Journal of Kufa for Chemical Sciences
ISSN(P) :2077-2351 ISSN(E): 3006-3280
Journal Homepage:
https://journal.uokufa.edu.iq/index.php/jkcs/index

review article

Discovering The Techniques For Obtaining Humic Acid and Its Power on Plants and Soil Health: A Comprehensive Review

Al- Kubissi Kh. Y.[1], Abdulrahman M. F.[2] Hameed A. T[3]

1Lec., Department of Chemistry, College of Sciences, University of Anbar, Al Anbar, Iraq

2Lec., Department of applied chemistry, Applied Science- Hit, University of Anbar, Al-Anbar, Iraq

3Assit. Lec., Department of Chemistry, College of Sciences, University of Anbar, Al-Anbar, Iraq

*E-mail: 1Khdyaralkubysy@uoanbar.edu.iq,

2mayfahmi2015@uoanbar.edu.iq

3abdullah.thamer@uoanbar.edu.iq

Abstract:

Concern about the spread of diseases associated with the use of chemicals and hormones in food production has accelerated the shift in developed countries toward agriculture using humic acid. This orientation stems from the desire to achieve economic stability and improve public health. Humic acid agriculture, which avoids the use of chemical fertilizers, is considered a safer and more sustainable alternative. These countries aim to reduce the health risks associated with exposure to chemicals, while supporting environmental sustainability at the same time. This article discusses the global need for sustainable agricultural practices that enhance productivity and the production of environmentally friendly materials. It aims to provide technologies that improve agricultural production per unit area and ensure a healthy ecosystem for humans, plants and animals. This article focuses on humic acid, It is an important organic substance that enhances plant growth and productivity. Humic acid plays a pivotal role in soil, converting fertilizers into nutrients that are easier for plants to access. This acid is characterized by its diverse applications, including adsorption and inhibition processes for some microorganisms present in the soil, and it is very effective in enhancing soil fertility.

Keywords: Humic Acid, Plants and Soil Health,

1. Introduction

Soil is a limited, non-renewable natural resource that plays a crucial role in supporting plant growth and development by providing essential nutrients and water [1]. However, human activities have long threatened soil resources, primarily through the application of synthetic fertilizers, pesticides, and herbicides, leading to declines in soil fertility, health, and biodiversity [2,3]. The soil fertility and quality degradation processes are becoming a major concern as they lead to decreased crop productivity, which poses great challenges in ensuring food security for the growing world population. Therefore, soil reclamation or remediation is gaining increasing interest among researchers as it helps to restore the original fertility of the soils. In this regard, HA, a naturally occurring aromatic organic matter produced through the humification of organic substances, has garnered significant attention among scholars and researchers worldwide. [4,5]

Humic acid (HA) is a complex mixture of various organic compounds produced during the humification and mineralization of organic matter through the action of microorganisms, fungi, and plants [6]. It accumulates naturally in soil, sediments, freshwater, and marine environments as a byproduct of decomposition processes. HA-based preparations are generally regarded as safe in terms of environmental and ecological impact, thus making them favorable candidates as natural growth promoters. HA compounds significantly influence soil properties and can remediate degraded soils. HA promotes the growth of a wide range of plants, weeds, and crops, enhancing the plant growth in treated soils with improved crop yield and fruit size and quality [7-9]

1.1. Introduction to Humic Acid (HA)

Humic acid (HA) is a dark brown-black gray natural polymeric organic compound and is one of the components of humic substances (HS). It is an important part of the earth's biosphere, and its existence and functions on the earth have attracted the attention of geoscientists, soil scientists, ecologists and chemists. It has a wide range of functions, such as maintaining soil fertility, purifying water quality, improving the ecological environment, and alleviating global warming. HA is also a class of natural organic compounds that exist in soil, water, and the atmosphere during the process of biological decomposition. [10,11].

Humic acid is one of the main organic macromolecules present in humic substances, accounting for about 50%–80% by weight of the total humic substances. It is an important component of soil organic matter (SOM) [12]. They are naturally found in humics, soils, peat, shale, lakes, and marine sediments, either in a form that sticks to the surfaces of soil and sediment particles to protect them or as a form that forms clumps together [13]. HAs, as natural organic substances, are also found like fulvic acids (FAs) in the effluent from bioreactors. HAs are characterized by a variety of polar and non-polar groups, such as carboxy, phenolic, and alcohol hydroxyls, ketones, amino acids, polysaccharides, polypeptides, quinones, esters, aromatic hydrocarbons, etc., which form a spectrum of both acidic and basic functional groups. The complexity and amphiphilic nature of HAs determined how they interacted with surfactants that were either cationic or anionic, as well as how they grouped together and precipitated with metals. HAs are water-insoluble and form stable dispersion in alkaline media at $pH > 8.0$, self-stabilized by the high charge density and other repulsive forces due to their amphiphilic nature. HAs are also precipitated with the addition of divalent or trivalent ions, low-molecular-weight polyelectrolytes, and nonionic surfactants. HAs have unique fluorescence spectral properties desirable for the environmental monitoring of natural organic matter, microbial biomass, and metals. HAs also effectively chelate and mobilizes metals due to the presence of a variety of hydroxyl, carboxyl, and phenolic groups. HAs with pKa between 4.9 and 5.1 are dissolved in water only under alkaline conditions and precipitated with acidification [14].

