intestine, allowing the villi to maintain their integrity against pathogenic agents, toxins, and dietary changes, according to this research. In addition, the development of gels causes increased viscosity in the small intestine, which results in slower rate of feed passage through the gut [20, 21], which increases the time of exposure of nutrients to digestive enzymes [22, 23].

The protective effect of HS on the digestive epithelium has been associated with increases in villus height, crypt depth, and epithelial surface area in mice [17], chickens [24–26], Japanese quails [27], and rabbits [28], and increased activity of digestive enzymes in the digestive mucosa of chickens [26, 29] and freshwater fish [30], and also to increased energy and protein digestibility in chickens [31, 32], organic matter, protein, and fiber in rabbits [28, 33] and protein in milking cows [34]. In addition, an increased weight of the gizzard and length of the duodenum and jejunum has been reported in chickens fed canola-based diets and added with HS [35], as well as the size and weight of the cecum of rabbits [28].

It has been proven that peat-based treatments can be used to relieve gastric, intestinal, and liver diseases [36]. Peat moss has been proven to minimize the size of ethanol-induced stomach ulcers in rats, speeding up the healing process of both gastric and duodenal ulcers [37]. Orally administered HS to rats has been shown to bind, presumably metabolize, and resorb toxins in the gastrointestinal system [38]. These results supported the orally administration of HS to animals for the treatment of diarrhea, dyspepsia, and acute poisoning [8, 39]. In recent research, pigs [40–42] and calves [43–45] fed with HS showed less severe diarrhea.

In addition to the benefits of HS observed on the mucosal surface of the digestive tract, healing and protective effects have also been reported on other types of epithelia. The restorative effect of HS on the epidermis has been demonstrated with balneotherapy, which is used for the treatment of various skin diseases such as chronic eczema, neurodermatitis, and psoriasis in various parts of the world [2], in which the activation of skin metabolism and regenerative processes has been observed [5].

On the other hand, the plethoric benefits that have been widely documented in the immune response, antioxidant status, digestive microbiology, and nutrient metabolism in humans and animals added with HS suggest that the mechanisms of action go far beyond its mucosal protective effect. It has previously been suggested that the absorption of orally administered HS is very low, at 0.05 to 0.07% [8]. But in subsequent evaluations, it has been reported that HS can be absorbed and transported to other tissues of the body. In young pigs, HS particles were observed in all segments of the small intestine and in lymph nodes associated with the intestine, and in urinary bladder and trachea [46]. In adult pigs, it was not possible to confirm these findings [47]. However, in older studies using  $^{125}\text{I}$ -HA, distribution of HA was observed in several tissues, notably the skin, blood serum, liver, muscle, and digestive tract of rats [48].

In several studies in which hepatotoxicity was induced using lipopolysaccharide (LPS), carbon tetrachloride, and ethanol in rats given HS orally by gavage, the protection against liver injury was confirmed, due to reduced serum levels of aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) [49–51]. The medical benefits of HS offered orally against different types of musculoskeletal and gynecological diseases as well as for the treatment of heart and liver conditions have been reviewed in other documents [2, 5]. In the following sections, the main results of the use of HS on the antioxidant status, immune response and inflammation, digestive microbiota, use of minerals, proteins, and energy, and the growth performance parameters in animals are reviewed. For information about the benefits of HS in aquaculture, it is recommended to review other scientific publications in this area [52–54].

### 3. Antioxidant status

The redox properties of HS have been investigated in *in vitro* and *in vivo* studies, with quinones being classified as reducible fractions and phenols being classified as electron-donating fractions with antioxidant properties in comparison with electron-accepting quinones, respectively [55, 56]. The neuroprotective, cardioprotective, and renoprotective properties of HS in rats have been already described [2]. In all cases, the total oxidative status and oxidative stress index levels were significantly decreased, and total antioxidant status was increased in the HS experimental groups. The hepatoprotective properties of HS have also been long recognized. In studies with rats in which hepatotoxicity was created using different challenge models, such as administration of LPS, carbon tetrachloride and ethanol, the oral feeding of HS elicited increased glutathione (GSH) and superoxide dismutase (SOD) activities and decreased a marker of lipid peroxidation, malondialdehyde activity (MDA) [49–51].

In broiler chickens supplemented with HS, a potent antioxidant activity such as increased glutathione reductase (GSH-Rx), total antioxidant capacity (T-AOC), and catalase activity in the blood has been reported [57]. In broilers supplemented with increasing dietary FA, increased SOD and glutathione peroxidase (GSH-Px) activity and decreased MDA levels in the blood were also found in a recent study [29]. On the contrary, in the liver of HS-added broilers, reductions of the SOD were found, whereas in the mitochondria of the muscle lower MAD, SOD and higher catalase were found [58]. It was suggested that the difference of SOD activity in serum versus the liver and mitochondria from muscles may be due to their ability to recombine with intermediate free radicals that may lead to partly inactivation of the enzyme [59]. In broilers supplemented with HS and subjected to transportation stress, increased

SOD and GSH-Rx activities in the mitochondria of the liver were observed [60]. In breast meat and thighs of HS-added chicken, decreased lipid oxidation was reported, especially after the fourth day of storage after slaughter [61]. Similarly, an increase of MDA, lightness and yellowness were observed in breast meat after 7 days of storage [62]. In red blood cells of HS-added hens, reduced levels of MDA, GSH-Px, glutathione-S-transferase,  $\gamma$ -glutamyltransferase and oxidized glutathione and increased levels of GSH-Rx, reduced glutathione and the ratio of reduced glutathione/oxidized glutathione were found [58].

In HS-added weaned pigs, reduced T-AOC and MDA were observed [63]. In piglets born from HS-added sows 2 weeks before and 1 week after farrowing, reduced TBA-active products concentration, lipid hydroperoxides, and protein carbonyl groups and increased SOD and catalase activity were found; after re-feeding HS in the older piglets, reinforced positive impact on the antioxidant defense system and free-radical processes were reported [64]. In HS-added weaning pigs, a decrease in oxidative stress was also reported after a challenge with an *Escherichia (E.) coli* LPS [65]. In HS-added calves, higher serum GSH-Px and T-AOC activities and lower diamine oxidase (DAO) and MDA concentrations were observed [44]. Later on, higher total SOD and T-AOC activities and lower MDA concentrations were confirmed in HS-added calves [45].

It has been suggested that decreased activity of SOD was connected with the antioxidant properties of HS that decreased the concentration of oxidized products, including superoxide anion [58]. Another possible way to activate the enzymatic activity of the antioxidant system is to increase the concentration of metals in the liver, which are part of the active centers of metal proteins: Zn—catalase, Mn (Cu/Zn)—superoxide dismutase [59]. The lower fat content in the meat of HS-added broilers and pigs may elicit a higher proportion of antioxidant components and could have an effect on the higher oxidative stability [62].

#### **4. Immune response and inflammation**

HSs have been shown to exhibit different immunostimulatory and immunomodulatory effects in patients with different infectious diseases, which have been associated with their anti-inflammatory properties [2, 5, 6]. It seems that HSs form solid complexes with carbohydrates, which allow the formation of glycoproteins with the ability to bind to natural killer cells and T lymphocytes and allow subsequent communication between these cells [66].

HS has been shown to stimulate the immune response in broilers [8, 67, 68]. For example, elevated lymphoid tissue distribution and density in the bursa of Fabricius and thymus [25] increased concentration of antibodies against infectious bursal disease [69], avian influenza [70], and Newcastle disease virus (NDV) [71, 72], and greater lymphocytes and leukocytes counts, globulins ( $\alpha$ ,  $\beta$ , and  $\gamma$ ), phagocytosis, and phagocytic index have been found in HS-added broilers [73]. In laying hens, supplementation HS significantly increased the serum IgG and IgM level [74].

In weaned and growing pigs supplemented with HS, increased percentage of lymphocytes and activity of neutrophils has been reported [41, 75]. In HS-added weaned pigs, increased level of IgM and IgG and reduced concentration of inflammatory factors such as tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-6 (IL-6) and IL-1, myeloperoxidase, and DAO were observed [63]. In HS-supplemented calves, higher IgA and IgG concentrations, lower TNF- $\alpha$  [43, 44] and higher serum IgA, IgG, and IL-4 concentrations, and lower IL-6 and TNF- $\alpha$  were observed [45].

The enhanced immune response in HS-added animals may be the cause of the reduced severity of diarrhea reported in pigs [40–42] and calves [43–45] and to the strong tendency of lower mortality reported in broilers [25, 67, 76, 77], Japanese quails [27], and pigs [41, 78].

## 5. Digestive microbiota

Reports from the microbial, geo, soil, and environmental sciences indicate that HS stimulates the growth and diversity of soil and environmental fungi and bacterial communities [79, 80]. A review of the main ways of action of HS on biota was previously reported [81]. The most outstanding mechanisms of HS on the activity of microorganisms are a) as a source of substrates, providing carbon, nitrogen, phosphorus, trace elements, and vitamins [82], and b) as natural surfactants, increasing the permeability of cell membranes in bacteria, due to their amphiphilic character, which enhances the absorption of nitrogen and other micronutrients [83]. Examples of some aerobic HS-degrading bacteria are *Pseudomonas* spp., *Streptomyces* spp., *Bacillus* (*B.*) *brevis*, *B. cereus* and *Alcaligenes faecalis*, among others [81, 84]. In one report, the anaerobic decomposition of HS by *Clostridium* (*C.*) spp. was also reported [79]. The recovered HS from the bacterial cultures show some modifications such as losses of aliphatic structural units and gains of aromatic structures under aerobic conditions [85], while under anaerobic conditions removal of carboxyl groups and polysaccharide-related substances and gains of aliphatic components, amide and aromatic groups have been observed [79]. Furthermore, it has been suggested that differences of the microbial degradability of HS are associated with differences of their chemical composition and the bacterial species.

All these factors are probably associated with the contrasting microbiological results reported under *in vitro* experiments in which different types of bacteria are cultured with HS or in *in vivo* experiments in which the feed or water of broiler chickens are added with HS. In an *in vitro* study, in which natural and synthetic HS were tested, the spectrum and degree of antimicrobial activity against many human pathogenic bacteria varied according to the origin, extraction mode of the HS, and the tested bacterial strain [86]. In another *in vitro* report, natural HS showed insignificant inhibition of the growth of bacteria such as *E. coli* and *Salmonella* (*S.*) *Enteritidis*, but the modified HS caused reductions from 78 to 80% and 58 to 70% of the number of colonies of *E. coli* and *S. Enteritidis*, respectively [87].

In HS-added broiler, no significant effects of HS have been reported on the total Gram-negative bacteria [15] or the anaerobic bacterial populations in the ceca [88, 89], while reductions of enterobacteria in the small intestine and cecum were found in one study [18]. Lower *E. coli* counts in the digesta content from the small intestine and ceca [71] and higher *E. coli* counts (between 10 and 100 times) in the ceca content [88] have been also reported. Furthermore, the addition of HS in broilers did not reduce the counts of *Clostridium perfringens*, *S. Enteritidis*, and *E. coli* in the gut [15, 90–92]. Opposite to this, in Japanese HS-added quails, reductions in *E. coli*, coliforms, and *C. perfringens* of the intestinal content were found [27].

Some differences observed on the antimicrobial effects of HS in *in vitro* studies may be due to the use of different sources and dosages of the tested products, the length of the incubation period, and hence, the duration of the bacterial exposure to the products, the temperature and pH conditions, and the nutritional composition of the culture broth. In contrast to this, the total passage time of feed through the

gastrointestinal tract in chickens lasts between 3 and 4 h [93], and only during this short period of time, the bacteria is exposed to HS; the core temperature of chickens is about 40°C and the pH varies in the range 4.5–5.9, 2.0–3.0, and 5.5–7.0 in the crop, proventriculus, and intestine, respectively [94]. The microbiota that resides in the digestive tract feeds on the nutrients released from the feed as digestion proceeds and on the endogenous secretions associated with the different compartments. In *in vitro* experiments, a cytotoxic effect of HS against many mammalian and bacterial cells has been demonstrated due to the accumulation of free radicals during long-term culture times, inducing lower oxygen uptake, lower electron transfer to acceptors, and lipid peroxidation in cell membranes [95]. It is probably that in some long-term *in vitro* experiments, the antimicrobial activity of HS was due to the accumulation of toxic metabolites in the culture. This effect may be precluded in the gastrointestinal tract.

In a recent study, the addition of HA extracted from worm compost in an *in vitro* chicken digestive system caused, in the simulated crop, increasing counts of *E. coli*, *C. perfringens*, *Lactobacillus (L.) salivarius*, and *B. subtilis* as the concentration of HA increased. In the simulated proventriculus, the counts of *S. Enteritidis*, *E. coli*, and *B. subtilis* were enhanced at the higher level of HA. In the simulated intestine, HA increased the counts of *S. Enteritidis*, *E. coli*, *C. perfringens*, and *B. subtilis*. The results suggest that HA can be used by bacteria as substrates, since they are organic sources of carbon, nitrogen, phosphorus, and other nutrients. HA can also improve nutrient assimilation, as probably occurred during the experiments as bacterial counts were increased. It is probably that in the simulated digestive system the rapid transit time through the digestive compartments, the addition of the buffering solutions and the presence of several dietary components in the intestine overcome the possible accumulation of toxic metabolites [96]. In two recent *in vivo* studies, increased lactic acid bacteria counts were reported in the gut of HS-added broilers [77, 91].

