CHAPTER I

INTRODUCTION

1. INTRODUCTION

During the past decades, the growth of nuclear energy industry and of the nuclear weapons testing has added large amounts of radioactive nuclides into the environment. The main sources of radioactive nuclides in the environment are either natural or artificial. These radionuclides are dispersed in the atmosphere, the hydrosphere, rocks, soils, marine and terrestrial biota [1]. It has become apparent that the characterization of the dissolved organic carbon is effective for understanding the carbon cycle in the hydrosphere [2]. The major organic fraction in soils and water is humic materials. The interaction of humic substances with nuclide ions has a multitude of consequences that are reflected in the physical, chemical and biological properties of the soil matrix. Practically, every aspect of trace element chemistry and reactions in soil is related to the formation of stable complexes with humic substances.

Natural Radionuclides:

The phenomenon of natural radioactivity was first discovered by Becquerel just before the end of the nineteenth century. It soon becomes apparent that natural radioactivity is useful tool to study the structure and properties of matter, both on a microscopic and macroscopic scale. The phenomenon of natural radioactivity has been used, for example, to reveal the structure of atomic nucleus, to estimate the age of the earth, and to measure the rate of sediment formation on ocean bottoms.

About 340 nuclides have been found in nature, of which about 70 are radioactive and are found mainly among the heavy elements [3]. All elements having an atomic number greater than 80 possess radioactive isotopes and all isotopes of elements heavier than atomic number 83 are radioactive. Alpher and Herman [4] call attention to the surprising uniformity of the ratios of elements not only on the earth but also in the atmosphere, and on the surfaces of the planets in meteorites, in interstellar matter, and even in extragalactic nebulas.

Artificial Radionuclides:

More than 350 artificial radionuclides are observed in the environment, most of them are being products released from nuclear bomb tests in the period 1955-1962[1]. Estimates of the amounts produced by bomb tests appear to exceed those released by nuclear power plants by a factor of a thousand. Among these radionuclides are ^{137}Cs, ^{60}Co and $^{(152,154)}Eu$.

1.1 Chemistry of the Investigated Elements:

The investigated radionuclides are associated with nuclear activities and may be used as indicators for the chemical behavior of the radionuclides in the environment. In the following, some of the essential properties of the investigated radionuclides and lead, as a toxic element, are presented.

1.1.1 Cobalt

Cobalt was discovered by Brandt (Sweeden) in 1735[5]. It is one of the transition elements and always occurs in association with copper and nickel, also, it is found in a small quantity in seawater, mineral sources, soil and organisms of animals and plants. Cobalt-60 ($T_{1/2}$ = 5.272 Y)[6] is formed in a nuclear fission process during the operation of a nuclear power reactor by activation of ^{59}Co present as an impurity in the metal for which the structural parts of the fuel assemblies are made. The decay energies of ^{60}Co are E_β = 0.314MeV, and E_γ =1.173 and 1.332 MeV [6]. Because of higher energies of γ-radiation and long half-life, large quantities of cobalt-60 are used as γ-ray sources for various applications in research, industry and medicine [7]. The decay scheme shows only one β transition followed by a cascade of two γ-rays. Natural cobalt has one stable isotope, ^{59}Co. A large number of radioactive isotopes are available for tracer studies. Good production of cobalt isotopes is obtained from deuteron bombardment of iron, a mixture of isotopes being produced with half- lives ranging from 18 hours to 270 days. The isotopes used are mainly ^{59}Co and ^{58}Co, as a mixture from bombarded iron targets.

The atomic weight of cobalt is 58.93. The electronic configuration is [Ar] $3d^7$ $4s^2$. It has a density of 8.9 g/cm^3 and melts at 1495 °C. The boiling point of cobalt metal is 2870 °C. The chemical properties of cobalt occur predominantly in the +2 oxidation state. In some complexes it is readily oxidizable to Co^{3+}. It also shows a valance of +1 in a few complex nitrosyls and carbonyls and a valance of +4 in some fluoride complexes.

In the simple cobalt compounds, the divalent forms are more stable. Cobaltous ion, Co^{2+} is basic and generally exists in a hydrolyzed form, yet it is not easily hydrolyzed in aqueous solutions. Cobalt is of biological significance because it forms organic complexes under favorable conditions. The release of cobalt into the environment includes the deposition and retention in the sediment which could be due to a part fixed by the humic materials present and a part deposited as a coagulate[8].

Cobalt is associated with vitamin B_{12}[9], a fact probably of significance in relation to the well-known production of abnormality erythropoetic activity brought about by cobalt deficiency. Large amounts of cobalt inhibit growth and respiration of microorganisms. Histidine and histidine–like compounds overcome this inhibition. It appears that this may be ascribed to the chelation of cobalt by histidine, which results in cobalt – histidine complex. This complex shows the interesting property of combining reversibly with molecular oxygen [10]. Medical interest in cobalt is concerned mainly with its use as a radium substitute [11].

1.1.2 Cesium

Cesium was discovered in 1860 by Bunsen and Kirchhoff in course of spectroscopic examination of the mineral constituents of certain spring waters. In minute amounts cesium occurs in nature in association with other alkali metals. Cesium also occurs in detectable amounts in plant and animal organisms, mineral waters and soil. Metallic cesium is usually obtained by reduction of its salt like cesium hydroxide, chloride or carbonate with metallic magnesium, aluminum, calcium or barium at 700 °C or with iron at 1000 °C. Cesium finds major application in the manufacture of vacuum tubes and photoelectric cells. The compounds of cesium resemble those of rubidium and are prepared under similar conditions. Cesium possesses only one stable isotope with mass number = 133 (atomic weight 132.905), but offers a very wide range of radioactive isotopes. Twenty one of these, with masses distributed between 123 and 145, as well as one isomer are known. The isotopes with masses lower than 133 are positron emitters or decay by (e^--capture); the isotopes of masses higher than 133 are fission products and β^- - emitters. The nuclides of ^{123}Cs to ^{130}Cs have relatively short half-lives. ^{131}Cs and ^{132}Cs decay by (e^--capture) with half–lives of 9.6 and 6.3 days, respectively. Natural cesium has a neutron capture cross-section of 31 barn, and forms ^{134}Cs (2.1 years; β^-; γ). The most important of the fission products are ^{135}Cs ($2.6\text{x}10^6$ years; β^-), ^{136}Cs (13 days; β^-), and above all ^{137}Cs (29.68 years;β^-, 0.514 MeV; γ, 0.661 MeV), which are formed in yields of 6.41%, 0.0065 %, and 6.15%, respectively.