According to Calderin-Garcia *et al.* (2016) [15], HA is an amorphous macromolecular natural organic compound that consists of a carbon skeleton of aliphatic and aromatic carbon chains that are joined together by covalent bonds, van der Waals, and hydrogen bonds, forming agglomerates of variable sizes. Nitrogen, oxygen, and sulfur entities can both form covalent bonds (-N, -OH, -COOH) and complementarities (-O, -S) with the complex carbon skeleton. Aliphatic chains may contain saturated ($–nCH_2–$) and unsaturated ($–nCH_2=$) zones, and a high number of aromatic rings may form with many hydrogen atoms substituting the aromatic carbon, and a lower number of hydrogen may be present if these carbon atoms are linked to –O, –OH, or –N groups. Stability is provided by a high number of electrons delocalizations through the electron forming pi bonds in the double bounds C=C and C=O. It is not biodegradable and is highly recalcitrant to microbial attacks in natural environments. Humic substances (HSs) are made up of different kinds of organic compounds that break down plant matter and man-made waste from industries in land, water, estuaries, and the ocean. HA is a main part of these substances. It takes part in the conversion of large complex organic molecules into simple mineralized products and in the aggregation and stabilization of sediments and soil particulates. It also plays a key role in global carbon, nitrogen, and sulfur cycles and in mitigating contaminants in the environment [16-19].

Multiple diverse structural models of HA have been proposed. The Stevenson's model is a widely used model of HA. This model exhibits a significant concentration of aromatic compounds Fig 1. The compound possesses several phenolic groups, some of which are unbound while others are connected as bridge units. Additionally, it includes carboxyl groups, predominantly bonded to aromatic rings. This model comprises oxidized lignin, a combination of phenol and amino acid, a hydroxyl-quinone, and a structural unit of lignin consisting of six carbon atoms and three carbon atoms. Carbohydrate and protein, like in Dragunov's structure, are also components of Stevenson's model structure [20].

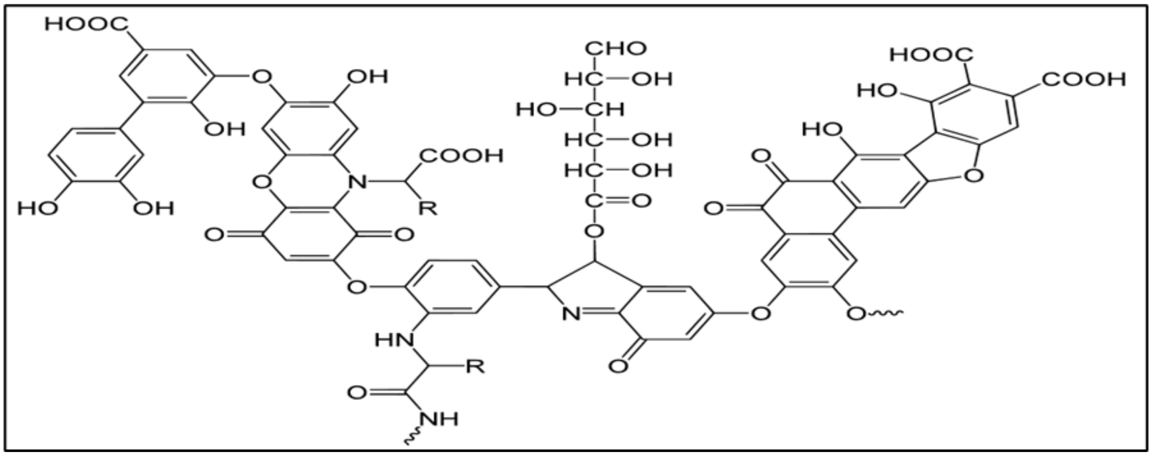

Figure 1: The Model proposed by Stevenson for Humic acid [20]

HA, with its different properties, has a wide range of uses. It can be used as a slow-release fertilizer, a compound for soil conditioning and quality improvement, and an agent for heavy metal ion adsorption. Due to the lower solubility of the natural HA in the aqueous solution, its application in practical production has not received much attention. Therefore, it is essential to extract HA with a high concentration from soil and keep it stable in the aqueous solution. An appropriate modification of the extracted HA can increase its solubility in water or shift its solubility to a specific pH area [12]. This has strong potential applications in the field of agricultural soil improvement and remediation.

1.2. Natural Sources of Humic Acid

Peat and lignite deposits have been traditionally recognized as natural reservoirs of humic acid, which is now also found in coal, soil, and river deposits. Such organic materials have been used for centuries as fertilizers to improve soil qualities. Further discoveries have expanded these materials on an industrial scale to manufacture “bio-activated” compounds included in agricultural products for more productive plant cultivation [11]. As a natural source of humic acid, the greatest attention is paid to an oxidized variety of lignite coals – leonardites. As a geochemical substance, leonardite is a soft brown-black sedimentary rock composed largely of humic acids and, to a lesser extent, of fulvic acids. Naturally, it occurs at the shallowest depths of coal-bearing sedimentary basins in regions of warm and humid climate, where deposition of fossil remains of plants took place. Coalification processes, under appropriate technogenic conditions, formed brown coals and then – black coals [11]. The process of peat transformation into coals is very slow and requires millions of years. In geological and geographical history, brown coals are of the most recent age, no more than 100 million years ago. They are formed from the youngest fossil materials of Underar when bogs with forest-floor flora of trees and shrubs predominated near oceanic basins. According to the biological recomposition and morphological structural features of molecules, HAs provide a spectrum of coloration of brown coals from dark brown through brownish to yellowish brown [21].

