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HUMIC SUBSTANCES AND THE MECHANISM OF THEIR INFLUENCE ON THE PRODUCTION OF HIGHER GREEN PLANTS

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SUMMARY

The latest research proposed a new point of view about the material composition of so-called 'humic substances,' isolated from natural objects (soils, peats, sapropels, and composts) by alkaline solutions, and presented the conceptual model of direct influence of 'humic substances' on biochemical and biophysical processes into plants. 'Humic substances' are essentially black liquor, i.e., a product artificially obtained because of the alkaline hydrolysis of organic material from natural objects. Black liquor consists of a complex mixture of a variety of organic compounds. According to the proposed model, the biological activity of black liquor connects with accelerating the circulation of nutrients within plants, increasing the permeability of cell membranes, exhibiting de-toxicological properties, optimizing the ratio of organic and mineral anions in plants, and using some HS components as organic nutrients, inducing gene expression. Therefore, humic substances solutions have multifaceted effects on green vascular plants due to their direct influence on biochemical and biophysical processes.

Keywords: Humic substances, black liquor, alkaline hydrolysis, higher green plants, plant production process, colloidal systems of natural polymers, organo-mineral compounds, plant organic nutrients

Key findings: 'Humic substances' are the same as black liquor, artificially produced from the alkaline hydrolysis of organic material from natural objects. Black liquor composes a complex mixture of a variety of organic compounds. Hence, 'humic substances' solutions exhibit multifaceted effects on green vascular plants due to their direct influence on biochemical and biophysical processes.

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INTRODUCTION

The restoration of the links of the soil-plant trophic system functioning, lost in the agricultural use of the territory, needs attention to effectively address the problems associated with sustainable crop yields. This direction becomes especially acute under conditions of strong anthropogenic pressure on agroecosystems (Popov and Chertov, 1997). One of the effective and economically feasible methods of influencing the production process of green vascular plants is the so-called humic substances (HS) (Khristeva, 1953; Guminsky, 1968; Nardi *et al*., 2002; Popov and Sukhanov, 2002; Prisa, 2020).

Most often, HS are isolated from various natural objects (soils, composts, lignite, and peat) using aqueous alkaline or neutral salt solutions, as well as a combination of aqueous alkaline solutions and sodium pyrophosphate (Kononova, 1963; Flaig *et al.*, 1975; Aleksandrova, 1977; Schnitzer, 1978; Swift, 1996; Orlov *et al*., 2004; Olk *et al*., 2019). According to Hayes (2006), so far, no satisfactory solvent system has been found that would be capable of isolating all substances of humic nature (if, of course, they are present in natural objects) absorbed by inorganic colloidal systems.

An opinion that the isolation of so-called 'humic substances' by any aqueous alkaline solutions and their further fractionation is incorrect because together with HS, non-humic substances, such as, components of living organisms and their postmortem residues, as well as simple and complex individual compounds also transferred into the liquid phase (Lehmann and Kleber, 2015; Paul, 2016). The disadvantage of all types of HS isolation and further fractionation is that the same materials end up in different fractions, which is undesirable (Olk *et al*., 2019). Moreover, HS is generally accepted as isolated from natural objects (soils, peats, sapropels, and composts) that correspond to those present in the same objects. But is it so?

The latest study aimed to characterize the material composition of the so-called HS solutions isolated from natural objects by alkaline solutions and to explain the possible mechanism of the HS solutions' action on the production process of higher green plants based on the analysis of past scientific literature.

ANALYSIS OF THE PROBLEM

During the alkaline isolation of HS from soil organic matter (SOM), individual compounds get released with them (Orlov *et al.*, 1996). In the course of further separation of HS, likely accompanying reactions occur, i.e., hydrolysis of high-molecular-weight components to form low-molecular-weight compounds, as well as the incorporation of part of the low molecular weight compounds - degradation products - into molecules of relatively high-molecular-weight biopolymers. The relatively low molecular weight organic compounds represent the prevailing part of fulvic acids (FA), appearing in solution during analytical procedures because of partial hydrolysis of various high molecular weight compounds, including HS and humin, which exists as an opinion (Orlov, 1999).

