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INFLUENCE OF HUMIC ACID AND SULFUR ON THE BIOAVAILABILITY OF SOME MICRONUTRIENTS IN CALCAREOUS SOILS

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Abstract

The field experiment has behaved at two locations, Qlyasan and Kanypanka in Sulaymani governorate, Iraq. The aim of this research is, to evaluate the effect of adding different levels of Humic Acid (HA) and Sulfur (S) on the bioavailability of some micronutrients in calcareous soils and improvement of their availability in rhizosphere. The experimental treatments were arranged in a factorial experiment and laid out in a complete randomized block design with three replicates. The treatments were included four grades of humic acid (0, 25, 50 and 100 kg ha^{-1}), and four grades of sulfur (0, 500, 1000 and 2000 kg ha^{-1}) were incorporated into the soil. Results revealed that different humic acid and sulfur significantly affected in the soil reaction (pH) at ($P<0.05$) of calcareous soil, however, the results showed that affected significantly on the availability of some micronutrients in the rhizosphere soil of a maize plant while the results showed that the humic acid and sulfur level application affected significantly of micronutrients uptake by shoot and grain of maize plant and the sequential chemical extraction (SCE) of micronutrients in the rhizosphere soil, partitioning of Cu, Zn, Fe and Mn as determined by SCE from Qlyasan and Kanypanka locations.

***Keywords*:** Sequential chemical extraction, rhizosphere, uptake, soil reaction.

Introduction

The bioavailability of a mineral or micronutrients is acquainted as the fraction of the take in a nutrient that is absorbed and subsequently utilized for normal physiological functions. It is essential to comprehend the bioavailability of some micronutrients which depends on its form in the soil, rather than on the total amount accumulated (Mateusz *et al.*, 2013). The conditions at the root-soil interface overwhelmingly referred to as the rhizosphere, are frequently different from those present some extent from the root system. For this cause, many researchers have concentrated on this part in addressing the problem concerning mineral speciation and bioavailability using different kinds of cultivation devices (Berg and Smalla, 2009). Micronutrients deficiencies in soil and food are critical issues in the developing countries (Welech and Graham 2000), and are widespread; many millions of hectares of arable land worldwide are incomplete in one or more micronutrient elements, since micronutrients play important functions in plant growth (Kabata-Pendias, 2010). The bioavailability and solubility of micronutrients may indicate their availability to plants. Micronutrients are generally related to the main components of soils (Rutkowska *et al.*, 2014). The association Bureau of Reference (BCR) manner is an ordinarily used sequential procedure that distinguishes micronutrients into weak acid soluble, reducible (oxide-bound), oxidizable (OM-bound) and imperishable fractions (Bakircioglu *et al.*, 2011), in the order of lessening bioavailability. To date, little studies have been behaved to illustrate the response of bioavailability of micronutrients to enhanced humic acid and sulfur levels.

Today, there is a renowned and increasing use of humic acids for their beneficial impact on the growth and cultivation of crops particularly in soils (Baigorri *et al.*, 2009). Humic acid does not directly provide nutrients to plants, and it is not a fertilizer but is a compliment to fertilizer. The increase root vitality, improved nutrient uptake, better formation and stability of aggregates, increased both water and fertilizer retention, and stimulate beneficial microbial activity by the addition of organic matter to organically-deficient soils (Wahdan *et al.*, 2006). Using humic acid to improve nutrient uptake is dating back to a long history of many years (Mackowiak *et al.*, 2001). Humic acid possesses extremely high ion-exchange capacities, which allow them to hold cations in a way that makes them more readily available to plant roots and thus improve the transfer of some micronutrient to the plant's deliberation system (Ebtisam *et al.*, 2012).

Sulfur is the important one of the primary essential plant nutrients in the soil, and by improving the use efficiency of other essential plant nutrients and providing direct nutritional value its take part to an increase in crop yields (Catherine, 2009). Ayup *et al.* (2007) found that the application of sulfur rates have an essential role in reducing soil pH slowly from (8.5 to 7.5). Orman and Kaplan (2011) reported that the application of 200 ppm sulfur to calcareous soil after three weeks resulted in 0.18 unit decrease in soil pH. Elemental sulfur is of particular interest to increase plant nutrient availability in the soil system, as a soil amendment, since is readily available and it possesses a slow release acidifying characteristic (Chien *et al.*, 2011). It is of particular interest in increasing soil nutrient mobility (Jankowski *et al.*, 2015) as it is commonly available and is slow release acidifying (Chien *et al.*, 2011). The acidifying function of S originates from its microbial oxidation to sulfuric acid over time (Vidyalakshmi *et al.*, 2009). However, according to some authors, application of elemental sulfur in soil amendment in their studies did not show a significant change in soil chemical properties such as acidity and nutrient availability (Skwierawska *et al.*, 2012). This might be due to both unsuccessful oxidations of applied sulfur as well as high carbonate content of the soil. However, successful oxidation of elemental sulfur and a significant change in soil chemical properties and nutrient availability are well documented for some soils (Khalid *et al.*, 2012).

The Rhizosphere is the most critical soil part affecting plant growth and development, and it's a zone of soil surrounding the root which is affected by it but its size differs spatially and temporally, ranging from a fraction of a millimeters for microbial populations and immobile nutrients

to tens of millimeters for mobile nutrients and water to several tens of millimeters for volatile compounds and gases released from roots and it is the soil-root interface consisting of a soil layer varying in thickness between 0.1 and up to a few millimeters depending on the length of root hairs. Availability of some micronutrients in the rhizosphere zone is controlled by the combined effects of soil properties, plant characteristics, and the interactions of plant roots with microorganisms and the surrounding soil (Dotaniya and Meena, 2015). This means that the interface between the root and the soil is complex and it is the most chemically and biologically, an active microsite in the soil and its ecological importance is widely recognized (Ma *et al.,* 2014).

The root exudation, nutrient uptake, microbial activity, and differences in water relations have an important role in changing chemical conditions in the rhizosphere compare with those of the bulk soil. Characterizing nutrient availability in soils is essential and affected by knowledge of rhizosphere chemistry and rhizosphere processes. In the last decade, much progress has been made toward a better understanding of the role of rhizosphere processes in plant nutrition, particularly the chemical, physical, and biological properties of the rhizosphere soil (Hinsinger *et al.,* 2009). Farmers apply fertilizers in most of the cases without knowing the rhizosphere role in particular nutrient chemistry about its availability to plants. It enhances crop yield by increasing plant nutrient availability. Through the agronomic and breeding approaches, the nutrient use efficiency can be enhanced by the manipulation of the rhizosphere environment (Dotaniya and Meena, 2015).

