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Agro-environmental Applications of Humic Substances: A critical review

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IMPROVING crop productivity in a sustainable manner represents one of the most important future challenges to provide sufficient food, feed and fiber supplies with minimum deterioration of soil quality. The term "ecological intensification" is recently introduced as an eco-friendly approach for improving crop productivity (maximizing water and nutrient supply potentials of soil) with minimum application of synthetic additives (e.g. mineral fertilizers). Humic substances (the final components of organic matter decomposition) are among the most important natural products that can help in promoting sustainable intensification of crop productivity given their high nutrients content, huge surface area, active biostimulators, stability against microbial decomposition and large number of active functional groups. Consequently, humic substances receive considerable interest for improving soil quality indices, modulating plant physiological responses and maximizing quantitative and qualitative yield parameters. On the other hand, the unique physicochemical characters of humic substances support its application as an efficient soil amendment toward stabilizing potentially toxic elements. In this review, we will shed the light on the agro-environmental applications of humic substances taking into consideration the state-of-the-art in this regard. Additionally, we will highlight the future perspectives of the ongoing-research about novel techniques of humic substances fabrication/functionalization in several agro-environmental applications.

Keywords: Humic substances extraction, Plant physiology, Soil physicochemical characterization, Soil quality, and Soil remediation.

Introduction

The term humic substances refers to the transformed products or organic matter biomass, which have an amorphous phase and bear no morphological resemblances to the structures from which they were derived (El-Ramady et al., 2015 and Kononova, 1961). Another definition of humic substances have been introduced as the category of the naturally generated heterogeneous organic substances with a high molecular weight, yellow to black color and refractory nature (Aiken, 1985). Humic substances have a large carbonaceous chain with a high resistance against microbial decomposition. The elemental composition of humic substances is mainly O, C, N, H and S, which arranged in a long carbon chain. Humic substances contain a wide variety of organic components including polypeptides, polysaccharides, esters, ethers, phenols, lipids, quinones, several combinations of benzene, ketal, acetal, lactol and furan ringed compounds as well as various aliphatic compounds (Kononova, 1961).

Humic substances are divided into three fractions based on their solubility in the acidic and/or alkaline solutions: (i) humic acid, (ii) fulvic acid and (iii) humin. Humic acid (HA) consisted of a combination of weak aromatic (about 35%) and aliphatic organic acids (65%) with a molecular size of about 10,000 - 100,000 (Schnitzer, 1978). Fulvic acid, however, is soluble in aqueous solution at different pH values with smaller molecular weight (1,000 - 10,000) and the double oxygen content of that in humic acid (Mao et al., 2013). Unlike humic acid and fulvic

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acid, humin is neither soluble in acidic nor in alkali aqueous solutions due to its large molecular weight (100,000 - 10,000,000) (Li et al., 2015).

Dissociation of active functional groups enables humic substances to be negatively charged with a high affinity to positively-charged ions/molecules (Wang et al., 2019). The unique chemical structure of humic substances supports its beneficial effects in several applications (*e.g.,* improving soil fertility/water retention in the rhizosphere, improving root architecture/nutrients uptake, modulating plant physiological responses/yield productivity as well as remediation of contaminated soil and wastewater resources) (Fig. 1). In this review we will shed the light on the importance of humic substances and its applications in several agro-environmental purposes. All reports introduced in this review were extracted from peer-reviewed resources (scientific journals, books and book chapters), which had been published only in English language.

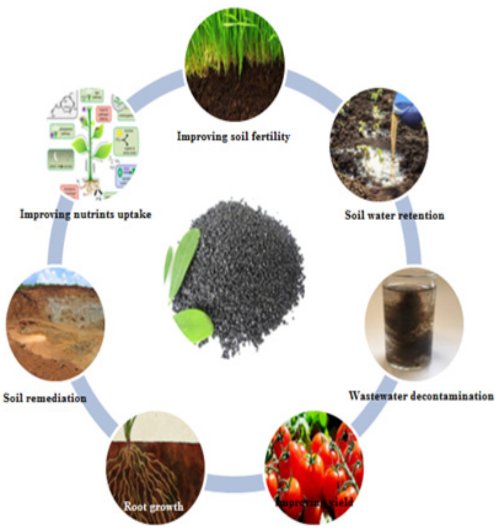

Fig. 1. Agro-environmental applications of humic substances

Humic substances extraction

Humic substances are the key constitution of natural organic matter where it can be isolated from several feedstock at different maturation degrees. An old study was carried out by Posner (1966) to investigate the effectiveness of various reagents for extracting humic fractions from soil. These reagents were (i) NaOH (0.5*M*), (ii) pyrophosphate (0.1 *M* at pH 7) and (iii) 0.5 *M* $Na(CO_3^{2-}/HCO_3^{-})$. Results revealed that extraction by these reagents was efficient to extract significant amounts of humic substances from soil. Soil pretreatment with 0.1 *M* HC1 increased the yield of humic acid with the aforementioned extractants. To maximize the efficiency of humic substances extraction, a modified method was introduced by Serra and Schnitzer (1972) using a mixture of alkali and chelating resin. The standardized International Humic Substances Society method is most widely applied for humic substances extraction (Aiken, 1985). In this classical method, the feedstock material is dried at 40°C, homogenized and mechanically ground using a ball mill. Humic acids extraction is carried out using 0.2 *M* NaOH under N_2 conditions, then acidified by HCl and purified using the mixture of (0.1 M HCl + 0.3 M HF) and centrifuged to isolate fulvic acid. The precipitated humic acid fraction was redissolved using NaOH and centrifuged at under N_2. Humic acid was precipitated using HCl centrifuged under N_2 conditions. In Netherlands, Veeken et al. (2000) studied alterations and characterization of humic substances extracted from biowaste during composting process. Humic acid content was low, and the rich aliphatic compounds were the dominant at early phase of composting; however, it increased steadily after 20 days owing to the conversion of aliphatic compounds into aromatic ones. Humic acid was also extracted efficiently from volcanic and alluvial soils in Italy (Baglieri et al., 2007). These soils were treated twice with aqueous glycerol (50%), once with citric acid (0.5 M) and once with the mixture of NaOH (0.5 M) and $Na_4P_2O_7$ (0.1 M). The extract ability of humic acids varied significantly among soils (21.2 *vs.* 8.3% for volcanic and alluvial soils, respectively). Humic acid (HA) extracted from volcanic soil exhibited more aromatic nature than HA extracted from the alluvial soil.

