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Humic Acid and Micronutrient Effects on Wheat Yield and Nutrients Uptake in Salt Affected Soils

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Abstract

A field experiment was conducted to evaluate interactive effect of HA, Cu and Zn on wheat (*Triticum asetivum* L cv. Tatara) yield and bioavailability of nutrients under salt- affected soils (silty clay loam, ECe 5.10 dSm^{-1} and SAR 9.99). Treatments were possible combinations of two levels of HA (0 and 2.0 kg ha^{-1}) with Zn, Cu and Zn+Cu (each 5.0 kg ha^{-1}). All treatments were applied with equal doses of NPK at 60:45:30 kg N: P_2O_5: K_2O ha^{-1}. Results showed that grain and biomass yields of wheat increased with HA, Zn and Cu applications. The highest grain yield was observed with 2.0 kg ha^{-1} HA applied with Cu+Zn showing 20.2% increase over control. When grain yield was averaged across Cu and Zn levels, HA application produced 8.07% increases over no HA while Cu and Zn alone induced 6.52 and 7.52% increases as compared to no Cu and Zn, respectively. Biomass yield of wheat increased by 17.1% over control with 2.0 kg ha^{-1} HA applied with Cu+Zn. Total nutrient accumulation by wheat crop (conc. × biomass) increased with application of HA, Cu and Zn. Highest N, P, K, Zn and Cu accumulation was recorded in treatments which produced highest yields. Post-harvest soil analyses showed that addition of Zn and Cu increased the AB-DTPA extractable levels of Zn and Cu. The increase in soil Zn concentration with addition of 5.0 kg Zn ha^{-1} was 3-fold whereas addition of Zn plus 2.0 kg ha^{-1} HA further enhanced soil Zn. Similarly, highest Cu of 5.25 mg kg^{-1} soil was observed with Cu+HA compared to pre-sowing levels. Values of ECe and SAR in the post-harvest soils drastically decreased to non-saline and non-sodic levels due to leaching with rainfall and minimum input of saline irrigation during the season. These results suggested that application of HA along with Cu and Zn seems economical in wheat production on salt affected soils and needs replication in saline calcareous soil.

Keywords: Humic Acid; Micronutrient Effects; Wheat Yield; Salt affected soils

Introduction

Humic acid (HA) is a complex molecule of polymeric organic acid of aromatic structure substituted by carboxyl, phenolic, hydroxyl and alkyl groups linked together through ether linkage (Sutton and Sposito, 2005). A typical HA molecule consists of polymers of a basic aromatic ring structure of di-or-tri-hydroxypenole linked by -O-, -NH-, -N and –S bonds containing both free OH group and the double quinines ($O=C_6H_4=O$) linkage (Stevenson, 1982). Humic acid is relatively stable product of organic matter decomposition (Stott and Martin, 1990) and hence accumulates in environmental systems. Humic acid might benefit plant growth by chelating unavailable nutrients and buffering pH (Julie and Bugbee, 2006). It contains 51-57% organic C, 4-6% N and 0.2-1% P, that may stimulate microbial activity, soil enzymatic activities thereby improves physicochemical and biological environment of soil (Brannon and Sommer, 1985), which enhances plant growth (Zancani *et al*., 2009; Khattak *et al*., 2013). Humic acid increases the availability of elements due to its ability to form complexes (Vaughan and MacDonald, 1976). Humic acid from wheat straw leachate can inhibit the formation of insoluble Ca phosphates and thus may enhance P bio-availability (Grossl and Inskeep, 1991). Humic acid forms complexes (chelates) with Na, K, Mn, Zn, Ca, Fe, Cu, and various other elements to, regulate their bioavailability in soil (Barron and Wilson, 1981) and overcome the element deficiency (Yingei, 1988). Most soils contain ample inorganic Fe for plant growth but in alkaline calcareous soils the concentrations of plant-available nutrients are extremely low. Humic compounds (humic and fulvic acids) can incorporate insoluble form into chelated complexes that promote their uptake by plants (Cheryl *et al*., 2001). Humic acid may be utilized in agriculture as fertilizer, plant growth promoter, nutrient carrier and soil conditioner (Nisar and Mir, 1989).

