

Effects of Humic Acids from Different Sources on Sodium and Micronutrient Levels in Corn Plants

(Kesan Asid Humik daripada Punca yang Berbeza ke atas Natrium dan Paras Mikronutrien dalam Tumbuhan Jagung)

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ABSTRACT

*The use of activated humic acids (HAs) in agricultural applications is a relatively recent development. Corn (*Zea mays L.*) is a major food and silage crop in Turkey and yields are reduced in calcareous soils by sodium (Na) toxicity and carbonate (CO_3) induced deficiencies of some micronutrients. In this study, the effects of two HAs extracted using the wet-alkali technique and activated with nitrogen (N_2) and oxygen (O_2) gases, on the Na and micronutrient (iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) and boron (B)) concentrations in the homogenised stems and leaves of corn plants were investigated. The experiment was conducted with a completely randomized design with factorial arrangement (2 HA types \times 2 fertilisation regimes \times 5 HA concentrations), with three repetitions, in a calcareous soil in pots in a greenhouse. The humic acid was applied at 100, 200, 400, and 800 mg/kg of soil before sowing the corn seed. The variance analysis showed that increasing HA levels decreased the Na concentration in the corn plants. The lowest plant Na concentrations were obtained with the addition of HA activated with N_2 and O_2 to both the fertilised and unfertilised treatments. The highest Zn and Cu uptakes, and Fe, Mn, and B uptakes, were associated with wet alkali extraction and gas activation, respectively. Overall, HA extracted with N_2/O_2 was more effective under unfertilised conditions and wet extracted HA was more effective under fertilised conditions.*

Keywords: Corn; humic acids; microelements; sodium

ABSTRAK

*Penggunaan asid humik diaktifkan (HAs) dalam aplikasi pertanian merupakan pembangunan terbaharu. Jagung (*Zea mays L.*) ialah makanan utama dan tanaman silaj di Turki dan penghasilannya telah berkurang dalam media tanah berkapur disebabkan oleh ketoksikan natrium (Na) dan karbonat (CO_3) dengan kekurangan mikronutrien di dalam media tanah teraruh. Dalam kajian ini, kesan penggunaan dua HA yang diekstraks menggunakan teknik basah-alkali dan diaktif dengan gas N_2 dan O_2 telah dikaji ke atas kepekatan Na dan mikronutrien (besi (Fe), mangan (Mn), zink (Zn), tembaga (Cu) dan boron (B)) dalam batang dan daun tumbuhan jagung terhomogen. Reka bentuk uji kaji ini dijalankan sepenuhnya secara rawak dengan susunan faktorial (2 jenis HA \times 2 regim persenyawaan \times 5 kepekatan HA), dengan tiga ulangan, di dalam tanah berkapur di dalam pasu di rumah hijau. Asid humik diaplikasikan pada 100, 200, 400 dan 800 mg/kg tanah sebelum penyemaian benih jagung. Analisis varians menunjukkan bahawa peningkatan tahap HA akan menurunkan kepekatan Na dalam tanaman jagung. Kepekatan Na yang terendah di dalam tanaman telah diperoleh dengan penambahan HA yang diaktifkan dengan N_2 dan O_2 untuk kedua-dua rawatan yang telah disenyawakan dan tidak disenyawakan. Pengambilan tertinggi Zn dan Cu, serta pengambilan Fe, Mn dan B, masing-masing dikaitkan dengan pengekstrakan alkali basah dan pengaktifan gas. Secara keseluruhannya, HA yang diekstrak dengan N_2/O_2 lebih berkesan di bawah keadaan yang tidak tersenyawa dan HA yang diekstrak secara basah adalah lebih berkesan di bawah keadaan yang tersenyawa.*

Kata kunci: Asid humik; jagung; mikrounsur; natrium

INTRODUCTION

Corn (*Zea mays L.*) is one of the most commonly cultivated cereal crops worldwide. It is also one of the most important silage plants because it provides both high biomass and energy (Roth et al. 1995). In most agricultural soils, maintaining the optimum concentration of plant

nutrients is the most difficult task for farmers and plant nutritionists (Jones 2001). In soil, organic matter (OM) is the key component regulating chemical, physical, and biological processes (Saruhan et al. 2011a, 2011b). Humic substances can chelate plant nutrients, preventing their leaching by rainfall or irrigation, and therefore, facilitate

their uptake by plants. However, these beneficial effects of humic substances are reduced when the OM content of a soil is too low or too high where the low level of cationic microelements in calcareous soils and soils containing high levels of OM become problematic (Garcia-Mina et al. 2004; Tan 1998).

The OM level of Turkish soils is generally low (Eyüpoğlu 1998) because many farmers burn crop residues or use them for animal food, and also excessively cultivate the soil, exposing the OM to accelerated oxidation.