In weaning pigs supplemented with HS, lower relative abundance of *Firmicutes*, *Bacteroides*, *Anaerovibrio*, *Oscillospira*, and *Ruminococcus* and a trend toward higher abundance of *Prevotella* in feces were reported compared to control pigs [41]. Also in HS-added weaned pigs, reduced short-chain fatty acids (SCFAs), gas formation, and microbial alpha diversity and no changes in the microbiome of the cecal and colon content were found [97].

In weaned Holstein HS-added calves, increased ratio of *Firmicutes* to *Bacteroidetes* and relative abundance of *Firmicutes* and decreased relative abundance of *Bacteroidetes* were reported [43]. In addition, a correlation analysis indicated that *Bifidobacterium*, *Lactobacillus*, and *Olsenella* were positively correlated with IgG, weight gain, and GSH-Px and negatively correlated with MDA, DAO, and fecal score, respectively, which might explain that HS inclusion could improve growth performance, anti-inflammatory, and antioxidative status and alleviate diarrhea of weaned calves via increasing the abundance of intestinal beneficial microbiota, and that upregulated fatty acid metabolites were positively correlated with increased beneficial intestinal microbiota [43]. In two additional research, HS supplementation increased the abundances of *Bifidobacterium* and *Lactobacillus* but decreased the abundance of *E. coli* in feces of calves [44, 45]. In HS-added goats, for 14 days prepartum and 56 days postpartum, increased ruminal acetate and propionate concentrations but reduced protozoa counts were observed [34, 98]. In growing HS-added rabbits, the total bacteria and *E. coli* counts in cecum were decreased [28]. Also in HS-added rabbits, increased concentrations of propionic and butyric acids were found [33].

It was previously suggested that within the body, HSs stimulate the good microbes while suppressing the bad microbes, and that HSs stabilize the intestinal flora thus ensuring an improved utilization of nutrients in animal [67]. Recent results in broiler chickens, pigs, calves, goat and rabbits seem to confirm this suggestion. Several of the aforementioned authors agree in that HSs cause a shift in the digestive microbiota by stimulating the growth of probiotic-type bacteria and modifying the microbial fermentation in nonruminant and ruminant animals, leading to the formation of a greater amount of SCFA, which reduces the pH of the medium and probably inhibits the growth of potentially pathogenic bacteria. In addition, some of the SCFAs found have a trophic effect on the digestive mucosa. The increased beneficial flora, reduced potentially pathogenic bacteria, and stimulated growth of intestinal villi have been associated with better intestinal health and improvements in the processes of digestion, including increased activity of different digestive enzymes. This provides excellent nutritional feed conversion efficiency which aids in body weight gain without increasing the amount of feed consumed.

### 5.1 Antiparasitic effects

In the area of aquaculture, experiments with goldfish (*Carassius auratus*) and common carp (*Cyprinus carpio*) under pond culture conditions, a HS bath caused reduction of infections of skin and gills by protozoan parasites and dropping of infections of the gills caused by *Ichthyophthirius* spp. [52]. In other studies, similar findings in HS exposed fish, ornamental fishes, and Nile tilapia were reported [53, 99]. In experimentally challenged mice with *Trypanosoma (T.) brucei brucei* and *T. brucei gambiense*, the administration of HS for 21 days in the drinking water induced adequate protection and significantly reduced the mortality rate, while all non-treated control mice died within 10 days after the challenge [100]. In HS-added goats and dairy cows, reduced ruminal protozoa counts were observed [34, 98]. Using liquid or solid extracts of HS from worm compost, the count of coccidia eggs in the excreta of broilers was reduced, but the results were generally inconsistent [91, 101]. More information is required on this topic.

### 5.2 Antifungal effects

It has been reported that HSs have a fungicidal effect on *Candida (C.) albicans* under *in vitro* conditions [86] and over that inhabiting the gastrointestinal tract of animals and humans [102]. HS was recently found to decrease the toxic effects of aflatoxicosis in broilers [103, 104]. Results of *in vitro* binding studies showed that HS has a high mycotoxin adsorption capacity; in HS-added broilers and fed aflatoxin-contaminated feeds, a protective effects against liver damage, stomach, and heart enlargement as well as reduction of some of the hematological and serum biochemical changes associated with aflatoxin toxicity were shown [103]. In HS-added broilers, provided with aflatoxin-contaminated feeds, improved feed efficiency and reduced liver and bursa damage as well as reduction of serum AST,  $\alpha$ -glutamyl transferase, and lactate dehydrogenase were observed [104, 105]. Reduced aflatoxin B1 residues in liver and increased NDV antibody titers in 28- and 35-day-old birds [105, 106] were also reported in HS-added broilers. In HS-added weaned pigs, improved weight gain and increased elimination of zearalenone in fecal samples (from 64, 77, and 92%) in a dose-dependent manner as well as recovery of the secretion of  $\beta$ -estradiol were observed [107].



The protective effect of HS against mycotoxicoses seems to involve the sequestration of aflatoxins in the gastrointestinal tract, reducing their bioavailability and increasing their excretion through feces, which reduces damage to the liver and other vital organs, thus promoting better immune response and greater growth in animals that consume feed contaminated with mycotoxins.

## **6. Metabolism of minerals, proteins, and lipids**

### **6.1 Minerals**

HSs are considered as the natural ligands with the highest complexation capacity, giving them a strong potential to form chelates with various ions, which have been linked to better mineral utilization in plants and animals [108]. Increases in ash and Ca content in tibia bone have been reported in broilers fed HS [25, 109, 110]. In the same way, supplementation with liquid extracts of HS from worm compost caused an increase in ash retention in growing chickens [31] and increments in the tibia ashes, Ca and P percentage in 21-day-old broilers as well as in tibia dry matter percentage, and Ca and P content in 42-day-old broilers [111].

The plasma concentrations of Cu, Mn, and Cu and the liver concentration of Cu, Fe, and Se were higher in under normal rearing conditions in HS-added broilers [112]; however after the transportation to the slaughter house, the plasma concentrations of Zn and Mn were decreased, while the Fe and Se were increased as well as the liver concentrations of Zn Cu, Mn, and Se [112]. In HS-added broilers, increases in Ca, P, Fe, and Cu concentrations in the meat [113–115] and increases in Ca, Mg, Zn, and Fe in the thigh and breast have been reported [116]. These results also agree with the increased percentage, thickness, and hardness of eggshells reported in HS-supplemented laying hens and pheasants [117–120]. In HS-fed weaned pigs increased serum phosphatase alkaline, Ca, P, and Mg [121]. In the milk of HS-added cows, increased Ca and Fe content [122] and serum Ca levels [123] were found.

In HS-added rabbits, an early increased content of Fe and Ca and late increased activity of ALP and Cu content in serum were observed; additionally, in HS-added rabbits, increased number of layers of osteons and osteoblasts in the bone tissue of the femur, number of osteons and osteoblasts in the bone tissue of the sternum, number of columns of chondrocytes, and number of chondrocytes in the column in the cartilaginous tissue of the sternum were found [124].

### **6.2 Proteins**

It has been suggested that protein utilization efficiency is improved in nonruminant and ruminant animals supplemented with HS. In HS-added broiler chickens, increased total body mass [125, 126] and serum and tissue protein contents [127] were previously reported. These results agree with the higher protein efficiency ratio [128], breast and thigh meat and total protein content [62, 102, 129], and higher carcass and breast yield [35, 91, 102] recently found in HS-added broilers. The addition of HS has also caused increases in protein retention and digestibility in broiler chickens [31, 32] and protein digestibility rabbits [28, 33] and milking cows [34]. In laying hens, HS linearly increased serum total protein, albumin, and globulin [130]. In Holstein-Friesian HS-added cows, the protein and casein contents in milk were increased [122].

In HS-added rabbits, a significant decrease in the concentrations of urea and increase in total blood protein, albumen, and globulin levels have been observed [33, 131].

### **6.3 Ammonia emissions**

Nitrogenous waste, ammonia emissions, and bad odors are reduced in animals supplemented with HS due to improved feed efficiency, digestibility, and nitrogen retention [15, 78, 132]. In HS-added broilers and pigs, reductions of aerial and feces ammonia were observed [15, 78, 133]. It was suggested that the reduction of aerial ammonia in pigs supplemented with HS could be attributed to the inhibition of the urease activity in manure [78]; it is possible that the reduction of the urease activity inside the intestine may have contributed to the greater nitrogen retention and digestibility in HS-added animals. In aquaculture systems, the addition of HS improved the water quality by decreasing the total ammonia nitrogen and nitrate-nitrogen concentration and increased the nitrogen utilization efficiency by changing the microbial communities and strengthening nitrification [54].

Using a rumen stimulation technique and in HS-added goat and dairy cows, linear reductions in ruminal ammonia concentration have been reported [34, 98, 134]. In addition, in HS-added rabbits, the concentration of ceca ammonia decreased sharply in a dose response manner [33]. The lower ruminal or ceca ammonia concentrations may be linked with the effectiveness of HS to reduce ammonia accumulation and also to their strong nitrogen-binding properties of HS. In this way, in ruminant animals, HS may enhance rumen crude protein utilization by decreasing ammonia loss owing to reduced solubility under the inhibitory effects of HS on urease activity [78]. This might cause a shift in nitrogen excretion from urine to feces due to reduced solubility. The ability of HS to alter ruminal fermentability may be linked to ammonia sequestration and then slow release for microbial growth; additionally, reducing protozoa number may increase microbial crude protein flow to the small intestine [34, 98].

### **6.4 Lipids**

In humans, HA and FA preparations are promoted as supplements to increase energy and to reduce fatigue. In a rodent model, a product containing 60–80% HS reversed the negative behavioral symptoms of depression and anxiety caused by chronic fatigue syndrome and also stabilized the HPA axis stress response by reversing the drop in corticosterone levels and adrenal gland weight; it was suggested that the effects of HS were partially due to the regulation of mitochondrial bioenergetics by increasing the activities of mitochondrial NADH dehydrogenase, succinate dehydrogenase, cytochrome oxidase, and ATP synthase.

In the majority of the published research, it is evident that HS modulates the use of energy toward reductions of the lipid content in chicken and pig meat but increases the milk fat content in cows and goat. In HS-added broilers, reductions of the total lipid content of liver [125] fat content in the breast meat [62, 114, 129] and blood cholesterol levels [68, 110, 135–137] have been reported. In HS-added broilers and laying hens, HS linearly decreased serum triglycerides and very low-density lipoproteins concentrations [68, 130] and also reduced the cholesterol content in the eggs yolk [138]. In HS-supplemented pigs, reduced backfat has been observed [75, 78, 139]. The findings in pigs seem to be associated with increased activity of the hormone-sensible lipase and reduced activity of lipoprotein lipase in adipose tissue, as has been shown in pigs added with increasing dietary FA [140].

Opposite to this, in dairy cows, increased fat milk content has been found [122, 123, 141, 142] but decreased serum nonesterified fatty acids and blood beta-hydroxybutyric acid levels [123] and serum cholesterol [34] have been seen. In HS-added goats, significantly reduced serum cholesterol concentrations [98, 143] have been reported. Also in HS-added rabbits, reduced total and low-density lipoproteins cholesterol and triglycerides [28, 33, 144, 145] but increased high density lipoproteins [28] have been observed.

## **7. Productive response in animals supplemented with humic substances**

### **7.1 Poultry**

In two recent published articles, more descriptive effects of HS in poultry have been given [68, 96]. In several experiments, enhanced final body weight and weight gain and reduced feed conversion ratio have been reported in HS-added broilers using different commercial sources of HS [24, 68, 113, 114]. In agreement with these results, using a liquid or dry extract of HS from worm compost, higher final body weight and weight gain and lower feed conversion ratio have been reported in broiler chickens [19, 31, 77, 146].

Greater carcass weight and yield have been also observed in HS-added broilers [35, 113, 114, 128], including those supplemented with worm compost-derived HS [91, 146]. In breast and thigh meat, lower water loss and increased lightness and yellowness were observed [62, 129].

In laying hens and partridges supplemented with HS, benefits in feed intake, egg production, egg weight, and feed conversion ratio have been observed [118–120, 130, 147]. Improved Haugh unit values and egg yolk color in HS-added laying hens [74] and increased albumen height, albumen index, and Haugh units of the egg in partridge [148] were observed. A strong tendency of lower mortality have been reported in HS-added broilers [25, 67, 77] and Japanese quails [27].

### **7.2 Pigs**

In HS-added weaned pigs, improved body weight, weight gain, feed intake, and feed conversion ratio have been reported [41, 42, 63, 121, 149, 150]. In HS-added growing pigs, higher weight gains and feed efficiency have also been reported [75, 78, 139]. Reduced severity of diarrhea [40–42] and lower mortality [41, 78] were reported in HS-added pigs.

### **7.3 Ruminants**

In HS-added calves, higher body weight and weight gain [43–45, 142] while in HS-added dairy cows, increased milk production, milk fat, and milk protein were found [122, 123, 141], as well as in dairy goats [145]. Reduced severity of diarrhea has been reported in HS-added calf [43–45].

