# Effect of Humic Substances on Micronutrients Availability in Soils

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## ABSTRACT

During the last decades a large area of irrigated lands in Syria has been shifted from extensive cropping to intensive agriculture. Many kinds of soils became incapable to supply high yield crops by their micronutrients requirements. Therefore, the deficiency of Zn, B, Mn and Fe was mentioned in this soil. Humic substances influence micronutrients availability through chelating, which can either increase or decrease the availability of the micronutrients (MN). In this framework, two pot experiments were carried out in a greenhouse at the University of Plymouth (2006) to investigate the effect of a commercial humic substance (HS) on the micronutrients availability of: Fe, Mn ,Zn & Cu in two composite surface soil samples collected from *Vertic Cambisols* ,southwest of Syria & from *Dystric Cambisols*, southwest of England. Seedlings of cos lettuce *(Lactuca romana)* were growing in 23 May under irrigation , the drainage water was re-used for irrigation. First experiment consisted of reference, control and four low dose (HS) treatments: (50, 100, 200 & 400 mg kg$^{-1}$ soil). Soil samples were collected on 26 June &26 July for chemical analysis. The results of (MN) analysis by ICP-MS shows no significant differences in (MN) concentration between the control & the treated soil samples. Second experiment consisted of reference, control & three high dose (HS) treatments: (1500, 3000 and 4500 mg kg$^{-1}$soil). Soil samples were collected and analysed 20 days after (HS) treatment .The result showed a significant decrease in the concentration of the available micronutrients of Fe, Mn, Zn, and Cu in the treated samples compared with the control. This decrease, perhaps, occurred due to chelating the (MN) by (HS) in the early stage of application, and a 20-day period seems not to be enough time to decomposition of the humic-metal complexes. This decrease still observed after 50 days of soil samples preservation in lab conditions.

**Key Words:** Available Micronutrients, Humic Substances, Chelating, *Cambisols.*

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**chelating**

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**(2006)**

:

*Vertic Cambisols*

.        *Dystric Cambisols*

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**Control**     **Blank**    :        :

**(**   **1-**    **400,200,100,50)**

.    **48=2*4*6 (     )**

**ICP-MS**        .

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.        **AB-DTPA**     **0.01 M. CaCl$_2$**

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**50**        **20**

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**AB-DTPA**     .

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.AB-DTPA

# Introduction

During the last few decades the food demand sharply increased across the world, therefore, there are a large extensive agricultural lands has been shifted to intensive agriculture, it put the natural resources, including the land, under a high pressure. Many kinds of soils became incapable to supply the high yield crops by their micronutrient requirements.

With intensive cropping the best natively fertile soils, stress eventually occurs in the proper procedures are not followed to replace crop- removed nutrient elements, and to maintain the proper nutrient element balance for optimum plant growth (Jones, 2001)

The mechanism controlling soil micronutrient availability or deficiency is often complex and depends on soil properties including: soil texture, pH, CEC, $CaCO_3$ and organic matter, clay, redox; fertilization system and crop species and their demand.

Micronutrients have rather complex chemical relationships controlling their availability in soils (Stevenson, 1991; Tan, 1998; McBride *et al.*, 2003).

Overall, however, the relative availabilities are controlling by the equilibria that exist between the soil solution, the soil organic matter and cation exchange capacity and insoluble soil compounds (Stevenson, 1991; Chen and Stevenson, 1986).

The micronutrient deficiency in different steps became more common in many countries including the soil samples of the fields of wheat and corn in Syria .

Sillanpaa, (1982) reported  that, due to high soil alkalinity , the availability of Mn and Zn to plants are low, and the most likely micronutrient problems in Syria are shortage of these elements. At some locations a response to B fertilization may be obtain.

The deficiency of iron, zinc and boron in irrigated soil in southwest of Syria was mentioned (Abu Nukta, 1995). While the vineyard trees of table grapes in this soil were significantly responded to Zn and B fertilization (Abu Nukta  & Bat'ha ,2005).

It has long been recognized that humic substances have many beneficial effect on soil & on plant growth (Chen and Aviad, 1990; Abu Nukta, 1994; Stevenson, 1994; Nardi *et al*, 1996; Tan, 1998).