A novel technique for humic acid extraction using urea was introduced by Hemati et al. (2013) given the full water solubility of urea and the alkaline nature of its aqueous solutions (pH > 8.3). Vermicompost was used as a feedstock for humic acid extraction using different urea concentrations (0.1, 0.5 and 1.0 *M*). The concentration of urea (0.1 *M*) was unable to isolate humic acid during the initial 24 hr. However, the concentration of 0.5 *M* was the most efficient concentration to extract the maximum yield of humic acid. In Brazil, an organic-mineral fertilizer was developed based on the alkali extraction of humic substances from peat (Saito and Seckler, 2014). Exploratory extraction procedures involved mixing peat with KOH solutions (1:5 v:v) at different concentrations (0.5, 1.0, 1.5 or 2.0 *M*) and two extraction periods (12 and 24 hr). Results showed that peat extraction using KOH (1.0 *M*) at extraction time of 12 hr is a favorable technique for producing organo-mineral fertilizers, which meets the Brazilian legislations of organic farming. Another investigation was conducted in Tunisia for humic acid isolation from Tunisian lignite (Wali et al., 2019). The extraction technique involved using NaOH at different concentrations (0.1 and 0.8 M), extraction temperatures (ranged between 25 and 85 °C) and agitation times (ranged between 3 and 24 hr). Under the controlled conditions of the investigation, 0.8 *M* NaOH at temperature of 85 °C and agitation time of 6 hr were the optimum conditions for extraction of humic acid from Tunisian lignite.

The traditional alkaline extraction of humic substances requires large amounts of chemical solutions, which generate huge quantities of disposals. Consequently, attention has been directed toward additional modified methods to minimize the generated sludge/disposals. An eco-friendly technique for the extraction of water soluble humic substances has been introduced by Guigue et al. (2015) for the simple extraction of humic substances with the minimum quantity of disposals. In this method, soil samples were extracted using ultrapure water (100°C) at a pressure of 10 MPa. Focused ultrasound solid liquid extraction was also introduced by Raposo et al. (2016) for better extraction of humic substances with lower amounts of generated disposals. Extraction procedures (ultrasound power, cycling, timing, NaOH concentration and extraction ratio) were investigated using multivariate analysis and analysis of variance. Results demonstrated that the optimum conditions to extract the highest humic acid yield were using NaOH (1.0 M) atultrasound power of 20 kHzat 29 min/10 cycles. Additionally, Mn^{2+} ions application during extraction has proved its efficacy to prevent humic substances degradation during extraction. Another investigation was introduced to co-recover proteins and humic acid from dried sewage sludge for animal feed and soil fertilization (Wei et al., 2016). Firstly, protein was recovered through precipitation by adjusting the pH value to 1.0, 2.0, 3.0, 4.0 and 5.0 using H_2SO_4 (2.0 *M*) and centrifugation at 7000 rpm for 30 min. Thereafter, humic acid was separated from the acidic sludge after raising the pH value and filtration through a porous ceramic membrane (1.0 MPa working pressure).

As mentioned in the previous reports, there are numerous methods that can be used for efficient extraction of humic substances including the standardized IHSS method, alkaline extraction using Na_2SO_4 and 0.1 M NaOH mixture (Ikeya and Watanabe, 2003), as well as hot water extraction (Guigue et al., 2015). However, these methods often achieved fragmented and contradictory information. For example, the classic alkaline extraction technique cannot distinguish between humic substances derived from natural humus and those derived from non-humified materials (Kleber and Lehmann, 2019). Recently fluorescence excitation-emission matrix coupled with parallel factor analysis has been introduced to identify with high accuracy of characterization soil humic and humic-like substances. Mohinuzzaman et al. (2020) studiedthe solubility of humic substances based on their fluorescence as well as alterations in their nutrients content as affected by time of extraction, type of extracting solutions, and the solid to liquid ration. Results of this important investigation could be summarized as follows: (i) solubility of dissolved humic substances and nutrients availability increased with extraction time, (ii) salt extraction method using KCl showed a low efficacy for humic substances extraction, (iii) alkaline extraction technique was efficient for maximizing humic substances yield and nutrients solubility, and the high solubility of Si indicated the high efficacy of alkaline method to extract humic substances bound to phytolith and silicate minerals, and (vi) the solubility of humic substances and nutrients showed a high increase at the low soil to liquid ratio (1:100 - 1:100); and, exhibited the highest yield at high solid to liquid ratio (1:10).

Spectroscopic analysis of humic substances

Spectroscopic investigations provide baseline information regarding the chemical identity of carbonaceous materials based on the wavelength of their electromagnetic spectrum. Among them, FT-IR spectroscopy is considered as an important technique, which is used intensively for humic substances characterization to understand the nature, reactivity and potential alterations of their oxygen-containing functional groups. FTIR spectra analysis of humic substances extracted from compost revealed the presence of several active functional groups such as –OH (~3337 cm^{-1}), *C*–H stretching (3000 cm^{-1}), C=O stretching and —NH_2 bending (1650 - 1750 cm^{-1}), C–O stretching (1244 - 1114 cm^{-1}), S=O stretching (1030 - 1080 cm^{-1}) and C–H bending (700 - 950 cm^{-1}) (Abido and Omar, 2020). Humic acid extracted from a non-tilled soil by different alkali extracts (NaOH, $Na_4P_2O_7$ and Na_2CO_3) was studied by FTIR technique (Tatzber et al., 2007). The FTIR spectra of humic acid extracted by NaOH exhibited high aromatic and carbonyl groups and reduction of its amide groups as the soil depth increased. Conversely, aliphatic groups showed the opposite trend. Another investigation demonstrated that composting of sewage sludge affected significantly the functional groups, which appeared in a non-homogenous combination of etherified aromatic structures bearing peripheral long-chain peptidic, lipidic and carbohydrate structures at initial stage of composting. Meanwhile, the aromatic nature and the presence of phenolic, methoxylic and carboxylic groups were presented during composting process due to the oxidation of readily accessible moieties of lipidic and peptidic structures (Amir *et al*., 2004). The terrestrial humic acid exhibited structural differences compared to those extracted from aquatic natures and the aromaticity of humic acid showed an increase following ozonation process with a presence of aluminosilicate traces (Rodríguez et al., 2016).