1.3. Importance of Humic Acid (HA) in Agriculture and Environment

Soil fertility and plant growth are critical portion of agricultural production. HA, a significant factor in soil fertility and plant growth, is responsible for determining the efficiency of soil use, ensuring environmental sustainability, and promoting sustainable agriculture. It contributes to the prevention of leaching, runoff, and denitrification of nitrogen fertilizers from the soil. Because of the need to increase crops to provide food, obtain renewable energy, and conserve the environment, soil scientists must concentrate on methodologies to improve soil fertility in the years ahead. HA has been found to be beneficial in these circumstances. The environmental implications of HA benefit from its adsorption and retardation of toxic substances in soil and water. Humic materials (HM), and hence HA, regulate the soil environment and sustain soil use effectiveness [22].

There are many reasons and benefits of using HA in agriculture. First, it can enhance soil by improving soil properties, fertility, and productivity of soils and crops [16]. HSs are viewed as the precursor of soil organic matter that partitions into humin, HA, and fulvic acid fractions. In agriculture, HA is normally added to soil to enhance and maintain plant nutrient content and availability. Second, HA can promote plant growth directly by altering physiological responses and improving seed germination [23]. HSs have the ability to impact root growth and modify the patterns of gene expression in certain genes that regulate basic metabolism, plant growth, development, and defense. Third, HA can mitigate the toxic effects of NaCl concentrations in plants. Such a response is mediated by the good antioxidant ability of HA and their ability to modulate phytohormone levels. Fourth, HA can improve shoot and root elongation, biomass, yield, and nutrition in plants. This nutrient uptake might also be supported by the production of root exudates such as mucilage, which create a greater root-soil contact surface area [24].

Due to its significance, numerous researchers have dedicated their efforts to extracting HA from different materials such as soil, peat, compost, and charcoal, employing a range of methods for laboratory or field experiments. These procedures can be categorized as chemical, thermal, and atmospheric pressure (low-temperature), or acid-base extraction redistributions. The objective of this review is to examine commonly employed techniques for obtaining HA and emphasize its importance for soil and plants [25].

Besides their agricultural application as soil conditioners in cropland, humic acids have been used in aquaculture, bioethanol production, and municipal wastewater treatment [26]. Humic acid has high phenolic hydroxyl and carboxyl functional groups, which can be oxidized into chelating agents for the solubilization of heavy metals into corresponding humic acid. Water-soluble humic acid can effectively maintain the concentration of heavy metals in water [27, 28].

2. Conventional Extraction Methods of Humic Acid

A number of methods have been suggested for extracting HA from natural sources, including anthracite coal, lignite coal, peat, and soil. Nevertheless, low-quality HA is usually obtained after a long time of processing (extraction), which results in low yields and degradation of the substance [29]. According to the types of solvents employed, HA extraction methods can be divided into physical, chemical and biological extraction processes. Chemical extraction methods include: alkaline, acid treatment, and hydrogen peroxide treatment, while biological extraction methods involve the use of microorganisms. [30, 31]

The extraction of HAs involves separating them in an alkaline solution, causing them to precipitate. In this extraction process, oxidation is often performed to enhance the initial quantity of HA present in the carbonaceous matrix. This can be achieved using several types of oxidizing agents, which may originate from different source materials. Now, we shall examine several methods that can be employed to extract HAs.

2.1. Physical Extraction Methods

Due to the knowledge that HA is a complex organic matter with a macromolecular structure, different approaches and techniques have been employed to attempt extraction with the aim of fully characterizing this complex organic material. Physical extraction methods generally consist of a gelatinous mass of brown coal, lignite, peat, etc. as a raw material. The char mass formed is then treated with specified solutions to extract soluble fulvic acids. [32]. This type of technology has been used in a number of countries for a long time, and the characteristics of the extracted acids from different raw materials have been presented. The material being considered in this study is Hongu-bronwn coal (inertinite type under the new classification). Coal was subjected to extraction with a 6M NaOH-solution in different temperature ranges (20 degrees, 50 degrees, 140 degrees, 230 degrees, and 20+50 degrees) with the addition of a liquid/solid ratio in one case. Both fulvic acid and crude humic acids were produced, and yield and compounds present after extraction were characterized. [33, 34]

A physical extraction method has been chosen that probes the physical aspects of dissolution. A gelatinous mass of sauerkraut (raw material) is submerged in distilled water and heated to 100 degrees with a salt addition. Thereafter, the mass is cooled, and electrolytes are cleared from it with a number of distilled washings. Then the flocculent precipitate that contains humic acid is formed by alkalizing (pH 12) the clear super. HA can be filtered from this solution, freeze-dried, and used for further studies. [35- 37]

