Metal Binding Studies of Soil Extracted Humic Acid with K (I), Zn (II) and Fe (III) ions

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(Received on 3rd February 2023, accepted in revised form 11th January 2024)

Summary: Physico chemical parameters of soil samples collected from agriculture lands of Taluka Sobhodaro District Khairpur, Sindh, Pakistan were examined to check the quality of soil. Moisture contents percentage, pH and EC values indicated that texture of soils is mostly silt- loam type having water holding ability hence can be considered as good fertile land, beneficial for crops. Humic acids (HAs) were extracted from soil samples by International Humic Substance Society(IHSS) method. HAs form biogeochemical cycle due to presence of electron clouds resultant macro and micro nutrients, transferred to the plants. HAs obtained were characterized by UV-Visible spectroscopy. The optical parameter E_4/E_6 values illustrated that HAs possess high molecular condensation and proved to be hydrophilic and aromatic in nature. To analyze the binding ability with metal ions, isolated HAs were complexed with potassium, zinc and ferric ions under optimized conditions. The concentrations of selected metal ions were detected in complexed HAs samples by Atomic Absorption Spectrophotometer (AAS). The data obtained indicated that the maximum concentration of complexation of metal ions was found for K^{1+}at pH 8, Zn^{2+}7 and Fe^{3+}6. The data was correlated and compared with literature.

Keywords: Humic acids; IHSS; AAS; Kinetic studies; Water holding capacity.

Introduction

Natural organic compounds known as humic substances emerge spontaneously from the biological breakdown of organic materials. Humic substances are the primary source of organic matter in soil and surface water. They have a significant impact on the physico-chemical makeup of soil and play a role in the majority of soil surface phenomena [1]. A chemical process known as humification turns organic materials into HS.Humin, humic acids (HA), and fulvic acids are three types of humic compounds that are differentiated based on their varied levels of solubility in water. HA are the principal carriers affecting the mobility of contaminants in the environment and are the percentage soluble at neutral and alkaline pH [2, 3]. One of largest and essential constituents of soil is an organic matter [4].Physiochemical properties of organic matter are due to present of carbon with oxygen possessing functional groups such as phenolic, carboxyl, alcoholic hydroxyl, phenolic hydroxyl, carbonyl, methoxy and quinone groups [5, 6].HAs is a polymer organic compound with molecular weight near about 5,000 – 100,000 Daltons. HAs is dark brown complex compound soluble in alkaline medium and insoluble in acidic solution [7]. The primary purpose of HAs is to increase the fertility of soils with low levels of organic matter [5]. HAs have significant roles like as to enhance soil biochemical and physical properties by promoting structure texture, microbial population and water holding capacity [8-10]. Particular micronutrients chelate with them and are transported to plants by HAs, which play a significant role in increasing the availability of nutrients in soil [11]. It lessen the transformation of heavy poisonous metals to precipitate them so that plants aren't exposed to these dangerous elements [12]. HAs enhance plants growth as well as crops growth resultant hormonal production promote which lead to increase cytokinase and auxin so the photosynthesis and nutrients metabolism become more active [9, 13]. Phenolic and carboxylic are major functional groups of HAs perform various function in soil [14]. The color of soil may become brown to black due to presence of HAs. The attachment of the soil grain and the formation of the aggregate soil structure are significantly influenced by the structure of HAs [15]. Which enhance the porosity, viscosity, soil compaction and air – water relations. HAs possess greater water holding ability in soils which improve the retention abilities. This factor is very important when the nature of soil is sandy and presence of HAs enhance the compactness of soil as well as maintain require amount of water for plants [16]. These ions are insoluble in water but can be soluble under certain particular conditions. Due to this process essential nutrients are provided to the plants and at the same time protect the plants from poisonous metals like as Cd, Pb, Hg and radioactive nuclides shifting them in to insoluble form this is the approach of powerful management. HAs containing soil

