

**EXTRACTION OF HUMIC SUBSTANCES FROM TANNERY
EFFLUENT TO STUDY THEIR COMPLEXATION WITH
CHROMIUM AND MANGANESE**

BY

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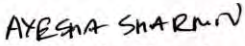
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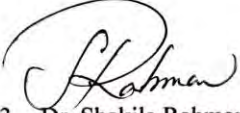
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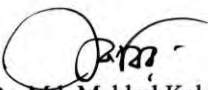
The thesis entitled "Extraction of Humic Substances from Tannery Effluent to Study Their Complexation with Chromium and Manganese" submitted by MD. Mainul Huda, Roll No.:0417032705, Session: April 2017, has been accepted as satisfactory in Partial fulfillment of the requirement for the degree of Master of Science on. May 28, 2019

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MD.Mainul Huda

I DEDICATE THIS THESIS TO.....

MY BELOVED MOTHER

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List of Abbreviations of Technical Symbols and Terms

1. Humic Acid (HA)
2. Tannery Humic Acid (THA)
3. Pure Humic Acid (PHA)
4. Chromium (Cr)
5. Manganese (Mn)
6. Tannery humic acid - chromium (THA-Cr)
7. Tannery humic acid - manganese (THA-Mn)
8. Purehumic acid - chromium (THA-Cr)
9. Tannery humic acid - manganese (THA-Mn)

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Abstract

Tannery effluent contains large amount of toxic chemical compounds. Effluents were collected from tanneries near Savar, Dhaka, Bangladesh. The concentration of Cr and Mn was traced from the point source towards the mixing zone in the nearby river. Flame-AAS and ICP-OES analysis showed concentrations of Cr ranged from 4213.908 mg/L to 0.015 mg/L and Mn: 1.723 mg/L to 0.010 mg/L in four different points towards the river direction. The attenuation of the discharged manganese and chromium was studied through complexation with the humic substances present in the effluent. The extracted Humic substances from the effluent were characterized with Fourier Transform Infrared spectroscopy (FTIR), Energy dispersive (EDX), proton magnetic nuclear resonance ($^1\text{H-NMR}$), GC-MS and CHN analyzer. A comparison of Humic-metal complexation was done between Humic substance extracted from uncontaminated swamp soil and tannery effluent. These complexes were characterized by FTIR and SEM-EDX. FTIR spectra indicated that Cr and Mn interacted with Humic substances primarily through oxygen containing functional groups. Metal percentage calculated from EDX data showed humic acids obtained from both the sources has higher affinity for Mn than Cr. Humic acid from tannery showed higher metal content compared to swamp humic acid. Present study suggests that the unique composition of humic substances from different natural set-ups can affect their complexation capacity for different metals.

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Chapter 1

Introduction

1.1 General Remarks

Humic substances are a series of relatively high- molecular- weight, light-brown- to black-colored, complex and heterogeneous organic polymers [1]. These are humic acids (HA), which comprise the alkali-soluble but acid-insoluble fraction; fulvic acids (FA), the fraction soluble in both alkali and acid; and the humin fraction, which cannot be extract by either dilute base or acid [2]. Chemically, the three fractions are similar, but they differ in molecular weight, ultimate elemental analyses [i.e., carbon (C), hydrogen (H), oxygen (O), nitrogen (N) contents], and functional group content [3] . Therefore humic substances are likely to affect the environmental fate of the pollutants even their potential for use in human health is being explored. Their molecular structure provide numerous benefits to crop production. They help break up clay and compacted soils, enhance water retention and slow down water evaporation from soils, increase seed germination rates and penetration [6]. HA and FA does not possess a clearly defined and consistent chemical structure. Humic acids and FAs from different sources and from the same source can also vary considerably in structure (e.g., degree of aromaticity / aliphaticity). Indeed, HAs produced from sequential extractions from the same source have been shown to have significant chemical and structural differences [3, 4]. Humic acids and FAs are extracted in large quantities from humates, which include a variety of naturally occurring organic lithologies with high HS content. On the background, an objective study was planned to characterize extract humic acid from tannery effluent. They are environmentally important because they are known to solubilize and transport organic and inorganic pollutants [5, 6]. The present work provides a detailed characteristic information about the humic acids.

1.2 Environmental impacts of the Tanning Industry

Leather industry plays an important role in Bangladesh Economy due to its large potential for employment, growth and export. At the same time, it poses serious environmental threats by discharging liquid effluents and solid wastes directly into surrounding low lying areas without proper treatment. Industrial wastes are major sources of pollution in all environments which require onsite treatment before discharge into sewage system [12].

In Bangladesh, there is a progressive increase in industrial wastes and due to rapid industrialization such waste products have been causing severe contamination to the air, water and soils thus pollutes the environment [10]. Around 20,000 peoples are presently living in the slums in the study area, under extremely densely populated and unhygienic conditions [13]. The quality of water deteriorates significantly after the discharge of industrial effluents into the river [11]. Chrome tanning is the most common type of tanning where maximum amount of chromium is used [17]. Tannery pollution is one of the most serious environmental problem. The tannery effluent, if not treated properly, can cause serious threat to soil and water bodies. The major public concern over tanneries has traditionally been about odours and water pollution from untreated discharges. Major pollutants combined with the tanning industry contain chloride, tannins, chromium sulphate, trace organic chemicals and finishing agents. These chemical substances are more toxic chemical and persistent and affect both human health and the environment [16]. Tanning industry wastes attitude serious environmental influence on water (with its high oxygen demand, discoloration and toxic chemical constituents) [11, 13]. Dissolved organic matter, especially proteins consumes away a large amount of dissolved oxygen into their chemical matrix during their microbial breakdown. Thus, the amount of dissolved oxygen in water is lowered and the water then becomes incapable of supporting fish or other aquatic life, which need dissolved oxygen for their survival. The discharged chemicals present in the effluent cause changes in ph, the acidity or basicity of the effluent and also influence the saturation of dissolved oxygen termed Chemical Oxygen Demand or COD. A single tannery can cause pollution of groundwater around a radius of 7-8 km. The high amount of salt contained in the effluents can increase soil salinity, reduce fertility and damage farming in large areas. Tannery pollutant water make various skin diseases.



Figure 1.1 Discharge of Effluent Directly into the River

1.3 Toxic metals as pollutants

Heavy metal pollution in the soil has become a serious issue due to a number of human activities, such as those related to the mining, mineral, smelting, and tannery industries [40]. Heavy metals are dangerous because they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment [41]. Heavy metals can enter a water supply by industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater. Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs. Long-term exposure may result in slowly progressing physical, muscular, and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis [42].

1.3.1 Toxic metal Chromium as pollutant

Chromium (Cr) is the 17th most abundant element in the Earth's mantle [14, 16]. Cr is widely used in industry as plating, alloying, tanning of animal hides, inhibition of water corrosion, textile dyes and mordants, pigments, ceramic glazes, refractory bricks, and pressure-treated lumber [16]. Due to this wide anthropogenic use of Cr, the consequent environmental contamination increased and has become an increasing concern in the last years [18, 21]. Chromium responsible for the toxic effects in humans it causes allergenicity and carcinogenicity in humans and animals also [20]. Chromium has three oxidative states Cr (0), Cr (III) and Cr (VI). Cr (0) is metallic form and two stable oxidation states, the trivalent Cr (III) and the hexavalent Cr (VI). Cr (VI) is higher toxic at lower concentration than Cr (III). Cr (VI) tend to stable complex form in soil. [19, 20]. Hexavalent chromium is mainly responsible for all carcinogenic activity in comparison to trivalent chromium [15]. With all the toxic effects there are clear evidence of asthmatic responses sometimes with respiratory exposures. It is responsible for dermatitis allergy; perforation in nasal septum and some cases of lung cancer is also evident. Due to the exposure to chromium some genetic alteration also takes place which is harmful for human health [18, 20]. It has been stated earlier that in mice excess of chromium cause patches on skin, lung cancer and liver damage [24]. But there is still no clear evidence of activity of chromium ions as an allergen to humans.

Cr affects several processes in plants namely seed germination, growth, yield and also physiological processes as photosynthesis impairment and nutrient and oxidative imbalances. Also, it has been shown that Cr is able to induce genotoxicity in several plant species [25]. Insolubility of chromic oxides in soil limited the formation of chromate and reduce environmental risk [22, 23].

1.3.2 .Toxic metal Manganese as pollutant

Mn is essential mineral for human body but excess issue lead to toxicity and harmful health effects. Excess manganese exposure changes in behavior, weakness, speech problems headaches, tremors, stiffness, balance problems and bronchitis. Hepatobiliary excretion of Mn represents a primary route of Mn clearance from the body, accounting for 80 % of Mn elimination. Thus, severe liver damage, owing to various chronic liver diseases, can result in an excessive accumulation of Mn in brain with ensuing signs and symptoms clinically called Mn hepatic encephalopathy. With weakened liver function, there is also an increased risk of neurodegeneration with continued Mn exposure [30, 34]. In those patients with chronic hepatic encephalopathy, liver transplant has proven to be effective in reducing brain Mn concentrations [32].

Manganese is an essential element for plants, intervening in several metabolic processes, mainly in photosynthesis and as an enzyme antioxidant-cofactor [27]. Nevertheless, an excess of this micronutrient is toxic for plants [26]. Mn phytotoxicity is manifested in a reduction of biomass and photosynthesis, and biochemical disorders such as oxidative stress [33]. Thus, Mn has two roles in the plant metabolic processes: as an essential micronutrient and as a toxic element when it is in excess [25, 28]. A reducing environment can be produced when there is an excess of water, poor drainage or applications of organic material [35]. Different organic molecules can dissolve solid Mn oxides through transfer of electrons, transforming them into an available Mn form for plants [36, 29]. The distribution of Mn excess in both roots and shoots is dependent on plant species and genotype. Early research associated Mn tolerance in some plants with a greater retention of Mn excess in the roots [39]. The root retention of heavy metals has been attributed to the formation of metal complexes in roots [38]. Leaf apoplast in the expression of Mn toxicity: formation of brown spots, induction of callose formation and an enhanced release of phenols and peroxidases into the apoplast [37].

1.4 Humic substances

Humic substances which make them very fertile. Humic substances are complex mixtures of decomposed organic compounds from incomplete breakdown of plant materials, animal's corpses and microorganisms [1]. Humic substances are sub-divided into three categories – fulvic acids, humic acid and humin. Humic acids are mainly a collection of hydroxyl and carboxyl groups hooked to the carbon skeleton which collectively acts as natural ligand capable of complex formation with various transition metals [2, 3]. Humic acid represent the dominant part of dissolved organic matter in freshwater supplied. Bioavailability of trace metals present in natural settings would mostly depend on the complexing capacity of these humic substances [5, 6]. Humic substances have been documented to interact in some manner with over 50 elements from the periodic table [2, 4].



Figure 1.2. Image of humic acid

Humic substances play an important role in the fate and transport of other chemical species of interest [7, 8]. Complexation of metal ions by humic and fulvic acids reduces the free metal ion concentration and hence changes their bioavailability and toxicity [9]. The nature and composition of humic substances are unique for different natural setups and this can affect their complexation capacity for different metals.

1.5 Our approach

HA has a great importance in mobilization and precipitation of certain metal ions. However, the structure and function of humic acids as metal complexing agent depends on the natural set-up from where it is obtained. In particular, the properties of the HA fraction were of interest because of almost lack of information about it from tannery effluent.

Taking into account the polyfunctionality of HA, the metal complexation with HA fractions may give information about the structure of different HA fractions and HA in general. HA may form sufficiently stable and negatively charged chelates with many different metal ions.

Therefore, the aims of the study are the following:

- a. Extraction of humic acid from tannery wastewater and swam soil.
- b. Characterization of humic acids by functional group analysis.
- c. Identification of elements present in tannery effluent.
- d. Complexation of humic acids with, Cr and Mn.
- e. Understand the nature of interaction among humic acids with Cr and Mn.
- f. The present study investigated the complexing ability of humic acids extracted from tannery waste water.