The humic substances have been long used as organic micro-fertilizer around the world .In the last decade the use of humic substances have been widely increased in intensive agriculture in Syria, particularly in greenhouses, vegetables and vineyards (Abu Nukta, 1995).

Humates are the most of humic substances are used, they are commercial products, usually prepared from leonardite, which may contain up to 60 % humic & fulvic acids. Humates most likely to be a mixture of humates, fulvates, humin and other components that may exist in the organogenic mine. Special properties of this material result from the extreme heterogeneity and their chemical reactivity.

These materials are composed of complicated organic mixtures which are linked together in random manner, resulting in extraordinarily complex materials (Stevenson, 1994; Tan, 1998; Garcia-Mina *et al.,* 2004).

Soil organic matter or humic substances influence micronutrients availability through chelating, which can either increase or decrease the available micronutrients (Mortvedt *et al*.,1991; Macwiak *et al.*, 2001), Although the solubility of chelated metals is generally increased over the inorganic form, the process usually decreases the activity of the ion in solution. Copper is particularly affected by the formation of chelates. Manganese and zinc are similarly affected at very high organic matter levels (Boyd and Somme, 1981; Reichman, 1994; McBride *et al*. 2003; Sanchez *et al*., 2006).

A chelate complex is formed when two or more coordinate positions about the metal ion are occupied by donor groups of a single ligand to form an internal ring structure. In soil role of ligands fulfilment simple organic compounds and function groups of humic substances (Tan, 1998). The order of decreasing affinity of organic groups for metal ions is approximately as follows: $-O->-NH_2>-N=N->=N>-COO->C=O$. While the order of decreasing ability of metal ions to chelating with humic acids as follows : $Fe^{3+}>Cu^{2+}>Ni>Co>Zn>Fe^{2+}>Mn^{2+}$ (Stevenson, 1994).

Humic substances have an important ability to be chelating –agents –they are in fact excellent in this role as strong enough to protect the micronutrients from leaching, but weak enough to release micronutrients to plants when required. The cationic micronutrients are most likely deficient on calcareous soils or soil high in organic matter where strong chelating decreases availability (Tan, 1998;

Garcia-Mina *et al*, 2004). Humic substances have been most effective in soils with less than two percent organic matter.

Micronutrients, especially iron may be made more available to plants in the presence of humic substances. Inorganic iron compounds are very unstable in soil and tend to become insoluble and unavailable, especially in calcareous soil. HS can incorporate iron into chelate, maintaining its availability to plants, although still in insoluble form (Pinton *et al.,* 1999; Ramasamy *et al.,* 2006).

Essential micronutrient cations that might normally be expected to precipitate at pH ranges found in most soils are maintained in solution through complexation with many organic compounds.

Enhanced plant growth following addition of humic substances has sometimes been related to increased micronutrient availability especially iron and zinc. There are also numerous reports of metal concentration being reduced to non- toxic levels following addition of complexion humic substances. Soil pH and OM content significantly affect the solubilities of Fe, Mn, Zn &Cu (Prasad and Sinha, 1982).

Organic carbon is significantly positive correlated with available Zn & Mn whereas available Mn significantly correlated with pH (Tan, 1998).

## Materials and methods

### Soil samples:

*Syrian soil:* A composite soil sample consisted of 16 sub-samples which were collected from representative soil sites, at depth of 0-25 cm, from irrigated land in southwest of Syria (Dar'aa governorate). Soil classification is: *Vertic Cambisols; Typic Haploxerepts*; and *Vertic Cinnamon*, according to FAO, USA Soil Taxonomy and Russian systems respectively,(Abu Nukta, 2004). Table 1 shows characteristics of the composite soil sample of Syria. Table 2 shows the available micronutrient destribution in soil profiles of three new irrigated fruit farms; olive, vineyard and pear trees, in *Vertic Cambisols*, southwest of Syria (Dar'aa governorate). The land was cultivated by rainfed crops for centuries. Deficiency symptoms of iron, zinc and boron were clearly seen on grape trees in many seasons (Abu Nukta, 1995; Abu Nukta, and Bat'ha, 2005). This table indicates the following levels:

Zn and B are very low and low, Cu is high, in all samples, Fe is low in the upper 50 cm and medium in the depth, in the contrary Mn is medium in upper 50 cm and becomes low in the depth.