### **7.4 Rabbits**

In HS-added rabbits, enhanced body weight, weight gain, and carcass weight but reduced feed conversion ratio have been found [28, 33, 144].

## **8. Conclusions**

The present review reinforces the promoted protective action of HS on the digestive mucosa, their antioxidant properties, immunomodulatory, and anti-inflammatory attributes, the suggested microbial shift to a probiotic-type bacteria in the gut, including antiparasitic and antifungal effects, as well as their influence on the higher efficient of minerals, proteins, and lipids utilization within the body. The sum of all of these effects result in improved health, lower diarrhea and mortality rates, and increased growth rate, feed conversion, meat and milk production, as well as improved animal product quality.

The information reviewed does not allow to clearly define the main mechanism(s) of action of HS due to the plethoric benefits found. It is likely that, in order of importance, the main benefits of HS derive from their ability to form protective barriers on the digestive mucosa, blocking the passage of pathogenic bacteria and bacterial toxins or metabolites that could otherwise damage or invade the mucosal cells of the digestive tract. This also includes other compounds such as mycotoxins, anti-nutritional factors, and other contaminants that could enter the intestine through the diet.

The other no less important benefit is the ability of HS to stimulate the establishment of a beneficial microflora, and a pattern of microbial fermentation that promotes intestinal health, which could reduce the development of potentially pathogenic bacteria, creating a hostile environment, for example, through the production of SCFA, such as lactic, butyric, and propionic acid, which may acidify the intestinal content.

The rest of the benefits of HS within the body, such as enhanced antioxidant and immune status, increased efficiency of mineral, protein, and lipid utilization and improved health and growth and are likely to be dependent on the proposed effects of the digestive tract. However, since the effects on the gut may vary depending on the factors associated with the animal (species, age, and physiological and health status) to the tested HS (origin, age, concentration of functional groups, and composition and length of the side chains) and the experimental design and treatments (dosage, route and form of administration, length of supplementation, type of facilities, and sanitary conditions), the additional effects within the body may significantly differ.

The current research also demonstrates that numerous theories have been presented to account for the infinite number of effects of HS reviewed in this literature survey. With the existing evidence, it is not possible to reach consensus conclusions. The truly molecular mechanisms of action of HS in the intestine and throughout the body remain unknown. However, outstanding improvements in health, productivity, and meat and milk quality obtained from animals supplemented with HS are common features in the majority of available research.

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## **Conflict of interest**

The authors declare no conflict of interest.

## **Author details**

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## Chapter 3

# Tropical Soil Humus

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### Abstract

In strongly weathered tropical soils, humus and humic substances (HSs) appear to play an important role in soil fertility because they represent the dominant reservoir and source of plant nutrients. As the refractory organic carbon form of soil, HSs play a vital role in the atmospheric CO<sub>2</sub> sequestration. Detailed classification of humus forms in tropical ecosystems and the dynamics and function of humus are still poorly understood. Nevertheless, in tropical environment many studies indicated that it is very difficult to differentiate between tropical humus, at least in normally drained soil. Moders, mulls, and Amphimull are the dominant humus forms in the topsoil of tropical environment. Knowing the mechanisms of formation, the dynamics and the methods of characterization of humus in tropical zones are a scientific challenge. This chapter aims to share recent findings from a broad humus in tropical soil and research related to this theme.

**Keywords:** tropical soil, humus forms, humic substances, fertility, climate

### 1. Introduction

Soil organic matter (SOM) is a main reservoir and source of plant nutrients, which controls soil's fertility in tropical soil. It also plays a major role in various soil functions in influencing soil chemical and physical properties and carbon storage [1]. Although, in tropical soils, SOM represents only about 1% of the soil mass, the humus is one of the most important fractions of these soils. Approximately 60–70% of organic matter in soil is composed of humic substances (HSs) [2]. As the refractory organic carbon form of soil, HSs play a vital role in the atmospheric CO<sub>2</sub> sequestration [3]. Previous studies summarized the benefits of soil humus and several functions have been assigned to them. These functions include physical, chemical, and biological control, retention of nutrients, metal complexation, and carbon storage [4]. Humus composition is an essential characteristic of HS in SOM [5]. Traditionally, HSs are separated into humic acid (HA), fulvic acid (FA), and humins (HN) based on the solubility characteristics of each fraction, and the humus composition is an essential characteristic of HS in SOM.

Humification is a global process that is implemented in soils [6, 7]. This process of transforming precursors of humification and polymerization of oligomer and monomer molecules into dark-colored, high-molecular-weight macromolecules has been described in terms of organic chemistry [8], environmental dynamics [9, 10], and various zonal soils dynamics, parent rock, vegetation, soil organisms [11, 12]. The

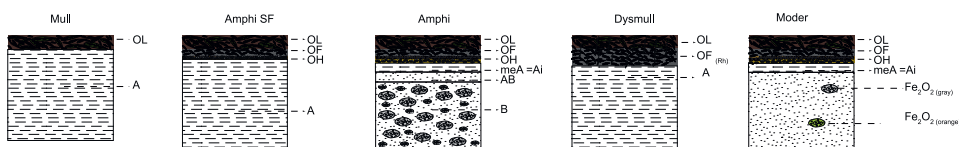
humus profile comprises different scales, which may be integrated: regional climate. Detailed classification of humus forms has been made for over a century in temperate regions, whereas, in tropical ecosystems, the dynamics and function of humus are still poorly understood.

Although previous works have studied the dynamics and function of humus forms in tropical forest ecosystems, however, these aspects are still poorly understood [5, 13, 14].

The aim of this chapter is to review the current state of knowledge on humic substances in tropical soil, with special emphasis on data concerning humus forms, the factors that control.

## 2. Humus forms in tropical soil

The humus form reflects the processes of heterotrophic decomposition, nutrient cycling and release [15–17], soil microbial and faunal activity [18, 19], stabilization of soil organic matter, and release of carbon dioxide CO<sub>2</sub> [19, 20]. Humus forms are thus crucial to the functioning of a forest ecosystem, being a key indicator of plant-soil interactions [21]. Under temperate forests, the humus has been studied for over a century, and its three main forms, mull, moder and mor, are well established [22]. In tropical environment, a few investigations have studied the dynamics and function of humus forms [14, 23, 24]. Amphimulls exhibit mixed features of moders and mulls and are widely represented tropical forest ecosystems (**Figure 1**) [14, 25]. Dabin [26] indicated that it is very difficult to differentiate between tropical humus, at least in normally drained soils, on the basis of morphological characters, since we are most often dealing with Mull-type humus with very thin, if not nonexistent, litter, which rests on a mineral horizon where the humus is well decomposed and strongly incorporated. The main criteria are related to the intensity of humic accumulation, which is manifested by the color of the horizon, possibly by its structure, and by the humic penetration in depth [26]. In tropical environments, [14] has evidence that humus forms are more varied and depend on parent rock, litter quality, and millipede activity. For instance, these authors observed two different humus forms in secondary forests in North Grande-Terre (Guadeloupe): a calcareous Amphimull and a Dysmull (**Figure 1**). The first is characterized by a 1.5 cm-thick OH horizon, which has a granular structure and consists of fecal pellets of millipedes. The second has a 7 cm-thick root mat [14]. In tropical humid lowlands, [5] has shown that forest soils generally exhibit the mull humus type and transitions to moder due to the favorable conditions for litter decomposition. According to [27], a great diversity of humus form was found in Atlantic forest (Mesotrophic Tropical Mull, Tropical Ologotrophic mull, Eumoder, Moder-Mull Dysmoder, Mesotrophic Mull), which is a reflection of



**Figure 1.** Representative humus profiles showing Main humus forms identified in tropical soils adapted from [14, 24]. These humus forms reflects the processes of heterotrophic decomposition, nutrient cycling and release, soil microbial and faunal activity, stabilization of soil organic matter, and release of carbon dioxide CO<sub>2</sub>.

the complex environmental conditions. In accordance with recent study in tropical environment, the classification of humus forms resulted in the identification of three humus systems: Mull, Moder, and Amphimull [24]. The attribution to an Amphimull system depends on the quality of the A horizon.

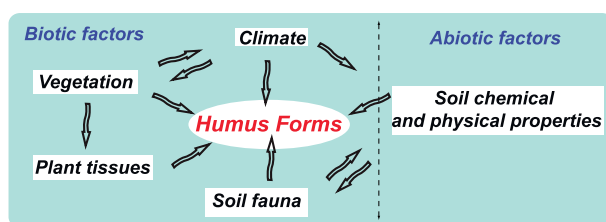
### 3. Humus horizon structure and composition

In tropical soils, humus horizons present some specific characteristics related to decomposition rate and nutrient absorption. For example, [27] found a dark color horizon between organic superficial layers and the first organic-mineral horizon. This horizon, which is of biological origin, was named Ai and had a significant amount of roots [27]. Moder humus forms are characterized by a structured with a juxtaposition of organic and mineral grain, named miA [24]. In opposition, [24] indicated that large organic mineral aggregates are found in Mull humus forms. Amphimull system displayed distinct characteristics similar to that of moder due to the formation of an OH horizon one the one hand and similar to that Mull system due to the presence of organo-mineral part [24]. Others studies indicated that fine roots have been found between horizon OF, which plays a role the development of the humus [14, 23].

### 4. Factors influencing humus form development in tropical environment

Humification, as well as litter decomposition, is primarily microbially mediated process, mainly controlled by site-specific variables such as temperature, soil water regime, pH, and available nutrients [5]. Biotic and abiotic factors can affect the development of the humus profile by constraining the dynamics of its humus horizons, leading to consequences for the global carbon cycle, climate change mitigation, and forest productivity (Figure 2) [28–30]. However, what drives the morphological organization and characteristics of humus horizons in tropical forests is still poorly understood [14, 23, 24].

At the regional scale, due to the effects of temperature and moisture, climate is the best predictor for the decomposition rate and consequently for the formation of humus forms [27]. In tropical environment, the length of the dry season governs the processes of humification [31]. Among these factors, soil moisture is a major factor affecting microbial activity [32]. Previous studies focused on the effects of different soil moisture content on the quantitative and qualitative characteristic of humic fraction, showed that humic fractions decreased with increasing soil moisture [33]. The chemical composition of plant tissues has implications for the recalcitrance of the



**Figure 2.**  
Factors controlling humus formations in tropical soils.



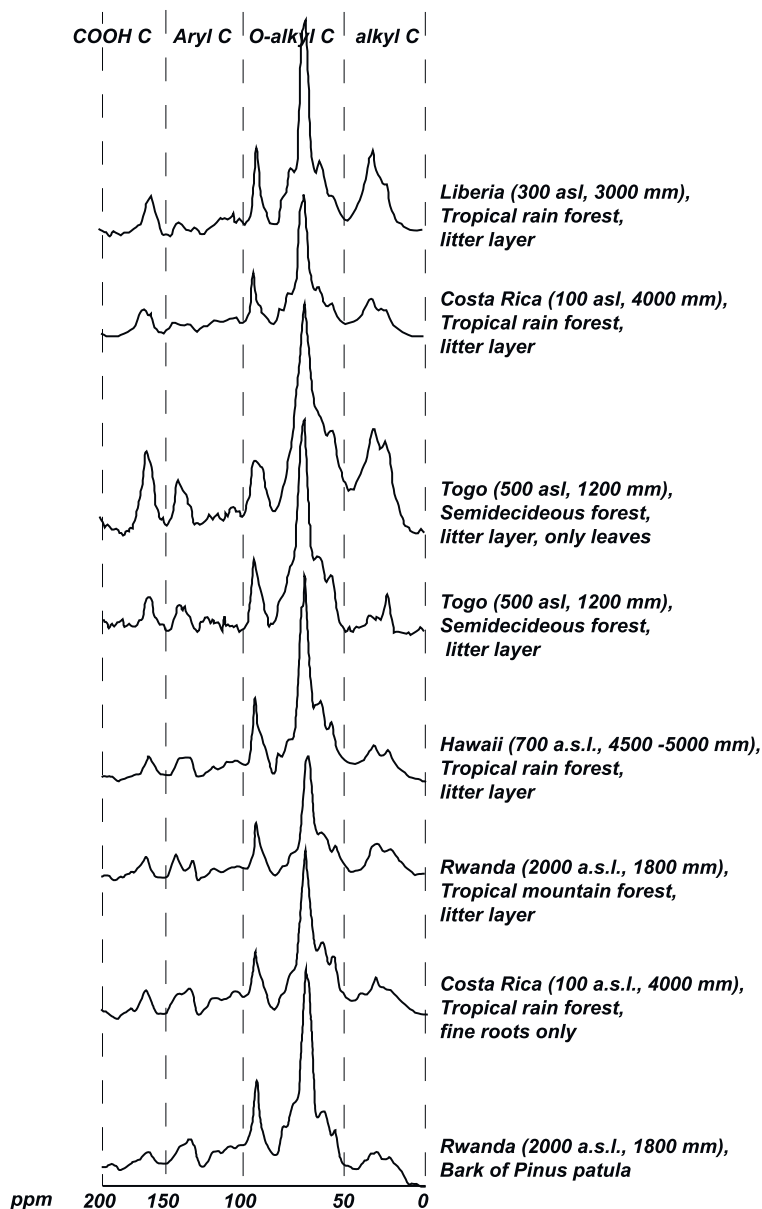
be applied. Because, biological activity could be similar between soil textures but less visible in sandy condition [24]. Regarding vegetation, [14] highlighted an accumulation of organic matter in humus horizons of secondary tropical forests compared with natural forests, which also occurred in forest restoration in bauxite mining areas in the Amazon [42]. For instance, grassland soils are known to possess relatively large amounts of humic acid (~70%) compared with fulvic acid (~30%), whereas forest soils have vice versa [43].

