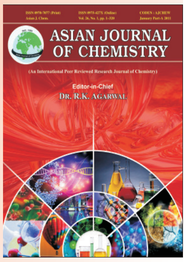
Asian Journal of Chemistry; Vol. 26, No. 13 (2014), 3977-3980

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16234



Influence of Humic Acid on Availability of Zn, Cu, Mn, Fe in Soils

CAFER TÜRKMEN* and ALI SUNGUR

Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Çanakkale Onsekiz Mart University, 17020 Çanakkale, Turkey

*Corresponding author: Fax: +90 286 2180545; Tel: +90 286 2180018/1316; E-mail: turkmen@comu.edu.tr

Received: 24 August 2013; *Accepted*: 17 December 2013; *Published online*: 23 June 2014; AJC-15386

The objective of this study was to investigate the influence of an organic substance, humic acid, on availability of extractable Zn, Cu, Mn and Fe in soils. Humic acid was applied in four different doses (0, 3, 6 and 12 L/da) and all treatments were incubated. During the incubation, soil samples were taken at four different times (I, II, IV and VIII weeks). In these soil samples Cu, Fe, Mn and Zn ions were extracted through diethylene triamine pentaacetic acid method and flame atomic absorption spectrometer was used for elemental analyses. Statistical analyses were performed by using MINITAB-16 statistical software program. Results revealed significant differences in Fe, Mn and Zn concentrations of dose-time interaction at $p < 0.01$ level and in Cu concentrations at $p < 0.05$ level. The differences in pH values of dose-time interaction were also found to be significant at $p < 0.01$ level. With regard to sampling times, the differences in electrical conductivity values were found to be significant at $p < 0.01$ level.

Keywords: Diethylene triamine pentaacetic acid extraction, Organic substance, Soil, Zn-Fe-Cu-Mn.

INTRODUCTION

In agriculture, various inorganic and organic amendments are frequently used to improve the soil properties. Many of them may modify significantly the mobility of metals (and also nutrients) in soils. The organic matter of soil with a key role in metal mobility throughout soil profile consists mainly of humic substances-humic and fulvic acids[1]. Humic substances have an interaction capacity with metal ions. Thus, they are commonly used as the indicators (agents) of metal mobility in soils. Humic substances, therefore, expose a strong control over metal behaviours in environmental media[2]. Transport of organic and inorganic pollutants and/or nutrients in soil and water are significantly affected by the mobility of natural dissolved organic substances[3]. Determination of total or almost total amounts of metals in soils provides information about metal accumulation in soils. But, total metal contents of soil does not indicate mobile amounts or the available amounts for plants. Therefore, different extraction methods are employed to determine the mobile or available fractions of the metals in ions[4].

Diethylene triamine pentaacetic acid (DTPA) extraction is commonly used by the researchers to determine the available elements for plants in environmental samples like soils[5-10] since DTPA extraction provides a chemical evaluation of the amount of metals that are available for plant uptake[7,11]. DTPA soil test was originally described by Lindsay and Norvell[5] for extraction of Cu, Fe, Mn and Zn in near neutral calcareous soils. Its use was extended in time, with relative success, to other soil conditions. Nowadays, DTPA is worldwide accepted after international standardization and incorporation into international soil analysis testing programs[12].

Basic objectives of the present study were set to investigate the effects of a liquid humic substance applied under controlled conditions to an agricultural soil on available copper, iron, manganese and zinc concentrations; to evaluate the effects of sampling time and dose of application and to put forward the variations of effects with same sampling. The soil samples with humic substance treatment were incubated and re-sampled in different times. Then, samples were exposed to DTPA extraction for available copper, iron, manganese and zinc and elemental analyses were performed with a flame atomic absorption spectrometer.

EXPERIMENTAL

The humic substance used in this research is produced by the General Directorate of Turkish Coal Enterprises (TKI-Hümas). It has a pH of between 11-13, organic matter content of 5 % and total (humic + fulvic) acid content of 12 %. All chemicals were of analytical reagent grade (Merck, Germany). In all experiments, double deionized pure water (TKA, GenPure, 18.2 MΩ/cm resistivity) was used. A SANYO model incubator, a Heidalph Model UNIMAX 2010 shaker, an M-tops Model HP 330 hotplate, a inoLab Model WTW pH meter and a HACH Model HQ 40d conductivity meter were used throughout the analyses. Copper, iron, manganese and zinc concentrations of samples were analyzed with an Analytic Jena Model novAA-350 flame atomic absorption spectrometer (FAAS).