Soil OM includes humic and fulvic acids which are termed humin materials (Schnitzer 1992). Humic and fulvic acids are highly recalcitrant, nitrogenous compounds that contain amino acids and aromatic complexes (Andriessse 1988). The carboxyl (-COOH) and phenolic (OH-) groups on these organic complexes affect the physicochemical characteristics of soils and therefore the nutrient uptake and growth performance of plants (Boyle et al. 1989; Schnitzer 1992).

The mechanism(s) by which humin materials stimulate plant development are not definitively understood but it has been reported that they increase oxygen uptake, respiration, photosynthesis, cell membrane permeability, phosphate uptake, and stem cell growth (Russo & Berlyn 1990). Furthermore, humic materials mediate the uptake of microelements such as Fe, Zn, Cu, Mn, and B (Chen et al. 1999; Manas et al. 2014). Due to their chelating ability, humic materials can have a very strong effect on plant development and mineral content (Pettit 2004). The effectiveness of humic materials in enhancing plant growth depends on their type, concentration and molecular weight (Saruhan et al. 2011a, 2011b). Humic materials chelate some micronutrients (Fe, Mn, Zn, and Cu), and alkali cations such as Na and potassium (K), are exchangeably adsorbed by COOH groups to form -COONa and -COOK, respectively (Baigori et al. 2009; Ghabbour & Davies 2001). Therefore, they can stimulate the uptake of certain elements by plants or decrease their toxicity to plants via a regulatory effect.

The agricultural usage of humic acid (HA) has become common in the last decade, especially through foliar application (Manas et al. 2014). 50, 500, and 1000 mg/kg of HA addition to soil in a pot experiment enhanced the nutrient uptake (N, P, K, Mg, Ca, Zn, Fe, and Cu) and growth performance of teak (*Tectona grandis* L.F.) seedlings while a decrease in Mn uptake was noted. Humic acid induced enhancement and/or changes in vegetative growth, and nutrient uptake and partitioning between the roots and the above ground biomasses of different plants grown in hydroponic culture, depending on the rate at which HA was applied (David et al. 1994; Sözüdoğru et al. 1996). The foliar application of HA resulted in an increase in the micronutrient concentration in corn leaves (Çelik et al. 2010). Moreover, Yıldırım (2007) reported that foliar HA application at a rate as low as 20 mL/L was more

effective than soil application in increasing the yield and quality of tomatoes. In addition, it has been reported that HA can alleviate the effects of saline soils on plants by decreasing Na uptake (Khaled & Fawy 2011).

The magnitude of the effects of HA on plant properties is highly dependent on the characteristics of the HA types, the application method and rate, and the plant species (Ferrara et al. 2007; Lobartini et al. 1997). Corn is an extremely important food and silage crop in Turkey, with yields varying considerably due to soil constraints, among other factors. Therefore, the purpose of this study was to examine the effects of two humic acids, obtained by wet-alkali extraction and activated with N₂/O₂ gas, on the Na, Fe, Mn, Zn, Cu, and B concentrations of fertilised and unfertilised corn plants grown in calcareous soil under greenhouse conditions.

MATERIALS AND METHODS

Peat material was collected from the Arifiye peatland in Sakarya Province, Turkey (40°42'26" N - 30°20'31" E), and then activated with two methods, wet alkali and N₂ and O₂ saturation, that produced two different liquids where both contained 15% humic acid. The activation process was performed in a closed reactor by saturating it with O₂ and N₂ gases at 150 to 250 °C and 4 bars of pressure (Butuzova et al. 1998).

The soil used in this greenhouse study was collected from a field in Kurupelit township of Atakum, a suburb of Samsun, Turkey (41°21'52" N - 36°11'18" E). The experiment was performed in a completely randomised design, with factorial arrangement and replicated three times. The experiment consisted of 2 fertiliser regimes (fertiliser and without fertiliser), 2 HA sources (wet alkali and N₂/O₂ gases) and 4 HA concentrations (100, 200, 400, and 800 mg/kg of soil). Plastic pots (19 × 23 × 15 cm - height × top diameter × bottom diameter, respectively) containing 4 kg of oven-dried soil were used to grow the corn plants. The 'no fertiliser' treatment pots were not amended with any fertiliser. In the fertilised treatment, the soil in each pot was amended with 200 mg/kg N (urea, 46% N), 80 mg/kg P (triple super phosphate, 42% phosphorus pentoxide (P₂O₅)), 50 mg/kg K (potassium sulphate (K₂SO₄)), 50% potassium oxide (K₂O)), 60 mg/kg Mg (magnesium sulphate pentahydrate (MgSO₄·5H₂O)), 20 mg/kg Fe (Iron(II) sulphate heptahydrate (FeSO₄·7H₂O)), 15 mg/kg Mn (manganese(II) sulphate dihydrate (MnSO₄·2H₂O)), 10 mg/kg Zn (zinc(II) sulphate heptahydrate (ZnSO₄·7H₂O)), 5 mg/kg Cu (copper(II) sulphate pentahydrate (CuSO₄·5H₂O)), and 0.5 mg/kg B (boric acid (H₃BO₃)), based on the soil analysis results and plant requirements. All of the fertilisers, except N, were mixed with the pot soil before sowing. The nitrogen was supplied in two equal parts, firstly as 100 mg/kg just before sowing and then 100 mg/kg 3 weeks after plant emergence. Five corn seeds of the variety Sakarya F1 (FAO-600) were sown in the pots on 20 May