The formation of humic substances (i.e., humification) is primarily a microbially mediated process [5]. In tropical environment, humus forms are determined by the composition of soil macrofauna rather than by edaphic properties [12]. In tropical forest soils, where faunal-mediated mixing between plant/microbial necromass and soil is intense [44]. Animal microbial in the soil fauna are known to influence SOM content in particular in humid climates where HS contributes to the soil moisture and nutrients. Many authors associated microbial and soil animal communities to humus forms [14, 22, 44] showed that mull is associated with high plant, biodiversity, and productivity. Conversely, mor has low productivity and biodiversity and organic layers (OL, OF, and OH) are well identified. Moder, which is an intermediate position, both is characterized by a high level of biological activity [14] showed that tropical semi-evergreen forests, as temperate forests, the activity of endoanecic earthworms gives mull humus profiles, whatever the quality of the litter [5] showed that in biologically active soils with high earthworm population, the mull humus type develops in L-A h horizons only. Many studies have shown that in tropical environments, humus forms contribute to diagnosing both the forest succession and the restoration of degraded areas [14, 24]. For instance, [14] highlighted an accumulation of organic matter in humus horizons of secondary tropical forests compared with natural forests, which also occurred in forest restoration in bauxite mining areas in the Amazon [42]. This accumulation in humus horizons indicates a collapse in the process of organic matter decomposition and incorporation, which results in a decrease in the stabilization of carbon in the soil, as suggested by [42] and [45].

## 5. Chemical nature of soil humified fractions

The primary contributors to SOM are various plant tissues, which are highly variable among tropical tree species [5]. The chemical composition of the secondary resources (soil fauna and microorganisms) is even more complex, but these sources are quantitatively less important than plants in surface layers of tropical forest soils (**Figure 4**) [46]. So, the structure, nature, chemical composition, and stages humification of the organic material determine molecular size and chemical structure of humic substances [47]. Humic substances in soils consist of heterogeneous insoluble macromolecular compounds, which form complexes with soil mineral surfaces and metal cations [48]. According to their solubility in aqueous solution at different pH values, humic substances can be divided into three main fractions, namely humic acid (HA), fulvic acid (FA), and humin [5].

Stevenson and Olsen [8, 49] studied the humus composition in tropical soils and showed that the bulk of the organic matter in most soils consists of a series of HA, FA, and humin. These authors have found that humified fraction from SOM had high content of insoluble fraction and predominance of fluvic acids. Moreover, the chemical nature of humic and fluvic acids varied with the soil depth. The HA concentration was higher at top soil [49]. The highest biological activity on the surface probably promotes



**Figure 4.** Carbon distribution of some primary resources of tropical forests according to CPMAS<sup>13</sup>C NMR spectroscopy adapted from [5].

the formation of condensed alkali-soluble humic substances with greater stability [50]. Studies carried out in Ivory Coast and Senegal showed that fluvic acid content attains and sometimes even exceeds that of humic acids [31]. Using CPMAS <sup>13</sup>C NMR analysis, [5] showed the presence of carboxyl fraction groups, aromatic carbon, and O-alkyl carbon in plant tissue of tropical forest. Among them, O-alkyl carbon was shown to be higher (75%) in wood or roots than leaf litter (50%). Moreover, carboxyl and aromatic carbon are low and represent about 5–10% and 10–15%, respectively, of total carbon.

## **6. Characterization of humic substances: a varied methodological approach**

In chemical terms, organic matter consists of three fractions of humic substances (HSs): humin, fulvic acids, and humic acids [51]. The humic substances eventually form between 80 and 90% of all SOM and consist of heterogeneous molecular compounds containing different functional groups [52]. Humic substances (HSs) have received attention from scientists in a wide variety of disciplines [53]. The main precursor of an ecosystem approach for the study of HS in soils has been provided by the work of [48, 54, 55].

Basic information on HS could be accessed through the chemical characterization of SOM. So far, the study of HS composition has been carried out under the action of strong oxidants (alkaline solution) or heat to determine the single structural units. Alkaline extraction remains the most common method for detecting the solubility of HS from soil, according to the International Humic Substances Society (IHSS) [56]. Other extraction procedures using organic solvents are used [57].

Recent methods such as spectroscopic such as infrared, electron spin resonance, and nuclear magnetic resonance (NMR), microscopic, pyrolysis, ionization techniques have also enabled to elucidate various structural characteristics of humic acids, and NMR has recently brought about considerable progress in the study of humic acids [47, 51, 58]. Machado et al. [51] used infrared spectroscopy and nuclear magnetic resonance (NMR) to characterize humic and fulvic acids in aggregates collected from areas under different crop and soil management systems in Brazil. These methods allowed new aspects of research in organic soil chemistry and have been extensively used to quantify the proportions of functional groups as well as the aliphatic and aromatic contents of HS. In an review, [59] stated that the CPMAS technique provides a quantitative measure of aromatic, paraffinic, carboxylic acids, and other groups in fulvic acids (FA) and humic acids (HA). Using solid-state  $^{13}\text{C}$  spectroscopy, [5] showed that there is considerable variability in the overall chemical composition of the various plant tissues of tropical forest trees (**Figure 4**). Due to the increasing demands for rapid and quantitative assessments of soil organic matter quality, thermal analysis techniques are a unique means to characterize the complete continuum that comprises soil organic matter. Among the most common thermal techniques, Rock-Eval pyrolysis [60, 61] has been increasingly applied to geologically recent sediment and soils [58, 62–65]. Details of the application of Rock-Eval to soils are provided elsewhere [58, 62, 64, 66, 67]. Rock-Eval provides information on quantity and quality of organic matter without sample preparation. It also gives information on stoichiometric of organic carbon [58, 68]. Disnar et al. [58] provided essential information on the amount and composition of tropical SOM. In addition to information on the abundance of SOM, Rock-Eval provides insight into the composition of SOM and even into its structure [58, 65]. In a recent review on pioneering works on SOM, [53] pointed out the great value of RE pyrolysis for soil scientists.

## **7. Humics substances and metal micronutrients**

Humic substances are able to form stable complexes with metal micronutrients, due to the presence in their structure of oxygen-, nitrogen- and sulfur-containing functional groups [69]. Organic associations of the metals play an important role in storing and stabilizing SOM [e.g. 70, 71]. In the case of Fe, highly stable HS complexes

mainly involve O-containing groups (carboxylic and phenolic groups) [72, 73]. More recently, it was shown that carboxylic acids in aliphatic domains are also involved in Fe(III)-HS complexation [74]. Stability and solubility of the complexes are both affected by pH and molar ratio between micronutrients and HS [75, 76]. Colombo et al. [77] showed that pH controls Fe-humic substances complexes stability and/or solubility. Therefore, the presence of insoluble complexes may explain plants growth in calcareous soils characterized by limited Fe availability [78]. The stabilization of amorphous Fe oxides HS, which limited Fe availability, has been reported in previous studies [77, 79]. This is the result of co-precipitation of the poorly crystalline Fe phases and its maintenance for a long period in this form [77, 79]. This form would increase Fe reservoir for plant nutrition. Urrutia et al. [80] reported that the ability of HS to complex Fe can also be important for phosphorous nutrition, since phosphate can be bound to HS by Fe bridges. This process would increase phosphate availability; in fact, complexation of Fe by ligands released by plant roots could promote uptake of both nutrients.

HSs are known to be redox reactive and capable of chemically reducing metals including  $Fe^{3+}$  [81, 82]. Very acidic pH values cause the reduction of  $Fe^{3+}$  conversely, this reduction process is limited by formation of  $Fe^{3+}$ -HS complexes. Bioreduction of minerals soils can be accelerate by HS dissolved and solid-phase due to shuttling electrons from bacteria to oxide surfaces [83].

As recently reviewed by [47], the classical view on Al-humus complexes is based on complexation of  $Al^{3+}$  and  $Fe^{3+}$  ions by carboxylic and phenolic groups of humic substances. The degree of "metal-humus" complexation is quantified by ratios of Al, C, and Fe determined in pyrophosphate extracts. Although  $Al^{3+}$  and  $Fe^{3+}$  ions doubtlessly form complexes with carboxylic and phenolic groups [84].

## **8. Future challenges to humus analysis in tropical soil**

Tropical environments share some similarities at the global scale (high productivity, rapid nutrient turnover, highly weathered soil, and low soil pH), but they also exhibit wide variation in soils and associated plant communities. In particular, different tropical regions possess distinct geological histories and plant communities [85]. Variation in climate, geology, and topography can cause diverse patterns and processes of plants, soils, and their interactions [86]. Similarly, the patterns of decomposition process reflected in humus form are highly variable. Most humus studies have been conducted in Amazonian forests [14, 24, 44], few studies have focused on humus in tropical soils in Africa in general and the Congo basin in particular. SOM humification and soil C accumulation are sensitive to climatic and local environmental fluctuations and changes in land use and soil management [87], comparison between tropical regions can provide variation in plant species, soil types, and availability of nutrients with which to investigate roles of soils and functions in tropical environment. Such knowledge is indispensable for the establishment of a sustainable management of the carbon budget maintaining or even improving at the same time major humus functions. In African tropical regions, previous studies focused on classification of humus forms. However, quantitative characteristic of humic substances is poorly studied. These studies can help to assess the role of humic substances in fertility and carbon sequestration in these very sensitive ecosystems.

Complexity of HS and their remarkable properties in agricultural applications has attracted and continues to attain the attention of many investigators, bringing



over the years new knowledge on their structure, physicochemical, and biological properties. The effects of HS on plant growth depend on the source, concentration, and molecular weight of humic fractions and mainly on different chemical. Since humus substances might be a source of nutrients for plants, increasing tropical soil OM stocks are also beneficial for soil fertility in these regions known to be poor in nutrients. Many efforts to identify if it is mainly the chemical structure in terms of compounds or the molecular weight of HSs to influence plant growth and development must be carried out. The influence of soil humus in contributing to improve soil quality in its physical stability and capacity to provide nutrients to plants can also be studied.

Regarding tropical soil, humic substances can act as carbon, nitrous oxide, and others greenhouse gases sink playing a role in climate change. This role depends on its quality, quantity, and interaction with soil organo-mineral. Understanding chemical composition structure of humic substances in an ever-evolving environment (changes in land use, agricultural practices, climatic or edaphic conditions, etc.) is essential. To this purpose many research groups are addressing these scientific challenges while striving to overcome scientific knowledge gaps in these mechanisms.

The effect of HS in improving nutrient assimilation and plant metabolism is well recognized. Previous studies indicated that humic fraction increases cell growth, metabolism, and nitrate uptake [88, 89]. Herder et al. [90] has reported that root architecture and nutrients uptake are directed affected by humus, enhancing plant yield. In tropical countries, soils are poor in nutrients leading to the excessive use of fertilizers whose prices are constantly increasing [91]. In order to give the best route for this enormous amount of residues, what are humic substances, new technologies are needed, which in turn could help farmers to cope with the high cost of imported fertilizers.

On the methodological level, analytical techniques such as fluorescence, electron spin resonance, and size exclusion chromatography at high pressure are not yet applied to study humic substances in tropical soil. These techniques can be applied to investigate the molecular changes of humic molecules when in interactions with metals and organic compounds, for example.

## **9. Conclusions**

The dynamics and function of humus forms in tropical forests are still poorly understood. The aims of this chapter were to review new approach tools, methods for qualitative and quantitative evaluation of humus in tropical environment attempting to provide a better understanding of humus forms in tropical. Diverse tools and methods for qualitative and quantitative evaluation of humus coming from diverse sources have been adopted so far to express transformation processes. For the characterization of humus and humic substances, the analytical techniques are applied. Mull, moder, and Amphimull are the dominant humus forms in the topsoil of tropical ecosystems. They differ by the distribution of organic and mineral-organic horizons. The quantity and quality of humic substances formed in soil depend on biotic and abiotic factors. The role and importance of organic matter in soils, and in particular of humus in tropical soils, have been proven. However, few studies have been undertaken in Africa in general and in the forests of the Congo Basin, which is the second largest carbon reservoir in the world. This is why the study of humus in these ecosystems must be carried out.