Beneficial effect of HA on crop production on normal soil have been frequently reported (Sharif, 2002; Sharif *et al*., 2002a; 2002b) but little attention have been given to investigate the degree and extent to which HA can promote crop growth in salt-affected soil. Shaaban *et al.* (2013), Khattak *et al.* (2013) attributed the beneficial effect of HA on crop production in salt affected soils to its role in promoting microbial growth, CEC and enzyme activity. Shaaban *et al*. (2013) reported sizeable ameliorative effect of commercial HA when applied with gypsum at an unusual high rate of 24 and 48 kg ha^{-1} in rice paddy system but alone application of HA showed non-significant effect on soil EC and SAR. Keeping the role of HA in bioavailability of micronutrients, the present study was planned to investigate the interactive effect of Zn, Cu and HA in salt-affected soils for better nutrient management and higher yields of wheat.

Materials and Methods

Field Experiment

The experiment was conducted on wheat (*Triticum asetivum* L) using *cv*. Tatara in salt-affected soils at Nasimabad, Lachi district, Kohat. Treatments were eight possible combinations of two levels of HA (0 and 2.0 kg ha^{-1}) with Zn, Cu and Zn+Cu each applied at 5.0 kg ha^{-1}. Treatments were arranged in split-plot randomized complete block design with three replications. Main plots were assigned to HA levels whereas subplot to micronutrients. All treatments were applied with equal doses of NPK at 60:45:30 kg $N:P_2O_5:K_2O$ ha^{-1} equivalent to half NPK recommended dose for the area, using urea, triple super phosphate (TSP) and sulfate of potash (SOP) as sources for N, P and K, commercially available $ZnSO_4.7H_2O$ (23% Zn) and $CuSO_4.5H_2O$ (25% Cu) were used for Zn and Cu, respectively. All fertilizers were applied before seed bed preparation. Humic acid was applied through spraying its water suspension obtained by dissolving required amount of HA in 10 L of irrigation water. Plot size was 7 ×3.5 m^2. Crop was sown with hand drill keeping seed rate of 120 kg ha^{-1} and 20 cm row space. Before HA or fertilizer application, soil samples were collected from 0-20 cm depth for pre-sowing analysis and after crop harvest for post-harvest soil analysis. Leaf samples, (3rd leaf) at maturity were collected for nutrient concentrations and accumulation as influenced by HA and micronutrients application.

Analyses of Soil Samples

Pre- and post-harvest soil samples were analyzed to evaluate impact of HA, Cu and Zn on soil chemical characteristics and nutrient status following standard procedures. Soil pH and EC were determined in saturation extract following procedure of Thomas (1996) and Rhoades (1996). Soil saturation extracts and water samples were analyzed for Na and K concentrations by flame photometer (Jenway-PFP7) using required standards. Calcium and magnesium by titration with EDTA and lime ($CaCO_3$) content of soils by the procedure of Richard (1954), soil organic matter by Nelson and Sommers (1996). Sodium adsorption ratio (SAR) was calculated using the concentration of Na and Ca + Mg in soil extracts and in water samples using the formula.

$$SAR = \frac{[Na^+]}{\sqrt{\frac{[Ca^{2+} + Mg^{2+}]}{2}}}$$

Where, [Na], [Ca], and [Mg] are in $mmol_{(+)}$ L^{-1}.

Soil samples were analyzed for AB-DTPA extractable P using spectrophotometer (Lamda, 35) and K, Fe, Mn, Cu and Zn following a standard analytical procedure as described by Soltanpour and Schwab (1977), with atomic absorption spectrophotometer (Perkin Elmer, 2380). Mineral N was determined by the procedure of Mulvaney (1996).

Analyses of Plant Samples

Plant samples collected were washed with distilled water, air dried in oven at 60-70ºC for 48 h. After grinding, the samples were digested with nitric acid (HNO_3) and perchloric acid ($HClO_4$) as described by Walsh and Beaton (1977) and samples were then analyzed for total N after Bremner (1996), P using Lambda 35 spectrophotometer, Na and K with flame photometer (Jenway PF7) and micronutrients (Zn, Cu, Fe and Mn) using atomic absorption spectrophotometry (Perkin Elmer, 2380).

