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*Review*

# Macromolecular Size and Architecture of Humic Substances Used in the Dyes' Adsorptive Removal from Water and Soil

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**Abstract:** Humic substances are naturally occurring materials composed of complex biogenic mixtures of substituted aromatic and aliphatic hydrocarbon core materials derived from the degradation and decomposition of dead plant and animal matter. They are ubiquitous in both terrestrial and aquatic systems constituting biotic pools and are characterized by unique properties; they are amphiphilic redox compounds with exceptional chelating features. Humic substances play a crucial role in both agriculture and the environment as carbon sequestrators, soil improvers, plant health promoters, as well as stabilizers of soil aggregates and regulators of organic/inorganic nutrients bioavailability. This review article attempts to summarize current knowledge about the molecular nature and characterization techniques employed for the study of humic substances worldwide as the chemistry of their components differs markedly and depends on natural processes, several abiotic and biotic factors, the origin of the organic matter, and their complexation with inorganic, e.g., metal-ion, compounds. This work is equally concerned with the association of humic substances with dyes, a notorious pollutant, responsible for various environmental issues generally arising from the discharge of untreated effluents into soils and water bodies. Azo dyes, in particular, negatively affect soil microbial communities, as well as plant germination and growth. The aim is to feature the potential contribution of humic substances as novel materials for environment-friendly and sustainable processes.

**Keywords:** polymer characterization; humic substances; fulvic and humic acids; molecular size; supramolecules; textile dye; hydrophobic and hydrophilic sites; chelation; dye adsorption

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## 1. Introduction

Humic substances (HS), a decomposition product of plant and animal tissues in the environment, are classified as a unique chemical class of organic compounds [1]. According to the International Humic Substances Society (IHSS), HS are complex and heterogeneous mixtures of polydispersed materials formed in soils, sediments, and natural waters by biochemical and chemical reactions during the decay and transformation of plant and microbial remains; humic substances are major components of natural organic matter (NOM) and can be divided into three fractions. Humic acids (HA), insoluble in acidic environments ($pH < 2$) but soluble at higher pH values, and fulvic acids (FA)—soluble in water at all pH values—are their main fractions, and humin is the fraction that is water-insoluble at all pHs (Figure 1), solubility reflecting the traditional alkali-extraction separating methods from the original material. Several chemically reactive groups (e.g., carboxyl and hydroxyl) are present. Humic substances exhibit a wide range of molecular weights and sizes [1], from a few hundred Da to several hundred kDa; FA are of lower molecular mass than HA, and soil-derived molecules are larger than those from aquatic

NOM, also composed of mixtures containing humic substances [1,2]. Macromolecular-structure models of HS have been developed based on size exclusion chromatography (SEC) [3,4].

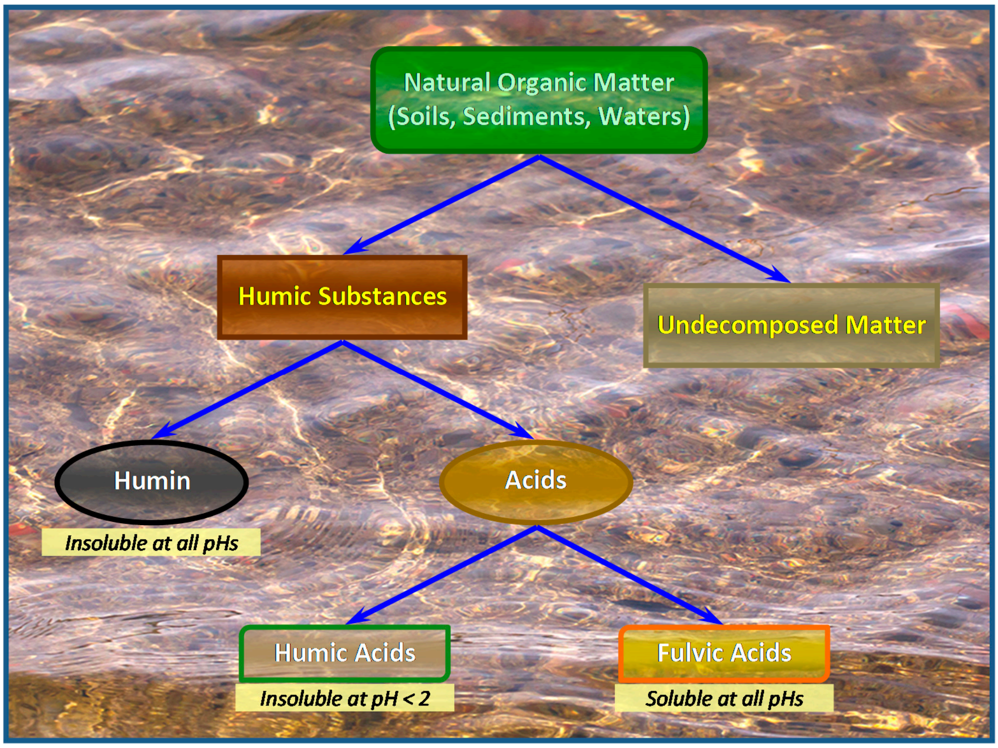

**Figure 1.** Traditional scheme of natural organic matter components separation.

Small and heterogeneous humic molecules [5] are self-assembled in supramolecular conformations, bound by weak van der Waals, $\pi$–$\pi$, CH–$\pi$, and hydrogen forces. However, as HS tend to aggregate in aqueous systems through physical and chemical crosslinking [6], different intra- and intermolecular interactions are observed; also, HS are negatively charged, accumulate at interfaces, and contain both hydrophobic and hydrophilic functional groups attached to their aliphatic–aromatic backbones [7], forming micelles that solubilize otherwise insoluble organic compounds [8]. As a result, due to their molecular complexity, it seems difficult to identify HS molecules and structures [6] by size exclusion chromatography, light scattering (LS), or viscosity measurements that commonly provide precise information on polymers.

Although polymer concepts such as the glass transition temperature ($T_g$) have been applied to support the presence of both glassy and viscoelastic domains within the HS [9], the available evidence does not support the formation of large-molecular-size and persistent HS in soils. Instead, soil organic matter (SOM) seems to be a continuum of progressively decomposing organic compounds [10]. These initial degradation compounds and microbially-produced small molecules undergo condensation reactions and hydrolytic or oxidative processes to generate humic substances (i.e., refractory materials that resist breakdown by the organisms themselves), the formation of which may involve chemical alterations of biomolecules, e.g., lignin, occurring randomly and leading to an increased system heterogeneity [11]. Subsequently, experiments relying on the alkali and acid extraction and functional-group chemistry to describe the prevalence of humic and fulvic acids and humin in the soil have led to the conclusion that HS, comprising complex and bulk macromolecules, are the largest and most stable SOM fraction. However, these components represent only a small fraction of total organic matter (TOM); direct observations verify the existence of smaller, simpler molecules [12], and the reported molecular masses are in the region of low hundred and low thousand Da. Thus, HS do not appear to be true polymers but molecular species with a tendency to congregate, supermixtures, and supramolecules. Ultrafiltration has been used to fractionate HS into ranges that refer to congregations rather than to large covalently linked molecules [13].