From studies carried out by Schreiner and Shorey (1910) at the beginning of the 20th century, HA includes, i.e., resin acids and their esters, fatty acid glycerides (fats), agrosterol, phytosterol, paraffinic, lignoceric, and agroceric acids, FA (crenic and apoceric acids) dioxystearic acid, picolinic carboxylic acid, pentosans, xanthine, hypoxanthine, cytosine, histidine, and arginine. The HA of one soil may have a different component composition than those of another soil. Similarly, the composition of FA varies from one soil to another. At that, organic compounds separated from SOM by aqueous alkaline solutions can be divided into two groups, i.e., one group comprises compounds poorly degraded by microorganisms (resins, wax-like substances, partly fats, pentosans), and the second group consists of intermediate products of the degradation of proteins (amino acids, and dihydroxystearic acid), nucleoproteins (cytosine, xanthine, hypoxanthine), and alkaloids and essential oils (Glinka, 1932). As considered by Trusov (1917), researchers created HS from any soil organic compounds that were not found as such in the soil. For example, resin acids, which are defined in HA, are not their constituents but the result of the action of alkaline solutions on SOM. Thus, some of the brown substances isolated from soils by alkaline solutions underwent formation by the action of alkali on SOM rather than being part of the soils, while others (tannins, chlorophylls, and encrusting substances) became brown. Additional facts confirming Trusov's (1917) point of view appeared only at the end of the 20th century and the beginning of the 21st century.

A review by Oriez *et al.* (2020) followed, stating that the HS resulted from the alkaline action on the main components of non-timber phytomass, such as, lignin, polysaccharides, and other substances. Lignin and saccharide oligomers pass into the aqueous liquid phase, forming a dark-colored colloidal solution, the so-called black liquor. However, black liquor contains products not only of lignin degradation but also of its subsequent depolymerization (Thring *et al.*, 1990; Tarabanko *et al.*, 1997; Cordoso *et al.*, 2009). Moreover, adding mineral acid caused precipitation to all lignin derivatives (Oriez *et al.*, 2020). Alkaline hydrolysis of biological material containing proteins, nucleic acids, carbohydrates, and lipids results in a coffee-colored aqueous solution containing peptide molecules, amino acids, oligo- and monosaccharides, as well as soaps - sodium or potassium salts of higher carboxylic acids (Thacker, 2004).

Furthermore, alkaline hydrolysis of proteins leads to the formation of lanthionine and lysinoalanine residues, as well as the degradation of arginine, lysine, and cystine (Maksimyuk and Maryanovskaya, 2009) and the chemosynthesis of glycine and α-amino-n-butyric acid (Bremner, 1951). Interestingly, such high-molecular-weight compounds as DNA and Fe-inositphosphates lose their aggregation stability upon acidification of the alkaline isolate ending up in the HA fraction, while low-molecular-weight acid-soluble components, such as, saccharophosphates and Ca-inositphosphates end up in the FA fraction (Makarov, 1997). Notably, the separation of HS into HA and FA depends on the concentration (Popov, 2004). Thus, if the concentration of HS is lower than the critical concentration of micelles (CCM), then HA retains its aggregative stability in an aqueous solution with a pH of 1-2. Conversely, the higher the concentration of HS above CCM, the more HA precipitates in a strongly acidic aqueous solution.