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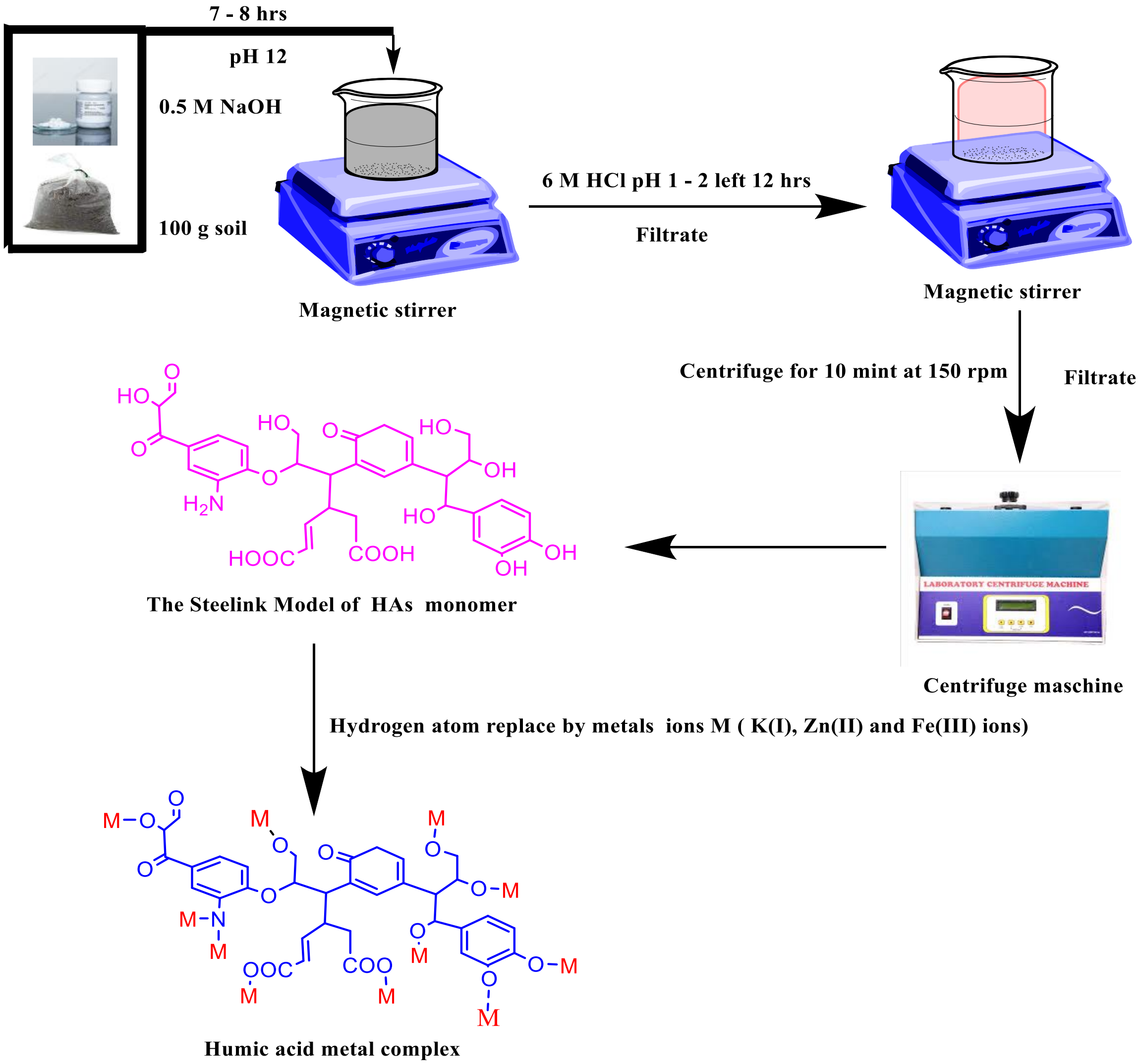
play significant role in suppressed the solubility of those nutrients which are present in excess quantity. Phosphors assimilation occurs when Al and Fe are absorbed by plants. When HAs react with Fe resultant its available as nutrient for plants while on other hand Al remain insoluble form [17]. HAs also play important role to remove the metal cation from water and enhance its consistency [18]. The HAs' chemical action is intimately related to their structure. HAs are divided into many functional groups and subgroups based on the type and degree of conversion.The structure and chemical reactivity of HAs are affected by several functional groups, aliphatic structure, and the amount of aromatic components. There is significant confusion over the structure of HAs despite the existence of numerous studies. The functional groups, structure, and molecular weight of HAs may alter as compounds age and come from different sources [16, 19]. This is over all depends on the process of humification which convert the organic matter in to HAs [16]. The structure of HAs possess various functional groups such as = C= O, C- OH, ArOH, NH_2, and COOH. These functional groups have ability to donate electron when interact with metal cation to form complex compounds [20, 21]. Due to this result different types of mechanism are generated like as complexation with electron donating groups, ion exchange complexation by the formation of M e^{n+} - HA bond which are formed due to existence of free pi electron on its surface [21]. Pakistan is basically agriculture country with huge population, to solve the issue of food it is necessary to enhance crop production in order to solve the matter of agriculture because of that it acts as economical national interest. HS specially HA play essential role in this regard [22].