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## **Conflict of interest**

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## Chapter 4

# Properties of Humic Acid Substances and Their Effect in Soil Quality and Plant Health

*Nitin Vikram, Ankita Sagar, Chetna Gangwar, Raja Husain and Raj Narayan Kewat*

### Abstract

During aerobic and anaerobic decomposition of plant and animal residue a complex aggregate of brown to dark coloured amorphous substances is obtained which is called as Humus. It includes humic substances and resynthesizes products of microorganisms. These products are stable and a part of the soil. Humus is categorised according to their molecular weights and solubility into humus, humic acids and fulvic acids. Humic substances are the organic material naturally present in soil. Humic substances positively effect's soil quality and fertility by increasing its water holding capacity, stabilisation of soil structure, soil microbial activity, plant physiology. It also influence nutrient uptake and root architecture act like phytohormones for phosphorus acquisition, and improving plant adaptation to saline condition. Humus is the primary microhabitat for microorganism such as dictyostelids, myxomycetes, some species of protostelids, members of the genus Copromyxa etc. Other than that auxin like activity of Humic Substances has also been demonstrated in recent studies. The research suggested that it could be the main biological factor that exhibits positive effect on plant physiology. Based on that fertiliser factory also trying to produce are bio- stimulants, based on humic substances and other organic compounds.

**Keywords:** amorphous, biostimulants, microorganism, nutrient uptake

### 1. Introduction

The word humus comes from the Latin word meaning soil or soil which refers to living things in the soil. Humus is a black organic matter found in the topsoil that is formed by decomposition by the action of soil microbes such as bacteria and fungi, which divide animal and vegetable material into smaller inorganic particles that can be used to grow plants and plants as well. It is an integral part of the biological life cycle of fertile soil. Technically, humus as a final result of this process is less important for plant growth than products produced during active rot. Humus has a high carbon content and is usually acidic due to its humic acid content. It increases the water storage capacity of the soil and produces carbonic acid, which transports minerals. Regardless of the soil group, the most important indicator of soil fertility is humus

content; both in the topsoil and in the entire soil profile [1, 2] because all the essential elements in organic material exist. In humus. It contains a lot of carbon so it is still organic, but micro-organisms can decompose further. Humus is so stable that it can withstand the soil for hundreds of years.

Humification is the formation of humus and affects the development of the soil by determining its colour. Dark brown to dark brown is usually high in humus content. The dark colour of humus helps to warm the cold soil in the spring. Humus plays an important role in sustaining living organisms in the soil which is essential for healthy plant growth. There are so many benefits of humus such as:

- The conversion of organic matter into compost provides micro-life food.
- Mineral storage: Solid humus absorbs minerals and prevents loss.
- Humus is important for storing and binding ionic forms of nutrients and keeps it safe from water extraction and provided for plant absorption.
- Humus helps hold water up to 80 to 90% of its weight. So it can prevent drought stress.
- Humus plays an important role in maintaining a good pH by adding high acidity or alkaline soils.

## **2. Soil organic matter (SOM)**

Bacteria, fungus, algae, actinomycetes, earthworms, nematodes, and arthropods are just a few of the living things that live in soil. All are important in the biological and microbiological activity that results in the decomposition of plant material, roots, dead creatures, and other organic residues that build up in soils. Soil organic matter is created as this organic material decomposes. Soil organic matter has a wide range of chemicals originating from algal, plant, and microbial sources, each with a unique structure and set of properties [3]. The major functional groups in SOM are O- and N-containing groups that donate electrons, fix nitrogen, and go through processes to generate humic substances (HS), a subclass of SOM made up of high molecular weight biopolymers that react strongly with both inorganic and organic pollutants. Soil organic matter makes up only 1–5% of the total soil mass, but it plays a vital role in soil health and fertility because it has an energetic effect on the characteristics, texture, and function of the soil.

SOM is an important component in the creation and stability of aggregates, as well as a wonderful storage for all necessary nutrients [4]. Soil organic matter absorbs nearly all of the nitrogen in the soil and, in most cases, creates it. Apart from that, SOM can remove hazardous substances from the soil, such as Al ions, lowering soil toxicity and providing numerous benefits to plants [5]. Thus, soils with a high SOM content are of higher quality and fertility than soils with a low SOM content, and they provide a wide range of positive benefits to plants and crops, such as improved rooting conditions, critical nutrients for plant growth, and increased soil water holding capacity. Even if the soil has a low pH, it can still deliver enough levels of N, P, and other nutrients if it has a lot of SOM. Overall, SOM has integrated a number of practical, chemical and biological activities that contribute to the cation exchange network, soil conservation, water flow and ventilation and

productive forests. Soil organic matter plays an important role in the functioning of the ecosystem by preserving the natural and chemical properties of the soil by incorporating nutrients [6]. Soil organic matter is protected from the soil by three main processes: biochemical recalcitrance, chemical stabilisation and physical protection.

### **3. Sources of soil organic matter**

Soil organic matter is a major source of carbon in the soil [7], and it can improve chemical, physical, and biological soil properties, as well as serve as a source of carbon and energy for microorganisms and have an impact on greenhouse gas emissions [8]. SOM is a mixture of molecules from plants, animals, and microorganisms with varying compositions, levels of availability, and environmental activities [1]. Plants are the principal source of organic matter in the soil. Organic matter is mostly added to the soil in the form of fallen leaves and plant parts that have died [9]. The crowns and roots of trees, shrubs, grasses, and other native plants provide a high volume of organic matter during the natural phase. Following harvest, a substantial quantity of crop plant parts are left in the soil, which acts as a source of organic matter in the same way as primary minerals serve as the parent materials of soil mineral components. The cytoplasm, cell membranes, and cell walls are the three elements of a plant that can be divided into three categories based on their chemical composition. The cytoplasm is rich in simple sugars, organic acids, amino acids, and enzymes, all of which are necessary for metabolic activity to continue. Proteins are sandwiched between two lipid bilayers in cell membranes. Hemicelluloses are abundant in plant cell walls. Cellulose, lignin, proteins, cuticle, and root wax are all components of the plant. Lignin is the second most prevalent organic compound in plant residues, accounting for about 5Vo of the mass of grasses and up to 6Vo of the mass of hardwood forest species. This component transforms into simple nutritional forms and increases soil organic matter. Animals are also regarded as secondary organic matter suppliers. Insects, millipedes, nematodes, and arthropods are among the soil creatures that contribute to the rise of organic matter. After they die, soil microorganisms contribute a significant amount of organic matter to the soil [10]. The dead bodies of soil organisms will be attacked by other organisms, which will decompose them into simpler products such as carbohydrates converted to simple sugar and proteins converted to simple amino acids. Sugar, starches, and protein decompose quickly, while fat, waxes, and resins decompose slowly. Because they are confined by microbial attack, a fraction of organic elements will survive in the soil for prolonged durations after all decomposable organic molecules have vanished. Decomposition is primarily carried out by microorganisms. Because of their ability to create specialised enzymes and the ability to access novel substrates via hyphae, fungi are regarded as key contributors [11].

Lignin, fat, waxes, and other materials make up the majority of this section. This proportion makes up the majority of humus. For instance, consider manure. Farmyard manure, compost, green manure, fish, meal, oil cake, and other organic matter sources are essential sources of organic matter in the soil, and their addition enhances the mineral content and other nutrients in the soil. Microbe-produced oil enzymes are essential for the biochemical processes of organic matter breakdown. SOM quality regulates SOM degradation and the release of carbon, nitrogen, phosphorus, and other macro and microelements (**Table 1**) [12].

SOM component	Description
<b>Living Component</b>	
Phytomas	Dead trees are also considered as phytomass
Microbial Biomass	Organic matter associated with cells of living soil microorganism
Faunal Biomass	Organic matter associated with living soil fauna
<b>Non-living Component</b>	
Particulate organic matter	usually dominated by plant derived materials
Litter	Organic materials devoid of mineral residues located on the soil surface
Macro-organic matter	Fragments of organic matter >20 µm or > 50 µm (i.e., greater than the lower size limit of the sand fraction) contained within the mineral soil matrix and typically isolated by sieving a dispersed soil

**Table 1.**  
*Component of SOM with their description.*

#### 4. Characteristics of humic substances

Humic substances are a complex mixture of biotic-derived organic molecules that have undergone extensive alteration since they were first created by plants. Humic substances, often known as “gelbstoff” or “yellow compounds,” are the end products of organic decomposition in marine, freshwater, and soil environments. They are highly transformable in their combinations of organic molecules (carbohydrates, amino acids, and fatty acids) as polymer compounds, and they are also highly resistant to further microbial degradation. Humic substances can be divided into humic acid (insoluble below pH 2), fulvic acids (soluble at any pH), and humin based on their solubility (insoluble in water) [13]. We also know that humic compounds are formed by the breakdown of plant material and can originate on land or in the sea (e.g., phytoplankton), and are a major source of organic C and N in aquatic systems.

#### 5. Humic substance calcification

##### 5.1 Fulvic acids

Carbon, hydrogen, oxygen, and nitrogen make up the majority of fulvic acid. Fulvic acid has a much lower molecular size than humic acids, which have molecular sizes ranging from 1000 to 10,000. They are water soluble in all pH ranges. The dissolution of protons in fulvic acids causes a progressive array of negative charges with rising pH. Over the lower pH range, say below pH 7, it is now well established that carboxylic type groups are mostly responsible for this behaviour. Polysaccharides and low molecular weight fatty acids are the main sources of fulvic acid.

##### 5.2 Humic acids

Humic acid has a molecular size of 10,000 to 1,000,000, which is larger than other humic substances. They are dark in colour, ranging from brown to black, and are made up of a mix of carbon chains and rings. In an acidic environment, humic acid is not soluble in water, but it is soluble in an alkaline solution.

### 5.3 Humin

Because it is strongly bound to inorganic soil colloids, humin is an insoluble component of humic material. Humins are humic material fractions that are insoluble at all pH levels. Because of their molecular weight, humin complexes are classified as macro organic compounds. The humin fraction increases the capacity of the soil to store water.

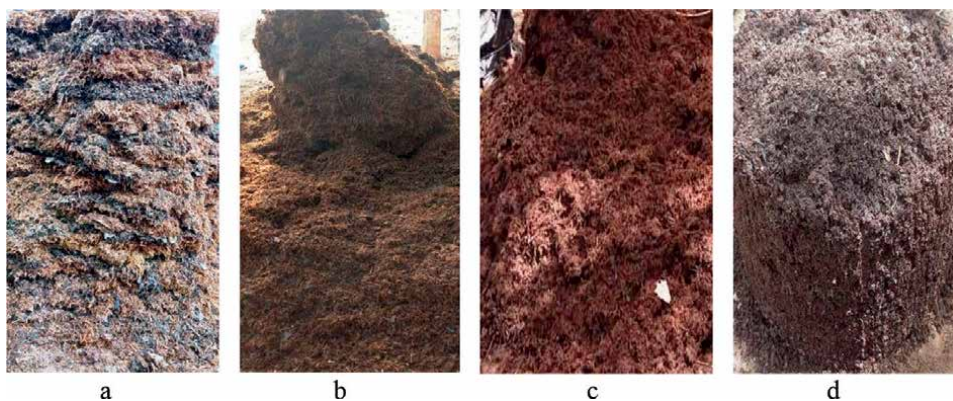
## 6. Mechanisms governing humus formation

One of the least understood features of humus is the generation of humic compounds. Decomposition and synthesis are two types of chemical reactions that have resulted in the production of humus. Plant and animal bio-chemicals are broken down by soil microbes during decomposition. The second of the humus-forming processes involves the breakdown of other basic organic compounds. Following that, the humus synthesis process begins with the polymerisation of monomers, resulting in the formation of polyphenols and polyquinones.

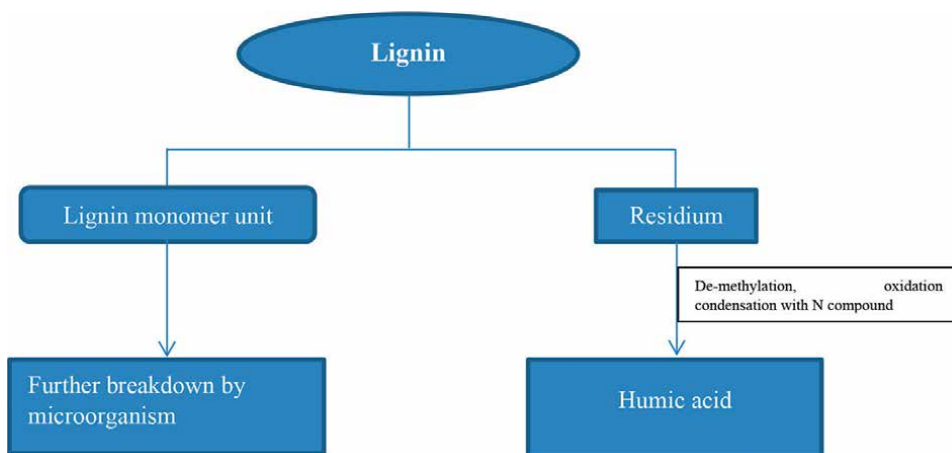
These high-molecular-weight molecules interact with N-containing amino acids and make up a large part of resistant humus. The synthesis of these polymers is aided by colloidal clays. The humic group and the nonhumic group are the two main groupings of chemicals that make up humus. According to humus formation theories, there are numerous avenues for the synthesis of humic compounds during the decomposition of plant and animal leftovers in soil. All of these processes, including sugar amine condensation, must be evaluated as possible mechanisms for the production of humic and fulvic acids in nature (**Figure 1**).