The study's view of the isolation and separation of humic substances from natural objects appears in Figure 1. In other words, humic substances (more precisely, black liquor) comprise a complex mixture consisting of a) mopes- and oligomers (products, which are inherited from initial material and/or formed resulting from the alkaline hydrolysis of natural polymers), b) colloidal systems of natural polymers (lignin, polysaccharides, fats, waxes, resins, and kerogen), c) organo-mineral compounds, including colloidal systems of natural polymers, and d) inorganic ions and a variety of compounds, including colloidal systems. That is, the black liquor solution is a vinaigrette of a variety of versatile structural and functional blocks. These blocks are also the basis of the nutrition of living organisms, following the opinion of Ugolyov (1991). From the study's point of view, this is the explanation of the biological activity of humic preparations. Understanding the mechanism of action of humic preparations on plants allowed researchers to develop a conceptual model reflecting the influence of organic compounds belonging to HS on biochemical and biophysical processes occurring in plants.

The model basis combines the analysis of scientific literature and this study's experimental data. According to the proposed model, the biological effects of HS on plants were due to their versatile properties (Guminsky, 1968; Vakhmistrov *et al*., 1989; Ovchinnikova, 1991; Nardi *et al.*, 1996; Ermakov and Popov, 2001; Popov, 2004; Quaggiotti *et al.*, 2004). Black liquor (HS) can affect plant physiology through complex transcriptional (gene-regulatory) networks (Trevisan *et al*., 2010). By regulating the expression of several genes, most of which are involved in the cell cycle, as well as in meristem and cytoskeletal organization, these compounds influence plant physiology and metabolism.

Developing the scientific statements related to the disclosure of the mechanisms of biological activity of black liquor (HS), the study developed a conceptual model of HS participation in biochemical and biophysical processes occurring in green vascular plants (Figure 2). According to the proposed model, the conditioning of the physiological activity of black liquor results from the presence of various functional groups in these compounds, colloidal properties, and component composition. The diverse functional groups of these compounds determine their participation in chemical oxidation-reduction reactions and the reactivity of these compounds in general. The reactivity of black liquor shows in the possibility of formation by these compounds of various organometallic complexes, including chelated ones; in the influence on osmotic pressure; in enzyme-substrate interactions; in detoxification of xenobiotic substances and compounds, as well as in some other processes (Yarchuk and Bulgakova, 1991; Popov, 2004).

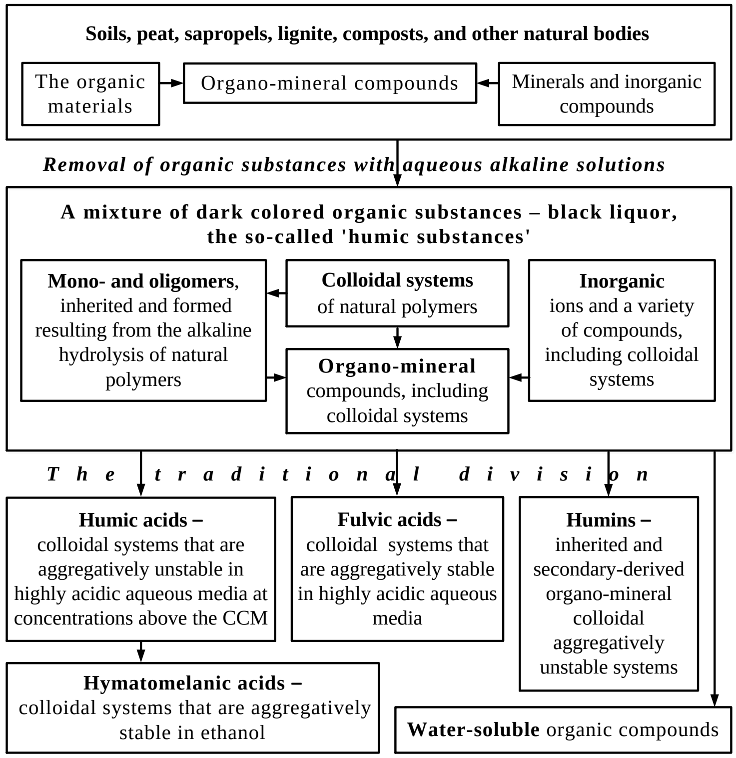

Figure 1. The scheme of traditional isolation and division of humic substances obtained from natural objects: CCM — critical concentration of micelles.