^{137}Cs is an important constituent of radioactive fall out. It decays in 92% of cases into ^{137}Ba, which is deexcited by the emission 0.661 MeV γ-radiation, with a half-life of 2.3 min; thus it rapidly achieves radioactive equilibrium with its precursor. ^{137}Cs accounts for 30% of the γ -activity of fission products after storage for one year, 70% after two years, and 100% after five years. ^{137}Cs finds numerous applications e.g. measurement of thickness, density, flow rates level control, etc. Owing to its nuclear characteristics, it is a very suitable material for industrial radiography and for the control of welds. Cesium, is a member of the alkali metals group, and possesses only one valence electron, its electronic structure being

[Xe] $6s^1$. After francium, it is the most electropositive of all elements. The ionic radius of Cs^+ is 1.67 Å[12].

1.1.3 Europium

Europium was discovered in 1901 by Democracy, by fractionation of the double nitrate of samarium and magnesium. The element has two stable isotopes, ^{151}Eu, which has an abundance of 47.77%, and ^{153}Eu with abundance of 52.23% [13]. Its atomic weight is 151.96. The thermal- neutron fission of ^{235}U yields 0.31% of ^{152}Eu and 0.085% of ^{154}Eu [14]. The analogy of europium in chemical behavior with the other lanthanide elements usually gives some information on the environmental behavior of the other members of this series. As pointed out by Greenwood and Earnshaw [15], the lanthanides are very electropositive and reactive metals. ^{152}Eu is a β^--emitter with a half-life of 1.7 years, and is used as a carrier-free radioactive indicator [12]. It is also possible to use the mixture of the two isomers of ^{152}Eu (9 h and 12.7 years) and Eu^{154} (16 years) formed in Eu (n,γ) reactions. ^{147}Eu is an α-emitter, and also decays by (e^--capture).

The electronic configuration of europium is [Xe] $4f^7$ $6s^2$. It readily forms +3 ion in solids like oxides and different complexes. However, europium can give the Eu^{2+} ion. The existence of +2 state was simply ascribed [16] to the extra stability associated with the formation of stable half-filled $4f^7$ subshell.

Europium metal is prepared by electrolysis of its fused salts or by reduction of the oxide with lanthanum. It crystallizes in the face-centered cubic lattice. It has a density of 5.24 g/cm^3 and an ionic radius (Eu^{3+}) of 95 pm; it has paramagnetic properties and melts at 822°C. The boiling point of europium metal is 1597 °C. The abundance of europium in the earth's crust is $2x10^{-5}$ % and $3.3x10^{-5}$ % in stony meteorite [12].

1.1.4 Lead

The metal lead is one of the seven metals known to man from the earliest times, the old Egyptians have been familiar with the metal at the time of Rameses III. According to the estimates of Vogel [17], lead is less commonly distributed than some of the elements usually regarded as rarer and scarcer, e.g., zirconium, vanadium, cerium, and yttrium.

There are few methods of extraction of lead from its ores, e.g., carbon reduction process and self-reduction process. It was found that galena, cerussite, anglesite, plumbo- jarosite and mimetesites are of interest as sources of lead. In the combined form it is found associated with the pyrite ores of copper, zinc, antimony and iron. The electronic configuration of lead is [Xe] $4f^{14}$ $5d^{10}$ $6s^2$ $6p^2$ [18]. Lead is ordinarily a bluish – gray metal but has a silver white luster in the pure state. Its melting point is 327.5°C, boiling point is 1740°C and density is 11.4 g/cm^3. It is the softest of the common metals so that it marks paper and can be scratched easily. It is a heavy and malleable metal so that it can be rolled into foil and pressed into pipes. Lead is not affected by dry air but in moist air it gets covered with a protective layer of basic carbonate. When strongly heated in air, it gives monoxide and finally red lead, Pb_3O_4.

Rose [19] reported that the salt of lead nitrate is stable in air and dissolves in water with a marked cooling effect. Mailfert[20] found that lead nitrate is slowly converted by ozone into the dioxide. The solution of salt in water was acidic to prosodic, and alkaline to litmus. Strömholm[21], and Athanasescu[22] studied the formation of basic nitrates by the action of aq. ammonia on solution of lead nitrate. The basic lead nitrates are usually obtained by the action of alkali or ammonium or other hydroxides on a solution of lead nitrate.

Lead finds numerous applications because of its cheapness, low melting point, high density, softness and inertness under ordinary conditions. It is used for making pipes, protective sheet for roof's drains, covering of electric wires, and as lining in sulphuric acid manufacture. Lead is used in accumulator plates and in the manufacture of white lead.

1.2 Ion Selective Electrode: -

Membrane electrodes are relatively simple electrochemical devices consist of electrochemical membranes which are composed of either a solid or a liquid phase permeable only to one ion or species [23,24]. The phase is dependent on its composition and the velocity of permeation to the various ions. The electrode normally contains an internal reference electrode and an inner- filling electrolyte to constitute an electrochemical half–cell. When the ion – selective electrode (ISE) is placed in a solution containing the particular ion (to which the membrane is selective), a small number of ions passes from the solution of higher concentration through the membrane to that of lower concentration, thus producing an electric potential difference known as liquid junction potential or diffusion potential [23]. This potential electromotive force (EMF) is measured against a standard external reference electrode e.g. Ag/AgCl (the other half- cells). These two half-cells form a complete galvanic cell represented by the following cell notation:

Ag /AgCl / KCl (saturated) / salt bridge sample

$\longleftrightarrow$

(reference electrode half–cell)

/membrane / internal solution /AgCl/Ag

$\longleftrightarrow$

(ion– selective electrode half-cell)

The measurement principle is simple: two reference electrodes of fixed potential are separated by the ion selective membrane. The solution on one side of the membrane is of known, constant composition and includes the ions to which the internal reference electrode and ISE membrane respond. The sample solution is in contact with the other side.

The measured EMF of this galvanic cell is the sum of a number of potential differences arising at the boundaries:

$$EMF = E_\circ + E_M + E_J$$

Where $E_\circ$ is the reference potentials in the cell; it is constant at a fixed temperature. E_J is the liquid junction potential; it is diminished to zero by using an electrolyte where the mobility of anions and cations has nearly equal values.

E_M is the membrane potential; the measured EMF of the cell reflects only the membrane potential.

1.3 Humic Substances:

Humic substances, are widely distributed in soils, natural water, marine and lake sediments, peat, carbonaceous shales, lignite, brown coals, and miscellaneous other deposits. These materials are best described as a series of acidic, yellow-to-black-colored polyelectrolytes that have properties dissimilar to the biocolloids of living organisms [25]. They may range in molecular weight from several hundreds to perhaps 300,000 [26-29].