### 6.1 Lignin theory

According to this theory, the residue of incomplete lignin breakdown by microorganisms becomes part of the soil humus. Loss of methoxyl (OCH<sub>3</sub>) groups with the formation of o-hydroxyphenols and oxidation of aliphatic side chains to create COOH groups are two examples of lignin modification. The changed product undergoes unknown alterations in order to create humic and fulvic acids. According to this notion, humic chemicals were transformed to lignin breakdown earlier by cellulose



**Figure 1.**  
*Physiological changes in humic acid formation.*



**Figure 2.**  
*Lignin theory.*

degrading mycobacteria. Lignin can be broken down into low molecular weight compounds in usually aerobic soils (**Figure 2**).

### 6.2 Polyphenol theory

According to this theory, the condensation of phenolic compounds produces humic chemicals. Lignin polyphenols are oxidised to quinones. To generate humic molecules, these quinones are condensed with low molecular weight microbial products. Amino acids, nucleic acids, and phospholipids are among the microbial products. Enzymatic conversion of phenolic aldehydes and acids released from lignin during microbiological attack to quinones, which polymerise in the presence or absence of amino compounds to create humic-like polymers. The synthesis of brown-coloured compounds by reactions involving quinones is a well-known phenomenon that occurs during the production of melanine. Lignin, microbes, and lignin decomposition are all possible sources of phenols for humus production. The phenols that are generated during decay are also used to make humus.

### 6.3 Sugar-amine condensation theory

According to this concept nonenzymatic polymerisation of reducing sugars and amino acids created as by-products of microbial metabolism results in brown nitrogenous polymers similar to those produced after dehydration of certain food products at moderate temperatures. The amine is added to the sugar's aldehyde group to generate the n-substituted glycosylamine in the first step of the sugar-amine condensation reaction. The N-substituted-1-amino-deoxy-2-ketose is then formed from the glycosylamine. Dehydration and creation of reductones and hydroxymethyl furfurals; fragmentation and formation of 3-carbon chain aldehydes and ketones, such as acetol, diacetyl, etc. All of these molecules are very reactive, and in the presence of amino acids, they quickly polymerise to generate brown-coloured products.



## 7. Effects of humus on soil invertebrates

Invertebrates play a crucial part in the soil's physical, chemical, and biological activities, although they are often overlooked. According to studies, invertebrates have self-organising systems and perform various types of ecosystem engineering [14]. Nematodes, collembola, and mites are major soil invertebrates in coniferous forests, while earthworms and termites are prevalent in temperate deciduous and tropical forests [15]. Invertebrates Griffiths *et al.* [16] have shown that both trophic (decomposition) and non-trophic (moment) effects are involved in the production of soil humus. Wolters [17] examined soil invertebrates' internal (ingestion and associated transformations) and outward (defecation, construction) regulatory mechanisms, concluding that these systems contribute to the stabilisation and destabilisation of soil organic matter. Microbial decomposition is aided by invertebrates, which also operate as real decomposers, breaking down dead organic waste with their own endogenous enzymes, which has an impact on the soil environment and plants. While macro invertebrates consume humus and redox active components (iron minerals and humic compounds), they change soil organic matter [18, 19]. In the case of arthropods, species richness is around 85%, and they make up a major component of the soil's meso and macrofauna. Arthropod excrement is the basis for the production of soil aggregates and humus. It physically stabilises the soil, increasing its nutrient storage capacity. Culliney, [20] During intestinal transit, macro invertebrates like soil-feeding termites virtually totally decrease acid soluble Fe (III) and humic acids. In the alkaline intestines of scarab beetle larvae, the same process occurs. The gut homogenates of *Pachnoda ephippiata* no longer converted Fe (III) to Fe (II) after sterilisation, suggesting the importance of the gut microbiota in the process. At pH 7 and 10, the PeC11 strain demonstrated a significant ability to reduce dissimilatory Fe (III) [15]. The full polymerisation of phenols and proteins is aided by some fly (*Bibio marci*) larvae and earthworms (*Aporrectodea caliginosa*). Using <sup>14</sup>C-labelled phenols and proteins, invertebrates were able to understand the biodegradation of organic materials and the creation of humic compounds in lab conditions. The same organic chemicals were employed as a control without the impact of invertebrates (phenols and proteins). In the excrements of *Bibio marci*, the fraction of the <sup>14</sup>C isotope in the humic acids was higher than in the control substances. The earthworm *A. caliginosa* excrement showed a substantial favourable response to proteins [19].

## 8. Effect of humus on soil fertility

Because of their complex and heterogeneous structures, soil organic matter, which is made up of 80% humic acid and the remainder un-decomposed matter, plays a vital function and has a direct impact on soil fertility and textures. Carbon, oxygen, hydrogen, nitrogen, and sulphur are all significant elements found in humic compounds. It is composed of 80% organic matter derived from the soil [7, 21]. Fulvic acid and humic acid, which are both soluble in sodium hydroxide, are categorised as humic compounds. Humin, on the other hand, is insoluble in sodium hydroxide [7, 21]. After solubilising in sodium hydroxide, humic compounds are treated with acid, which dissolves the fulvic acid portion while precipitating the humic acid portion [7].

Decomposition of corpses and debris produces humic chemicals. Total soil nitrogen, available nitrogen, total phosphorus, available phosphorus, total potassium,

available potassium, and organic matter levels were all improved, as were enzymes like urease, sucrase, and phosphatase, as well as bacteria and fungi. Granular maize straw was added to soil in an experiment to improve soil humus carbon, fulvic acid carbon (FAC), and humic acid carbon (HAC). After applying granular straw to the soil, HEC, FAC, and HAC all increased by 17.59%, 8.32%, and 26.51%, respectively. The application of straw makes the structure of humic acid aliphatic, simple, and younger, as well as promotes the continuous renewal of humus and making soil younger and more fertile, according to techniques like infrared spectrum and fluorescence spectrum used to analyse soil humic acid and principal components analysis (PCA) [22]. Zhang et al. [23] investigated HA molecular structure variation after a 35-year long-term experiment in black soil. The researchers used methods such as Fourier transform infrared spectroscopy (FTIR), <sup>13</sup>C nuclear magnetic resonance spectroscopy (NMR), and fluorescence spectroscopy. The findings revealed that the molecular structure of HA in black soil is more simple, aliphatic, and younger as a result of manure application, but inorganic fertilisers make the HA structure more complicated due to increased condensation. Manure can be used alone or in combination with inorganic fertilisers to increase crop yield and improve soil organic matter structure. The use of humus in the soil was also found to be statistically significant in increasing corn N uptake in salty land, although the use of foliar humic acids did not increase P, K, Mg, Na, Cu, and Zn uptake but did increase P, K, Mg, Na, Cu, and Zn intake. Wheat has been subjected to similar findings. Under salt stress, the first dosages of humic compounds applied to the soil and foliarly boosted nutrient absorption in corn and wheat [24, 25].

## **9. Effect of humus on toxic pollutants in soil**

In aqueous solutions, humic substances (HS) demonstrate the property of detoxifying agents. It has the ability to eliminate free contaminants (indirect bio-effects) as well as trigger organisms' protective responses (direct bio-effects). Bondareva and Kudryasheva will get married in 2021 [26]. Bacteria, such as Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes, as well as fungi from the phyla Basidiomycota and Ascomycota, were discovered to be the predominant degraders in the interactions between microbes and humic compounds. Laccase, peroxidase, and dehydrogenase enzymes in the soil were also found to be positively influenced. In the presence of sufficient nitrogen, humic acid and fulvic acid promote microbial activity. The breakdown of oils into fatty acids and sugars by humic substances includes enzyme production, chemical reactions, and microbial degradation, as well as some biological technologies. The oxidative binding of organic contaminants to humic compounds can be catalysed by these enzymes. Humic compounds and microorganisms are employed as terminal electron acceptors to promote organic pollutant biodegradation or lower metal bioavailability by suppressing electron transfer to CO<sub>2</sub>, reducing CH<sub>4</sub> production in anoxic settings. On the other hand, fulvic acid was discovered to be a powerful chelating agent capable of extracting metal ions from salt molecules. The use of humates as a remediation strategy has a number of advantages over the use of microorganisms, including increased soil water wettability, lower oxygen requirements, simplicity of application, and lower cost. [27–29]. During short-term (45-day) experiments in mining and metallurgical places in the Kola Peninsula, Russia, the effects of exogenous coal humates, peat humates, and their combinations with nitrogen fixers and mycorrhizae forming fungi showed promising effects on

environmentally friendly and effective solutions for soil health restoration caused by long-term air pollution with sulphur dioxide and heavy metals. pH, HM mobility, dissolved organic carbon content, and microbial activity all improve when coal is present. It reduces HM mobility (from 4 mg/kg to 12 mg/kg for Ni and Cu, respectively, to 1 mg/kg and 2 mg/kg) and raises pH from 4.1–5.0 to 5.5–6.0. It does not necessitate the use of lime and has a beneficial impact on the growth of test cultures and microbiological activity. Inoculation of humates with nitrogen fixers has no effect, whereas mycorrhizae-forming fungi operate well with coal humates and promote the development of the test culture's root system [30]. The number of bacteria, rate of oxygen consumption, and generation of carbon dioxide in soils supplemented with fulvic acids followed a normal sigmoid curve, showing that mineralisation occurred promptly with no lag phase. The ability to oxidise aromatic chemicals, such as vanillin and phydroxybenzoic acid, increases as fulvic acids and microorganisms decompose. The structural study of humic acids and aromatic compounds is done using IR spectra and chromatographic examinations of their hydrolysates. The findings revealed that soil samples pre-incubated with glucose had a higher rate of fulvic acid decomposition than untreated samples, as well as a link between fulvic acid metabolism and aromatic chemical metabolism Kunc et al. [31]. In the treatment of arsenic (As)-contaminated soil, humus can be employed as a green agent to improve EKR (electrokinetic) technology. During electrokinetic remediation, electromigration controlled the distribution of As in the soil, and the addition of humus considerably increased the release of As from the soil (EKR). When 4 g of humus was added to the mix, the efficiency of the cleanup was increased by 20%. The presence of multiple oxygen-containing functional groups in humus was revealed by functional group analysis, which desorbed as from soil particles under both acidic and basic conditions. At pH 10, approximately 11.63% of bound as was desorbed as free as, indicating that EKR [32] was correct.

## **10. Effect of humic substances on agricultural productivity**

Humic substances (HS) are known to be natural, effective growth promoters because they induce beneficial local and systemic physiological responses via hormone-like signalling pathways. The dosage, origin, and molecule size, as well as the degree of hydrophobicity and aromaticity, as well as the spatial distribution of hydrophilic and hydrophobic domains, all have a role in defining the effect. Low-molecular-weight HS can enter root cells and evoke intracellular signals directly, but high-molecular-weight HS bind to exterior cell receptors to activate molecular reactions. Nutrient transporters, plasma membrane H<sup>+</sup>-ATPases, hormone pathways, and genes/enzymes involved in nitrogen absorption, cell division, and development are all affected by HS.

Humic acid and humic substances, when used in the soil and in the laboratory, can improve soil structure, increase fertiliser utilisation, and act as growth enhancers by promoting morphogenesis, lateral root formation, and root hair initiation in intact plants, as well as stimulating root and shoot development in treated cell calluses. It improves nutrient utilisation efficiency, macro and microelement assimilation, and carbon, nitrogen, and secondary metabolism induction. Encourage crop growth as a result to increase production and income [33–35]. External application of plant-derived humic acid (PDHA) and coal-derived humic acid (CDHA) to wheat growing in two alkaline calcareous soils in pots resulted in considerable spike weight increases

in both clayey and sandy loam soils. Serenella et al. [36] discovered that applying PDHA and CDHA at rates of 50 mg/kg to both soils increased grain yield by 21 and 11 percent, respectively, over control. Physiological and molecular techniques were used to test nitrate assimilation in *Zea mays* seedlings. The gene expression of *Z. mays* plants was affected by HS treatment at the transcriptional level, and its regulation was highly influenced by the availability of nitrate in the growing medium [37]. In flowering plant like *Gladiolus* (*Gladiolus grandiflorus* L.) applications of HA and NPK gives early and uniform sprouting, more foliage growth per plant, greater leaf area, and total leaf chlorophyll contents, earlier spike emergence, greater number of florets per spike, longer stems and spikes, and greater diameter of a spike, higher flower quality, longer vase life, higher number of cormels per clump, and greater cormel diameter and weight applied Iftikhar *et al.*, [38]. To counteract the negative effects of salt on flax seed. Opal, Giza-8, and Mayic flax types were grown and treated with humic acid and/or foliar-applied chemical additions. The results revealed that the Giza-8 variety responded positively to these chemical additions, allowing it to more effectively withstand the harmful effects of salinity. Bakry et al. [39] found that foliar spraying of proline improved seed yield, straw yield, and oil yield at humic acid.

## **11. Conclusions**

Crop production productivity is the basis of certain nutrients for human life which depends on amount of available nutrient in soil. This review has revealed that humus and humic acid substances has significant roles in soil quality, plant health, soil environment, plant-soil-microbial interactions and as well as productivity. This review counted a lot of factors that affect humic acid substances performance in soils, plants and also crops. Humic acid substances brings benefits in terms of agricultural yield, improves plant growth and the uptake of nutrients; but its economical application levels should be also determined. More research is needed to optimise the combined effect of different humic acid substances application and soil quality parameters under defined field conditions.