The primary objective of this study was to investigate micronutrients speciation in soil in an attempt to obtain a better understanding of its availability and subsequent uptake by maize, in this regard, the study focused on root-induced change of various micronutrients species in the maize rhizosphere and to improve the quality of the seeds and increase the concentration of these micronutrients in the gains of maize by the effect of different humic acid combined with sulfur.

Materials and Methods

To evaluate the influence of humic acid and sulfur on the bioavailability of some micronutrients in calcareous soils, field experiments were carried out at two different locations. The first one at Qlyasan Agricultural Research Farm (45° 21' 29" E, 35° 34' 36" N 757m above sea level) and the second one at Kanypanka Agricultural Research Farm (45° 42' 57" E, 35° 22' 56" N 578 m above sea level) in Sulaimani governorate, Iraq, during spring growing season of (10th and 11th Apr. 2017 to 29th and 27th Jul. 2017) respectively, to elucidate the influence of humic acid (HA) and sulfur (S) incorporated into the soil on some micronutrients availability in the rhizosphere and maize (*Zea mays* L.) cv. Gloria growth in calcareous soil.

The experiment comprised 16 experimental units combinations in a factorial experiment and laid out in complete randomized block design (RCBD) with three replications, include four levels of humic acid as humate potassium (Humic 85%), obtained from AFICO factory in Jordan, (H_0=0, H_1= 25, H_2=50 and H_3= 100 kg HA ha^{-1}) and four levels of sulfur, obtained from LAWA factory in Sulaimani–Iraq, as agricultural sulfur which contained 99% S (S_0=0, S_1= 500, S_2=1000 and S_3= 2000 kg S ha^{-1}). The experiment was conducted on the 622.5 m^2 area (15 m X 41.5 m), the area of each experimental unit was 6 m^2 (2 X 3) m, each experimental plot included 3 rows in 3 m length, 0.70 m between rows and 0.30 m within rows between the individual plants, to obtain a mean density of 50,000 plants ha^{-1} and the distance between the experiment units was 0.5 m while the distance between blocks was 2 m.

Treatments were as follows:

T_1= (HA and S 0kg ha^{-1})

T_2= 500 kg S ha^{-1}

T_3= 1000 kg S ha^{-1}

T_4= 2000 kg S ha^{-1}

T_5= 25 kg HA ha^{-1}

T_6= 25 kg HA ha^{-1}+500 kg S ha^{-1}

T_7= 25 kg HA ha^{-1}+1000 kg S ha^{-1}

T_8= 25 kg HA ha^{-1} +2000 kg S ha^{-1}

T_9= 50 kg HA ha^{-1}

T_{10}= 50 kg HA ha^{-1}+500 kg S ha^{-1}

T_{11}=50 kg HA ha^{-1}+1000 kg S ha^{-1}

T_{12}=50 kg HA ha^{-1}+2000 kg S ha^{-1}

T_{13}=100 kg HA ha^{-1}

T_{14}=100 kg HA ha^{-1}+500 kg S ha^{-1}

T_{15}=100 kg HA ha^{-1}+1000 kg S ha^{-1}

T_{16}=100 kg HA ha^{-1} +2000 kg S ha^{-1}

Fertilizers were applied, 200 kg N ha^{-1} from urea 46% N was added and divided into two equal doses the first dose was applied after 20 days of germination and the second dose was applied at the testing stage, 200 kgP_2O_5 ha^{-1} as triple superphosphate (TSP) and 150 kgK_2O ha^{-1} as KCl was applied at the seeding time. The crop was irrigated through a surface irrigation system every 5-7 days at a required time. Besides, all required management practices were done at proper times, and the standard practices were used for weed control.

Soil samples were taken before planting from a depth of 0-40 cm of the soil used in the field experiments. The soil samples were air-dried, passed through a 2 mm sieve, and stored in plastic bottles before analysis. Some physical and chemical properties of the soils are given in Table1.

Rhizosphere soil samples were collected from the study locations. The soil around the plant roots is selected, and soil samples were collected in the rhizosphere regions. Four soil samples for each treatment were collected, mixed thoroughly, homogenized, air dried and sieved to remove larger particles, placed separately in fresh polythene bags, labeled and brought to the laboratory and stored at 4C° for analysis.

Determination micronutrients in the rhizosphere soil were extracted by Diethylenetriaminepentaacetic (DTPA) test, Lindsay and Norvell (1978) developed this test for assessing the availability of micronutrients, such as Zn, Mn, Fe and Cu. In this test, 10 g of soil was weighed into a 50 ml plastic centrifuge tube, where 20 ml of the DTPA solution were added. This solution (DTPA) was prepared by mixing 9.835 g of 0.005 M DTPA, 7.4 g of 0.01 M $CaCl_2$ and 74.5 g of 0.1 M triethanolamine (TEA) and taking the solution to 5

L volume, after the pH of the solution was adjusted to 7.3 with a few drops of concentrated HCl.

The mixture was shaken in an end-to-end shaker for 2 hours at 21 C° at 20 rpm and was filtered through a Whatman No 42 filter paper. The solution was kept at 4 C° until analysis.

Three plants of each plot were tagged and harvested. Plant kernels, leaves, stems and roots were separately washed in deionized water, then dried at 65C° in an oven for 72 hours and weighed and ground (Jones, 2001). Then samples were analyzed.

Rare micronutrients species in soil, the following procedure used in this study was based on the four-step method developed by Tessier *et al.* (1979).

Step 1. Exchangeable (F_1)

5ml of 1 mol L^{-1} Mg $(NO_3)_2$ were added to 2.5g of soil in a 40 ml centrifuge tube. The tube was shaken for two h at 25°C on a mechanical shaker. The extract was separated from the solid residue by centrifugation at 3000 rpm. The residue was washed with 5mL of distilled water by shaking for 5 min, centrifuged and the washings added to the extract.

Step 2. Bound to Fe Mn oxides (F_2)

10 ml of 0.04 mol L^{-1} NH_2OH•HCl (25%V/V HCl) were added to the residue from Step1. The tube was warmed at 96±2°C in a water bath for eight h. The separation procedure was repeated as described in Step 1.

Step 3. Bound to organic matter (F_3)

Six mL of 30% H_2O_2 (adjusted to pH 2.0 by HNO_3) was dropped slowly to avoid a violent reaction with the residue from Step 2. Digestion was continued at 85±2°C in a water bath for about five hours. Three mL of H_2O_2 was added to the cool residue and treated in the water bath again. The residue was extracted by 15 mL 1 mol L^{-1} NH_4Ac (pH 5.0). The extraction procedure was the same as Step 1.