Statistical Analysis

Data recorded on plant yield, plant composition and soil analyses were subjected to statistical analysis for comparison (Steel and Torrie, 1980).

Results

Effect on Wheat Grain and Biomass Yield

Grain yield was significantly affected with HA application while micronutrient's effect and their interaction were non-significant (Table 1). Grain yield of wheat increased with HA and improved when applied with Zn and Cu combinations (Table 2). The highest grain yield of 2171 kg ha^{-1} was observed in treatments receiving 2.0 kg HA along with Cu+Zn at 5.0 kg ha^{-1} as compared to the lowest yield in control. When data were averaged across Cu and Zn levels, HA application revealed 8.07% increases in grain yield over control. Similarly, when data were averaged across HA levels, sole application of Cu and Zn induced 6.52 and 7.52% non-significant increases while Cu+Zn produced 11.66% more yield as compared to control (0 kg Cu and 0 Zn) and 20% increases with HA when supplemented by Zn, Cu or both. Sole application of HA increased grain yield of wheat by 12.8% as compared to control (Table 2).

Biomass of wheat significantly increased with application of HA however effect of Zn and Cu and their interaction with HA was non-significant (Table 1 and 2). The highest biomass yield of 6927 kg ha^{-1} was obtained with 2.0 kg HA ha^{-1} along with 5.0 kg ha^{-1}Cu+Zn showing

Table 1: ANOVA showing F values for grain and biomass yield of wheat as influenced by interactive effect of HA, Cu and Zn in salt-affected soils

SOV	D.F.	Grain yield	Biomass yield
HA	1	49.08*	74.22*
Error	2	-	
Cu or Zn	3	Ns	ns
HAx(Cu or Zn)	3	Ns	ns
Error	12		
CV%	-	7.02	11.59

Table 2: Wheat grain and biomass yield as influenced by HA, Cu and Zn alone and in combination in silty clay loam salt affected soils

Treatments ($kg\ ha^{-1}$)		+HA ($kg\ ha^{-1}$)		% increase Over control
Cu	Zn	0	2.0	-
		Grain Yield ($kg\ ha^{-1}$)		
0	0	1781	2009	12.8
5	0	1970	2067	16.1
0	5	1942	2133	19.8
5	5	2061	2171	22.1
Mean		1938 b	2095 a	-
		Biomass ($kg\ ha^{-1}$)		
0	0	5920	6472	9.4
5	0	6573	6620	11.9
0	5	6360	6907	16.7
5	5	6467	6927	17.1
Mean		6330 b	6731 a	-

Means followed by different letters are significant at $P<0.05$

Table 3: Nutrient concentrations in wheat leaves as influenced by HA applied alone or combined with Cu or Zn in a silt clay loam salt affected soil

Treatments ($kg\ ha^{-1}$)			N	P	K	Cu	Fe	Zn	Mn
Zn	Cu	HA	-------- $g\ kg^{-1}$ ----------			------------------ $mg\ kg^{-1}$ ------------			
-	-	-	4.28	0.66	14.60	6.53	311.27	7.40	88.73
5.0	-	-	5.33	0.76	14.50	7.67	450.80	13.13	99.73
-	5.0	-	4.88	0.60	15.06	6.85	366.27	10.13	85.73
5.0	5.0	-	4.65	0.71	12.62	8.13	389.47	20.27	91.33
-	-	2.0	4.52	0.64	15.12	6.33	424.87	13.60	89.53
5.0	-	2.0	4.61	0.66	13.38	6.13	523.00	13.27	88.67
-	5.0	2.0	4.59	0.59	15.68	7.73	335.60	17.53	85.80
5.0	5.0	2.0	4.62	0.56	12.64	7.50	425.00	18.80	102.47
Averaged across Cu and Zn									
No HA			4.79	0.68	14.20	7.30	379.45	12.73	91.38
With HA			4.58	0.61	14.21	6.93	427.12	15.80	91.62
% change with HA			-4.20	-9.96	0.07	-5.09	12.56	24.08	0.26
Averaged across HA levels									
No Cu or Zn			4.40	0.65	14.86	6.43	368.07	10.50	89.13
Cu or Zn			4.78	0.65	13.98	7.34	415.02	15.52	92.29
% change			8.70	-0.39	-5.93	14.04	12.76	47.83	3.54

increases of 17.01% over control (Table 2). As evident, increases in biomass yield ranging from 9.4 to 17.01% show additive effect of Cu, Zn and Cu+Zn on the beneficial effect of HA on wheat biomass (Table 2).

