We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Humic Substances: Its Toxicology, Chemistry and Biology Associated with Soil, Plants and Environment

*Rajneesh Kumar Gautam, Dimuth Navaratna,
Shobha Muthukumaran, Amarendra Singh, Islamuddin
and Nandkishor More*

Abstract

In recent decades, scientists in different disciplines have been increasingly concerned about the fate of natural organic matter, and in particular of humic substances (HS). The term humic substances (HS) incorporates refractory autochthonous and terrestrial organic matter in the soil and aquatic ecosystem, and are one of the key fractions of natural organic matter. These substances are important chelators of trace elements constituting complex class of molecular structures that occur naturally, consisting of aggregation and assembly processes in which biomolecules derived from plant and animal residues are gradually transformed through biotic and abiotic tracts. Since these organic compounds are bound by or linked with soil mineral fractions, they must be physically or chemically separated from the inorganic components by an extraction method before their physico-chemical study. This chapter focuses on the chemo-toxicological, molecular aspects of humic compounds and their derivatives such as humins, fulvic acids, humic acids etc., with their agricultural, biomedical, environmental and biochemical applications. In addition to studying their impact on plant physiology and soil microstructure to expand our understanding about humic compounds.

Keywords: humic compounds, Humins, fulvic acids, humic acids

1. Introduction

Humic substances are a structurally specified fraction of soil organic matter that are thought to be an important component of the biosphere, as they are responsible for many physical and chemical processes in soil. Organic carbon accounts for approximately 62 percent of global soil carbon, with at least half of this carbon classified as the chemically resistant portion known as humic substances (HS) [1–3]. Plant and microbial biomass are the primary sources of organic matter production in soil, and their composition and properties are important major constraints for humification processes. When looking at the structural aspects of HS, we must understand the contributions of the different compound groups that make up those tissues. These compound types include intracellular (i.e. protein) and structural (for example, polysaccharides, lignin and aliphatic) compounds that

shape membranes and cell walls, including membrane lipids and plant cuticles [4]. Due to their intimate relationships with soil mineral phases, they cannot be easily decomposed and are chemically complex to be used by microbes. One of the most notable properties of HS in soil is their ability to interact with metal ions, oxides, hydroxides, mineral and organic compounds [5], including toxic contaminants [3], to form water-soluble and water-insoluble complexes [4, 5].

Humic substances (HS) are important elements of natural organic matter (NOM) in soil, water, as well as geological organic substances including shales, brown coals, peat and lake sediments. They contribute to the brown or black color of surface soils and make up much of the characteristic brown color of rotting plant waste. They are major components of NOM in surface waters and can impart a dark color at higher concentrations, especially in brown fresh water ponds, lakes, and streams. The color of composts due to leaf-litter can range from yellowish-brown to black, depending on the degree of decay and concentration. It is well understood that HS are the most significant source of transient refractory organic carbon in the earth's crust. Conventionally, the mixtures are processed into humic acids (HA), fulvic acids (FA), and humin (Hu). HA is recognized as the fraction precipitated from an aqueous solution at $\text{pH} < 2$ (under acidic conditions), FA residues in solution after HA precipitation, and the Hu fraction is insoluble in aqueous solvents [6].

2. Nature and origin of humic substances

Humic substances are soil complexes produced during humification phase, are responsible for fossil coal, oil deposits, and other geological deposits. It is the Earth's second most important organic phase, after photosynthesis. It is estimated that photosynthesis binds more than 5000 tons of atmospheric carbon per year, while the transformation of dead living organisms results in the creation of approximately 4000 tons of carbon [7], which is deposited annually on the Earth's surface. Humic substances are naturally occurring organic material that are formed due to biochemical, chemical, and physical changes in dead plants, microbial residues, and fauna debris. Humic material formation is linked to the behavior of microorganisms, resulting in the accumulation of recalcitrant macromolecular compounds with unique properties [7]. Due to death of microbes a recalcitrant humic mass is created due to their assimilation. These progressive decay leads to biotic and abiotic transformation processes which ultimately produces diverse and heterogeneous mixtures of polydisperse materials, which are a major component of natural soil organic matter. Humic material can take hundreds or thousands of years for their formation, resulting in a wide range of humic substances. Humic substances are extremely heterogeneous in composition, structure, and are derived from a variety of organic matter sources. This sets them apart from naturally occurring biomolecules such as proteins, carbohydrates, lipids, and others.