UV–Vis spectroscopy is also an important technique, which provides important details regarding the origin and chemical constitution of humic substances (Mecozzi et al., 2002). In this context, classical parameters such as E_4/E_6 (the ration between absorbance at 465 nm and 665 nm) and $\Delta \log K$ coefficient ($\log Abs_{400nm} - \log Abs_{600nm}$) provide insights regarding the humification degree of humic substances extracted from several organic feedstock (Peuravuori, 2000). The ratio of humic acid/ fulvic acid increased with increasing Q4/6 measured in visible spectral range, and the highest absorbance of visible spectral was noticeable in *Luvic Chernozem* and *Haplic Chernozem* (FasurovÁ and PospÍŠIlovÁ, 2010). Spectral alterations of humic acids during photocatalytic oxidation was further studied using UV–vis absorption (Uyguner and Bekbolet, 2005). Photocatalytic degradation of humic acid resulted in originating small fractions of lower molecular size humic substances. In addition, for fractions below 10 kDa, UV254 absorbing moieties in treated humic acid samples became higher than that of raw humic acid. As mentioned earlier in the study reported by Rodríguez et al. (2016) regarding the spectroscopic alteration of humic substances during ozonation, UV–Vis parameters showed that the fitted correlations with aromaticity were $SUVA_{254}$, $SUVA_{280}$, A_{280}/A_{350} ratio and A_{250}/A_{364} ratio. In addition, the best correlations with molecular weight were $SUVA_{254}$, $SUVA_{280}$ and A_{280}/A_{350} ratio.

^{13}C NMR has been employed to understand the functionality of humic substances and to differentiate between carbon nuclei (Fernandes et al., 2010). ^{13}C NMR spectroscopy was performed to characterize humic substances extracted from soils of two Siberian Arctic islands (the degree of humification and the molecular structure) (Abakumov et al., 2015). One of the investigated soils (Bolshoi Lyakhovsky Island) exhibited both modern and buried horizons; however, the other one (Wrangel Island) exhibited only the modern humus horizon. The degree of aromaticity was higher (2-3 folds) in fulvic acid relative to humic acid and it showed high oxygen-containing functional groups. In Saudi Arabia, ^{13}C NMR spectroscopy was employed to characterize humic acids extracted from agricultural byproducts (agricultural wastes, date palm fronds and poultry manure) (Al-Faiyz, 2017). ^{13}C NMR spectroscopy demonstrated that humic acid extracted from agricultural wastes recorded the lowest carbohydrate content as compared with other feedstock. The aromaticity of humic acid was higher in that extracted from date palm fronds; however, humic acid extracted from poultry manure showed the highest content of aliphatic compounds. Humic acid samples showed high intensity of hydroxyl groups and did not show absorbance for carbonyl groups.

Role of humic substances in improving soil quality indices

Soil physical properties

Humic substances are important soil constitutions that have several key-roles in improving soil fertility in terrestrial ecosystems. Therefore, studies undertaken regarding humic substances supplementation either as soil application or as foliar spraying showed a progressive increase in the last decade (Table 1). The unique composition of humic substancesis interlinked within the aggregation of soil particles and improving biogeochemical reactions in the rhizosphere. Sandy soils are the most vulnerable to the potential deteriorations of environmental conditions (*e.g.* erosion) due to their poor texture. Piccolo and Mbagwu (1990) demonstrated that humic acid application led to increase large-size aggregates for the most fragile soils. Agricultural soils located at Mediterranean climates, especially those exposed to wetting and drying cycling, are also subjected to significant reductions inaggregate stability and runoff. To overcome this environmental problem, a silty-clay loam soil exposed to wetting and drying cycling in Venetia, Italy was treated with humic acid supplementation (0-10.00 g/kg of soil). Soil amendment with humic acid not only improved aggregate stability but also reduced the disaggregating effects of wetting and drying cycling and structural stability of soils (Piccolo et al., 1997a). A typical watershed in the Southern Red Soil Hilly Region (ShaoyangStation, Hunan, China) was studied to explore dissolved organic matter characterization in response to water erosion (Zhang et al., 2019). Findings obtained from this study demonstrated that the concentration of dissolved organic matter in the deposited topsoil (0–5cm, $0.69gkg^{-1}$) was higher than that in the eroded soil ($0.27gkg^{-1}$). The high aromatic, hydrophobic and high molecular weight moieties nature of dissolved organic carbon was concentrated in the eroded topsoil relative to those in the deposited topsoil.

Not only by runoff soils are eroded but also by raindrop splash, which act as small bomb destroying soil aggregates. A rainfall simulator (2 × 0.05 × 0.01 m with a slope of 15%) was packed with soil (bulk density of 1.20 Mg/m^3) and subjected to artificial rainfall of 400 mm h^{-1} for 1 hr (Piccolo et al., 1997b). Humic acid (originated from Sardinia coal under N_2 atmosphere with 1 M NaOH at 100°C for 7 h) were applied at rates of 0, 0.05, 0.10, 0.50 and 1.00 g/kg and amounts of soil erosion, starting time of runoff and drainage were determined.