Chapter 2

Experimental

2.1 Materials and instruments

2.1.1 Chemicals and reagents

The chemicals and reagents used in this research were analytical grade and used without further purification. Deionized water was 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. Glacial acetic acid (99.8%) (Merck, Germany)
- ii. Sodium hydroxide(NaOH) (Merck, Germany)
- iii. Ethanol (99.0%) (Merck, India)
- iv. Isoamyl alcohol (99.0%) (Merck, Germany)
- iv. Humic acid(Merck, India)
- v. Hydrochloric acid (98%) (Merck, Germany)
- vi. Chromium Sulphate (Merck, India)
- vii. Manganese Sulphate (Merck, India)
- ix. Acetone (Sigma-Aldrich)
- x. Sodium Deuterioxide (NaOD) (Sigma-Aldrich)
- xi. Deuterium hydroxide (D₂O) (Sigma-Aldrich)
- xii. Dimethyl sulfoxide-d₆ (99.9%) (DMSO-d₆) (Merck, Germany)

2.1.2 Instruments

Analysis of the samples was performed using the following instruments:

- i. Fourier Transform Infrared Spectrophotometer (SHIMADZU FTIR-8400)
- ii. Scanning Electron Microscopy (JSM-7600F, Tokyo, Japan)
- iii. Energy-dispersive X-ray spectroscopy (EDS) (Philips, Expert Pro, Holland)
- iv. Nuclear magnetic resonance (NMR)
- v. Centrifuge machine (Hettich, Universal 16A)
- vi. pH meter (Hanna, HI 8424, Romania)
- vii. Digital Balance (AB 265/S/SACT METTLER, Toletto, Switzerland)
- viii. Freeze dryer (Heto FD3)
- ix. Oven (Lab Tech, LDO-030E)
- x. Atomic Absorption Spectroscopy (AAS) (Shimadzu,AA-7000,Japan)

2.2 Study area

The effluent was collected from different outlet of tannery industries namely of Bay Tanneries, Fancy Tanneries and Sathi tanneries were selected for collecting the samples tannery tank, drainage and river side at Savar near Dhaka city. The sample used in this study was collected from in 10 litre polyethylene containers and stored with preservatives prior to its use in the experiments.



Figure 2.1. Discharge of Effluent Directly into the River

2.3 Sampling

The Samples were collected in two sets: one, used for determining Chromium and manganese constituents, and another for humic acid extraction process. In order to determine trace metallic concentration of the samples, instantly after collection, 1 ml of concentrated HNO_3 (65%) was poured into each of the samples.

The samples were then mixed properly to bring the pH below 2 and minimize precipitation and adsorption on container's walls. To prevent the likelihood of hydrolysis and oxidation, samples were conserved at 4°C in a refrigerator.



Figure 2.2. Sample collection from tannery area

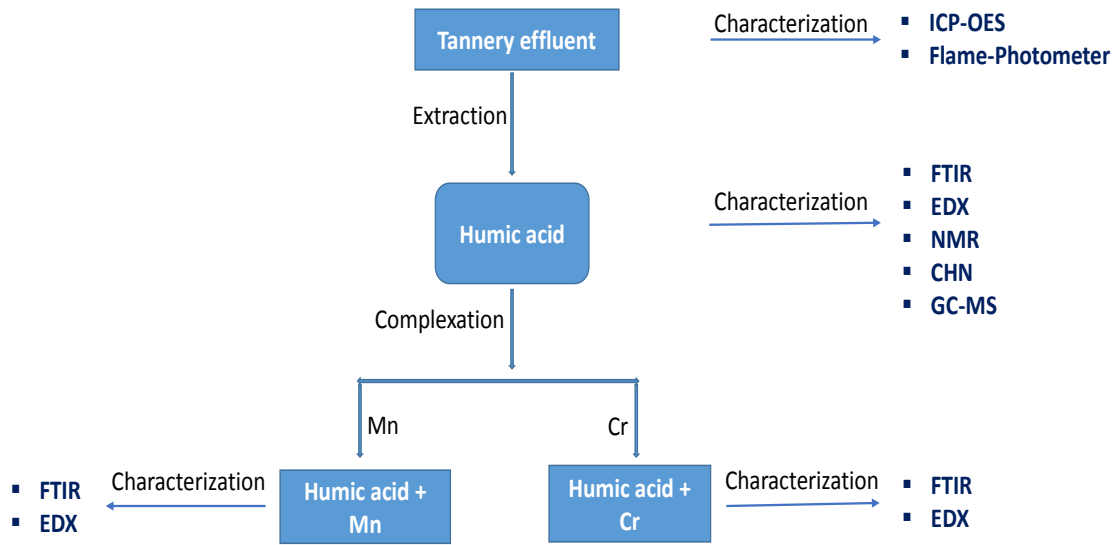
2.4 Method of preparation

2.4.1 Extraction and purification of HA from tannery wastewater

In order to extract humic acid from tannery wastewater, three steps were involved. First, 100-ml water sample were taken in a 250-ml separation funnel and 5 ml of glacial acetic acid was added. The mixture was shaken vigorously and 15 ml isoamyl alcohol was added with continuous shaking. The mixture was allowed to stand until the two layer separated and the humic acid was precipitated at the interface [43].

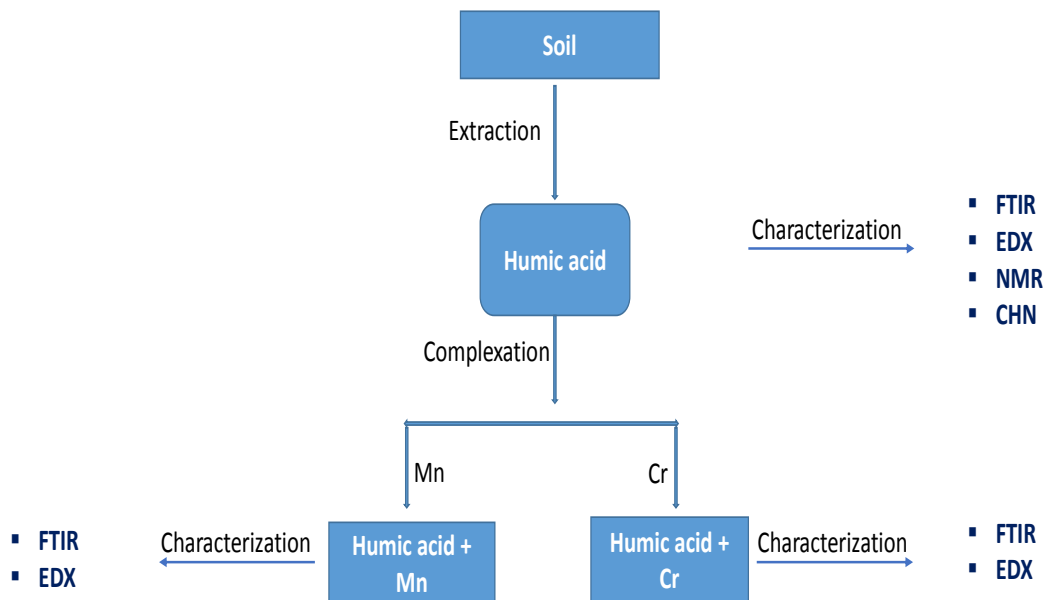
Second, the contents of the separatory funnel were filtered through a sintered-glass filter funnel under vacuum. The humic acid precipitated was collected on the sintered-glass, washed with distilled water and 95% ethanol to remove any humatomelanic acid present and air dried. General scheme for humic acid extraction from both sources are outlined below.

General Scheme



1

General Scheme



12



Figure 2.3. Schematic illustration of humic acid extract process

2.4.2 Extraction and purification of HA from soil

The sample was washed with HCl solution and NaOH solution was 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 was precipitated. The precipitated was collected and washed with distilled water to remove impurities[52].



Figure 2.4. Schematic illustration of humic acid extract process

2.4.3 Preparation of HA-Metal Ion Complex

HA-Mn Complex: Prepared by dissolving 0.998g of HA 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. $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (3.3804 g) dissolved in 20 ml of water and added slowly (drop wise) into 200 ml of the humic acid 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 HA-Mn complexes in solution from the precipitated HA-Mn complexes. The complexes were washed with distilled water to remove sulphate ion. Finally, the precipitates were dried in an oven at 95°C and stored in a desiccator under vacuum.

HA-Cr Complex: Chromium hydroxidesulphate $\text{Cr}_4(\text{SO}_4)_5(\text{OH})_2$ (14.446 g) dissolved in 20 ml of water and added slowly (drop wise) into 200 ml of the humic acid (0.998 g) solution using the above procedure.



Figure 2.5. Schematic illustration of complex process

2.5 Sample characterization

2.5.1 Flame atomic absorption spectroscopy (FAAS)

Tannery effluent were analyzed by flame atomic absorption spectroscopy (FAAS) to find the concentration of manganese and Chromium present in the supernatants. A spectrometer of Atomic Absorption Thermo Scientific model ICE 3000 Series was used and analyses were made in triplicates.

2.5.2 Fourier transform infrared (FTR) 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.

2.5.3 Energy-dispersive X-ray spectroscopy (EDS) analysis

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 ~ 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 10.0 kV. The system was computer interfaced and thus provides recording in the computer file for its use as hard copy.

2.5.4 Elemental analysis

The elemental analysis (CHN) was conducted in an elemental analyzer Leco Truspec, model CHN628, which detects carbon, hydrogen and nitrogen. Overall, 3 mg of sample were used and analyses were conducted in triplicates.

2.5.5 Nuclear magnetic resonance (NMR) analysis

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.

2.5.6 Gas chromatography-Mass spectroscopy analysis

All analyses were performed on an Agilent Technology (USA) HP 5973 mass spectroscopy (MS) system. The GC was fitted with a HP-5Ms capillary column (60m* 0.25mm id, 0.25 μ m film thickness). Helium was used as the carrier gas at a flow rate of 1.7 ml/min. The following temperature program was employed: initial temperature of 80°C for 4 min; increased at 10 °C

/ min to 150 °C, held for 1 min; then another increase at 5 °C/min to 200 °C; yet another ramp 30 °C/min to 250 °C, held for 2 min. The injector temperature was 280 °C and all injections were made in the splitless mode.

Chapter 3

Results and Discussion

3.1 Analysis of Tannery waste water

3.1.1 Element analysis in tannery waste samples by ICP-OES

ICP-OES is based on emission technique. By using ICP-OES technique we can determine minimum 10 ppb levels. The quantity of elements observed in tannery wastewater. Figure 3.1 shows the relative concentration of different elements present in discharge point of tannery wastewater obtained by ICP-OES. Table 3.1 shows the total quantity of element found in each of the tannery samples using ICP-OES.

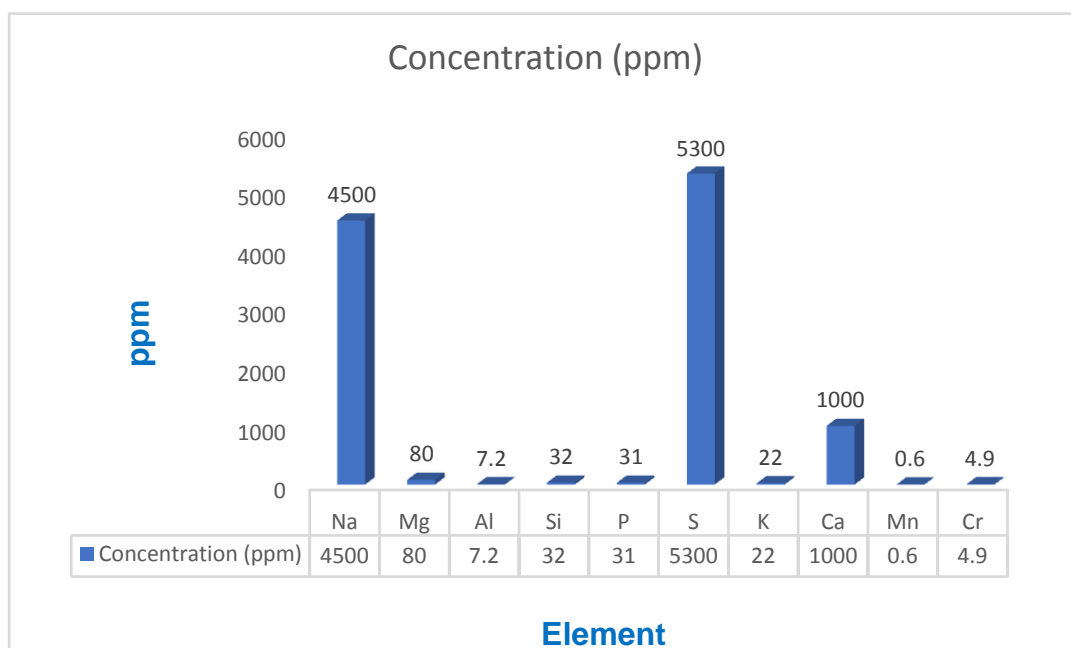


Figure 3.1 Chart of element concentration in tannery wastewater

It shows that the metals such as Na, Mn, K, Ca, Cr, and S are present in larger quantity in all samples. The highest concentration of S (5300 ppm), Na (4500 ppm) were found in Tannery sample.

