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Abstract

The aim of this study was to examine the effects of the soil application of humus substances on the dry weight and the nutrient uptake of selected elements in maize grown under salt stress in greenhouse conditions. Sodium chloride was added to the soil to obtain 0, 15, 30, 45 or 60 mM NaCl. Three different doses of solid humus (0, 1 or 2 g kg^{-1}) were applied to the soil one month prior to planting. High levels of salt (45 and 60 mM NaCl) had negative impacts on the dry weight and the N, P, K, Ca, Mg, Fe, Cu, Zn and Mn uptake of the maize plants. The highest mean dry weight, Mg and Mn uptake were observed for the 1 g humus kg^{-1} treatment and the highest mean Cu content was in the 2 g humus kg^{-1} treatment. On the contrary, the highest mean uptakes of N and P were found in the soils in which humic substances was not added. The interactions of NaCl and the soil humus content were significant for the uptake of Cu ($p \leq 0.01$), and we found that adding humus increased the content of Cu in maize plants under slight salt stress (15 mM NaCl) ($p \leq 0.01$).

Keywords: humic substances, maize, nutrient elements, salinity

Introduction

Soil salinity affects agricultural productivity in many parts of the world, particularly in irrigated lands (Allakhverdiev *et al.*, 2000; Zörb *et al.*, 2004). The genesis of saline soils may be natural or accelerated by the extension of irrigation in agricultural practices, the intensive use of water resources combined with high evaporation rates and human activity (Lambers, 2003; Arzani, 2008). In addition to uncontrolled irrigation, continuous cropping, excessive fertilization and poor-quality water may also cause salinity problems with reduction in the yield and quality of product (Maas and Grattan, 1999; Cansev and Ozgur, 2010).

Several studies have evaluated the effect of the organic matter content on the fertility of soils (Loveland and Webb, 2003; Pan *et al.,* 2009). The humic substances, the major component of soil organic matter, have both direct and indirect effects on plant growth (Sangeetha *et al.*, 2006). The direct effects are those that require the uptake of humic substances into the plant tissue resulting in various biochemical outcomes, whereas the indirect effects involve the improvement of soil properties, such as aggregation, aeration, permeability, water holding capacity, micronutrient transport and availability (Tan, 2003). Chen and Aviad (1990) and Varanini and Pinton (1995) have summarized the effects of humic substances on plant growth and mineral nutrition, pointing out the positive effects on seed germination, seedling growth, root initiation, root growth, shoot development and the uptake of macro and microelements.

The agricultural areas that are affected by salt need amendments, such as a determination of the most suitable salt-tolerant plant species or the application of different substances in order to reduce the effects of salinity (Bartels and Sunkar, 2005; Yamaguchi and Blumwald, 2005). Xudan (1986) and Kulikova *et al.* (2005) have indicated that humic substances might provide anti-stress effects to plants under abiotic stress conditions (i.e., unfavorable temperature, extreme pH, and soil salinity) by reducing the uptake of some toxic elements.

Considering that salinity is a major problem in the cereal growing basins in Turkey there is a need to determine the effects of salinity and soil applications of humic substances on the growth of plants. In this context, the objective of the current study was to determine the relations between the application of humic substances and the growth/nutrient uptake of maize (*Zea mays* L.), a moderately salt-sensitive plant, under salt stress.

Materials and methods

The soil used in this study was collected from a depth of 0-20 cm of the field located in the Agricultural Research and Application Center of Uludag University, Bursa, Turkey. The soil was classified as Vertisol (Typic haploxerert) according to Soil Taxonomy and in the unit of Eutric Vertisol according to the FAO/UNESCO classification systems (Aksoy *et al.*, 2001).

Selected physical and chemical properties of the soil were analyzed. The texture was determined using the hydrometer method (Tan, 2005). The pH and EC were measured in a 1:2.5 water extract, and the lime content was determined according to Richards (1954). The organic matter content was analyzed according to the modified Walkley-Black method (Nelson and Sommers, 1982). Total nitrogen was determined with a Buchi K-437 / K-350 Digestion/Distillation Unit according to the metod of Kjeldahl (Bremmer, 1965). The available P was determined using a Shimadzu UV 1208 model spectrophotometer according to the method of Watanabe and Olsen (1965). Exchangeable cations (Na, K, Ca and Mg) were extracted with ammonium acetate at pH 7.0 (Jackson, 1958) and determined using an Eppendorf Elex 6361 model Flame Photometer. Available Fe, Cu, Zn, Mn were extracted with DTPA (0.005M DTPA+0.01M $CaCl_2$+0.1M TEA pH 7.3) (Lindsay and Norwell, 1978) and determined with a Philips PU9200x model Atomic Absorption Spectrophotometer. Selected chemical and physical properties of the soil used in this research are shown in Tab. 1. The soil used in the experiment had a neutral pH, it was classified as sandy clay with a low salt and content, and the content of organic matter and was inadequate in terms of nitrogen, phosphorus and zinc.