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# The Contribution of Humic Substances in Improving Agriculture and Livestock Sector in African Great Lakes Region: A Review

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## Abstract

The economy of the Great lakes region (GLR) depends on livestock and agricultural production. Although the region boasts massive diverse natural resources, such as humic substances (HSs), it is yet to benefit from this valuable natural resource. The current review sought to establish the contribution of HSs in improving the agriculture and livestock sector in the GLR. The outcome of the review establishes a positive relationship between the use of HSs and the improvement of the agriculture and livestock sector in the GLR. These substances stimulate the growth of plants, enhance soil fertility, and increase the availability of nutrients to plants. Conversely, HSs are vital components in controlling mycotoxins in animal feed. While the GLR is reputable for its massive agricultural production, this review affirms that the region has yet to fully explore HS's benefits. Further research is necessary to specifically explore ways to maximize the use of HSs in boosting agricultural and livestock production in the GLR.

**Keywords:** humic substances, great lakes region (GLR), agriculture, and livestock sector

## 1. Introduction

According to MacArthur Foundation, the African Great Lakes (Swahili: *Maziwa Makuu*, Kinyarwanda: *Ibiyaga Bigari*) region encompasses eleven countries: Burundi, Democratic Republic of Congo (DRC), Ethiopia, Kenya, Malawi, Mozambique, Rwanda, South Sudan, Tanzania, Uganda, and Zambia [1]. Burundi, Rwanda, Malawi, and Uganda are fully covered, while the other seven countries are partially covered. There are varied definitions of the GLR based on the different geographical locations [2].

The GLR encompasses regions with some of the world's largest freshwater systems, comprising diverse ecosystems. Also, the region is rich in a significant quantity

of natural resources such as land (peatland), water (lakes and rivers), and others rich in organic matter [3]. Humic substances are essential components of organic matter, widely distributed in surface soils, sediments (Sapropel, peat, charcoal), rivers, lakes, and marine waters [4]. Previous research has highlighted the application of HS in both agriculture and livestock rearing. Some sources report that farmers use this component to improve rhizome growth and speed up seed germination [5]. HS facilitates the transport of oxygen and nutrient utilization by plants and accelerates respiration. These findings have motivated scientists to evaluate the characteristics of HS and their potential use in improving the well-being of animals and plants [5].

A similar study by Yasar et al. [6] established a positive correlation between increased use of humic acid and significant weight gain in rats. On the other hand, Islam et al. [7] concluded that HS can improve how animals utilize nutrients in their feed. Notably, HS can achieve this by forming a protective layer on the gastrointestinal tract, which protects the host against toxins and infections.

Stakeholders in the agricultural sector have extensively utilized HS to spur growth and production. The benefits attributed to the use of HS on agricultural soils are enormous, mainly in areas with limited organic matter. HS form an integral part of global ecosystems and are valuable in cycling both carbon and nutrients [8].

Although GLR has abundant humic substance sources, these organic compounds are not exploited and utilized. According to FAO [9], agriculture is the principal economic sector in the Great Lakes region of Africa. This sector represents a significant source of income for rural populations in Burundi, the Democratic Republic of Congo (DRC), Rwanda, and Uganda [9].

Therefore, the application of HSs may play a vital role in improving agriculture and livestock. This review seeks to highlight the role of humic substances from sediments (Sapropel and peat), waters, and surface soil in improving the agricultural land and livestock sector in African GLR (great lakes countries). The paper also covers the primary sources of HSs in the GLR and explains the extraction methods of HSs from different sediments, soil, and water.

## **2. Definition and primary sources of humic substances in GLR**

In the environmental chemistry book, humic substances were defined as “a general category of naturally occurring, biogenic, heterogeneous organic substances that can generally be characterized as being yellow to black in color, of high molecular weight and refractory” [10].

HSs comprise a combination of heterogeneous organic compounds of biotic origin that have been transformed since plants first produced them. They are heterogeneous refractory since they resist decomposition and breakdown [11]. They have attracted widespread interest due to their applications in different sectors and have long been studied. Many famous scientists from the 18th century until now are involved in researching soil humic substances. Humic substances can originate from terrestrial or marine (e.g., phytoplankton) [12].

### **2.1 Sapropel**

Sapropel is a resource of organic origin characteristic of limnetic areas of eutrophic freshwater bodies. The term “sapropel” means “decayed sludge.” The first work referring to “gyttja” was published in 1862 by a Swedish scientist Hampus von Post

who accurately described it as a decomposed mass consisting of mineral particles with inclusions of plankton remains mollusk shells, chitin from the exoskeletons of insects, pollen, and spores [13, 14].

Sapropels are notable sources of humic acid. These are natural organo-mineral formations formed from dead plant and animal organisms, minerals of biochemical and chemical origin, and mineral components. Sapropel humic acids possess the properties of biogenic stimulants, stimulate the macrophage defense reaction, and promote tissue repair [15].

Lake Kivu is one of the African Great Lakes. It lies on the border between the Democratic Republic of the Congo and Rwanda [16]. The sapropel deposits in Kivu lake of the rare mineral monohydrocalcite interlaid with diatoms. Under these deposits, there are sapropelic sediments with high pyrite content [16]. The layer of sapropelic deposits in Lake Kivu contained organic matter from primarily algal sources [16].

The group composition of the organic matter of sapropel is represented by bitumoids, humic substances, easily hydrolyzable (carbohydrate complex) and difficult to hydrolyze (cellulose and lignin components) substances, and non-hydrolyzable residue [17]. Various nitrogenous and hormone-like compounds, enzymes, pigments, organic acids, alcohols, and others make up the pool of the biologically active component of sapropel [14]. The mineral component of sapropel contains silicon dioxide, calcium oxide, and compounds of iron, magnesium, potassium, aluminum, sulfur, phosphorus, and other macro- and microelements [14, 17]. In small doses, it was revealed that sapropels contain such substances as crude protein, digestible protein, quickly hydrolyzable carbohydrates, and vitamin A [18].

An essential feature of the organic part of sapropel is the high content (up to 50%) of humic compounds, which largely determine the attributes and nature of silts. The humic acids included in the composition of sapropel have a different degree of chemical activity, and the bactericidal effect of sapropel depends on it [14]. Humic acids of silica sapropels have a more pronounced antimicrobial effect [18].

Using sapropel as a feed additive increases the number of microorganisms in the rumen of lactating cows, resulting in the optimal acidity of the contents being established more than in the control variants [19]. In comparison with the control group increases by 8.2%, the number of protozoa in the experimental variant increases by 20.3% and is 647 thousand/ml. The high content of microorganisms in the rumen promotes better digestion of plant material [19].

Currently, the volumes and range of use of various humic fertilizers are constantly increasing. A comparison of different humic fertilizers showed their high efficiency in growing potatoes. The most significant increase in yield was provided by the use of stimulated fertilizer, the increase in yield over 3 years of research varied within the range of 14.6–27.3% [4, 20]. An increase in the starch content in potato tubers and a decrease in the accumulation of nitrates in plants were found [21].

## **2.2 Peat**

The great lakes region hosts million and millions of tons of peat deposits. The first study on the peatlands of Rwanda and Burundi was carried out between 1958 and 1964 [22]. It was estimated around 14,000 ha of peatlands along the valley of the Akanyaru river, which lies between Rwanda and Burundi.

For instance, Rwanda hosts millions of tons of peat deposits, and the western province is important as it is close to Kivu Lake (which lies between DRC and

Rwanda). About 77% of peat reserves are near the Akanyaru river (which lies between Rwanda Burundi), the Nyabarongo river, and the Rwabusoro Plains [23].

According to Pfadenhauer [24], 7% of Uganda (14,350 km<sup>2</sup>) is covered with peatlands. They are located around lakes and rivers to cite Kego, Albert, and Victoria lakes and the Nile, Victoria, and Okok rivers [25].

In DRC, peat deposits are located in different areas (**Table 1**). For instance, the mountainous area of Bukavu in the eastern province, west of Lake Kivu. The peatland area is around 0.5 to 10km<sup>2</sup>. **Table 1** shows the peatlands area in DRC.

Peat is an accumulation of decayed plant material over thousands of years. It is generally found in wetland areas, and the type of decayed plants determines the type of peat (grasses or heathers) and the location (inland or maritime). Peat is composed of water (88–92%), carbon (50–60%), hydrogen (5–7%), nitrogen (2–3%), phosphorus (< 0.2%), oxygen, and mineral nutritional elements [26]. Besides elemental composition, peat has four groups of organic compounds. The first group is bitumen, a natural compound found in peat. The second group consists of water-soluble matter, easily hydrolyzed matter, and cellulose. The third group is humus or humic substances, which includes humic acid, fulvic acid, and humin; and the fourth is a mixture of lignin, lignin-like matter, cutin, suberin, etc. [27].

According to Lehtonen et al. [28], HSs are common organic constituents in soils and peats. HSs in peat comprise multiple structures derived from plants. The formation of these substances occurs during organic matter’s chemical, physical, and microbial degradation.

### 2.3 Aquatic humic substances

Aquatic HSs are water-derived organic acids that are colored and polyelectrolytic. They constitute between 40 and 60 percent of dissolved organic carbon and make up a significant amount of aquatic natural organic matter.

Area	Extent (in km <sup>2</sup> )
Upemba	8500 (W), 4500 (D)
Kamulondo depression	11,800 (W) 7040 (D)
Tumba	767
Chesi	41
Maji Ndombe	2294
Kifukula depression	1502
Mweru (Luapula River)	4580
Middle Congo swamps	40,550
Malebo Pool	ca. 300
Ruzizi River	?
Semliki delta	?

**Table 1.** Potential peatland areas in the Democratic Republic of Congo (Howard-Williams and Thompson 1985). W = extent during wet season and D = extent during dry season (= permanent).

Aquatic HS has multiple functional groups, including carboxylic acids, phenolic hydroxyl, carbonyl, and hydroxyl groups. Aquatic humic substances are comprised of two components; humic and fulvic acids. Humic acid component precipitates at pH 2.0 or less, while fulvic acid remains in solution at pH 2.0 or less [29].

The differences in functional and elemental groups and average molecular weights among other characteristics mark the distinction between aquatic fulvic acids and humic acids. Also, there is a significant difference.

Aquatic humic and fulvic acids also differ from their corresponding soil counterparts. The typical average molecular weights of aquatic fulvic acids are 800–1000 daltons, and those of aquatic humic acids are 2000–3000 daltons. In contrast, Suffet and MacCarthy [30] postulate that the molecular weight of soil humic acids can measure several hundred thousand Daltons.

## 2.4 Surface soils

Humic substances are the main organic compounds of soil, and they are the markers of healthy soil. HSs are considered a vital component of the terrestrial ecosystem, responsible for many complex chemical reactions in soil [12].

Typical surface soils have up to 30% organic matter (OM), of which HSs can amount to 50–60%. The sources of soil humic substances are lignin and lignin-like materials; leaf polyphenols; cellulose and other polysaccharides; lipids; and proteins and amino acids.

Dead biomass mixed in and on top of soil consists of 50–60% cellulose and other polysaccharides, 15–20% lignin, and 15–20% fatty lipid molecules. Depending on the water, oxygen supply, temperature, and other environmental factors, up to 80% of the carbon in the biomass is converted to CO<sub>2</sub> and returned to the air within a year. But with the aid of fungi, the remaining carbon is converted into humus (humified) and protected, resulting in some HSs that are thousands of years old! Eventually, however, these HSs form CO<sub>2</sub> by reacting with oxygen. Such a respiration process prevents the HS soup from covering the earth's surface. Also, it is crucial in completing the carbon cycle. Notably, the long-lived HS occurs after the loss of most of the CO<sub>2</sub> during the respiration process highlighted above [31].

## 3. Composition, structure, and properties of humic substances

Humic substances contain carbon, hydrogen, oxygen, and nitrogen with a small amount of sulfur and phosphorus. These elements are always present regardless of their origin and country [32–34].