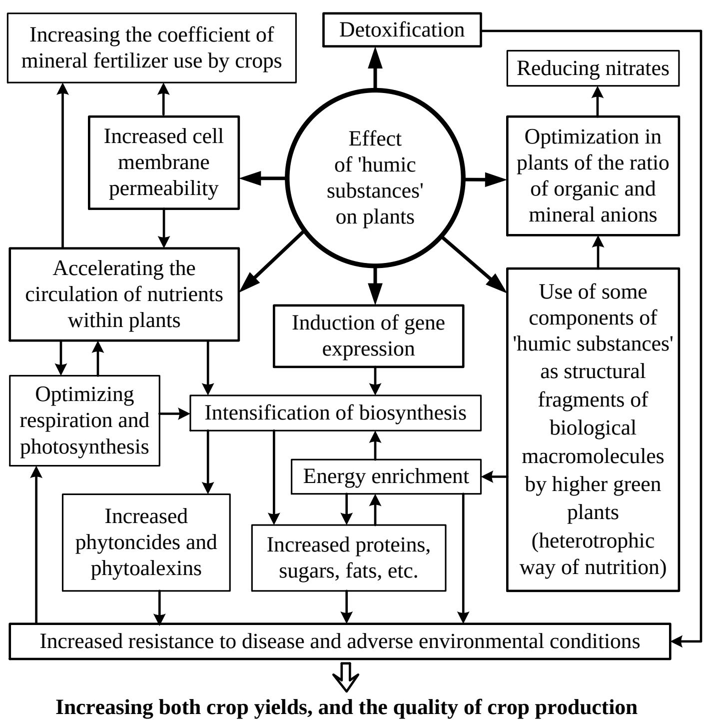

Figure 2. Effect of humic substances on higher green plants.

The participation of HS in various redox processes optimizes plant respiration and partially photosynthesis (Khristeva, 1953; Prát, 1965; Guminsky, 1968; Komissarov and Klimova, 1971; Gorovaya *et al.*, 1995). At the same time, the activation of photosynthesis may not be due to the manifestation of the redox properties of black liquor as to the acceleration of transport and circulation of food substances within plants (Popov, 2004). As a result, the more intensive plant respiration and photosynthesis, the more active the biosynthesis of various organic compounds. Experimentally establishing HS led to the enhancement of reactions with the formation of macroergic bonds (Řeřábek, 1963). Potassium humate intensified endogenous respiration and cytochrome oxidase activity in homogenate from roots of *Triticum vulgare* Vill. (Šmidová, 1962).

Organic compounds included in the HS showed capable of forming chelate compounds, and these substances contribute to the supply of biophilic elements to plants in the form of cations, in particular iron, potassium, copper, calcium, magnesium, and other macro- and microelements (Prozorovskaya, 1936; Khristeva 1953; Gorovaya *et al.*, 1995), and in the form of, i.e., phosphate, nitrate, sulfate (Vaughan and MacDonald, 1971; Maggioni *et al.*, 1987). The influence of HS on nitrate uptake by plants can be explained both by hormone-like action (Dell'Agnola and Nardi, 1987) and by genomic modifications induced by humic acids ('HA' + FA) (Attinà *et al.*, 1992). Thus, the effect of HS on the expression of nitrate carrier proteins gained discovery (Vaughan *et al.*, 1985). As was shown in maize (*Zea mays* L.) (Quaggiotti *et al.*, 2004), HS can directly affect the transcription of genes responsible for nitrate ion entry into plant roots.

The HS can serve as suppliers of organic anions, and for the favorable growth of plants in their cells, a certain ratio of organic and mineral anions should be observed (Osmolovskaya and Ivanova, 1989). Probably, organic anions included in black liquor (HS) can better perform the role of counterions for cations in plants (K^+, Na^+, and NH_4^+), optimizing the ratio of anions and leading to the reduction of nitrates in plant products (Ermakov and Popov, 2001).

