STUDY OF COMPLEXATION OF LEAD, CHROMIUM, MANGANESE AND ZINC WITH FULVIC ACID EXTRACTED FROM BATTERY–CONTAMINATED SOIL

by

MAHABUB ISLAM
STUDENT ID: 0417032701

MASTER OF SCIENCE IN CHEMISTRY

Department of Chemistry
BANGLADESH UNIVERSITY OF ENGINEERING AND TECHNOLOGY

The thesis entitled "STUDY OF COMPLEXATION OF LEAD, CHROMIUM, MANGANESE AND ZINC WITH FULVIC ACID EXTRACTED FROM BATTERY–CONTAMINATED SOIL" submitted by Mahabub Islam, Roll No.:0417032701, Session: April 2017, has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Master of Science on. July 13, 2019.

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It is hereby declared that this thesis or any part of it has not been submitted elsewhere for the award of any degree or diploma.

Mahabub Islam

I dedicate this thesis to.....

My Beloved Parents
& Sister

List of Tables and Figures

Tables

List of Abbreviations of Technical Symbols and Terms

1. Energy Dispersive X-ray spectroscopy (EDX)
2. X-ray fluorescence spectroscopy (XRF)
3. Nuclear magnetic resonance (NMR)
4. Fulvic acid (FA)
5. Hydrochloric acid (HCl)
6. Sodium hydroxide (NaOH)
7. Nitric acid (HNO_3)
8. Fourier Transform Infrared (FTIR)
9. Lead (Pb)
10. Zinc (Zn)
11. Chromium (Cr)
12. Manganese (Mn)
13. Negative logarithm of the hydrogen ion concentration (pH)
14. Alkalinity (Alk)
15. HS (Humic Substance)
16. Battery waste contaminated (BWC)
17. Tangor Haor (TA)

Acknowledgement

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Abstract

Humic substances are considered as the most important part of soil. Different types of humic substances are humic acid (HA), fulvic acid (FA) and humate. Humic substances (HS) are produced from microbial degradation, chemical polymerization and oxidation of organic matter generated from plants and animals. Both composition and structure of humic substances(HS) are different from each local area to other and characterizations such as XRF, EDX-SEM, FTIR and NMR exhibit better understanding of their impact on soil chemistry. Due to polyfunctionality, HS have many different functional groups. FA is lighter and more soluble compare to HA and plays a vital role to transport chemical species. HS work as the most powerful chelating agents among natural organic substances. Only a few scientific literatures are found on HA-metal interaction in Bangladesh and none on FA-metals interactions. FA are extracted by alkali treatment from battery waste contaminated (BWC) and Tangoar haor (TA) region soil. The structure and composition of FA extracted from soil of the TA and BWC region are not well studied. The quantity of metal in the FA fractions was determined. It is found that FA from the BWC soil contain more metal than FA from the TA soil.

Soil experiments of metal/FA complexes showed that FA fractions have the best precipitation ability for heavy metals. The obtained results suggested that FA contain mainly phenolic, methyl, carboxylic, carbonyl functional groups with smaller amount of sugars and nitrogen bases attached to a fused aromatic backbone. These functional groups allow them to interact with metal ions and complex formation. Metal-FA can influence removal and transportation of heavy metal from soil and water. During this study the ability of FA (isolated from BWC) to reduce toxic metals' concentration of contaminated water was examined. More specifically, the effect of these compounds was tested on water contaminated (at various concentrations) with Mn, Zn, Cr and Pb. Therefore the knowledge of FA composition and complexation with metals will help us to understand the application in metal removal.

Introduction

Experimental

Result and Discussion

CHAPTER 1

Introduction

1.1 General Remarks

Humic substances (HS) are produced   in soil, peat, coal, water, sediments, and   other natural materials as a product of biological and chemical method of transforming organic matter. HS are supposed to be complex heterogeneous and polydisperse mixtures of nonstoichiometric composition [1]. HS are produced through aerobic   and   anaerobic decomposition of plants. Chemical and morphological complexity of the matter that makes the HS difficult to characterize and thus, the exact structure of HS cannot be defined [2,3]. The classical distinguish of HS is based on solubility differences at different pH values. According to that, HS are defined as highly transformed amorphous dark coloured materials that can be divided into four fractions: 1) humic acid (HA) - soluble in water at higher pH values (Over pH=11); 2) fulvic acid (FA) - soluble in water under all pH conditions (Higher soluble over pH=9) ; 3) humin - not soluble in water at any pH value. On the other hand they get precipitated at different pH : 1) HA precipitated around pH=1-2; 2) FA precipitated around pH=4.5-5. The color of fulvic acid is light yellow and the color of humic acid is dark brown [1,2]. The functional groups of HS are hydroxyl groups of phenols and alcohols, carbonyl groups of ketones/quinones, aldehydes, carboxylic groups, amino- and nitro-groups, and sulphur containing entities from thiols, sulfates and sulfonates [5,6]. These group provide HA the capability to be chelating agents. Therefore they can easily bind with heavy metals.

1.2.1 Humic substances

Humic substances are formed through the process of degradation of plants and animals materials [1]. Different types of HS are humic acid (HA), fulvic acid (FA) and humin. FA is lighter and more soluble compare to HA and plays a vital role to transport chemical species [2]. HA is soluble in basic solution on the other hand humin is insoluble in aqueous solution. FA, HA and humin have similar element composition but different structure and molecular weight.
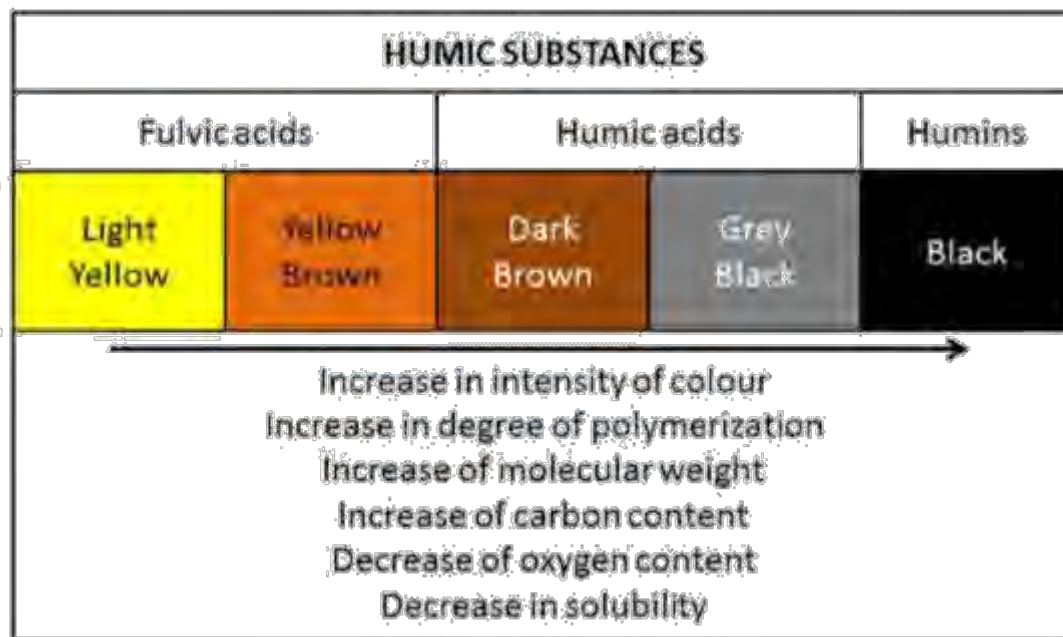
Figure 1.1 Chemical Properties of humic substances (Wikipedia)

1.2.2 Fulvic acid structure

The exact structure and molecular weight of humic substances remain unfamiliar and unknown though many analytical technique like EDX-SEM, NMR, FT-IR have been performed on humic substances to find out real structure [3]. According to literature elements composition of fulvic acid C(40-60%)>O(30-50%)>H(4-5%)>N(1-4%)>S(1-2%) by weight [4].

In the past, it seemed that humic substances were high molecular weight polymers [5], but at present it is suggested that humic substances are heterogeneous supramolecules.

Both composition and structure of humic substances are different from each local area to other and hence need to be characterized for better understanding of their impact on soil chemistry. The condensation of refractive phytyl side chains of chlorophyll molecules in plants are considered as the main source of these substances, although contributions from other organic sources also take place . They have mainly phenolic, methyl, carboxylic, carbonyl functional groups with smaller amount of sugars and nitrogen bases attached to a fused aromatic backbone [6].

Although humic and fulvic acid have almost same structure and element composition but clear differences exist between these two substances.

Fulvic acids contain lower molecular weight, less aromatic structure, lower pKa value, lower carbon content and higher hydrophilic, properties than humic acids [6].

1.2.3 Application of fulvic acid

Humic substances contain positive impacts on soil fertility, crops yield and plant growth. So humic substances work as common and important portion of fertilizers in agriculture industry [7].

Atiyeh and his colleagues [8] mixed humic substances with crops growth materials ,then they observed that the growth of cucumbers and tomatoes showed significant growth in terms of plant heights, leafs areas and so on.

Another study was done by Eyheraguibel in 2008 showed same impact on maize. [9]. Fertilizer rich with humic substances can increase crop yields. The positive impacts increase nutrient and soil fertility. [10]

Humic substances can be also added with animals feed as growth promoting agents of chickens and pigs [11].

Another important use of humic substances is to remove heavy and toxic metals like Zn, Pb, Cr, Mn, Cu and so on. At present heavy and toxic metals are utilized casually and hugely in tannary, pharmaceuticals, mining, textile, Lead-acid battery, metallurgical, electronic and metal finishing industries or in many other industries. When water is released from their waste treatment plants , then this polluted water by toxic metals is mixed with natural water and soil and in this way they increase pollution in our environment. Because most of the waste treatments plants have less efficiency. So in that case industrial effluents can be easily removed by using humic substances [12].

So humic substances are considered as the most valuable portion of soils as well as environment.

1.2.4 Fulvic acid as a binding agent

Fulvic acids are organic materials produced by oxidative decomposition of complex organic molecules such as carbohydrates, lignins, proteins, lignans and fats from litter, dead organism, root and the excrements of living organism [13].

The oxygen containing group such as phenolic hydroxyl and carboxyl allow fulvic acids to easily bind and produce complexes. In this way Varity of compounds are formed. The high binding potential ability of fulvic acid helps to easily bind toxic and other harmful substance.

Substance like heavy metals [14], pesticides [15], herbicides [16], organic pollutants [17] can be binded by fulvic acids.

Some researcher have reported that organic materials can be used to removed trace toxic metals by adsorption because they have high surface area [18] which increase the property of binding trace metals.

1.2.5 Fulvic as heavy metals reducing agents

Various types of environmental methods like ion exchange, chemical precipitation, solvent extraction, coagulation process, reduction process, adsorption, zar test, and so on are use to remove heavy metals from water [19]. In our country zar treatment and coagulation process are mostly used in water treatment plant. But along all these process the adsorptions process is the most facile and cost-effective method [20,21]. The knowledge of fulvic acid established a comparatively low cost and highly effective method to reduce the concentration of heavy metals in water [22,23]. The fulvic acids reduce oxidation number of heavy metals by reacting with them [24]. So fulvic acids are called reducing agents. In water heavy metals have mobility [25-27]. But when they are binded with fulvic acid, then acids are also called isolating agent. Fulvic acids can easily be isolated from cheap coal, lignite, soil, water by using alkaline method [28].

So fulvic acids can be used to isolate and remove heavy metal from water with[29] investing low cost.

1.2.6   Two metals simultaneous binding ability

Organic Fulvic acids show different types of binding capability when two heavy metals are present in complexation procedure. Whether it will increases binding capability of fulvic acid or it will reduce binding capability of fulvic acid.