Tab. 1. Physical and chemical properties of the soil

Texture	Sandy clay	Exchangeable cations, me $100g^{-1}$	
Sand, %	45.15	Sodium (Na)	0.17
Silt, %	15.22	Potassium (K)	0.45
Clay, %	39.63	Calcium (Ca)	19.26
pH	7.24	Magnesium (Mg)	2.35
EC, mS cm^{-1}	0.83	Available microelements, mg kg^{-1}	
$CaCO_3$, %	0.22	Iron (Fe)	5.56
Organic matter, %	1.30	Copper (Cu)	1.30
Total nitrogen (N), %	0.08	Zinc (Zn)	0.20
Available phosphorus (P), mg kg^{-1}	7.96	Manganese (Mn)	10.44

The experiment was conducted in a greenhouse in a completely randomized factorial design with three soil application doses of humus, 0 (control) 1 or 2 g kg^{-1}, and five NaCl doses, 0 (control), 15, 30, 45 or 60 mM. Each application consisted of three replications. The humus that was used obtained from Delta Chemicals Co. (Ankara, Turkey) and was a derivate of leonardite (65% w/w, pH 4.87, EC: 5.80 mS cm^{-1}) and commercially known as Deltahumus.

Air-dried soil samples were passed through a 4 mm sieve. For humus applications, Deltahumus was placed into a large bowl and the total weight of the soil was adjusted to 5 kg. The mixture was homogenized and put into polyethylene covered plastic pots. Sodium choride was added to the pots according to the final salt levels and the pots were incubated for 30 days. As a basal fertilizer, nitrogen (100 mg kg^{-1}, as NH_4NO_3), phosphorus (80 mg kg^{-1}), potassium (100 mg kg^{-1}, as KH_2PO_4), zinc (0.5 mg kg^{-1}, as $ZnSO_4$) were applied to the pots before planting. Six maize (*Zea mays* L. 'Fleuri AG 92149') seeds were sown into each pot which was thinned to four after emergence which had a diameter of 20 cm and a depth of 18 cm. All pots were irrigated with deionized water during the experiment.

After two months of vegetative growth, the plants were harvested, dried at 65°C and the dry weights were determined. The plant samples were wet digested by using a HNO_3+$HCIO_4$ (4:1) mixture. The nitrogen level was determined by the Kjeldahl method (Bremmer, 1965), and the P was determined by the vanadomolybdophosphoric acid method using a spectrophotometer (Kacar and Inal, 2008). The K, Na and Ca values were determined by flame emission (Horneck and Hanson, 1998). The contents of Mg, Fe, Mn, Zn and Cu were determined by atomic absorption spectrometry (Philips PU 9200x, Pye Unicam Ltd. GB) (Hanlon, 1998).

The pot experiment was arranged in a completely randomized design (CRD) with three soil application doses of humus, five NaCl doses and three replicates (n=45). Analysis of variance (ANOVA) of data for all parameters was computed using TARIST computer package (Tarist, 1994). The mean values were grouped with LSD multiple range test ($p \leq 0.01$).

Results and discussion

The effects of NaCl on plant growth and the uptake of plant nutrients

The effects of the soil application of humus on plant growth, the uptake of mineral nutrients and their interactions with NaCl levels are given in Tab. 2, 3 and 4. According to the results, an increase in the NaCl level gradually decreased the growth, expressed as dry weight, of the maize plant (Tab. 2). Reductions in dry weight, even at the

Tab. 2. The effects of soil application of humus on plant dry weight under increasing NaCl levels (g pot^{-1})

NaCl mM	Humus levels, g kg^{-1}						
	0		1		2	Mean	
0	21.94		24.22		21.53	22.56	a
15	22.51		21.27		20.33	21.37	a
30	18.08		17.73		14.97	16.93	b
45	15.96		14.46		13.11	14.51	c
60	10.77		12.79		11.06	11.54	d
Mean	17.85	A	18.09	A	16.20 B		
Humus $LSD_{0.01}$ 1.277		NaCl $LSD_{0.01}$ 1.649			Humus x NaCl ns		

The differences between values indicated by different letters are significant. Capital letters indicate rows and small letters indicate columns. ns: not significant

Tab. 3. The effects of soil application of humus on macronutrients uptake of maize under increasing NaCl levels (mg tdw^{-1})