Experimental design and sampling: Humic substance was applied to experimental soils in 4 different doses. Experiments were performed in randomized block design with 5 replications over 20 plots (4 doses × 5 replications) (Table-1). Humic substance doses were calculated for 1.5 kg soil and applied as 0, 3, 6, 12 L/da. Control treatment had 0 L/da implementation. Doses were arranged as control, the dose recommended by producer firm (6 L/da), half of the recommended dose (3 L/da) and double of the recommended dose (12 L/da). Liquid substance was applied to soil with pre-determined field capacity through irrigations with distilled water. Samples were incubated at 27 °C. Experimental pots were covered with stretch films to prevent abrupt moisture losses and a total of 15 holes were opened with a pen-nib. Pots were weighed weekly and moisture deficit was brought to field capacity with distilled water accordingly.

Soil samples were taken during the I, II, IV and VIIIth weeks of incubation to evaluate the changes in time. pH and EC values of entire samples were determined, DTPA extractions were performed and Cu, Fe, Mn and Zn concentrations were determined with FAAS right after extraction.

Analysis: The pH and electrical conductivity (EC) of soil samples in 1:2.5 (sample: water ratio, w/v) suspension was determined by a pH meter[13] and a conductivity meter[14]. In addition, calcium carbonate content was obtained by means of a calcimeter[15]. Soil grain distribution (texture) was determined with a hydrometer in accordance with the principles specified by Bouyoucos[16]. Total Cu, Fe, Mn and Zn contents were determined by hot-plate digestion technique with a aqua regia solution. Diethylene triamine pentaacetic acid (DTPA) extraction method was employed to determine available Cu, Fe, Mn and Zn concentrations of the soil samples[5]. For extractions, 10 g soil sample was placed into 250 mL Erlenmeyer flask; 20 mL DTPA solution was added and shaken at 180 rpm for 2 h. Then, the samples were filtered through Whatman-42 filer paper and made ready for analysis. Pearson correlation was applied to data to determine the relationships among available Cu, Fe, Mn and Zn concentrations, applied humic substance doses and their variations in time. Statistical analyses were performed by using MINITAB-16 statistical software.

RESULTS AND DISCUSSION

Characteristics of experimental soil are provided in Table-2. Experimental soil is almost neutral with pH values between 7.28-7.33 (average 7.30), unsaline with electrical conductivity values between 351-399 μS/cm (average 374 μS/cm). The soil has sandy-loam texture (SL) with low lime content (1.5 %). Total Cu varied between 41.42-45.26 ppm (average 43.44 ppm), Fe between 17895-17990 ppm (average 17946 ppm), Mn between 735.6-750 ppm (average 742 ppm) and Zn between 53.85-58.24 ppm (average 55.86 ppm). Results revealed that experimental soils were within typical ranges of world soils[17,18].

TABLE-2
DESCRIPTIVE STATISTICS OF GENERAL SOIL CHARACTERISTICS (n = 3)

Soil characteristics	Min. value	Max. value	Mean ± SD*
pH	7.28	7.33	7.30 ± 0.03
EC (μS/cm)	351	399	374 ± 24
% $CaCO_3$	0.9	2.1	1.5 ± 0.6
% Clay	17	19	18 ± 1
% Silt	19	22	21 ± 2
% Sand	60	64	62 ± 2
Total Cu (ppm)	41.42	45.26	43.44 ± 1.93
Total Fe (ppm)	17895	17990	17946 ± 48
Total Mn (ppm)	735.6	750.4	742.6 ± 7.4
Total Zn (ppm)	53.85	58.24	55.86 ± 2.22

*Standard deviation

Results revealed significant effects of dose x sampling time interaction on pH, available Fe, Mn and Zn concentrations at $p < 0.01$ and on available Cu concentrations at $p < 0.05$. On the other hand, sampling time alone had significant effects on EC values at $p < 0.01$ significance level.