Table 3.1 Concentration of elements in tannery wastewater

Element	Tannery-1 mg/L	Tannery-2 mg/L	Tannery-3 mg/L	Tannery-4 mg/L
B	1.2	0.160	0.100	0.200
Na	30	1500	4500	saturated
Mg	2.9	21	21	80
Al	7.2	1.9	1.1	1.100
Si	32	28	27	5
P	20	0.960	31	0.720
S	5300	940	3700	2400
K	22	5.1	19	9.9
Ca	150	230	17	1000
V	1.4	0.300	0.029	0.050
Cr	4.9	25	1.4	0.280
Mn	0.6	0.160	0.096	0.020
Fe	4.0	6.0	2.0	0.490
Ni	1.9	0.074	0.110	0.085
As	8.6	0.430	0.410	0.710
In	1.7	3.9	2.7	8.6
Sb	43	0.620	0.220	0.410
I	12.0	0.540	15	0.600
Pt	2.0	0.180	0.140	0.280
Pb	2	0.320	1.6	0.410
Bi	4.4	0.290	0.210	0.410

3.1.2 Concentrations of manganese and chromium in tannery waste samples

Figure 3.2 and 3.3 shows the concentration of Cr and Mn in tannery effluent collected from different distances from the discharge point respectively. Table 3.2 gives the quantity of manganese and chromium in tannery wastewater using Flame photometer which were collected from tannery industry area. The result show that the concentration of manganese and chromium in the tannery wastewater were in the following order Cr > Mn.

One effluent samples contains extremely high concentration of chromium (4213.908 mg/L) and manganese (1.723 mg/L), due to the fact that they were collected directly from the tannery industry tank.

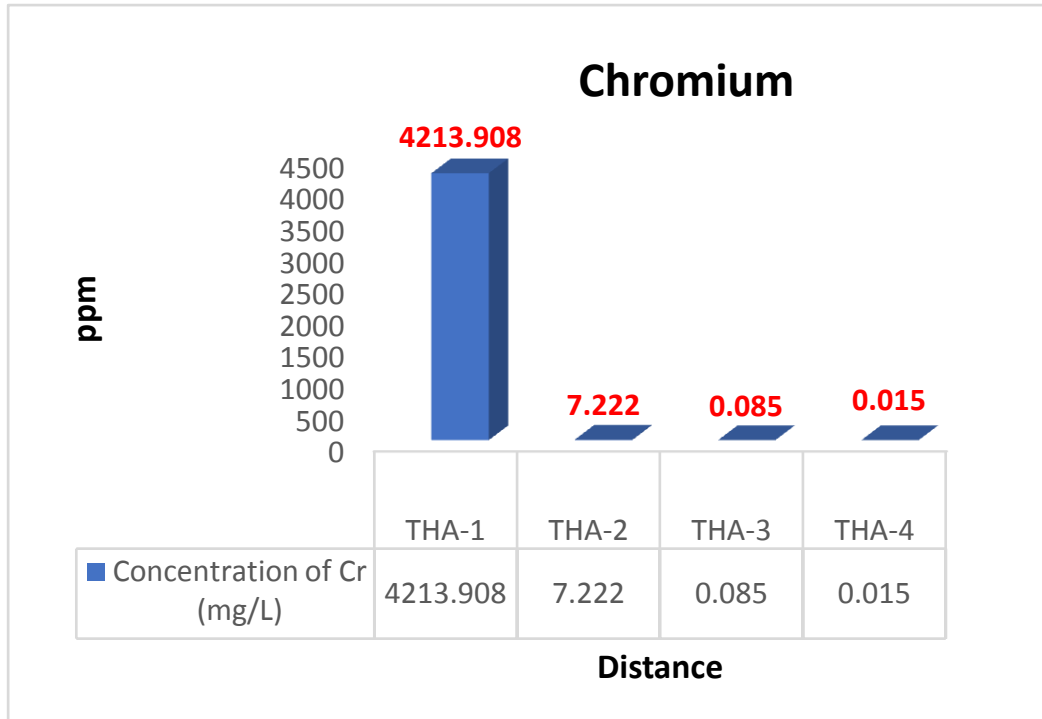


Figure 3.2 Chart of chromium concentration in tannery wastewater

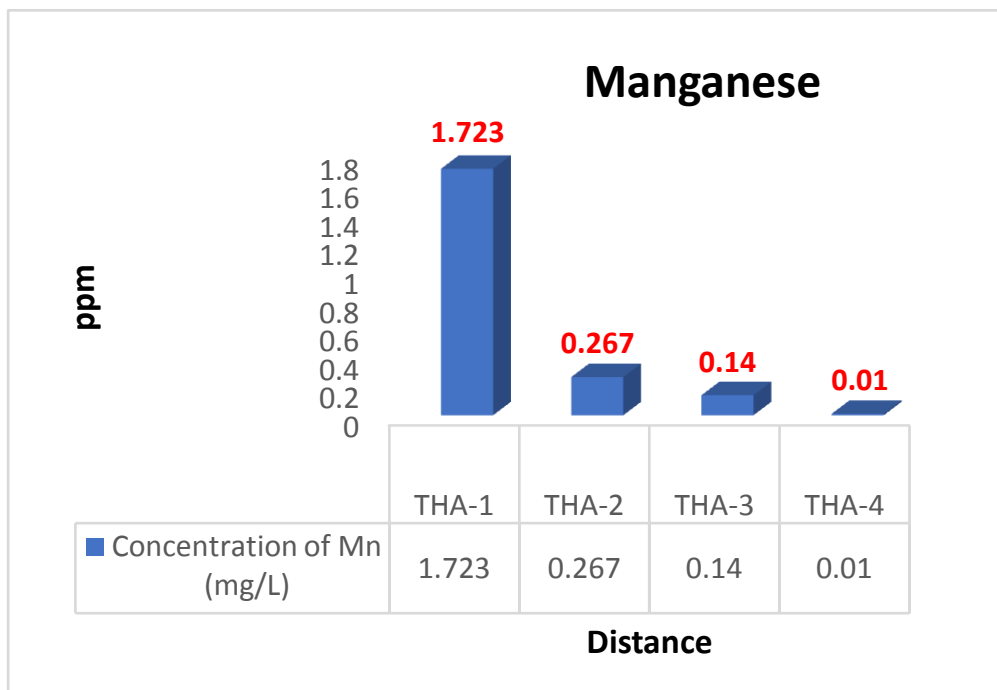


Figure 3.3 Chart of manganese concentration in tannery wastewater

The other samples were collected from different drain water of different tanneries, with their chromium concentration (7.222 mg/L, 0.085 mg/L, 0.015 mg/L) and manganese concentration (0.267 mg/L, 0.140 mg/L, 0.010 mg/L) was low compared to the first sample. This may be due to the dilution of the samples with drain water, sedimentation, coagulation and precipitation of metals in the effluent samples [44].

Table 3.2 Concentration of Cr and Mn in tannery wastewater

Sample ID	Concentration of Cr (mg/L)	Concentration of Mn (mg/L)
THA-1	4213.908	1.723
THA-2	7.222	0.267
THA-3	0.085	0.140
THA-4	0.015	0.010

3.2 Characterization of extracted humic acid

3.2.1 Functional group analysis using Fourier transform infrared (FTIR)

The IR spectra of humic acids are shown in fig. 3.4. The Infrared spectrum showed the structural composition of the humic acid. The summarized results are presented in the table 3.3. From both IR spectrum, major spectra bands are observed around at 3394 cm^{-1} (-OH stretch), 2926 cm^{-1} (-CH stretch), 1651 cm^{-1} (-C=O stretch from -COOH), 1455 cm^{-1} (C=O stretching vibration, mainly of -COOH), 1525 cm^{-1} (-C=O stretch from H-bonded conjugated ketones, -COO- asymmetric stretch, aromatic -C=C- rings), 1223 cm^{-1} (-CO stretch and -OH bending), 1033 cm^{-1} (-CO stretch in carbohydrates), 669 cm^{-1} (-C=C- aromatic disubstituted). Both spectra show the presence of aliphatic (bands $2900\text{-}2860\text{ cm}^{-1}$) and aromatic components (1600 cm^{-1}).

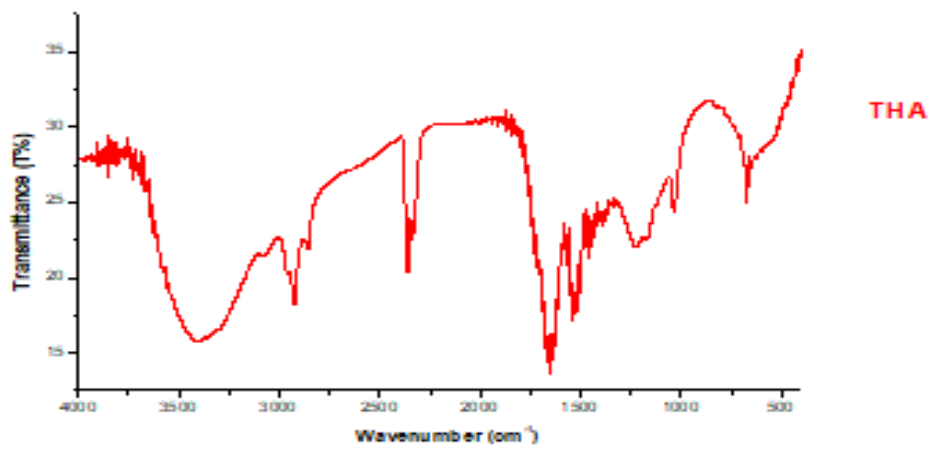


Figure 3.4 FTIR spectra of tannery humic acid

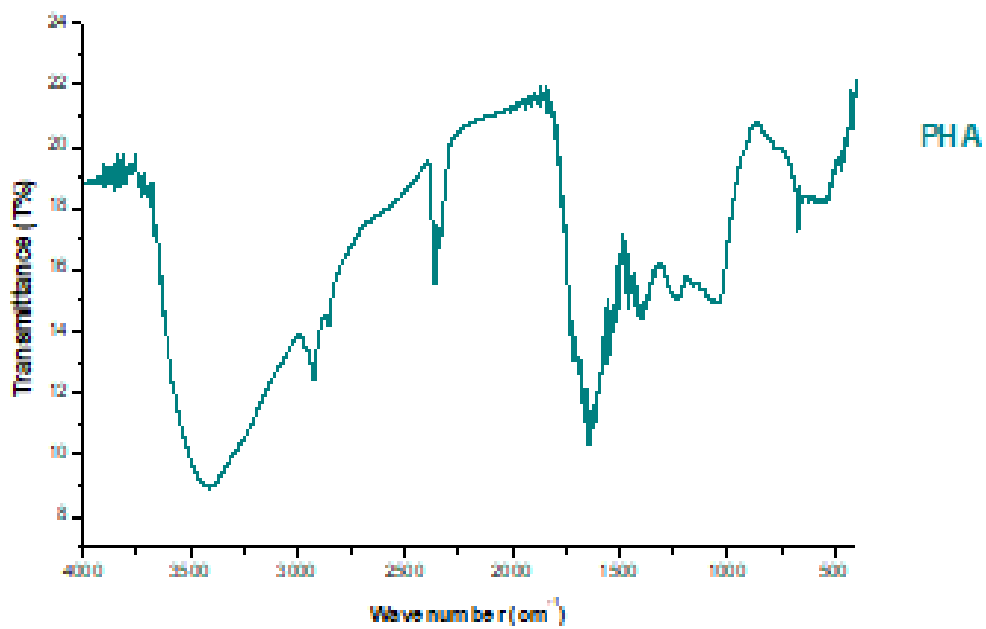


Figure 3.5 FTIR spectra of pure humic acid

The result indicated that both humic acid might contain the Phenolic hydroxyl (-OH), alcoholic (-OH), aromatic family (-C=C-), and free carboxyl group (-COOH). The IR spectra of the two humic acids are shown in Fig 3.6. The spectra indicate a number of similarities and differences in absorption peaks and their strengths. It is clear from the spectra that tannery HA is characterized by strong absorption peak around 2920 cm^{-1} (aliphatic C-H stretching), which implies the high aliphatic capacity than pure HA.