Step 4. Residual (F_4)

The residue was dried at 105±2°C. The 0.125g residue was digested by Aqua Regia solution (3:1 HCl: HNO_3 mixture) following the procedure recommended by the International Organization for Standardization (1995).

Table 1 : Soil Physico-chemical properties of soil used in field experiments

Soil properties	Qlyasan	Kanypanka
Physical properties		
Sand g kg^{-1}	59.68	37.40
Silt g kg^{-1}	619.17	500.30
Clay g kg^{-1}	321.15	462.30
Textural Class	Silty clay loam	Silty clay
Bulk density *M*g m^{-3}	1.40	1.50
Chemical properties		
pH	7.42	7.46
EC dSm^{-1} At 25 C°	0.38	0.27
Cation Exchange Capacity (CEC) cmolc kg^{-1}	49.82	46.50
Organic matter (OM) g kg^{-1}	19.59	22.50
Total $CaCO_3$ equivalent g kg^{-1}	215.68	215.50
Available Fe mg kg^{-1}	2.08	2.54
Available Mn mg kg^{-1}	9.86	16.35
Available Cu mg kg^{-1}	3.01	3.07
Available Zn mg kg^{-1}	0.45	0.42
Ca^{2+} mmol L^{-1}	2.0	4.20
SO_4^{2-} mmol L^{-1}	0.79	0.89
Mg^{2+} mmol L^{-1}	0.81	0.90
Na^{+} mmol L^{-1}	0.46	0.80
K^{+} mmol L^{-1}	0.156	2.70
HCO_3^{-} mmol L^{-1}	2.51	4.20

The micronutrients (Fe, Cu, Mn and Zn) in the all sequential solutions (F_1, F_2, F_3, and F_4) and the concentration of them in rhizosphere soil and plant samples were determined by using Atomic Absorption Spectrometer, Perkin Elmer, Analyst 800.

Nutrients uptake in the grain and straw of maize plant were calculated by the following formulas.

Nutrients uptake by (grain or straw kg ha^{-1}) = Nutrients content in (grain or straw %) × Grain or straw yield (kg ha^{-1}) / 100

Data were subjected to analysis of variance (ANOVA) performed by XLSTAT (2016) Package, and the differences were compared by Duncan Multiple Range Test (DMRT) at 5% significance level.

Results and Discussion

pH of calcareous Soil

Soil reaction (pH) is an important chemical property because it affects the availability of nutrients to plants and the activity of soil microorganisms. Soil reaction is a measure of soils' acidity or alkalinity. The pH represents a measure of H^+ activity in a soil solution which is in a dynamic equilibrium with a negatively charged solid phase. H^+ ions are strongly attracted to these negative sites and have sufficient power to replace other cations from them. A diffuse layer in the vicinity of a negatively charged surface has higher H^+ activity than the bulk soil solution. Generally, soil pH regulates the solubility, mobility, concentration of ions in solution and acquisition of elements by plants (Fageria *et al.*, 1997). In low pH (acid) soils, most of the micronutrients are at their peak availability (Figure1). Further, low pH favors free metal cations and protonated anions and higher pH favors carbonate or hydroxyl complexes. Thus, the availability of micronutrient and toxic ions, which are present as cations in the soil solution, increases with increasing soil acidity. The availability of Cu, Fe, Mn, and Zn usually decreases as soil pH increases.

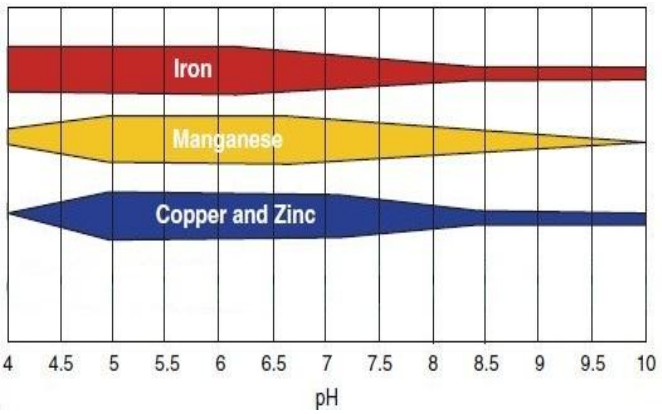

Fig. 1 : Relative availability of micronutrients at different soil pH (Source: Truog 1946).

The effect of humic acid applied combined with sulfur on soil pH are presented in Table (2). The data showed that the soil pH decreased with increasing the levels of humic acid and sulfur. The maximum value of soil pH (7.420 and 7.447) was recorded by T_3 and T_1 from both of locations Qlyasan and Kanypanka respectively, while the minimum value of soil pH (7.307 and 7.313) was recorded by T_{16} from Qlyasan and Kanypanka locations respectively. These results are in agreement with the results obtained by El-Galad *et al.* (2013) and Ebtisam *et al.* (2012) they observed that the value of soil pH decline after the use of humic acid. These results are in line with the general opinion of the positive effect of soil acidification on soil nutrient availability (Wang *et al.*, 2006 and Bolan *et al.*, 2003), and signifies that with decreasing soil pH the soil nutrient release was increased.

Influence of humic acid and sulfur rates on the availability of some micronutrients in the rhizosphere soil

The available of micronutrients concentrations of experimental soil after maize harvesting are presented in Table 2. The data representing available Cu, Zn, Fe and Mn as affected by humic acid and sulfur statically analysis revealed that humic acid and sulfur rats application significantly at ($P<0.05$) increased the soil available Cu, Zn, Fe and Mn in both locations. The corresponding relative increase of mean values soil Cu, Zn, Fe and Mn availability as affected by humic acid combined with sulfur compared with control treatment.

Table 2 : Influence of humic acid and sulfur rates on the soil pH and availability (DTPA- extractable) of some micronutrients in the Rhizosphere soil (mg kg^{-1}) from Qlyasan and Kanypanka locations:

Treatments	Qlyasan					Kanypanka				
		Available nutrients (mg kg^{-1})					Available nutrients (mg kg^{-1})			
	pH	Cu	Zn	Fe	Mn	pH	Cu	Zn	Fe	Mn
T_1	7.413^{a}	2.60^{e}	1.59^{g}	2.66^{efg}	15.87^{g}	7.447^{a}	2.60^{fg}	1.61^{g}	2.74^{gh}	17.05^{g}
T_2	7.417^{a}	2.68^{e}	1.65^{fg}	2.56^{fg}	14.87^{h}	7.440^{a}	2.43^{g}	1.79^{f}	2.79^{fgh}	16.41^{h}
T_3	7.420^{ab}	2.66^{e}	1.76^{efg}	2.64^{fg}	14.71^{h}	7.447^{a}	2.66^{fg}	1.56^{g}	2.74^{gh}	16.90^{gh}
T_4	7.400^{bc}	2.64^{e}	1.66^{fg}	2.48^{g}	15.91^{g}	7.447^{a}	2.74^{ef}	1.53^{g}	2.71^{h}	16.71^{gh}
T_5	7.393^{cd}	2.77^{de}	1.77^{ef}	2.73^{ef}	16.24^{g}	7.437^{a}	2.77^{ef}	1.56^{g}	2.79^{fgh}	17.84^{f}
T_6	7.383^{cd}	2.92^{bcd}	1.77^{ef}	3.02^{d}	16.23^{g}	7.433^{a}	2.95^{de}	1.81^{ef}	2.88^{efg}	17.73^{f}
T_7	7.390^{de}	2.82^{cde}	1.77^{ef}	2.87^{de}	16.93^{f}	7.417^{b}	3.04^{cd}	1.84^{def}	2.94^{def}	18.17^{f}
T_8	7.373^{ef}	3.00^{abc}	1.83^{ef}	3.05^{d}	17.83^{e}	7.403^{b}	2.97^{de}	1.90^{def}	2.93^{def}	19.00^{e}
T_9	7.363^{fg}	3.00^{abc}	1.88^{de}	3.29^{bc}	17.83^{e}	7.417^{bc}	3.05^{cd}	1.89^{def}	2.95^{def}	19.34^{de}
T_{10}	7.363^{fg}	3.09^{ab}	1.90^{de}	3.05^{d}	17.96^{e}	7.393^{cd}	3.08^{bcd}	1.96^{de}	3.00^{de}	19.39^{de}
T_{11}	7.353^{g}	3.22^{a}	2.01^{d}	3.10^{cd}	18.98^{d}	7.390^{cd}	3.25^{abc}	1.97^{d}	3.07^{cd}	19.73^{d}
T_{12}	7.350^{gh}	3.07^{ab}	2.20^{c}	3.36^{b}	19.74^{c}	7.383^{d}	3.31^{ab}	2.15^{c}	3.19^{c}	20.43^{c}
T_{13}	7.347^{gh}	3.10^{ab}	2.45^{b}	3.37^{b}	20.95^{b}	7.343^{e}	3.37^{a}	2.24^{c}	3.66^{b}	21.76^{b}
T_{14}	7.337^{h}	3.18^{a}	2.82^{a}	3.62^{a}	21.19^{b}	7.347^{e}	3.47^{a}	2.43^{b}	3.99^{a}	22.40^{a}
T_{15}	7.310^{i}	3.18^{a}	2.75^{a}	3.65^{a}	21.85^{a}	7.313^{f}	3.40^{a}	2.92^{a}	4.09^{a}	22.85^{a}
T_{16}	**7.307^{i}**	3.19^{a}	2.73^{a}	3.74^{a}	22.19^{a}	7.313^{f}	3.29^{abc}	3.04^{a}	3.98^{a}	22.59^{a}
Pr > F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

The results show that significantly increased the mean values soil Cu, Zn, Fe and Mn availability from 2.60, 1.59, 2.48 and 14.71 were recorded from (T_1, T_1, T_4, and T_3) to 3.19, 2.82, 3.74 and 22.19 were recorded from (T_{16}, T_{14}, T_{16}, and T_{16}) respectively for Qlyasan location and from 2.43, 1.53, 2.71 and 16.41 were recorded from (T_2, T_4, T_4, and T_2) to 3.29, 3.04, 4.09 and 22.85 were recorded from (T_{16}, T_{16}, T_{15}, and T_{15}) respectively for Kanypanka location.

These results are in agreement with the results reported by Shaban *et al.* (2012) and Mackowiak *et al.* (2001) who indicated that application of humic acid positively influenced micronutrients availability, in soil and indicated that the availability of micronutrients in soil depends on the change of soil pH. While our results showed plant nutrient availability from the soil to be significantly affected by the addition of sulfur for Qlyasan and Kanypanka locations (Table 2), there are also contrasting reports on the effect of elemental S on nutrient availability (Klikocka, 2011 and Safaa *et al.*, 2013). The effectiveness of elemental sulfur application on plant nutrient availability was not observed in some soils (Shenker and Chen, 2005 and Skwierawska *et al.*, 2012). At the same time, the positive effect of elemental sulfur on plant nutrient availability that is in line with our results was reported by Cui *et al.* (2004).

Micronutrients uptake by shoot and grain of a maize plant

The results presented in Table 3 and 4 refer to the significant effect of humic acid and sulfur rates fertilizer on the Cu, Zn, Fe and Mn uptake by shoot and grain of a maize plant. The results showed that the increasing humic acid and sulfur fertilizer application increased the Cu, Zn, Fe and Mn uptake significantly. The highest Cu, Zn, Fe and Mn uptake (0.227, 0.801, 1.493 and 1.619 kg ha^{-1}) by shoot were obtained in treatments (T_{16}, T_{16}, T_{15}, and T_{15}) and the lowest Cu, Zn, Fe and Mn uptake (0.165, 0.574, 1.046 and 1.284 kg ha^{-1}) by shoot were obtained in treatments (T_2) of Qlyasan location.

Table 3 : Influence of humic acid and sulfur rates on the uptake of nutrients in the maize plant from Qlyasan location:

Treatments	Nutrients uptake (kg ha^{-1})							
	Cu in shoot	Cu in grain	Zn in shoot	Zn in grain	Fe in shoot	Fe in grain	Mn in shoot	Mn in grain
T_1	0.169 ^{g}	0.037 ^{hi}	0.580 ^{i}	0.145 ^{fg}	1.052 ^{h}	0.395 ^{gh}	1.292 ^{gh}	0.422 ^{fg}
T_2	0.165 ^{g}	0.035 ^{hi}	0.574 ^{i}	0.142 ^{fg}	1.046 ^{h}	0.376 ^{gh}	1.284 ^{h}	0.412 ^{fg}
T_3	0.174 ^{fg}	0.047 ^{fg}	0.577 ^{i}	0.180 ^{de}	1.047 ^{h}	0.494 ^{f}	1.306 ^{fgh}	0.524 ^{cd}
T_4	0.180 ^{efg}	0.040 ^{hi}	0.583 ^{i}	0.147 ^{fg}	1.047 ^{h}	0.413 ^{gh}	1.337 ^{f}	0.441 ^{ef}
T_5	0.174 ^{fg}	0.033 ^{i}	0.626 ^{h}	0.128 ^{g}	1.088 ^{h}	0.365 ^{h}	1.391 ^{e}	0.386 ^{g}
T_6	0.172 ^{fg}	0.039 ^{hi}	0.633 ^{gh}	0.147 ^{fg}	1.092 ^{h}	0.394 ^{gh}	1.386 ^{e}	0.424 ^{fg}
T_7	0.186 ^{ef}	0.041 ^{gh}	0.666 ^{fg}	0.161 ^{ef}	1.152 ^{g}	0.426 ^{g}	1.445 ^{d}	0.441 ^{ef}
T_8	0.194 ^{de}	0.047 ^{fg}	0.681 ^{ef}	0.180 ^{de}	1.175 ^{FG}	0.498 ^{f}	1.329 ^{FG}	0.484 ^{de}
T_9	0.187 ^{of}	0.052 ^{of}	0.722 ^{cd}	0.197 ^{cd}	1.204 ^{f}	0.519 ^{of}	1.425 ^{de}	0.524 ^{cd}
T_{10}	0.209 ^{cd}	0.056 ^{code}	0.715 ^{de}	0.228 ^{b}	1.288 ^{e}	0.604 ^{bc}	1.456 ^{d}	0.591 ^{b}
T_{11}	0.205 ^{cd}	0.063 ^{b}	0.711 ^{de}	0.227 ^{b}	1.327 ^{de}	0.610 ^{b}	1.452 ^{d}	0.606 ^{b}
T_{12}	0.213 ^{bc}	0.054 ^{de}	0.756 ^{bc}	0.195 ^{cd}	1.356 ^{d}	0.533 ^{def}	1.500 ^{c}	0.525 ^{cd}
T_{13}	0.213 ^{bc}	0.059 ^{bid}	0.770 ^{ab}	0.209 ^{bc}	1.430 ^{c}	0.560 ^{cde}	1.550 ^{b}	0.556 ^{bc}
T_{14}	0.220 ^{abc}	0.051 ^{ef}	0.773 ^{ab}	0.181 ^{de}	1.444 ^{bc}	0.483 ^{f}	1.608 ^{a}	0.481 ^{de}
T_{15}	0.231 ^{a}	0.071 ^{a}	0.795 ^{a}	0.254 ^{a}	1.493 ^{a}	0.679 ^{a}	1.619 ^{a}	0.670 ^{a}
T_{16}	0.227 ^{ab}	0.061 ^{bc}	0.801 ^{a}	0.220 ^{b}	1.479 ^{ab}	0.581 ^{bcd}	1.611 ^{a}	0.576 ^{b}
Pr > F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

However, the highest Cu, Zn, Fe and Mn uptake (0.265, 0.967, 1.720 and 1.993 kg ha^{-1}) by shoot were obtained in treatments (T_{14}). The lowest Cu, Zn, Fe and Mn uptake (0.160, 0.599, 1.053 and 1.337 kg ha^{-1}) by shoot were obtained in treatments (T_5, T_3, T_1 and T_5) of Kanypanka location, while data revealed that the Cu, Zn, Fe, and Mn uptakes were affected significantly at ($P<0.05$ level) by the application of humic acid and sulfur rate to the soil. The maximum of the Cu, Zn, Fe and Mn uptake by grain (0.071, 0.254, 0.679 and 0.670 kg ha^{-1}) were produced by (T_{15}), while the minimum Cu, Zn, Fe and Mn uptake by grain (0.033, 0.128, 0.365 and 0.386 kg ha^{-1}) were produced by (T_5) from Qlyasan location. However, the maximum of the Cu, Zn, Fe and Mn uptake by grain (0.073, 0.314, 0.664 and 0.698 kg ha^{-1}) were produced by (T_{16}, T_{16}, T_{13} and T_{16}), while the minimum Cu, Zn, Fe and Mn uptake by grain (0.045, 0.187, 0.475 and 0.503 kg ha-1) were produced by (T_5, T_5, T_1, and T_1) from Kanypanka location.

The application of humic acid led to increasing nutrients in the plant due to increased absorption and transfer of nutrients in plants by enhancing metabolism. So, humate with its positive effects on physiological processes including photosynthesis and facilitating the transfer of materials within the plant can improve the grain growth, and the humates enhance nutrient uptake, improve soil structure, and increase the yield and quality of various crops (Cordeiro *et al.,* 2011). Similar results were obtained by Hakan *et al.* (2011) and Awwad *et al.* (2015) for humic acid, and were obtained by Khan *et al.* (2006) and Habtamu (2015) for sulfur rates application, they reported that humic acid and sulfur rate had beneficial effects on nutrient uptake by plants, and was particularly crucial for the transport and availability of micronutrients due to the better developed root systems.

Sequential Chemical Extraction (SCE) of Micronutrients in the rhizosphere soil

Extractions were performed on samples from sites of Qlyasan and Kanypanka locations for elements (Cu, Zn, Fe, and Mn). The results of micronutrients chemical fractions (F_1-Exchangeable; F_2-Bound to Fe Mn oxides; F_3 -Bound to organic matter; and F_4 -Residual) concentrations in the different locations, in the rhizosphere soil, were plotted on separate charts for each of the considered metals (Cu, Zn, Fe and Mn), as shown in Figures 2 and 3.

Partitioning of Cu as determined by SCE:

Concentrations of different fractions of Cu extracted by sequential extraction from the soil in Qlyasan area was shown in Figure (2). The results indicated that the amount of exchangeable Cu extracted in (F_1) was varied between the treatments; Cu values were ranged from (0.951 to 1.215 mg kg^{-1}). The highest value was found in T_{12}, while the lowest value was found in T_4. Cu displayed its moderated recovery in the HA-HCl extraction compared to the HOAc extraction, indicating its principal presence in reducible solids. The recoverable ranges of Cu, liberated in this stage were ranged from 15.391 to 13.697 mg kg^{-1}. The highest Cu liberated during HA-HCl extraction was found in T_{16}, while the lowest value liberated was found in T_3 for Qlyasan location and the recoverable ranges of Cu, liberated in this stage were ranged from 15.398 to 13.410 mg kg^{-1}. The highest Cu liberated during HA-HCl extraction was found in T_{16}, while the lowest value liberated was found in T_3 for Kanypanka location.