Within the scope of experiments, doses and sampling times had significant impacts on pH values of entire samples. Despite the irregular distribution of pH values, the lower pH values were observed at the highest dose of 12 L/da. Therefore, humic substance supplementation may be considered as a significant implementation in structural improvement of alkaline agricultural soils and to provide support in nutrient intake in such soils. On the other hand, it was remarkable that electrical conductivity values of entire samples increased with sampling timing rather than with humic substance doses (Table-3).

Copper is the least mobile heavy metal in soils[18] and Cu was mostly correlated with organic fraction in several researches[19,20]. Results revealed relatively increased Cu mobility and increasing available levels through the end of incubation period (in IVth week sampling). Such a case indicates the 4th week as the optimum period with regard to impacts of humic substances on available Cu levels. Despite the irregular distribution of Cu values, the highest dose (12 L/da) and the longest sampling time (VIII. week) had the lowest Cu levels (Table-3). Such a case indicates increasing immobility levels of Cu with increasing humic substance dose and incubation period. Copper has also high affinity over organic matter and therefore present results may be explained by complex formation of Cu with increasing humic substance. Some authors reported that organic matter applications to soil did not increase plant tissue Cu contents[21].

TABLE–1
EXPERIMENTAL DESIGN

4* (0 L/da HS**)	13 (6 L/da HS)	1 (0 L/da HS)	8 (3 L/da HS)	19 (12 L/da HS)
12 (6 L/da HS)	2 (0 L/da HS)	16 (12 L/da HS)	3 (0 L/da HS)	10 (3 L/da HS)
9 (3 L/da HS)	20 (12 L/da HS)	7 (3 L/da HS)	14 (6 L/da HS)	15 (6 L/da HS)
18 (12 L/da HS)	6 (3 L/da HS)	11 (6 L/da HS)	17 (12 L/da HS)	5 (0 L/da HS)

4*: Pot number, HS**:Doses of Humic Substances

TABLE-3
DTPA-EXTRACTED Cu, Fe, Mn AND Zn CONCENTRATIONS (ppm ± SD), n = 5

Elements	Doses (L/da)	Sampling Time			
		1st week	2nd week	4th week	8th week
Cu	0	1.97 ± 0.18	2.32 ± 0.11	1.96 ± 0.08	1.72 ± 0.16
	3	1.95 ± 0.26	1.97 ± 0.25	1.92 ± 0.13	1.69 ± 0.15
	6	1.92 ± 0.46	1.84 ± 0.07	2.00 ± 0.09	1.60 ± 0.08
	12	2.24 ± 0.77	1.67 ± 0.08	2.17 ± 0.17	1.58 ± 0.10
Fe	0	11.33 ± 1.89	13.66 ± 0.66	9.72 ± 0.45	5.49 ± 0.92
	3	11.47 ± 2.19	11.03 ± 2.06	9.91 ± 0.76	5.46 ± 0.80
	6	8.85 ± 0.88	9.40 ± 0.70	10.09 ± 0.78	4.95 ± 0.16
	12	9.53 ± 0.66	9.16 ± 0.50	11.88 ± 0.84	4.84 ± 0.33
Mn	0	5.89 ± 1.02	7.73 ± 0.39	12.07 ± 0.24	12.74 ± 0.26
	3	6.00 ± 1.04	6.17 ± 1.05	12.02 ± 0.60	13.26 ± 0.52
	6	5.65 ± 2.24	5.26 ± 0.33	12.24 ± 0.23	12.65 ± 0.57
	12	4.83 ± 0.33	4.77 ± 0.16	13.16 ± 0.46	12.92 ± 0.29
Zn	0	0.96 ± 0.09	1.11 ± 0.04	0.82 ± 0.03	0.77 ± 0.05
	3	0.99 ± 0.12	0.97 ± 0.11	0.85 ± 0.05	0.78 ± 0.04
	6	0.81 ± 0.04	0.87 ± 0.07	0.90 ± 0.06	0.78 ± 0.11
	12	0.86 ± 0.03	0.85 ± 0.03	0.94 ± 0.07	0.77 ± 0.07