Humic substances are made of humic acids (HA), fulvic acids (FA), and humins (HM). Humic acids are a fraction of humic substances, readily soluble in alkali and less aggregately stable than the other humic fraction and fulvic acids. Humic and fulvic acids also differ in molecular weight and degree of aromaticity: fulvic acids contain more aromatic structures with a molecular weight of two orders of magnitude [35]. The difference between humic acids and fulvic acids is present in **Table 2**. The degree of polymerization of fulvic acids by hydrogen bonds, cation bridges, and other methods may be lower due to the relatively low ability of the benzene nucleus to enter into chemical reactions. Humins are insoluble components, and their insolubility and intractable nature have made them difficult to study [42]. Research on humins depicts similarities between the substance and humic acids. However, humins are generally

Humic acids (HA)	Fulvic acids (FA)
HAs are dark (carbon rings and chains) [36]	FAs are tight yellow to golden yellow [37]
HAs are not soluble in acidic conditions, and they are soluble in alkaline conditions[36]	FA are soluble in acidic and alkaline conditions [36]
HAs unlock nutrients and bind to them, thereby making them available for uptake by plants [36]	FAs bind to nutrients and transfer them to plants [37]
HAs have a higher molecular weight (1500–5000 Daltons in streams and from 50,000–500,000 Daltons in soils) [38]	FAs are characterized by relatively low molecular weight [39]
HAs contain oxygen, nitrogen, hydrogen, and phosphorus [33]	FAs are more biologically active: the oxygen is twice that of Humic acids [37]
HAs have aromatic rings in a molecule [40]	FAs have many functional groups (hydroxyl and carbonyl groups) [37]
They are less polar and more hydrophobic than fulvic acids [40]	These groups make FA polar [40]
The physical shapes of HAs molecules are determined by pH value, ionic strength of a solution and metal ions. HAs are negatively charged [41]	FAs are negatively charged. They are the most effective carbon-containing compound known due to their small molecular size and electric charge [37]
HA molecules are susceptible to photodegradation (abiotic) [41]	FA are resistant to microbiological degradation [37]

**Table 2.**  
*The comparison between humic acids and fulvic acids.*

insoluble components because they bind firmly to metals and clays, rendering them insoluble components [43].

Different studies have shown that humic substances vary in their composition. The difference in the composition depends on the source, location, and mode of their extraction. However, the similarities are higher than the differences [44]. The above chapter discussed the source of humic substances, while the following section of the review will discuss the extraction mode.

In 2014, Hou and his colleagues analyzed the humic substances in sediments separately in different fractions: humins (HM), humic acid (HA), and fulvic acid (FA). The results of the study revealed that the concentrations of HM, HA, and FA in sediments were detected in the range of 6.40–58.16 g kg<sup>-1</sup>, 0.27–3.50 g kg<sup>-1</sup>, and 0.27–4.26 g kg<sup>-1</sup>, respectively. HM was the dominant form and accounted for 74–94% of total organic compounds (TOC) and 80–90% of humus [27].

#### 4. Extraction and quantification of humic substances

Humic substances can be extracted from the soils, sapropel, peat, and other sediments with alkaline solutions [45]. After their extraction, they can be operationally fractionated into humic acids and fulvic acids based on their different water solubility, [46].

The IHSS method for humic substance extraction from soils has been developed by the *International Humic Substances Society (IHSS)*. The advantage of this method is that high yields are obtained and this method is the applicable gold standard in making lab-based comparisons. The use of an adsorbent resin in the purification process is the main feature of this method [47].

The second method is the NAGOYA method developed in Japan at the University of Nagoya. This method is different from the IHSS method. Among the differences, we can highlight the volume of 0.1 N NaOH used for both methods. The IHSS method uses a 0.1 N NaOH solution with 10 times the volume of the soil weight (g) to extract the humic substances while the NAGOYA method used the same solution with 300 times the volume of the carbon content (g) in soil. The second difference is the Fulvic acids (FA) purification process. According to Kuwatsuka et al. [48], Fulvic Acids fractions contain brown polymeric materials designated as humic substances and other organic substances such as carbohydrates, peptides, and lipids, designated as non-humic substances. IHSS considers and designates only humic substances in the FA fractions as "FAs." In the IHSS method, the polymeric-colored materials are collected from FA fractions using the hydrophobic adsorption resin XAD-8. However, NAGOYA considered that those FAs consist of humic and non-humic substances. Also, it is challenging to distinguish between humic substances and non-humic substances in the FA fractions. In the NAGOYA method, FAs do not exclude non-adsorbed materials on resins such as XAD. Those materials are also important constituents of FAs in their quantity and role in the soil environment [48, 49].

The third method to discuss is ultrasound-assisted extraction with 0.1 N KOH solutions. At RAS Limnology Institute (Russia), Mityukov and others have developed a new way of extracting ultra-disperse humic sapropel suspensions (UDHSS). The latter was extracted with alkaline extraction and ultrasound treatment of air-dry sapropels from the Seryodka deposit (Pskov region, Russia). UDHSS was derived from the hot method extraction at 40°C (104°F) and the cold method at 20°C. In his dissertation, Nsengumuremyi used the IHSS method to extract and quantify humic acids from UDHSS [50].

## 5. Application of humic substances

Humic substances in agriculture are primarily due to their environmental safety, physiological activity, immunomodulatory properties, and ability to bind toxic compounds.

A promising direction for using humic substances is the reclamation of contaminated environments. Composition of carboxyl, hydroxyl, and carbonyl groups in a complex with aromatic structures promotes the formation of ionic and donor-acceptor interactions and active participation in sorption processes. The binding of toxicants leads to a decrease in the concentration of their free form and, consequently, a reduction in toxicity. Therefore, humic substances act as natural detoxifying substances [51].

According to Stevenson et al. [46], approximately 60% of soil organic matter is humic substance (HS). HSs are critical components of the terrestrial ecosystem, responsible for many complex chemical reactions in the soil. Research shows that humic substances enhance root, leaf, and shoot growth and stimulate the germination of various crop species.

The interaction between HSs on one hand and metabolic and physiological processes on the other underpins the positive effects of sapropels. There is a positive relationship between humic substance and soil fertility. Such a positive impact implies that increased HS content in the soil is likely to increase the level of nutrient availability to plants. In other cases, HS can act on certain physiological targets to hasten the signaling pathways and metabolic processes in plant development [12, 46].

The positive physiologic effects of humic acid make them viable for the cultivation of low-lose plants. They have effects on plant physiology, including the promotion of root growth. Humic acids induce the plasma membrane proton (H<sup>+</sup>)-ATPase activity in root cells in the same way that growth is induced by exogenous auxin [52].

HSs are valuable components in plant physiology. They improve the structure and fertility of soil and influence nutrient uptake and root architecture.

Due to the presence of oxygen-, nitrogen- and sulfur-containing functional groups in the structure of humic substances, the latter can form stable complexes with metal micronutrients [20]. However, according to [53], the stability of metal-HS is lower than the complexes between iron and synthetic chelating agents (such as EDTA, EDDHA) or organic compounds of biological origin (such as organic acids, siderophores, and phenols).

Molar ratio and pH between micronutrients affect HS stability and solubility of the complexes. High stability may be favored when the pH range is 5–9 and by low metal: HS ratio [54]. Therefore, plants growing in calcareous soils with limited iron availability could benefit from the formation of stable and soluble iron-HS complexes and insoluble complexes with high molecular weight HS [20].

Sapropel humic acids cannot only remove ecotoxins from the body but also introduce the necessary biogenic metals into it in an easily accessible complex form, which makes it possible to consider them as a biologically active food additive.

In studies carried out by Ismatova with colleagues, it was found that humic substances isolated from peat and sapropel have anti-inflammatory activity, which in some cases is comparable with the effect of diclofenac (a nonsteroidal anti-inflammatory drug with an analgesic effect). The object of the study was purified sodium humate.

The antimicrobial activity was assessed on test cultures of *museum strains Proteus mirabilis* N 132, *Citrobacter diversus* N 244, *Klebsiella pneumoniae* N 251, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853., wild yeast [55].

The bactericidal effect of humic substances is based on the action of active groups included in humic acids and is comparable to the action of synthetic antibiotics [56]. Biological testing of various sapropel preparations was tested for *Staphylococcus Aureus*, *Escherichia Coli*, *Corynebacterium diphtheriae gravis*, and wild *Candida* yeast to establish a significant bactericidal effect of sapropel preparations comparable to that of synthetic antibiotics [13, 57].

The bactericidal properties of sapropel preparations can ensure stability during the storage of grain, used for preparing food products (flour, bread, cereals, etc.) or feeding animals [58].

Fulvic acids are a group of humic acids with a more pronounced peripheral part and a less pronounced aromatic core. FAs dissolve well in alkaline solutions, acids, and water. The humification process begins with forming fulvic acids [59]. The concentration of fulvic acid can be determined by the spectrophotometric method [31].

Fulvic acid also affects enzyme activity during grain germination. The chemical composition of fulvic acid (FA) with a molecular weight below 500 (FA-500) has been analyzed and its activity in promoting the germination of wheat seeds has been studied in that article [60]. FA-500 was obtained using membrane separation technology and analyzed qualitatively and quantitatively using gas chromatography–mass spectrometry coupled with the retention index. The best concentration was 0.5%, and an inhibitory effect appeared with increasing concentration. During seed germination, FA-500 can influence seed growth by affecting the activity of amylase, associated with respiration [60].



Humic (non-hydrolyzable residue) is a combination of humic and fulvic acid compounds; currently, saponins are used to produce ceramic products and chipboards, in drilling, and manufacture fabrics in the foundry. Based on saponins, preparations were obtained that can bind heavy metals and remove them from soil and water. Saponins are used in medicine and veterinary medicine. Saponins are essential in agriculture as plant growth activators (fertilizers) and in animal husbandry as animal growth stimulators and immunomodulators (biological active feed additives). Doctors have established that the humic complex from saponin is for the body and has a wide spectrum of action; the harmless complex has antimicrobial properties. Saponin peloids (medicinal mud) have antiseptic properties [14].

Purifying saponin is fairly cheaper than other sorbents because it is a natural substance. Besides, the substance has a characteristic high efficiency in absorption compared to other absorbents in the contemporary market, including clays or activated carbons.

The specific properties of humic acids make it possible to use them in industry, agriculture, ecology, and biomedicine [61].

It is important to conduct research on the use of extracts obtained from saponin in the food industry; to expand the field of application of saponin [17, 62].

Humic substances have shown a strong affinity for binding various substances, including various mutagenic, herbicides, heavy metals, *Bacillus subtilis* bacteria, and other substances [63].

Experimentally, Santos, et al. [64] proved the ability of polymers of humic acids to adsorb mycotoxins such as ochratoxin A and zearalenone. Humic acid polymers have also been found to be able to absorb more than 96% of both mycotoxins at pH 3.0.

In the study by Van Rensburg et al. (2006), oxygumate (pure, high-quality humic acids from bituminous coal) proved to be much more effective in attenuating aflatoxicosis in broilers. Oxygumate showed a high affinity for aflatoxin B1 in vitro.

Humic substances are present in all-natural environments, including river water and lakes, soil, peat, coal, and saponin. The complex composition of humic substances is caused by the absence of a unique dependence on the properties of the composition [65, 66]. Despite the known inhomogeneity of the chemical composition, the use of modern Physico-chemical methods of research has confirmed the independence of humic substances as a special class of natural organic high-molecular heterogeneous nitrogen-containing compounds [67].

Humic substances also can increase the yield of grain and fodder crops, stimulating photosynthesis and the respiration of plants. They are involved in plant lipid metabolism and in nonspecific plant responses to stress to restore membrane structure damage due to membrane ruptures and lipid peroxidation [68].

The antibacterial properties of humic compounds are due to their influence on the metabolism of proteins and carbohydrates of bacteria. Acting as catalysts, they accelerate the direct destruction of bacteria or viruses, thereby reducing antibiotics and the cost of treating animals [69–71].

## 6. Conclusion

Humic substances are essential organic compounds found in surface soils and aquatic environments. These degraded substances benefit agriculture in several ways. The current study sought to establish the contribution of humic substances in improving the agriculture and livestock sector in the Great Lakes Region. This

study establishes a positive correlation between HSs and improvement in livestock and agricultural production. In agriculture, HSs significantly decrease the toxicity as detoxifying agents, stimulating the root growth and germination of various crop species, soil fertility, and increasing nutrient availability for plants. Whereas in livestock, they are used against mycotoxins in animal feed. While the GLR is reputable for its massive agricultural production, this review affirms that the region has yet to fully explore HS's benefits.

### **Authors' contribution**

Writing–Original Draft Preparation, DN, NVB and SH Writing–Review & Editing, DN and SH; Supervision, NVB.

### **Competing interests**

“The authors declare that they have no competing interests”.

### **List of abbreviations**

ATPase	Adenosine Tri-Phosphatas
CO <sub>2</sub>	Carbon Dioxide
EDDHA	Ethylenediamine- <i>N,N'</i> -bis(2-hydroxyphenylacetic acid)
EDTA	Ethylenediaminetetraacetic acid
FAs	Fulvic acids
H <sup>+</sup>	Hydrogen ion
HAs	Humic acids
HM	Humin
HSs	Humic Substances
IHSS	International Humic Substances Society
KOH	Potassium Hydroxide
N	Normality
NaOH	Sodium Hydroxide
°C	Degree Celcius
°F	Degree Fahrenheit
pH	Potential Hydrogen
RAS limnology institute	Russian Academy of Sciences limnology institute
UDHSS	ultradisperse humic sapropel suspensions
XAD & XAD-8	Resin & Resin category

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Besides live biomass and decaying dead biological matter, humus constitutes an important component of organic matter in the soil. Humic substances are heterogeneous polymers formed during the process of decay and degradation of plant, animal, and microbial biomass. The humic fraction of organic matter is a stable nutrient base that serves as a slow-release source of carbon and energy for indigenous, slow-growing microorganisms in the soil. This book discusses recent advances and applications of humus and humic substances in agriculture, industry, and the environment. Chapters address such topics as the chlorination and phenanthrene sorption for humic and fulvic acids extracted from different soils, humic substances as growth promoters for farm animals, dynamics and function of humus from tropical forests, and much more.

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