Humic substances in soil have properties almost similar to those found in other natural systems. They have the ability to form stable complexes with polyvalent cations. The presence of these substances facilitates the mobilization, transport, segregation and deposition of trace metals in soil, sediments, sedimentary rocks and biogenic deposits of various types [26]. Organic complexing agents play a key role in the chemical weathering of rocks and minerals, and they function as carriers of metal cations in natural waters. Complexation of metal ions and trace anions (e.g phosphate) by humic substances decreases the toxicity of certain metals toward microorganisms and increases the availability of some metals, but decreases phosphorus resources [30].

There are three major fractions of humic substances, they defined in terms of their solubility [31] as the following:

Humin: the fraction of humic substances that is not soluble in water at any pH value.

Humic acid: the fraction of humic substances that is not soluble in water under acidic conditions (below pH 2), but becomes soluble at higher pH.

Fulvic acid: The fraction of humic materials that is soluble under all pH conditions.

1.3.1 History:

Achard[32] was the first one who attempts to isolate humic substances from soil, he extracted peat with alkali and obtained a dark, amorphous precipitate upon acidification. This alkali-soluble, acid-insoluble material latter comes to be known as humic acid. Achard observed that larger amounts of humic material could be extracted from the lower, more humidified layers of the peat than from the upper less decomposed layers.

The first comprehensive study of origin and chemical nature of humic substances was carried out by Sprengel[33, 34]. He developed many of the procedures for the preparation of humic acids became generally adopted, such as pretreatment of the soil with dilute mineral acids prior to extraction with alkali. Sprengel concluded that, for soils rich in bases, humic acid was in a bound form and consequently, the soil had a neutral reaction (contains "mild humus"). This soil was regarded as highly fertile. On the other hand, for soils poor in bases, the humic acid was delivered to be in the free form with the result that the soil was acidic and unproductive (contains "acid humus"). Thus Sprengel felt that the different forms of humus were of considerable importance in soil fertility.

De Saussure[35] is usually credited with introduction describes the dark colored organic material in soil. He noted that dark humus was rich in carbon (C) and poore in hydrogen (H) and oxygen (O) than the plant material from which it was derived. In 1882, Döbereiner [36] designated the dark colored component of soil organic matter as "humussaure'' or "humus acid", although Waksman[37] concluded that "humic acid" was applied to the precipitate obtained from the alkali extract by acidification. This definition of humic acid has persisted through the present time.

Berzelius [38] isolated two high-yellow colored humic substances from mineral waters and as limy mud rich in iron oxides. They were obtained from the mud by extraction with alkali, which was then treated with acetic acid and copper acetate. A brown acetate was obtained called "copper apocrenate". Copper crenate was obtained when the acetic acid solution was neutralized. The free acids "apocrenic" and "crenic" were then brought into solution by decomposition of the Cu complexes with alkali. These newly described humic substances were examined in considerable detail, including isolation, elementary composition and properties of their metal complexes (Al, Fe, Pd, Mn, etc.).

1.3.2 Occurrence:

Humic substances are widely distributed in soils, natural water, marine sewage, peat bogs and lake sediments. As observed by Swanson and Palaces [39], humic substances are accumulated in surface and subsurface soil layers, in beach sand of bays and bayous and shore. These constituents are best described as a series of acidic, yellow- to black colored polyelectrolytes. They represent an extremely heterogeneous mixture of molecules, which in any given soil or sediment, may range in molecular weight from as low as several hundreds to perhaps over 300,000 [26].

1.3.3 Formation

There are several pathways existing for the formation of humic substances during the decay of plant and animal remains in soil. Waksman[37], concluded that humic and fulvic acids were formed by a multistage process that included:

(1) Humic substances, the major organic substituents of soils and sediments, are widely distributed over the earth's surface occurring in almost every aquatic and terrestrial environment. The total soil carbon contains approximately 60-70 % humic materials that were estimated by Griffith and Schnitzer [40]. The decay of organic matter provides the largest CO_2 input into the atmosphere [41], this illustrates the importance of the role of humic substances in the carbon cycle as a major source of CO_2.

(2) Decomposition of all plant components, including lignin, into simpler monomers, metabolism of the monomers with an accompanying increase in the soil biomass, repeated recycling of the biomass carbon (and nitrogen) with synthesis of new cells and concurrent polymerization of reactive monomers into high – molecular weight polymer [42,43].

(3) Humic substances in nature arise from the chemical and biological degradation of plant and animal residues and from synthetic activities of microorganisms. The product so formed tends to associate into complex chemical structures that are more stable than the starting materials.

The formation of humic substances may results from oxidative alteration of organic fragments, microbial synthesis or chemical condition after biological degradation or autolysis of living biomass [44].

There are three stages for the formation of humic substances; the first stage is formation of "primary humic substance" from the deformation of the product of cellular constituents of dead organisms. These substances have fairly small stable molecules, such as amino acids and carbohydrates or macromolecules resulting from condensation reaction. The second stage is formation of "secondary humic substances" through chemical and biological oxidative degradation, which results in a decrease in the humin fraction and increase in the fulvic acid fraction. The third stage is incorporation into the sediment and buried. The smallest and most soluble fraction is the fulvic acid due to diffusion and mineralization. Humic acid undergoes insolublization by defunctionalization and evolves with humin into the kerogen (soil bituminous material in some shales), which yields petroleum when heated.

1.3.4 Extraction of humic substances

The extraction of humic substances is often the first task that confronts the investigator. The ideal extractant should remove practically all of humic material without altering its physical and chemical properties. The dilute aqueous sodium hydroxide solution remains the most commonly used and quantitatively the most effective reagent for extracting humic substances from soil and sediments.

Achard [32] was the first who described the use of aqueous solutions of sodium hydroxide for the extraction of humic substances. Such solution has been the solvents of choice by most workers since that time. Achard shown that the basic solvent systems, 0.5M NaOH, extracted more humic substances from a H^+ exchanged organic soil, because of the highly alkaline conditions which prevailed all the acid groups in macromolecules were dissociated and the repulsion of charge gave the fully expanded random soil structure.

1.3.5 Separation

Separation is a necessary step in the characterization of soil matter. The primary objective of separation is to facilitate the application of analytical techniques by reducing heterogeneity of the isolated material. Humic substances are classified into three major fractions of different acidifies, and these fractions once isolated from the environment, are operationally defined in terms of their solubility as a function of pH.

1.3.5.1 Fulvic acid

It is the fraction of humic substances that is soluble under all pH conditions or the material, which remains soluble in the extract at pH1.0[45]. It has a straw–yellow color at low pH values and turns to wine–

red at a pH near 3.0. At very low pH, fulvic acid has the highest particle weight and particle volume. With increase in pH, the two parameters decrease to a minimum at pH 3 and then began to increase moderately.