While the world natural production of HS is 10 Gt $a^{-1}$, relatively little is known of their exact structures [14], which have yet to be unambiguously determined, as HS formation in soil and aquatic environments strongly depends on both biotic and abiotic parameters, e.g., parent materials, microbial consortia, and environmental conditions. In this work, the fundamental knowledge of HS nature is presented; the aim is to demonstrate the unique HS functional properties—i.e., amphiphilic character, colloidal constitution, molecular size and structure, surface accessibility, and porosity—that play a crucial role in soil–plant systems and are related to the chemical and biochemical behavior of HS. Adsorption onto HS is also discussed, as these natural organic compounds are useful for the immobilization of soil and water contaminants, mostly heavy metals and synthetic dyes. The latter, especially azo dyes, being toxic and persistent, entering the food chain, creating harmful byproducts, and causing agricultural pollution, need to be eliminated from terrestrial and aquatic ecosystems.

## 2. Structure and Characterization of Humic Substances

### *2.1. Macromolecular Nature and Chemical Retrosynthesis*

Natural HA are functional polymers with uniform, independent of their source and origin, unusual for a polymer structural properties (e.g., pH and redox buffering effectiveness, strong binding of water and ions, surface activity, interaction with minerals, hydrophobic and hydrophilic species carrying, as well as improvement of soil performance and plant growth by enhancing microbial and fungal activity) [14]. Structural and functional characteristics of different HA matrices are crucial for the reactivity with heavy metals and organic contaminants, e.g., dyes, to be determined [15]. The size of HA molecules depends on the number of solvent-accessible or surface functional groups, altering the HA chemistry by affecting the density of reactive sites. This chemistry suggests that multiple acid and phenoxide species are present on the HA [16], as well as acidic and basic functional groups, optical chromophores that bind metals via several photoreduction mechanisms. Determination of the molecular weight of HA fractions by high performance size exclusion chromatography (HPSEC) is a debated topic, as the complex HA mixtures behave differently in SEC columns [17].

Thermal investigation of the NOM molecular structure provides evidence of glass transition phenomena in peat HA and stream FA identified with temperature modulated differential scanning calorimetry (TMDSC) and thermal mechanical analysis (TMA). The $T_g$ of both soil- and stream-derived humic substances suggests a macromolecular structure for HA and FA in NOM. A more aromatic humic acid shows a higher $T_g$ than a river fulvic acid. Thermal analysis techniques may determine thermodynamic parameters related to molecular-scale interactions between NOM and contaminants [18]. As already stated, HS may not be regarded as polymers, but rather as supramolecular associations of relatively small heterogeneous molecules. Such supramolecules (either aggregates or self-assemblies) are clearly sizable; in any case, HA fractions that exhibit the highest molar masses (in the region of 15–25 kDa [19] obtained from HPSEC) are expected to be of macromolecular construction. As water molecules tend to exclude from humic molecular aggregations, the latter are held together and stabilized by dispersive—mainly hydrophobic—binding forces [19]. High-resolution in situ X-ray spectromicroscopy reveals that the sizes and shapes of HS macromolecular structures [20] depend on their origin, solution chemistry, and soil mineralogy. Geometries of these natural organic polyelectrolytes determine the role of HS in biogeochemical processes, and any changes in structure affect the solubility of pollutants, biotransformation, and the carbon cycle in soil [20].

Synthetic humic acids (SHA), made by hydrothermal polycondensation [14] or in the presence of peroxidases and phenoloxidases [21–23], are also useful for technical applications. Natural HA and SHA have been examined using fluorescence spectroscopy (FS) and atomic force microscopy (AFM) [5]. $MnO_2$ and $O_2$ catalysts promote the polyphenol–amino acid polymerization, and accelerate both the transformation of FA to HA and the evolution of HS [24]. Consistent with the view of 3-D macromolecular HS matrix, the Flory–Huggins theory has been used as the preferred thermodynamic framework for describing hydrophobic organic compounds (HOC) binding to dissolved HS [25]. Mimicking natural humification, fast and efficient chemical processes produce SHA that optimize desirable properties (e.g., amphiphilicity and redox potential); cheap and sustainable artificial humic polymers made from biomass waste, having a negative carbon footprint, i.e., lowering atmospheric carbon dioxide, can be exploited in industry and agriculture. Esterification and copolymerization with flexible monomers may improve the plasticity properties or optimize the advantages of these specialty polymers in environmental remediation [14]. Molecular modeling and computational chemistry have been used to visualize and determine intra- and intermolecular physicochemical properties of HA, SOM and dissolved organic matter (DOM) in water (e.g., aromaticity, polarity, bonds, long- and short-range forces, SOM and DOM oligomer models, and reactions with polar biological molecules) [26,27].

### 2.2. Characterization Techniques, Supramolecules, and Molecular Weights

Many analytical methods of qualitative and quantitative HS characterization have been applied; analysis of HS has been focused on standardization (e.g., elemental compositions, or ash and water contents of IHSS standard humic and fulvic acids). Physicochemical and elemental analysis, titration, UV–vis and fluorescence spectrophotometries, NMR spectroscopy, mass spectrometry, fractionation methods (i.e., gel permeation chromatography and flow field flow fractionation), as well as degradation methods (e.g., oxidation, pyrolysis, and hydrolysis) have been employed. Data obtained from physical, chemical, and spectroscopic methods provide detailed information on HS structure and supramolecular architecture [28].

In HPSEC systems, a variable wavelength UV–vis detector is used to detect the chromophoric composition of HS, which contain chromophores with unique molar absorptivities for a given wavelength; thus, molecular weights and their distributions [29] may depend on the wavelength chosen. Molecular weights of selected HA and FA increase with increasing detector wavelength, and, as the number average molecular weight ($M_n$) is more sensitive to wavelength changes than the weight average molecular weight ($M_w$), HS appear less polydisperse at higher wavelengths [29]. Lower light absorption due to a change in solution compositions is attributed to HS disaggregation and is confirmed by UV–vis spectrophotometry at wavelengths from 250 to 450 nm [19]. Intermolecular hydrophobic interactions [30], the degree of hydrophobicity, and the extent of changes in self-association are important factors [19,30].

Nuclear magnetic resonance spectroscopy, pyrolysis studies, X-ray absorption near-edge structure spectroscopy, and electrospray ionization mass spectrometry show that soil HS are dynamic associations organized into micellar structures in aqueous media. Molecules associated with HS (e.g., biomolecules bound in humic fractions) are, therefore, humic components, as they cannot be separated by physicochemical techniques [31]. These constituents are linked together via intermolecular interactions to form supramolecules, segregate on the scale of nm, and display a unique molecular motion [31]. Readily aggregated at low pHs [32] and dispersed (as functional groups dissociate) at higher pHs, HA and FA—behaving like linear, flexible polyelectrolytes—are associations of phenolic and benzene-carboxylic molecules (i.e., building blocks that originate from microbes, polyphenols, lignin, and condensed lignin) held together by weak linkages [32]. However, none of the above methods alone is sufficient to elucidate the complex structures of humic molecules. Chemical fractionation may remove molecules from the humic matrix, and advanced analytical tools may reveal their molecular structure. This “humeomics” approach, i.e., a progressive isolation of components from the humic suprastructure (by breaking intermolecular bonds without destructing carbon–carbon chains) followed by molecular characterization, allows their supramolecular organization to be identified [33].