2013, and after emergence, the three most vigorous plants were left in each pot. The field capacity (FC) of the soil used in this study was calculated by using the saturation percentage of soil, according to Grewal et al. (1990). The pots were then continuously kept near the FC with gravimetric irrigation (Fontenelli et al. 2016).

On 3 August 2013, after 75 days, the corn plants were cut just above the soil surface. After the samples were washed with deionised water and dried at 65 °C, they were ground in a stainless-steel mill to obtain homogenised plant samples (15.5% moisture content) for analyses (Kacar 1984). The Na, Fe, Mn, Zn, Cu, and B concentrations in the plant samples were determined with the use of an atomic absorption spectrophotometer (Perkin Elmer AA-400) after digestion with 4:1 nitric acid (HNO₃):perchloric acid (HClO₄) (Kacar & İnal 2008).

The pH and EC of the soil used in the experiment were determined in 1:1 soil:water extract (Soil Survey Laboratory 1992), and the texture was determined according to the Bouyoucos hydrometer method (Bouyoucos 1951). The saturation percentage of the soil was determined according to the methods of

Labuschagne et al. (1995) while calcium carbonate equivalent (CCE) was determined with a calcimeter (Soil Survey Staff 1993). The organic matter (OM) level was determined with a modified Walkley-Black method. The available phosphorus (P) was determined spectrometrically in 0.5 M sodium bicarbonate (NaHCO₃) extract, total nitrogen (N) was determined with the micro-Kjeldahl method, and available potassium, calcium (Ca), magnesium and sodium were determined in 1 N ammonium acetate (NH₄OAc) extract. The cation exchange capacity (CEC) was determined with the sodium saturation method whereas available iron, manganese, copper, and zinc were determined with an Atomic Absorption Photometer (AAS) (Perkin Elmer AA-200), after extraction with 0.005 M diethylenetriamine pentaacetate (DTPA), and available B was determined according to the Azometin-H method (Kacar 2009). The physical and chemical characteristics of the experimental soil are provided in Table 1.

The data set obtained from the experiments was subjected to ANOVA in the SPSS 17.0 program and separation of the means of the treatments was performed with the Duncan Multiple Range Test ($p < 0.05$).

TABLE 1. Physical and chemical properties of the experimental soil

Parameters	Values
pH _{1:1}	7.89
EC, dS/m	1.44
CCE, %	8.79
OM, %	1.75
Sand, %	26.2
Silt, %	18.1
Clay, %	55.7
Texture class	Clay (C)
CEC, cmol/kg	48.5
Saturation, %	87.3
Field capacity, %	54.6
Total nitrogen (N), %	0.03
Available P, mg/kg	1.56
Available K, cmol/kg	0.68
Available Ca, cmol/kg	38.3
Available Mg, cmol/kg	8.55
Available Na, mg/kg	27.6
Available Fe, mg/kg	16.0
Available Mn, mg/kg	5.93
Available Zn, mg/kg	0.21
Available Cu, mg/kg	2.18
Available B, mg/kg	0.47

EC: Electrical Conductivity; CCE: Calcium Carbonate Equivalent; CEC: Cation Exchange Capacity;

OM: Organic Matter

RESULTS AND DISCUSSION

The Na and micronutrient concentrations of the homogenised stems and leaves of corn plants treated with wet alkali extracted and N₂/O₂ activated humic acid (HA), under fertilised and unfertilised conditions, were given in Table 2. Humic acid type significantly increased Fe and Mn concentrations under unfertilised conditions ($p < 0.05$) whereas only the Cu concentration significantly increased under fertilised conditions ($p < 0.01$). Humic acid application had significant effects on the Na, Zn ($p < 0.05$) and B contents of corn plants under both fertilised and unfertilised conditions ($p < 0.01$), but the Fe, Mn and Cu concentrations were only significantly affected under fertilised conditions ($p < 0.01$). Specifically, the effects of the HA application rate were highly dependent on the micronutrient in question and the fertilisation regime. In addition, the interaction of HA type and rate was significant for Zn under unfertilised conditions ($p < 0.05$), and also significant for Cu under fertilised conditions ($p < 0.01$).