Scheme-1: Formation of complexed HAs with metals.

Experimental

Collection of soil samples

Samples of soil were collected from fertile lands of TalukaSobhdaro District Khairpur, Sindh Pakistan. These samples shifted to the laboratory and dried for three days at room temperature, subsequently using pestle and mortar to mechanized sieved through 2 nm mesh size and sieves, finally humic acid was extracted from powdered soil samples and stored for further analysis.

Sampling stations

Agriculture soil samples considered to be the source of humic acid were collected from the different stations of fertile lands of Taluka Sobhodaro:

1.Hingorja 2. Lal bux Khan 3. Wada Bhellar 4. Khaki 5. Ranipur 6. Watni 7. Gadiji 8. Ali Marda Tunio 9. Village Ghulam Mohammad 10. Village Liquat Ali Qureshi 11. Village Badal Shah 12. Sobhodero Town 13. Lower Setharja 14. PiloSharef. Standard Humic acid (Aldrich).

Physicochemical analysis of soil samples

pH and EC of soil samples were measured by applying APHA standard methods with Eutech Instruments Bench pH meter Cyber Scan pH/conductivity/TDS/ meter, Singapore [28]. Soil water suspension 1:1 was prepared and allowed to left at room temperature for 15 to 20 minutes. In order to standardized the conductivity meter 0.1 N KCl used as standard solution. Reading was noted after immersed the conductivity meter in to the suspension of soil. Instrument were calibrated before every reading after complexation of HA with K, Zn and Fe metal ions. All chemical HCl, NaOH, KNO_3, $ZnCl_2$ and $FeCl_3$ used of analytical grade.

Extraction of HAs from soil samples

HAs extracted from soil followed as prescribed by International Humic Substance Society Method (IHSS) [23]. Take 100g of soil which is dried and filtered dissolved in 10% hydrochloric acid for 1-2 hrs then decalcination and allow to dissolve in 0.5M NaOH maintain pH 12 and shake for 7-8 hrs. In order to obtain the alkaline solution left the suspension for 12 hrs at room temperature then centrifuged for 10 min at low speed 150 rpm. Dark brown solution is treat with 6M HCl at pH 1 with constant stirring and left for 12 hrs. The suspension was again centrifuged for 10 mints at 40 °C1500 rpm. Extracted floating material was discarded, HAs obtained in coagulated form were washed several times with DI water to remove the chloride ions and finally purified samples were preserved for further analysis [24].

HA-Metal complexation

Complexed were formed by combination of K, Zn and Fe ions with HAs, The process was followed by preparing stock solutions (50 mg dm^{-3}) of each HA by dissolving them in deionized water with small addition of 0.1M NaOH to final pH 8 and under N2 atmosphere. For K(1), Zn(II) and Fe(lll) stock solutions (1000 mg dm^{-3}) were prepared in deionized water from KNO_3, $ZnCl_2$ and $FeCl_3$ of analytical grade purity. The pH of the solution was adjusted to 8, 7 and 6 with 0.1M HCl and NaOH [25].

UV-Visible spectrophotometric studies of humic acid

Ultra violet-visible spectroscopic measurement of HAs were made between 200-700nm by using Birchrom Libra 522 Spectrophotometer. Wave length was set at 200, 254, 436, 465, 665 and 700 nm for measuring the spectral absorbance. Optical parameter E_4/E_6 ratio was obtained at 465 and 665 nm. Every sample for UV-Visible analysis was prepared taking 1mg of HAs dissolved in 5 ml of 0.1M NaOH [26, 27].

Results and Discussions

Moisture contents in soil samples

Presence of moisture in soil enhance the physical and chemical properties and provide the nutrients to plants for performing the enzymatic functions in soil [28, 29]. Moisture content in soil is influenced by the organic matter, texture, morphology, and mineralogy of the soil [30]. According to [31] soil having significant percentage of clay has greater amount of moisture as compare to the soil having less quantity of clay. The moisture contents of all the soil samples are shown in Table-1, with Sample 3 having the greatest value and Sample 11 having the lowest value at room temperature. It indicates that sample 3 contains a higher amount of clay.

