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Role of Humic Substances on Plant Nutrition

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ABSTRACT

The scientific community is rigorously examining the multifaceted influence of humic substances on plant metabolic processes and growth physiology. Contemporary research underscores that the efficacy of these substances is modulated by variables including fractionation, source, concentration and molecular weight. Notably, low molecular weight fractions exhibit a superior capacity to traverse the plasmalemma of plant cells, thereby facilitating direct absorption, whereas higher molecular weight fractions predominantly engage with the cell wall. Humic substances are recognized for their ability to augment nutrient assimilation, particularly nitrates; however, their roles in intermediary metabolic pathways, such as respiration and photosynthesis, are not yet comprehensively elucidated. The hormone-mimetic properties of humic substances may stem from their intrinsic chemical configurations or from the presence of hormones of microbial origin within their composition. Collectively, humic substances recurrently demonstrate advantageous effects on plant cellular growth and developmental processes.

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INTRODUCTION

Humic substances (HS) are the predominant component of soil organic matter, constituting approximately 60% of its total composition and are recognized as essential elements within terrestrial ecosystems due to their involvement in numerous complex chemical interactions within soil matrices (Stevenson, 1982). Due to their intricate association with soil mineral phases, HS are resistant to decomposition, rendering them chemically too complex to be readily metabolized by microorganisms. This characteristic contributes to their persistence in the soil for extended periods, making them a vital component in maintaining soil health.

The exceptional capacity of humic substances to interact extensively with metal oxides, ions, hydroxides and other minerals and organic molecules is one of their major characteristics (Albers *et al.*, 2008). These interactions encourage the production of both water-soluble and water-insoluble complexes in addition to binding harmful contaminants (Cattani *et al.*, 2009). These complexes facilitate the metals and organic substances to dissolve, mobilize and transport, in both soil and aquatic environments. Alternatively, they can also enable the accumulation of these substances within soil horizons, affecting nutrient distribution and availability.

The importance of humic substances extends beyond soil structure to plant nutrition and growth. Humic substances, being produced by the decomposition of plant and microbial residues, significantly improve the qualities of soil, such as microbial diversity, water retention and nutrient availability (Goel and Dhingra, 2021). One of their most important roles is the mobilization of essential nutrients, such as phosphorus and converts them into bioavailable forms; thus improving plant uptake and acquisition (Yuan *et al.*, 2022). Moreover, humic compounds improve the nutrient digestion and encourage the overall plant growth by stimulating a variety of biochemical processes within plants, such as hormone control and enzyme activity (El-Tahlawy and Ali, 2022; Navya *et al.*, 2021). They also improve the permeability of cell membranes, which facilitates improved nutrient absorption and toxic chemical detoxification, enhancing plant resistance under abiotic stress conditions (Popov *et al.*, 2022; Goel and Dhingra, 2021).

Beside these benefits, the application of humic substances offers a sustainable solution for improving agricultural productivity. Their capability towards enhancing the nutrient efficiency, promoting microbial activity and reducing dependency on synthetic fertilizers are making them an eco-friendly alternative for modern farming practices (Navya *et al.*, 2021).

Humus

Humus is a carbon-rich complex compound, coloured as dark brown to black, with a fine molecular structure that is not detectable under light microscopy, produced from the breakdown of organic matter over time. It is distinguished from non-humic substances by its absence of specific chemical formulas and its exceptional persistence in soil, often enduring for several centuries. It is essential to maintain the fertility and structure of the soil and also for long-term stabilisation of soil organic matter.

Humic Substances

Humic substances (HS) are the key component of humus and represent relatively large and stable organic complexes that significantly enhance the soil properties. They improve the soil structural integrity, porosity and water retention, while also increase the cation and anion exchange capacities. Because of these characteristics, humic compounds are very much essential for soil fertility and nutrient cycling.

The major chemical components of humic compounds are carbon, oxygen, hydrogen, nitrogen and sulphur, which are arranged in the intricate molecular structures with C-C, C-N and C=O bonds. Humic compounds are divided into three primary categories based on their solubility in water at different pH levels:

- Humin: Components those are insoluble in both alkaline and acidic environments.
- Humic Acids (HAs): Components that dissolve in alkaline environments, but precipitate in acidic conditions.
- Fulvic Acids (FAs): These components are soluble in both acidic and alkaline environment.

These subdivisions reflect their varying roles and behavior in soil chemistry. Table 1 provides a summary of some of the key characteristics of humic compounds.