The uptake of micronutrients by plants is suppressed to a large extent in calcareous soil (Kacar & Katkat 2009; Turan & Horuz 2012). While HA reduces salt uptake from the soil, at the same time it chelates with micronutrients that enhance their uptake by plants (Akıncı 2011; Khaled & Fawy 2011). Sharif et al. (2002) reported that treatment with HA increased the Zn, Fe, Mn, and Cu concentrations in corn plants when compared with the control. Similarly, it has been reported that HA regulates the bioavailability of micronutrients, decreasing plant deficiency by forming complexes (chelates) with Mn, Zn, Ca, Fe, Cu, and other elements (Ca, K, and Na) that facilitate its plant uptake especially in the calcareous soils (Tan 2003; Yingei 1988).

SODIUM CONTENT

The HA dosage had a significant effect on the plant Na concentration ($p < 0.05$) (Table 2). The highest Na

concentrations were in the wet alkali and N₂/O₂ activated HA control treatments under fertilised and unfertilised conditions at 96.6 and 99.0 mg/kg, respectively (Table 2). The lowest Na concentrations were 60.5 and 58.0 at 800 mg/kg of soil for wet alkali and N₂/O₂ activated HA use, respectively, under unfertilised conditions, and 40.5, and 34.2 mg Na/kg at 800 mg/kg for wet alkali and N₂/O₂ activated HA use, respectively, under fertilised conditions. Fertilisation decreased the plant Na concentration, due possibly to dilution, and it was especially apparent for N₂/O₂ activated HA. In effect, there were 59.1 and 65.4% decreases in the Na concentration for the 800 mg/kg dosage for the wet alkali and N₂/O₂ HA treatments, respectively (Table 3). That meant N₂/O₂ activated HA was slightly more effective in reducing Na uptake and hence the plant concentration (Figures 1 & 2).

There were negative correlations (-0.934*, -0.876 and -0.917*, -0.970**) between Na concentration and HA application for both HAs for the non-fertilised and fertilised treatments (Table 3). The reason for a lower Na concentration may be antagonistic relationships between soil Na and soil nutrients, especially micronutrients (Horuz et al. 2013; Kacar & Katkat 2009; Turan & Horuz 2012; Yingei 1998). Under both fertilisation treatments, there was a negative correlation between HA application and the plant Na concentration, and also between the Na concentration and Fe, Mn, Zn, Cu, and B concentrations (Table 3). Masciandaro et al. (2002) reported that humic substances moderated the effects of abiotic stresses on plants, such as unsuitable temperature and pH, and the decreased nutrient uptake induced by salinity. Moreover, Khaled and Fawy (2011) reported that under salt stress the first soil and leaf applications of 2 and 4 g/kg of solid humus and 0.1 and 0.2% doses of humic acid, respectively, increased nutrient uptake in corn plants.

TABLE 2. Corn stem + leaf Na and micronutrient concentrations obtained with two humic acid treatments

Treatments	HA mg/kg	mg/kg											
		Na		Fe		Mn		Zn		Cu		B	
		Fertilisation											
		-	+	-	+	-	+	-	+	-	+	-	+
	0	96.60	98.99	16.39	50.98	11.20	33.69	8.45a	29.88	7.43	22.06ef	6.39	19.18
	100	87.41	89.00	16.24	78.74	12.47	39.73	8.96a	35.59	9.02	32.44d	6.14	24.12
Wet alkali extracted HA	200	78.98	60.84	16.83	70.32	12.54	46.77	8.58a	40.49	8.38	39.42bc	8.43	38.25
	400	66.47	45.81	19.02	89.82	13.51	54.93	8.66a	46.28	9.84	48.04a	8.32	36.96
	800	60.46	40.48	20.43	83.39	12.89	66.07	9.68a	51.91	10.35	34.30cd	8.02	32.57

	0	96.60	98.99	16.39	50.98	11.20	33.69	8.45a	29.88	7.43	22.06ef	6.39	19.18
N ₂ /O ₂ activated HA	100	86.05	87.72	16.68	52.68	11.75	40.77	5.62b	33.90	8.90	19.07f	6.49	25.87
	200	73.72	69.77	18.02	69.78	9.39	61.99	8.40a	39.52	9.25	26.37f	7.17	34.64
	400	65.56	55.14	17.86	93.82	11.39	68.26	8.96a	44.56	9.46	44.96ab	7.97	39.63
	800	58.03	34.21	18.23	61.31	11.55	52.75	9.56a	39.02	11.09	34.23cd	8.45	37.23
Combined means for the two HAs	0	96.60A	98.99A	16.39B	50.98C	11.20	33.69C	8.45AB	29.88B	7.43	22.06C	6.39B	19.18C
	100	87.24B	88.36B	16.46B	65.71C	12.11	40.25C	7.29B	34.74AB	8.96	25.75C	6.31B	24.98B
	200	76.36C	65.30C	17.42AB	70.04B	10.97	54.37B	8.49AB	40.01AB	8.31	32.89B	7.80A	36.44A
	400	60.03D	50.48D	18.33AB	91.82A	12.44	61.59A	8.81A	45.42A	8.65	46.50A	8.14A	38.97A
	800	58.04D	38.84E	19.33A	72.35B	12.22	59.41A	9.62A	45.47A	9.38	34.26B	8.23A	34.90A
<i>F</i> : HA type		ns	ns	*	ns	*	ns	ns	ns	ns	**	ns	ns
<i>F</i> : Application rate		*	*	ns	**	ns	**	*	*	ns	**	**	**
<i>F</i> : HA type x application rate		ns	ns	ns	ns	ns	ns	*	ns	ns	**	ns	ns