Effect on Nutrient Concentrations and Total Accumulation of Wheat

The nutrients concentrations in wheat leaves were non-significantly affected by HA, Cu, Zn and their combinations (Table 3). When averaged across Zn and Cu levels, HA treatments increased Fe and Zn concentrations in leaves by 12.5% and 24.08% over non-HA treatments. When averaged over HA levels, the micronutrients (Zn and Cu) induced changes of 8.7, -5.93, 14.04, 12.76, 47.83 and 3.54% in N, K, Cu, Fe, Zn and Mn in leaves tissues over no Cu or Zn, respectively (Table 3). These changes were mainly associated with variations in plant yield induced by HA and Cu or Zn as corroborated by the total accumulation (uptake) of nutrients by wheat biomass (Table 4). Highest N, P, K, Zn and Cu uptake were found in HA and Zn and Cu treatments producing highest yields.

Post-harvest EC, SAR and Nutrient Concentrations

Initially the soil before wheat sowing had ECe of 5.91 $dS\ m^{-1}$ and SAR 9.99, silt clay loam texture being irrigated with saline water of ECiw of 5.82 $dS\ m^{-1}$(Table 5). The soil was low in organic matter, deficient in P, Cu and Zn (Table 5). Values of ECe and SAR in the post-harvest soils were improved when compared to pre-sowing levels. Post-harvest ECe varied between 0.72 and 1.02 $dS\ m^{-1}$ and SAR 4.14 to 5.88 (Table 6) showing drastic decreases compared to pre-sowing values (Table 5). Addition of Zn and Cu elevated the AB-DTPA extractable levels of Zn and Cu, respectively, in post-harvest soils (Table 7). The increase in soil Zn concentrations with addition of 5.0 kg Zn ha^{-1} were 3-fold whereas addition of Zn plus 2.0 kg HA ha^{-1} further enhanced soil Zn with highest values of 2.83 and 2.71 $kg\ ha^{-1}$ (Table 7). Similarly the highest Cu of 5.25 mg kg^{-1} was recorded with Cu+HA combination, suggesting additive effect of HA on micro-nutrient availability in soils. When averaged over Cu and Zn, HA increased AB-DTPA extractable soil P level by 27.49% over no HA.

Discussion

This study focused on evaluating the impact of coal derived humic acid (HA) on wheat yield and nutrients uptake applied alone and in combination with Zn and Cu. The results showed increases in wheat grain and biomass yield from 12 to 22.1% and 9.4 to 17.1%, respectively.

The beneficial effect of HA on grain and biomass yields of wheat are supported by findings of Mishra and Srivastava (1988), Hai and Mir (1998), Sharif (2002) and Sharif *et al.* (2002b) and Khattak *et al.* (2013). The degree and extent of crop response to HA depends on variation in soil, climatic conditions, crop species, cultural practices and sources. Sharif *et al.* (2002a, 2002b) reported 25 to 40% increases in wheat and maize crop in non-saline calcareous soils of Peshawar valley with application of coal derived HA. The effect of HA further improved when applied in combination with other nutrients. HA applied with Zn and Cu increased maize yields in alkaline calcareous soils (Naheed, 2008). Coal derived HA enhances plant growth (Nisar and Mir, 1989) that could be associated to its effect

Table 4: Nutrient accumulation (concentrations x biological yield) as influenced by HA applied alone or combined with Cu or Zn added to salt affected soils