Increasing humic acid application delayed runoff and reduced the erodability of tested soils through forming humus-polyvalent metal-clay complexes between active functional groups of humic acid and surface polyvalent cations (*e.g.* Al^{3+}, Ca^{2+} and Mg^{2+}) of clay minerals. In Turkey, liquated humic substances were tested at levels of 5-40 mL L^{-1} as soil spraying on erosion pans (1.5 m^3 at slope of 9%) to reduce soil losses and runoff caused by artificial rainfall conditions (40 mm h^{-1} for 1 hr) (Sinkpehoun and Yönter, 2018). The highest humic substances level reduced splash (37%), water runoff (45%) and soil losses by 97%.Soil aggregation indices (macroporosity, bulk density and microinfiltration) were measured to explore the ameliorative effect of sludge derived humic and fulvic acids (0-40 Mg ha^{-1}) on a fragile soil (Norambuena et al., 2014). The macroporosity increased as the amount of humic and fulvic acid levels increased. Additionally, the use of liquid humic substances in combination with calcium sulphate was the most efficient treatment for improving soil aggregation.

In China, the effect of bentonite-humic acid applied at a rate of 30 Mg ha^{-1} was investigated by Zhou et al. (2019) for seven years under the field conditions. They found that addition of bentonite-humic acid mixture improved soil physical properties (soil aggregate distribution, soil porosity and water holding capacity). Moreover, maize productivity increased significantly by about 50% after five years of application and remained steady thereafter. Different humic substances (*i.e.* fulvic acid, humic acid, insoluble humin, iron-linked humin and clay-combined humin) were investigated for their feasibilities for improving the characteristics of heavy textured soils in different typical zones *Luvisols*, *Alisols*, *Acrisols*, *Plinthosols*, and *Ferrosols* (Wei et al., 2020). Aggregate-associated fulvic acids were positively interlinked with clay content (illite and kaolinite) and sesquioxides; however, it was negatively correlated with soil pH, silt content and vermiculite. These physicochemical characters, however, showed opposite effect on aggregate-associated humic acids and humins. Another long-term field experiment was carried out in China (8-years field experiment) showed that humic substances promoted structural stability binding silt + clay fractions to form secondary microaggregates and then larger aggregates (Zhang et al., 2018).

A high number of widely cited research articles pointed out to the high efficiency of humic substances on improving water retention in soil matrix. To highlight the remarkable effect of humic substances on water retention, 44 soil profiles were sampled from State of Santa Catarina, Brazil (Costa et al., 2013). Results of this investigation demonstrated that water retention increased with increasing soil organic matter content, especially those exhibited humic characters.In Egypt, Selim and Mosa (2012) studied the effect of humic substances applied at the rate of 120 L ha^{-1} on water and nutrient supply potentials of a sandy soil. The unamended treatment showed prevalence of the vertical movement caused by gravity force, which led to substantial water losses from the root zone. The application of water soluble humic substances improved the lateral movement and the capillary force of sandy soil due to water retention *via*

TABLE 1. Former investigations for studying humic substances application in terrestrial ecosystems.

Humic feedstock	Application rate/ method	Cultivated plant	Soil texture	Significant finding	References
leonardite	1.50 t ha^{-1} (soil application)	Rapeseed	sandy1 clay loam	Improving soil physical and chemical properties and maximizing productivity of rapeseed, and ameliorated saline-sodic soil	(Nan et al., 2016)
Commercial	80 $g{\cdot}m^{-2}$ (Foliar application)	Potato	Clay	Increasing N and P availability in soil and their uptake by potato as well as inhibition of hollow heart	(Suh et al., 2014)
Commercial	5%	Faba bean	Sandy	Improving growth responses, photosynthetic pigments, seed yield quantity and quality	(Dawood et al., 2019)
lignite	30 Mg ha^{-1} (soil application)	Maize	Sandy	Improving soil structure the formation of water stable aggregates as well as water holding capacity of soil	(Zhou et al., 2019)
lignite	6.2 g pot^{-1} (soil application)	Celery and leek	Sandy	Improving sorption complex characteristics, organic matter content and dehydrogenase activity	(Ciarkowska et al., 2017)
Commercial	20 kg ha^{-1} (soil application)	Thai basil	Sandy	Enhanced the soil organic matter, microbial activity, essential oil quantity, and quality	(Dehsheikh et al., 2020)
Commercial	200-600 kg ha^{-1}	Potato	Clay loam	Increasing tuber weight, dry matter, specific gravity, starch content, protein, and nutrients concentration	(Ekin, 2019)
Commercial	10-40 ml/L (Soil and foliar application)	Pepper	Clay	Increasing total and reducing sugars, chlorophyll content and marketable yield	(Karakurt et al., 2009)
Humalite	4 g C kg^{-1}	*Medicago sativa* and *Elymustrachycaulus*	Laomy	improving macro aggregation, organic carbon, and macronutrients and showed a long-term effect on maximizing the yield	(Szczerski et al., 2013)
Soil	140-560 mg kg^{-1}	Wheat	Silty loam	Increasing plant biomass yield	(Chen & Zhu, 2006)
Vermicompost	2.24 g/l	Winter wheat		Increasing grain yield	(Bezuglova et al., 2017)
Soil, podrite and compost of clover straw	25-100 mg kg^{-1} (soil application)	Sorghum	Sandy	Maximizing quantitative and qualitative yield characters of sorghum as well as calcium and iron use efficiency by sorghum plant	(Hamad & Tantawy, 2018)
Compost and Biogas manures	47.6 and 95 Lha^{-1} (foliar application)	Faba bean	Loamy sand	Increasing NPK uptake and the dry weight yield of faba bean	(Samie et al., 2018)
Commercial	1 g $plant^{-1}$ (soil application)	Potato	Clay	Improved potato growth, specific gravity of tubers and nutrients uptake by plants.	(Abd-Elrahman & Taha, 2018)
Commercial	2g L^{-1} (foliar application)	Cowpea	Sand clay loam	Increased plant growth parameters, chemical composition of leaves and quality of seeds	(Faiyad et al., 2019)
Commercial	4% (foliar application)	Tomato	Clay	Enhancing vegetative growth indices and quality of fruits in addition toinhibiting the symptoms of blossom end rot in tomato fruits	(Abou El Hassan & Husein)