The different letters in the same column indicate significant differences at $p < 0.05$, ** %1, * %5 and, ns: not significant Fertilised; -: Unfertilised

TABLE 3. Correlations between stem+leaf nutrient concentrations of micronutrients after the application of humic acid from two sources

HA type	Factor	Unfertilised						Fertilised					
		Na	Fe	Mn	Zn	Cu	B	Na	Fe	Mn	Zn	Cu	B
Wet alkali extracted	HA application	-.934*	.967**	.643	.837	.878	.611	-.876	.663	.981**	.954*	.407	.532
	Na		-.929*	-.857	-.653	-.918*	-.782		-.765	-.951*	-.970**	-.762	-.862
	Fe			.653	.714	.855	.628			.749	.805	.818	.652
	Mn				.382	.864	.684				.994**	.573	.682
	Zn					.770	.148					.657	.742
	Cu						.494						.887*
Activated by N ₂ /O ₂ gases	HA application	-.917*	.797	.220	.614	.940*	.953*	-.970**	.328	.481	.599	.647	.757
	Na		-.944*	.023	-.570	-.938*	-.967**		-.512	-.678	-.766	-.744	-.891*
	Fe			-.348	.631	.846	.885*			.910*	.914*	.890*	.791
	Mn				-.201	.095	.091				.975**	.795	.929*
	Zn					.392	.713					.868	.968**
	Cu						-.888*						.822

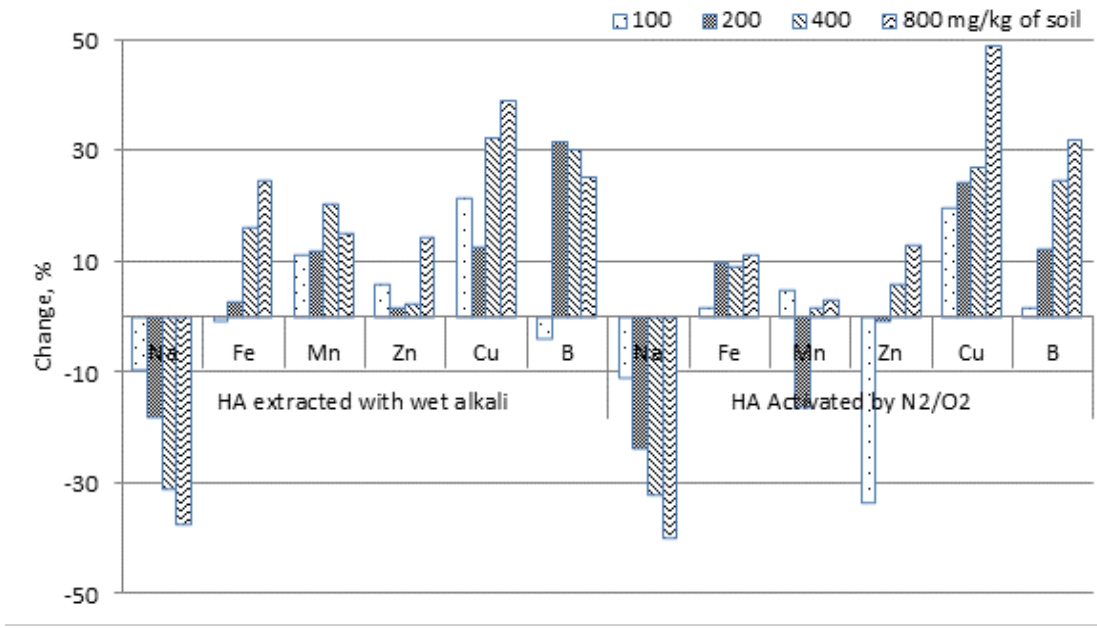


FIGURE 1. Homogenised stem and leaf Na and microelement concentrations of corn plants with two types of humic acid applied under unfertilised conditions