Treatments (kg ha^{-1})			N	P	K	Cu	Fe	Zn	Mn
Zn	Cu	HA	---------- kg ha^{-1}---------			------------------ g ha^{1}------------			
-	-	-	25.36	3.87	86.30	38.12	1838	43.61	524.45
5.0	-	-	35.02	5.06	95.95	50.72	2910	84.37	660.59
-	5.0	-	30.52	3.81	94.97	43.27	2313	67.31	546.53
5.0	5.0	-	29.81	4.55	81.54	52.78	2493	128.11	587.68
-	-	2.0	29.22	4.18	97.95	41.04	2735	87.12	578.69
5.0	-	2.0	30.45	4.35	88.56	40.63	3472	87.90	586.73
-	5.0	2.0	31.64	4.04	109.04	53.54	2287	116.79	599.97
5.0	5.0	2.0	32.00	3.91	87.53	51.59	2893	125.71	707.66
Averaged across Cu and Zn									
No HA			30.18	4.32	89.69	46.22	2389	80.85	579.81
With HA			30.83	4.12	95.77	46.70	2847	104.38	618.26
%increase with HA			2.15	-4.59	6.78	1.03	19.17	29.10	6.63
Averaged across HA levels									
No Cu or Zn			27.29	4.02	92.13	39.58	2286	65.36	551.57
Cu or Zn			31.57	4.29	92.93	48.75	2728	101.70	614.86
% increase			15.70	6.50	0.87	23.18	19.31	55.59	11.48

Table 5: Pre-sowing soil pH, EC, SAR and AB-DTPA extractable nutrient concentration of wheat fields used for the interactive effect of HA and Zn and Cu under salt affected soils

Parameter	pHe	ECe	SAR	Min. N	P	K	Cu	Fe	Zn	Mn
Unit	-	dS m^{-1}	-	-------------------- mg kg^{-1}--------------------------						
Values	8.16	5.10	9.99	32.37	0.56	137.67	1.72	3.10	1.10	0.32
SD	0.07	0.36	0.92	3.59	0.11	10.65	0.56	0.68	0.14	0.09

on root enzymes and rhizosphere soil (Vaughan and MacDonald, 1976). Improvement in microbial activities, enzymes activities, CEC and moisture retention mainly account for the HA induced increases in plant growth and yield (Khattak *et al*., 2013).

Nutrients (N, P, K, Cu, Fe, Zn and Mn) concentrations in wheat tissue showed variable response to HA but when the concentrations were converted to uptake [concentration x yield], consistent increases were noted with application of HA, which further increased with Zn and Cu (Table 4). These increases in nutrient uptake followed the trend exhibited by wheat plant bio-mass.

The availability of micronutrients to plants is governed by a variety of reaction that includes complexion with organic and inorganic ligands, ion exchange and adsorption, precipitation and dissolution of solids and acid-base equilibrium (Lindsay, 1979). Humic acid plays a key role both in enhancing the bioavailability of nutrients to plants and in reducing the adverse effect of some of the free ions due to its chelate forming capability (Bloom *et al*., 1979). Micronutrients that would ordinarily be converted to insoluble precipitates as carbonates, sulfides or hydroxide at higher pH are undoubtedly maintained in solution through chelation (Sposito, 1989). According to Yingei (1988) HA attracts positive ions, forms chelates with micronutrients and releases them slowly when required by plants. Application of HA to soil and nutrient solutions have produced significant growth responses. Among other mechanisms, it has been suggested that plant growth is enhanced by increasing the uptake of micronutrients by the plants, thereby affects metabolic relationships (Mylonas and McCant, 1980). Sharif (2002) concluded in pot experiment that beneficial effect of HA on plant growth and nutrient uptakes of maize are mainly associated with the potential of HA to improve the physio-chemical and biological environments of the soil. In a recent study, Khattak *et al.* (2013) demonstrated in a series of laboratory experiments that HA applied to soil at the rates equivalent to the field studies (2 kg HA ha^{-1}) promoted soil microbial activity, cation exchange capacity, water holding capacity and

Table 6: Post-harvest soil pH, EC and SAR as influenced by HA applied alone or combined with Cu and Zn in salt affected soils