**Table (1). Characteristics of composite soil sample (Syria).**

**Water Extraction**

| pH | EC1:5 dS m⁻¹ | Salt % | HCO₃ | Cl | SO4 | Ca | Mg | Na | K |
|----|------|------|------|----|-----|----|----|----|---|
| | | | cmol kg⁻¹ | | | | | | |
| 7.86 | 0.47 | 0.15 | 1.31 | 0.72 | 0.16 | 0.80 | 0.56 | 0.80 | 0.03 |

**Exchangeable cations (cmol kg⁻¹) , calcium carbonate and humus %**

| Ca | Mg | Na | K | CaCO₃ | Active Lime | Humus |
|----|----|----|---|-------|-------------|-------|
| 31.29 | 10.00 | 6.66 | 1.68 | 13.60 | 6.80 | 1.03 |

**Microelements (DTPA and Hot Water) mg kg⁻¹**

| Fe | Mn | Zn | Cu | B |
|----|----|----|----|---|
| 12.57 | 13.16 | 0.92 | 3.62 | 0.95 |

**Soil Texture %**

| Clay | Silt | Sand | Texture |
|------|------|------|---------|
| 39.50 | 40.50 | 20.00 | Clay Loam |

**Table (2). The available micronutrients in irrigated *Cambisols* in southwest of Syria (DTPA ext., mg kg⁻¹ soil).**

**A-  Field of olive trees.**

| Soil depth, cm | pH | CaCO₃% | Fe | Mn | Zn | Cu | B |
|----------------|----|--------|-----|------|------|------|------|
| 0-25 | 8.0 | 19.56 | 9.59 | 10.00 | 0.37 | 1.76 | 0.61 |
| 25-50 | 8.0 | 20.47 | 10.30 | 8.32 | 0.23 | 1.92 | 0.63 |
| 50-75 | 7.9 | 22.74 | 11.53 | 9.02 | 0.24 | 1.69 | 0.57 |
| 75-100 | 7.8 | 25.01 | 11.92 | 7.72 | 0.12 | 1.45 | 0.71 |

**B-  Field of vineyard trees.**

| Soil depth, cm | pH | CaCO₃% | Fe | Mn | Zn | Cu | B |
|----------------|----|--------|-----|------|------|------|------|
| 0-25 | 7.8 | 17.28 | 8.02 | 11.71 | 0.73 | 2.58 | 0.42 |
| 25-50 | 7.8 | 18.19 | 9.12 | 10.27 | 0.37 | 2.14 | 0.46 |
| 50-75 | 7.8 | 20.47 | 11.48 | 7.69 | 0.27 | 1.75 | 0.67 |
| 75-100 | 7.8 | 22.74 | 10.67 | 8.07 | 0.25 | 1.54 | 0.71 |

**C-  Field of pear trees.**

| Soil depth, cm | pH | CaCO₃% | Fe | Mn | Zn | Cu | B |
|----------------|----|--------|-----|------|------|------|------|
| 0-25 | 7.8 | 15.01 | 9.55 | 9.87 | 0.48 | 2.39 | 0.56 |
| 25-50 | 8.0 | 15.92 | 10.85 | 8.11 | 0.31 | 2.11 | 0.46 |
| 50-75 | 7.9 | 22.74 | 13.86 | 7.37 | 0.20 | 1.81 | 0.50 |
| 75-100 | 7.8 | 27.29 | 14.71 | 6.38 | 0.22 | 1.69 | 0.60 |

*English soil:* A composite soil sample was collected from representative soil sites of southwest of England (Devon district). Soil classification is: *Dystric Cambisols* (Soil atlas of Europe, 2005); *Dystrochrepts* , and *Meadow Cinnamon* ,according to FAO, USA Soil Taxonomy and Russian systems respectively,(Abu-Nukta, 2004). Table 3 shows characteristics of the composite soil sample of England.