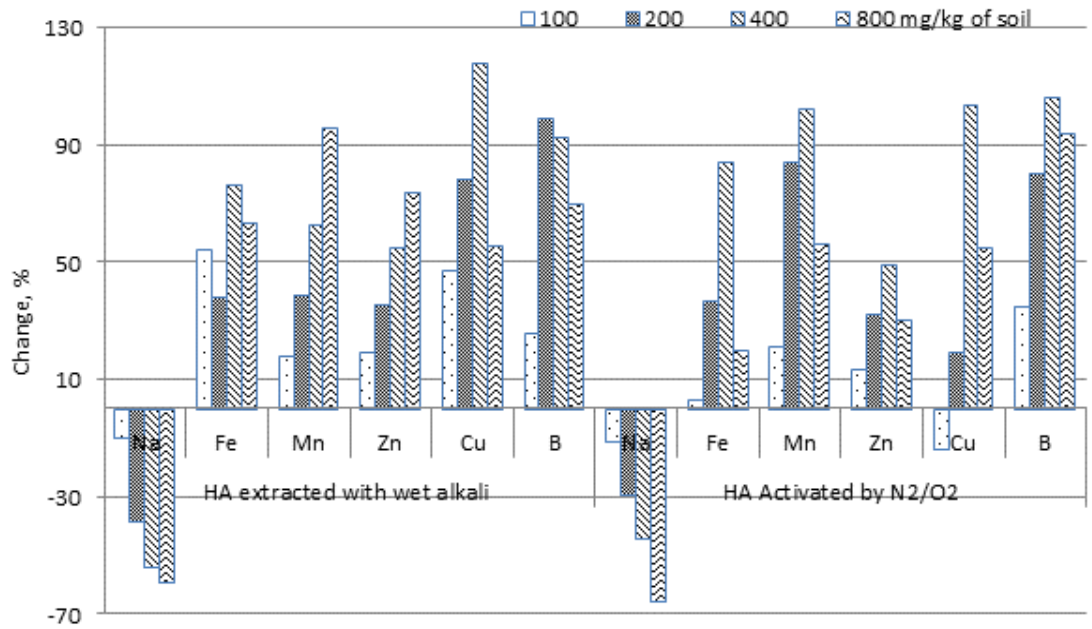


FIGURE 2. Homogenised stem and leaf Na and microelement concentrations of corn plants with two types of humic acid applied under fertilised conditions

IRON CONTENT

There was a HA application induced increase in the plant Fe concentration that was significant in combination with chemical fertilisation ($p < 0.01$). However, the HA type \times application rate interaction was not significant ($p > 0.05$). The plant Fe concentration for the 800 mg/kg HA treatment ranged from 18 to 20 mg/kg without fertilisation. In comparison, the concentration increased about 4.5 fold for the 400 mg/kg HA treatment in combination with chemical fertilisation (Table 2). The N_2/O_2 activated HA was more effective in increasing the Fe concentration in the fertiliser treatment. Moreover, there was a HA induced Fe concentration increase of up to 24.6% in the non-fertilised treatment whereas it was as high as 84.0% in the fertilised treatment (Table 3). That means that the HA treatment had a greater efficacy when used in combination with chemical fertilisation (Figures 1 & 2).

The correlations between both HA types and Fe and Na concentrations were significant and higher in treatments without fertilisation (Table 3). The reason for this may be the dissolution of existing Na compounds and $FeCO_3$ because of HA addition to the soil and its effect on the high pH and carbonate content of the soil. Kacar and Katkat (2009) reported that the available Fe concentration of calcareous soils could be increased by organic matter amendment. In the present study, positive correlations were also found between the Fe concentration and Mn, Zn, Cu, and B concentrations of corn plants in combination with both fertilisation regimes and the wet alkali HA treatment. For the HA activated with N_2/O_2 , there was a negative correlation with Mn concentration and positive correlations with the other micronutrients. In general, stronger correlations were evident for the activated HA treatment.

Similarly, Çimrin et al. (2019) reported that the application of 1000 mg/kg of HA to the soil increased the Fe, Zn, and Mn concentrations in corn plants. Normally, despite an accumulation of iron oxide minerals during the development of soils, the plant available concentration of Fe in calcareous soils is extremely low (Kacar & Katkat 2009; Turan & Horuz 2012). The incorporation into the soil of humic compounds can chelate the iron and facilitate plant uptake (Cheryl et al. 2001; Lobartini et al. 1997).

MANGANESE CONTENT

There was apparent HA induced Mn uptake by plants, possibly in the chelated form. The effect was more obvious for the combination of HA and chemical fertiliser ($p < 0.01$). The highest Mn concentration was 13.5 mg/kg of plant tissue at 400 mg/kg of soil application of wet alkali HA and without fertilisation; whereas it was 66.1 and 68.3 for 800 mg/kg application of wet alkali HA and 400 mg/kg application of N_2/O_2 activated HA with fertilisation, respectively (Table 2).