From the position of colloidal chemistry, black liquor serves as a set of various colloidal systems, which have electrosurface, and surface-active properties. The electrosurface properties of organic compounds mobile in HS worked, for example, in their sorption and ion-exchange abilities, as well as in their electrostatic interactions. These compounds also exhibit surface-active compounds (Vakhmistrov *et al.*, 1989). In this regard, HS, once in plants, hydrophilizes the walls of the conductive system and facilitates pinocytosis, affects bioelectrical reactions and osmotic pressure, and exerts ionophore effects, which ultimately accelerate the movement and circulation of nutrients in the plant. The better the transport and circulation of nutrients in plants, the higher the rate of photosynthesis, the more mineral nutrient compounds enter the plants, and therefore, the faster the growth and development of crops. Consequently, crops more fully use the fertilizers applied to the soil (NPK fertilizer utilization rate increases) (Popov, 2004).

The bio-stimulatory activity of HS functioned greatest in fractions with relatively low molecular weight (Vaughan *et al.*, 1985, Post *et al.*, 1988, Nardi *et al.*, 2002). Moreover, relatively low molecular weight HS entered plant cells more readily than their higher molecular weight counterparts. The low molecular weight HS fraction moves along the symplast and directly affects plant metabolism, whereas the higher molecular weight HS fraction moves along the apoplast and affects processes related to plant cell wall functioning (Nardi *et al.*, 2002). However, the relatively low molecular weight HS fraction can also move along the apoplast, affecting the plasmalemma (Vaughan *et al.*, 1985). Based from the review (Popov, 2004), HS moves along the apoplast and has a variety of effects on cellular plasmolemmes: they counteract alkalinization, increase the electrochemical proton gradient, form humic-protein complexes, have a direct ionophore effect, and enhance the permeability. An experimental setup took place that the value of the elastic modulus of plant root cell walls depends on the content of water-soluble HS in the plant root system (Ermakov *et al.*, 2000).

The component composition of HS undoubtedly affects not only the trophicity but also some other biological properties of these specific compounds. Some components of HS representing structural fragments of biological macromolecules can also be absorbed and assimilated by plants. In this case, plants 'save' energy, and the greater the value of 'saved' energy, the more phytoncides, and phytoalexins - active means of plant protection - are produced, resulting in less disease for crops (Popov, 2004).

Some studies reported an analogy in the favorable effect of HS on plants with the action of phytohormones, such as, auxins (to a greater extent), gibberellins, and others (Bottomley, 1917; Hillitzer, 1932; Řeřábek, 1963; Cacco and Dell'Agnola, 1984), and HA have pronounced an auxin-like activity in a wider concentration range than the auxins themselves (Chukov *et al.*, 1995). The HS can synergistically interact with many plant growth regulators (Poapst and Schnitzer, 1971; Tichý, 1982). In addition, HS can activate the synthetic auxin reporter DR5:GUS and enhance transcription of the early auxin-dependent gene IAA19 (Trevisan *et al*., 2010). The HA, as well as the indoleacetic acid induce lateral root development through a concerted plasmalemma and tonoplast H^+ pumps activation (Zandonadi *et al.*, 2007). Beyond the auxin-like HS activation, gibberellin-like signaling to break seed dormancy can be involved (Cha *et al.*, 2017).

Reports also state that HS can reduce salt-induced stress in some plants, i.e., *Arabidopsis thaliana* L., *Phaseolus vulgaris* L., and *Zea mays* L. (Khaled and Fawy, 2011; Aydin *et al.*, 2012). Thus, HS delays the salt-mediated degradation of a sodium-influx transporter HIGH-AFFINITY K^+ TRANSPORTER 1 (HKT1), resulting in the facilitation of sodium flux (Khaleda *et al.*, 2017). All the earlier-mentioned examples of the impact of black liquor on physiological and biochemical processes in plants will improve the growth, development, and productivity of plants, as well as increase their resistance to stresses. Overall, the 24 years revealed the results of field production experiments with HS solutions prevailed in different climatic zones (Figure 3).