Treatments (kg ha^{-1})			pHe	ECe	Na	Ca+Mg	SAR
Zn	Cu	HA		---dS m^{-1}	-------mmol$_{(+)}$ L^{-1}		
-	-	-	8.58	1.02	8.12	5.33	5.00
5.0	-	-	8.46	0.72	6.53	4.83	4.15
-	5.0	-	8.53	1.03	8.99	5.00	5.68
5.0	5.0	-	8.43	0.85	5.77	4.33	4.14
-	-	2.0	8.51	0.89	6.40	4.08	4.51
5.0	-	2.0	8.52	1.00	6.96	5.75	4.09
-	5.0	2.0	8.56	0.88	6.75	4.58	4.46
5.0	5.0	2.0	8.49	0.93	6.74	4.25	4.84
Averaged across Cu and Zn							
No HA^{0}			8.50	0.90	7.35	4.88	4.74
With HA^{2}			8.52	0.93	6.71	4.67	4.47
% change with HA			0.22	2.49	-8.75	-4.27	-5.69
Averaged across HA levels							
No Zn or Cu			8.54	0.95	7.26	4.71	4.76
Zn or Cu			8.50	0.90	6.96	4.79	4.56
% change			-0.53	-5.20	-4.16	1.77	-4.12

Table 7: Post-harvest soil nutrient concentrations (AB-DTPA extractable) as influenced by wheat crop, HA and micronutrients

Treatments (kg ha^{-1})			P	K	Cu	Fe	Zn	Mn
Zn	Cu	HA	-------------------------mg kg^{-1}-----------------------------					
-	-	-	2.31	124.92	3.33	10.47	0.60	4.51
5.0	-	-	2.08	126.73	3.03	9.69	1.92	4.36
-	5.0	-	2.26	128.54	3.86	9.95	0.85	3.93
5.0	5.0	-	1.54	129.35	4.20	11.19	2.18	4.57
-	-	2.0	2.82	128.60	3.25	11.00	1.06	5.20
5.0	-	2.0	3.31	129.03	3.72	10.16	2.71	5.49
-	5.0	2.0	2.69	128.29	5.25	11.23	0.89	4.49
5.0	5.0	2.0	1.61	126.68	3.99	11.00	2.83	4.48
No HA			2.04	127.38	3.61	10.32	1.39	4.34
With HA			2.61	128.15	4.05	10.85	1.87	4.92
% increase with HA			27.49	0.60	12.29	5.06	34.79	13.18
No Cu or Zn			2.56	126.76	3.29	10.73	0.83	4.86
Cu or Zn			2.25	128.10	4.01	10.53	1.90	4.55
% increase			-12.38	1.06	21.87	-1.86	127.69	-6.28

enzymes phosphatase and nitrogenase activity in normal and salt-affected soils.

The values of ECe, SAR and nutrients (Table 6 and 7) in the post-harvest analysis exhibited changes in relation to pre-sowing data (Table 5). The values of pH increased and those of EC decreased by several fold. The decreases in ECe and SAR could not be associated wih HA application rather these changes could be caused by leaching of salts due to rainfalls during winter and also because of limited irrigation with saline waters having ECiw of 5.10 ± 0.07 dSm^{-1}, which minimized salt input. The increases in pH could be associated with the removal of salts and with dissolution of $CaCO_3$'s and subsequent removal of HCO_3 with rain waters (Lindsay, 1979; Sposito, 1989).

Shaaban *et al*. (2013) reported similar decreases in post-harvest. ECe and SAR in paddy soil when treated with combined application of HA, gypsum and farm yard manure but observed no effect on EC and SAR even with high doses of 24 and 48 kg ha^{-1} HA when applied alone. The observed increases in P and micronutrients might be associated with release of insoluble P from the dissolution of carbonates of Ca, Mg, Fe and Mn (Sposito, 1989; Bohn, 2001), as HA promotes the solubility of otherwise insoluble nutrients (Vaughan and MacDonald, 1976; Grossl and Inskeep, 1991; Barron and Wilson, 1981) and their uptake by plants (Cheryl *et al*., 2001).

It can be concluded from this study that biomass and grain yield of wheat increased with application of HA combined with Cu and Zn. Soil nutrients availability improved which resulted in enhanced uptake by wheat in response to HA plus Zn and Cu addition. Marked improvement was observed in lowering post-harvest soil salinity-sodicity as shown by decreasing EC and SAR values. Application of HA when supplemented with micronutrients could be an economical practice for crop production in salt-affected and or degraded soils.

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