hydrophilic properties of humic substances. This noticeable improvement of water holding capacity of soil increased water retention in the root zone and reduced the number of irrigations. A novel functionalized humic material (poly (acrylic acid-*co*-acrylamide) $AlZnFe_2O_4$/potassium humate) was applied at rates of 0.1 to 0.4 w/w% to explore its positive effect on maximizing water holding capacity of soil (Shahid et al., 2012). The functionalized humic materials significantly increased soil moisture retention and seeds germination and delayed the wilting of seeds by about 6–9 days.

Improving soil chemical properties

The unique chemical composition of humic substances, which appeared in its recalcitrant nature, huge surface area, high content of reactive functional groups and nutrients concentration, reinforces its stimulating effect to soil chemical properties.The ameliorating effect of soil humic additives at the rate of 25 kg ha^{-1} on availability of Cd, Pb, Ni, and Zn was investigated under a 180-day incubation experiment (Karaca et al., 2006). Humic substances reduced the availability of heavy metals in soil following their stabilization at low-available forms. Several investigations were carried out to illustrate the short-term effect of humic substances applications on soil chemical properties. However, the long-term effect of humic substances application in terrestrial ecosystems is not fully understood. A 3-yr consecutive experiment was carried out in the North China Plain under continuous peanut cropping to investigate the beneficial effect of humic substances application on physicochemical properties, microbial diversity, and enzyme activities of soil (Li et al., 2019). The beneficial effect of humic substances application led to increase in total& available NPK concentrations, soil organic matter content by the third year. However, the stimulating effect of humic substances application showed early responses on urease, sucrase, and phosphatase activities after the first year of application.

Soil salinization has become a worldwide serious and complicated problem, especially in arid and semi-arid zones(Sary and Elsokkary, 2019)**.** The accumulation of sodium ions inside the soil matrix caused several deteriorative impacts on soil physical properties, which hindered water and air movement across the soil profile (Bano and Fatima, 2009). Furthermore, high salinity stress can impose ion toxicity and osmotic stress leading to cause several harmful effects on soil microbial activities and plant nutritional status (Alshaal et al., 2017). The excessive accumulation of sodium in soil solution and active soil components (soil colloids) negatively affect physicochemical properties of soil (*e.g.* swelling/dispersion of clay particles, disruption of soil aggregates and water-salt movement) (Clark et al., 2007; Tejada etal., 2006; Yu et al., 2014). Former investigations showed an ameliorative effect of humic substances application toward leaching the excessive Na^+acrossthe soil matrix, thereby reducing salinity stress on plant and soil microorganisms. The amending effect of humic acid derived from sewage sludge on saline–alkali soil was investigated under column experiments (Motojima et al., 2012). Humic acid application reduced the pH and increased the cation exchange capacity of soil. According to Liu et al. (2019), humic substances application to a saline coastal soil located at Shandong Province, China reduced soil salinity due to developing soil macroaggregates, which facilitated the leaching of salts from soil matrix. Another field experiment was carried out in Chile to investigate the reclamation effect of humic substances on saline–sodic soils (Alcívar et al., 2018). Humic substances application at the rate of 5 kg ha^{-1}signifcaintly reduced electrical conductivity (ECe), sodium adsorption ratio (SAR), and exchangeable sodium percentage (ESP). In the eastern coastal area of China, a 3-yr field investigation was conducted at saline-sodic soil to evaluate the reclamation effect of humic acid (1.50 Mg ha^{-1}) on physicochemical properties of soil (Nan et al., 2016). Humic acid application reduced soil reaction (pH), exchangeable sodium percentage (ESP), sodium adsorption ratio (SAR). Meanwhile, soil electrical conductivity (ECe) and saturated hydraulic conductivity (*Ks*) showed slight increments.

Incorporation of humic substances into the soil matrix was an effective approach for improving soil chemical properties including total carbon/nitrogen, pH, EC, and cation exchange capacity (Pal, 1992). Several types of compost originated from different feedstock (coal, compost and peat) were tested for their function in modulating soil pH, EC, CEC and the adsorption of K^+ and NH_4^+ into soil and their availability to maize plants under pot experiments (Mindari et al., 2014). Humic acid application reduced soil pH and EC and increased exchangeable Ca^{2+}, Mg^{2+} and K^+ and NH_4^+ .Soil acidity is another major limiting factor for sustainable crop productivity in the tropical soil subjected to high leaching and weathering (Zheng, 2010). Several reports pointed out to the beneficial effect of humic substances on improving chemical properties of acidic soils (Zheng, 2010). Potassium humate derived from lignite improved the chemical properties of acidic soil including the increase of soil reaction, soil buffering capacity, cation exchange capacity, phytoavailability of plant nutrients and immobilization of heavy metals because of its alkaline nature and the abundance of active functional groups (Shujrah et al., 2010). Acidic soils decreased the availability of phosphorus due to the high content of soluble Al species. To overcome this problem, Yang et al. (2013) reported that fulvic acid increased soil pH (from 5.83 to 6.27) after 21 days of soil application. Additionally, the extractability of Al^{3+} ions underwent substantial reduction, and this in turn increased phosphorus availability in soil. The ameliorative effect of humic substances on phosphorus availability is also reported under alkaline soil conditions. Humic acids and their derivatives improved phosphorus availability due to their beneficial effect on binding active Ca^{2+} ions (El-Ghamry et al., 2009; Mosa et al., 2016; Wang et al., 1995).