Fulvic acid are reducing agent. When two metals are present, the reduction ability of
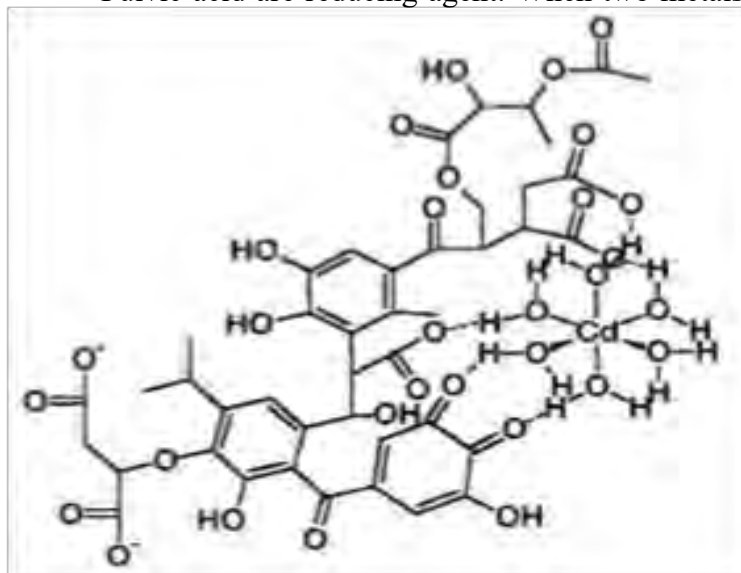
etal by lab complexation or natural

lead acid battery waste, then the

ong connection with metals or old

by binding with new metal. So this

d. If two metals try to take place in

between metals atoms are observed
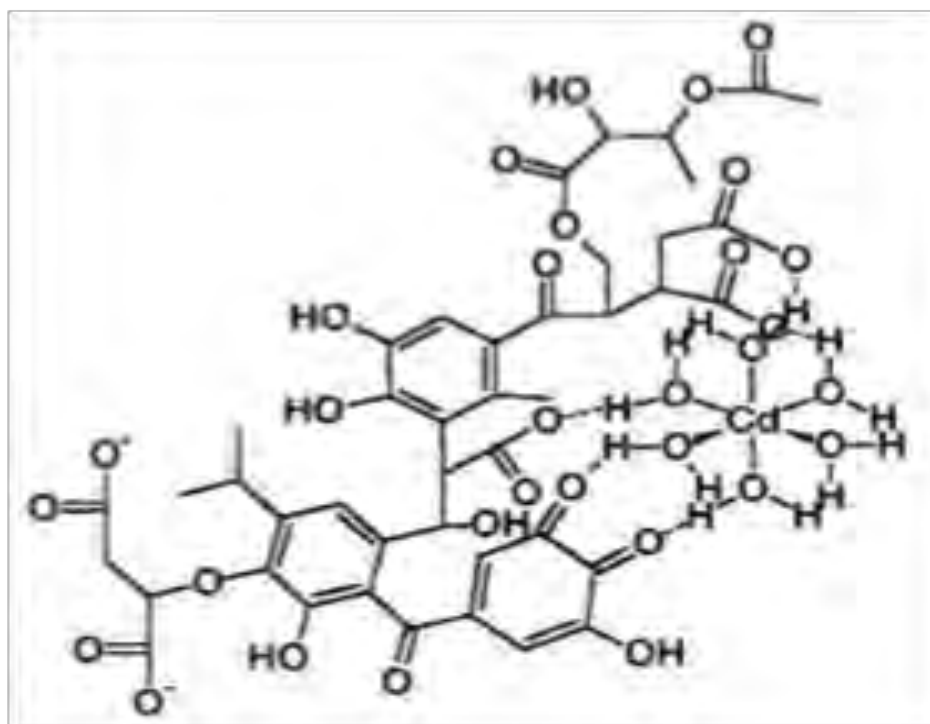
Figure. 1.2 Fulvic acid binding with metal.

1.2.7 Removal of heavy metals by fulvic acids

The capability of fulvic acid to interact with cations has long been recognized because of strong association of fulvic acid with organic and inorganic compounds in soil and water, acting as both storage and transport agents for these species [33]. Due to the polyfunctionality as well as oxygen containing group of FA, they have a strong affinity toward metal cations and therefore can interact strongly with various metal ions to form FA complexes and affect the adsorption/desorption and mobility behavior of metals [34, 35, 36]. The mobility, capturing, and transport of metal ions in the environment is strongly influenced by their complexation with FA [37,38]. FA have a great influence for reactivity with many natural and anthropogenic chemicals. Knowledge about metals in FA is useful for better understanding of mobilization and transport mechanisms of trace metals for environmental remediation efforts and soil nutrition studies, as well as their toxicity and bioavailability. The migration property of metallic elements in natural ecosystems is significant because of severe problems of environmental pollution. Lately, adsorption of metal ions on the FA of various origins was studied.

FA is a valuable of natural environment. It is known that the addition of cations, especially heavy metals, to a FA solution changes the intramolecular forces and FA molecules have a tendency to bind up with relatively hydrophobic and hydrophilic surfaces. Also, the formation of aggregates is depend on metals. Because of the size changes induced by conformational rearrangement and aggregation/dissociation arising from intermolecular hydrogen bonding, FA can form soluble complexes that can migrate long distances or precipitate, carrying bound cations with them. The migration/precipitation abilities depend on some criteria such as the metallic ion, the cation charge, the degree of ionization of the organic molecule, the ionic strength of the media, and the location of the metal ion [39].

1.3.1 Heavy metal as pollutant

Heavy metals have a relatively high density compared to water. Heavy metals also include metalloids such as arsenic, that are able to induce   toxicity at low level of exposure. Heavy metals have several industrial, agricultural,   domestic and technological applications.   Sources of heavy metals are   environment gynogenic, industrial, agricultural, battery, tannary, pharmaceutical, domestic   effluents, and atmospheric sources. Environmental pollution is increasing for mining, foundries and smelters, and other metal-based industrial operations [40, 41].

Although heavy metals are produced by environmental process, but environmental contamination and human exposure result from anthropogenic activities such as mining and smelting operations, industrial production and use, and domestic and agricultural use of metals and metal-containing compounds [42]. Environmental contamination can also produce through metal corrosion, atmospheric deposition, soil erosion of metal ions and leaching of heavy metals, sediment re-suspension and metal evaporation from water resources to soil and ground water [43].

Heavy metals can take place   in trace   concentrations (ppb range to less than 10 ppm) in various environmental matrices, so they are also regarded as trace elements [44]. Their bioavailability is influenced by both physical (such as temperature, phase association, adsorption and sequestration) and chemical factors (such as thermodynamic equilibrium, complexation kinetics, lipid solubility and octanol/water partition coefficients). Biological factors such as species characteristics and biochemical/physiological adaptation, also play an important role. The essential heavy metals show biochemical and physiological functions in plants and animals. They are important   part of several key enzymes and play vital roles in various oxidation-reduction reactions. Copper for example work as an essential co-factor for several oxidative stress-related enzymes including catalase, superoxide dismutase, peroxidase, cytochrome c oxidases, ferroxidases, monoamine oxidase, and dopamine β-monooxygenase. Copper is a vital   nutrient that is incorporated into a number of metalloenzymes involved in   hemoglobin formation, carbohydrate metabolism, catecholamine biosynthesis, and   cross-linking of collagen, elastin, and hair keratin. The efficacy of copper to cycle   between an oxidized state, Cu (II), and reduced state, Cu (I), is done by cuproenzymes through redox reactions [45]. However, the superoxide and hydroxyl radicals compound of copper can bring toxicity [46]

Also, excessive presence to copper causes cellular damage leading to Wilson disease in humans [47]. Similar to copper, several other essential elements are required for biologic functioning, however cellular and tissue damage leading to a variety of adverse effects and human diseases will carry by an excess use of such metals.

There is a very narrow range of concentrations between beneficial and toxic effects of toxic metals [48]. Non-essential metals metals such as aluminum (Al), antinomy (Sb), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd), gallium (Ga), germanium (Ge), gold (Au), indium (In), lead (Pb), lithium (Li), mercury (Hg), nickel (Ni), platinum (Pt), silver (Ag), strontium (Sr), tellurium (Te), thallium (Tl), tin (Sn), titanium (Ti), vanadium (V) and uranium (U) and they have no established biological functions.[49].

Figure. 1.3 Heavy metals pollution (Beribad area, Dhaka)

1.3.2 Toxicity of lead-acid batteries

Lead acid batteries are used in cars, trucks, motorcycles, boats and other motor equipments. Lead acid batteries are important power supply devices. They are commonly used in telecommunication systems, solar panel, uninterruptible power supply (UPS) and so on. According to statistics, approximately 3 million tons waste batteries are taken place every year and the production of lead acid batteries increases that number for development of economy [50].

Lead acid batteries generally contains four parts, they are electrolyte, lead and lead alloy grid, lead paste , organic material and plastics [51]. These chemical can bring toxic, hazard, flammable, explosive substance that can easily bring potential risk.

Fires, poisoning, explosive and leaks are taken place by lead acid battery explosion. These contaminate environment and damage environment ecosystem. So lead acid batteries may bring lots of pollution [52].

The main chemical composition are given below.

Materials	Risks	Physical state	Sources
Lead and Lead compounds	Toxicity	Solid	Electrode and grid
Antimony	toxicity	Solid	Plates
Hydrogen	Explosiveness	Gas	Water electrolysis
Sulfuric acid	Corrosion	Liquid	Electrolyte

Table. 1.1. Risk assessment of lead acid batteries (*Environ. Geochem.* Health 295 1999, 21, 257-272)

So lead acid batteries contains compounds and antimony. These compound and antimony are toxic. This toxicity affects both humans and the environmental system.

1.3.3 Lead as environmental pollutants

Environmental pollutants cause of environment pollution. At this present world, it is an alarming issue all over the world. Now a days it is a burning question that how to reduce environmental pollution.

A substance exists in environment more than abundance due to human activity, which ultimately effect on the environment and therefore on living organism and mankind is called pollutants. eg Pb, Hg, SO_2, CO_2, etc are environmental pollutants .

Environmental pollutants bring adverse effect on physical and biological components of the earth system. These pollutants can be produced by natural process or human made activities. Any use of natural substances at a rate of greater than nature, capacity to restore itself can bring in pollution of air, water, and land.

Environmental pollutants can hamper ecosystem chain, bring a lot of diseases, reduce plants growth, reduce human and other animals life time and so on.

Such pollutant is lead. Lead is a toxic and heavy metals. It affects acutely and chronically on humans, especially on children. It can affect the nervous system and ultimately lead to reduce IQ levels. Pb contamination is preliminary generated by lead acid batteries, combustion of coals, and vehicles and smelting of non-ferrous metals. But mostly now a days Pb pollution is occurred from combustion of coals. And on the other hand rapid increase of the use of   lead acid batteries also increase lead pollution. So it is regarded as global. problem at present .