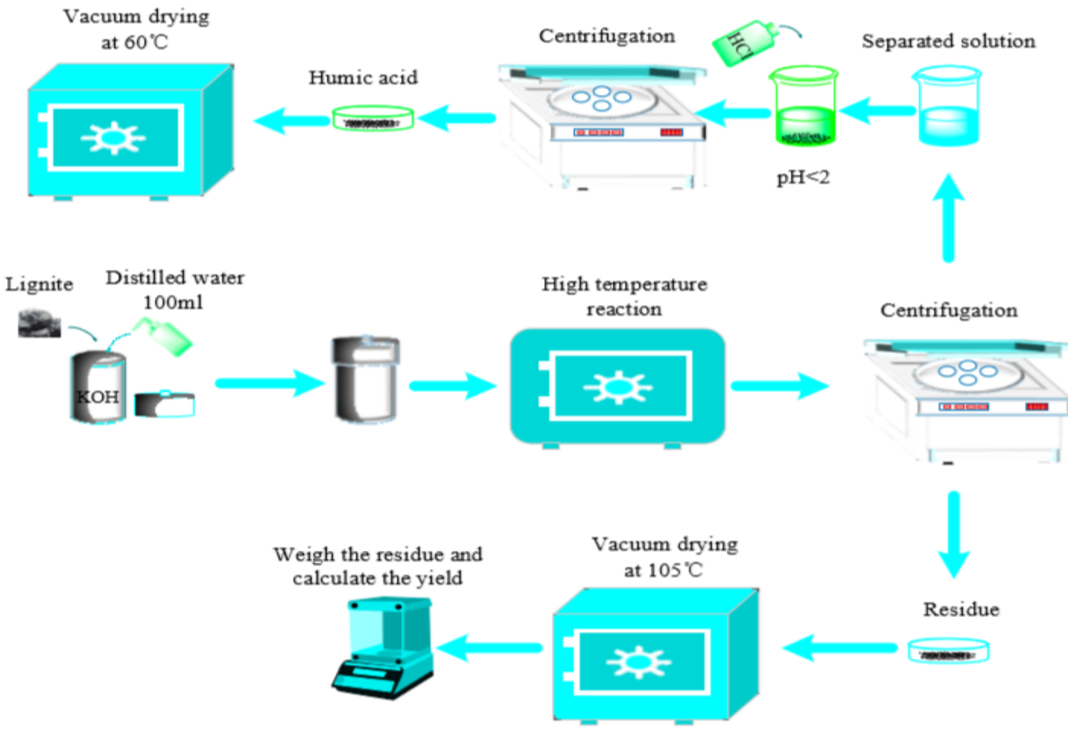

Figure 2: humic acid (HA) extracted by hydrothermal method [38]

2.2. Biological Extraction

Biological extraction methods are explored as a relatively new, more environmentally friendly, and sustainable means of obtaining humic acid. They use microorganisms that can produce enzymes that complete the polymerization of humic acids from plant sources. In particular, studies on fungi and the use of lignocellulose by Trichoderma species are examined in detail. A mention of how the fungi can live off lignocellulose sources with the free availability of nutrients and humic acid production to provide a model biological extraction system is included [39].

Fungal species have been studied to isolate a new indigenous soil species, Trichoderma species, from Bangladesh. It was found that they can produce HA from lignocellulose. This lignocellulose is utilized as the carbon source since Trichoderma species are able to do this in the agro-climatic conditions of

Bangladesh [40]. The ability to produce HA from various lignocellulosic sources by an indigenous soil fungus mentioned above indicates the scope for a biological system in the production of HAs. The combined advantage of low-cost and high production is significant, particularly in less-developed and developing nations [41]. The influence of HA on soil is also discussed, highlighting how they can play an ecological and biogeochemical role in the soil environment, thus influencing soil processes like mineral nutrient, acid, and metal solubility according to the ion's surface effect on nonabsorbent pH [42].

2.3. Chemical Extraction Methods

Chemical synthesis is one of the most widely studied methods for the synthetic production of HA. There are various chemicals, including sodium hydroxide, sulfuric acid, sodium sulfite and black liquor were used to chemically synthesize HA. These chemical synthesis methods are mostly done in laboratory and small scale [43]. In the chemical synthesis of HA, adequate research is not done regarding the characterization of the product HA. Synthetic HAs been characterized spectroscopically by various chemical characterization techniques like NMR and FT-IR to confirm the formation of HA. The characterization of product HA is essential to understand the nature and properties of synthetic HA and to compare it with other natural sources of HA.

Chemical synthesis of HA from sodium sulfite treatment has been studied and elemental and chemical characterization of HA has been done. According to the study, HA was successfully synthesized from sodium sulfite treatment of dealbook and Indus timber plant waste fibers. Masa-NaOH treatment has been reported to produce HA-like product, but there is very little or no characterization is reported regarding the product. Chemical synthesis of HA using black liquor lignin and characterization of HA is documented [44]. In the formed HA, a series of polymerization and oxidation, condensation of aromatic structures has taken place. The chemical composition of synthetic HA is variable and depends on the concentration of chemical reagent, oil shale and type of oxidized slime residues.

2.3.1. Solvent Extraction Methods

In the solvent extraction method, the commonly used solvents are water, alcohol, KOH-alkaline solution, t-butyl methyl ether, methyl isobutyl ketone, ethyl acetate, n-butanol, chloroform, n-butyl alcohol, petroleum pomace, and methylene dichloride. It was concluded that the solvent extraction method using low molecular weight organic solvents accounts for the largest proportion, and the extraction rate was in the order of lower molecular weight pore-forming agents, aprotic solvents, alcohols, and acid-base organic solvents. High-yield extraction can produce HA products at lower costs because the extraction rate of the solvent extraction method was higher than that of the ethanol-alkaline extraction method. In addition, alkali extraction, supercritical fluid extraction, and enzyme-assisted extraction can also be used to extract HA. Superfine grinding, ultrasonic mixing, microwave, and other treatment methods were used to pretreat samples from natural sources. The results showed that pretreatment can significantly improve the extraction of HA. In conclusion, the methods mentioned above can extract HA from natural substances. The process is feasible, but the extraction yield or extraction rate of the methods is different [45].