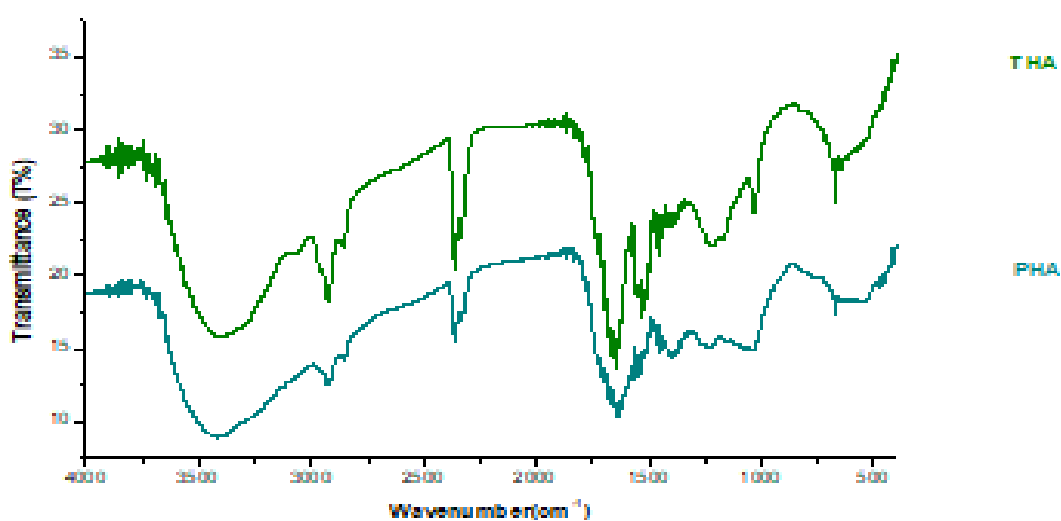


Figure 3.6 FTIR spectra of tannery humic acid and pure humic acid

The spectrum of tannery HA is also characterized by one additional peak present at around 1525 cm^{-1} (N-O stretching). On the other hand, pure HA is characterized by strong absorption around 1633.73 cm^{-1} (stretching of aromatic, C=C), which implies the high aliphatic capacity than tannery HA. The results of IR spectra indicate that tannery HA is more aliphatic than pure HA and pure HA is more aromatic than tannery HA.

Table 3.3 Summarized FTR spectra of tannery HA and pure HA in the zone 4000 cm^{-1} . 400 cm^{-1} .

Tannery HA Wave no.(cm⁻¹)	Pure HA Wave no.(cm⁻¹)	Functional Group
3394.83	3417.01	OH stretching vibration of phenolic –OH, N-H stretching (trace)
2926.11	2924.18	Asymmetric stretching vibrations of aliphatic C-H bonds in methyl and/or methylene units.
2855.71	2853.18	symmetric stretching vibrations of aliphatic C-H bonds in methyl and/or methylene units
1651.12	1633.73	COO ~ asymmetric stretching and stretching of aromatic (C=C)
1525.74		C=C stretching within the ring
1455.34	1410.01	C=O stretching vibration, mainly of -COOH
1223.87	1228.70	C-O stretching in protonated carboxylic acids
1033.88	1037.74	C-O stretching in polysaccharides
699.32	668.36	C=C-H vibrations of the aromatic ring

3.2.2 Energy dispersive x-ray (EDX) spectra analysis

The EDX spectra of extracted humic acid is present in the figure 3.7 and 3.8. The EDX spectra gives us the type and weight percent of each element present in the selected point [48] . From the figure, it is observed that extracted humic acid contain carbon, oxygen, nitrogen, sodium, respectively.

Both sample had very similar energy-dispersive X-ray spectra. However, there is no peak of chromium and a little manganese peak was observed in this spectrum.

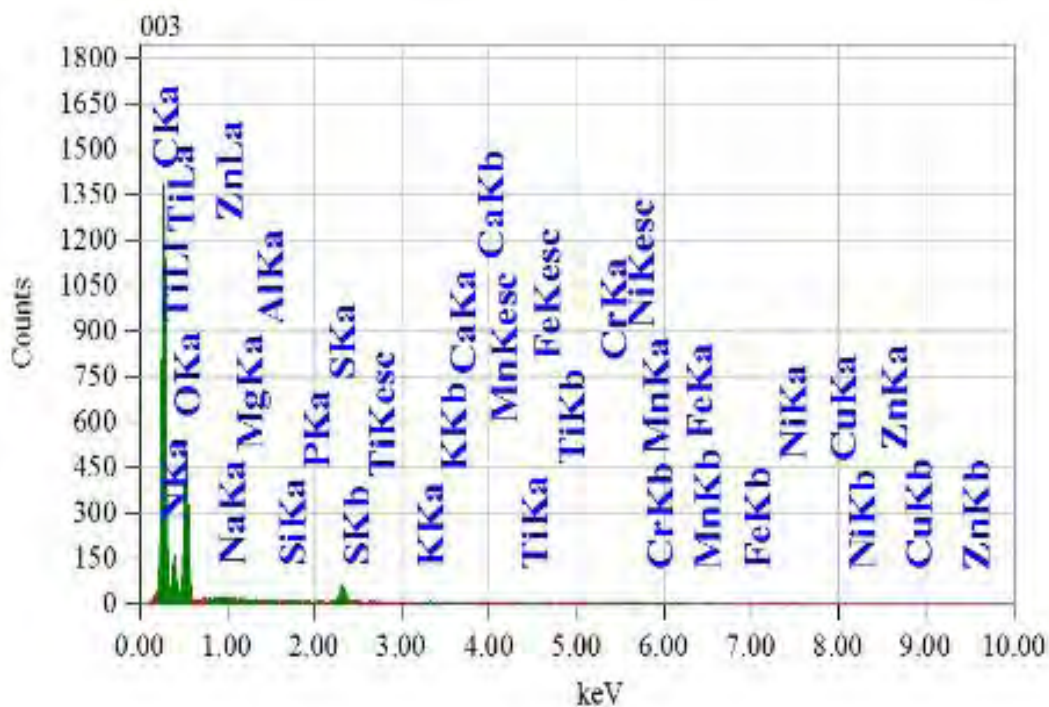


Figure 3.7 EDX spectra of tannery humic acid

Table 3.4 Normalized % mass of elements in tannery humic acid

Element	Mass%	Atom%
C	50.50	56.95
N	18.46	17.86
O	28.71	24.31
Na	0.12	0.07
Mg	0.00	0.00
Al	0.01	0.00
Si	0.03	0.02
P	0.00	0.00
S	1.40	0.59
K	0.02	0.01
Ca	0.16	0.05
Cr	0.00	0.00
Mn	0.53	0.13
Fe	0.00	0.00
Zn	0.00	0.00

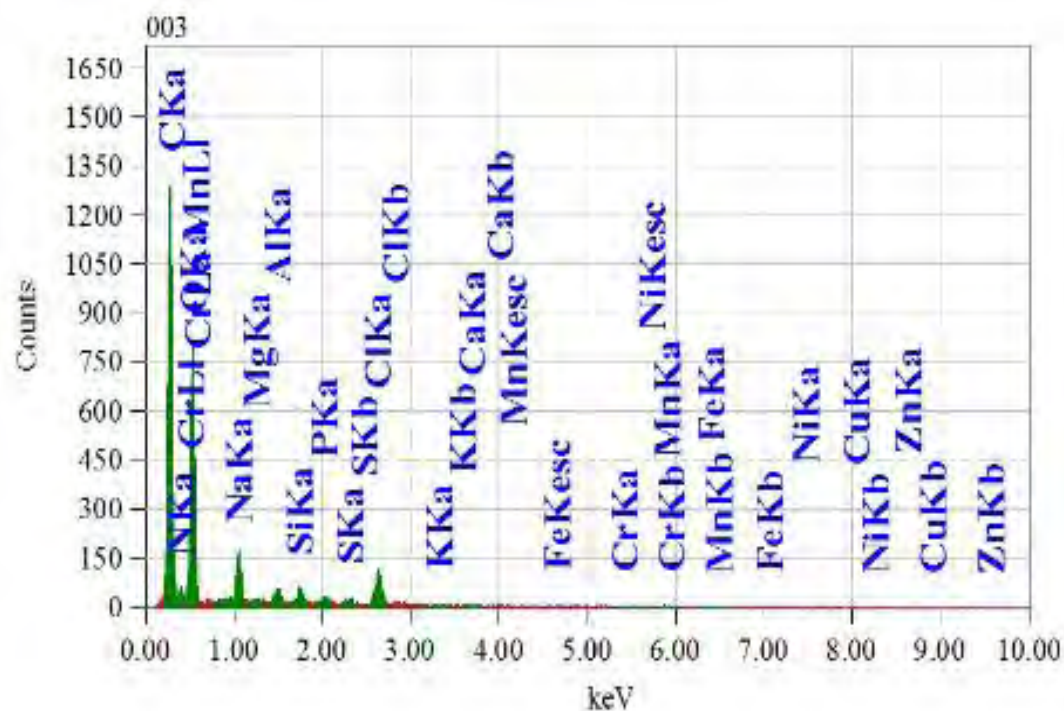


Figure 3.8EDX spectra of pure humic acid

Table 3.5 Normalized % mass of elements in pure humic acid

Element	Mass%	Atom%
C	51.04	59.22
N	8.73	8.69
O	32.92	28.68
Na	2.04	1.24
Mg	0.02	0.01
Al	0.60	0.31
Si	0.69	0.34
P	0.41	0.19
S	0.27	0.12
K	0.11	0.04
Ca	0.10	0.03
Cr	0.01	0.00
Mn	0.29	0.07
Fe	0.18	0.05
Zn	0.00	0.00

3.2.3 CHN analysis of extracted humic acid

The elemental composition of humic substances, as well as their properties, may vary according to the source of organic matter and place of formation. The elemental analysis is important since it provides an idea about the general composition of the molecule. The element composition of extracted humic acid were analyzed with CHN analyzer. The percentage of C, H, and N is presented in table. C, H, N content in tannery extracted humic acid was higher than Soil extracted humic acid. The ratio of H/C and N/C values are close to both humic acid.