Sample collecting

Water wasted by lead

Soil wasted by lead
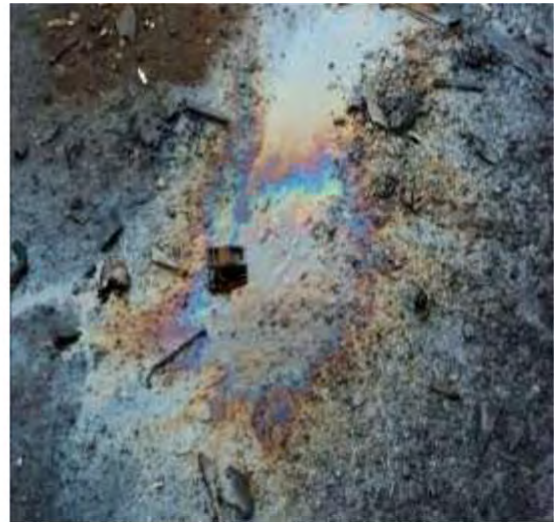
Soil & water wasted by sulfuric acid of battery

Figure. 1.4 Lead acid battery waste contaminated soil & water

1.4.1 Aim of the study

FA were extracted from soil of haor region and battery waste contaminated soil of battery industries of Dhaka, korotoya river of Bougra. Still, there is very little information of the FA and their fractions of the used sediments. In particular, the properties of the FA fraction were of interest because of almost lack of information about it. There was hardly any information about metal ion complexation with FA. Taking into account the polyfunctionality of FA, the metal complexation with FA fractions may give information about the structure of different FA fractions and FA in general. FA may form sufficiently stable and always negatively charged chelates with

many different metal ions.

Therefore, the aims of the study are the following:

1. Extraction of HA and FA from soil contaminated with battery waste.

2. Characterization of humic substance by functional group analysis.

3. Identification of metals present in soil contaminated with battery   waste.

4. Complexation of FA with Pb, Mn,Cr, and Zn.

5. Characterization and study of competitive binding of Pb with HA and FA.

6. Improve the current knowledge of humic substances present in battery-waste contaminated sites in Bangladesh.

7. Understand the impact of fulvic acid on mobilization of lead in soil and water.

8. Understand the nature of interaction between metal and humic substances.

CHAPTER 2

Experimental

Materials and instruments

2.1 Chemicals and reagents

The chemicals and reagents used in this research were analytical grade and used without further purification. Distilled and double distilled were used as solvent to prepare most of the solutions of this work. The chemicals and reagents which were used in this research are given below:

i. Nitric acid (Merck, Germany)

ii. Hydrochloric acid (Merck, Germany)

iii. Sodium Hydroxide (Merck, India)

iv. Fulvic acid (Merck, India)

v. Lead sulphate (Merck, India)

vi. Chromium sulphate (Merck, India)

vii. Manganese sulphate (Merck, India)

viii. Ethanol (Merck, Germany)

ix. Deuterium oxide (Merck, Germany)

x. Choloroform (Merck, Germany)

xi. Zinc sulphate (Merck, Germany)

2.2 Instruments

Analysis of the samples was performed using the following instruments:

i.   Fourier Transform Infrared Spectrophotometer (SHIMADZU FTIR-8400)

ii.   Field Emission Scanning Electron Microscopy (JSM-7600F, Tokyo, Japan.)

iii. Energy Dispersive X-ray spectroscopy (Jed-2300 analysis station)

iv.   Nuclear Magnetic Resonance (NMR)

v.   X-ray Fluorescence Spectroscopy (XRF)

vi.   Centrifuge machine (Hettich, Universal 16A)

vii. pH meter (Hanna, HI 8424, Romania)

viii. Digital Balance (AB 265/S/SACT METTLER, Toleto, Switzerland)

ix.   Freeze dryer (Heto FD3)

x.   Oven (Lab Tech, LDO-030E)

xi.   UV-visible Spectrophotometer (Shimadzu-1800)

2.2.1 Sample collection

Soil samples were collected from lead-acid battery recycling plants, near lead-acid battery production industries and haor region in Sylhet. The lead-acid battery recycling plants is located at a remote area named South-Fulbari, in Bogura Shadar. The lead-acid battery production industry is Panna Group of Volvo battery industry which is located at Beribad area in Dhaka city. And the haor is Tanguar haor in syhlet. From each area 10 to15 soil samples were collected.

Figure. 2.1. Soil sample collecting

Method of preparation

2.3.1 Extraction and purification of Fulvic acid (FA)

Soil were collected from battery industries, battery recycling plants and haor region. The sample will be washed with 1 N HCl solution and NaOH solution will be added to dissolve humic substances. Conical flasks containing sample in NaOH solution was shaken in a rotatory shaker. After that the solution was centrifuged and the supernatant was taken. Supernatant's pH was adjusted to between 1 and 2 with HCl. The solution was settled for enough time, the insoluble humic acid will be precipitated. The precipitate was collected and the pH of the supernatant was again adjusted between 4.5 and 5 using NaOH solution. The fulvic acid fraction was precipitated and settled for enough time. Both the humic substances and fulvic acid fractions were washed with distilled water to remove impurities. Finally black coloured fine light brown crystals of humic acid and coloured crystal of fulvic acid were dried in air for further study.

General Scheme

Extraction of Fulvic acid from soil

Soil $\xrightarrow[\text{grinding for pass through 2mm sieve}]{\text{air dried}}$ **Grinded soil** $\xrightarrow[\text{HCl}]{\text{dissolved into}}$ **Washed soil/ Sample + Inorganic compounds**

$\downarrow$ Centrifuge

Humic substance

HA + FA $\xleftarrow[\text{at 3500 rpm}]{\text{Centrifuge}}$ **Humin $\downarrow$ + HA+ FA** $\xleftarrow[\text{shaking over night}]{\text{pH=11 by NaOH \&}}$ **Humic substance**

$\downarrow$ pH=(1-2) for 18hr

HA $\downarrow$ + FA $\xrightarrow{\text{Centrifuge}}$ **FA** (Dissolved) $\xrightarrow{\text{pH=(4.5-5)}}$ **FA $\downarrow$** (lite brown)
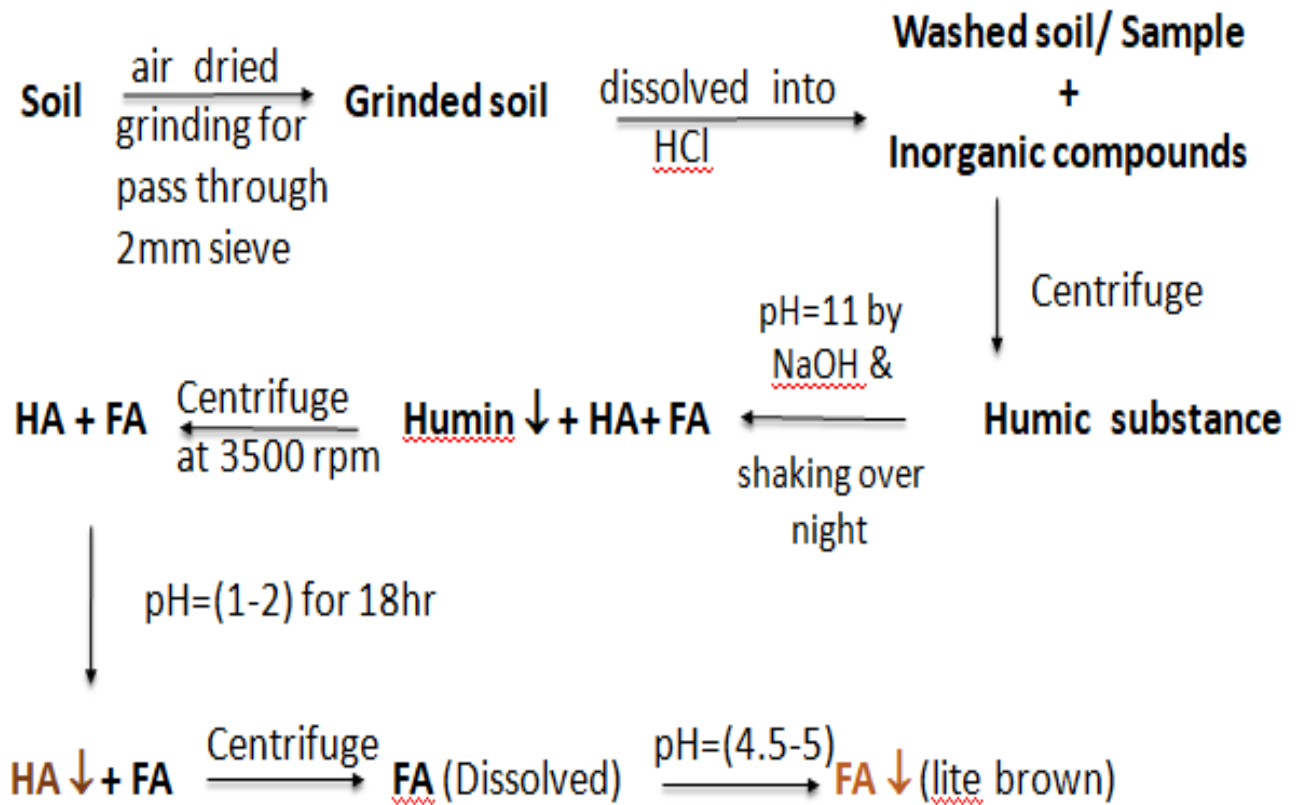
General Scheme

Complexation procedure

$$FA \xrightarrow[\substack{pH=(>10.5) \\ \text{with stiring}}]{\text{Controlling}} \textbf{dissolved FA} \xrightarrow[\substack{\text{metal (TM) solution} \\ \text{with continuous} \\ \text{string}}]{\text{droppwise Toxic}} \textbf{FA + M}$$

FA + M $\xrightarrow[\substack{\text{vigorous} \\ \text{string for} \\ \text{14hr}}]{pH=(8-9)}$ FA - M

FA - M (Complex) $\xleftarrow[\text{3500 rpm}]{\text{string 15min}}$ FA - M
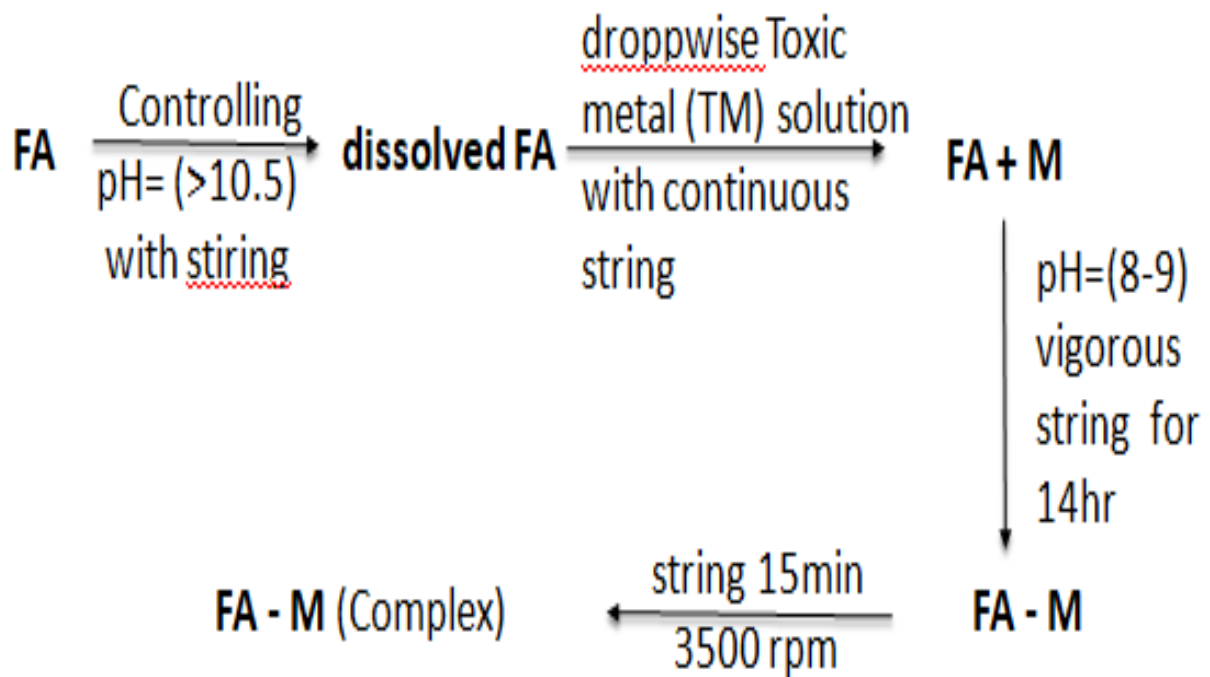
2.3.2 Preparation of FA-Metal Ion Complex