Humic substances happens to be the most common of the various organic substances found in nature. They account for approximately 25% of total organic carbon on Earth and can also be found in a variety of ecosystems such as lakes, rivers, sea sediments, soil, and coal composites. Humic substances influence the chemistry, cycling, and bioavailability of chemical elements in terrestrial and aquatic environments, as well as the distribution and degradation of xenobiotic and organic chemicals of natural origin [7]. For decades, scientists have discussed the formation mechanisms of HS [8, 9]. The lack of repeated sequences, as well as the variety of chemical and biological reactions involved in their formation, render HS very complex and multifaceted molecules capable of important signaling and

nutritional functions in the soil–plant system. The polymeric origin of HS has a high molecular mass (100–300 kDa) [10], derived from lignin decomposition and abiotic catalysts such as primary minerals and layer silicates. Humic compounds are classified into three categories: (1) Humin, (2) Humic Acids (HAs), and (3) Fulvic Acids (FAs) [11]. **Figure 1** summarizes several of the major chemical characteristics of humic compounds.

Figure 2, illustrates the hypothetical model structure of fulvic acid (Buffle’s model) [13], which contains aromatic and aliphatic structures, both extensively substituted with oxygen containing functional groups [15, 16].

2.1 Humus

Humus is generally brownish or black colored complex variable of carbon-containing compounds that does not show cellular organization in the form of animal bodies or plant under a light microscope. Humus is distinguished from non-humic substances such as carbohydrates (a primary component of carbon), fats, waxes, alkanes, peptides, amino acids, proteins, lipids, and organic acids by the fact that distinct chemical formulae for these non-humic substances can be published. The majority of small molecules of non-humic compounds are easily destroyed by soil microbes. Soil humus, on the other hand, decay slowly under natural soil conditions. When combined with soil minerals, soil humus can survive in the soil for centuries. Humus is the primary component of soil organic matter, accounting for 65 to 75 percent of the total. Humus plays an important role as a fertility factor in all soils, far outweighing its percentage contribution to total soil mass.

2.2 Humins

Humins are derived from humic compounds that are not soluble in alkali, not soluble in acids, and are insoluble at neutral pH ranges. However, the physical and

Figure 1.
Chemical characteristics of humic compounds (modified and adapted from [12–14]).

Figure 2.
Model structure of fulvic acid [13].

chemical properties of humins are partly known. Humin complexes are also recognized as macro organic substances due to their large molecular weights (MW) ranging from 1×10^5 to 1×10^7 . Conversely, the molecular weights of carbohydrates range between 5×10^2 and 1×10^5 [17]. The humins are highly resistant to decomposition of all humic substances found in soil. Humins are known for enhancing the soil's water holding capacity, improving the soil fertility, taming soil structure, retaining soil stability, and acting as a cation exchanger.