**Table (3). Characteristic of soil sample of *Dystric Cambisols*, England**

| pH | EC$_e$ dS m$^{-1}$ | O.M. | Fe | Mn | Zn | Cu | Texure |
|----|------|------|------|------|-----|-----|--------|
|    |      |      | mg kg$^{-1}$ |||| |
| 5.8 | 0.22 | 3.4 | 61.5 | 26.4 | 4.2 | 7.1 | Silt Loam |

Table 4 shows characteristics of humic substances used in experiment.

**Table (4). Characteristics of humic substance.**

| pH 1:10 susp. | EC 1:10 dSm$^{-1}$ | Moist. | P | K | O.M | Ash | Total Acidity | COOH | OH (phenol) | C=O |
|------|------|------|------|------|------|------|------|------|------|------|
|      |      | % |||| | Meq g$^{-1}$ |||| |
| 9.2 | 19.0 | 5.43 | 1.91 | 2.60 | 70.74 | 29.26 | 2.81 | 1.14 | 1.67 | 0.42 |

The concentration of micronutrients in irrigation water is negligible.

# Methods

*Greenhouse experiment*: Two pot experiments in randomised complete block design in six treatments in the first experiment and five treatments in the second and four replications for each were carried out in glasshouse at university of Plymouth (2006), to investigate the effect of humic substances (HS) on the concentration of available micronutrients in composite surface soil samples from Syria and England. Tables 1&2 showed the characteristics of soil samples.

Small plastic pots ($\approx$ 400 mL) were used, holes in the bottom of pots were covered with filter paper, pots were partly filled ($\approx$75mL) with perlite to excess irrigation water to drain off. Two hundred grams of fine earth were filled in each pot, and enough space was left in the top of pot for water irrigation.

Two cos lettuce seedlings were transplanted in every pot, soil was irrigated to moisture field capacity level, irrigation was done 2-3 times

per week depening on weather conditions. Drainage water was collected in dish beneath each pot to re-use for irrigation. Dish was washed to re-use that water in irrigation.

A commercial dry (HS), perhaps, (k-humate) was added one time in each experiment in solution form according to doses shown below after the seedlings transplant. Table 4 shows characteristics of the (HS) used.

*First experiment consisted of:*

-Reference: Air-dry soil samples without any treatment.

-Control: Soil samples with lettuce seedlings, untreated.

-Four low dose (HS) treatments (50,100, 200,and 400 mg kg$^{-1}$soil).

The experiment was lasted from 23 May to 26 July.

*Soil sampling*

From each pot a small soil sample was collected twice, on 26 June and on 26 July for chemical analysis.

*Second experiment consisted of:*

-The same reference and control of the first experiment.

-Three high dose (HS) treatments (1500, 3000 and 4500 mg kg$^{-1}$ soil), on 26$^{th}$ of July.

From each pot a soil sample was collected one time, 12 days after (HS) application and analysed twice after collection in 14$^{th}$ of August and 4$^{th}$ of October.

*Laboratory Analyses:*

Three collections of soil samples were air-dried, sieved through non metallic 2-mm sieve and submitted to laboratory analyses in July for first collection, in August for two other collections and again in October for the third collection.

*Micronutrients extraction:*

Four extract solutions were used :

-DTPA, (Lindsay, 1977) for available micronutrients in *Cambisols* of Syria.

-0.01 M. Ca Cl$_2$, (Houba *et al.*, 2000)

-AB- DTPA, (Soltanpour, 1991)

- 0.1 M. Ca Cl$_2$, (Smilde *et al.*, 1992)

*Humic substance:*  It was dissolved in water and filtered before analysis.

### *Instruments used:*

Inductively Coupled Plasma –Mass Spectrometry (ICP-MS), FISONS Instruments was used to determine, Mn, Zn and Cu in low concentration extract, (0.01 and 0.1 M.CaCl$_2$). ICP is a type of a mass spectrometry that is highly sensitive and capable of analysis many metals and non-metals at below one part in $10^{12}$. It is based on coupling together an inductively coupled plasma as a method of producing ions (ionization) with a mass spectrometer as a method identifying and detecting the ions.

THGA Graphite Furnace, SIMAA 6000was used for iron determination.