Solvent extraction is one of the earliest methods used to extract HM from soil and sediment samples. It consists of stirring a pre-insoluble sample treated with sodium pyrophosphate for 48–72 h with a buffer solution containing 0.1 M HCl and 0.1 M NaOH for 3 h. The buffer solution is then dried and poured into the KOH. The obtained solid is then washed with distilled water until a neutral reaction is reached. The precipitate is then treated with hot, concentrated HNO_3 and placed in a sand bath to carbonize the carbonaceous material. After cooling, the solution is filtered, and the residues are washed several times with distilled water. The solid material is then treated in an oven for 8 hours to obtain a white powder. The final product is a brown powder of HM that contains either iron or aluminium oxides extracted from the original material [46]

The extraction of HA involves separating them in an alkaline solution, causing them to precipitate. In this extraction process, oxidation is often performed to enhance the initial quantity of HA present in the carbonaceous matrix. This can be achieved using several types of oxidizing agents, which may originate from different source materials. Now, we shall examine several methods that can be employed to extract HAs. The above methods are described in detail in the following sections:

2.3.2.1 Acid Extraction Methods

Focusing specifically on acid extraction, the section proceeds to explore this method in greater detail. Acid extraction, frequently regarded as the gold standard in HA isolation, utilizes acids to precipitate out HAs from HA-free samples or lignites. Numerous studies have addressed this topic, but researchers often use disparate methods to define certain variables. Thus, the goal of this work is to present a more systematic approach. Special attention will be paid to the extraction of HAs using strong acids (4M–7M HCl) [11, 68].

To put this subject into perspective, the appropriate preparation of hematite and/or humate soil samples prior to extraction is discussed. The recommended sedimentation times and acid wash conditions are presented. Particular emphasis is placed on the subsequent purification of the isolated HAs. It is noted that the HAs produced can differ enormously in their degree of humification [47]. Potential causes for this variability are provided in the discussion, along with the desired properties of representative samples. This work ultimately serves as a reference for the systematic extraction and purification of HAs, heightening the reproducibility of results in further studies.

2.3.2.2 Alkaline Extraction Methods

Alkaline extraction is the most widely used method for extracting HA. This method is based on the negative charge on the HA molecule, which inhibits precipitation at alkaline pH. The proposed alkaline extraction process has many advantages, such as being environmentally friendly, simple and easy, low-cost, and effective for separating HAs from different sources. However, a sodium hydroxide treatment at high temperature and pH may be required. Practical considerations regarding the alkaline extraction of HA include its adsorption on insoluble residues after effluent separation, contamination of obtained products, by-products formed, target samples, solid-to-liquid ratio, and total extraction time [48].

Following the principles of alkaline extraction on HA to obtain a nontoxic and easily purifiable product, this work used deep eutectic solvents (DESs) composed of choline chloride with urea or glycerol to extract HA from different natural sources in a very simple procedure without any purification steps, allowing for the rapid analysis of HA. After being separated from the starting material by filtration, the extracts were directly analyzed by infrared spectroscopy, which is the most common method of characterization. Comparison of the spectra with a publicly available database of standard fractions from soil HA and previous studies of HA extracted from other natural sources showed that the constituents of HAs extracted were similar, confirming new broad-spectrum methods of obtaining the industrial mass of HA [46].

HA is obtained through the extraction of alkaline acids or other solvents. It is typically found in partly colored solutions or granules that contain sodium or ammonium humate. When exposed to acidic solutions, HA precipitates as a gelatinous and amorphous substance.