Size exclusion chromatography has been used to characterize HA in natural waters, domestic and treated wastewaters, effluent from night soils, landfill leachates, pig slurry, sediments, soils, and oxidized coal [34]. Molecular weights determined by HPSEC are consistent with those from vapor pressure osmometry (VPO) and field flow fractionation (FFF). Analyses indicated that aquatic HS are smaller with a lower polydispersity than believed in the past. Both spectroscopy and molecular weight measurements provide information on the bulk properties of aquatic humics from different sources [35,36]. A comparison of ultrafiltration fractionation (UF) and HPSEC has revealed that neither technique leads to absolute molecular weight values [37]. Refractive index detection (RID)-based HPSEC has been used to determine the molecular mass of HA. RID is less selective than UV absorbance detection (UVAD) for structurally heterogeneous HA and, thus, is better for characterizing the molecular weight distributions of HA. From HPSEC-RID chromatograms, higher $M_w$ and lower $M_n$ values are estimated, as well as a higher polydispersity for the HA samples. The fraction of HA exhibiting the highest molecular weight is more aliphatic with fewer UV-sensitive bonds, e.g., C=C, compared to the low-molecular-mass component [38]. As mentioned, HPSEC does not always provide reliable results for HS; procedure standardization leads to reproducible measurements [36].

Physical parameters of HA fractions—isolated by basic (HAb) and pyrophosphate (HAp) extractions from several types of peat—have been analyzed with UV–vis, fluorescent, infrared (IR), and electron paramagnetic resonance (EPR) spectroscopies. The average molecular weights of fractions range from 17.2 to 39.7 kDa, and their humification index (HIX) varies from 0.49 to 1.21. The HAp fractions show higher contents of both aromatic structures and phenolic OH groups compared to the HAb fractions. The EPR signals are consistent with the presence of semiquinone-type free radicals, the number of which can be determined from the HIX [39]. Soil organic matter humification increases the concentration of semiquinone-type free radicals (SFRC), and electron spin resonance (ESR) spectroscopy is a useful humification indicator. Simpler and more sensitive compared with ESR, FS is an effective low-cost tool for studying the humification of soil HA by detecting the changes in HS structure [40].

### *2.3. Structural and Compositional Architecture of Size Fractions*

UV–vis and fluorescence spectroscopic indices, e.g., $A_{465}/A_{665}$ ($E_4/E_6$) [41] and $A_{250}/A_{365}$ ($E_2/E_3$) ratios, have been correlated with basic HS properties such as molecular weight, acidity, and aromaticity [2]. In samples of HA—derived from original coal oxidized by $H_2O_2$—the $A_{446}$-to-$A_{665}$ ratio, used to estimate the molecular weight and aromaticity of HA, has been determined by UV–vis spectrophotometry at 446 nm and 665 nm. $^{13}C$ nuclear magnetic resonance ($^{13}C$ NMR) spectroscopy clarified the detailed characteristics of the HA carbon structure, and Fourier transform infrared (FTIR) spectra showed both the existence and the types of HA bonds [42]. Infrared (IR) analysis reveals the active groups and, also, the macromolecular structure of HS. The spectra of FA (typically containing larger amounts of carboxyl groups) differ from those of HA, which contain more aromatic rings [43]. Quantification of coal-derived humic components from mixtures in aqueous solutions has been carried out via ATR FTIR spectroscopy by exploiting the carboxylate bands at 1570 and 1383 $cm^{-1}$ [44]. Peak intensities of diffuse reflectance infrared Fourier transform (DRIFT) spectra are not useful to calculate HS concentrations. However, the relative intensity ratios are constant across a range of concentrations; therefore, the method can be used to quantitatively determine functional groups [45]. Aggregate sizes of $\geq$2 mm produce higher NMR, UV–vis, and FTIR spectral magnitudes in aromatic and aliphatic structures than those of <2 mm. Structural features of the HA and FA fractions are related to organic matter on the soil surface, especially in agricultural systems with species diversity [46]. An increase in HS particle size during the oxidation of ferrous iron to ferric hydrous oxides has been observed, also aggregating the HS that are present in the pore waters of contaminated sediments [47].

Aquatic HS contain both aromatic and aliphatic components, as well as polynuclear aromatic and fused-ring structures. The major aliphatic segments are composed of two to four short saturated chains, i.e., methylene units, and the aromatic rings bear three to six alkyl substituents; branched structures are also present [48]. Structural and functional properties of sodium humate (SH) and commercial lignohumate (LH) have been studied by UV–vis, FTIR, steady-state fluorescence, and $^{13}$C NMR spectroscopies. Samples of SH isolated from brown soil, compost, or lignite are poly-condensed, humified, unsaturated, oxidized, and aromatic while LH samples are characterized by low levels of conjugated chromophores and fluorophores, are less condensed, less humified, and contain simple heterogeneous structural components of low molecular weight and size [49] (Table 1).

**Table 1.** Relative distribution (%) of different humic carbons as obtained from $^{13}$C CPMAS NMR spectra [50].

| **Extract (Origin)** | **Carboxyl-C** | **O–Aryl-C** | **Aromatic-C** | **O–Alkyl-C** | **$CH_3O$/C–N** | **Alkyl-C** |
|---|---|---|---|---|---|---|
| HA (leonardite) | 5.3 | 5.5 | 35.7 | 14.7 | 7.8 | 31.0 |
| HA (oak forest soil) | 9.7 | 3.2 | 15.7 | 26.0 | 13.0 | 32.4 |
| HA (compost) [a] | 11.5 | 4.1 | 14.8 | 22.8 | 11.5 | 35.2 |
| HA (treated lignite) | 5.6 | 4.8 | 64.5 | 6.6 | 2.3 | 16.3 |
| HA (compost) [b] | 11.3 | 4.0 | 14.2 | 28.5 | 11.0 | 31.0 |
| HA (oxidized coal) | 7.7 | 8.6 | 53.1 | 8.8 | 6.1 | 15.8 |
| HA (forest soil) [c] | 6.7 | 3.8 | 15.8 | 26.1 | 11.8 | 35.8 |
| HA (peat) | 9.6 | 5.6 | 20.6 | 16.9 | 10.0 | 37.3 |
| HA (vermicompost) | 10.3 | 4.8 | 15.8 | 23.7 | 13.5 | 31.9 |
| HA (lignite) | 6.7 | 6.1 | 39.9 | 9.2 | 2.3 | 35.7 |
| FA (agricultural soil) [d] | 10.8 | 4.4 | 15.7 | 42.4 | 10.2 | 16.4 |
| FA (agricultural soil) [e] | 15.2 | 3.8 | 7.8 | 32.4 | 13.5 | 27.4 |
| FA (volcanic forest soil) | 13.0 | 3.7 | 15.0 | 40.2 | 11.3 | 16.8 |

[a] After 150 d maturation, [b] after 30 d maturation, [c] fir and beech, [d] loamy, and [e] sandy loam.