The biological activity of HS plays an important role both in providing high biological productivity of the soil-plant system and in increasing the resistance of this system to adverse influences. The following regularity was revealed in the application of foliar treatments with HS solutions (Popov, 2014) i.e., a) at high production potential of soils and favorable agrometeorological conditions a relatively low increase in crop yield was observed, and b) at extreme agrometeorological circumstances, however, with good provision of soils with basic elements (NPK) of plant nutrition, the effect proved maximal. Additionally, the foliar treatment of mother plants with HS solutions indicated one of the ways to improve the sowing properties of cereal seeds. A study result found that foliar treatment of spring barley with HS solutions exhibited an improvement in the dynamic properties of seeds germination and germination energy (Stefanova and Popov, 2002).

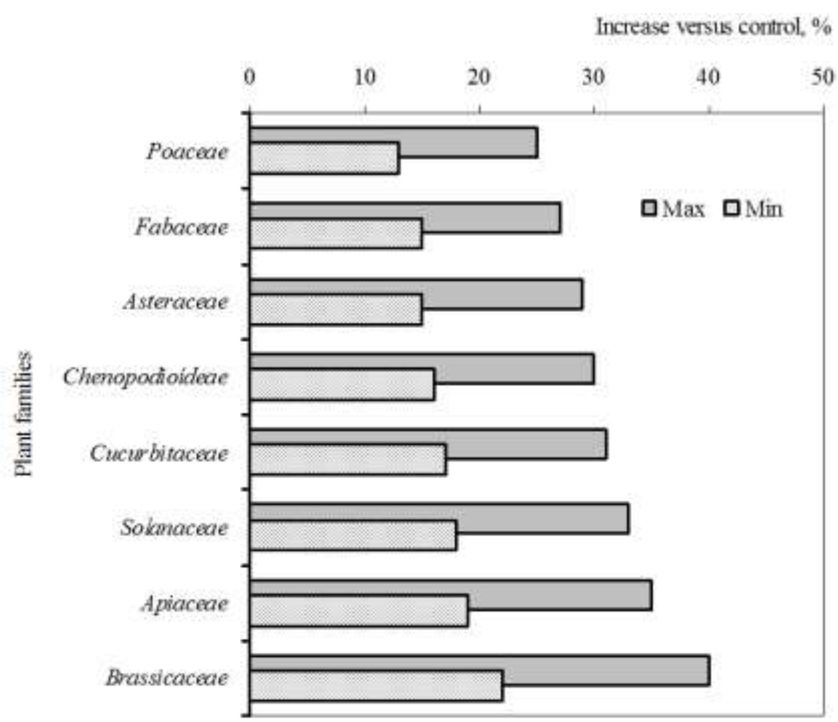

Figure 3. Crop yield increase (%) relative to control.

CONCLUSIONS

Humic substances, confirmed as black liquor, were artificially obtained as a result of the alkaline hydrolysis of organic materials from natural objects (soils, peats, sapropels, and composts). Black liquor is a vinaigrette consisting of organic compounds which are versatile structural and functional blocks, following the opinion of Ugolyov (1991). Therefore, the solution of humic substances has a versatile effect on green vascular plants. The HS, due to their direct influence on the biochemical and biophysical processes of plants, allows fast growth and development of crops. The biological activity of HS results in its presence in compounds of various functional groups, colloidal properties, and component composition. Humic substances can hasten the circulation of nutrients inside plants, increasing the permeability of cell membranes; facilitate the entry and movement of nutrients in cultivated plants; relieve crop stress after pesticide application; optimize ratios of organic and mineral anions in plants, using some components of HS that are structural fragments of biological macromolecules; and induce gene expression.

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