Table-1: Moisture contents in soil at different temperatures.

Sample No.	25 °C	105 °C	LOI 550 °C
1	10	5	4.0
2	15	5	2.5
3	20	4.5	2.5
4	12.5	4	2.0
5	13.5	5	3.0
6	10.5	6	4.5
7	10	5	2.5
8	7.5	4	3.0
9	10	3.5	5.0
10	12.5	5	3.0
11	7	8	2.0
12	12.5	3	1.5
13	10	2.5	2.0
14	11.5	5	3.0

In comparison to soil with less clay, soil with more clay has a greater amount of moisture.The soil has a sizable amount of clay, which has a better ability to retain moisture and is more compact, as well as a sizable amount of organic matter. Sample 11 contrasts with sample 10, being more sandy in character and containing less organic content[32]. At 105 °C, the maximum moisture loss from 11 samples indicates the presence of a sizable amount of organic matter. Samples 9, 12, and 13 have negligible amounts of soil moisture, in contrast. Due to the soil's ability to hold more water due to the presence of organic matter, the amount of moisture in the soil has increased [33].

pH of soil

Table-2 pH of Soil column lists the average pH of all the soil samples. Materials' solubility and the availability of nutrients for plants are both impacted by pH [34] pH play key role for the nutrients availability [35].Samples 2, 11, and 12 are slightly more acidic in nature than samples 1, 4, and 7, which all have pH values of 7. While samples 5, 10, and 14 are basic in nature, samples 3, 6, 8, 9, and 13 are weakly alkaline. The pH value indicates that the soil is fertile, and different crops can grow easily and more healthily since the soil is soluble in considerable nutrients between pH values of 6.5 and 7.5. Micronutrients such as Ca, Mg, K, N, P, and S are present at pH values of 6.5 to 8, whereas Zn, Al, Mn, Cu, and Fe are given to plants at alkaline pH values[36].

Table-2: pH and EC soil samples.

Sample No.	pH	EC (dS/m)
1	7.0	9.0
2	6.8	10.0
3	7.1	13.0
4	7.0	9.0
5	8.5	14.0
6	7.6	12.5
7	7.0	14.0
8	7.3	9.5
9	7.1	8.5
10	8.0	13.5
11	6.9	11.0
12	6.7	14.5
13	7.2	13.5
14	8.1	17.0

Electrical conductivity (EC)

EC of soil is due to presence of total dissolved salt in soil through which type of soil may be saline or non-saline [37]. EC conductivity measures the different factors which directly affected the fertility of soil such as texture, clay content and salinity. So for crop management EC is act as quick indicator [38]. EC values of all soil samples are shown in table 2 which are in range of 8.5 to 17 dS/m which indicate that mostly soil is non-saline, normal class and possesses excellent fertility [39].

UV-Visible Spectroscopic analysis

Table-3 represent the spectral absorbance E4/E6 at different wave length of HAs extracted from soil, which is in the range of 1.63-5.81.The optical parameter E4/E6 of samples 1-14 as well as standard is < 5. E4/E6 reflects the degree of aromaticity. Whenever, optical parameter ratio increase consequently decrease in aromaticity and molecular mass [40].Samples 1 to 14 and standard possess greater aromatic character as well as molecular mass [41]. Whereas, sample 7 having higher value > 5 show that HAs is more aliphatic with low molecular mass and aromaticity [39]. Higher absorbance in UV-Visible spectroscopic measurement due to present of oxygen containing functional groups like as –OH, CO- COOH and $–CONH_2$ [17]. Absorbance at 665 nm shows formation of complexation with non humic substances [25].

Table-3: Spectral absorbance and their ratio at specific wave length by UV-Vis spectrophotometric studies of Has.