Iron availability significantly decreased with increasing humic substance dose and sampling time (Table-4). High organic matter in soils may convert soil Fe into a chelate form[18,22]. Also, mineral and organic Fe compounds transform into each other easily and organic matter has significant impacts on Fe oxide transformations. Effects of Fe with own characteristics on other elements are also worth to mention[22]. Therefore, present findings may be explained by regular fixation of Fe to humic substance in time and stable chelate formation of Fe in such cases. Current findings indicate that humic substance supplementation to sites with critical Fe levels may decrease available Fe levels and humic substances should not be supplied in such sites. On the other hand, humic substance supplementation to Fe-polluted sites may provide significant outcomes for plant culture. Such positive impacts should definitely be investigated in further researches. Also in sites where iron can easily transfer into solution, humic substance treatments may prevent continuous Fe losses and preserve the available levels for plants.

Available Mn levels clearly increased with increasing humic substance doses and sampling times. Especially the dose-timing interaction significantly increased available Mn levels (Table-4). Manganese had exhibited differences from the other metal (Cu, Fe and Zn) because of lower affinities of Mn especially in organic environments. Amounts of elements complexed with organic substance were in the order of: Mn < Fe < Cu < Zn[23]. It was also reported in researches that manganese toxicity is likely with plants that are fertilized with acid-forming fertilizers[24]. Relative decrease in pH of soil with humic substance treatment and incubation and constant 27 °C

TABLE–4
EFFECTS OF HUMIC SUBSTANCE ON SOIL pH, EC AND AVAILABLE Cu, Fe, Mn, Zn LEVELS

Properties	Doses (L/da)	Sampling Time (weeks)							
		1st week		2nd week		4th week		8th week	
pH	0	7.65	a[*]	7.56	abc	7.65	a	7.52	bc
	3	7.51	bcd	7.31	g	7.60	ab	7.50	b-e
	6	7.57	abc	7.39	efg	7.49	b-f	7.59	ab
	12	7.38	fg	7.37	fg	7.40	d-g	7.45	c-f
EC (μS/cm)	ns[**]	342.50	d	433.25	c	503.90	b	607.15	a
Cu (ppm)	0	1.97	bcd	2.32	a	1.96	bcd	1.72	def
	3	1.96	bcd	1.97	bcd	1.92	b-e	1.69	def
	6	1.93	b-e	1.84	c-f	2.00	a-d	1.60	ef
	12	2.24	ab	1.68	def	2.17	abc	1.58	f
Fe (ppm)	0	11.34	bc	13.66	a	9.72	de	5.49	f
	3	11.48	b	11.03	bcd	9.91	de	5.46	f
	6	8.85	e	9.40	e	10.09	cde	4.95	f
	12	9.53	e	9.16	e	11.88	b	4.84	f
Mn (ppm)	0	5.89	e	7.73	d	12.07	c	12.74	abc
	3	6.00	e	6.17	e	12.02	c	13.26	a
	6	5.65	ef	5.26	ef	12.24	bc	12.65	abc
	12	4.83	f	4.77	f	13.16	ab	12.92	abc
Zn (ppm)	0	0.96	bcd	1.11	a	0.82	fgh	0.77	gh
	3	0.99	b	0.97	bc	0.85	fgh	0.78	gh
	6	0.81	fgh	0.87	def	0.90	c-f	0.78	gh
	12	0.86	efg	0.85	e-g	0.94	b-e	0.77	h

a[*]: Columns labeled with the different letters are significantly different at $p < 0.05$ or $p < 0.01$ according to Duncan test, ns[**]: not significant temperature also indicate Mn mobility. Likewise, Alloway[17] indicated that Mn toxicity was commonly associated with acidic soil and warm climates. It was concluded herein that humic substance may provide significant contribution to plant Mn intakes from alkaline soils and therefore humic substance supplementation to such soils was found to be a significant implementation.

Available Zn levels decreased with increasing humic substance doses and incubation periods (Table-4). On the other hand, immobile zinc characteristics were indicated in previous researches and it was reported that zinc supplementations to soil showed maximum availability immediately after application and decreased availability later on[25]. Considering the current findings, zinc immobility may be explained by stable complex formations of zinc with organic matters.