Table 4 : Influence of humic acid and sulfur rates on the uptake of nutrients in the maize plant from Kanypanka location:

Treatments	Nutrients uptake (kg ha^{-1})							
	Cu in shoot	Cu in grain	Zn in shoot	Zn in grain	Fe in shoot	Fe in grain	Mn in shoot	Mn in grain
T_1	0.172 ^{de}	0.048 ^{gh}	0.634 ^{ef}	0.193 ^{hi}	**1.053 ^{cd}**	**0.475 ^{d}**	1.362 ^{g}	**0.503 ^{e}**
T_2	0.178 ^{cde}	0.047 ^{gh}	0.649 ^{ef}	0.204 ^{fghi}	1.064 ^{cd}	0.476 ^{d}	1.415 ^{efg}	0.521 ^{de}
T_3	0.175 ^{cde}	0.050 ^{fgh}	**0.599 ^{f}**	0.213 ^{efghi}	0.996 ^{d}	0.485 ^{d}	1.322 ^{g}	0.519 ^{de}
T_4	0.181 ^{cde}	0.057 ^{cdef}	0.626 ^{ef}	0.231 ^{def}	0.990 ^{d}	0.548 ^{bc}	1.395 ^{fg}	0.573 ^{c}
T_5	**0.160 ^{e}**	**0.045 ^{h}**	0.667 ^{e}	**0.187 ^{i}**	1.063 ^{cd}	0.506 ^{cd}	**1.337 ^{g}**	0.508 ^{de}
T_6	0.172 ^{de}	0.056 ^{def}	0.782 ^{d}	0.217 ^{efgh}	1.121 ^{cd}	0.511 ^{bcd}	1.516 ^{d}	0.517 ^{de}
T_7	0.176 ^{cde}	0.053 ^{efg}	0.754 ^{d}	0.208 ^{efghi}	1.131 ^{cd}	0.505 ^{cd}	1.469 ^{def}	0.525 ^{de}
T_8	0.192 ^{cd}	0.053 ^{efg}	0.767 ^{d}	0.200 ^{ghi}	1.184 ^{c}	0.506 ^{cd}	1.495 ^{de}	0.507 ^{de}
T_9	0.197 ^{c}	0.064 ^{bc}	0.893 ^{c}	0.269 ^{bc}	1.437 ^{b}	0.647 ^{a}	1.674 ^{c}	0.644 ^{b}
T_{10}	0.236 ^{b}	0.057 ^{cdef}	0.951 ^{ab}	0.234 ^{de}	1.540 ^{b}	0.560 ^{b}	1.910 ^{a}	0.555 ^{cd}
T_{11}	0.225 ^{b}	0.060 ^{cde}	0.880 ^{c}	0.227 ^{defg}	1.520 ^{b}	0.558 ^{b}	1.763 ^{b}	0.570 ^{c}
T_{12}	0.243 ^{b}	0.062 ^{cd}	0.894 ^{c}	0.248 ^{cd}	1.509 ^{b}	0.562 ^{b}	1.818 ^{b}	0.623 ^{b}
T_{13}	0.235 ^{b}	0.072 ^{a}	0.901 ^{bc}	0.312 ^{a}	1.551 ^{b}	**0.664 ^{a}**	1.772 ^{b}	0.703 ^{a}
T_{14}	**0.265 ^{a}**	0.071 ^{a}	**0.967 ^{a}**	0.305 ^{a}	**1.720 ^{a}**	0.641 ^{a}	**1.993 ^{a}**	0.694 ^{a}
T_{15}	0.232 ^{b}	0.070 ^{ab}	0.877 ^{c}	0.290 ^{ab}	1.564 ^{b}	0.638 ^{a}	1.741 ^{bc}	0.687 ^{a}
T_{16}	0.240 ^{b}	**0.073 ^{a}**	0.908 ^{bc}	**0.314 ^{a}**	1.593 ^{ab}	0.646 ^{a}	1.777 ^{b}	**0.698 ^{a}**
Pr > F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

The results indicated that the amount of Cu released during the H_2O_2 extraction was varied between the treatments; Cu values were ranged from (10.502 to 13.55 mg kg^{-1}). The higher amount of Cu liberated was found in T_{16}, while the lowest value was found in T_3 for Qlyasan location while Cu values were ranged from (10.797 to 12.094 mg kg^{-1}). The higher amount of Cu liberated was found in T_{15}, while the lowest value was found in T_3 for Qlyasan location. The aqua regia extraction was designed to extract metals remaining in the soils after the first three stages. However, it should be noted that it was not a total dissolution and likely did not extract all remaining metals, but most likely did not extract all remaining in well-crystalline oxides and some silicates (Ryan *et al.*, 2002). The value ranges of Cu, in this stage were ranged from 1.557 to 0.908 mg kg^{-1}. The highest Cu value was found in T_{16}, while the lowest value was found in T_1 for Qlyasan location and ranged from 1.401 to 0.915 mg kg^{-1}. The highest Cu value was found in T_{16}, while the lowest value was found in T_3 for Kanypanka location. These results were in agreement with those reported by (Wesolowski, 2003; Caillaud *et al.*, 2009).

Partitioning of Zn

Figures (2 and 3) showed no amount of exchangeable Zn extracted in step1 (F_1) by using HOAc can be detected, indicating that no exchangeable Zn was found, in Qlyasan and Kanypanka locations. The amounts of Zinc, extracted by using HA-HCl displayed it is the highest recovery in the HA-HCl extraction compared to the HOAc extraction, indicating its principal presence in reducible solids. The recoverable ranges of Zn, liberated in this stage were ranged from (7.249 to 7.485 mg kg^{-1}) for Qlyasan location and ranged From (8.448 to 11.177 mg kg^{-1}) for Kanypanka location. The highest Zn librated during HA-Hcl extraction was found in T_{16} and T_{15}, while the lowest values were determined in T_1 for Qlyasan and Kanypanka locations respectively. The distributions through treatments showed the values increases with increasing the humic acid and sulfur rates fertilizer. The amounts of Zn released during the H_2O_2 (F_3) extraction were relatively higher than the Zn extracted in the F_2 stage. The amounts of Zn released in this stage ranged from 13.990 to 12.349 mg kg^{-1}. The highest amount of Zn liberated was in T_{16}, with mean value 13.990 mg kg^{-1}, while T_2 contained the lowest amount of Zn determined with a mean (12.349 mg kg^{-1}) for Qlyasan location.

On the other hand, the amounts of Zn released at this stage ranged from 16.240 to 14.335 mg kg^{-1}. The highest amount of Zn liberated was in T_{16}, while T_2 contained the lowest amount of Zn for Kanypanka location. Total aqua regia extractions were also shown in Figures (2 and 3). The results showed that the values of Zn extracted by aqua regia were ranged from 46.428 to 45.400 mg kg^{-1}, the highest values were found in T_{15}, while the lowest values were found in T_5 for Qlyasan location. So, the results showed that the values of Zn extracted by aqua regia were ranged from 55.975 to 53.231 mg kg^{-1}; the highest values were found in T_{16}, while the lowest values were found in T_1 for Kanypanka location. These results were in agreement with the results reported by Wesolowski, (2003).