2.3 Humic acids

Humic acids (HA) are organic acids that are a combination of weak aliphatic (carbon chains) and aromatic (carbon rings) organic acids that are insoluble in acidic water but soluble in alkaline water [18]. Humic acids are composed of humic compounds that precipitate from aqueous medium when the pH drops below 2. Due to their variable chemical composition, humic acids (HA) are also known as polydisperse. These complicated carbon-containing compounds are assumed to be 3-dimensionally flexible linear polymers that reside in the form of random coils with cross-linked bonds. On average, 35 percent of humic acid (HA) molecules are aromatic (carbon rings), with the remaining components being aliphatic (carbon chains) [17]. Humic acids (HAs) have molecular sizes ranging from 1×10^4 to 1×10^5 . Clay minerals readily bind to humic acid (HA) polymers, forming stable organic clay complexes. Natural and synthetic-organic chemicals can be accommodated in the polymer's peripheral pores in a lattice (clathrate) arrangement. Humic acids (HAs) readily combine with trace inorganic mineral elements to form salts. It is reported in the literature that there exists more than 60 distinct mineral elements in naturally occurring humic acids (HAs). These trace elements are bound to humic acid molecules in a form that is easily assimilated by a variety of living organisms. As a result, humic acids (HAs) play an important role in ion exchange and metal complexing (chelating) [19].

2.4 Fulvic acids

The proportion of humic substances that are soluble in water at all pH levels. They are known as fulvic acids because they remain in solution after humic acid has been removed by acidification (FA). Fulvic acids range in color from light yellow to yellow-brown. The elemental composition of various FA and HA reveals that C, H, O, N, and S are the main elements in their composition. These major elements are always present, regardless of their birth, country of origin, or continent of origin. An examination of a broad range of HS reveals that the percentage composition of C, O, H, and N varies as follows: C (45–60),

Substances	% Dry ash-free basis			
	C	H	N	O
Lignin	62–69	5.0–6.5	—	26–33
Proteins	50–55	6.5–7.3	15.0–19.0	19–24
Humic acids	52–62	3.0–5.5	3.5–5.0	30–33
Fulvic acids	44–49	3.5–5.0	2.0–4.0	44–49

Table 1.
Composition of several plant material and humic substances (by Kononova) [16, 21].

O (25–45), H (4–7), N (10–13) and inorganic elements (ash) 0.5–5 **Table 1** [20].
summarises the composition of humic substances.

3. Preparation of fulvic and humic acids: a toxicological evaluation

The general properties for HA, and FA relate to their basic constituents (carbon, hydrogen, nitrogen and sulfur) and their oxygen-containing functional groups (hydroxyl, carboxyl, carbone and phenolic) which give rise to their overall chemical properties, including their ability to react with ionic particles in solution. Because of their properties, HAs and FAs have been used in a variety of agricultural applications, including improving nutrient and water use, soil quality, and carbon sequestration [11]. Although a variety of mechanisms have been proposed and/or studied for the effects on humic substances on plant life, much can be learned from the imperfect understanding of these mechanisms. One such process is ion exchange, which allows the use of plant minerals by prevention of the development of insoluble mineral complexes in the soil and then enhances humic substances in exchange for hydrogen and carbonic acid, which can produce absorbable mineral ions for plant roots. In sequestration of radioactive metals in soil, the ion-exchange process also works [22]. As humic substances play a role in maintaining plant life and their ability to attach or sequester potentially harmful environmental toxicants, they can also easily be extrapolated such that they can improve human nutrition and provide some protection from unintended ingestion of dietary toxic elements.

Two active New Dietary Ingredient Notices (NDIN) for use in a dietary supplement containing additional minerals to improve the status of minerals and trace elements were subjected to a specific preparation of HA and FA derived from Hungarian peat [20]. The NDIN has recorded 9 unpublished and one published clinical evaluations in which mineral status in humans has been improved and/or absorption is inhibited and toxic element excreted [23]. However, it is not clear if these effects can generally be extrapolated to humic substances due to the possible variations between this and other humic preparations as well as the inclusion of exogenous minerals. The use of clinical or mechanical research on other humic preparations, important for humans, is restricted and there are no other studies of the above-mentioned results, although it was shown that the addition of a humic extract to the broiler feed was intended to improve the growth of chickens. Due to the heterogeneous existence of the humic substances, an extrapolation for the whole community has not been deemed sufficient toxicological evaluation of one particular unique material, while attempts have been made for the identification of an appropriate model. As a result, a number of toxicological studies have been reported on humic materials of diverse origin even though many of them have been performed to examine the mutagenic potential of byproducts produced during water disinfection because of the normal occurrence of low levels of humic material

in surface waters. Chlorination of organic non-volatile substances found in HA and FA is known to lead to the development in bacterial mutation tests of compounds (such as mucochlorine acid and 2, three, three-trichloropropenal) under conditions of decreasing pH and sufficient chlorine and HA and chlorine.