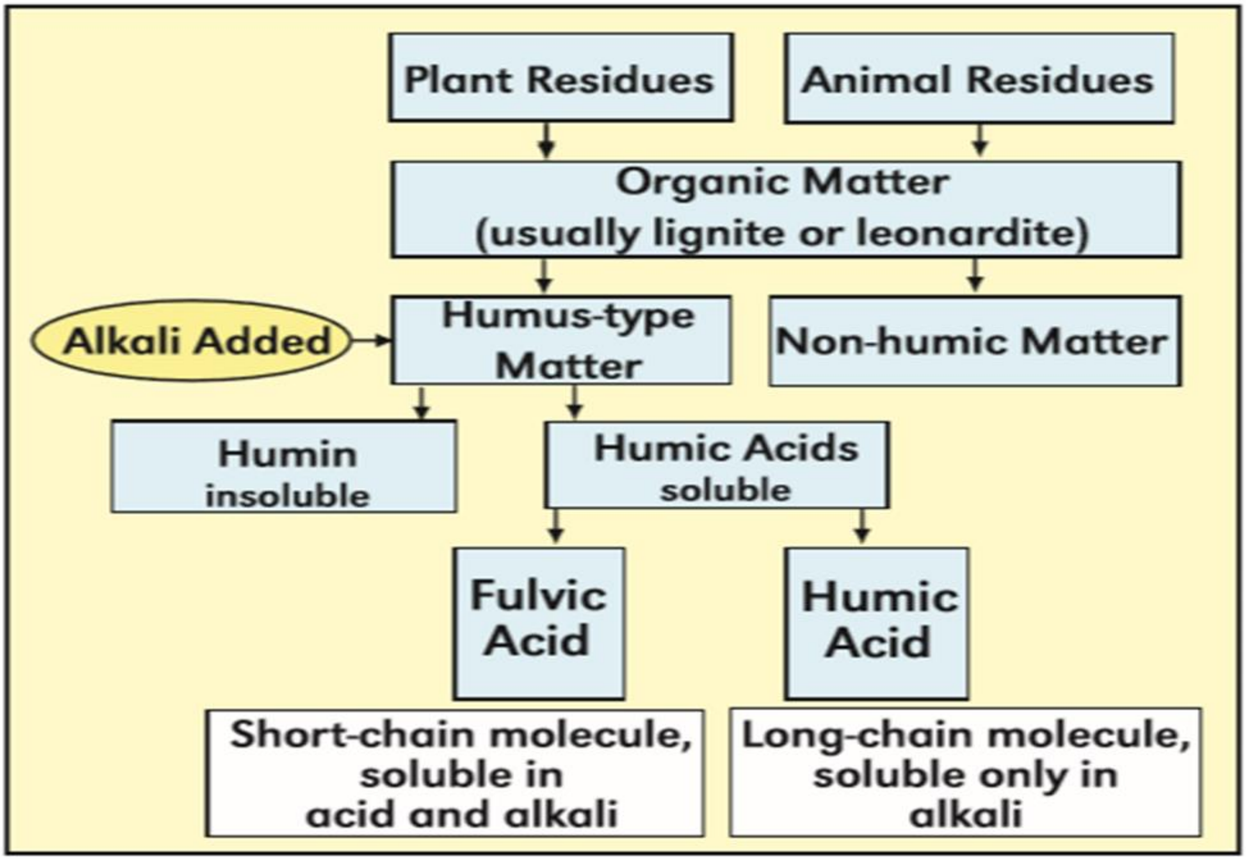

Figure 3: Common chemical extraction technique for extracting organic materials into different fractions commonly [49]

3. Purification Methods

HA in sodium hydroxide or potassium hydroxide extracts can be purified by fractional precipitation with acid alcohols. This process involves gradually increasing the concentration of acid alcohol and allowing the solution to stand for 24 hours after each increment of saline additions. Such disinfectations allow mineral components of the extracts to coagulate and precipitate. Stabilization of HA can also be done using PO_4 particles. A filter (e.g. a glass wool plug) modified with amorphous $FePO_4$ or $AlPO_4$, on which HA were immobilized, was placed in a chromatographic reactor [50].

Alternatively, HA can be fractionated using chromato-settling columns. Such columns contain mixtures of Fe_3O_4 and polyacrylamide that set as beads with a high porosity. HA, which are less dense than water or saline solutions, pass through these columns, whereas humin and minerals are retained [51]. HA can also be precipitated with ferric hydroxides from alkaline extracts containing iron salts. The precipitate is soluble in NaOH and H_2O_2 and can be reprecipitated from the salt-free solution with acidified alcohols [52].

3.1. Fractional Precipitation

Fractional precipitation is a widely applied method to purify and analyze humic acid, whose aim is to separate the impurities from the acidic humic acid aqueous extract [53].

After the first partial neutralization with LiOH, fractions with high metal ion affinities and higher molecular weights were obtained. Precipitation using Li-OAc removed most of the alkalis and alkaline earths, but the majority of both humic acid types remained in the supernatant. Further precipitation with LiCl removed humic acids with lower binding capacities for cationic metal ions and pesticides, while LiHMDS precipitated high molecular weight and easily solvated humic acids [54]. The precipitation was carried out at ambient temperature in the presence of nonpolar solvents (ethanol, dioxane, and hexane). After the initial humic acid precipitation with LiCl, the humic acid-Ca fraction was heated, and molecular weights were determined using gel permeation chromatography.

3.2. Chromatography Techniques

In addition to extraction processes, chromatographic methods are also used for the separation and purification of HS. Due to differences in the nature, chemical composition, and size of HS, it is possible to separate and purify different HS fractions using chromatographic methods. Polymer-based size exclusion chromatography (SEC) with linear or cross-linked polystyrene gel columns has been one of the most widely used chromatographic techniques for separating and purifying HS since the early 1980s [2]. SEC separates organic compounds based on particle size and assumes that larger organic compounds elute first due to being excluded from smaller pores in the column packing material. A significant drawback of this method is that the elution profile is not truly commensurate with the molecular size in cement pore water, since different types of HS and humic-like (HS) molecules can have vastly different shapes (branched, globular, or linear) that influence their apparent molecular size in water [55].

The HS size distribution can be evaluated using either high-performance size exclusion chromatography (HPSEC) or high-performance liquid chromatography (HPLC). HS is separated based on the average equivalent size of their molecules with respect to molecular weights (MW) in polystyrene sulfonate units (PSSu) in the range of 250-2500 Da. Folins-Ciocalteu analysis can be carried out at the end of chromatographic separation to determine the concentration of benzoic compounds with different equivalent sizes of HS molecules. Gradient and isocratic chromatographic separation modes can be applied to determine HS compounds with different properties. Since the development of this technique, various HS separation modifications have been suggested. However, the primary limitation of HPSEC is that complete fractionation cannot be achieved, as some higher molecular weight (MW) and bulk polycyclic aromatic hydrocarbons (PAHs) can elute together with the low MW organic and ionic aqueous fractions [56].