Sample No	200 nm	254 nm	436 nm	465 nm	665 nm	700 nm	E_4/E_6
1	0.706	0.747	0.094	0.075	0.023	0.091	3.26
2	1.111	1.011	0.432	0.380	0.218	0.211	1.74
3	0.335	0.415	0.320	0.251	0.109	0.103	2.30
4	1.111	1.021	0.423	0.188	0.052	0.046	3.61
5	0.427	0.514	0.678	0.521	0.137	0.121	3.80
6	1.121	1.09	0.806	0.659	0.224	0.198	2.94
7	1.111	0.353	0.437	0.536	0.093	0.077	5.76
8	0.506	0.538	0.085	0.074	0.047	0.073	1.57
9	1.121	1.301	1.561	1.215	0.350	0.294	3.47
10	1.131	1.121	0.378	0.318	0.156	0.152	2.03
11	1.101	1.301	2.801	2.246	0.931	0.851	2.41
12	1.151	1.121	0.472	0.372	0.103	0.093	3.61
13	1.151	1.248	0.471	0.379	0.132	0.122	2.87
14	1.080	1.201	0.498	0.405	0.154	0.144	2.62
St	1.08	0.27	0.481	0.550	0.120	0.055	4.58

Concentration of K complexed with soil extracted HA

At pH 8, Fig. 1 depicts the development of a potent combination between HAs and K. One of the most important metal cations that is crucial to plant growth is potassium[42]. K causes a variety of enzymes to become active, which causes sugar transfer, carbon and nitrogen metabolism, photosynthesis, and protein synthesis to occur[43]. Potassium is essential for plants' biophysical and metabolic processes; a lack of potassium can reduce their levels of chlorophyll, photosynthetic activity, and fixed carbon transport[42]. Availability of K in soil protect the plants from diseases, pests and reduce the toxicity of Na^+ [44]. K promote the initial growth of plants and enhances the production of protein and keep safe plants against diseases and insects [42]. Opening and closing of stomata, movement of leaf and tropism of plants are due to the K^+ production of torpor pressure [45]. Whenever, K ion accumulate within cell which provide osmatic pressure consequently,cellular and leaf expansion [46]. At pH 8, the maximum complexation between K and HAs occured, indicated in Table-4. Samples 1 and 13 show the highest and lowest levels of complexation of K with HAs, respectively. Samples 2 and 9 exhibit considerable complexation, whereas samples 3, 4, 10, and 14 have less bound with HAs.

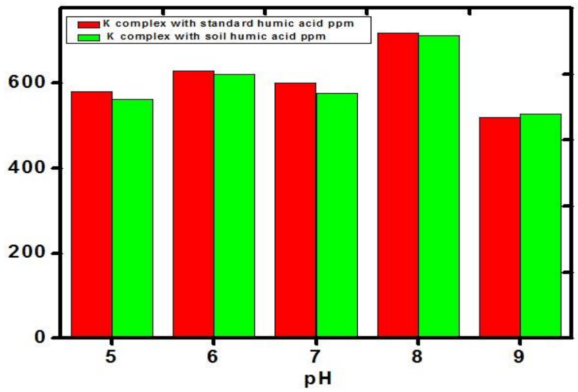


Fig. 1: Potassium complexed with standard and soil HA at different pH.

Complexed zinc concentration with HA from soil.

Zn is act as most important micronutrient for all vital system which is available in form of divalent metal cation [47]. Zinc humate contain Zn which is essential nutritional element for plants and animals [48]. It acts as micronutrient and necessary for the growth of plants. Zn is used for the formation of tryptophan which is raw material for the synthesis of Indole – 3- acetic acid and this compound is used for the synthesis of auxinharmone in plants [49]. Excess amount of Zn cause toxic effect on the growth of plants [50]. The optimal pH for compound formation between Zn and HAs is 7, as demonstrated in Fig 2. Table-5 demonstrates that chelation between Zn and HAs is greatest in 5 samples and least in 10. Chelation is moderate occured in samples 1, 2, 7, and 8, however the smallest amount of Zn associated with HAs was found in samples 3, 4, 6, 9, 11, and 14. HAs chelate with Zn, its solubility increases and it becomes more available to the plants. When this complex reaches the surface of the roots, it can easily split from the chelate structure and enter in the roots of plants, or it can penetrate in the form of chelate inside the roots. Zinc metal exists in soil as a divalent cation and an anion, forming a complex ion [51].

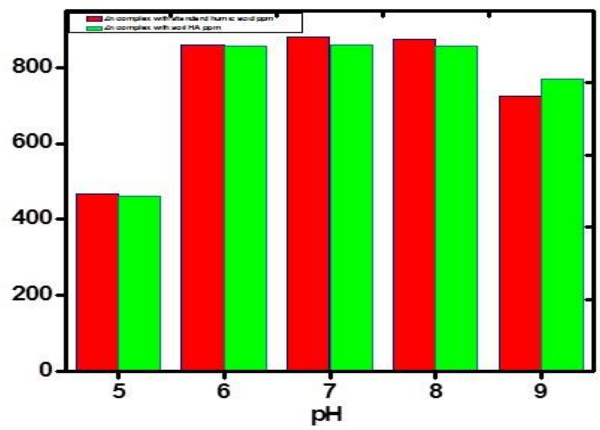


Fig. 2: Concentration of Zinc complexed with standard and soil HA at different pH.