FA-Pb, FA-Cr, FA-Mn, FA-Zn complexes were prepared by using same procedure. So FA-Mn complex preparation are described here. 1.00g of FA was dissolved in 200 ml distilled water. pH was maintained around 10.5 with 10 M NaOH. The solution was stirred for 4 h and centrifuged for 15 min at 7000g. Metal salt was dissolved in 20 ml of water to make 1M solution and was added slowly (drop wise) into 200 ml of the FA solution under vigorous stirred and continuous pH control (8-9) with 10 M NaOH. The solution was diluted to 500 ml, stirred for 14 h and then centrifuged for 15 min at 700g to separate the FA-Mn, FA-Pb, FA-Zn, FA-Cr complex from solution. The complexes was washed with distilled water to remove negative sulphate. Finally, the precipitates were dried in an oven at 95^0C and stored in a desiccator under vacuum

2.4 Sample characterization

1. Fourier transform infrared (FTIR) analysis

The infrared spectra of humic acid and their metal complexes were recorded on FTIR spectrometer the region of 4000 – 400 cm-1. All sample had dried. A small portion of samples were taken into vial and oven dried at 95°C to confirmed their dryness. Metal complexes is hard so it was grinded into a mortar with a pestle to get powder. HA samples were not grinded because they were physically granule/powder in shape after completely drying. Around 0.5 mg of dried HA and metal complex was grinded with 100 mg KBr till it became homogenous. The powder was then placed in the path of IR beam for measurements.

2.   Field emission scanning electron microscopy (FE-SEM)

The surface morphology of the synthesized FA-Pb, FA-Mn, FA-Cr, FA-Zn complexes and fulvic acid (FA) was adopted using Field Emission Scanning Electron Microscopy (FE-SEM). The completely air-dried samples were put on a conducting carbon strip. The sample loaded strip was then mounted to a chamber that evacuated to $\sim 10^{-3}$ to 10^{-4} torr and then a very thin platinum layer (~few nanometers thick) were sputtered on the sample to ensure the conductivity of the sample surface. The sample was then placed in the main SEM chamber to view its surface. The microscope was operated at an accelerating voltage of 5.0 kV. The system was computer interfaced and thus provides recording of the surface images in the computer file for its use as hard copy.

3.   Energy dispersive X-ray (EDX) spectra

Elemental analyses of the fulvic acid and FA-Pb, FA-Cr, FA-Mn, FA-Zn complexes were performed by EDX spectra. The dried powder of FA and FA-Pb, FA-Cr, FA-Mn, FA-Zn complexes were placed on a 1cm $\square$ 1cm conducting steel plate. The steel plate was then placed on a conducting carbon glued strip. The sample was then placed in the main FE-SEM chamber with the EDX instrument.

4.   Nuclear magnetic resonance (NMR)

1H-NMR was used to estimate the relative proton content from aromatic and aliphatic group present in sample. The spectra were recorded with Bruker 400MHz. About 5 mg of sample was dissolved in 0.5 ml DMSO and spectra were recorded in δ range 0-14ppm.

5. X-ray fluorescence spectroscopy (XRF)

Lead acid battery contaminated soil composition analyses were performed by XRF spectra. The dried powder of soil samples were placed on a 1cm $\square$ 1cm conducting steel plate. The steel plate was then placed on a conducting carbon glued strip. The sample was then placed in the main XRF chamber with the XRF instrument.

CHAPTER 3

Results and Discussion

3.1   Contaminated soil analysis :

# 3.1.1 Soil composition analysis by XRF :

Lead acid batteries are renewable batteries that are widely used in all over the world. The use of lead acid battery is increasing day by day. Though these batteries are commonly used in motor vehicles, on the other hand these batteries are also utilized in different sources like solar system, IPS system and so on. Lead acid batteries commonly contain plastic cover, sulfuric acid, and lead plates. Around all of these lead plates play a vital role because they have the ability to store energy or to release energy.

But when a lead acid battery has crossed its life time, then these lead plates no longer able to store energy source. Then they are called weak lead plates and the battery is called waste battery. Because the battery can no longer be recycled or reused. These waste batteries are converting into toxic materials.
Though the lead plates in waste battery are weaker but they can be reused. In fact most of the new batteries are made of recycled or purified lead plates. The market of recycled lead plates are increasing at present.

In low and middle income countries like Bangladesh are common market of renewable lead plates. Because poor and uneducated people can take risk for money, so though they work with toxic materials during extraction process, this is their main income source. During extraction process lead dust and sulfuric acid are mixed with soil and water and change composition of soil and water.

So by using X-ray fluorescence spectroscopy (XRF) composition of battery waste contaminated soil can be determined. And it will also give the harmful effect of lead acid battery toxicity.
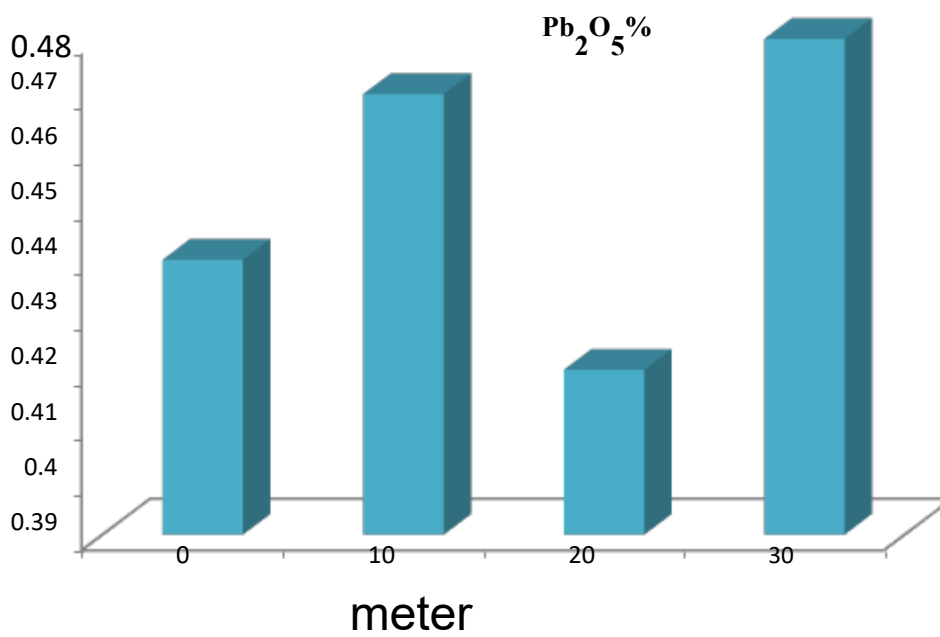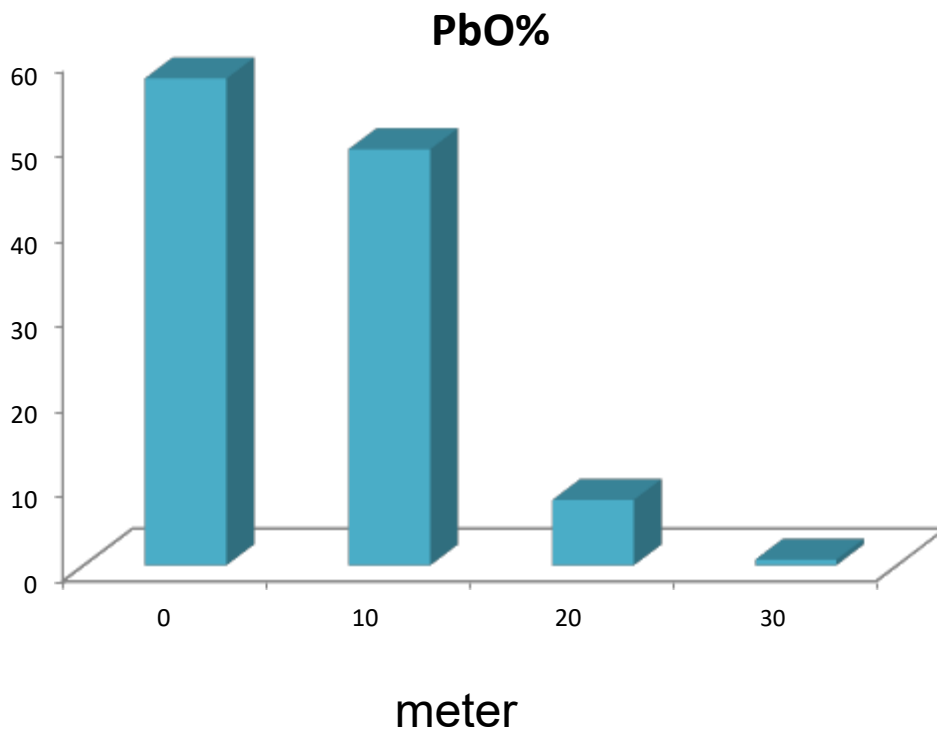
Figure 3.1: XRF analysis of contaminated soil

Heavy metals	Tolerable level (mg/Kg)
Cadmium (Cd)	1.5
Lead	100
Nickel (Ni)	40
Zinc (Zn)	300
Copper (Cu)	50
Arsenic (As)	6
Mercury (Hg)	4

Table : 3.1. Tolerable level of lead into soil

Alarming !

- 67 times more Pb is present in soil.(It is 20m away from plant)
- So pollutants are spreading.

3.2   Characterization of FA & BFA :

3.2.1 Surface analysis by Scanning electron microscopy :

The surface morphology of the extracted FA & BFA complexes and fulvic acid (FA) was adopted using Field Emission Scanning Electron Microscopy (FE-SEM). SEM is used to produce image of a   surface by scanning the surface with a focused beam of electrons. Although, SEM is not conclusive for assessing the existence of a true inclusion compound in the solid state, it can be of some utility to prove the homogeneity, similarity or different of the solid phases. Fulvic acid shows fibrous structure at SEM image (According to other literature review).
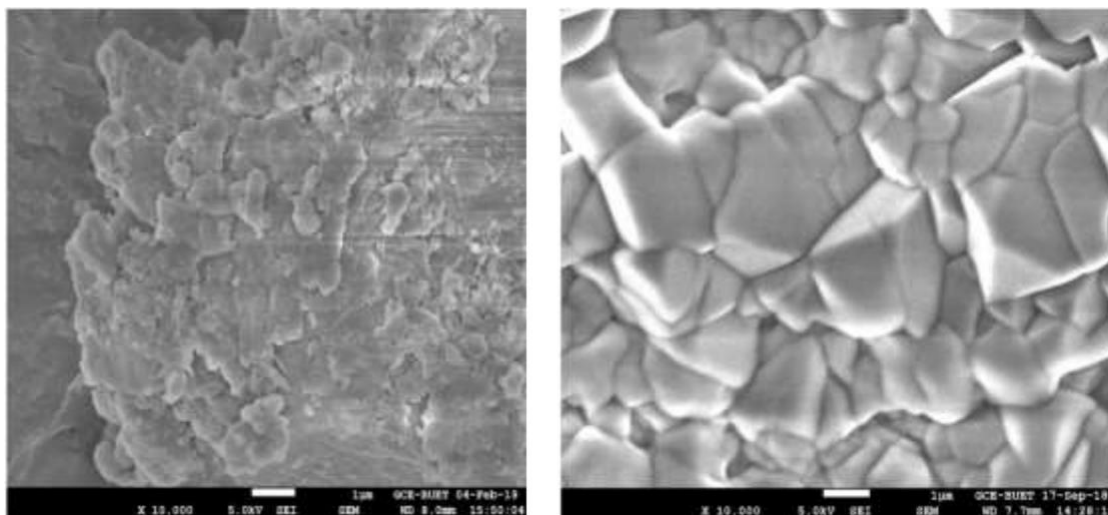
FA
Resolution: ×10,000

BFA
Resolution: ×10,000

Figure 3.2 SEM spectra of (a) FA & (b) BFA

Both FA & BFA are fulvic acid but they don't look same compound. Although they are not look same compound but there is a similarity, the similarity is both have fibrous structure and this is their homogeneity.