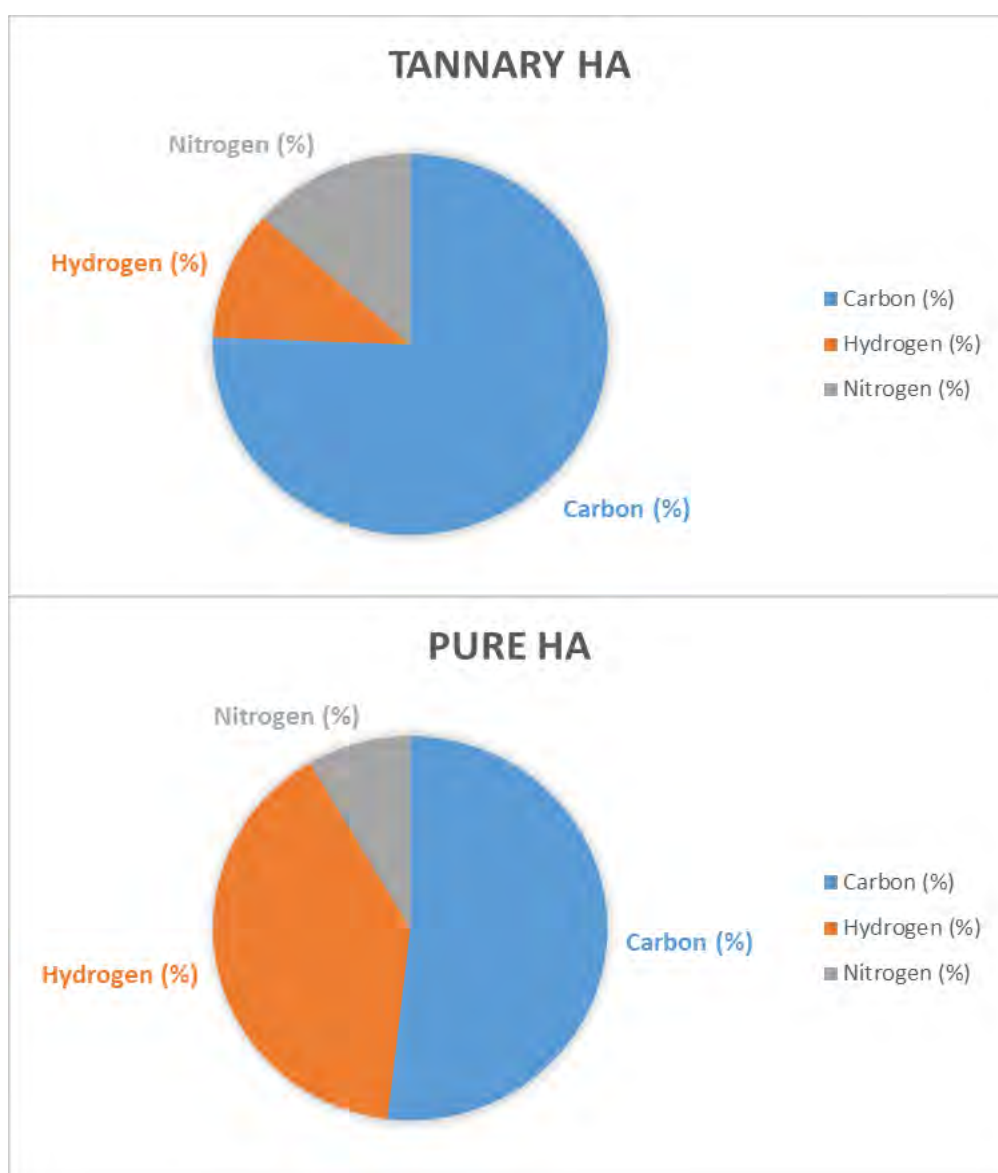


Figure 3.9 Chart of CHN composition in extract humic acid

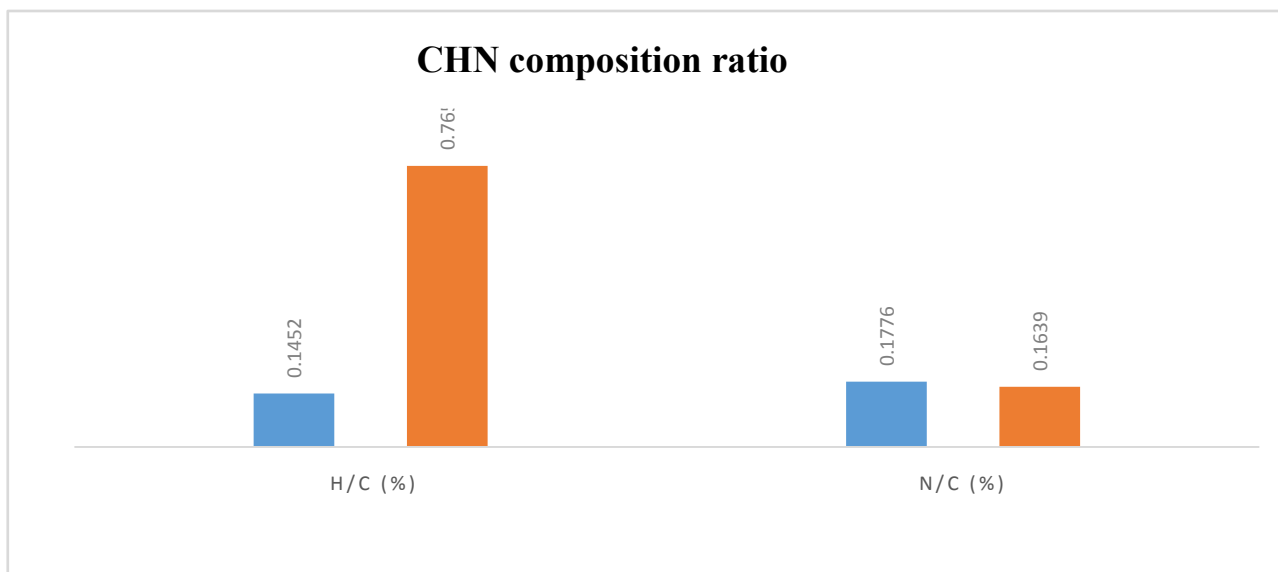


Figure 3.10 Chart of CHN composition ratio in extract humic acid

Table 3.6 CHN composition of extracted humic acid

Run	Carbon (%)	Hydrogen (%)	Nitrogen (%)	H/C (%)	N/C (%)
Tannery HA	46.29%	6.72%	8.22%	0.1452	0.1776
Pure HA	1.83%	1.40%	0.30%	0.7651	0.1639

3.2.4 Nuclear magnetic resonance (NMR) study

¹H-NMR-based analysis was used to estimate the relative contents of aromatic and aliphatic protons present in the samples. The ¹H-NMR spectra (Figure) obtained show the peaks from 0 to 2.5 ppm region were assigned to the presence of the aliphatic protons in the HA samples. The sharp peak at around 0.9 ppm was attributed to the branched methyl proton; also the presence of carboxylate in HA samples [46, 47]. The peaks between 1.1 and 2.5 ppm can be explained by the presence of methylene and methyl protons, which are attached to electronegative groups, such as carboxyl group or an aromatic ring. The peaks from 6 to 8.5 ppm were attributed to the aromatic region of the protons. The sharp peak which appears at around 2.5 and 3.32 ppm can be attributed to the traces of DMSO [49].

INARS,BCSIR,1H spectrum, TH in DMSO

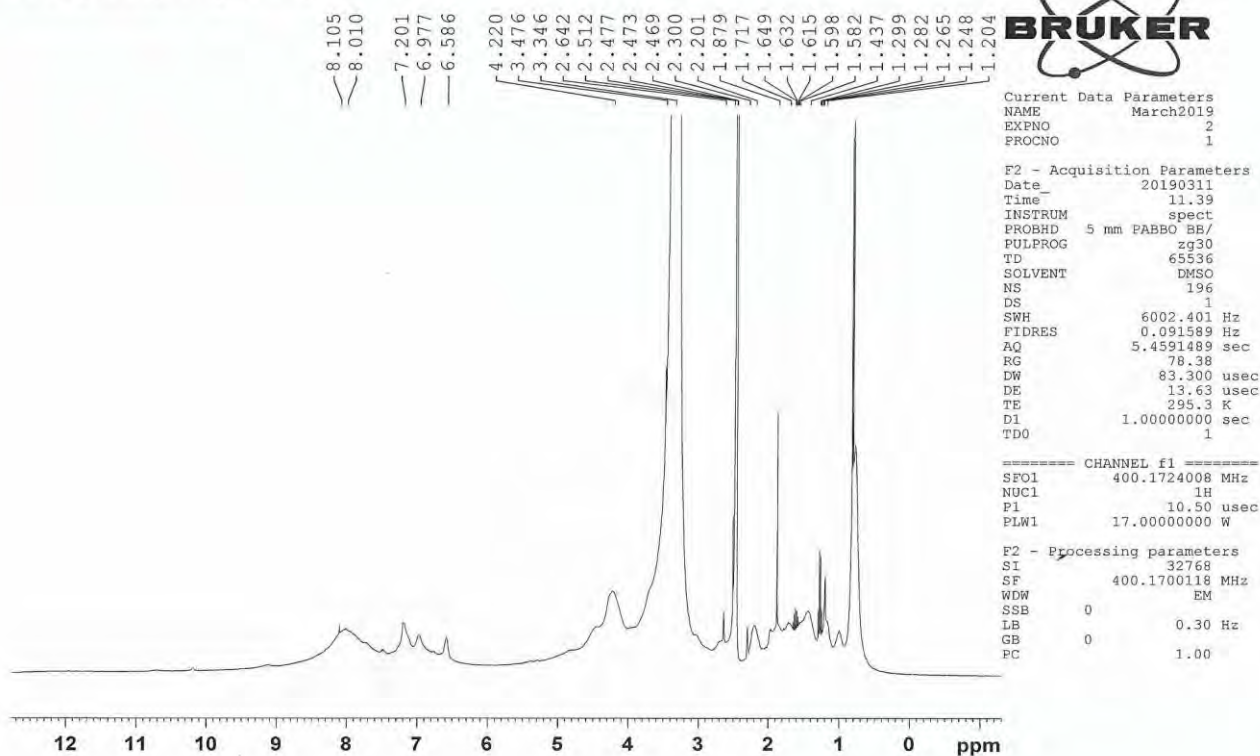


Figure 3.11 NMR spectra of tannery humic acid

INARS,BCSIR,1H spectrum, PHA in DMSO

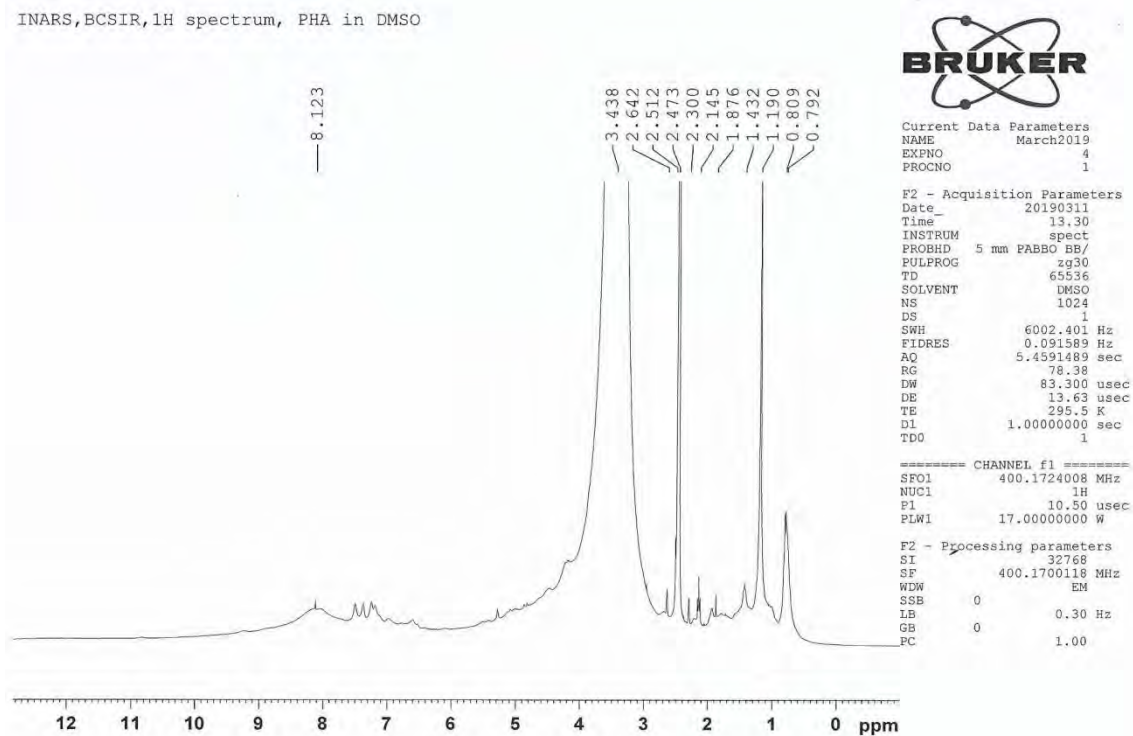
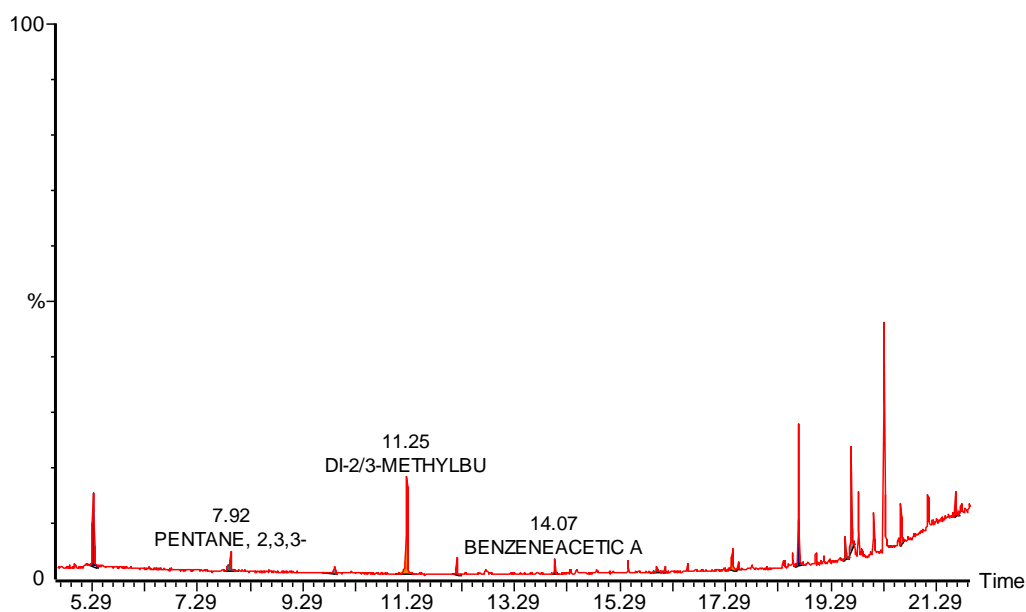


Figure 3.12 NMR spectra of pure humic acid

3.2.5 Gas chromatography – Mass spectroscopy (GC-MS) analysis study

Chemical composition was determined through GC-MS technique. One typical fragment molecule was monitored. Humic acid naturally oxidized, which gives them a net negative charge [45, 50]. It can easily bind to other organic molecules. From GC-MS result get many organic molecules are present with humic acid.