4. Plant physiology and humic substances

Various authors have assumed that even though high molecular weight (HMW) and small molecular weight fractions (LMW) appear to be behaving differently, HS may be adsorbed by the root plant [24]. The mechanisms by which HS interacts with the root cells affecting the plant physiology and growth have not been significantly researched until today. Changes in size and growth were the first to be analyzed among the modifications caused by HS on treated plants. HS will promote plant growth with a view to increasing plant length and dry and fresh weight under specific conditions. These effects are apparently dependent on the dosage and source of the drug, the plant species and age and the conditions of the trial cultivation [25]. Many studies have recently confirmed the assumption that HS has direct effect on plant physiology, especially in regard to the development of root hair and side root [26, 27]. The impact of HS has been thoroughly evaluated on metabolic processes in plants. For example, several studies have shown that HS affects breathing, protein synthesis and the function of the enzyme in higher plants [28, 29]. With regard to the photosynthesis process, few reports are available which focus on the contents of chlorophyll and transport of electrons.

4.1 Humic substances and abiotic stress regulation in plants

The productivity of crop plants will benefit significantly from regulation that mitigates the impact of abiotic stress on the growth and production of crops during ontogenesis. There are insufficient information currently available on control mechanisms and techniques. Optimizing plant growth conditions and providing regulators for water, nutrients and plant growth will help in avoiding abiotic pressure [30]. Bio-stimulants such as HS are also used in production systems with the aim to regulate physiological events in plants in order to improve productivity as well as these traditional strategies.

In the last two decades, scientists have increasingly utilized HS from natural materials [31]. In addition, HS provides new methods to alter physiological activity in plants to increase plant growth and abiotic stress tolerance. A number of examples show the ability of HS to improve plant abiotic stress tolerance. Treatment with hydrated leaves, anti-oxidants, radical growth and shooting under dry conditions, increased hydration of the leaves (*Agrostis palustris*) and increased fescue (*Festuca arundinacea*) [32]. Recent studies have shown that the blocking of cell wall pores by fouling has been responsible for the effects induced by HS that could be related to temporary mild water stress (a beneficial stress, 'eustress') and the downstream effects of HS on hormonal signals and plant growth [33].

4.2 Humic substances and improved mineral nutrition in plants

HS in the soil promotes root and spring growth through the improvement of mineral nutrition below the soil surface. In terms of plant production and active development, the function of these substances can be assessed [34], by their complementary and possibly diverse effects. HS regulates plant growth and mineral assimilation. In general, these effects are classified as direct and indirect.

HS activities rely mainly on the structural features, functional groups and their inclination to associate with inorganic, organic and soil substratum molecules [35]. Furthermore, HS primarily affects the bioavailability of nutrients through its ability to form complexes containing metallic ions, thus enhancing micronutrient availability (zinc, manganese, copper and iron). On the other hand, HS targets plant cell membranes that can cause biochemical and molecular processes at post-transcriptional levels in the roots and shoot [32]. Targeted HS typically increase macronutrient and micronutrient consumption in plants.

Jackson [36], used ^{14}C HS-listed substances, and found a small portion of them entering the root apoplastic pathway, especially those of low molecular weight. Therefore, the effect of HS to promote molecular systems and signal pathways of nutrient absorption present in cell membranes can be controlled. The full advantage of this direct influence is still unknown. Non-specific HS activity on the leaf and root can, however, be speculated on by controlling events at both the transcriptional and post-transcriptive stages, to modify molecular and biochemical processes.