Spiteller and Schnitzer [46] concluded that polymaleic acid was found to resemble fulvic acid in a number of characteristics (water solubility, rich in COOH groups, dark brown color). It is significantly different from fulvic acid in a number of respects (UV, fluorescence, NMR spectra as well in oxidation products). Compared to fulvic acid, polymaleic acid appeared to be better organized and more homogeneous at the molecular level, richer in olefinic structures and less aromatic. But the chemical structures of both polymaleic acid and fulvic acid remain largely unknown.

1.3.5.2 Humic acid

It is the fraction of humic substances that is not soluble in water under acid conditions (below pH 2), but becomes soluble at greater pH. It is a dark brown to black material has a carbon content of about 58%[47]. Springer [48] subdivided humic acids into brown humic acid and gray humic acid on the basis of their behavior toward electrolytes. This was accomplished by redissolving the original humic acid in alkali and adding electrolyte (KCl), the gray humic acids were easily coagulated, and exhibited a low degree of dispersion. A high proportion of the oxygen in humic acids occurs as structural component of the nucleus (e.g in ether or easter linkages). In scanning electron microscope the effect of pH on the humic acid structure was similar to that observed on fulvic acid except that, because of low solubility in water, the pH range had to be narrowed to between 6 and 10 and the pH at which the major transitions occurred was higher. In viscosity measurements [49] the humic acid at pH 7.0 behaved like uncharged polymers, but at higher pH levels (pH 10.5), humic acid exhibited strong polyelectrolytic characteristics. But in case of fulvic acid it is in contrast to humic acid due to the low molecular weight, which contains considerably more acidic functional groups, particularly COOH. Another important difference between humic acid and fulvic acid is that practically all the oxygen in fulvic acids can be accounted for in known functional groups (COOH, OH, C= O); a high proportion of the oxygen in humic acids occurs as a structural component of the nucleus (e.g, in ether or ester linkages).

1.3.5.3 Humin

It is the fraction of humic substances that is not soluble in water at any pH value. Humin is commonly defined as the class of sedimentary humic matter that remains insoluble when sediments are treated with dilute alkali to extract the soluble humic and fulvic acids. Humin has been the least studied of all humic fractions because of its insolubility and macromolecular nature [50].

1.3.6 Fractionation of humic acid

The behaviour of humic substances in solution is strongly influenced by the presence and concentration of background electrolyte (salt) like any other charged macromolecule (polyelectrolyte). At very low concentrations of background electrolyte the diffused double layers extend some distance from the surface of the charged macromolecule. If the background electrolyte concentration is increased the extension of the double layer is suppressed much closer to the surface of the molecule. This allows the macromolecules to approach one another more closely so that intermolecular attractive forces predominate and coagulation or precipitation can occur. Suppression of charge interaction to the extent where precipitation occurs is referred to as salting-out. The best known use of salting–out as a method of fractionation is the splitting of humic acid into gray and brown humic acid fractions by the addition of a salt, usually KCl, to a solution of humic acid [48]

1.3.7 Molecular weight

One of the first questions asked about an organic compound is its molecular weight. A pure compound has a single molecular weight that can accurately be determined. Macromolecules are of two types: (1) Those that are homogeneous and where all particles have the same molecular weight, and (2) Those that are polydispersed and thereby exhibit a range of molecular weights. Humic and fulvic acids fall into the second category. As pointed out by Stevenson [26], molecular weights of humic substances may vary from as law as a few hundreds for fulvic acids to as much as several hundred thousands for humic acid. The determination of molecular weights for humic substances is difficult because most humic substances are not discrete chemical entities but are a complex mixture of organic substances with a wide range of molecular sizes. Soil humic substances appear to have higher molecular weights than their aquatic counterparts. The measurements of the molecular weight for humic substances depend on pH, concentration and ionic strength [51].

1.3.8 Properties of humic substances

Humic substances have been the subject of intense research because of their significance in agricultural and environmental processes. In addition to the properties outlined above, some other important properties of humic substances are summarized in the following:

1) Solubility characteristics

Humic and fulvic acids form both soluble and insoluble complexes with polyvalent cations, which depend on the degree of saturation. It was found that the metal complexes of fulvic acid are more soluble than those of humic acid; this is the result of their lower molecular weights and higher content of acidic functional groups [52]. The ability of humic and fulvic acids to form complexes with metal ions can be attributed to their high content of oxygen – containing functional groups, including COOH, phenolic – alcoholic and enolic –OH, as well as C=O structure of various types. The formation of these complexes facilitates the mobilization, transport, and deposition of trace metals in soil.

2) Association with organic solutes:

Stream humic substances can interact with other organic solutes by hydrogen bonding, cation and anion exchange, hydrophobic adsorption, and partitioning within the humic molecules, this is because humic substances engage in self–association. The association with polycyclic aromatic hydrocarbon [53], and with petroleum hydrocarbon [54], markedly increases the apparent water solubility of these compounds.

3) Reduction properties of humic substances:

Humic substances have the ability to reduce oxidized forms of certain metal ions as the reduction of Fe (III) [55,56]. Other examples include reduction of Mo(VI) to Mo(V) and Mo(III), and Hg (II) to Hg (0) [57]. The polyelectrolyte nature of humic molecule leads to very strong complexing ability, which increases in strength with increasing the degree of ionization of the carboxylate groups.

4) Ion exchange and protonation:

Adsorption through ion exchange is due to those organic chemicals that either behave as cations or become positively charged through protonation. Depending on the protonation supplying power of humic colloids, bonding mechanisms for the retention of organic chemicals by soil humic substances include ion exchange, hydrogen bonding and coordination through an attached metal ion [26]. Both clay and organic matter contribute to the cation exchange capacity of the soil, the contribution from humic and fulvic acids is due largely to the ionization of COOH groups, although some contribution from phenolic OH and NH groups are expected [58].

1.3.9 Characterization of humic substances:

The characterization of various humic fractions and that of humic substances from different sources is of considerable importance to understand the chemistry of humic matter. Many different chemical and physical analysis of humic substances have been reported during the last few decades. Ultimate analysis of humic materials has provided information on the distribution of the major constituent elements. Functional group analysis have thrown light on the occurrence of major functional groups (COOH, OH and C=O groups) in humic substances and their reactivity. On the other hand, the use of physical methods for the characterization of humic substances appears more attractive because of the complexity of many chemical procedures. Thus spectroscopic methods, potentiometric titration and molecular weight measurements have provided valuable information on the nature and properties of humic substances of widely different origins. In the following sections, a resume of the published characterization results is given.