Though they are fibrous, they have different surface , because they are extracted from different location soil.

3.2.2 Composition analysis by energy dispersive X-ray (EDX)

What types of elements are present in a compound and also their amount can be identified by EDX. Though only hydrogen atom cant be identified by EDX spectroscopy. For characterization of FA & BFA , it is important to know the composition . Fulvic acid is extracted from humic substance, so it is organic compound. So carbon (C), nitrogen (N), hydrogen (H) and oxygen (O) are common elements of fulvic acid.

But for pollution or other environmental different , heavy metals or other elements can bind with fulvic acid and it can change the composition of fulvic acid.
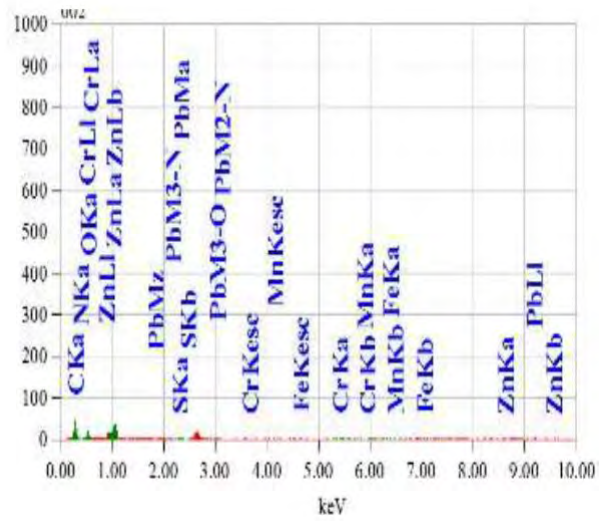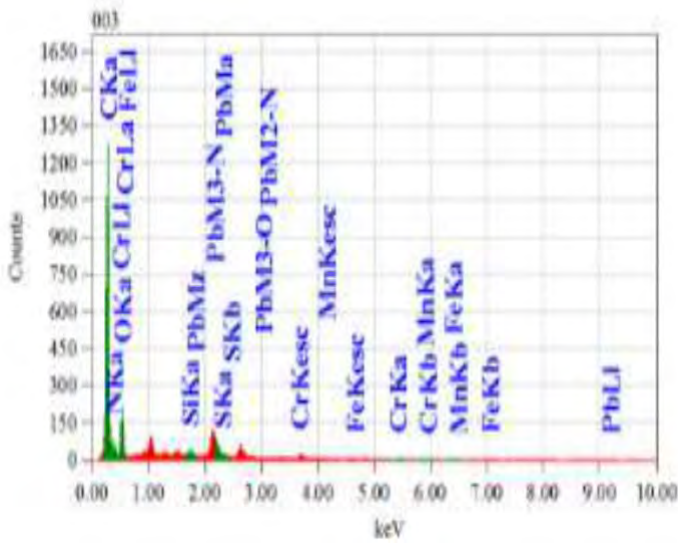
a Elements	Amount in percentage (%)
Carbon (C)	59%
Nitrogen (N)	21%
Oxygen (O)	15%

b Elements	Amount in percentage (%)
Carbon (C)	53%
Nitrogen (N)	8%
Oxygen (O)	29%
Lead	5%

Figure 3.3: EDX spectra of (a) FA & (b) BFA

According to EDX spectra of FA and BFA , both are organic compound so both have carbon, nitrogen and oxygen as their main elements, but in battery waste contaminated soil fulvic (BFA) acid has lead (5%). So the composition of BFA is different then natural fulvic acid. So binding, fertilizing, nutrient carrier capability and other properties will change. This impact will hamper environmental chain system. Due to lead acid battery pollution , lead is easily blinded with fulvic acid. That is why even soil was washed with HCl (conc.) , lead was found in BFA.

3.2.3 Functional group analysis using Fourier Transform Infrared spectroscopy of FA & BFA (FTIR)

FTIR measurement were carried out in order to confirm the presence of functional group in FA & BFA. According to FTIR spectra, both compounds have common groups. Such as carboxylic (-COOH), phenolic (-OH), alcoholic, aliphatic (-CH), aromatic, cyclic, acyclic (-C=C-), ketons (-C=O of –COOH) and many other groups. FA & BFA compounds FTIR spectrum are over lap to each other, so both compounds show same group at same wave number.
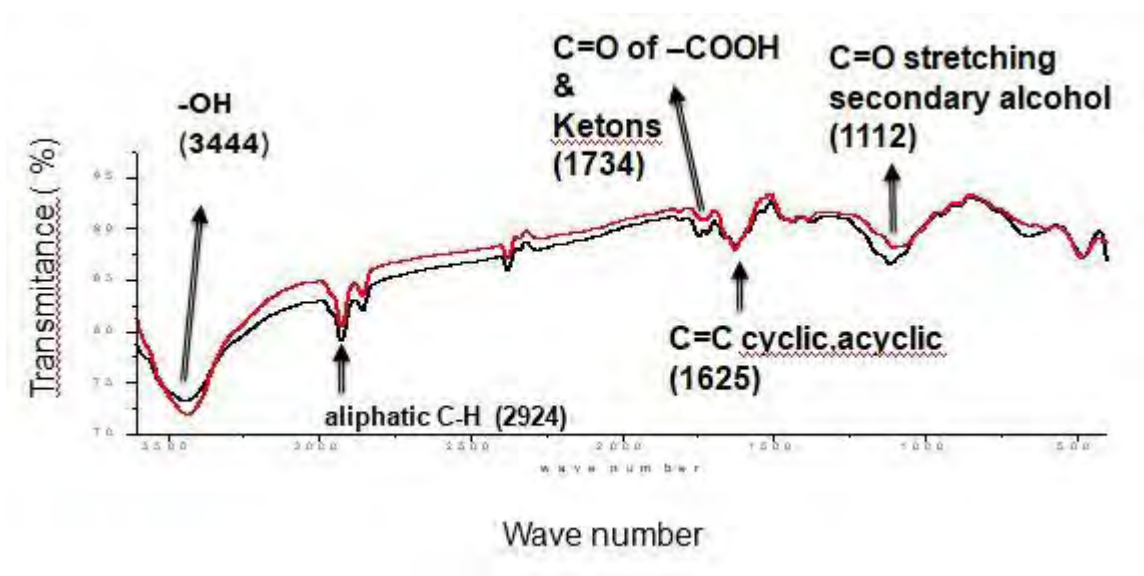
Figure 3.4 FA &BFA spectra of FTIR

Table 3.2 Characteristic peak and interpretations correspond to FA and BFA compounds :

FA(cm^{-1})	BFA(cm^{-1})	Functional Group
3431	3444	H-bonded OH, Free OH, Intermolecular bonded OH
2924	2924	Aliphatic C-H, C-H2, C-H3, stretching
1739	1734	C=O stretching of carboxylic acids, cyclic and acyclic aldehydes and ketons, quinines
1625	1625	C=O stretching vibration of double bonds of cyclic and acyclic compounds, benzene ring substitution
1099	1112	C-O stretching, secondary alcohol

From that table , it is understand that both BFA and FA have almost same functional group at same wave number. Though they were collected from different area but they are same compounds. So that is why they have same functional groups.

3.2.4 Nature of proton analysis by nuclear magnetic resonance (NMR) :

1H NMR spectra just provide the information of the nature of proton in a organic compound. The NMR spectra of FA and BFA provide the information about the nature of protons which are available in FA and BFA.

(0 – 1.7) shows Aliphatic proton (branched methyl proton.

(1.7 – 3.00) shows Methylene and methyl groups attached to an aromatic ring or carboxyl group. (3 .0 – 5.0) shows aprotons on carbons attached ssigned to to O or N atoms (polysaccharides, carbohydrates, methoxyl groups of lignins, amines or amino groups of proteins). (5.0 – 6.5) corresponding to protons of olefins. These peaks are available in FA and BFA, so these types of proton are available in FA and BFA.
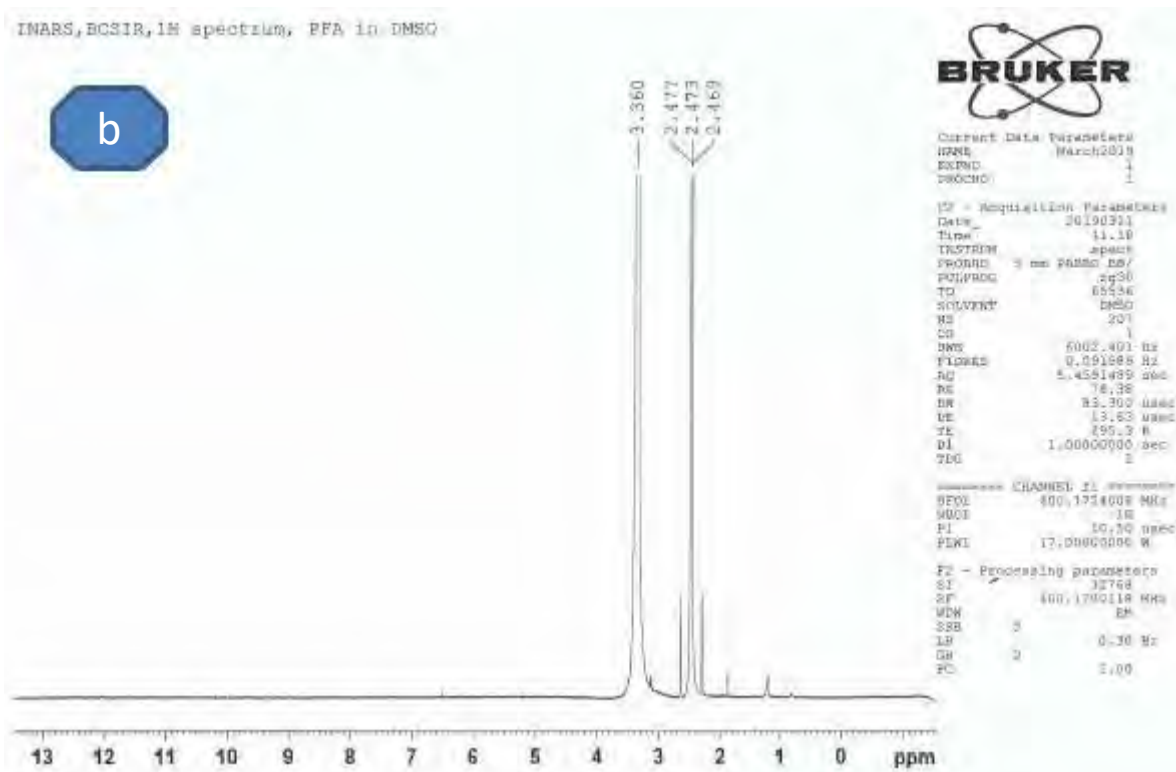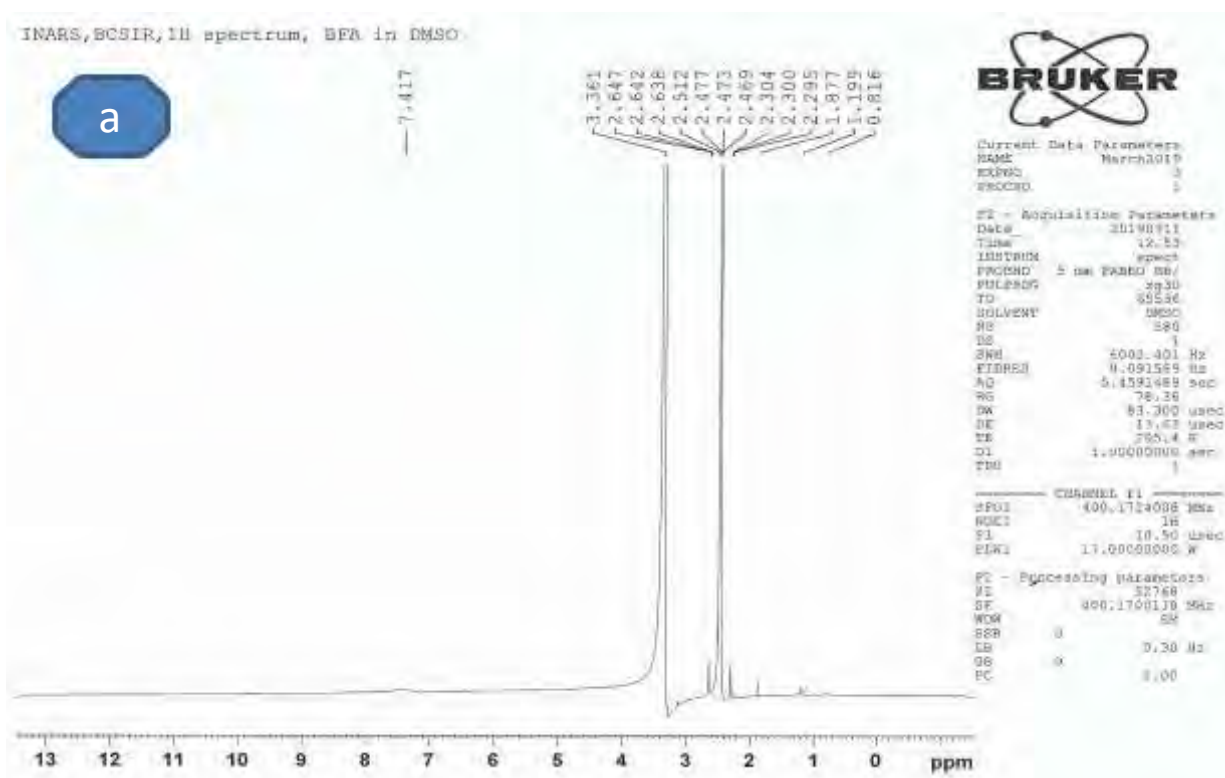
Figure 3.5: (a) BFA & (b) FA NMR spectra