Humic substances constiute 50-80% of soil organic matter and are considered to be the most stable fractions because of their complex chemical composition, strong interactions with clay minerals and metal cations, and incorporation in soil aggregates (Theng *et al.*, 1989). Because of their stability, humic compounds will continue to enhance soil fertility and promote plant growth for a long time.

Table 1: Characteristics of humic substances

Humic substances	Elemental composition (%)					Functional group (cmol kg^{-1})			E_4/E_6
	C	H	N	S	O	Total acidity	-COOH	Phenolic-OH	
Humic acids	54-59	3-6	1-4	0.1-1.5	33-38	500-900	150-600	200-600	3-5
Fulvic acids	41-51	4-7	1-4	0.1-3.5	40-50	700-1400	500-1100	100-600	6-9

(Source: Schnitzer, 1991)

HUMIC SUBSTANCES AND THEIR WORTH AS FERTILIZER CONSTITUENTS

Humic substances are frequently found as carbon-rich minerals, including brown coal, low-grade lignite and leonardite, within soil, aquatic environment and peat formation, typically manifesting in three forms: humin, humic acids (HAs) and fulvic acids (FAs) (Ghani *et al.*, 2021). Fertilizer-grade humic substances occur naturally in these carbon-rich minerals, particularly in lignite and leonardite deposits, which are distributed globally. Leonardite, specifically, is termed as the highly oxidized form of low-grade lignite with a comparatively high level of smaller molecules, notably fulvic acids (Lodhi *et al.*, 2013). Traditional humus-building methods, while beneficial, are often slow, labor-intensive and potentially costly. As a more efficient alternative, the direct application of mined humic substances (humates) to soil, or their use in liquefied form as foliar fertilizers, offers a rapid and practical approach to enhancing soil fertility (Khadel and Fawy, 2011). The incorporation of humic substances into soil management practices can optimize the utilization of residual nutrients from plants, lower fertilizer expenses and facilitate the release of essential nutrients currently trapped in mineral and salt formations.

HUMIC SUBSTANCES AND THEIR CONTROL ON SOIL FERTILITY

Soil flora and fauna utilize both humic and non-humic compounds as primary sources of energy and carbon, thereby exerting a significant influence on soil fertility and plant health. A critical role of humic substances present in soil ecosystems is their exceptional water retention capability (Piccolo *et al.*, 1997). On a statistical perspective, water represents the most essential resource that plants take up from the soil. Humic substances act as natural sponges because of their substantial surface area and internal electrical charges, promoting a favorable soil structure that allows water to enter and stay in the root zone. These sponge-like materials have a greater capacity that exceeds the soil clays, retaining up to seven times their volume in water (Piccolo and Mbagwu, 1999). The availability of water at the soil's surface and subsurface is indisputably the most vital component of fertile soil, serving as a channel for the transportation of nutrients essential for plant roots and soil organisms.

Soils enriched with high quantities of humic substances help in retaining water effectively, enabling crops to access water during periods of drought. Consequently, the application of humate-based fertilizers, when integrated with sustainable agricultural practices, often supports successful crop production even under dry conditions. Humic substances are also fundamental to the development of stable soil aggregates, or 'crumbs' and creation of a friable (loose) soil structure (Imbufe *et al.*, 2005). Soil aggregates are formed when humic compounds and complex carbohydrates produced by bacteria combine with clay and silt particles. As the mineral fraction of the soil becomes more intimately linked to humic substances, colloidal complexes of humus-clay and humus-silt aggregates form through electrostatic processes, enhancing the cohesive forces that draw clay components and fine soil particles together. These aggregates contribute to an ideal crumb structure in the topsoil, promoting friability, improved tilth and the formation of porous spaces. These pores improve water infiltration and facilitate gaseous exchange with the atmosphere, both of which are essential for strong soil health.

The mean residence period of these organo-mineral complex aggregates vary significantly among various humic compounds, as evidenced by radiocarbon dating of extracts from undisturbed soils. Estimates indicate residence times of 1,140 years for humin, 1,235 years for humic acid and 870 years for fulvic acid. However, anthropogenic activities, including excessive fertilization and soil tillage, have accelerated the decomposition of humic compounds by intensifying soil weathering processes. In ideal conditions, it takes around 30 years for the organic carbon added annually from plant and animal waste to cycle over. To sustain the existence of humic substances in soil, it is imperative for growers to adopt agricultural practices that inhibit their degradation and maintain their natural residence time. Essential practices include avoiding excessive fertilization, implementing crop rotation, minimizing pesticide use, restricting deep plowing and incorporating crop residues into the topsoil through minimal tillage methods.