5. Bimolecular fragments in soil humic substances

Humic soil fractions comprise bio-molecular fragments that were explicitly omitted from conventional humic concepts. Many of these bio-molecular movements are closely linked to the humic fraction by seven covalently and cannot be effectively isolated [37]. Why should a whole group of constituent molecules be excluded? Simply because it can be classified as biomolecules if we consider humic material as a super-molecular association of constituent molecules. It is inappropriate and deceptive for these nuanced, heterogeneous mixes to purge humic fractions of closely related, identifiable bimolecular components [38]. A description that is applicable to experimental studies of the humic component should include all molecules which are closely linked within a humic fraction, i.e. those which cannot be eliminated without substantial changes in the chemical characteristics of the fraction. That will necessarily include biomolecules that are identifiable and play important structural roles. Will this wider concept of humic molecules be contrary to the recognized refractory nature?

Soil humic compounds are typically aged between hundreds and thousands of years on average and many biomolecules easily decomposed by energy-seeking microbes and nutrients. However, it can lead to biomolecules being protected against microbial degradation by binding them with humic fractions, creating identifiable but refractory biological molecules [39], is alternatively a “two-part” view of the stability of humic substances. Although the majority of humic material is permanent, some humic elements may be transient. The persistent material contributes to ^{14}C , while the transitional components will contribute mainly to the C turnover rate. A significant portion of the transient, often refilled humic components may be made up of closely related, but easily degraded biomolecules. While most humic materials are permanent, some humic elements may be temporary. The continuous material contributes to ^{14}C , while the intermediate components are primarily responsible for the C turnover rate [40]. A large part of the frequently refilled transient humic components can consist of closely related, but easily degraded biomolecules.

6. Application of humic substances

Humus is one of the largest reserves of carbon in the world. To date, industrial uses of humus and goods extracted from humus have been rare. In the second half of the 19th century and the first half of the 20th century the use of coal was more

abundant and, in addition, it was a foundation of chemical production. The application Petroleum was also considered the key raw material of 20th-century chemical industry. HS applications can now be broken down into four major categories: agricultural, industrial, environmental and biomedical.

6.1 Agriculture applications

From an agronomic and agricultural point of view, HS plays a significant role as they have a huge impact on soil quality and productivity. HS also shows a high Base Exchange potential, which is essential to soil fertility, in addition to enhancing the soil's physical properties and humidity. Many researchers and humates have observed the growth-promoting effect of humic substances in various preparations for plant growth improvement. Soil productivity in the presence of humic materials can be improved by various methods. However, in some case the consistency and fertility of the soil is greatly affected. In addition to the improvement of physical properties and moisture of the soil, HS shows a high Base Exchange potential, which is important for soil fertility. In various preparations for plant growth enhancement, several investigators have studied the growth-promoting effects of humic substances.

6.2 Environmental applications

Natural organic colloids are important because they form water solutions with a large number of metals, including radionuclides. It is well known that the humic substances affects the absorption of natural solids into the surface and ground waters of radionuclides. Thus, these organics may be essential as a transport agents in the environment for radionuclides. In environmental chemistry, the key role of humic substances is to extract radioactive metals, organic chemical substances of anthropogenic nature and other contaminants from water [18]. Calcium humate ion exchange products were considered suitable for the extract from the water and removal of radioactive elements from water discharges from nuclear power plants for heavy metals such as iron, nickel, mercury and cadmium and copper [11]. They also use their selective bonds to destroy ammunition and chemical warfare agents [41]. For the last 30 years, interactions between humic and micro-organic materials has been widely studied and researched. Fermenting bacteria have been found capable of reducing humic materials. Many investigators have been studying the use of humics as energy source for various bacteria, fungi and higher microorganisms, but they have not exploited them. Many researchers including [42, 43], investigated the use of humic as an energy source for specific bacteria, mushrooms and higher micro-organisms but pointed out that humic materials cannot be exploited as a source of food.