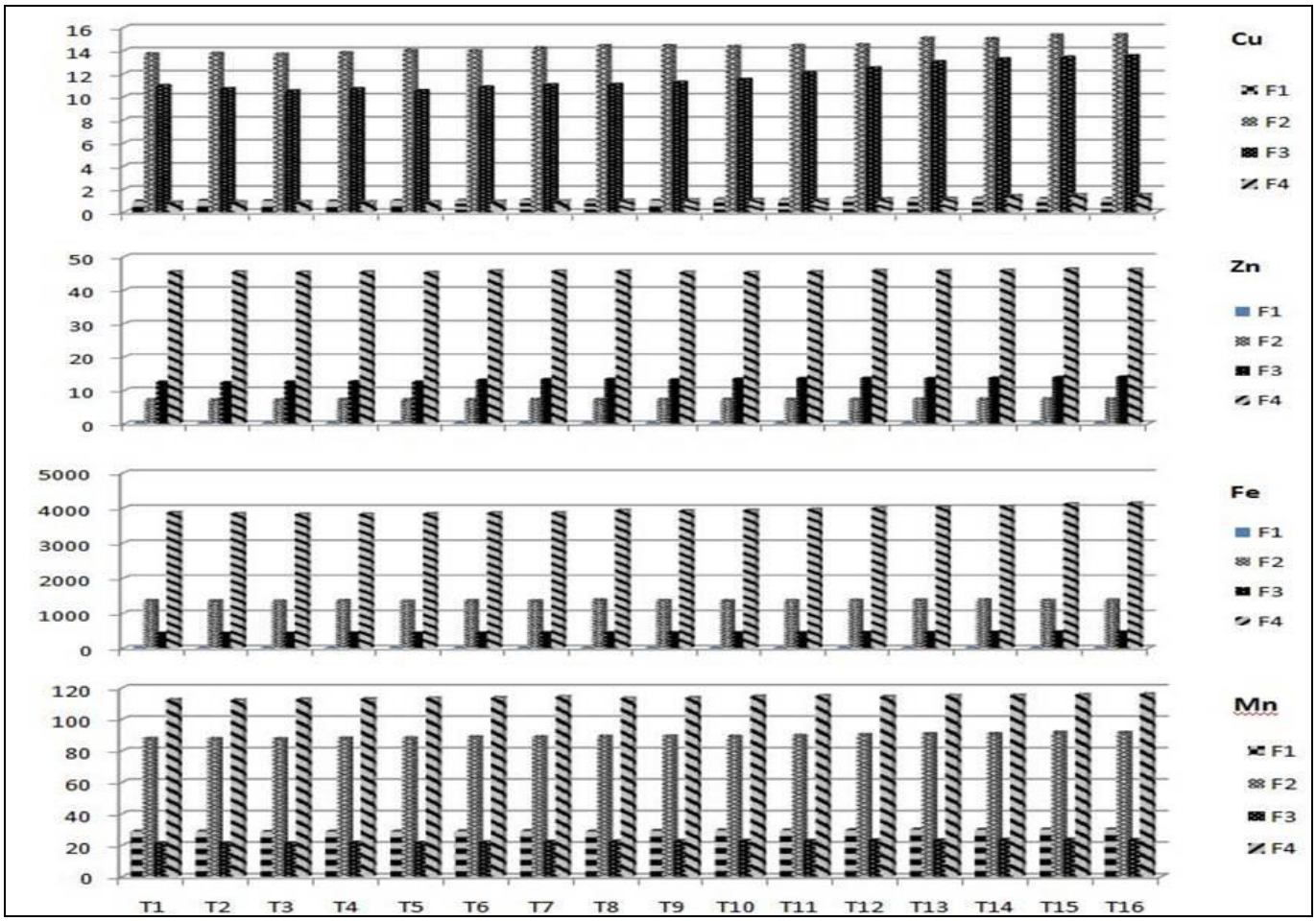

Fig. 2 : Influence of humic acid and sulfur on the distribution of some micronutrients in rhizosphere soil from Qlyasan location (mg kg^{-1} soil).

Partitioning of Fe

Figures (2 and 3) showed the different fraction of Fe extracted by sequential extraction from Qlyasan and Kanypanka locations. The results indicated that the amount of exchangeable Fe extracted by HOAc in (F_1), was low in all treatments compared to all stages, the range of exchangeable Fe was (6.556- 5.194 and 7.195 - 5.923 mg kg^{-1}) and the highest values were found in T_{15} and T_{16}, and lowest values were found in T_1 for Qlyasan and Kanypanka location respectively. Iron displayed its high concentration in the HA-HCl extraction compared to that of HOAc extraction. The recoverable ranges of Iron, liberated in this stage were ranged from 1385.613 to 1351.675 mg kg^{-1}, the highest Fe librated during HA. HCl extraction was found in T_{16}, while the lowest value determined in T_3 for Qlyasan location and Kanypanka location ranged from 1610.497 to 1561.873 mg kg^{-1}, the highest value was found in T_{16}, while the lowest value determined in T_2.

The amount of Fe released during the (H_2O_2) extraction was relatively lower than F_2 (HA-HCl extracted) stages. This implied that oxidizable sites contained a correspondingly low amount of Fe. The amount of Fe released in this stage ranged from 467.640 to 428.980 mg kg^{-1}. The higher amount of Fe liberated in T_{16} and the lower value liberated in T_2 for Qlyasan location. On the other hand, the amount of Fe released ranged from 532.110 to 495.042 mg kg^{-1}. Higher amount liberated in T_{16} and the lower value liberated in T_1 for Kanypanka location. The extraction of residual Fe in aqua regia was much more than the other three previous stages (F_1, F_2, and F_3) in all treatments ranged from 4150.743 to 3829.697 mg kg^{-1}, the highest value was found in T_{16}, and the lowest value was found in T_1 for Qlyasan location, and for Kanypanka location the values of Fe that extracted by aqua regia were ranged (4642.157-4428.741 mg kg^{-1}), and these values were much higher than the values of the other 3 steps. The highest value was found in T_{15}, while the lowest value was found in T_1. These results were in agreement with those by (Wesolowski, 2003 and Garnier *et al.*, 2009).