FAAS, Varian, Spectra AA 50, GBC 902 Double Beam AAS and GTA 100,VARIAN- SpecterAA  880 were used for Fe, Mn, Zn and Cu determination in AB- DTPA and DTPA extracts.

*Statistical analysis:* It was conducted using SAS statistical package for two factor analysis of variance, and by a single factor ANOVA. T-test was used for 0.01& 0.1 M. Ca Cl$_2$ extractions.

## Results and discussion

### *First experiment:*

In the first soil samples collection, micronutrients were extracted by 0.01 M.CaCl$_2$.

Table 5 shows that the concentration of these elements are very low, and measured by ppb in soil, and should conceder as a fraction of the available or adsorbed elements (Houba *et al*.2000).There were no significant differences in the elements concentration between the control and the treated soil samples, but at the same time the concentration of Fe and Zn decreased significantly in the control comparing with the reference, perhaps it occurs due to the absorption of these elements by crop. In English soil samples there are no significant differences in Fe and Zn concentration between the control and the treatment. Extractable Mn decrease significantly in the treatments 2and 3 comparing with the control, and no significant difference in other treatments, this decrease probably resulted from chelating.

**Table(5). Extractable concentration of micronutrients** $\mu g\ kg^{-1}$ **(0.01 M** *CaCl2***)**

**A-Syrian soil.**

|        | Fe    | Mn      | Zn    | Cu    |
|--------|-------|---------|-------|-------|
| 0      | 51.75 | 967.25  | 75.6  | 60.5  |
| 1      | 14.75 | 827.5   | 13.2  | 49.55 |
| 2      | 11.75 | 884.5   | 9.75  | 42.65 |
| 3      | 17.98 | 1238.5  | 25.7  | 37.3  |
| 4      | 32.75 | 1180    | 13.28 | 45.65 |
| 5      | 25    | 2071.5  | 34.98 | 43    |
| LSD 5% | 29.2  | 1411.2  | 34.0  | 28.0  |

**B-English soil.**

|        | Fe    | Mn      | Zn    | Cu    |
|--------|-------|---------|-------|-------|
| 0      | 12.15 | 1159.75 | 7.83  | 44.88 |
| 1      | 13    | 1601.75 | 22.25 | 57.48 |
| 2      | 14.5  | 997.25  | 32.05 | 50.5  |
| 3      | 32    | 968.75  | 13.75 | 48.4  |
| 4      | 19    | 1087    | 21.68 | 40.88 |
| 5      | 6.73  | 1282.5  | 10.95 | 41.68 |
| LSD 5% | 23.2  | 593.0   | 28.7  | 13.2  |

Concentrations of Mn in both soils and Fe in English soil are higher significantly in 0.1 M. CaCl$_2$ in comparison with 0.01 M. CaCl$_2$ extraction.

In the second soil samples collection, the micronutrients were extracted by tow extractants AB-DTPA and 0.01 M. CaCl$_2$.

Table 6 shows no significant differences in Fe, Mn and Zn concentrations extracted by AB-DTPA in all soil samples, Cu concentration significantly decreased in the treatments in comparison with the control in both soils, it is probably because Cu is capable to make chelate more than other elements (Stevenson, 1991). There is a decrease of Fe and increase of Mn in the reference in comparison with the control, it's perhaps due to cropping practices.

**Table (6).Extractable concentration of micronutrients** $mg\,kg^{-1}$ **(AB-DTPA)**

**A-Syrian soil**

|         | Fe    | Mn    | Zn    | Cu   |
|---------|-------|-------|-------|------|
| 0       | 9.38  | 12.10 | 8.11  | 4.88 |
| 1       | 13.5  | 10.21 | 10.48 | 5.49 |
| 2       | 13.32 | 10.09 | 9.37  | 5.45 |
| 3       | 13.14 | 10.78 | 10.35 | 5.30 |
| 4       | 12.06 | 9.76  | 11.13 | 5.22 |
| 5       | 12.78 | 9.08  | 12.99 | 5.17 |
| LSD 5%  | 1.4   | 3.8   | 2.9   | 0.2  |