Humic compounds also play a significant role in the inactivation and degradation of toxic compounds within the soil. They facilitate the stabilization or breakdown of various toxicants, including nicotine, aflatoxins, antibiotics, phenols and a majority of natural pesticides. Through microbial degradation processes, the carbon within these toxic substances is not entirely released as CO_2; rather, humic substances are complex polymers of highly stabilised aromatic ring compounds with high integration, more so than aliphatic compounds. The electrically charged sites on the surfaces of humic substances are instrumental in attracting and neutralizing pesticides and other toxins. Consequently, the Environmental Protection Agency (EPA) has endorsed the utilization of humates for the remediation of harmful waste sites (Lovley *et al.*, 1998). Several bioremediation companies also utilize humate-based compounds for the decontamination of toxic areas. Farmers interested in detoxifying their soils, particularly in reducing residues of toxic pesticides, increasingly apply humic substances for this purpose. In addition to these benefits, humic substances contribute to buffering soil pH and facilitating the release of carbon dioxide. Repeated field studies support the addition of humic substances as an effective means of stabilizing soil pH levels.

Soil enzymes, which are complex proteins, are stabilized by humic compounds through covalent bonding interactions within the soil matrix (Allison, 2006). These enzymes are less vulnerable to microbial destruction as a result of this stabilisation, while preserving their functional integrity. Nevertheless, a huge number of these covalent bonds are rather weak, allowing for the release of enzymes during shifts in soil pH. Some constituents of humic substances form robust bonds with soil enzymes, further enhancing enzyme stability and restricting the activity of potential plant pathogens. Enzymes secreted by pathogens to compromise a plant's defenses can bind with humic substances, thereby inhibiting the pathogen's capacity to infect host plants.

Humic compounds also play a significant role in moderating soil temperatures and reducing the rate of water evaporation (Pacheco and Havel, 2001). Their insulating properties promote a more stable soil temperature, particularly during abrupt climate events, including cold spells, heat waves, *etc*. While soil moisture is less likely to evaporate into the atmosphere, water retained within humic compounds serves to buffer temperature fluctuations. Furthermore, a number of chemical reactions in the soil are greatly influenced by the electrical characteristics of humic compounds, particularly through: (i) complex formation or chelation, (ii) electrostatic (Coulombic) attraction and (iii) water bridging. The electrostatic attraction, involving the binding of metal cations to anionic sites on humic substances, prevents the leaching of these cations into the subsoil, retaining them for plant uptake (Eshghi and Garazhian, 2015). This electrostatic interaction, also known as organo-metal chelation, keeps essential cations available within the soil ecosystem, facilitating their transport into roots of plant or exchange with other cations. Humic substances' charged sites are also integral in dissolving and binding trace minerals; a metal cation's affinity for fulvic acid (FA) or humic acid (HA) enhances the ease with which it dissolves from mineral surfaces, making these ions more accessible to plants (Visser, 1986, Nardi *et al.*, 2000a).

HUMIC SUBSTANCES AND THEIR CONTROL ON PLANT GROWTH AND DEVELOPMENT

Plant growth is regulated through both indirect and direct mechanisms mediated by humic substances. Indirectly, humic substances facilitate energy provision to beneficial soil organisms, optimize soil water retention, enhance structural integrity, mobilize plant nutrients from soil minerals, increase trace mineral bioavailability and broadly elevate the fertility of the soil. Direct effects encompass alterations in plant metabolic pathways triggered by the cellular absorption of organic macromolecules like humic acids and fulvic acids. Upon cellular entry, these compounds initiate a series of biochemical modifications across membrane structures and various cytoplasmic elements (Vaughan, 1986).

Humic substances effects some biochemical improvements in plant metabolism, summarized in figure 1.