NMR is a powerful technique useful in the characterization of heterogeneous matrices such as HS, mainly through its wide range of applications in liquid, semisolid, and solid states. NMR is primarily carried out in the solid state via cross-polarization $^{13}$C MAS NMR spectroscopy; on this basis, a variety of carbons in similar chemical environments are determined altogether, as shown in Table 1 [50] at the corresponding aliphatic, aromatic, phenolic, carboxylic, and quinone regions [51,52]. The composition of HA has been examined using solid-state $^{13}$C NMR. Direct-polarization magic-angle spinning (DPMAS) $^{13}$C NMR spectra peak areas, corrected by cross-polarization spin-lattice relaxation time experiments with total sideband suppression (CP/$T_1$–TOSS), have been used to obtain quantitative intensities [53]; the HA investigated consisted of more than 60% aromatic, carbonyl, and carboxyl carbons. A variety of NMR protocols, i.e., direct-polarization magic-angle spinning and DPMAS with recoupled dipolar dephasing, as well as $^{1}$H–$^{13}$C two-dimensional heteronuclear correlation (2D HETCOR) with or without $^{1}$H spin diffusion combined with spectral editing techniques, may be useful to determine the HS functional groups [54].

Total luminescence spectroscopy (TLS) has been applied to characterize the fluorescence of HS. The spectra discriminate between soil- and aquatic-derived HS, HA, and FA samples. Ionic strength (0–1 M KCl) does not affect the HS fluorescence characteristics, but acidity has a significant effect (pH = 6). At pH values of 2 or 10, good results can also be obtained, but these solution conditions do not represent the humic matrix natural environment [55] (Table 2).

**Table 2.** Mean and standard deviation for elemental compositions of HS isolated from locations all over the world [56].

| Substance (Number of Samples) | Elemental Composition (wt%) [a] | | | |
|---|---|---|---|---|
| | C | H | O | N |
| Unsegregated HS | | | | |
| HA (410) | 55.1 ± 5.0 | 5.0 ± 1.1 | 35.6 ± 5.8 | 3.5 ± 1.5 |
| FA (214) | 46.2 ± 5.4 | 4.9 ± 1.0 | 45.6 ± 5.5 | 2.5 ± 1.6 |
| Humin (26) | 56.1 ± 2.6 | 5.5 ± 1.0 | 34.7 ± 3.4 | 3.7 ± 1.3 [b] |
| HA from different sources | | | | |
| Soil HA (215) | 55.4 ± 3.8 | 4.8 ± 1.0 | 36.0 ± 3.7 | 3.6 ± 1.3 |
| Freshwater HA (56) | 51.2 ± 3.0 | 4.7 ± 0.6 | 40.4 ± 3.8 | 2.6 ± 1.6 |
| Peat HA (23) | 57.1 ± 2.5 | 5.0 ± 0.8 | 35.2 ± 2.7 | 2.8 ± 1.0 [c] |
| FA from different sources | | | | |
| Soil FA (127) | 45.3 ± 5.4 | 5.0 ± 1.0 | 46.2 ± 5.2 | 2.6 ± 1.3 |
| Freshwater FA (63) | 46.7 ± 4.3 | 4.2 ± 0.7 | 45.9 ± 5.1 | 2.3 ± 2.1 |
| Peat FA (12) | 54.2 ± 4.3 | 5.3 ± 1.1 | 37.8 ± 3.7 | 2.0 ± 0.5 |

[a] On an ash-free basis, [b] 24 samples, and [c] 21 samples.

UV–vis and FTIR spectroscopies, as well as elemental analysis [56] (Table 2), have been applied to characterize the HA and FA fractions of HS. The spectra showed humification, the generation of negative charges related to the cation exchange capacity (CEC), and the complexation with metal ions. Degree of humification depends on the C/N and $E_4/E_6$ ratios [57]. FTIR, UV–vis, and fluorescence spectroscopies indicate qualitative differences between resin- and alkali-extracted humic acid fractions isolated from bog soil. Resin-extracted HA fractions contain lesser amounts of aliphatics, carbohydrates, aromatics, and amides; are rich in carboxyl and phenolic groups; have higher $A_{254}/A_{436}$ and $E_4/E_6$ ratios; and exhibit a higher fluorescence intensity and longer wavelengths of emission maxima [58]. Potentiometric titration has been used to study the acid–base properties of size-fractionated HA. The acidic group contents increase as the molecular mass decreases and are related to the HA aromaticity [59]. In HA divided by ultrafiltration into fractions of 300, 100–300, 50–100, 10–50, and 1–10 kDa, and characterized by IR and $^{13}$C CPMAS NMR spectroscopies, the molecules of ≥100 kDa fraction are aliphatic while those of the ≥10 kDa fraction are aromatic, and an increase in carboxyl groups is observed as the molecular size decreases [60]. The heterogeneity of HS has been examined in relation to their molecular size. HA fractions with molecular mass ranges > 300, 100–300, 30–100, and 10–30 kDa have been characterized by SEC, elemental analysis [61] (C, N, H, and O data presented in Table 3), as well as spectroscopic techniques including UV–vis, $^{13}$C CPMAS NMR, FTIR, and FS.

**Table 3.** Elemental composition of FA, total HA, and HA size fractions of different weight average molecular weights [61].

| Sample [a] | Elemental Composition (wt%) | | | | $\overline{M}_w$ (kDa) |
|---|---|---|---|---|---|
| | C | H | O | N | |
| FA | 52.6 | 4.5 | 40.9 | 1.7 | 10.5 |
| HA | 55.2 | 5.3 | 35.8 | 3.3 | >16.8 |
| $HA_1$ | 58.4 | 5.8 | 31.2 | 4.0 | >63.0 |
| $HA_2$ | 57.0 | 5.0 | 34.4 | 3.0 | 24.8 |
| $HA_3$ | 56.6 | 4.6 | 36.0 | 2.4 | 15.7 |
| $HA_4$ | 48.5 | 4.2 | 45.5 | 1.6 | 8.5 |

[a] Extracted with IHSS standard procedures from a humic Gleysol in northern Switzerland.

The HA size fractions differ in their chemistry and composition; smaller fractions contain more chargeable functional groups and a higher amount of aromatic carbon, while aliphatic carbon increases with the molecular weight. Furthermore, the smallest HA fractions and FA fractions of similar molecular weights and carboxyl carbon contents have different chemical compositions; i.e., HA contain more aromatic and less aliphatic carbon [62].

Reverse osmosis (RO) combined with XAD-8/XAD-4 resin adsorption may be used for the identification of water NOM constituents, e.g., the humic material, which can be fractionated on the basis of its hydrophobic and hydrophilic properties. Results have shown that hydrophobicity is associated with a higher molecular mass and increased aromatic contents, while the hydrophilic character corresponds to higher amounts of nitrogen and oxygenated functional groups [63]. Fractions of a soil fulvic acid, a soil humic acid, and an aquatic NOM have been studied using total luminescence (TL) fluorescence spectroscopy. The fluorescence-producing moieties of all samples are in the same size fractions, suggesting that in situ fluorescence can monitor the movement of NOM in aquatic systems [64].