6.3 Biomedical applications

Veterinary and human medicine uses humic substances manufactured on a commercial scale. Various studies have been published on the medicinal properties of humic materials. The proliferation of gastric damage caused by ethanol was greatly reduced by humic acids administered prophylactically to rats [44]. TPP given to rats with gastric and duodenal ulcers improved the healing process considerably [44]. The interest in the use of humic materials in medicine and biology has developed in the past few decades. There is an investigation into the possibility that soil humus extract could be an applicant for cosmetic and pharmaceutical products with amino acid complexes and vitamin B analogues [11]. Anti-virals, profibrinolytic,

anti-inflammatory and estrogenic activities are the primary explanation for the increased attention given to humic acids [45].

6.4 Chelate balneotherapy capacity of humic substances

In many mammalian cells, humic acid was, on the other hand, shown to be a toxic factor, although its precise cytotoxicity mechanism remains uncertain. Humic acid can be reduced from Fe (III) to Fe (II) under the wide range of pH values in aqueous conditions (4.0 to 9.0) and iron from ferritin reduction and release, but the process is partially inhibited by superoxide scavengers. It was shown that iron released from ferritin accelerates the lipid peroxidation caused by humic acid. Humic acids are also capable of reducing, releasing and supporting lipid peroxidation from ferritin storage. HAs coupled to freed iron can therefore disrupt the redox balance of the biological system and evoke oxidative stress. This may be one of the main pathways for cytotoxicity caused by HA [46]. Now is the time for new applications for humic substances, particularly in biomedicine, in less conventional areas.

7. Conclusions

HS has been widely studied in different fields of agriculture, such as ground chemistry, fertility and plant physiology as the major component of organic soil content. HS plays an important role in the regulation of pollutants’ activity and mobility in the atmosphere and makes a major contribution to improving the status of global soil fertility. Together with a strong demand for safe food and sustainable agriculture, these features have led to increasing the environmental importance of

Figure 3.
Schematic representation of impact of humic substances on plant biology.

HS, recently recognized as a potential tool in facing global environmental problems. Many of its positive effects on soil structure and plant growth have been shown to depend on their chemical composition, but progress in HS research is known to be regulated by auxin. In addition, physiological and molecular evidence indicate that brassinosteroids may have a possible additional impact on the growth of plants. A critical review of literature shows that HS requires an auxin transduction pathway in order to determine its action in plant physiology but also show that the various signaling cascades in the global physiological response of plants to these substances (**Figure 3**). This could be used as a starting point for the clarification of mechanisms in the molecular response to HS in the plant. Further studies are required to evaluate the molecular goals and signaling trajectories involved in the intersection of the HS and the plant cells.

Author details

Rajneesh Kumar Gautam^{1*}, Dimuth Navaratna¹, Shobha Muthukumaran¹, Amarendra Singh², Islamuddin³ and Nandkishor More⁴

¹ College of Engineering and Science, Victoria University, Melbourne, Australia

² Department of Civil Engineering, Institute of Engineering and Technology, Lucknow, India

³ Department of Civil Engineering, UIET, Babasaheb Bhimrao Ambedkar University, Lucknow, India