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#	Name	RT	Area	Area %
1	3-METHYL-1-BUTANOL	5.31	103408	19.490
2	2, 3, 3-TRIMETHYL-PENTANE	7.92	20994	3.960
3	DI-2/3-METHYLBUTYL MALEATE	11.25	144570	27.250
4	2-PENTADECYL-1, 3-DIOXOLANE	12.20	20369	3.840
5	BENZENEACETIC ACID	14.06	19340	3.650
6	2, 3-ANHYDRO-D-GALACTOSAN	15.99	4855	0.920
7	1, 2-BENZENEDICARBOXYLIC ACID	16.14	6338	1.190
8	PROPANOIC ACID	17.43	16028	3.020
9	3-METHYLBUTYL DODECANOATE	18.67	94937	17.900
10	1-(1, 2-DIMETHYLPROPYL)-1-METHYL-2-NONYLCYCLOPROPANE	19.67	40623	7.660
11	DOCOSANE	20.61	18262	3.440
12	ACRYLIC ACID HEXADECANYL ESTER	21.64	17087	3.220

Figure 3.13 GC-MS spectra of tannery humic acid

3.3 Characterization of extracted humic acid and metal complexation

3.3.1 FTIR spectral analyses of humic acid -metal complexes

It was reported that the main functional groups found in HA are carboxyl, phenolic hydroxyls and alcoholic hydroxyls[51]. Therefore, we will focus on the most interesting FTIR bands of these main groups which might have a crucial role in the binding of metal ions. It was suggested that HA carries negative charges in weak acidic and basic media, which in turn promote the adsorption of cations via electrostatic interactions.

3.3.1.1 FTIR spectra for HA-Mn complex

It was reported that the origin of HA substances and their pre-treatments affects the sorption capacity of metal ions. Figure 3.14 and 3.15 shows the FTIR of HA-Mn complex spectra. The major changes apparent in all spectra of humic acid- Mn complexes are the decreased intensity of the peaks around at 3394 cm^{-1} , 2926 cm^{-1} , 1651 cm^{-1} and 1223 cm^{-1} . New peaks appear in the manganese- humic acid complex spectra at 1118 cm^{-1} , 623 cm^{-1} and 507 cm^{-1} . Peaks at 1651 cm^{-1} and 1223 cm^{-1} , which are typically assigned to the C=O and C-O stretching in protonated carboxylic acids.

Complexation of this humic acid with Mn ion does not completely remove nor shift the peak usually assigned to C=O stretching as normally occur carboxylic salt formation. However, the peak at 1223 cm^{-1} disappears, which is consistent with salt formation.

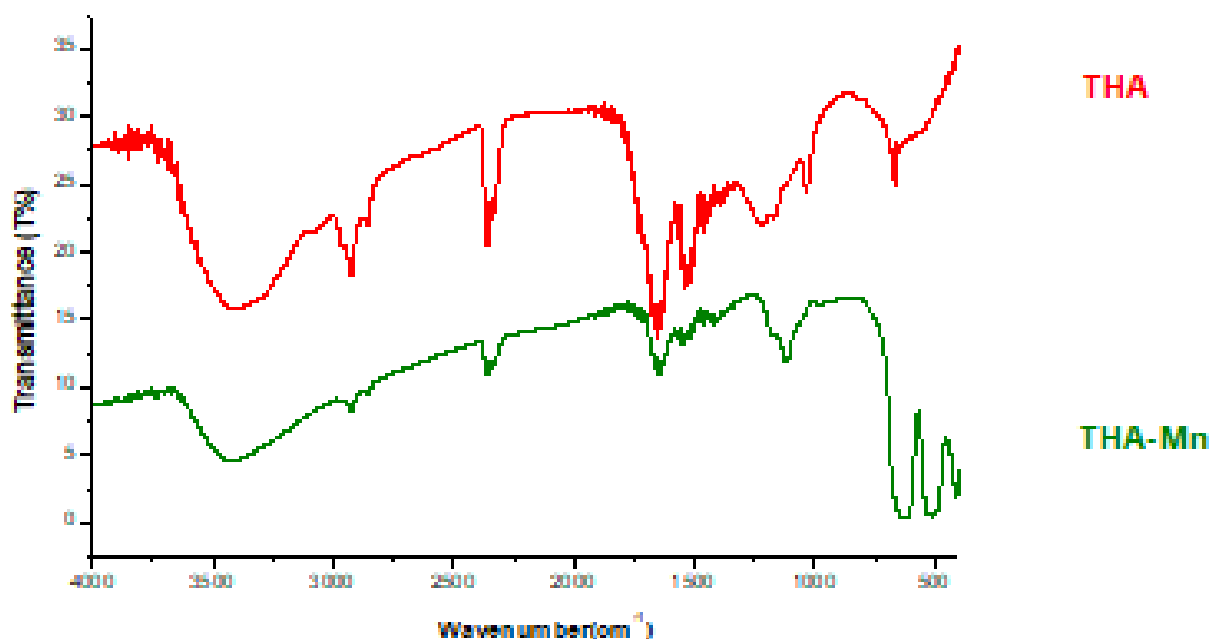


Figure 3.14 FTIR spectra of THA complex with Mn

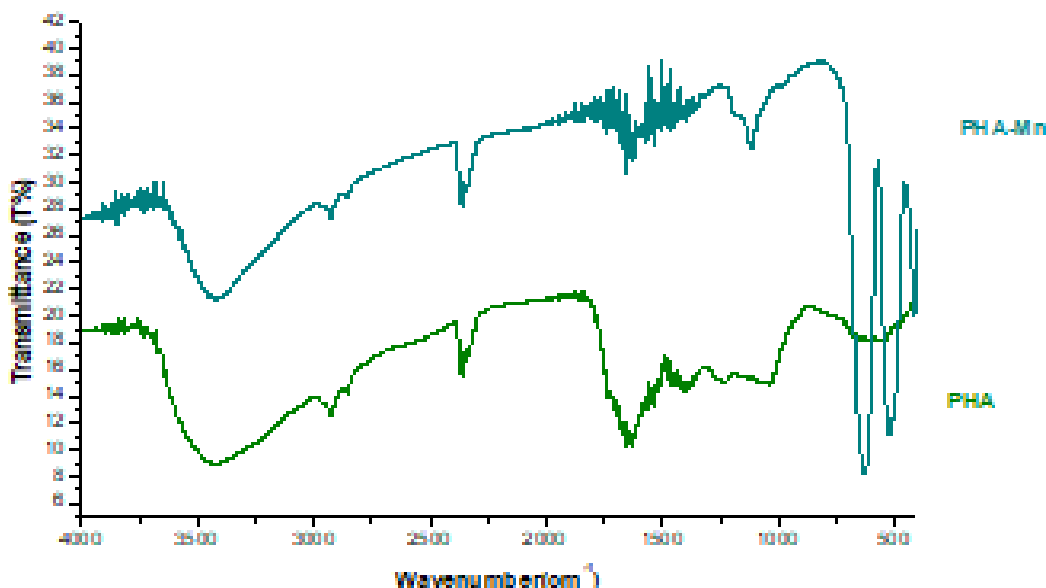


Figure 3.15 FTIR spectra of PHA complex with Mn

3.3.1.2 FTIR spectra for HA-Cr complex

Humic acid forms complexes with metals through the carboxylic acid functional group. This interaction can be concluded from the reduction of the absorption band of the COO-symmetric stretching to lower wavelength and the appearance of the COO-antisymmetric stretching. Figure 3.16 and 3.17 shows that the major changes apparent in all spectra of humic acid- Cr complexes are the decreased intensity of the peaks at 3394 cm^{-1} , 2926 cm^{-1} , 1223 cm^{-1} and 1651 cm^{-1} . New peaks appears in the chromium- humic acid complex spectra at 1118 cm^{-1} , 980 cm^{-1} , 840 cm^{-1} 617 cm^{-1} and 518 cm^{-1} . Peaks at 1651 cm^{-1} and 1223 cm^{-1} , which are typically assigned to the C=O and C-O stretching in protoned carboxylic acids.

Complexation of this humic acid with Cr ion does not completely remove nor shift the peak usually assigned to C=O stretching as normally occur carboxylic salt formation. However, the peak at 1223 cm^{-1} disappears, which is consistent with salt formation.

And the increased intensity of peaks at 1385 and 1617 cm^{-1} ~ due to deprotonation of simple carboxylic acids and asymmetric and symmetric stretching of cations coordinated to -COO-groups, respectively. These changes are very similar to those observed in the FTIR spectra when complexed with Cr. In addition to these changes, peaks appeared at 1118, 623 and 507 cm^{-1} in the spectra of two humic acid-Cr complexes when it complexed with Cr.

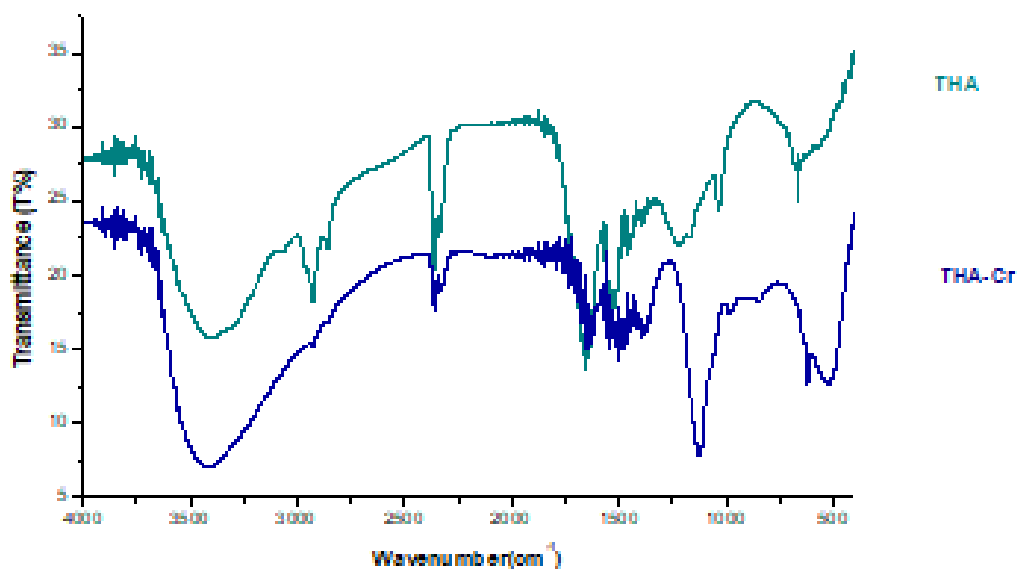


Figure 3.16 FTIR spectra of THA complex with Cr

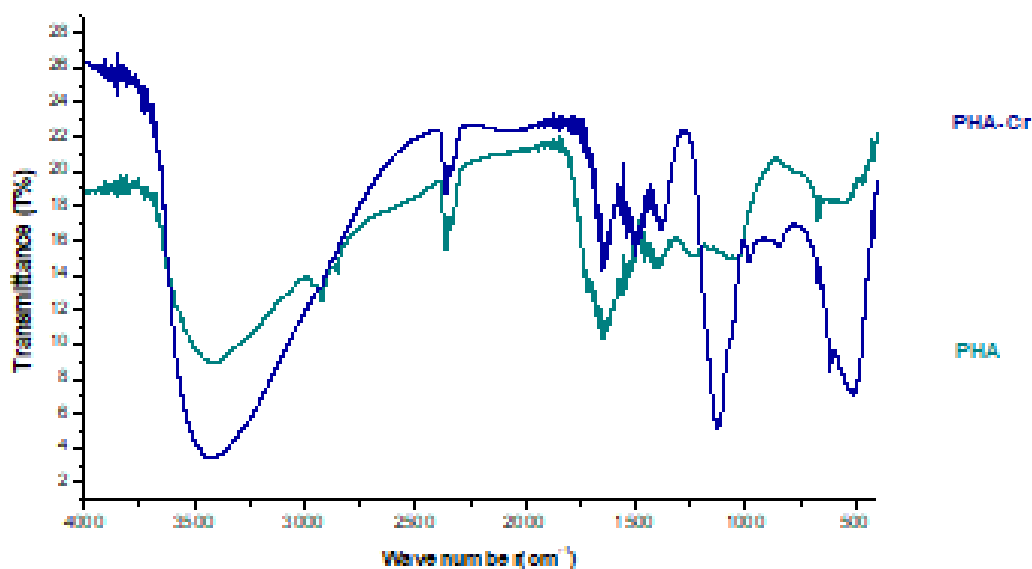


Figure 3.17 FTIR spectra of PHA complex with C

3.3.2 Energy dispersive x-ray (EDX) spectra analysis of HA- Mn complex

The EDX spectra of tannery humic acid and pure humic acid with manganese complex are shown in figures 3-18 and 3-19. Since the spectra contain clear peaks for manganese with other

constituents, this proves the formation of tannery humic acid –manganese and pure humic acid –manganese complex. The percent of each element after complexation is presented in table 3.7.