Table-4: Concentration of K in complexed and soil HAs samples.

Sample #	Quantity of HAs after complexation with K (g)	K in Soil HAs (ppm)	K in complex soil HAs (ppm)	Net Amount of K complexed with soil HAs
1	0.194	2.90	460	457.10
2	0.196	2.27	410	487.73
3	0.203	2.50	300	297.50
4	0.185	3.20	390	386.80
5	0.181	3.92	340	336.08
6	0.191	3.45	440	436.55
7	0.173	2.16	430	427.84
8	0.207	3.55	420	416.45
9	0.918	2.83	440	437.17
10	0.207	3.86	280	276.14
11	0.201	5.70	360	354.30
12	0.196	4.30	390	385.70
13	0.175	3.33	260	256.67
14	0.201	4.17	350	345.83
ST	0.213	4.20	344	339.80

Table-5: Concentration of Zn in complexed and soil Has.

S.NO	Quantity of HAs after complexation with Zn (g)	Zn in Siol HAs (ppm)	Zn in complexed soil HAs (ppm)	Net amount of Zn complexed with soil HAs (ppm)
1	0.234	0.60	804	803.4
2	0.236	0.48	768	867.5
3	0.253	0.46	620	619.6
4	0.575	0.54	696	695.5
5	0.175	0.45	860	859.5
6	0.813	1.14	696	694.9
7	0.626	0.42	720	719.6
8	0.761	0.31	736	735.7
9	0.124	1.28	648	646.7
10	1.137	0.65	696	495.4
11	0.268	3.75	644	640.2
12	1.211	1.19	580	578.8
13	0.998	0.48	508	507.3
14	1.891	0.66	632	631.4
St	0.213	4.21	840	835.8

Table-6: Concentration of Fe in complexed and soil Has.

S.No	Quantity of HAs after complexation with Fe (g)	Fe in soil HAs (ppm)	Fe in complexed soil HA (ppm)	Net amount of Fe complexed with soil HAs (ppm)
1	0.87	10.61	596.00	585.40
2	0.41	13.34	552.00	638.65
3	0.16	78.51	476.00	397.50
4	0.16	11.64	520.00	508.35
5	0.06	20.71	580.00	559.30
6	0.17	10.51	580.00	569.50
7	0.17	11.06	640.00	628.95
8	0.18	9.251	632.00	622.75
9	0.24	13.00	588.00	575.00
10	0.22	26.00	480.00	454.00
11	0.18	27.91	584.00	556.08
12	0.15	15.81	552.00	536.20
13	0.07	18.51	248.00	229.50
14	0.18	19.31	608.00	588.70
St	0.32	22.51	560.00	537.50

Table-7: Comparison of concentration of K, Fe and Zn complexed with soil HAs (ppm).

Sampling stations	Amount of K complexed with soil HAs (ppm)	Amount of Zn complexed with soil HAs	Amount of Fe complexed with soil HAs
1	457.10	803.4	585.40
2	487.73	867.5	638.65
3	297.50	619.6	397.50
4	386.80	695.5	508.35
5	336.08	859.5	559.30
6	436.55	694.9	569.50
7	427.84	719.6	628.95
8	416.45	735.7	622.75
9	437.17	646.7	575.00
10	276.14	495.4	454.00
11	354.30	640.2	556.08
12	385.70	578.8	536.20
13	256.67	507.3	229.50
14	345.83	631.4	588.70
ST	339.80	835.8	537.50