Two-dimensional correlation spectroscopy (2D-COS), based on the absorption and the synchronous fluorescence spectra at different irradiation times, has been employed to characterize the spectral responses of two HS upon UV-A irradiation [65]. The behavior and applications of HS are determined by the relationship between their properties and structures based on the interpretation of humic fractions as supramolecules; structural differences observed in the HS and HA fractions are due to their conformational and structural organizations [66]. Comparison of the HA and FA molecular compositions using pyrolysis gas chromatography mass spectrometry (Py-GC-MS) demonstrates that HA and FA differ only slightly in their characteristic chemical compounds, i.e., phenols, lignin phenols, benzenes, and carbohydrates; but within each sample, FA differ from the corresponding HA in the heterocyclic, mono- and polyaromatic hydrocarbon contributions. Therefore, the binding properties of HA and FA can be ascribed, in addition to their carboxyl and phenolic groups, to molecular architecture [67].

## 3. Interaction of Humic Substances with Soils and Pollutants

### *3.1. Soil Improvers, Chemical Regulators, and Chelating Agents*

Humic substances [68] constitute ~75% of the organic matter weight in most soils and ~50% of the organic carbon in surface waters. The contents of HS vary with the geographic area and soil type; in the case of infertile sandy soils, humic substances are expected to be reduced.

Humic substances perform multiple dynamic and interactive vital roles—protective, accumulative, and ameliorative—in soil physics, chemistry, and biology; they create a framework for sustainable agriculture by enhancing plant growth and nutrition, improving soil fertility, and maintaining edaphic quality. Also, HS increase the buffering and water-holding capacities, shear strength, bulk density, porosity, and aeration of soils. The dark brown color of HS enhances sunlight absorption and improves soil thermal properties, playing a crucial role during the plant growing period. Their organic groups, being mostly carboxyl and phenolic, deprotonate in neutral and alkaline environments, increasing the cation exchange capacity of soils and offering anti-inflammatory properties [69,70].

Additionally, HS promote chemical interactions and surface phenomena (i.e., adsorption of macro- and micronutrients), remove pollutants from waters and soils, act as antioxidants, protect against UV radiation [69], and they may be soil bactericides [71] or plant fungicides [72]; on the other hand, they provide a stabilized environment for microorganisms beneficial to soil fertility. Humic substances may act as stress alleviators, increasing the resistance of plants to both biotic and abiotic factors, e.g., salinity, heat, drought, or reactive oxygen species (ROS); they behave as phytohormones, inducing structural, physiological, and biochemical changes; they accelerate seed germination; and they stimulate the growth of plants. Humic substances have also been characterized as nutrient facilitators for N and P; the dissociation of carboxyl functional groups renders soil pH slightly acidic, favoring the solubility of these micronutrients. In short, HS increase the atmospheric carbon assimilation, enhancing nutrient supply and raising biomass yield, they therefore contribute to the mitigation of the greenhouse effect [70].

Bearing a plethora of organic groups, e.g., –$NH_2$, –OH, –COOH, –$CONH_2$, and –SH [73], HS are expected to be highly susceptible toward complex formation with both organic and inorganic compounds—polar and nonpolar—including anthropogenic organic chemicals and other biocidal substances; also, HS exhibit interesting specific properties, e.g., they are universal amphiphiles with ion-binding capacity nearly 20% of that of a battery storage material [14]. Both the colloidal character and the high surface functionality of HS facilitate the retention of either ionic or molecular pollutants regulating their mobilization and/or immobilization in natural environments [74–76]. Besides their surface functionality, HA amphiphilic colloid nanoparticles with a hydrophobic core have proved useful as solubilizing media, to transport hydrophobic active molecules and to encapsulate nonpolar compounds [69]. Acting as surfactants on this basis, HA can form—in neutral to acidic conditions—micelle-like structures, which are useful not only in agriculture and pollution remediation, but also in medicine and pharmaceuticals [69]. Nevertheless, in the water supply industry, HS can be a nuisance affecting water quality with an undesirable coloration, odor, and taste [77,78]. In soil washing, an in situ remediation method for removing contaminants from the soil by the use of an aqueous washing solution, HS are added to water and utilized as extraction or flushing solutions to recover metals and organic substances [79]. The dispersion and aggregation of HS in aqueous solutions are mainly dependent on pH and affect the HS reactivity, especially in the presence of both small and macromolecular moieties, e.g., metal ions and hydroxides, pesticides, carbohydrates, peptides, clays, and minerals, that tend to aggregate or interact with the humic building blocks [80].

The porous texture—which also assists the retention of xenobiotics by inclusion (Figure 2)—of different HS and their fractions studied via both experimental and computational techniques demonstrated that FA, made of small particles, have the largest apparent surface area (~80 $m^2\ g^{-1}$), most probably due to the fact that as the molecular mass is increased, intramolecular aggregation increases and polarity decreases. Fulvic acids also exhibit the narrowest microporosity distribution. For all humic fractions, the maxima of micro-, meso-, and macropore distributions are identical and independent of the humics origin, revealing that all fractions possess a textural similarity [76]. Here it must be noted that HA compositions may depend on their origin, process of obtainment, and functional groups (e.g., carboxyl, phenolic, and quinone) [69].

### *3.2. Complexation with Nutrient and Contaminant Metals*

A major contribution of HS to soil is their role as chelators associating with soil metal ions (both micronutrients and heavy metals). Metal uptake by HS depends on the type of soil, the metal, and the concentration of HS; it changes the speciation of metals in soils and regulates the mobility, bioavailability, and translocation of nutrients and toxics, the HS acting as geochemical barriers to the acute toxicity of the pollutants [81].

The presence of heavy metals in soils as a result of urbanization and industrialization constitutes a prime danger to the environment and to human health. Heavy metals, toxic and nonbiodegradable, can easily find their way into the environment and the food chain, threatening ecosystems and humans [82].

Owing to its simplicity and high efficiency, soil leaching is considered an important cleanup technique for contaminated soils. In this case, heavy metals are removed via their complexation with washing agents [82]. HA are perhaps the most widely encountered natural complexing ligand. Almost all metals detected in soil (both the natural components and those induced as pollutants) can interact with HS, including major and trace elements, alkaline earth and transition metals, as well as actinides. Metal ion–HS interactions are omnipresent in both aquatic and terrestrial ecosystems; in addition to complex formation and/or chelation (attachment of metal ions to HS surface groups and $\pi$-binding), they may include weaker interactions involving, e.g., electrostatic attraction. Complexation of the metal ions with the various nitrogen- and oxygen-containing HS functional groups, as well as carbon–cation $\pi$-association between HS aromatic rings, e.g., quinones, and metals enhance the effectiveness of metal immobilization and alter the electroactivity of HS. As a result, metal ions may remain dissolved or become coprecipitated with HS, especially when scavenged by humic-modified mineral surfaces (Figure 2). Soil pH markedly affects the process; in general, as metal ions are more soluble at high pHs, alkaline soils increase the metal-ion mobility [83,84]. Furthermore, appropriate amounts of HA added to soils may improve the efficiency of phytoremediation techniques [85].