1.3.9.1 Elemental composition

As indicated by Schnitzer and Khan [31], the elemental analysis provides a useful inventory of the distribution of the major elements in humic substances. Since most humic preparations are mixtures of many components, elemental analysis does not provide an absolute molecular formula but it does provide general compositional information. The predominant elements are carbon and oxygen. The carbon content of humic materials ranges from 50 to 60 %, the oxygen content from about 30 to 45 %; the percentage of hydrogen and nitrogen range from approximately 4 to 6 % and 2 to 4 %, respectively. The sulfur content may vary from close to zero to between 1 and 2%. Humic acids generally show a lower H/C ratio than fulvic acids indicating a higher degree of unsaturation in humic acids than fulvic acid. The oxygen content decreases in the order: fulvic acids > humic acids > humins. This order parallels the solubility of the humic fractions and may be important for metal ion transport in waters and soils. The elemental content of humic substances would be expected to be affected by such factors as pH, parent material, vegetation and age of the soil. For lake sediments, the C content of humic acids has been found to increase with increasing depth [59]. Table (1.1) illustrates the usual range for the elemental composition of humic and fulvic acids, as pointed out by Steelink [59].

Table (1.1): Usual range for the elemental composition of humic substance.

Element	Humic acid	Fulvic acid
Carbon	53.8-58.7%	40.7-50.6%
Oxygen	32.8- 38.3	39.7-49.8%
Hydrogen	3.2-6.2	3.8-7.0
Nitrogen	0.8-4.3	0.9-3.3
Sulfur	0.1-1.5	0.1- 3.6

1.3.9.2 Reactive functional groups

Methods for the determination of functional groups and their distribution in humic and fulvic acids are provided in several reviews[60-64]. Most methods for determining reactive functional groups in humic materials have been based on the acidic properties of the groups involved. Because of the complex nature of humic substances, the acidities of the various groups may overlap. For this reason, results obtained by methods dependent on ion–exchange or pK values must be interpreted with caution. Polycarboxylic acids, for example, exhibit a whole series of dissociation constants that decrease as successive protons dissociate. On the other hand, substituted phenols are often more strongly dissociated than the unsubstituted compounds. Some groups may be unreactive because of H- bonding or through stearic hindrance.

Other problems in the quantitative determination of functional groups include (1) insolubility of the material (particularly humic acids) in water and most organic solvents, (2) oxidation and reduction, (3) interactions with reagents used for forming derivatives, and (4) the nonstoichiometric nature of the reactions.

1.3.9.3 Spectroscopic characteristics

Spectroscopic measurements in the different regions of the electromagnetic spectrum are used by soil scientists for qualitative and quantitative investigations on soil humic substances. Attractive features of most of these methods are (a) they are nondestructive; (b) small sample weights are needed; (C) they are experimentally simple and do not require special manipulative skills; and (d) they often provide valuable information on molecular structure and on chemical interactions. A wide variety of spectroscopic techniques can be used in the study of humic substances such as ultra violet (UV), visible, infrared (IR), nuclear magnetic resonance (NMR), electron spin resonance (ESR), spectrofluorimetry and Raman spectroscopy. The most useful of these methods are IR and NMR spectroscopies.

1.3.9.3.1 UV-visible spectroscopy:

In several cases, UV-visible spectroscopy is a valuable tool in identification of chromophoric functional groups in discrete organic molecules. But even a simple two component mixture may make the interpretation of this spectrum difficult from a functional group point of view.

The aqueous solutions of humic substances of dark color have stimulated chemists to use color as a criterion for the analysis of these materials. Absorption in the UV (200-400 nm) regions involves the elevation of electrons from the ground state to higher energy levels. The ultra violet and visible regions are generally considered together, since both correspond to electronic transitions within the absorbing species, and they are also referred to as electronic spectra.

As pointed out by Schnitzer[62],while the absorption spectra of humic substances do not provide much detailed information on their chemical structure, the similarity of their spectra suggests that these compounds are of similar basic structures. The UV-visible spectroscopy has many useful applications for purposes other than determining functionality in the study of humic substances. For example estimation of the degree of humification using E_4/E_6 ratio [65,66], determination of the concentration of dissolved humic substances based on Beer's low plots and studying the interaction of metal ions with fulvic and humic acids [26].

**** *E_4 / E_6 ratio:***

The ratio of absorbencies at 465 and at 665 nm is often used for the characterization of humic substances. This ratio, referred to as E_4 / E_6, is independent of the concentration of the humic compound [66], but varies for humic substances from different types.

Campbel et al [67], found an inverse relationship between E_4/E_6 ratios and the mean residence of humic materials in soil. Chen et al [65] indicated a convincing case for a relationship between E_4/E_6 ratio and the molecular weight. Thus a low ratios means high degree of aromatic condensation and infers the presence of relatively more aliphatic structure [26] i.e. the magnitude of E_4 / E_6 ratio is inversely proportional to the degree of condensation or the molecular weight[26]. The ratios for fulvic acids are usually more than those for humic acids.

1.3.9.3.2 Infrared (IR) spectroscopy

The IR spectra of humic substances and their derivatives contain a variety of specific molecular structures; in contrast to the relatively few absorption bands observed in the visible and ultraviolet region [68]. Infrared spectroscopy is of considerable value in humus research for the following reasons [26]:

(a) Key information is provided regarding the oxygen containing functional groups; (b) The occurrence of protein and carbohydrate constituents can be established. (c) The presence or absence of inorganic impurities (metal ions and clay) in isolated humic fractions can be demonstrated; and (d) The technique is suitable for quantitative analysis. Other applications of IR spectroscopy have included studies on metal-organic and pesticide-organic matter interactions.

As pointed out by Stevenson [26], the main absorption bands, in the infrared spectra of humic substances, are in the regions of: 3300 cm^{-1} (H-bonded OH groups), 2900 cm^{-1} (aliphatic C-H stretching), 1720 cm^{1} (C=O stretching of COOH and ketonic C=O), 1610 cm^{-1} (aromatic C=C and H-bonded C=O), 1250 cm^{-1} (C-O stretching and OH deformation of COOH groups), and 1390 cm^{-1} (O-H deformation, CH_3 bending or C-O stretching).

Several authors reported surprisingly similar IR spectra of fulvic [69-72] and humic acids [45,73] from different soils. The similarity of IR spectra of fulvic and humic acids from these different soils suggests a similarity in their chemical structures that are related to their IR absorption. However, IR spectroscopy is not sensitive enough to uncover minor structural differences among fulvic and humic acid [74].

1.3.9.4 Potentiometric titration

The potentiometric titration data are important as a basis for comparing acidic properties of humic substances from various sources, determining total and exchange acidity (i.e. ≈ COOH) contents, determining charge densities and radii and determining metal-ion binding properties, including stability constant measurements. Humic substances are polybasic acids with at least two different types of oxygen-containing functional groups (COOH and phenolic OH). It is very difficult to distinguish between the two by titration because the dissociation of protons from the two groups overlaps. Potentiometric titration have been used to characterize acidic functional groups in humic materials. Numerous descriptions of methods of analysis of the acidic functional groups of humic substances have been published [26,31,75]. Potentiometric titration curves are usually sigmoidal, indicating an apparent monobasic character.