Overall, the combinations of wet alkali extracted HA without fertiliser and N_2/O_2 activated HA with fertiliser induced higher micronutrient uptake. When compared with the control, the highest increases in the plant Mn concentration without fertiliser application were 20.6 and 4.9% for 400 mg/kg of wet extracted HA and 100 mg/kg of HA activated with N_2/O_2 , respectively, and 96.1 and 102.6% for 800 mg/kg of wet extracted HA and 400 mg/kg of HA activated with N_2/O_2 , respectively, under fertilised conditions (Table 3).

Overall, the combinations of wet alkali extracted HA under unfertilised conditions and HA activated with N_2/O_2 under fertilised conditions produced higher increases in plant Mn content (Figures 1 & 2). Çelik et al. (2010) reported that foliar HA application had significant, positive effects ($p < 0.01$) on the Cu, Zn and Mn contents of corn plants. Under unsuitable soil conditions such as high pH and $CaCO_3$ levels, HAs cover the surface of micronutrient minerals like a membrane with two layers and enable the previously insoluble micronutrients to dissolve and then chelate (Tombacz & Rice 1999).

While there was a positive correlation between Mn content and HA application under both fertilisation regimes, there was a highly significant correlation (0.981**) between wet alkali extracted HA and Mn content under unfertilised conditions. The correlations between Mn and HA (0.643) and Na and Mn (-0.857) for wet alkali extracted HA under unfertilised conditions were lower and higher, respectively, than for the fertilised conditions at 0.981** and -0.951*, respectively. Overall, for HA activated with N_2/O_2 , lower correlation coefficients were found under fertilised and unfertilised conditions (Table 3). The results of this study showed that wet alkali extracted HA, under both unfertilised and fertilised conditions, was more effective in increasing the Mn content of corn tissues and decreasing Na content. While negative correlations were found between the Mn content and Fe and Zn contents for HA activated with N_2/O_2 under unfertilised conditions at -0.348 and -0.201, respectively, there was a positive correlation between the fertilisation regimes and HA type. While low correlations were found between Mn content and the Zn, Cu and B contents for HA activated with N_2/O_2 and under unfertilised conditions, high correlations were found under the other conditions. Thus, wet alkali extracted HA was more effective than the gas activated HA in facilitating micronutrient uptake under unfertilised conditions.

ZINC CONTENT

The Zn content of corn plants significantly increased ($p < 0.05$) for some HA application rates under fertilised conditions. The highest Zn contents were registered for 800 mg/kg of wet extracted and N_2/O_2 activated HA at 9.7 and 9.6 mg/kg, respectively, under unfertilised conditions, and at 51.9 and 44.56 for 800 mg/kg of wet alkali extracted HA, and 400 mg/kg HA activated with

N_2/O_2 , respectively, under fertilised conditions (Table 2). Furthermore, wet extracted HA produced a higher plant Zn content than the gas activated HA under both fertilised and unfertilised conditions. When compared with the control, the highest changes in Zn content were 14.6 and 13.2%, for 800 mg/kg of wet alkali and N_2/O_2 activated HA, respectively, under unfertilised conditions, and 73.8 and 49.2 for 800 mg/kg of wet alkali extracted HA and 400 mg/kg of HA activated with N_2/O_2 , respectively, with fertiliser use (Table 3). It is noteworthy that wet alkali extracted HA induced a higher increase in Zn content under both unfertilised and fertilised conditions (Figures 1 & 2). Manzoor et al. (2014) reported that HA increased the Cu and Zn content of wheat, and Asri et al. (2016) reported that the foliar application of 0, 0.15, 0.30 and 0.45% HA to tomato plants increased the leaf Mn and Cu contents, and that the most effective dosage was 0.30%.

While there was a positive correlation between Zn content and HA application under both fertilisation regimes and HA types, there was a negative correlation with Na content. In a specific case, the correlation with Zn content was application rate dependent for wet alkali extracted HA under fertilised conditions. Furthermore, the correlation between wet alkali HA application and Na content was much higher than for HA activated with N_2/O_2 at 0.970** and -0.766, respectively.

Under fertilised conditions, there was a high correlation between the Zn content and Fe and Mn contents at 0.805 and 0.994**, respectively, with wet alkali HA, and 0.914* and 0.975**, respectively, for HA extracted with N_2/O_2 . Similarly, there were high correlations between Zn content, and the Cu and B contents, for HA activated with N_2/O_2 , at 0.868 and 0.968**, respectively. Overall, fertiliser application in association with both HA types increased the plant Zn content more than non-fertilisation.

In this study, it appears that there was a positive effect between Zn, Fe, and Mn by means of HAs forming complexes with them. In addition, some researchers have suggested that there are positive correlations between HA use and the levels of micronutrients in plants because HA form complexes with them (Gezgin & Hamurcu 2006; Turan & Horuz 2012).