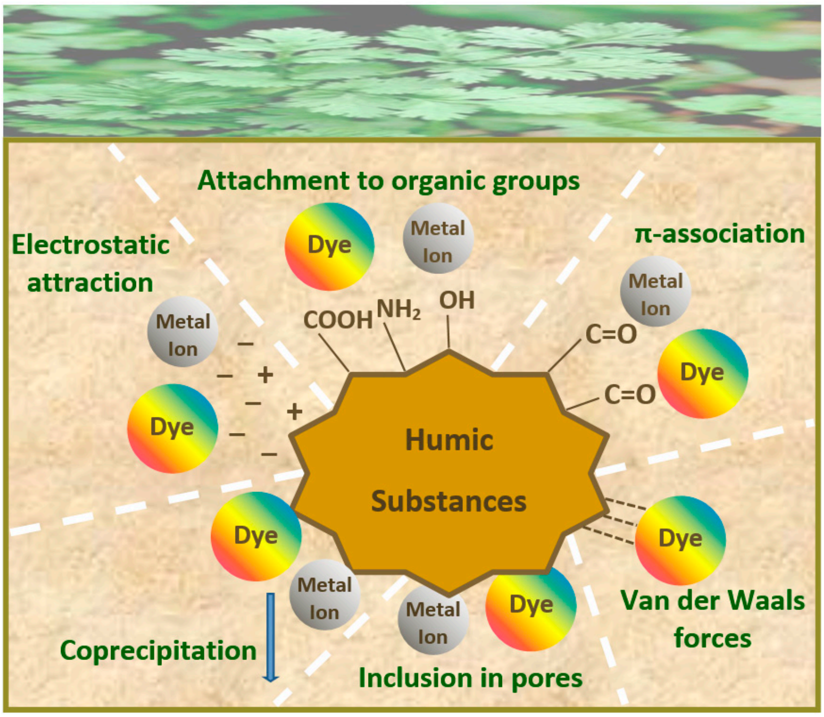

**Figure 2.** Association of metal ions and dye molecules with humic substances.

The changes in HA electroactivity after complexation with metal ions have been correlated with a different biological role of HA; in addition to reducing the toxicity and bioavailability of pollutants, HA may also catalyze their oxidation by both anaerobic and aerobic microorganisms by acting as electron-shuttling compounds. In this case, HA transfer electrons from humic-reducing bacteria to distant electron acceptors using the quinone and phenolic hydroxyl groups, as well as the N- and S-containing moieties [86].

In the case of $Ca^{2+}$, the metal ion–HA interactions can be adequately described using only electrostatic forces of attraction (Figure 2). In fact, theoretical calculations (counterion condensation–Donnan model) have demonstrated that the $Ca^{2+}$–HA association includes counterion condensation in the intraparticulate double layer, while the binding of the transition metals Cd(II), Pb(II), and Cu(II) with HA also involves inner-sphere complex formation [87].

Besides the regulation of the nutrient and toxic elements in ecosystems, complexation of HA [88] with metal ions has been proved, as mentioned, to be an asset in studying the HS aggregation properties and the formation of nanoparticles [88,89]. Metal (Cd, Cu, Pb) ion–humic material interactions have been examined via field flow fractionation–inductively coupled plasma mass spectrometry (FFF-ICP-MS). The sizes of HA aggregates (2.9 nm to 5.8 nm), their size distributions, and the diffusion coefficient of humic acids in solution ($1.51 \times 10^{-6}$ $cm^2$ $s^{-1}$) have been determined. Small and large humic aggregates display higher affinities for Cu and Pb, respectively [88]. Silver nanoparticles have been fractionated by flow field flow fractionation to examine their size change in the presence of HS. A reduction in size is observed and the nanoparticles are largely dispersed, as the humic matter improves their stability by forming a surface coating under environmentally realistic conditions, increasing Ag bioavailability [89]. The physicochemical behavior of metal ions in the natural environment has been examined in HA–metal ion–mineral ternary systems. AFM and scanning electron microscopy (SEM) have been applied to study the shapes of heterogeneous macromolecular structures of HA adsorbed onto the basal-plane surface of mica and silicon under different conditions. Spherical colloids of HA are formed at low pHs and high-ionic-strength conditions; networks or linear structures are observed at a high pH and low salt concentration [90].

## 4. Dyestuff and Color Removal by Humic Substances

### *4.1. Dye Adsorption*

Adsorption of contaminants on humics has been extensively investigated; e.g., polycyclic aromatic hydrocarbons (PAH) can undergo rapid sorption onto surfaces of organic matter (i.e., HA and FA) in soil matrix, resulting in a decreased PAH bioavailability [79]. Humic substances, presenting polyelectrolytic characteristics with structural units involving condensed aromatic rings connected with phenol groups and attached chelant groups [73], interact with all classes of ecotoxicants, including azo dyes [91]. The synthetic-dye industry and dyeing processes are amongst the top ten sources of pollution [92]. The presence of synthetic organic dyes in groundwater, soil, and surface water has been reported; dyes are considered to be micropollutants, are visible to the eye at very low concentration, i.e., 1 mg $L^{-1}$, in aquatic environments [93], and can poison living organisms (e.g., bacteria) at solution concentrations as low as 0.5 mg $L^{-1}$ [94]. Dyes from effluents of dyestuff manufacturing industries and the residual unfixed dyes present in exhausted aqueous dyebaths result in environmental pollution after their discharge into the receiving waters. Colorant removal is of crucial significance in relation to water of the desired quality. Retention experiments have demonstrated that HS (Figure 2) are efficient dye adsorbents. Therefore, HS–dye association leads to the removal of the sorbed dyestuff from both soils and waters; this process appears to be a promising remediation strategy [95].

Regarding the retention of dyes, humus-based filters originally prepared in the late 1990s for sewage purification proved useful in dye removal from wastewaters and other aquatic systems. It is worth noting that, initially, HS were employed in leather processing as a dye, a tanning agent, and an ingredient of finishing solutions; in the woodworking industry to prepare a “natural indigo” for wood veneer dyeing; and in the production of plastics as dyes or plasticizers [96].

Commercial HS have been found to associate rapidly with toluidine blue to produce a dye–HS complex peaking at 630 nm, used for HS determination at microgram levels in natural waters. At increased pH values (pH = 11), more dye molecules are bound to the HA matrices [97]. By application of the Langmuir model to the adsorption data, a total number of $1.45 \pm 0.04$ mmol $g^{-1}$ binding sites has been calculated [98]. A dye–HS complex is also formed when HA taken from an Andisol interact with an oxazine dye (oxazine-1 perchlorate). The amount of bound dye depends strongly on the ionic strength and less on the solution acidity. However, the number of adsorption sites in humic acid for binding oxazine increases with pH and decreases with the electrolyte concentration, affecting the amount of dye retained [99]. The complexation of C.I. Basic Blue 3 with commercial potassium humate on surface sites available for deprotonation and potential adsorption of cations has been verified by spectroscopic techniques [95]; the dye–HS interactions involve attraction between the positively charged dye and the dissociated carboxyl groups of HS favored at pH = 4. This complexation mechanism explains why potassium humate is an exceptionally efficient adsorbent towards C.I. Basic Blue 3. Anthraquinone disperse dyes, i.e., 1,4,5,8-tetraaminoanthraquinone and 1,4-diaminoanthraquinone, have also been shown to interact with HA, increasing their solubility in the HA solution, especially at low ionic strength and high temperatures [100].