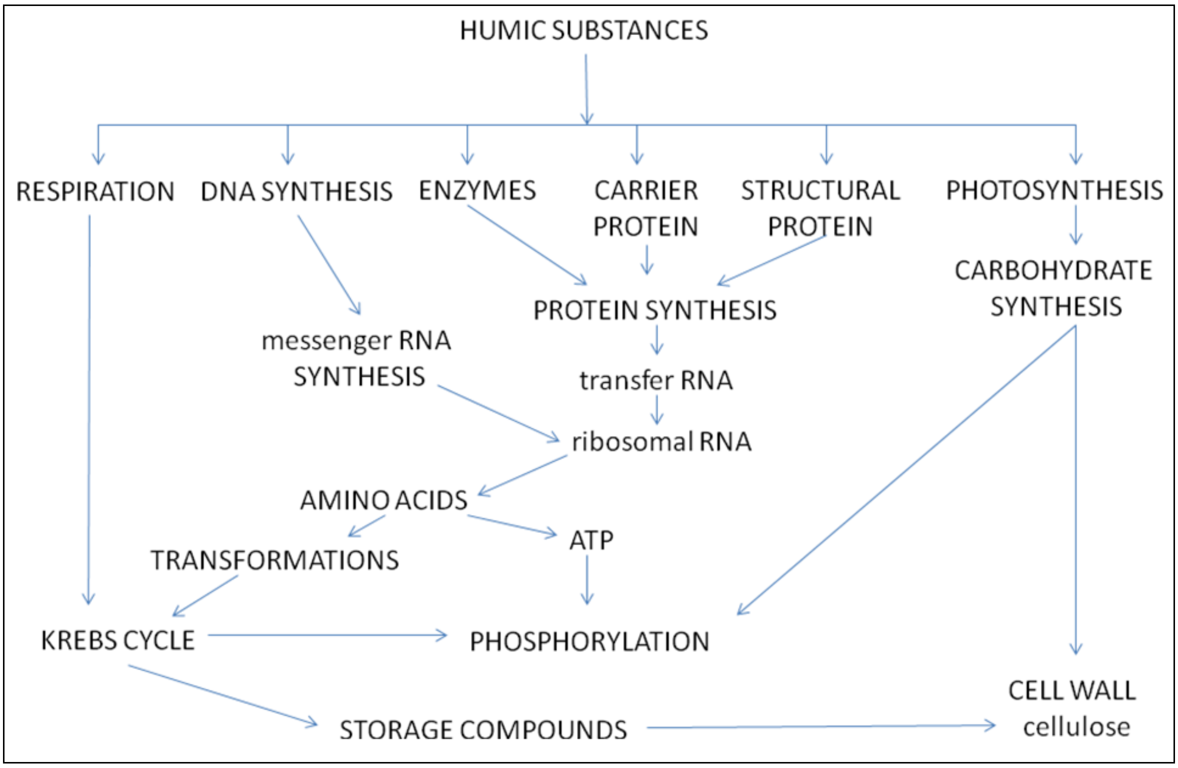

Figure 1: Effect of humic compounds on certain biochemical improvements of plant metabolism

RESPONSIBILITIES OF HUMIC MATTERS IN THE ION ADSORPTION

The pH of the surrounding medium and the content of humic substances (HS) both affect the selective variability in the modulation of ion absorption by humic compounds. In beetroot tissue assays, humic acid (HA) has been shown to significantly enhance the adoption capacity for Na^+ and Ba^{2+}, whereas the absorption of Ca^{2+} and Zn^{2+} remained mostly unaltered (Vaughan and MacDonald, 1976). HA similarly facilitates phosphate assimilation, while concurrently suppressing chloride uptake (Vaughan and MacDonald, 1971). Additionally, studies indicate that both humic acid (HA) and fulvic acids (FA) may have an impact on the uptake of NO_3^-, SO_4^{2-} and K^+ in barley and oat seedlings (Maggioni *et al.*, 1987). The intricate mechanisms by which HS influence ionic absorption in plant roots remain difficult to elucidate, given the complex and incompletely characterized nature of humic compounds. Comparative analysis is further complicated by variations in HS origin and extraction methodologies, which yield HS of differing properties. Through post-transcriptional regulation, HA has been found to stimulate the production of carrier proteins in barley roots (Dell'Agnola *et al.*, 1981), a finding later substantiated by measurements of elevated mRNA levels in maize seedlings with low-molecular-size (LMS) fraction treatment (Nardi *et al.*, 2000b).