Special difficulties are encountered in interpreting titration curves of humic substances. Among them; the proper selection of the end point. Posner[76], Torres[77] and Helal[73] applied the point where the rate of change of pH with the added titrant becomes maximum. This value was obtained at pH's between 6.0 and 8.0 depending on the ionic strength of the solution used. Martin and Reeve [78] selected pH 8.0 as the end point. To analyze titration curves of fulvic and humic acids; several attempts have been made using approaches known to be successful for week acid polyelectrolytes. A brief outline of one of these attempts [26] is given in the following: The dissociation of an acidic functional group (HA) from the molecule proceeds as:

$$HA \rightleftharpoons A^- + H^+ \text{ ....(1)}$$

For which the ionization constant (K) is given by:

$$K = [A^-] [H^+] / [HA] \text{ ....(2)}$$

From the titration data, (A^-) and (HA) can be estimated at any given pH, from which the ionization constant can be determined. Since K is normally of a low value, the negative logarithm is often recorded; the so-called pK of the acid group:

$$pK = - \log [A^-] [H^+] / [HA] \text{ ....(3)}$$

The ionization constant of an acid is of value because it reveals the proportion of the material, which occurs, in ionized and unionized forms at any chosen pH. From equation (3).

$$pK = pH - \log [A^-] / [HA] \;\ldots\ldots\ldots\ldots(4)$$

At half-neutralization, pK is equal to pH and this is commonly referred to as the pK_a of the acid or acidic group. A variety of equilibrium functions have been developed to characterize the acid–base behaviour of synthetic and natural polyelectrolytes; the most common one is the Henderson-Hasselbalch equation[79]

$$pH = pK_a + n \log [\alpha / 1\text{-}\alpha] \;\ldots\ldots\ldots\ldots\ldots\ldots(5)$$

Where α is the degree of ionization. pK_a and n are constants, for a given titration, depend on the concentration and ionic strength. A plot of pH versus log [α/1-α] yields a straight line with a slope equal to n and an intercept corresponds to the pK_a value. The carboxylate capacities of humic substances were determined by several authors [73,77-79] using potentiometric titration. The values of the total carboxylate capacities were found to be different depending on the source and method of isolation of the humic material.

Table (1.2) includes some of the total carboxylate capacities of fulvic and humic acids.

1.3.10 Structure

Because of the chemical complexity of humic substances, many chemists have used degradative methods to produce compounds that can be identified and whose structures can be related to those of the starting materials. On light of the degradation results and the other methods of analysis, many attempts have been made to devise a structural formula representative of humic acids, but non-has proved entirely satisfactory.

The structures shown in figures (1.1- 1.3) all show the presence of

Table (1.2): Total Carboxylate capacities of fulvic and humic acids.

Humic substances	**Coo^{-}capacity (meq/g)**	**Reference**
Fulvic acids:		
Bhorizon fulvic acid	12.4	[80]
Domino fulvic acid	7.9	[71]
Contech fulvic acid	4.7	[71]
Soil fulvic acid	8.2	[71]
Lake fulvic acid	5.41	[79]
Humic acids:		
Ismialia canal humic acid	3.6	[73]
Agricultural land humic acid	6.0	[45]
Soil humic acid	4.45	[81]

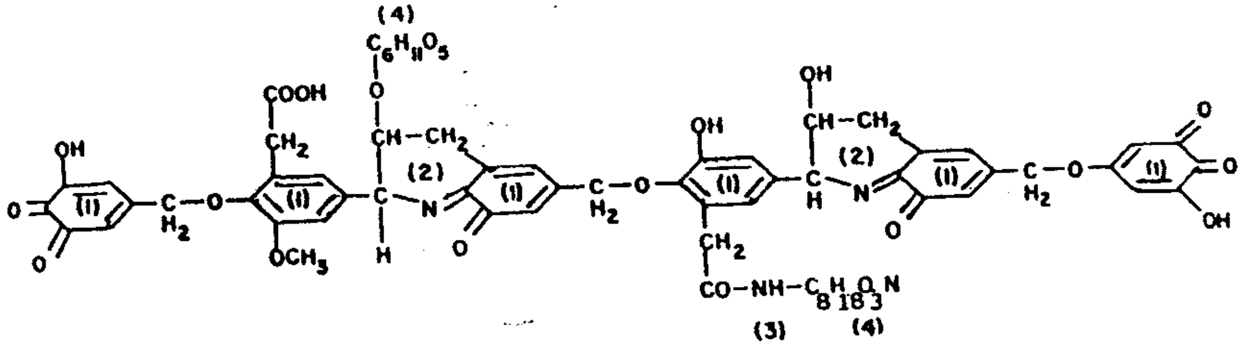


Fig. (1.1): Dragunov's structure of humic acid as recorded by kononova.

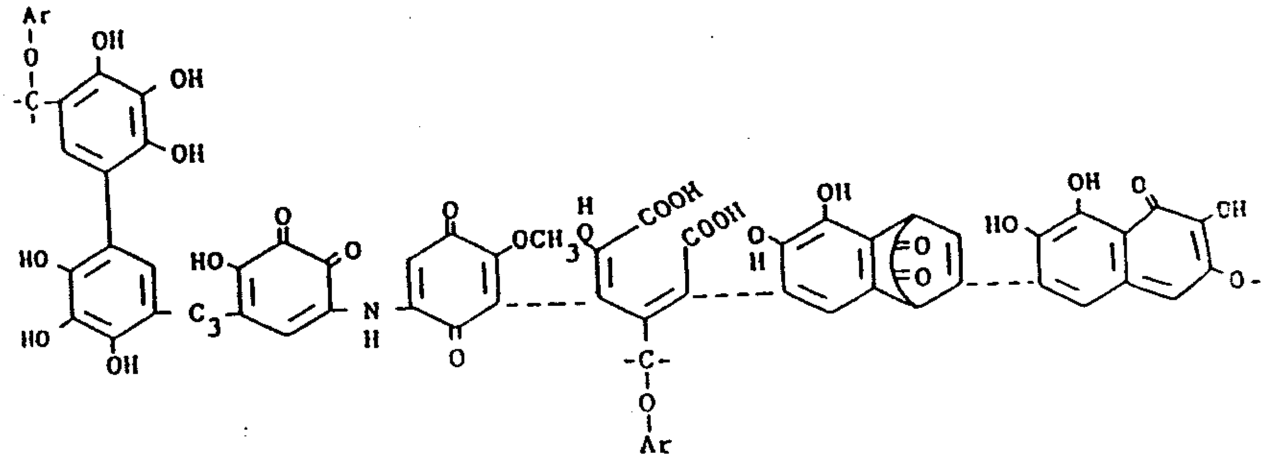


Fig. (1.2): Hypothetical structure of humic acid according to Flaig.