Improving soil biological properties

Healthy and fertile soils are crucial for ensuring the optimal profitability and sustainability of crop productivity. Among different carbon-based compounds, humic substances are the most efficient ones for improving soil health and biological indices because of their content of indol acetic acid (IAA) (Canellas et al., 2002). Humic substances (as organic additives) have a long-term motivating effect on soil microbial activities *via* raising soil organic carbon content (Qin et al., 2019). Other reports suggested the beneficial role of humic substances to the active functional groups, which act as electron donors for bacterial respiration (Coates et al., 2002). Besides, these active functional groups triggered the anaerobic oxidation of CH_4 (Tan et al., 2018). Additionally, these organic amendments stimulated the activities of bacterial heterotrophs and autotrophic nitrifiers, which in turn improve plant cell membrane permeability to nutrients and consequently enhance plants growth (Valdrighi et al., 1996). According to Maji et al. (2017), application of humic substances derived from vermicompost stimulated microbial biomass carbon, microbial biomass nitrogen and CO_2 respiration. Another recent study demonstrated that application of humic substances originated from spent mushroom substrate to paddy field olocated at south eastern China showed a remarkable effect on improving soil organic carbon, alkali-soluble humic carbon, microbial biomass carbon and microbial biomass nitrogen (Li et al., 2020). In additional study, Til'ba and Sinegovskaya (2012) reported that humic acid supplementation to soybean either as seed coating or foliar spraying with sodium humate maximized nodules number and nitrogen-fixing efficiency, thereby increased the produced yield by about 22% relative to the control (without humic substances supplementation). The beneficial effect of water soluble humic substances on growth of *Bradyrhizobium liaoningense* CCBAU05525 and its nodulation was further confirmed by Gao et al. (2015). Results of this study demonstrated that water soluble humic substances maximized cell density, nodules number, nodule fresh weight and nitrogenase activity of soybean. Authors attributed this stimulating effect to the flavonoid analogues that enhanced the expression of *nod* genes involved in symbiotic nitrogen fixation.

Humic substances might have an important role in stimulating soil enzymes. In support of this, the effect of humic acid supplementation on the microbial population in the rhizospheric sugarcane was studied (Sellamuthu and Govindaswamy, 2003). Their results indicated that bacteria were the most dominant population, which increased significantly as the application rate of humic acid increased in the rhizosphere. Furthermore, humic acid application stimulated alkaline phosphatase and dehydrogenase activities. Likewise, Shahriari and Higashi (2014) indicated that humic substances application to Andosols improved the protease enzyme activities. In contrary, the beneficial effect of humic substances application was not verified in volcanic soils. For example, humic acid application in volcanic soils of Hawaii strongly diminished the activity of soil enzymes (Allison, 2006).

A recent study is directed toward functionalizing modern humic substances with modified physicochemical properties compared to the pristine ones. Layered double hydroxides (LDHs) exhibited unique physicochemical properties (*e.g.* higher thermal stability, huge surface area and active functional groups (Wang et al., 2017). To support this hypothesis, a greenhouse experiment was conducted to discover the effect of several LDHs supported humic acid applications (0, 1, 3, 5 and 7%) on a mine soil cultivated with *Artemisia ordosica* (Xiang et al., 2019). Results demonstrated that LDHs showed an ameliorative effect on potentially toxic elements (Pb, Cr, Ni, Cd, Zn, and As) and improved soil enzyme activities (β-glucosidase, urease, and phosphatase).

The ameliorative effect of humic substances on polluted soil remediation

Due to the vast urbanization and industrialization, the rapid discharge of potentially toxic elements into the ecosphere caused several deteriorative impacts on the terrestrial ecosystems (El-Ramady et al., 2015; Moghanm et al., 2020; Mosa et al., 2016; Yin et al., 2019). Additionally, the transport of these potentially toxic elements across soil profile possesses a potential ecological threat to the water aquifers. Several carbonaceous materials (*e.g.* biochar) exhibited a high efficacy toward heavy metals immobilization/ stabilization (El-Banna et al., 2019; Fang et al., 2017; Mosa et al., 2018; Mosa et al., 2017; Song et al., 2019 and Mosa et al., 2020). Others found that humic acid minimized sorption of heavy metals, *i.e.* Cs and Sr on clay minerals (ElShazly et al., 2019). Soil washing is considered as one of the most efficient techniques for heavy metals removal from contaminated soils instead of its stabilization into the soil matrix. However, most of washing surfactants (*e.g.* EDTA, salts, strong acids and surfactants) are toxic to the biosphere microorganisms and soil fauna. Thus, the recent on-going research is looking for affordable and eco-friendly materials to serve as washing chemicals for contaminated soils, in which humic substances could have the potentiality to become an efficient one in this regard. In support of this, leonardite-derived humic substances were tested for their potential application as a washing material for Cd-contaminated soils (Meng et al., 2017). Humic substances showed a high ability toward Cd decontamination and the removal efficiency increased by increasing the concentration of humic substances, washing time and near neutral pH value.

Humic substances derived from either leonarditeor compost were evaluated for washing of synthetic contaminated soil aged with Cu, Pb, Zn, Cd, Cr for 4 and 12 months. Humic substances derived from compost exhibited a higher washing capacity for the removal of heavy metals compared to leonardite. Another washing experiment was carried out in Poland to investigate the potentiality of humic substances extracted from sewage sludge for the removal of Cu and Cd from highly polluted clay and sandy clay loam soils (Kulikowska et al., 2015). Single washing (one batch sorption) with humic substances showed a moderate removal efficiency of heavy metals (80.7% of Cu and 69.1% of Cd with the sandy clay loam soil and 53.2% of Cu and 36.5% of Cd with the clay soil). Meanwhile, the triple washing (triple batch sorptions) of contaminated soils removed 100% of Cu and Cd from the sandy clay loam soil and about 85% from the clay soil. Another investigation was conducted on a polluted soil in Nottingham, UK, which has been treated with sewage sludge for long decades (Yang and Hodson, 2019). Synthetic humic-like acid with high content of COOH was tested as a washing material and removed 45.2, 34.6, 42.2 and 15.6% of Cu, Zn, Ni and Pb, respectively. Humic acid was further subjected to a comparison with synthetic surfactants (sodium dodecylsulphate and Triton X-100) for decontamination of highly polluted soil close to chemical plantin Northern Italy (Conte et al., 2005). Humic acid showed a comparable removal efficiency compared to synthetic surfactants (~ 90%). It is therefore, recommended to use natural humic substances for the removal of heavy metals from contaminated soils given their additional role in improving soil health relative to other synthetic surfactants.