**B-English soil**

|         | Fe    | Mn    | Zn   | Cu   |
|---------|-------|-------|------|------|
| 0       | 63.75 | 27.10 | 4.35 | 7.17 |
| 1       | 75.94 | 14.89 | 3.97 | 7.15 |
| 2       | 76.94 | 13.47 | 3.77 | 6.85 |
| 3       | 74.73 | 13.06 | 3.89 | 6.99 |
| 4       | 73.23 | 13.59 | 3.82 | 6.71 |
| 5       | 72.90 | 12.26 | 4.72 | 6.66 |
| LSD 5%  | 4.6   | 3.71  | 1.0  | 0.23 |

Table(7) shows a significant increase of Fe, Zn and Cu concentration extracted by 0.01M $CaCl_2$ in July compared with extracted in June from the 5th and partly from the 4th treatment.

*Second experiment:*

It started at the end of the first experiment on 26 July and consisted of the same reference and control, soil samples were collected one time in 7th of August and analysed tow times in 14th of August and 4th of October. AB-DTPA was used to extract (MN).

**Table (7). Extractable concentration of micronutrients** $\mu g\,kg^{-1}$ **(0.01 M** $CaCl_2$**) June and July**

**A-Syrian soil**

|         | Fe     | Mn      | Zn     | Cu     |
|---------|--------|---------|--------|--------|
| 0       | 51.75  | 967.25  | 75.6   | 60.5   |
| 1       | 14.75  | 827.5   | 13.2   | 49.55  |
| 4 Jun   | 32.75  | 1180    | 13.28  | 45.65  |
| 5 Jun   | 25     | 2071.5  | 34.98  | 43     |
| 4 July  | 160.75 | 641     | 125.8  | 196.25 |
| 5 July  | 368.5  | 728     | 499.25 | 250.5  |
| LSD 5%  | 144.91 | 1357.37 | 124.01 | 59.34  |

**B-English soil**

|         | Fe    | Mn      | Zn     | Cu     |
|---------|-------|---------|--------|--------|
| 0       | 12.15 | 1159.75 | 7.84   | 44.88  |
| 1       | 13    | 1601.75 | 22.25  | 57.48  |
| 4 Jun   | 19    | 1087    | 21.68  | 40.88  |
| 5 Jun   | 6.73  | 1282.5  | 10.95  | 41.68  |
| 4 July  | 69.1  | 253.95  | 171.65 | 172.75 |
| 5 July  | 80.75 | 463.25  | 402.63 | 217.58 |
| LSD 5%  | 25.30 | 593.35  | 76.13  | 105.97 |

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Table 8 shows the concentration of the extracted (MN) in August. The concentration of extracted Fe, Mn, Zn and Cu decreased significantly in the treated samples in comparison with the control in both soils. This result indicated the transformation of the available (MN) to unavailable due to chelating by (HS) or precipitation under (HS) effect. Table 8 shows that the concentrations of Mn and Cu are approximately similar in both soils, while in Syrian samples the concentration of available Zn is three times higher and available Fe is six times lower in comparison with English soil samples, it is related to the pedogenesis differences between both soils. Table 9 shows (MN) analysis after 50 days of preservation. A significant difference still existed between the reference and the most of treated soil samples, except for Zn in Syrian samples. Table 10 indicates a comparison between August and October (MN) analysis. Preservation of Syrian soil samples makes the Fe, Mn and Cu less available, and Zn more available, while preservation of English soil samples makes Fe, Zn and Cu more available, and Mn less available. It may conclude that the (MN) availability in this experiment differs depend on the kind of element, chelating capability and soil type.

**Table (8). Extractable concentration of micronutrients $mg\,kg^{-1}$ (AB-DTPA), August**

**A- Syrian soil**

|        | Fe    | Mn    | Zn    | Cu    |
|--------|-------|-------|-------|-------|
| 0      | 9.38  | 12.11 | 8.03  | 4.88  |
| 1      | 13.5  | 10.46 | 10.48 | 5.49  |
| 2      | 7.38  | 7.45  | 8.06  | 4.4   |
| 3      | 8.5   | 8.37  | 8.61  | 4.45  |
| 4      | 11    | 11.07 | 8.12  | 4.36  |
| LSD 5% | 1.012 | 1.623 | 1.719 | 1.019 |