Concentration of iron complexed with soil extracted HAs

The quantity of chlorophyll present in the leaves of plants direct indication of iron nutrition (Require amount of Fe supply → greater chlorophyll quantity → increase greenery of plants) (deficiency Fe supply → low chlorophyll quantity → leaves of plants become yellow). Proper dose of iron supplied plants remain green and healthy [51].As illustrated in Fig. 3, complex formation between HAs and Fe at optimized at pH 6. Table-6 demonstrates that sample 7 has better chelating ability with Fe and sample 13 has less capacity to chelate Fe, but samples 1 and 12 show moderate chelation with the exception of sample 3, 10, which has less complexation with iron. Iron-chelated soil solution of soluble HAs.This type of chemical link is soluble and transportable. However, iron and organic substances are stored with low solubility. The redox state of the soil determines iron mobility. When the pH rises and the oxidation intensifies, the solubility of Fe falls [52]. Fe is a micronutrient that is required by both animals and plants but excess iron consumption can cause tissue damage. Metabolism occurs in the human body as a result of Fe. The literature reveals that Fe and other metals form complexes with HAs at pH 6 [53]. The complexation of metals and HAs is more important in understanding the availability and transport of (bio) metals in neutral aquatic environments. The earth's crust contains 4.2% iron, but most soils have between 2 and 60%. The availability of iron influences plant growth at all levels [54].

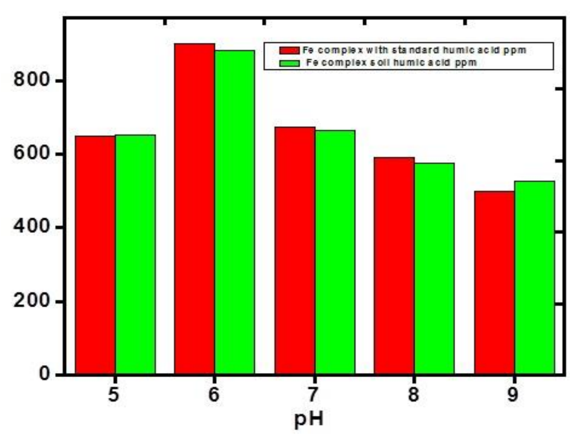


Fig. 3: Fe complexed with standard and soil HAs at different Ph.

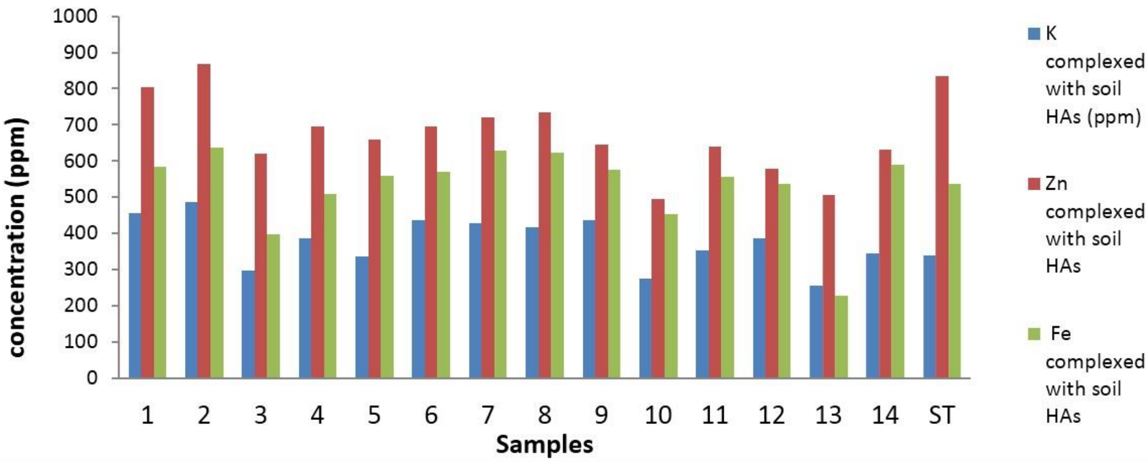


Fig. 4: Comparison of concentration of K, Fe and Zn complexed with soil Has.

Conclusions

Physico chemical parameters proved the excellent quality of agriculture soil having high water holding capacity. The optical parameters E_4/E_6 ratio values of isolated HAs < 5.0 illustrated the mature and humified structure of HAs having high molecular mass and hydrophilic in nature. K^{1+}, Zn^{2+} and Fe^{3+} metal cations showed significant chelation ability with soil HAs at pH 8, 7 and 6 respectively in order Zn > Fe > K.. It is also found that the interaction of HAs with metal cations increases with pH, decreases with metal ion concentration with increasing HA concentration which may be due to the differences in sorption ability of particular metal ions on HAs. The studies have revealed stronger metal ions complexation at higher pH was attributed to higher dissociation degree of functional groups present in HAs.

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