In particular, an increase in NO_3^- uptake was demonstrated only in LMS fractions with gibberellin-mimetic activity. Humic substances (HS) have been shown to strongly inhibit root H^+ extrusion and microsomal ATPase activity, mirroring the effects observed with gibberellic acid application. A vanadate-sensitive proton-pumping ATPase (H^+-ATPase) that establishes an electrochemical proton gradient across the plasma membrane is essential for primary active transport in plant cells (Morsomme and Boutry, 2000). This gradient, in turn, powers secondary active transport through carrier-proteins utilizing symport or antiport mechanisms. Specifically, nitrate (NO_3^-) uptake is mediated by a stimulated H^+/NO_3^- symporter with a 2:1 stoichiometry (Miller and Smith, 1996). Scientific consensus suggests that low-molecular-size (LMS) fractions of HS may interact directly with these transport proteins, modulating NO_3^- uptake through a "fine" regulatory mechanism (Vaughan, 1986; Prista, 2020). Moreover, humic compounds may mitigate the alkalinization typically associated with NO_3^- utilization as a nitrogen source, which otherwise inhibits the H^+/NO_3^- symporter, by reducing the pH at the plasma membrane surface of root cells (Raven and Smith, 1976). Remarkably, an increase in ammonium (NH_4^+) absorption frequently corresponds with a decrease in NO_3^- uptake, indicating a compensatory relationship between both the nitrogen sources (Reddy and Ulaganathan, 2015). Additionally, the plasma membranes of plant cells exhibit various redox processes that may further influence both nitrogen metabolism and nutrient uptake efficiency in plants (Berczi and Møller, 2000).

RESPONSIBILITIES OF HUMIC SUBSTANCES AS HORMONE

Specific molecular components of humic compounds are known to modulate plant growth hormones. By inhibiting the enzyme indole acetic acid oxidase (IAA-oxidase), humic acids and fulvic acids reducing the breakdown of indole-3-acetic acid (IAA), a key plant growth regulator. HS also affects enzymes critical for growth regulation and enhances the production of adenosine triphosphate (ATP), increasing cellular energy. Early work by Bottomley suggested that HS promotes growth by providing unique compounds termed "auximones" (Bottomley, 1914; 1917; 1920), a hypothesis supported independently by Hillitzer (1932) and Chaminade and Boucher (1940). Few more studies have shown that morphological variations identical to those triggered by IAA are only induced by the low-molecular-size (LMS, <3500 Da) fraction of humic compounds (Muscolo *et al.*, 1993).

Additionally, LMS fractions were found to enhance peroxidase and IAA oxidase activities; however, IAA itself, while increasing IAA oxidase activity, inhibits antioxidant enzyme activity. Nardi *et al.* (1994) further showed that *Nicotiana plumbaginifolia* roots are stimulated to develop by the LMS and IAA fractions, an effect counteracted by auxin inhibitors PCIB (4-chlorophenoxy-isobutyric acid) and TIBA (2,3,5-triiodobenzoic acid). More recent findings question the direct hormone-like function of humic compounds, suggesting instead a pronounced effect on plasma membrane H^+-ATPase activity (Varanini and Pinton, 2001) or enhanced bioavailability of essential micronutrients, such as Fe and Zn (Bondareva and Kudryasheva, 2021).

Auxin (Lebuhn and Hartmann, 1993) and gibberellin (Rademacher, 1992) levels are typically elevated in the rhizosphere compared to the bulk soil, likely because of the heightened microbial populations or accelerated metabolic activity driven by root exudates. The beneficial effects of organic materials, such as humus, compost and peat, are most pronounced following decomposition and biotic processing. Biological and biochemical transformations primarily occur during the degradation of these substrates, suggesting that the active compounds in humus are the byproducts of the soil microbiota's metabolism rather than the original parent molecules (Nardi *et al.*, 2002). Initial organic materials provide substrates or precursors that facilitate the microbial production of chemicals that are biologically active, such as hormone-like molecules. These plant growth regulators, embedded within humic substances, have notable ecological significance as they resist leaching and remain accessible to plants over time (Pizzeghello *et al.*, 2001).

CONCLUSION

Humic substances can produce a multifaceted influence on higher plant metabolism at various levels. Their effects may manifest as distinct, additive, overlapping, or, in some instances, mechanistically interconnected. The diverse targets of HS action can be attributed, in part, to their chelating capabilities and in part, to their hormone-like activities. To elucidate the beneficial impacts of humic compounds on higher plants, further research is essential, particularly focusing on the subsequent key areas: (i) the bioavailability of humus within the rhizosphere and soil solution; (ii) the correlation between active metabolites produced by different soil microorganisms and humus activity; and (ii) the application of more precisely defined humic compounds in experimental investigations concerning plant metabolic processes.

Conflict of Interest

The authors declare no conflict of interest.

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