**B-English soil**

|        | Fe    | Mn    | Zn   | Cu   |
|--------|-------|-------|------|------|
| 0      | 63.75 | 27.10 | 4.36 | 7.18 |
| 1      | 75.94 | 14.88 | 3.97 | 7.15 |
| 2      | 45.38 | 6.33  | 2.91 | 5.19 |
| 3      | 53.75 | 7.42  | 3.05 | 5.32 |
| 4      | 57.13 | 11.22 | 3.12 | 5.51 |
| LSD 5% | 3.02  | 1.94  | 0.95 | 1.74 |

**Table (9). Extractable concentration of micronutrients** $mg\,kg^{-1}$ **(AB-DTPA) Oct.**

**A-Syrian soil**

|  | Fe | Mn | Zn | Cu |
|---|---|---|---|---|
| 0 | 9.32 | 13.61 | 9.75 | 4.94 |
| 2 | 5.93 | 6.86 | 11.09 | 4.26 |
| 3 | 6.27 | 4.31 | 9.3 | 3.93 |
| 4 | 8.15 | 10.12 | 9.9 | 4.09 |
| LSD 5% | 0.77 | 2.09 | 2.33 | 0.24 |

**B-English soil**

|  | Fe | Mn | Zn | Cu |
|---|---|---|---|---|
| 0 | 61.6 | 35.19 | 4.9 | 6.27 |
| 2 | 59.62 | 4.75 | 3.88 | 5.52 |
| 3 | 64.58 | 7.60 | 3.73 | 5.7 |
| 4 | 51.4 | 3.74 | 3.58 | 5.43 |
| LSD 5% | 3.75 | 0.90 | 0.36 | 0.41 |

**Table (10). Extractable concentration of micronutrients** $mg\,kg^{-1}$ **(AB-DTPA) Aug.Oct**

**A-Syrian soil**

|  | Fe | Mn | Zn | Cu |
|---|---|---|---|---|
| 0 | 9.32 | 13.61 | 9.75 | 4.94 |
| 2 Aug | 7.38 | 7.45 | 8.06 | 4.4 |
| 3 Aug | 8.5 | 8.37 | 8.61 | 4.46 |
| 4 Aug | 11 | 11.07 | 8.12 | 4.36 |
| 2 Oct | 5.93 | 6.86 | 11.09 | 4.26 |
| 3 Oct | 6.27 | 4.31 | 9.3 | 3.93 |
| 4 Oct | 8.15 | 10.12 | 9.9 | 4.09 |
| LSD 5% | 0.92 | 1.66 | 1.96 | 0.18 |

**B-English soil**

|  | Fe | Mn | Zn | Cu |
|---|---|---|---|---|
| 0 | 61.6 | 35.19 | 4.9 | 6.27 |
| 2 Aug | 45.38 | 6.33 | 2.93 | 5.19 |
| 3 Aug | 53.75 | 7.42 | 3.05 | 5.32 |
| 4 Aug | 57.13 | 11.22 | 3.12 | 5.51 |
| 2 Oct | 59.63 | 4.75 | 3.88 | 5.52 |
| 3 Oct | 64.58 | 7.60 | 3.73 | 5.7 |
| 4 Oct | 51.4 | 3.74 | 3.58 | 5.43 |
| LSD 5% | 3.47 | 1.58 | 0.29 | 0.37 |

## Conclusions

- The treatment of soil samples by humic substance in low doses (50- 400 mg kg$^{-1}$soil) did not affect 0.01 M. CaCl$_2$ – extractable Fe, Mn, Zn and Cu in soil one month after the treatment date, however there is a significant increase two mouths after the application date in 5$^{th}$ and partly in 4$^{th}$ treatment.

- The treatment of soil samples by humic substance in high doses (1500- 4500 mg kg$^{-1}$soil) led to decrease the available Fe, Mn, Zn and Cu after 20 days of treatment date, in comparison with the control, it occurs probably due to chelating or precipitation.

- Fifty day period of soil samples preservation under lab. conditions was not enough time for decomposition of the organo-metal complexes (chelates). Zinc was the only element which released after the preservation period.

The use of humic substances in high doses in Syrian soil should be accompanied with micronutrients fertilizers.

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