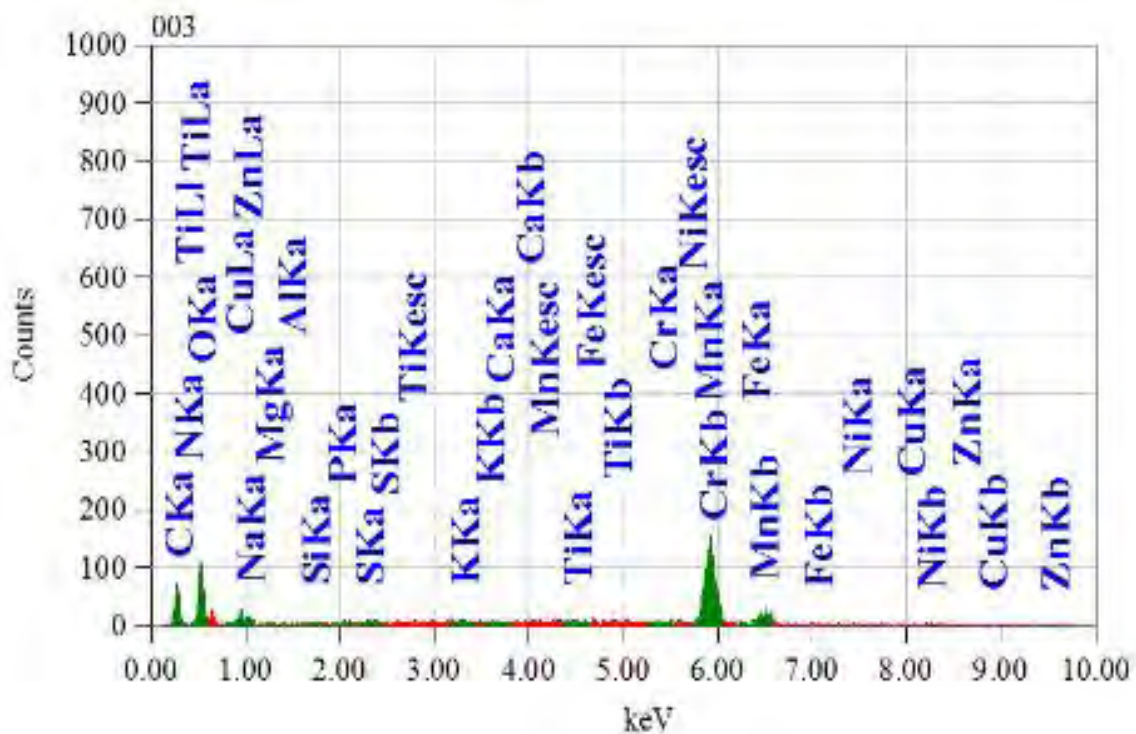


Figure 3.18 EDX spectra of tannery humic acid manganese complex

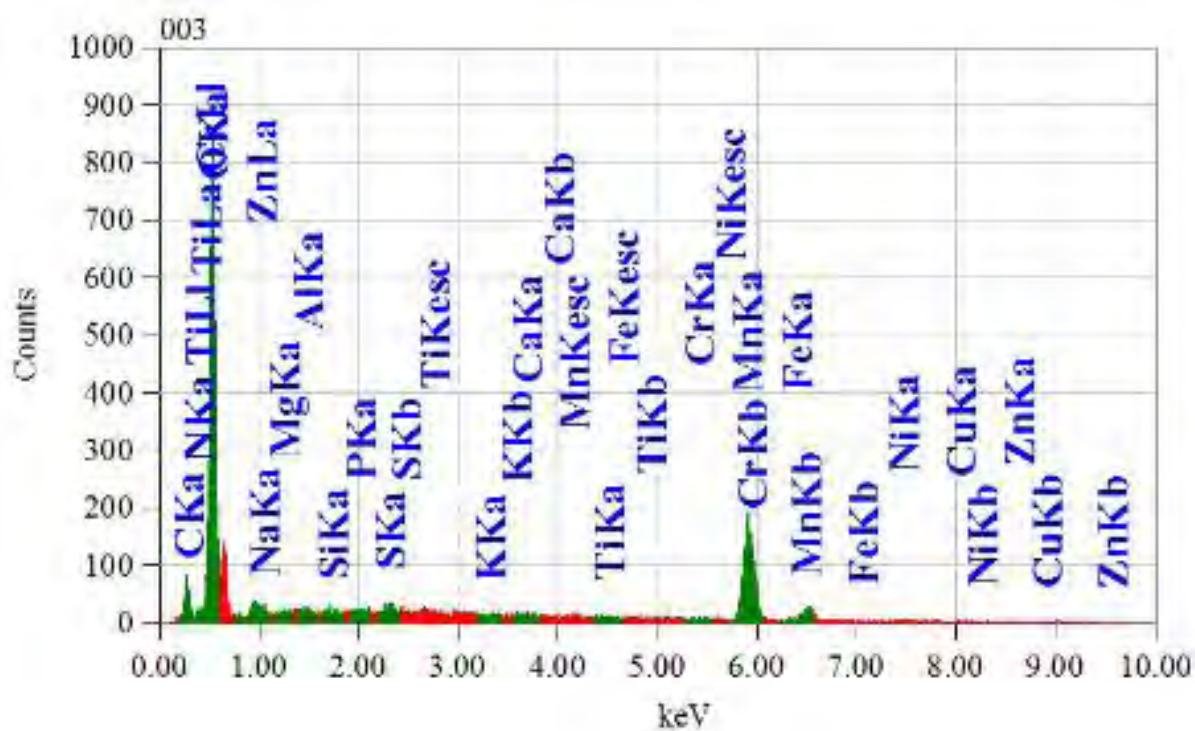


Figure 3.19 EDX spectra of pure humic acid manganese complex

Table 3.7 Normalized % mass of elements in Tannery Humic acid and its metal complexes.

Compound	C%	N%	O%	Mn%	Cr%
Tannery HA	50.50	18.46	28.71
Tannery HA+ Mn	8.25	0.23	5.44	84.04	...
Tannery HA+ Cr	4.97	0.10	53.98	30.22

Metal percentage calculated using EDX

3.3.3 Energy dispersive x-ray (EDX) spectra analysis of HA- Cr complex

Figure 3-20 and 3-21 represents the spectrum of tannery humic acid and pure humic acid complex. In this figure, the spectra of contain clear peaks for chromium with other constituents. This chromium containing spectra also proved this formation of tannery humic acid-chromium and pure humic acid - chromium complex. The percent of each element after complexation is presented in table 3.8.

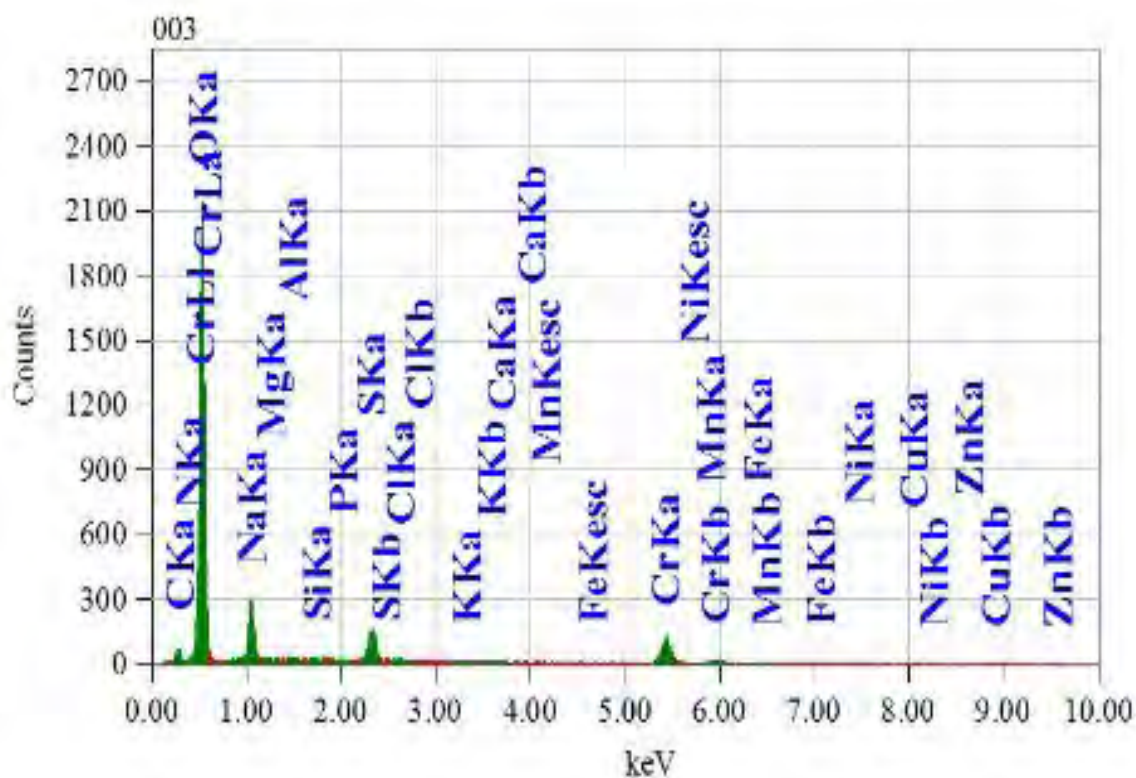


Figure 3.20 EDX spectra of tannery humic acid chromium complex

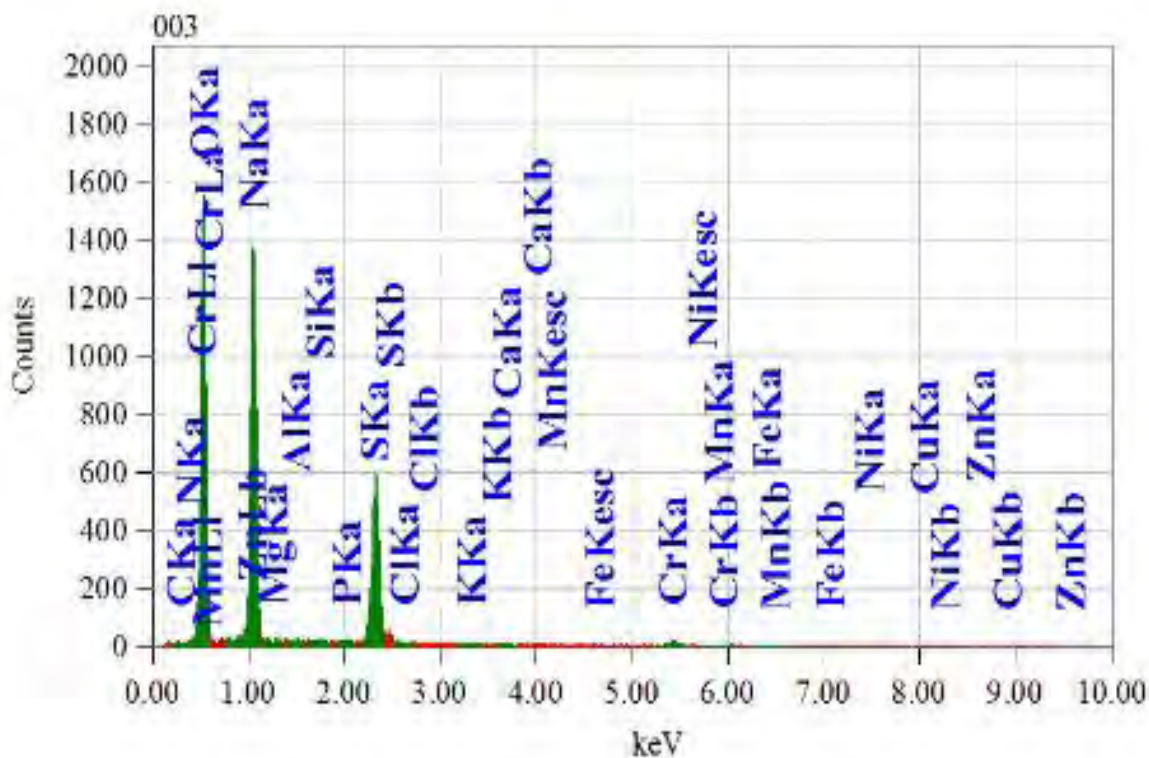


Figure 3.21 EDX spectra of pure humic acid chromium complex

Table 3.8 Normalized % mass of elements in Pure Humic acid and its metal complexes.

Compound	C%	N%	O%	Mn%	Cr%
Pure HA	51.04	8.73	32.92
Pure HA+ Mn	5.30	0.53	26.10	65.57
Pure HA+ Cr	0.80	0.00	50.30	2.55

Metal percentage calculated using EDX

3.4 Conclusion

In this work, we focused on understanding the structure and composition of Humic acids present in tannery effluent and their complexation ability towards two of the most prominent

heavy metal contaminants present. To best of our knowledge, this was the first time humic acids were extracted from any tannery waste water.