4. Analytical Methods for Humic Acid

Conventional analytical techniques include diverse methodologies that provide structural, physical and chemical information on humic substances (HS). The most commonly utilized techniques include spectroscopic and chromatographic methods. The spectroscopic methods mainly used for the analysis of humic substances are nuclear magnetic resonance (NMR) and ultraviolet-visible (UV-Vis) spectrometry. NMR spectroscopy is a rigorous technique for structural investigation of humic substances [57]. However, the complexity and the high concentration of humic substances make NMR a difficult technique for their analysis. In addition, unresolved signals due to the overlapping of peaks often obscures the understanding of the NMR spectra. UV-Vis provides information on the functional group's distribution of humic fractions. Like NMR, UV-Vis is also a qualitative method and therefore does not provide extensive details on the structure of humic materials [58]. Chromatographic methods, on the other hand, enhance the separation of humic materials and provide better understanding of their structural properties. The most commonly used chromatographic methods are size exclusion (SE) chromatography, liquid chromatography (LC) and high-performance liquid chromatography (HPLC). Currently, size exclusion chromatography (SEC) is the predominant technique used for the analysis of the size distributions of humic substances. SEC is based on the separation of molecules depending on their hydrodynamic volume. It relies on porous stationary phases that allow the passage of smaller molecules and restrict the passage of larger molecules. Therefore, SEC can only be used to distinguish the size distributions of humic materials [59].

The chemical structure of humic acid was investigated using Fourier transform infrared spectroscopy, nuclear magnetic resonance, UV–Vis spectroscopy, and gel permeation chromatography analyses. Experimental results reveal that the s-triazine ring most probably contributes to the formation of humic acid derivatives. Humic acid from s-triazine is satisfactorily characterized by spectroscopic and chromatographic methods and could be applied in wastewater treatment and other fields [60].

4.1. Spectroscopic Techniques

The most widely used methods for the characterization of humic acid are spectroscopic techniques such as UV-Vis spectrophotometry, fluorescence spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy.

The spectral absorbance of natural organic matter is commonly recorded from 200-800 nm using a double beam UV-Vis spectrophotometer. Samples are placed in a quartz cuvette and the baseline correction is performed using deionized water. The spectral characteristics of HA include the absorption maximum (λmax) at which the absorbance is highest, the specific absorbance (S650) calculated at 650 nm, and the area under the spectral curve (AUC). The λmax and S650 values represent the composition and complexity of different aromatic groups in HA and thus the extent of humification. HA of low humification has maximum absorbance at longer wavelengths ($\lambda max>300$ nm) and low throughout absorbance at longer wavelengths ($S650<2.74$). On the other hand, HA of high humification has λmax of less than 290 nm and greater S650 (>5.85) [61]. AUC is divided into 3 regions: VIS1 (300-430 nm), VIS2 (430-580 nm), and VIS3 (580-800 nm). The greater the proportion of AUC ratio vis-1:vis-3, the higher are the aliphatic carbon proportion and the degree of humification of HA. AUC ratios have a good correlation with the result of the C13-NMR spectra analysis [58].

In fluorescence spectroscopy, the excitation and emission spectra of HA are analyzed at different excitation wavelengths in tandem with EEM experiments. HA samples are diluted using a suitable ratio of deionised water and 1 M KCl solution. The correlation between the meta-excitation wavelength and the observed emission peak is applied to revolatilize the PARAFAC-MC-ALS model (FQS1).

5. Mechanisms of HAs in Increasing Soil Cation Exchange Capacity (CEC)

Humic substances (HS) are involved in providing soil CEC through various mechanisms. Both field and lab experiments have shown that the application of humic substances increases soil CEC Fig 4. The most commonly mentioned mechanisms for HS in increasing soil CEC include the adsorption of cations and the increase in soil organic matter (SOM). The effects of HS on soils in increasing nutrient retention and availability, soil structure, water-holding capacity, and microbial activity are very slow and not consistent in both short- and long-term conditions. This is because HS physically and chemically interacts with soil and functions in soil through short- and long-term processes, both abiotically and biotically. Also, HS in all soil supports living plants. There are real interactions between HS, plants, soil, microorganisms, and nutrients in soil. The complex effects of HS on raising soil CEC are caused by these interactions or changes in soil over time caused by crops using HS. Principal-component analysis addressed HS application effects on soil properties. [62, 63]