Certainly, the changes observed in the UV–vis spectrum of a dye in the presence of humic matter are the most valid proof of dye–humics association; it is worth noting that when brilliant blue (or C.I. Acid Blue 9) is sprayed onto turf grass, a green color is imparted to the field. The color shift from blue to green has been attributed to the interactions between the acid dye and the humic material present [101]. Additionally, to select suitable fluorescent dye tracers in hydrology and aquatic studies, the fluorescence quenching impacted by HS on polycyclic aromatic compounds has been employed to investigate the interactions of dissolved IHSS standard HS (humic and fulvic acids) with three fluorescent dyes, these being xanthene derivatives, i.e., fluorescein (C.I. Acid Yellow 73), rhodamine 6G (C.I. Basic Red 1), and rhodamine B (C.I. Basic Violet 10) [102]. Among the three dyes, the fluorescence of rhodamine 6G is largely quenched by HA, and a nonlinear Sterne–Volmer plot is obtained, caused by both electrostatic interactions and hydrophobic repulsions between the cationic dye and HA.

Commercial iron humate has been examined as a new low-cost sorbent for removing the basic dyes methylene blue (C.I. Basic Blue 9), malachite green (C.I. Basic Green 4), methyl violet (C.I. Basic Violet 1), crystal violet (C.I. Basic Violet 3), and rhodamine B from waters. Both the pH and the presence of inorganic salts have negligible impact on the adsorption, while the presence of an anionic surfactant increases dramatically the methylene blue adsorption. The two main mechanisms considered involve, firstly, the association of cationic dyes onto polar (or cation-exchange) active sites and, secondly, relatively small dye–surfactant aggregates onto nonpolar sites of the sorbent [103].

Interactions between HA extracted from a South Moravian lignite and two cationic dyes (methylene blue and rhodamine 6G) have been examined via diffusion and adsorption experiments to shed light on the mechanism of HA interactions with low-molecular compounds of amphiphilic nature, i.e., charged aromatic solutes; in this case, the dye–HA interactions involve electrostatic attraction between oppositely charged compounds and impact the rate of the diffusion process. Dye transport proved mostly dependent on both the affinity of the pollutant to HA/NOM and the strength of the HA active binding sites but is independent of the HA origin [104]. This effect is almost equally approved for both original and methylated HA obtained via esterification of the HA carboxyl groups. Humic acid methylation leads to higher surface area and greater microporosity without affecting the particle size distribution, a fact possibly explained by the different morphology adopted by the alkylated HA [105].

In addition to HA, humin has been employed in cleanup methods for the adsorption of reactive dyes, i.e., removal of C.I. Reactive Red 120 and C.I. Reactive Orange 16; dye retention is favored mainly at pH = 1 [106]. Peat, having a high abundance of HS, can also be utilized in textile wastewater remediation [107]. These results point to the flexibility of HS molecules, which swell (mainly at high pH and low ionic strength), increasing the availability of binding sites. Moreover, HS can strongly affect both the mobility and fate of dyes—and related pollutants—in the environment [99].

Standard and reference HA have also been reported [108] to assist the decolorization of an azo dye (C. I. Acid Red 27) under anoxic and anaerobic conditions in the presence of *Shewanella oneidensis* MR-1 bacteria; in this case, humic acids act as electron acceptors, i.e., redox mediators. The positive synergistic effect of HA has been found to increase with HA concentrations. In addition, HA have been shown to induce dye degradation besides dye adsorption via environmentally persistent semiquinone free radicals (hydroxyl and superoxide ones) generated on the biosorbent surface by irradiation and promoted by the presence of $O_2$ and $H_2O_2$. Thus [109], methylene blue, crystal violet, and malachite green elimination can be processed in two steps, namely, adsorption and decolorization of the dye, while the consumption of free radicals is proportional to the rate of dye degradation. Additionally, $Al_2O_3$ nanoparticles have been employed as immobilization matrices for the coaccumulation of humus-reducing microorganisms and HS to promote the biological treatment of dye wastewater in an upflow anaerobic sludge bed reactor, i.e., to achieve the reductive decolorization of the recalcitrant azo dye C.I. Reactive Red 2 [110,111]. Finally, when C.I. Acid Red 27 is decolorized by *Shewanella* algae in the presence of HA, this mediated degradation reaction produces less phytotoxic aromatic amines [112,113]. However, in the case of rhodamine B decomposition by $Co_xMn_{3-x}O_4$ particles via free radicals, the presence of FA has been reported to decrease the dye degradation. This reduction has been attributed to the fact that FA competed with rhodamine B for the reactive radicals [114,115].

### *4.2. Humic-Matrix Hybrid Materials in the Removal of Dyes*

Hybrid organo-inorganic materials have been prepared and used for colored wastewater remediation. In this context, $Fe_3O_4$ and chitosan, conventional adsorbents used for the retention of dyes and heavy metals, have been superficially modified with HS obtained from dry horse dung powder to achieve higher efficiency. The new adsorbent proved successful in retaining both methylene blue and Pb(II) over the pH range of 5 to 6 via ion exchange and chemical adsorption, respectively [116]. Humic acid (sodium salt) has been, also, immobilized onto $Fe_3O_4$ using the eco-friendly coprecipitation route (reaction of HA with $FeCl_3/FeSO_4$) to produce high-surface functionalized nanosorbents bearing extended magnetic saturation, applied to remove the carcinogenic dye malachite green from water. The highest dye removal has been achieved at pH = 6; sorption has been characterized as chemisorption, endothermic, feasible, spontaneous in nature, and involving the formation of multilayers [117]. With the use of the coprecipitation technique, humic acid extracted from the peat soil of South Sumatra has been deposited onto $Fe_3O_4$ and utilized in malachite green removal [118,119]; in fact, from the mixture of dyes employed—containing malachite green, methylene blue, and rhodamine B—the adsorbent demonstrated selectivity toward malachite green, attributed to the smaller size of this dye. Compared with the parent HA, $HA/Fe_3O_4$ is stable, with enhanced adsorption capacity, and can be regenerated [120]. Perlite, an inorganic aluminosilicate eco-friendly material, has been superficially modified with commercially available HA and tested for methylene blue retention via a reaction between acylated HA and amino-bonded perlite. The deposition of HA enhances dye adsorption compared to the original expanded perlite. Factors affecting the adsorption process are pH, ionic strength, and contact time [121]. A humic acid has been impregnated into Zr-pillared montmorillonite via inclusion into the interlayer space of the pillared clay at low pHs, and the adsorption of methylene blue, crystal violet, and rhodamine B onto the hybrid material produced has been tested [122]. The HA-modified clay displays an increased retention capacity compared with the unmodified sample, especially toward methylene blue. Kaolinite, illite, and smectite clays have been used to adsorb HA from water. Adsorption of HA onto clays proceeds via ligand exchange and electrostatic hydrophobic interactions; aliphatic humic fractions are initially absorbed, followed by molecules of higher molecular weight [77]. Clay–HA hybrid materials might be useful in removing organic dyes from irrigation waters.