3.3 Complexation study :
3.3.1 Surface study of Mn-complex by SEM :

The complex was formed between Mn-salt with FA and Mn-salt with BFA. Before complexation the surface of BFA and FA was like fibrous.

But after complex formation, the surface structure of FA and BFA is change. It is observed that aggregation is taken place on the surface of FA and BFA. BFA surface gets more aggregation than FA. So it proves that complex formation capability of BFA is more than FA with Mn atom.
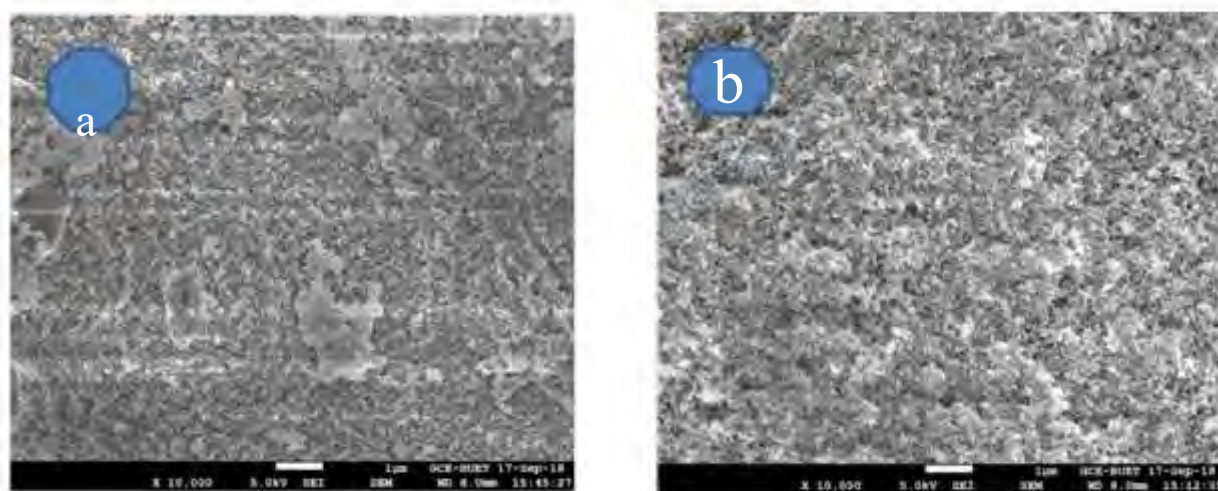
Figure 3.6. SEM spectra of (a) FA-Mn & (b) BFA-Mn (Resolution: ×10,000)

3.3.2 Functional group analysis of Mn-complex using Fourier Transform Infrared spectroscopy (FTIR)

FTIR measurement were carried out in order to confirm the presence of functional group in Mn-FA & Mn-BFA. According to FTIR spectra, after complexation, two change are observed at $1734 cm^{-1}$ (-COOH) and $1112\ cm^{-1}$ (-C=O) of FA and BFA. For Mn-FA complex $1734 cm^{-1}$ and $1112 cm^{-1}$ peak are shifted into $1718 cm^{-1}$ and $1149 cm^{-1}$. For Mn-BFA complex $1734 cm^{-1}$ and $1112 cm^{-1}$ peak are shifted into $1751 cm^{-1}$ and $1103 cm^{-1}$. It proves that bonds are formed at oxygen containing groups.
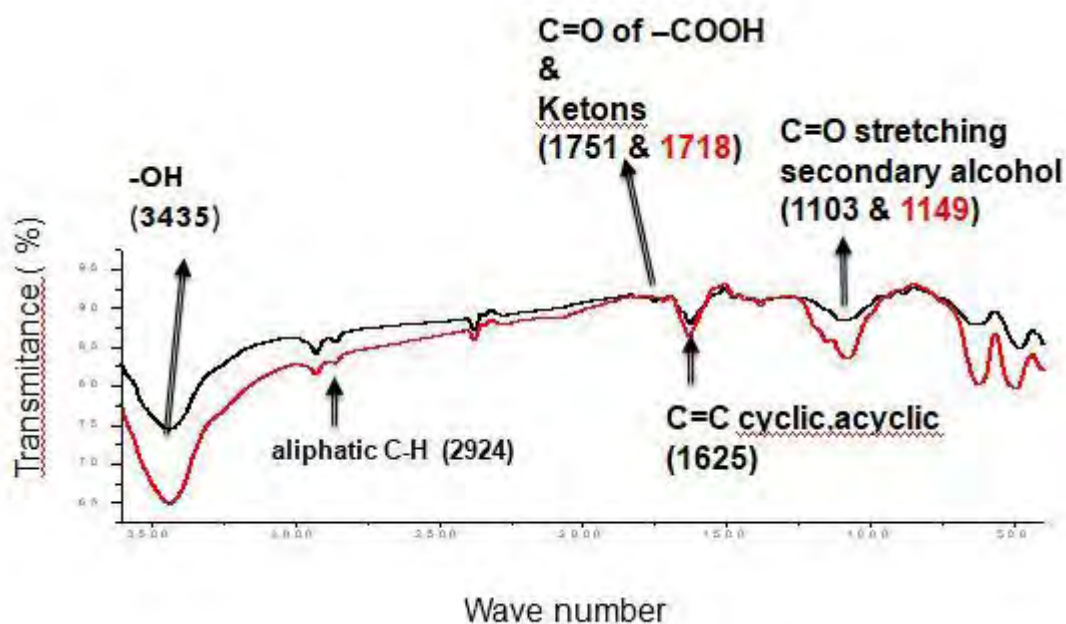
Figure 3.7 Mn-FA & MnBFA spectra of FTIR

Table 3.3 Characteristic peak and interpretations correspond to FA-Mn and BFA-Mn compounds :

Mn-FA(cm^{-1})	Mn-BFA(cm^{-1})	Functional Group
3433	3444	H-bonded OH, Free OH, Intermolecular bonded OH
2924	2924	Aliphatic C-H, C-H2, C-H3, stretching
1751	1718	C=O stretching of carboxylic acids, cyclic and acyclic aldehydes and ketons, quinines
1625	1625	C=O stretching vibration of double bonds of cyclic and acyclic compounds, benzene ring substitution
1149	1103	C-O stretching, secondary alcohol

3.3.3 Energy dispersive X-ray analysis of Mn-Complex(EDX) :

After complexation, it observed that 57% Mn is absorbed by BFA and on the other hand 5% Mn is absorbed by FA. So binding capability of BFA is higher than FA with mangage.
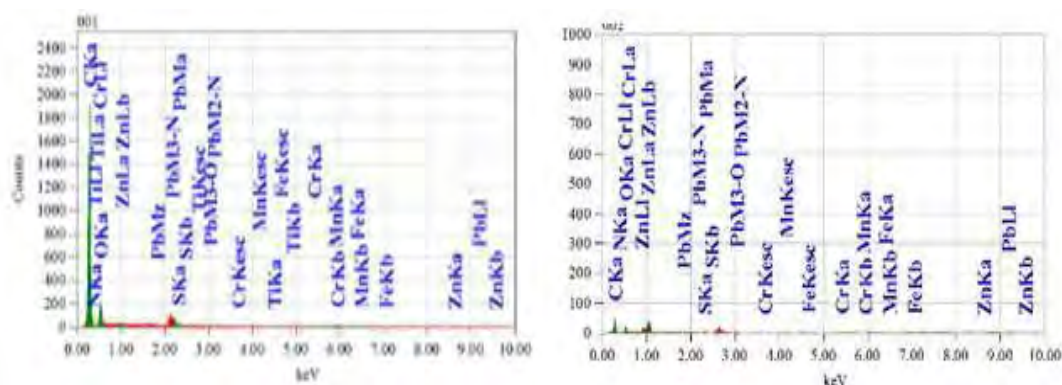
Elements a	Amount in percentage (%)	Elements B	Amount in percentage (%)
Carbon (C)	81%	Carbon (C)	3%
Nitrogen (N)	1%	Nitrogen (N)	3%
Oxygen (O)	13%	Oxygen (O)	37%
Manganese (Mn)	5%	Manganese (Mn)	57%

Figure 3.8 EDX spectra of (a) FA-Mn & (b) BFA-Mn

3.3.4 Surface study of Cr-complex by SEM :

The complex was formed between Cr-salt with FA and Cr-salt with BFA. Before complexation the surface of BFA and FA was like scales.