Role of humic substances on stimulating physiological plant system

The beneficial impacts of humic substances on plant growth are mainly related to their direct (stimulating physiological plant responses) and/ or indirect (improving the growth conditions in the rhizosphere) effects (Alshaal et al., 2017; Mosa, 2012; Selim et al., 2009). The assertion that humic substances might have a direct effect on physiological plant responses takes place probably through the potential uptake of these organic molecules by plants. Prat (1963) was among pioneers who studied this issue, and he relied on color alterationsin plant organs subjected to humic substances application. Later, the isotopic technique (^{14}C-labeled humic substances) revealed a considerable uptake of isotopic humic substances, especially fulvic acid (Vaughan and Ord, 1981). In general, plant response to the exogenous supplementation of humic substances differed widely according to the plant genotype (*e.g.* the response of monocotyledonous appears greater than dicotyledonous) and the origin of humic substances (*e.g.* lignite and leonardite feedstock are less effective than peat, composts or vermicomposts) (Canellas and Olivares, 2014). These humic substances contain active substances called "auximones" (previously known as "auxin-like activity") which can stimulate plant growth (Nardi et al., 1988; Piccolo et al., 1992). Moreover, humic substances induce carbon and nitrogen metabolism in which might promote physiological responses of plant. In view of this, enzymes linked to the pathways of nitrogen assimilation (*e.g.* nitrate reductase, glutamate dehydrogenase and glutamine synthetase) are significantly promoted *via* the exogenous application of humic substances (Hernandez et al., 2015 and Vaccaro et al., 2015). In this context, transcription regulation as well as large number of genes linked in developmental and metabolic processes are regulated by humic substances (Jannin et al., 2012). Additionally, humic substances are responsible for stimulating up to 80% of genes linked in sulfate metabolism (sulfate transporter, ATP sulfurylase and serine acetyltransferase) (Canellas et al., 2015)

Humic substances have further positive effects on modulating the induced resistance of plant linked to biotic and abiotic stress conditions through several mechanisms including: (i) improving cell membrane stability, (ii) stimulating the generation of active compounds linked to shikimic pathway, which serve as plant resistance promoters against non-enzymatic antioxidant, (iii) maintaining water uptake under ionic stress conditions, (iv) synthesis of hormones and proteins, (v) inducing enzymatic defense against stress conditions/plant pathogens and (vi) increasing nutrients uptake by plant roots (Aslam et al., 2016; Calvo et al., 2014; Saidimoradi et al., 2019 and Eid et al., 2019). According to Azevedo and Lea (2011), humic substances application modulated the capacity of plant to adjust osmotic stress through maintaining water uptake and cell turgor. In addition, humic acid application to rapeseed subjected to water stress promoted the activity of antioxidant enzymes (peroxidase, ascorbateperoxidase and catalase), improved PSII activity and decreased lipid peroxidation (Lotfi et al., 2015). Humic substances generated from biochar showed also a scavenging effect on Pb-induced oxidative stress on chicory plants grown in hydroponic system (El-Banna et al., 2018).

Several reports mentioned about the stimulating effect of humic substances on H^{+}-ATPase that is responsible for proton-pumping across plasma membrane of roots, thereby generating proton,which is the motive force necessarily for the active and passive transport of metal ions and metabolites through the symplastic pathway (Morsomme and Boutry, 2000). It is hypothesized that humic substances are able to motivate nutrients uptake by plants through interfering with the transcription of genes linked in meristem formation, cell cycle behavior and microtubule organization and cytokinesis (Trevisan et al., 2010). Uptake of ammonium and nitrate *via* energy-dependent process is generated by H+-ATPase proton pumping existed in the plasma membrane (Tegeder and Masclaux-Daubresse, 2018). According to Huertas Tavares et al. (2019), humic acid-derived compost showed stimulatory effects on nitrogen uptake and assimilation by rice grown under low and high nitrate and ammonium in the nutrient solution. The enhancement of nutrients uptake by plants is significantly affected by the improvement of soil health. For instance, humic acid improved nutrients availability and uptake by maize plants through improving the fungal/bacterial community structures (Liu et al., 2019) E . Regarding the stimulating effect of humic substances on micronutrients, it comes from generation of metal–humic complexes that protect micronutrients from leaching into the hydrosphere and/or fixation into insoluble fractions (Chen et al., 2004; El-Ghamry et al., 2018; Garcia-Mina et al., 2004; Selim et al., 2010). Humic substances are also responsible for stimulating genes encoding the Fe(II) root transporter (CsIRT1) and Fe(III) chelate-reductase (CsFRO1) that maximize the availability of Fe(II) and its uptake by plants (Elena et al., 2009). Other reports pointed out to the physiological mechanisms of humic substances, which induce alterations in root morphology and modulate plant membrane activities that regulate nutrients uptake, primary and secondary metabolic pathways as well as hormonal and reactive oxygen balance (Zanin et al., 2019).

The pre-harvest treatment of crops with humic substances can offer a cost effective and safety technique to reduce the post-harvest losses. The beneficial effect of humic substances on regulating nutritional plant system induces consequently the postharvest responses of plant (Faiyad et al., 2019) and this might be to enhancing calcium uptake and hormone-like activity of humic substances (Nikbakht et al., 2008). Physiological mechanisms of foliar application of humic acid on cut chrysanthemum flower postharvest vase life are attributed to the increments of chlorophyll content, antioxidant enzymes, synthesis of soluble sugars and proteins as well as reducing malondialdehyde content in chrysanthemum flowers (Fan et al., 2015). More recently, Sandepogu et al. (2019) reported that exogenous application of humic acid promoted the early growth of lettuce and spinach and reduced losses of marketable yield during storage stage.