The tannery HA contains aromatic, carboxylic acid, carbonyl, alcoholic and ether like functional groups. The extracted humic acids from tannery water and swamp soil showed similar functional groups according to FTIR and NMR studies; however the elemental analysis showed slightly higher N/C ratio for tannery HA. Investigation of the complexing ability of humic acids revealed that tannery HA has better binding affinity towards Cr and Mn compared to swamp HA. This study also suggested that Mn has a higher affinity for humic acids compared to Cr. Further study needed to be done for a better understanding of competitive binding of these metal ions with tannery HA. The complexation mechanism can be utilized to remove toxic metals from tannery wastewater.

References

1. Stevenson, F. J. 1982. *Humus chemistry: Genesis, composition, reactions*. John Wiley and Sons., New York.
2. Sparks, D.L. 1996. *Environmental Soil Chemistry*, Academic Press, New York.
3. Stevenson, F.J. 1982. *Humus Chemistry*, John Wiley & Sons, New York,
4. Wilson, M. A. 1987. *Humic substances. In NMR techniques and applications in geochemistry and soil chemistry*. Pergamon Press. New York.
5. Czinkota, I., Foldenyi, R., Lengyel, Z. and Matron, A., Adsorption of propisochlor on soils and soil components equation for multi-step isotherms," *Chemosphere*, Vol. 48, pp. 725-731, (2002).
6. Hayes, M.H.B., and Clapp, C.E., ..., Humic substances: considerations of compositions, aspects of structure, and environmental influences," *Soil Science*, Vol. 166, pp. 723–737, (2001).
7. Rahman, M. A., Hasan, M. A., Rahim, A., Alam, A. M., ..., Characterization of Humic Acid from the river bottom Sediments of Burigonga: Complexation Studies of Metals with Humic Acid," *Pak.J.Anal. Environ.chem.*, Vol. 11, No. 1, pp.42-52, (2010).
8. Dolmaa, G., Aleksandrova, G. P., Lesnichaya, M. B., Nomintsetseg, B., Ganzaya, G., Bayraa, B., Sukhov, B. G., Regdel, D., Trofimov, B. A., ..., Properties of humic substances isolated from different natural sources," *Mongolian journal of chemistry*, Vol. 14, pp. 51-56, (2013).
9. Antilen, M., Silva, K., Acevedo, S., Amiama, F., Faundez, M., Knicker, H., Pizarro,

- C., Characterization of humic acids extracted from biosolid amended soils” *Journal of Soil Science and Plant Nutrition.*, Vol. 14(4), pp. 1005-1020, **(2014)**.
10. Islam, M.R., Lahermo, P., Salminen, P., Rojstaczer, S., and Peuraniemi, V., “Lake leaching from tropical soils.” *Bangladesh Environmental Geology*, Vol. 39, pp. 1083-1089, **(2000)**.
 11. Nanda, S.N., and Tiwari, T.N., “Effect of discharge of industrial effluents on the quality of river Brahmani at Rourkela.” *Indian J. Environ. prot.*, Vol. 19, pp. 52-55, **(1999)**.
 12. Emongor, V., Nkegbe, E., Kealotswe, B., Koorapetse, I., Sankwasa, S., and Keikanetswe, S., “Pollution Indicators in Gaborone Industrial Effluent.” *Journal of Applied Sciences*, Vol. 5, pp. 147-150, **(2005)**.
 13. Zahir, H., Ahmeduzzaman, M., “A case study on the impact of leather industries on the ground water aquifer in Bangladesh.” *International Journal of Civil & Environmental Engineering IJCEE-IJENS*, Vol. 12, pp. 52-54, **(2012)**.
 14. Rai, D., Eary, L. E., Zachara. J. M., “Environmental chemistry of chromium,” *Sci Total Environ*, Vol. 86, pp. 15–23, **(1989)**.
 15. L’opez-Luna, J., Gonz’alez-Ch’avez, M. C., Esparza-Garc’ia, F. J., and Rodr’iguez-V’azquez, R., “Toxicity assessment of soil amended with tannery sludge, trivalent chromium and hexavalent chromium, using wheat, oat and sorghum plants,” *Journal of Hazardous Materials*, vol. 163, no. 2-3, pp. 829–834, **(2009)**.
 16. Avudainayagam, S., Megharaj, M., Owens, G., Kookana, R. S., Chittleborough, D., and Naidu, R., “Chemistry of chromium in soils with emphasis on tannery waste sites,” *Reviews of Environmental Contamination and Toxicology*, vol. 178, pp. 53–91, **(2003)**.
 17. Gauglhofer, J., “Environmental aspects of tanning with chromium,” *J Soc Leather Technol Chem*, Vol. 70 (1), pp. 11, **(1986)**.
 18. Zayed, A. M., and Terry, N., “Chromium in the environment: factors affecting biological remediation,” *Plant and Soil*, vol. 249, no. 1, pp. 139–156, **(2003)**.
 19. Becquer, T., Quantin, C., Sicot, M., and Boudot, J. P., “Chromium availability in ultramafic soils from New Caledonia,” *Science of the Total Environment*, vol. 301, no. 1–3, pp. 251–261, **(2003)**.
 20. Kimbrough, D. E., Cohen, Y., Winer, A. M., Creelman, L., and Mabuni, C., “A critical assessment of chromium in the environment,” *Critical Reviews in Environmental Science and Technology*, vol. 29, no. 1, pp. 1–46, **(1999)**.

21. Friess, S. L., "Carcinogenic risk assessment criteria associated with inhalation of air borne particles containing chromium (VI/III)," *Sci Total Environ*, Vol. 86, pp. 109–112, **(1989)**.
22. James, B. R., Bartlett, R. J., "Behaviour of chromium in soils. VI. Interactions between oxidation-reduction and organic complexation," *J Environ Qual*, Vol. 12, pp. 173–176, **(1983)**.
23. James, B. R., "The challenge of remediating chromium contaminated soils," *Environ Sci Tech*, Vol. 30, pp. 248–251, **(1996)**.
24. Long, L. L., Li, X. R., Huang, Z. K., Jian, Y. M., Fu, S. X., Zheng, W., "Relationship between changes in brain MRI and 1H-MRS, severity of chronic liver damage, and recovery after liver transplantation," *Exp Biol Med*. Vol. 234 pp. 1075–85, **(2009)**.
25. Kochian, L., Hoekenga, O., Piñeros, M., "How do crops plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorus efficiency," *Annu. Rev. Plant Biolog*, Vol. 55, pp. 459-93, **(2004)**.
26. Squitti, R., Gorgone, G., Panetta, V., Lucchini, R., Bucossi, S., Albini, E., et al., "Implications of metal exposure and liver function in Parkinsonian patients resident in the vicinities of ferroalloy plants," *J Neural Transm*, Vol. 116, pp. 1281–7, **(2009)**.
27. Agarwala, S. C., Sharma, C. P., and Kumar, A., "Interrelationship of iron and manganese supply in growth, chlorophyll, and iron porphyrin enzymes in barley plants", *Plant Physiol*, Vol. 39, pp. 603-609, **(1964)**.
28. Elarnin, O. M., and Wilcox, G. E., "Effect of magnesium and manganese nutrition on watermelon growth and manganese toxicity," *J. Amer. Soc. Hort. Sci*, Vol. 111, pp. 588-593, **(1986)**.
29. Godo, G. H., and Reisenauer, H. M., "Plant effects on soil manganese availability," *Soil Sci. Soc. Am. J.*, Vol. 44, pp. 993-995, **(1980)**.
30. Khan, K., Factor, L. P., Wasserman, G. A., Liu, X., Ahmed, E., Parvez, F., et al. "Manganese exposure from drinking water and children's classroom behavior in Bangladesh E," *Environ Health Perspect.*, Vol. 119, pp. 1501–6, **(2013)**.
31. Aschner, M., Guilarte, T. R., Schneider, J. S., Zheng, W., "Manganese: recent advances in understanding its transport and neurotoxicity," *Toxicol Appl Pharmacol*. Vol. 221, pp. 131–47, **(2007)**.
32. Guilarte, T. R., "Manganese and Parkinson's disease: a critical review and new findings," *Environ Health Perspect*, Vol. 118(8), pp. 1071– 80, **(2010)**.

33. Reaney, S.H., Kwik, U. C. L., Smith, D. R., "Manganese oxidation state and its implications for toxicity," *Chem Res Toxicol*, Vol. 15(9), pp. 1119–26, **(2002)**.
34. Zoni, S., Bonetti, G., Lucchini, R., "Olfactory functions at the intersection between environmental exposure to manganese and Parkinsonism," *J Trace Elem Med Biol*, Vol. 26, pp. 179–82, **(2012)**.
35. El-Jaoual, T., Cox, D., "Manganese toxicity in plants," *J. Plant Nutr*, Vol. 21, pp. 353-386, **(1998)**.
36. Laha, S., Luthy, R., "Oxidation of aniline and other primary aromatic amines by manganese dioxide," *Environ. Sci. Technol.* VOL. 24, PP. 363-373, **(1990)**.
37. Fecht, C. M., Braun, H., Lemaitre, G. C., Van, D. A., Horst, W., "Effect of manganese toxicity on the proteome of the leaf apoplast in cowpea," *Plant Physiol*, Vol. 133, pp. 1935-1946, **(2003)**.
38. Foy, C., Chaney, R., White, M., "The physiology of metal toxicity in plants," *Annu. Rev. Plant Physiol*, Vol. 29, pp. 511-566. **(1978)**.
39. Andrew, C. S., Hegarty, M.P., "Comparative responses to manganese excess of eight tropical and four temperate pasture legume species," *Aust. J. Agric. Res.* Vol. 20, pp. 687-696. **(1969)**.
40. Kasassi, A., Rakimbei, P., Karagiannidis, A., Zabaniotou, A., Tsiouvaras, K., Nastis, A., Tzafeiropoulou, K., "Soil contamination by heavy metals: measurements from a closed unlined landfill," *Bioresour Technol*, Vol. 99, pp. 8578–8584. **(2008)**
41. Voutsand, D., Samara, C., "Labile and bio accessible fractions of heavy metals in the airborne particulate matter from urban and industrial areas. *Atmospheric Environment*, pp. 3583-3590. **(2002)**.
42. Krishnaswamy, K., Kumar, B.D., "Lead toxicity," *Indian Journal of Paediatrics*, Vol. 35, pp. 209-216. **(1998)**.
43. Martin, D. F., Pierce, R. H., "A Convenient Method of Analysis of Humic acid in Fresh Water," *Environmental Letters.*, Vol.1 (1), pp. 49-52, **(1971)**.
44. Dejong, G. J., Brinkman, U. A. Jr., "Determination of chromium (III) and chromium (VI) in seawater by atomic absorption spectrometry," *Anal Chim Acta.*, Vol. 98, pp. 243–250. **(1978)**.
45. Stuczynski, T.I., McCarty, G.W., Reeves, J.B., Wright R.J., "Use of pyrolysis GC/MS for assessing changes in soil organic matter quality," *Soil Sci.*, Vol, 162, pp. 97–105. **(1997)**.

46. Wilson, M.A., „„Application of nuclear magnetic resonance spectroscopy to the study of the structure of soil organic matter,”*J. Soil Sci.* Vol. 32,pp. 167–186.(1981).
47. Sanders, J.K.M., Hunter, B.K., „„Modern NMR Spectroscopy,”*New York*,(1987).
48. Preston, C.M., Cade-Menun, B.J., Sayer, B.G., „„Characterization of Canadian backyard composts: chemical and spectroscopic analyses,”*Compost Sci. Util.* Vol. 6, pp. 53–66.(1998).
49. Preston, C.M., „„Applications of NMR to soil organic matter analysis: history and prospects,”*Soil Sci.* Vol. 161, pp. 144–166.(1996).
50. A. Piccolo, P. Conte, E. Trivellone, and B. Van Lagen, „„reduced heterogeneity of a lignite humic acid by preparative HPSEC following interaction with an organic acid. Characterization of size-separates by Pyr-GC-MS and H-1-NMR spectroscopy,” *db Environ. Sci. Technol.* Vol. 36 pp. 76–84.(2002).
51. Tan, K.H., „„Formation of metal-humic acid complexes by titration and their characterization by differential thermal analysis and infrared spectroscopy,”*Soil Biology and Biochemistry*, Vol. 10-2, PP. 123 – 129, (1978).
52. C. Steelink in E.A. Ghabbour, G. Davies, *Understanding Humic substances*, Royal society of Chemistry, Vol 1-8(1999),