⁴ Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, India

*Address all correspondence to: rajneesh.gautam@live.vu.edu.au

IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Lal, R., *Soil carbon sequestration impacts on global climate change and food security*. science, 2004. **304**(5677): p. 1623-1627.
- [2] Swift, R. and M. Hayes, *Humic Substances II. In Search of Structure*. John Wiley: Chichester, 1989. **449**.
- [3] Orlov, D., *Humic acids of soils and general theory of humification*. Moscow State University, 1990. **1**: p. 325.
- [4] Kögel-Knabner, I., *The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter*. Soil Biology and Biochemistry, 2002. **34**(2): p. 139-162.
- [5] Albers, C.N., et al., *Effect of different humic substances on the fate of diuron and its main metabolite 3, 4-dichloroaniline in soil*. Environmental science & technology, 2008. **42**(23): p. 8687-8691.
- [6] Frimmel, F.H. and R.F. Christman, *Humic substances and their role in the environment*. 1988: John Wiley and Sons Ltd.
- [7] Webe, P.J., *Humic Substances and their Role in the Environment*, in *EC Agriculture 2020*, EC AGRICULTURE Editor's Choice - 2020. p. 03-08.
- [8] Burdon, J., *Are the traditional concepts of the structures of humic substances realistic?* Soil science, 2001. **166**(11): p. 752-769.
- [9] Kelleher, B.P. and A.J. Simpson, *Humic substances in soils: are they really chemically distinct?* Environmental science & technology, 2006. **40**(15): p. 4605-4611.
- [10] Schulten, H.-R. and M. Schnitzer, *Chemical model structures for soil organic matter and soils*. Soil science, 1997. **162**(2): p. 115-130.
- [11] Peña-Méndez, E.M., J. Havel, and J. Patočka, *Humic substances-compounds of still unknown structure: applications in agriculture, industry, environment, and biomedicine*. J. Appl. Biomed, 2005. **3**(1): p. 13-24.
- [12] Piccolo, A. and F. Stevenson, *Infrared spectra of Cu²⁺ Pb²⁺ and Ca²⁺ complexes of soil humic substances*. Geoderma, 1982. **27**(3): p. 195-208.
- [13] Dalang, F., J. Buffle, and W. Haerdi, *Study of the influence of fulvic substances on the adsorption of copper (II) ions at the kaolinite surface*. Environmental science & technology, 1984. **18**(3): p. 135-141.
- [14] Hayes, M.H., R. Mylotte, and R.S. Swift, *Humin: its composition and importance in soil organic matter*. Advances in agronomy, 2017. **143**: p. 47-138.
- [15] Leenheer, J., et al., *Models of metal binding structures in fulvic acid from the Suwannee River, Georgia*. Environmental science & technology, 1998. **32**(16): p. 2410-2416.
- [16] Weber, J., *Definition of soil organic matter*. Humintech: Humic acids based products, 2002.
- [17] Pettit, R.E., *Organic matter, humus, humate, humic acid, fulvic acid and humin: their importance in soil fertility and plant health*. CTI Research, 2004. **10**: p. 1-7.
- [18] Boggs Jr, S., D. Livermore, and M.G. Seitz, *Humic substances in natural waters and their complexation with trace metals and radionuclides: A review*. 1985.
- [19] Pandey, A.K., S.D. Pandey, and V. Misra, *Stability constants of metal-humic acid complexes and its role in environmental detoxification*. Ecotoxicology and environmental safety, 2000. **47**(2): p. 195-200.