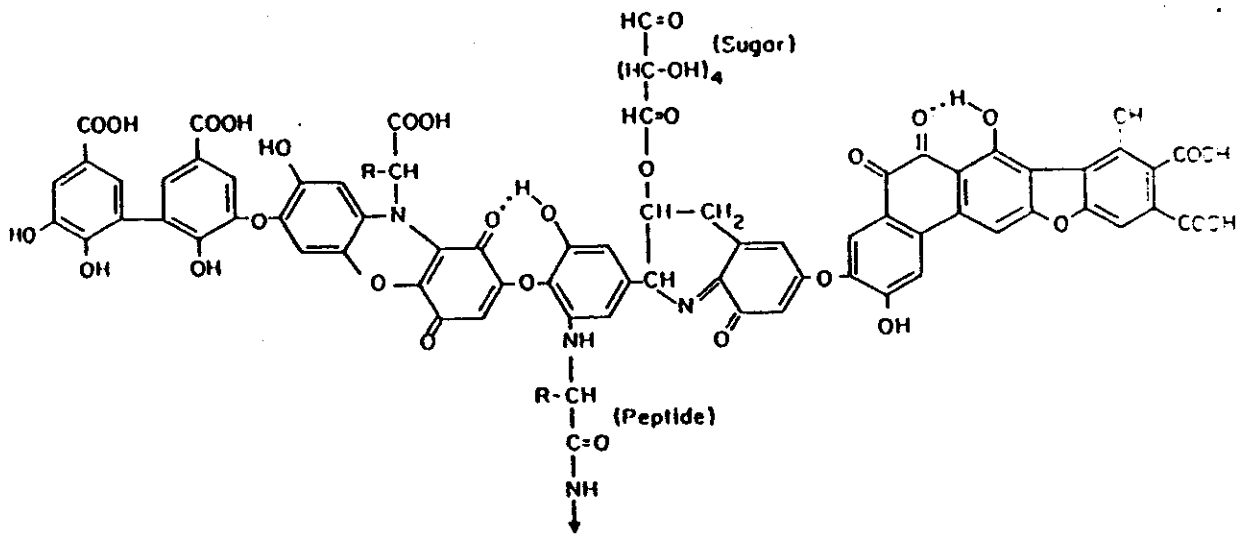


Fig. (1.3): Hypothetical structure of humic acid From Stevenson.

aromatic rings of the di and trihydroxy benzene type, as well as the presence of quinoine group. Dragunov's structure, Fig. (1.1)[66], as well as Flaig[28], Fig. (1.2), contains an insufficient number of COOH groups (relative to phenolic OH). All three structures show nitrogen as a structural component; Figs. (1.1) and (1.3) indicate the occurrence of carbohydrate and protein residues. Schnitzer and Khan[31] concluded that fulvic acids consist in part of phenolic and benzene carboxylic acids held together through hydrogen bonds, Fig. (1.4), to form a polymeric structure of considerable stability. The structure proposed by Buffle[82] for fulvic acid, Fig (1.5), contains both aromatic and aliphatic structures, both extensively substituted with oxygen containing functional groups.

The chemical structure of humic substances from different sources was not the same. For example, humic substances derived from terrestrial plants were found very different from those derived from aquatic plants. Terrestrial humic substances are largely aromatic [83], while the main distinguishing feature of humic substances derived from aquatic flora is their aliphatic structure [80]. Each fraction of fulvic and humic acids must be regarded as being made up of a series of molecules of different sizes, few having precisely the same structural configuration or array of reactive groups[26]. The lower molecular weight fulvic acids contain higher oxygen but lower carbon contents, and they contain more acidic functional groups, particularly COOH. All oxygen in fulvic acids can be accounted for known functional groups (COOH, OH, C=O). Schnitzer and Khan [31] concluded that fulvic acids consist in part of phenolic and benzene carboxylic acids held together through hydrogen bonds.

1.3.11 Peat humic substances

The formation of humic substances in a peatland environment is a complex humification process that is principally due to certain enzymatic and microbial activities. These organic matter transformation

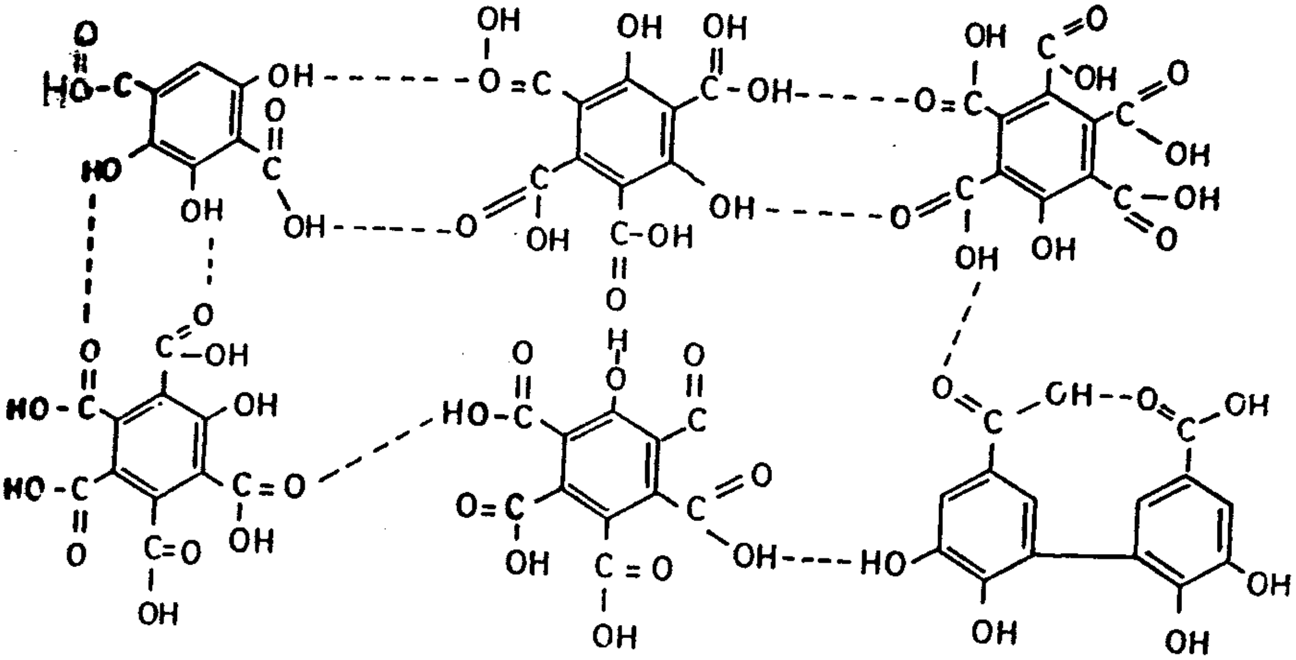


Fig. (1.4): Structure of fulvic acid as proposed by Schnitzer and Khan.