Surface-active silanized humic derivatives immobilized onto silica gel have been synthesized in aqueous solution; potassium humate from leonardite and low moor peat have been employed. These humic materials are natural hyperbranched polyelectrolytes (with self-assembling properties to form humic adlayers), capable of ionic, hydrophobic, and donor–acceptor interactions due to their surface functionality and amphiphilic properties. Silanized derivatives proved successful in azo dye (C.I. Direct Brown 1) and plutonium removal from contaminated waters [123]. Distinct HA (one commercial and the other isolated from Brazilian peat soil) have been attached onto aminopropyltrimethoxysilane-modified silica gel to produce two new adsorbents for indigo carmine (C.I. Acid Blue 74) uptake [73].

Owing to their polyfunctionality, HS can be immobilized onto various types of surfaces [124]. Alumina, superficially modified by the deposition of HA, has been prepared and tested using batch experiments on cationic dye (i.e., methylene blue) removal from aqueous solutions via a clean and eco-friendly process. The more HA loaded on the alumina surface, the higher the retention of methylene blue. Both pH and temperature assist in dye removal; contrarily, divalent cations and ionic strength inhibit dye adsorption [125]. Another adsorbent tested for methylene blue uptake is HA-crosslinked cellulose possessing an increased surface functionality. In this case, also, dye retention has been proved pH-dependent [126]. Immobilization of HA onto etherified wood pulp cellulose nanofibers bearing a positive charge followed by freeze-drying leads to the formation of a porous foam useful in crystal violet, methylene blue, and Cu(II) adsorption [127].

Hydrogels prepared from chemically pure sodium humate, polyacrylamide, and hydrophilic (laponite RD) clay are hybrid materials demonstrating an outstanding performance in methylene blue adsorption (~800 mg $L^{-1}$/g hydrogel), useful for wastewater cleanup [128]. The adsorption capacity of hydrogels is increased with a rise in the sodium humate or clay content, and less dye is desorbed as the amount of humate increases; both are attributed to the formation of an ionic complex between the imine groups of the dye and the ionized carboxyl groups of the humates. A hybrid biodegradable hydrogel composed of acrylic acid, sodium acrylate, acrylamide monomers, and sodium humate has been synthesized and tested against crystal violet and methylene blue adsorption. When swollen, this hydrogel demonstrates a greater adsorption capacity toward both dyes as compared to its dry state. The addition of sodium humate (2.40 wt%) increases both the swelling ratio and the adsorption capacity of this hydrogel [129]. Similar hydrogels prepared from acrylic acid, acrylamide, and sodium humate by free radical solution copolymerization also exhibit the non-Fickian diffusion and a swelling behavior dependent on the sodium humate content. These hydrogels display an increased adsorption capacity toward both Cu(II) and methylene blue [130].

A composite material suitable for dye adsorption has been synthesized from graphene oxide modified with HA followed by coprecipitation with $Fe_3O_4$ [131]. When this adsorbent is employed to remove methylene blue from wastewaters, it demonstrates a higher retention capacity compared with the precursor graphene oxide. The adsorption has been found to be spontaneously endothermic. The mechanisms behind methylene blue adsorption mainly include electrostatic attractions, $\pi$–$\pi$ interactions, and hydrogen bonding. A composite material fabricated from commercial HA and carboxymethyl cellulose deposited onto calcium hydroxide and ferric hydroxide precipitates [132] shows a high affinity toward methylene blue, retaining about 670 mg dye/g composite. The adsorption process essentially represents ion exchanging and $\pi$–$\pi$ interactions for both ionic and aromatic groups. SEM micrographs show that the composites are rougher than their precursors, exhibiting a larger surface area, a fact that explains their better adsorption performance. The synthesis of a highly effective nanocomposite adsorbent, i.e., $CoFe_2O_4$–HA, via the coprecipitation method for application to methylene blue and Remazol Red RR 133 (C.I. Reactive Red 198) adsorption has been reported [133]; the HA, extracted from peat soil, improve dye removal rates. Retention—fast and pH-dependent—of both dyes is attributed to a process of chemisorption. Deposition of HA onto hematite ($\alpha$-$Fe_2O_3$) particles results in a material used for crystal violet retention [134]. Dye adsorption proved to be temperature- and pH-dependent; theoretical calculations suggest that the dye–composite interactions occur on the outer part of the hematite–HA material and, mostly, at the active sites on composite surfaces.

Finally, a carbon composite based on HA has been synthesized by the partial carbonization of commercial HA in the presence of $Al_2(SO_4)_3$ and $(COOH)_2$ as an environmentally friendly and inexpensive alternative to remove anionic dyes, i.e., Congo red (C.I. Direct Red 28), from wastewaters. This mesoporous material possesses high surface area and many Al–O bonds, both enhancing its adsorption potential compared to the raw HA [135].

## 5. Conclusions and Future Perspectives

This work forms the basis for the use of HS in applications such as water and soil remediation. Humic substances, produced from biochemical weathering of animal and plant remains [20], have long been recognized as playing an important role in agriculture and the environment; present in both aquatic and terrestrial ecosystems, they constitute a large pool for organic carbon and nitrogen, contain redox moieties with excellent chelant properties, and are indispensable for soil and plant health. Researchers have employed all the modern instrumental analytical techniques to resolve the elusiveness regarding HS structure and characterization arising from the complexity and irregularity of HS due to differences in their origin, organic residue inputs, and pathways of their formation, but we are still far from considering HS as strictly defined molecules. Missing, also, is an understanding of how the chemical composition, the molar mass, and the architectural arrangement of HS molecular associations influence their behavior in natural systems.

Owing to their molecular size and architecture, amphiphilic colloidal nature, aromatic and ionic structure, ion binding capacity, surface reactivity and charge, degree of hydration, and porous texture, HS have proved remarkably efficient in adsorbing persistent organic pollutants, e.g., dyes; synthetic dyestuffs discharged into water bodies increase both the BOD and COD, reduce light penetration, and inhibit the photosynthetic process, therefore, their removal is of significant importance. When introduced into agricultural soils, dyes enter the food chain providing biomagnification, and can act as toxic and mutagenic agents, being extremely harmful to soil microorganisms and plants if not properly managed.

Humic substances are widely applied in agriculture as plant growth promoters exhibiting bactericidal and fungicidal properties—supporting both the sustainability of natural ecosystems and novel strategies for environmental protection—, in medicine, pharmaceutical, and cosmetic areas as solubilizing agents and to allow the transport of hydrophobic active compounds. Apart from that, the average properties of structurally heterogeneous HS, mostly their amphiphilic character and chelating functionality, have not yet been fully exploited and require further investigation. Future research may involve efforts to improve methods for the global standardization and chemical identification of HS. More importantly, much work should be undertaken to correlate the structural characteristics of HS molecules and fractions with the dye retention capacity; possible mechanisms for dye adsorption onto HS have not been thoroughly studied and must be confirmed on a case-by-case basis to establish specific tailored dye-pollution management systems. The diverse chemical and physical composition of HS is responsible for the facts that a single method cannot be utilized for the different HS–dye systems and that there is no universal mechanism that describes the manner in which HS–dye complexes are formed.

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