But after complex formation, the surface structure of FA and BFA is change. It is observed that aggregation is taken place on the surface of FA and BFA. BFA surface gets more aggregation than FA. So it proves that complex formation capability of BFA is more than FA with Cr atom.
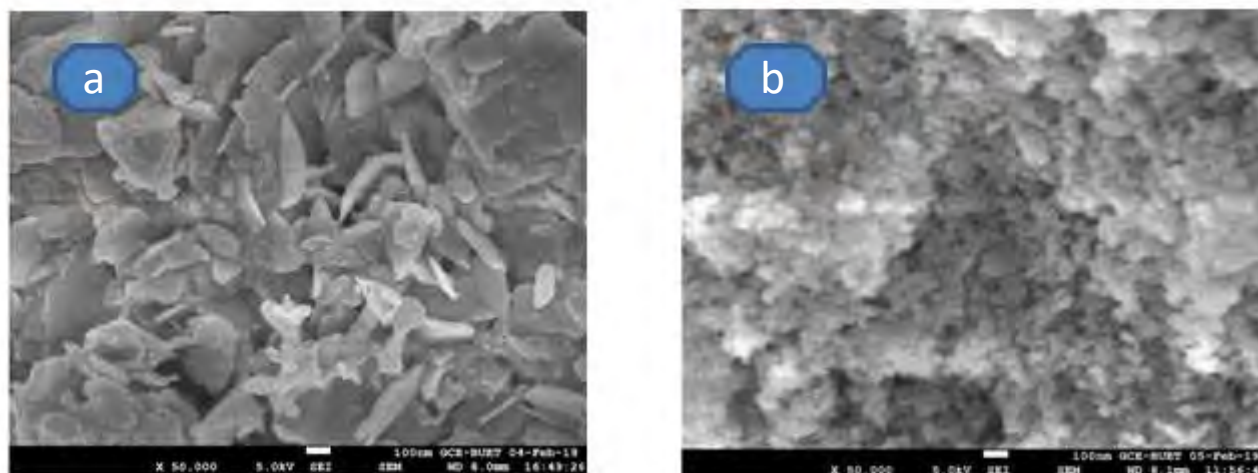
Figure 3.9 : SEM spectra of (a) FA-Cr & (b) BFA-Cr (Resolution: ×50,000)

3.3.5 Functional group analysis of Cr-complex using Fourier Transform Infrared spectroscopy (FTIR)

FTIR measurement were carried out in order to confirm the presence of functional group in Cr-FA & Cr-BFA. Arrording to FTIR spectra, after complexation, one change is observed at at $1734cm^{-1}$ (-COOH) of FA and BFA. For Cr-FA complex $1734cm^{-1}$ and $1112cm^{-1}$ are shifted into $1718cm^{-1}$. For Cr-BFA complex $1734cm^{-1}$ peak is completely gone.

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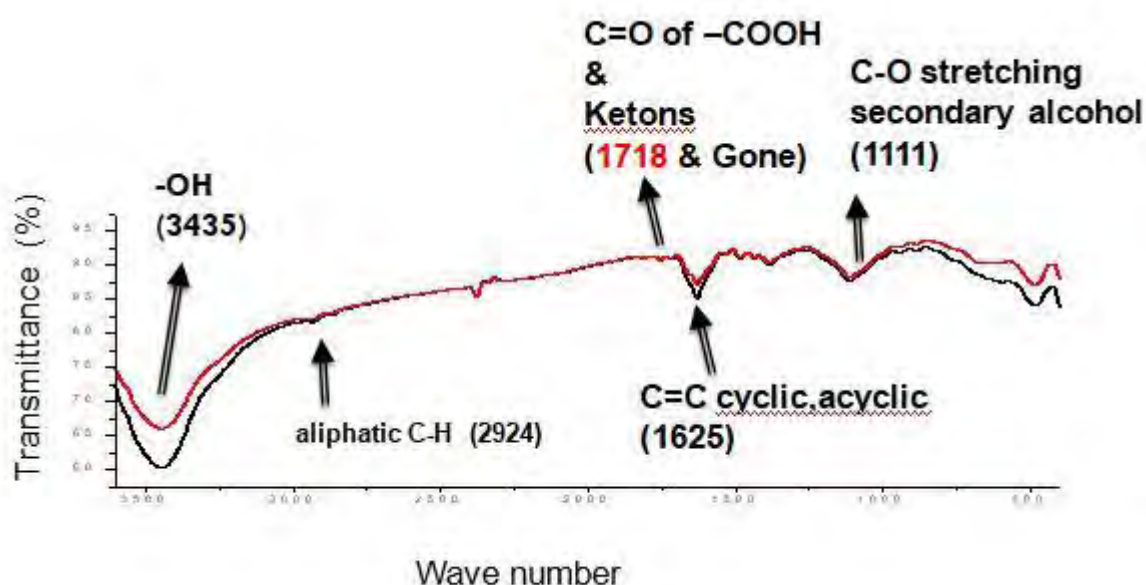
Figure 3.9 Cr-FA & Cr-BFA spectra of FTIR

Table 3.4 Characteristic peak and interpretations correspond to FA-Cr and BFA-Cr compounds :

Cr-FA(cm^{-1})	Cr-BFA(cm^{-1})	Functional Group
3433	3444	H-bonded OH, Free OH, Intermolecular bonded OH
2924	2923	Aliphatic C-H, C-H2, C-H3, stretching
1718	Gone	C=O stretching of carboxylic acids, cyclic and acyclic aldehydes and ketons, quinines
1625	1625	C=O stretching vibration of double bonds of cyclic and acyclic compounds, benzene ring substitution
1111	1113	C-O stretching, secondary alcohol

3.3.6 Energy dispersive X-ray analysis of Cr-Complex (EDX) :

After complexation, it observed that 2.03% Cr is absorbed by BFA and on the other hand 0.17% Cr is absorbed by FA. So binding capability of BFA is higher than FA with chromium.
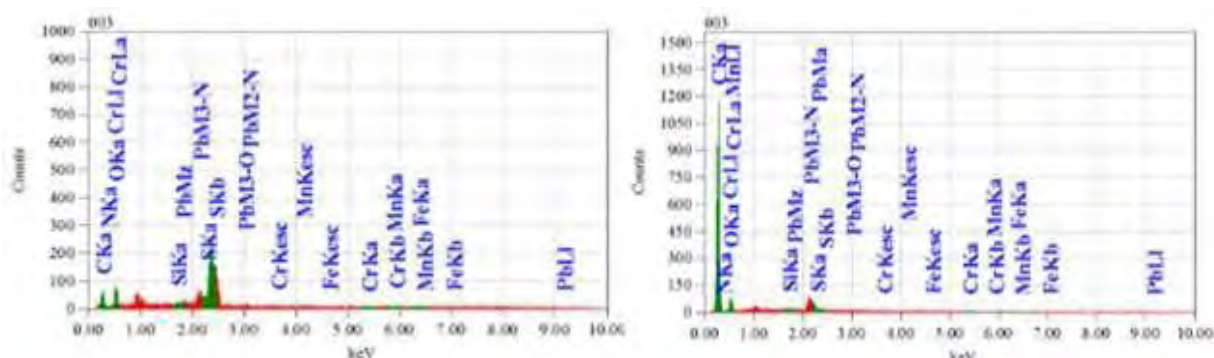
Elements a	Amount in percentage (%)
Carbon (C)	13.08%
Nitrogen (N)	1.15%
Oxygen (O)	9%
Chromium (Mn)	0.17%

| Elements b	Amount in percentage (%)
Carbon (C)	78%
Nitrogen (N)	8%
Oxygen (O)	10%
Chromium (Mn)	2.03%

Figure 3.10 EDX spectra of (a) FA-Cr & (b) BFA-Cr

3.3.7 Surface study of Zn-complex by SEM :

The complex was formed between Zn-salt with FA and Zn-salt with BFA. Before complexation the surface of BFA and FA was like scales. But after complex formation, the surface structure of FA and BFA is change. It is observed that aggregation is taken place on the surface of FA and BFA. BFA surface gets more aggregation than FA. So it proves that complex formation capability   of BFA is more than FA with Zn atom.

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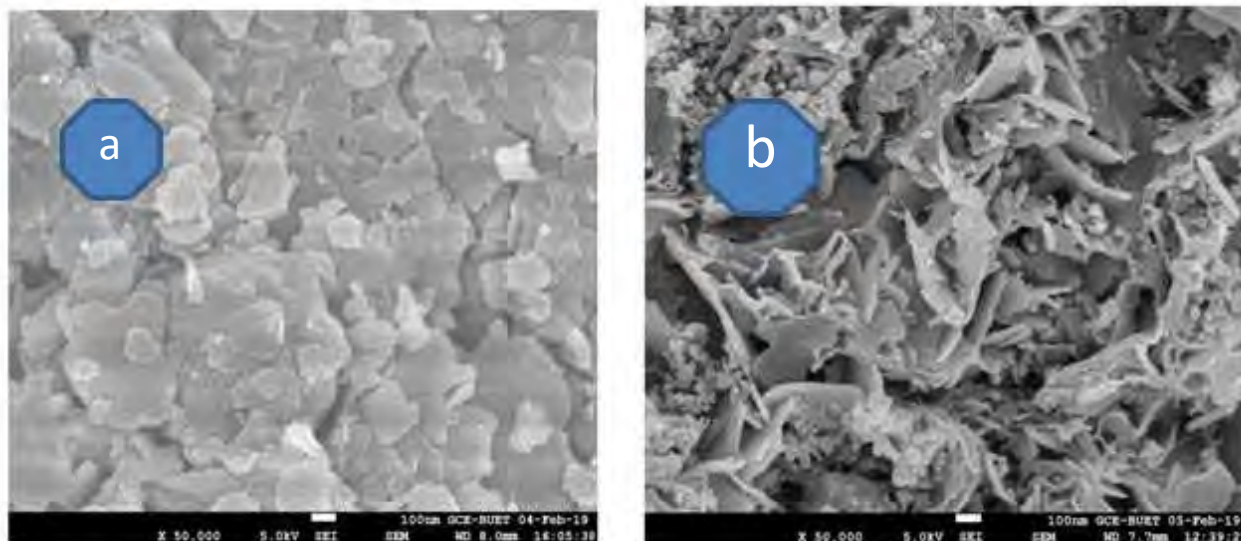
Figure 3.11 : SEM spectra of (a) FA-Zn & (b) BFA-Zn (Resolution: ×50,000)