Future prospective for the agro-environmental applications of humic substances

Our globe is changing at a rapid pace, driven by the progressive scientific knowledge. In view of this, the ongoing research regarding the agro-environmental applications of humic substances is looking for innovative techniques to maximize their efficacy. For example, an innovative technique has been introduced during the last decade in Egypt for the effective application of humic substances (Mosa, 2012; Selim and Mosa, 2012; Selim et al., 2010 and Selim et al., 2009). In this technique, humic substances are applied through the drip irrigation system. This technique provides a uniform distribution of humic substances *via* drip irrigation in which water and fertilizers use efficacy is improved significantly. In addition, humic acid combination with active microorganisms could be highlighted as a novel technique for improving the effectiveness of humic substances application. In this regard, the synergy of the combination between microalgae (*Scenedesmus subspicatus*) and humic substances has been introduced by Gemin et al. (2019). Results of this investigation demonstrated that onion seedlings immersion into the mixture of humic acid and microalgae solution improved growth, yield, nutrients uptake and quality parameters of onion yield. Seeds coating with humic acid is an efficient technique for increasing seeds vitality, improving the rhizospheric microbial community and increasing the nutritional status of plant. Maize seeds coating with humic acid was investigated for the potential maximization of plant growth and bacterial activity in the root zone (da Conceicao et al., 2008). Seeds coating stimulated the growth of maize and endophyticdiazotrophic bacteria *(e.g. Herbaspirillum seropedicae*). Likewise, the encapsulation of seeds with urease could substantially contribute to maximize nitrogen use efficiency at early growth stages of plant. Fabrication of stable humic-urease complex in barley seeds encapsulation for improving nitrogen use efficiency was studied (Mvila et al., 2016). The bioassay experiment revealed that humic-urease complexes restarted the conversion of NH_4^+ –N into NO_3^- –N, thereby increased the uptake of nitrogen by about 2-fold.

On the other hand, there is a noticeable progress in the area of using humic substances for aqueous pollutants removal/recovery. A novel biosorbent material was developed by coating cellulose extracted from rice husk with humic acid for the active removal of Cr and Ni (Basu et al., 2019). This novel biosorbent material showed a high efficiency for the removal of aqueous Cr(VI) and Ni(II).The bioavailability of aqueous organic pollutants can be mitigated by sinking to suspended particles. Coating suspended particles with humic substances showed also high efficiency for maximizing the removal of pharmaceuticals, estrogens, and phenolic compounds (Ra et al., 2008). A cost effective synthetic humic substances (humic acid-modified biochar) was designated via hydrothermal-assisted pyrolysis technique for the removal of aqueous mercury, and recorded about 95% remediation efficiency (Zhang et al., 2020).

Conclusion

Humic substances have a wide range of agro-environmental applications due to their beneficial roles on improving soil fertility, modulating plant physiological responses and controlling the accessibility of pollutants across the environment ecosystems. These beneficial roles together with the current global challenges toward maximizing safe food demands in a sustainable manner enlarge the agro-environmental significance of humic substances to be the ideal eco-friendly natural product for ecological intensification of terrestrial environments. We reviewed the structural properties of humic substances, their extraction and their spectroscopic characterization. We then highlighted their potential roles in improving soil fertility indices taking into consideration their stimulating effect to water and nutrient supply potentials, their reclamation effect to the polluted soil as well as their physiological effect on modulating plant organelles. Finally we should mention that, further systematic investigations should be provided to elucidate the relationships among extraction technique of humic substances and their potential impacts on the ecosphere.

Declaration

Authors declare that there is no conflict of interest regarding financial issues or personal relationships that might influence the work reported in this manuscript

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التطبيقات البيئية-الزراعية للمواد الدبالية: دراسة مرجعية نقدية

تعتبر عملية تطوير إنتاجية المحاصيل بطريقة مستدامة أحد أهم التحديات المستقبلية لتوفير ما يكفي من إمدادات الغذاء والأعلاف و الألياف أخذا في الإعتبار الحد من تدهور جودة التربة و حديثا تم عرض مصطلح التكثيف البيئي كمنهج صديق للبيئة لتطوير الإنتاجية المحصولية (تعظيم امكانيات إمداد الماء والعناصر الغذائية للتربة) مع الحد الأدني للإضافات المخلقة (مثل الأسمدة المعدنية). المواد الهيومية (المكونات النهائية لتحلل المادة العضوية) من بين أهم المنتجات الطبيعية والتي يمكن أن تساعد في تحفيز التكثيف البيئي للإنتاجية المحصولية عطفا علي محتواها العالي من العناصر الغذائية، سطحها النوعي الكبير، محتواها من المحفزات الحيوية النشطة، ثباتها ضد التحلل الميكروبي و محتواها الكبير من المجاميع النشطة الفعالة. ومن ثم، تلقي المواد الهيومية اهتماما كبيرا لتحسين معايير جودة التربة، تحفيز الإستجابات الفسيولوجية للنبات و تعظيم المعايير الكمية والنوعية للمحصول.علي الجانب الآخر, الخواص الفيزوكيميائية الفريدة للمواد الهيومية يدعم قابلية استخدامها كمحسن تربة كفء نحو تثبيت العناصر محتملة السمية.في هذا البحث المرجعي، سوف نلقي الضوء علي التطبيقات البيئية-الزراعية للمواد الهيويمة أخذا في الإعتبار الأبحاث الجارية في هذا الإطار. بالإضافة لذلك، سوف نشير إلي الآفاق المستقبلية للأبحاث الجارية حول التقنيات الحديثة لتوظيف المواد الهيويمة في العديد من التطبيقات البيئية الزراعية.