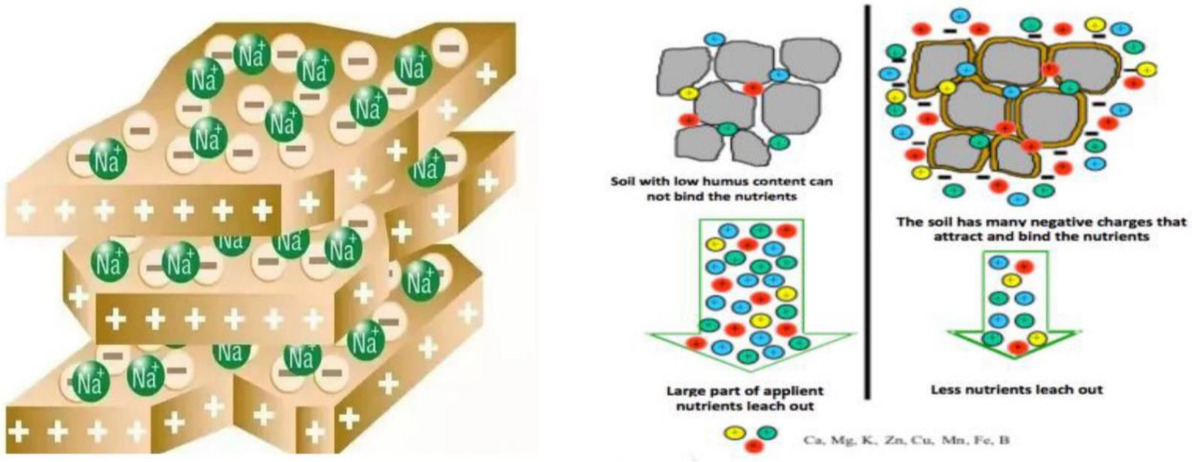

Figure 4: The cation exchange capacity (CEC)

6. Application of Humic Acid in Soil and Plants

It is apparent that the chemical extraction of HA is very large in volume, but only small portions of HAs are needed to achieve beneficial effects on soil or plant. Due to these reasons, the chemical extraction method of HA can be applied to various environments, including agricultural and environmental uses. The molecular weight of HA was reduced during the extraction process but still showed remarkable effects on soil and plants.

Soil structure and fertility improvement are the most important aspects of HA application. Numerous studies in different countries have been focused on this practical utilization of HA. Soil structure improvement was proved from detailed studies measuring the sedimentation rate of soil particles. Application of HA changed the sedimentation rate of soil particles from clay, silty to sandy loam, even though the soil was collected from various locations [15]. HA is known to have a high affinity for clay particles and several functional groups to enzymatically polysaccharides, improving soil structure [64]. HA encourages the formation of macro-pores by improving soil aggregation. The newly formed aggregates provide an environment favorable for microbial growth and activity, sufficing soil fertility improvement.

6.1. Improving Soil Structure and Fertility

HA enhances soil structure and fertility in several significant ways. First, HA contributes to improving soil structure by ensuring porous aggregates formation in relatively dry soils. It also promotes cohesive interactions between clay particles, resulting in a greater stability of soil aggregates [17]. Second, HA contributes to reducing the bulk density of cultivated soils. This prevents compaction and the free movement of air and water throughout the soil profile, as well as influences the entry of roots into the deeper soil layers [65]. Third, HA has a significant impact on the water retention capacity of soils. In a sandy soil, the addition of HA increases the soil water content at field capacity from 3.2 to 17.8% (w/w soil), while in a clayey soil the increase is from 22.3 to 30.2%. Fourth, HA improves soil nutrient holding capacity. It promotes an increase of 6.73-fold in the CEC of the sandy soil and 2.31-fold in the clay soil. Fifth, HA contributes to the fertility of different soils. In a field experiment conducted in Huai'an, Jiangsu Province, China, the application of HA stimulated the higher yielding rates of oilseed rape (BR) and winter wheat (WW) by 78.02 and 46.85 kg/ha, respectively. This is due to the increase of soil nutrient contents. It is worth noting that HA can effectively enhance the soil quality and productivity in sandy and clayey soils. The application of highly purified HA significantly improves the microbiological and biochemical properties of both soils. More importantly, HA seems to enhance resilience of soil biological properties to environmental stresses in the clayey soil.

6.2. Enhancing Nutrient Uptake in Plants

Plant growth-promoting substances have received a notice in recent years owing to the damage of the chemical fertilizers to the environment and the health. HA is also one of the natural plant growth-promoting substances. It is considered one of the main components of HS, which is known to promote plant growth. Enhanced root growth was observed in HA-treated plants. Electrolyte leakage was reduced in exogenous HA-treated plants. It was, however, more favorable to the uptake of potassium ions than sodium ions by the treated plants. The supply of HAs to the soil, compared with control, treated plants with HAs showed an increase in height, root length, and weight of the stems and roots. Soil could be improved with amendments containing humic substances obtained from different sources like composted peat, green waste compost, and cereal straw [66]. HAs, from different commercial sources, proved beneficial for the vegetative growth of seedlings grown in a salt-affected soil. Different concentrations of HA as an amendment with compost were proved to be effective in enhancing soil properties and seedling quality of seedlings grown in seawater-affected nursery media. Farmyard manure along with HAs and soil-illirigated saline groundwater were found to be effective in enhancing soil and plant properties of Gladiolus L [67].

7. Conclusion

In order to imitate these natural processes in the laboratory, numerous extraction methods have been developed to fulfill the HA extraction from both low-rank coals and lignites in an effective way. Although numerous researchers have used various techniques that share the same fundamental steps, extracting HA from natural samples is known to be a laborious, time-consuming, and expensive process. The efficiency comparison of these methods among themselves is difficult as well. Moreover, applied treatments will start to transform or degrade the extracted HA from natural coal or lignite samples into another compound, probably causing alterations in the extracted material's physicochemical properties. The aim of this study was to review different methods of HA extraction and their importance in different applications. The data introduced here might be helpful in various applications to obtain purified HA.

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