Conclusion

In present study, despite the irregular variations, humic substance decreased soil pH values and increased electrical conductivity levels. Increasing humic substance doses and incubation periods also increased Mn availability and clearly decreased Fe availability. Availability of Cu and Zn decreased with increasing humic substance doses and incubation periods. It was concluded that humic substance treatments may provide significant outcomes in Cu, Fe and Zn-polluted soils and in sites where slow release and long-term intake of these elements are desired. Humic substance treatments increased Mn availability and provided supports in short-term intake of Mn by plants. Humic substances may further be investigated in future studies by correlating them with plant intakes.

REFERENCES

1. P. Janos, J. Vavrova, L. Herzogova and V. Pilarova, *Geoderma*, **159**, 335 (2010).
2. C. Donisa, R. Mocanu and E. Steinnes, *Geoderma*, **111**, 75 (2003).
3. L. Weng, E.P.M.J. Fest, J. Fillius, E.J.M. Temminghoff and W.H. Van Riemsdijk, *Environ. Sci. Technol*., **36**, 1699 (2002).
4. M.K. Zhang, Z.Y. Liu and H. Wang, *Commun. Soil Sci. Plant Anal*., **41**, 820 (2010).
5. W.L. Lindsay and W.A. Norvell, *Soil Sci. Soc. Am. J*., **42**, 421 (1978).
6. M.C. Amacher, in ed.: D.L. Sparks, Nickel, Cadmium, and Lead, Madison, Wisconsin, USA, pp. 739-769 (1996).
7. A. Fuentes, M. Llorens, J. Saez, A. Soler, A.I. Aguilar, J.F. Ortuno and V.F. Meseguer, *Chemosphere*, **54**, 1039 (2004).
8. G. Wang, M.Y. Su, Y.H. Chen, F.F. Lin, D. Luo and S.F. Gao, *Environ. Pollut*., **144**, 127 (2006).
9. A.K. Gupta and S. Sinha, *J. Hazard. Mater*., **149**, 144 (2007).
10. Q.H. Zhu, D.Y. Huang, S.L. Lui, Z.C. Luo, H.H. Zhu, B. Zhou, M. Lei, Z.X. Rao and X.L. Cao, *Plant Soil Environ*., **58**, 98 (2012).
11. D.C. Su and J.W.C. Wong, *Environ. Int.*, **29**, 895 (2004).
12. R.C. Cancela, C.A. de Abreu and A. Paz-González, *Commun. Soil Sci. Plant Anal*., **33**, 2879 (2002).
13. T. Grewelling and M. Peech, Chemical Soil Test, Cornell University, Agr. Expt. Sta. Bull., p. 960 (1960).
14. L.A. Richards, Diagnosis and Improvement of Saline and Alkali Soils, U.S. Department of Agriculture Handbook 60, p. 94 (1954).
15. R.E. Nelson, in ed.: A.L. Page, R.H. Miller and D.R. Keeney, Carbonate and Gypsu, American Soc. of Agronomy, Madison, Wisconsin, USA, pp. 181-197 (1982).
16. G.S. Bouyoucos, *Argon. J*., **43**, 434 (1951).
17. B.J. Alloway, Heavy Metals in Soil, Wiley & Sons Inc., New York, p. 338 (1993).
18. I. Pais and J.B. Jones, Handbook of Trace Elements, St. Lucie Press, Boca Raton, Florida, USA, p. 222 (2000).
19. D.T. Cuong and N. Obbard, *Appl. Geochem*., **21**, 1335 (2006).
20. S. Saraçoglu, M. Soylak and L. Elçi, *J. AOAC Int*., **92**, 1196 (2009).
21. P.R. Warman and K.A. Havard, *Agric. Ecosyst. Environ*., **68**, 207 (1998).
22. A. Kabata-Pendias, and H. Pendias, Trace Elements in Soils and Plants, CRC Press LLC, Boca Raton, Florida, USA, p. 413 (2001).
23. D.C. Adriano, Trace Elements in the Terrestrial Environment, Springer-Verlag, New York, p. 501 (1986).
24. T. El-Jaoual and D.A. Cox, *J. Plant Nutr*., **21**, 353 (1998).
25. T.J. Logan and R.L. Chaney, Utilization of Municipal Wastewaters and Sludges on Land-Metals, Proc. Workshop on Utilization of Municipal Wastewater and Sludge on Land, University of California, Riverside, CA, pp. 235-323 (1983).