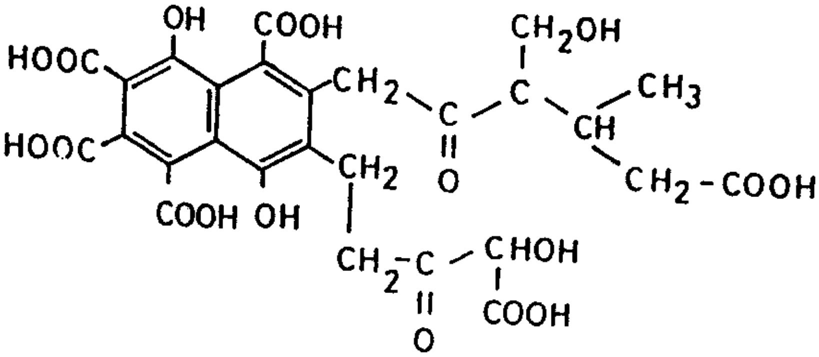


Fig. (1.5): Structure of fulvic acid as porposed by Buffle.

processes are influenced by the nature of the peat-forming plants and certain physical and chemical properties within a particular peatland. It has been indicated that sodium hydroxide extracts of organic soils, particularly their anaerobic zones, contain considerable amounts of nonhumic materials. In case of the extraction with pyrophosphate, the yield is less for nonhumified material but does not dissolve all the humus in the peat. If the sodium hydroxide extraction is preceded by an acid treatment, the humus removed tends to be richer in fulvic acid than in humic acid [84,85]. The characterization of peatland humic acids and those isolated from mineral soils, by elemental composition, functional group analysis and spectral properties, has indicated that peat humic acids are similar to those in mineral soils. The humic substances formed in peatlands tend to be more soluble than those in mineral soils due to the paucity of oxygen, metals and microbial activity that may induce or cause further aggregation and transformations. Some of the humic substances formed in peatlands may therefore be translocated by water movement.

Most peatlands are deficient in iron, manganese, zinc, copper, boron, and molybdenum due to lack of clay minerals and the leaching of some of these elements under wet acidic conditions. When virgin organic soils are drained, limed and fertilized for agronomic use, the humus content increases and with continued cultivation and resultant biological oxidation the mineral content increases. This tends to correct minor element deficiencies, the change in mineral content is often accompanied by an increase in soil pH and become suitable for plant availability of the nutrient metals [86]. Organic soils are considered a storehouse of plant nutrients and water, and provide a desirable medium for producing high quantity vegetable crops that grow grit-free in or on the ground.

1.4 Reaction of humic substances with metal ions

The ability of humic substances to form complexes with metal ions can be attributed to their high content of oxygen-containing functional groups, including COOH, phenolic, and enolic-OH, and C=O structures of various types, amino groups may also be involved. The interactions between metal ions and humic substances have been described as chelation, ion–exchange, surface-adsorption, coagulation and peptization reactions.

There are several evidences for the complexing of cations by fulvic and humic acids:

1) Selective retention of metal ions by fulvic and humic acids in presence of cation exchange resins [26].

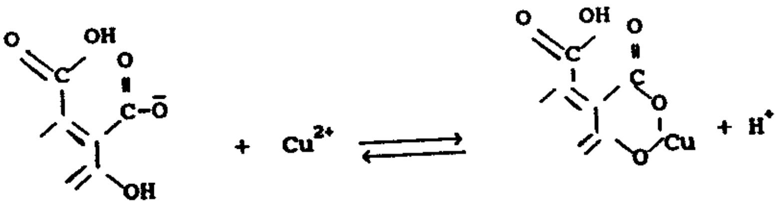
2) The ability of natural synthetic chelating agents to extract metals and organic matter from soils [87].

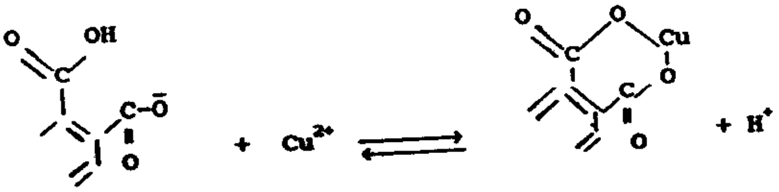
3) Correlation between humus content and micro nutrient retention by soil[26].

4) Inability of exchangeable cations such as Ba^{2+} and K^{+} to replace all Cu^{2+} and Zn^{2+} adsorbed by humic substance [87].

5) The binding of Cs^{+}, $Co^{2+}$and Eu^{3+} with the different sediment fractions was found to decrease in the presence of humic acid as a ligand [88].

Schintzer [89] and Gamble et al[90] Postulated that two types of reactions are involved in metal-fulvic acid interactions, the most important one involving both phenolic OH and COOH groups. The two reactions are:





The formation of phthalate -type complexes is likely because humic acids have been shown to contain COOH groups that are located on adjacent position of aromatic rings [26]. The probable presence of multiple complexing sites in humic substances has been suggested in several studies. There are three types of sites involved in the retention of $Zn^{2+}$by humic acids as concluded by Randhawa and Broadbent[91]. The less stable complex was believed to be associated with phenolic OH groups and weakly acidic COOH; the more stable complex involve strongly acidic COOH [26]. Khanna and Bajawa [92] obtained similar results for Cu^{2+}; they detected four peaks by elution chromatography.

There are several ways for detecting the formation of metal-fulvate and metal humate complex [93]:

a) Change in chemical behaviour, (b) absorption spectra, (c) electrical conductance, (d) pH effect, (e) solubility, (f) isolation of natural complexes,(g) oxidation potential, and (h) polarographic behavior. These and other criteria have been used by soil scientists to provide evidence for the formation of metal-fulvate and metal-humate complexes.

Metal–ion binding capacities

Methods used to determine the binding capacities of humic substances for metal ions include proton release [94,95], metal-ion retention as determined by competition with a cation-exchange resin [96], dialysis [97] and ion selective electrode or anodic stripping voltammeter measurements [98-100]. Other approaches include the determination of binding sites that are occupied using the techniques of ultraviolet and fluorescence spectroscopy [101,102].