3.3.8 Functional group analysis of Zn-complex using Fourier Transform Infrared spectroscopy (FTIR)

FTIR measurement were carried out in order to confirm the presence of functional group in Zn-FA & Zn-BFA. According to FTIR spectra, after complexation, one change is observed at $1734cm^{-1}$ (-COOH) of FA and BFA. For Zn-FA complex $1734cm^{-1}$ peak is gone. For Zn-BFA complex $1734cm^{-1}$ peak is shifted to $1749cm^{-1}$. It proves that bonds are formed at oxygen containing groups.
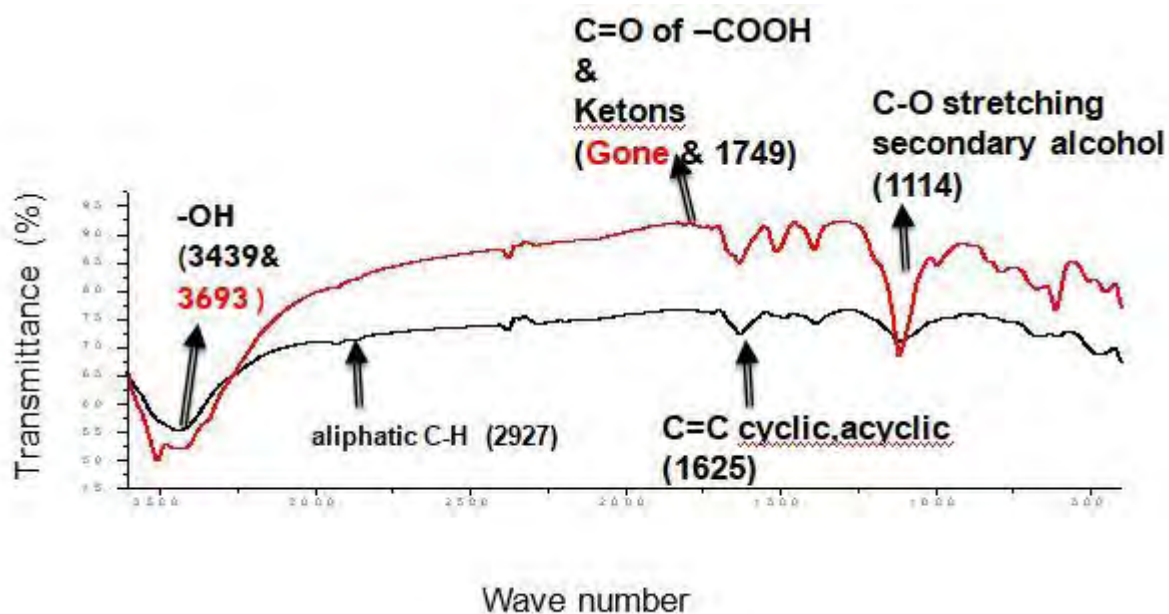
Figure 3.12 Zn-FA & Zn-BFA spectra of FTIR

Table 3.4 Characteristic peak and interpretations correspond to FA-Zn

Zn-FA(cm^{-1})	Zn-BFA(cm^{-1})	Functional Group
3433	3444	H-bonded OH, Free OH, Intermolecular bonded OH
2924	2923	Aliphatic C-H, C-H2, C-H3, stretching
Gone	1749	C=O stretching of carboxylic acids, cyclic and acyclic aldehydes and ketons, quinines
1625	1625	C=O stretching vibration of double bonds of cyclic and acyclic compounds, benzene ring substitution
1111	1113	C-O stretching, secondary alcohol

3.3.9 Energy dispersive X-ray analysis of Zn-Complex (EDX) :

After complexation, it observed that 30% Zn is absorbed by BFA and on the other hand 7% Zn is absorbed by FA. So binding capability of BFA is higher than FA with chromium.
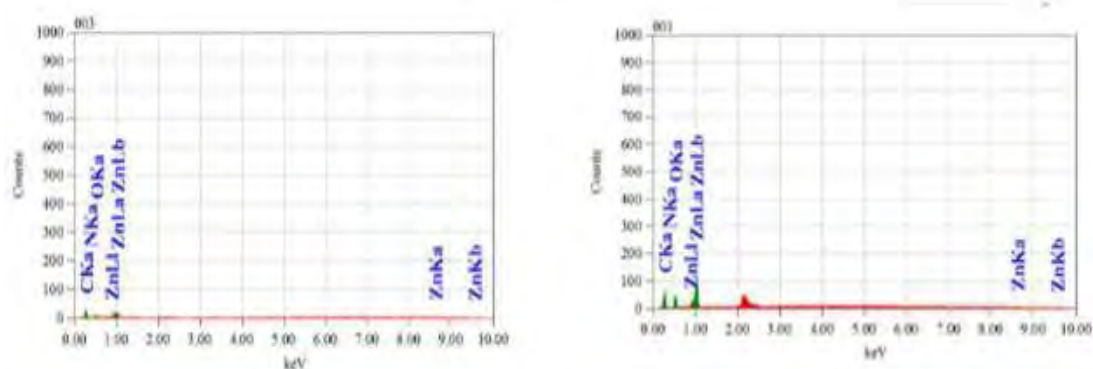

Elements (a)	Amount in percentage (%)
Carbon (C)	53%
Nitrogen (N)	14%
Oxygen (O)	24%
Zinc (Zn)	7%

Elements (B)	Amount in percentage (%)
Carbon (C)	45%
Nitrogen (N)	1.9%
Oxygen (O)	22%
Zinc(Zn)	30%

Figure 3.13 EDX spectra of (a) FA-Zn & (b) BFA-Zn

3.3.10 Surface study of Pb-complex by SEM :

The complex was formed between Zn-salt with FA and Zn-salt with BFA. Before complexation the surface of BFA and FA was like scales.

But after complex formation, the surface structure of FA and BFA is change. It is observed that aggregation is taken place on the surface of FA and BFA. FA surface gets more aggregation than BFA. So it proves that complex formation capability of FA is more than BFA with Pb atom.
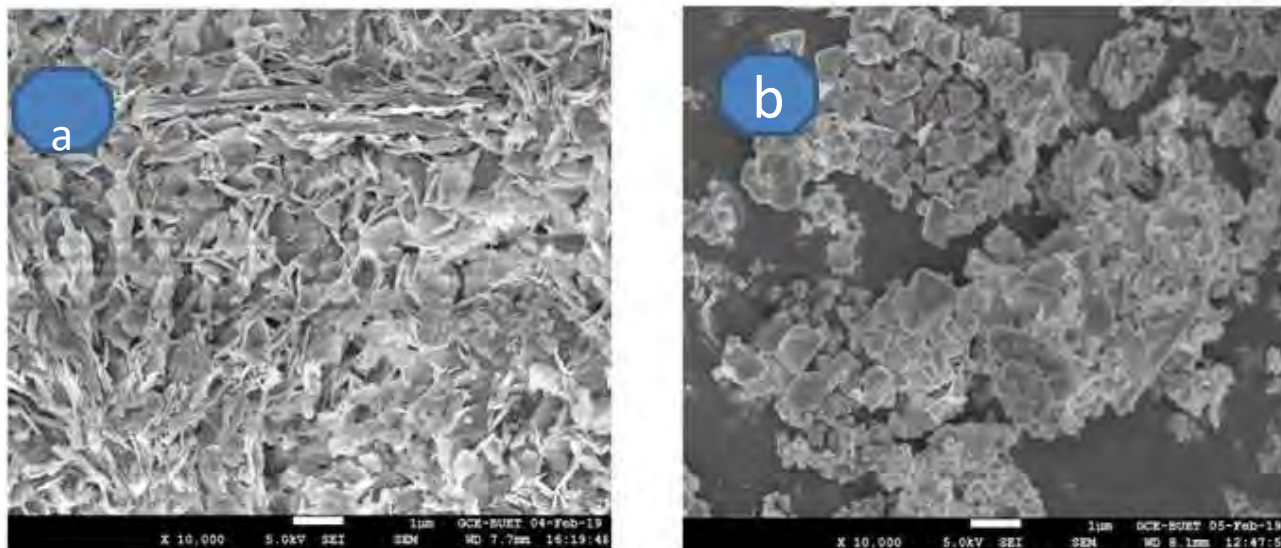
**Figure 3.14 SEM spectra of (a) FA-Pb & (b) BFA-Pb
(Resolution: ×10,000)**

3.3.11 Functional group analysis of Pb-complex using Fourier Transform Infrared spectroscopy (FTIR) :

FTIR measurement were carried out in order to confirm the presence of functional group in Pb-FA & Pb-BFA. According to FTIR spectra, after complexation, two change are observed at $1734cm^{-1}$ (-COOH) and $1111cm^{-1}$ (-C-O) of FA and BFA. For Pb-FA complex $1734cm^{-1}$ and $1112cm^{-1}$ peak are shifted into $1735cm^{-1}$ and $1111cm^{-1}$ For Pb-BFA complex $1734cm^{-1}$ and $1111cm^{-1}$ peak are shifted to $1745cm^{-1}$ and $1097cm^{-1}$.

It proves that bonds are formed at oxygen containing groups.
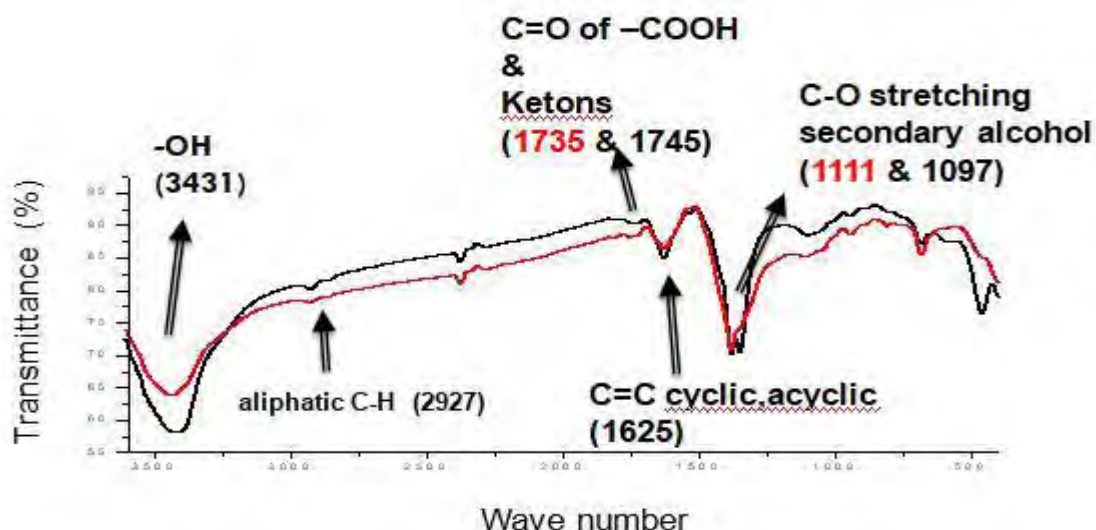
Figure 3.15 Pb-FA & Pb-BFA spectra of FTIR

Table 3.5 : Characteristic peak and interpretations correspond to FA-Pb and BFA-Pb compounds :

Pb-FA(cm^{-1})	Pb-BFA(cm^{-1})	Functional Group
3433	3444	H-bonded OH, Free -OH, Intermolecular bonded OH
2924	2923	Aliphatic C-H, C-H2, C-H3, stretching
1735	1745	C=O stretching of carboxylic acids, cyclic and acyclic aldehydes and ketons, quinines
1625	1625	C=O stretching vibration of double bonds of cyclic and acyclic compounds, benzene ring substitution
1111	1097	C-O stretching, secondary alcohol

3.3.12 Energy dispersive X-ray analysis of Pb-Complex(EDX) :

After complexation, it observed that 24% Mn is absorbed by BFA and on the other hand 22% Pb is absorbed by FA. So binding capability of BFA is higher than FA with lead.

Elements a	Amount in percentage (%)
Carbon (C)	9%
Nitrogen (N)	1%
Oxygen (O)	67%
Lead(Pb)	22%

Elements b	Amount in percentage (%)
Carbon (C)	60%
Nitrogen (N)	6.4%
Oxygen (O)	8.59%
Lead(Pb)	24%

Figure 3.16 EDX spectra of (a) FA-Pb & (b) BFA-Pb

3.4 Conclusion :

The complexation property is an important property of fulvic acid. By using this property, fulvic acid can show mineral transporter, super charge electrolyte and detoxification characteristics. So fulvic acid is an important compound for living organism. Without fulvic acid, fulvic acid, plants will not get proper nutrients.

But if fulvic acid adsorbs toxic metals from polluted environment, then it can carry it into living organism and can create toxicity into living living organism. So it can change the chain system of environment.

Fulvic acids are more capable to bind with manganese (Mn), lead (Pb), Zinc (Zn) but they can hardly bind with chromium (Cr). They have oxygen containing groups, for this they can easily bind with heavy metals.

When fulvic acid adsorbs heavy metals, this thing will improve or activate binding sites of fulvic acid.

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