Partitioning of Mn

The concentrations of four fraction of Mn released during the sequential extracted from Qlyasan and Kanypanka locations in treatments were shown in Figures (2 and 3). The results indicated that the amount of exchangeable Mn extracted in step1 (F_1) was varied, and the values ranged from 30.157 to 28.638 mg kg^{-1}. The highest amount of exchangeable Mn was found in T_{16}, while the lowest amount was found in T_3, for Qlyasan location, and the values ranged from 58.325 to 52.458 mg kg^{-1}. The highest amount was found in T_{16}, while the lowest amount was found in T_1, for Kanypanka location. Manganese displayed its highest recovery in the HA-HCl extraction than the HOAc extraction. The recoverable ranges of Mn, liberated in this stage ranged from 91.820 to 87.806 mg kg^{-1}. The highest amount of Mn librated during HA-Hcl extraction was found in T_{15}, while the lowest value liberated was determined in T_2 for Qlyasan location, and for Kanypanka location the recoverable ranges of Mn, liberated in this stage ranged from 177.924 to 170.405 mg kg^{-1}. The highest amount of Mn librated during HA-Hcl extraction was found in T_{16}, while the lowest value liberated was determined in T_2.

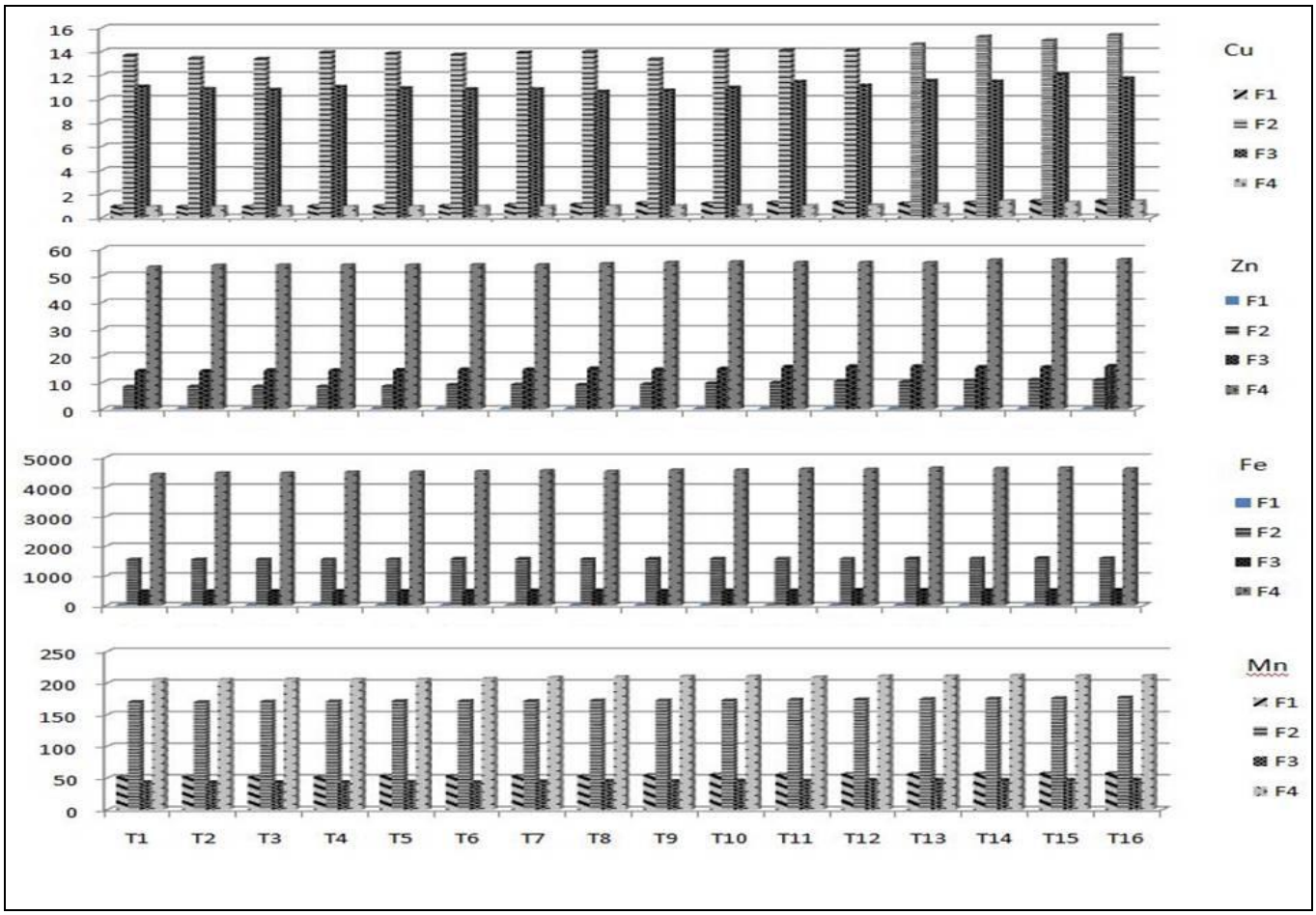

Fig. 3 : Influence of humic acid and sulfur on the distribution of some micronutrients in rhizosphere soil from Kanypanka location (mg kg^{-1} soil).

The amounts of Mn released samples during the (H_2O_2) extraction were relatively lower when compared to the other previous stages. The Mn released in this stage ranged from 23.786 to 21.462 mg kg^{-1}. The highest amount of Mn liberated in T_{15}, while T_1 contained the lowest amount of Mn determined for Qlyasan location. On the other hand, for the Kanypanka location, the Mn released ranged from 48.001 to 43.645 mg kg^{-1}. The highest amount of Mn liberated in T_{16}, while the lowest amount of Mn liberated in T_3. The amounts of Mn, extracted in the residual stage (F_4) were relatively high, and the values ranged from 116.343 to 112.471 mg kg^{-1}. The highest value extracted was recorded in T_{16}, while the lowest value liberated in T_2 for Qlyasan location, and for Kanypanka location the results showed that the values of Mn extracted by aqua regia were ranged (211.963 - 205.156 mg kg^{-1}), the highest value was found in T_{14}, while lowest value was found in T_2. These results were in agreement with those reported by (Wesolowski, 2003; Caillaud *et al*., 2009).

Conclusions

Applications of humic acid and sulfur at high rates to soils improved the soil properties of calcareous soil (pH). Also the humic acid and sulfur increased the available nutrients in the soil. As well as, the improved of micronutrients uptake by shoot and grain of maize plant. On the other hand, the sequential chemical extraction (SCE) of micronutrients in the rhizosphere soil increased with increasing rates of humic and sulfur application. In general, application of humic acid can decline, the need for chemical fertilizers and compare with other chemical and biological fertilizers. That application of humic acid not only increases the micronutrients uptake and available nutrients in the soil but also can play a significant role in achieving the goals of sustainable agriculture. Sulfur is an essential nutrient for plant growth. However, sulfur interactions with humic acid are directly related to the alteration of physiological and biochemical responses of crops, and thus required to be studied in depth. This would help to understand the nutritional behavior of sulfur about humic acid and provide guidelines for inventing balanced fertilizer recommendations to optimize yield and quality of crops and improved the soil properties.

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