- [20] Murbach, T.S., et al., *A toxicological evaluation of a fulvic and humic acids preparation*. Toxicology reports, 2020. 7: p. 1242-1254.
- [21] Rice, J.A. and P. MacCarthy, *Statistical evaluation of the elemental composition of humic substances*. Organic Geochemistry, 1991. 17(5): p. 635-648.
- [22] Kapoor, A. and T. Viraraghavan, *Fungal biosorption—an alternative treatment option for heavy metal bearing wastewaters: a review*. Bioresource technology, 1995. 53(3): p. 195-206.
- [23] Klontz, K.C., et al., *The role of adverse event reporting in the FDA response to a multistate outbreak of liver disease associated with a dietary supplement*. Public Health Reports, 2015. 130(5): p. 526-532.
- [24] Vaughan, D. and B. Ord, *Uptake and incorporation of ¹⁴C-labelled soil organic matter by roots of Pisum sativum L.* Journal of Experimental Botany, 1981. 32(4): p. 679-687.
- [25] Trevisan, S., et al., *Humic substances biological activity at the plant-soil interface: from environmental aspects to molecular factors*. Plant signaling & behavior, 2010. 5(6): p. 635-643.
- [26] Canellas, L., et al., *Humic acids crossinteractions with root and organic acids*. Annals of Applied Biology, 2008. 153(2): p. 157-166.
- [27] Canellas, L.P., et al., *Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence, and plasma membrane H⁺-ATPase activity in maize roots*. Plant physiology, 2002. 130(4): p. 1951-1957.
- [28] Nardi, S., et al., *Chemical and biochemical properties of humic substances isolated from forest soils and plant growth*. Soil Science Society of America Journal, 2000. 64(2): p. 639-645.
- [29] Vaughan, D., *The stimulation of invertase development in aseptically stored tissue slices by humic acids*. Soil Biology and Biochemistry, 1969. 1(1): p. 15-28.
- [30] Onaga, G. and K. Wydra, *Advances in plant tolerance to abiotic stresses*. Plant genomics, 2016. 2: p. 1-12.
- [31] Yakhin, O.I., et al., *Biostimulants in plant science: a global perspective*. Frontiers in plant science, 2017. 7: p. 2049.
- [32] Van Oosten, M.J., et al., *The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants*. Chemical and Biological Technologies in Agriculture, 2017. 4(1): p. 1-12.
- [33] Olaetxea, M., et al., *Abscisic acid regulation of root hydraulic conductivity and aquaporin gene expression is crucial to the plant shoot growth enhancement caused by rhizosphere humic acids*. Plant Physiology, 2015. 169(4): p. 2587-2596.
- [34] Zandonadi, D.B., et al., *Plant proton pumps as markers of biostimulant action*. Scientia Agricola, 2016. 73(1): p. 24-28.
- [35] Garcia-Mina, J., M. Antolin, and M. Sanchez-Diaz, *Metal-humic complexes and plant micronutrient uptake: a study based on different plant species cultivated in diverse soil types*. Plant and Soil, 2004. 258(1): p. 57-68.
- [36] Jackson, M., *New root formation in plants and cuttings*. Vol. 20. 1986: Taylor & Francis.
- [37] Frimmel, F.H., *Photochemical aspects related to humic substances*. Environment International, 1994. 20(3): p. 373-385.
- [38] Sutton, R. and G. Sposito, *Molecular structure in soil humic substances: the new view*. Environmental science & technology, 2005. 39(23): p. 9009-9015.

- [39] MacCarthy, P., *The principles of humic substances*. Soil Science, 2001. **166**(11): p. 738-751.
- [40] Campbell, C., et al., *Applicability of the carbon-dating method of analysis to soil humus studies*. Soil Sci, 1967. **104**(3): p. 217-224.
- [41] Talmage, S.S., et al., *The fate of chemical warfare agents in the environment*. Chemical Warfare Agents: Toxicology and Treatment, 2nd ed. Chichester, West Sussex, England: John Wiley & Sons Ltd, 2007: p. 89-125.
- [42] Gaur, A. and K. Bhardwaj, *Influence of sodium humate on the crop plants inoculated with bacteria of agricultural importance*. Plant and Soil, 1971. **35**(1): p. 613-621.
- [43] Sollins, P., P. Homann, and B.A. Caldwell, *Stabilization and destabilization of soil organic matter: mechanisms and controls*. Geoderma, 1996. **74**(1-2): p. 65-105.
- [44] Abshenas, J., R. Kheirandish, and A.R. Salary, *Gastroprotective effect of mummy on induced gastric ulcer in rats*. Comparative Clinical Pathology, 2014. **23**(2): p. 305-309.
- [45] Mishra, T., H. Dhaliwal, and P.K. Gill, *CHARACTERIZATION OF HUMIC ACID SYNTHESIZED FROM WHEAT BRAN THROUGH MICROBIAL STRAINS ASSOCIATED WITH SHILAJIT*.
- [46] Ho, K.-J., et al., *Humic acid mediates iron release from ferritin and promotes lipid peroxidation in vitro: a possible mechanism for humic acid-induced cytotoxicity*. Archives of toxicology, 2003. **77**(2): p. 100-109.