COPPER CONTENT

The Cu content of corn plants increased under both fertiliser regimes, with the increase being HA dose dependent. However, only fertiliser application caused a significant increase ($p < 0.01$). The Cu contents were 10.4 and 11.1 for 800 mg/kg of wet extracted and N_2/O_2 activated HA, respectively, under unfertilised conditions, and 48.0 and 45.0 for 400 mg/kg dose of wet extracted and N_2/O_2 activated HA, respectively, under fertilised conditions (Table 2). Compared to the controls, there was

a general increase in the plant Cu content for both HA types under both fertiliser regimes. The highest increases were 39.90, and 49.30% for 800 mg/kg of soil application of wet extracted and N_2/O_2 activated HA, respectively, under unfertilised conditions, and 117.8 and 103.81% for 400 mg/kg soil application of wet extracted and N_2/O_2 activated HA, respectively, under fertilised conditions (Table 3). In addition, there was a 125.0% increase in Cu content for 400 mg/kg of wet extracted HA and a 79.70% increase for 800 mg/kg of HA extracted with N_2/O_2 (Table 3). In a recent study, Danre et al. (2014) reported that maximum Cu concentration in garlic was obtained with 300 mg/kg of HA in soil. In the present study, there were significant correlations between the plant Cu content and HA activated by N_2/O_2 (0.940*) under unfertilised conditions, and also between Cu content and Fe content (0.890*) under fertilised conditions. There were also positive correlations between the Cu content and the plant concentrations of Mn, Zn and B, and negative correlations between the Cu content and Na concentration for the wet alkali extract (-0.918*), and for the N_2/O_2 activated HA (-0.938*) under unfertilised conditions. The correlations were higher under unfertilised conditions than under fertilised conditions, except for Fe, Mn and Zn and HA activated with N_2/O_2 . In addition, there was a significant, positive correlation between the Cu and B content of corn plants under fertilised conditions in combination with wet alkali extracted HA (0.887*), and a negative correlation for unfertilised conditions and the use of HA activated by N_2/O_2 (0.888*). Overall, HA extracted with N_2/O_2 was more effective under unfertilised conditions (Figure 1) and wet extracted HA was more effective under fertilised conditions (Figure 2).

BORON CONTENT

The B content of corn plants increased significantly ($p < 0.01$) under both fertilisation regimes, dependent on the HA application rate. The highest B contents were 8.4 and 8.5 mg/kg for 200 mg/kg wet extracted HA and 800 mg/kg of gas activated HA under unfertilised conditions, respectively, and 38.3 and 39.6 for 200 mg/kg of wet extracted HA and 400 mg/kg of gas activated HA, respectively, under fertilised conditions (Table 2). Under both unfertilised and fertilised conditions, the effects of HA activated with N_2/O_2 gases were higher on the B content of the corn plants. When compared with the control, the highest changes in B content were 31.9 and 32.2% for the 200 mg/kg wet extracted HA and 800 mg/kg of gas activated HA treatments, respectively, under unfertilised conditions, and 99.40% for 200 mg/kg soil of wet alkali HA and 106.60% for 400 mg/kg soil of gas activated HA (Table 3). Wet extracted HA induced a higher increase in B content under unfertilised conditions, while the gas activated HA produced a higher increase

in B concentration under fertilised conditions (Figures 1 & 2). The current study supports the result of Ekinci et al. (2015) who reported that HA applied as boron humate to tomato plants increased both the leaf and fruit B content.

In addition, significant correlations were found between the plant B content and the application of gas activated HA (0.953*), the Fe and Cu contents under unfertilised conditions in association with gas activated HA (0.885* and -0.888*), and also the Mn (0.929*) and Zn (0.968**) contents under fertilised conditions. Furthermore, there was a significant positive correlation (0.887*) between the B and Cu contents under fertilised conditions and with wet alkali extracted HA applied. In contrast, there were negative correlations for the relationship between Na content and fertiliser regime, specifically -0.967** under unfertilised conditions and -0.890* under fertilised conditions.

CONCLUSION

In this study, HA activated by N_2/O_2 was more effective than wet alkaline sourced HA, especially in decreasing the Na content and increasing the Fe, Mn, and B contents of corn plants. Thus, the gas activated HA is potentially more effective in reducing Na damage (salt effect) to the corn plants and increasing yields by boosting the plant levels of micronutrients. That would mean that less gas activated HA needs to be applied to calcareous soil to reduce Na toxicity and increase the uptake of some micronutrients by corn plants, compared to wet alkali extracted HA. The results of this study therefore justify new studies on the efficacy of the use of HA activated by N_2/O_2 on different agricultural crops.

ACKNOWLEDGEMENTS

The authors thank Dr. Gregory T. Sullivan for both the English editing and helpful comments on an earlier version of this manuscript.

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Received: 10 May 2019
Accepted: 4 March 2020