NaCl, mM	Nitrogen							
	Humus Levels, g kg^{-1}							
	0		1		2		Mean	
0	408.68		433.67		422.09		421.48	a
15	431.25		417.77		392.91		413.98	a
30	416.68		379.67		353.19		383.18	b
45	318.39		301.06		297.10		305.52	c
60	230.90		205.28		202.79		212.99	d
Mean	361.18	A	347.49	AB	333.62	B		
	Humus $LSD_{0.01}$ 22.161		NaCl $LSD_{0.01}$ 28.610		Humus x NaCl ns			

NaCl, mM	Phosphorus							
	Humus Levels, g kg^{-1}							
	0		1		2		Mean	
0	66.55		72.69		61.26		66.83	a
15	65.59		62.12		57.13		61.61	b
30	58.20		52.11		44.46		51.59	c
45	43.64		41.90		40.78		42.10	d
60	29.74		31.32		25.99		29.01	e
Mean	52.74	A	52.03	A	45.92	B		
	Humus $LSD_{0.01}$ 4.005		NaCl $LSD_{0.01}$ 5.171		Humus x NaCl ns			

NaCl, mM	Potassium							
	Humus Levels, g kg^{-1}							
	0		1		2		Mean	
0	478.80		491.87		468.66		479.78	ab
15	479.11		634.58		577.52		563.74	a
30	417.46		403.05		459.67		426.72	bc
45	394.06		312.21		401.34		369.20	cd
60	256.30		331.07		357.82		314.97	d
Mean	405.09		435.56		453.00			
	Humus ns		NaCl $LSD_{0.01}$ 103.333		Humus x NaCl ns			

NaCl, mM	Calcium							
	Humus Levels, g kg^{-1}							
	0		1		2		Mean	
0	89.72		88.63		89.11		89.15	a
15	100.38		96.55		95.44		97.46	a
30	96.83		98.62		87.11		94.19	a
45	93.19		87.53		84.53		88.42	a
60	65.69		73.56		73.68		70.97	b
Mean	89.16		88.98		85.97			
	Humus ns		NaCl $LSD_{0.01}$ 9.898		Humus x NaCl ns			

NaCl, mM	Magnesium							
	Humus Levels, g kg^{-1}							
	0		1		2		Mean	
0	67.80		88.82		70.20		75.61	b
15	79.96		94.95		81.68		85.53	a
30	76.88		89.46		68.62		78.32	ab
45	66.92		77.68		63.05		69.22	b
60	43.01		60.12		51.75		51.63	c
Mean	66.92	B	82.21	A	67.06	B		
	Humus $LSD_{0.01}$ 7.265		NaCl $LSD_{0.01}$ 9.379		Humus x NaCl ns			

The differences between values indicated by different letters are significant; capital letters indicate rows and small letters indicate columns; ns: not significant; tdw: total dry weight

Tab. 4. Effects of soil application of humus on micronutrients uptake of maize under increasing NaCl levels (mg tdw^{-1})

NaCl, mM	Iron			
	Humus Levels, g kg^{-1}			
	0	1	2	Mean
0	1.697	1.553	1.420	1.557 a
15	1.583	1.590	1.277	1.483 a
30	1.627	1.680	1.520	1.609 a
45	1.313	1.183	1.147	1.214 b
60	0.813	0.713	0.803	0.777 c
Mean	1.407	1.344	1.233	
	Humus ns	NaCl $LSD_{0.01}$ 0.258	Humus x NaCl ns	

NaCl, mM	Copper			
	Humus Levels, g kg^{-1}			
	0	1	2	Mean
0	0.087 bc B	0.210 a A	0.220 a A	0.172 a
15	0.143 ab B	0.173 ab B	0.240 a A	0.186 a
30	0.163 a A	0.163 ab A	0.190 a A	0.172 a
45	0.097 bc A	0.123 bc A	0.123 b A	0.114 b
60	0.083 c A	0.097 c A	0.083 b A	0.088 b
Mean	0.115 B	0.153 A	0.171 A	
	Humus $LSD_{0.01}$ 0.026	NaCl $LSD_{0.01}$ 0.033	Humus x NaCl $LSD_{0.01}$ 0.058	

NaCl, mM	Zinc			
	Humus Levels, g kg^{-1}			
	0	1	2	Mean
0	0.177	0.200	0.200	0.192 a
15	0.227	0.207	0.200	0.211 a
30	0.193	0.193	0.173	0.187 a
45	0.157	0.157	0.163	0.159 b
60	0.120	0.137	0.087	0.114 c
Mean	0.175	0.179	0.165	
	Humus ns	NaCl $LSD_{0.01}$ 0.025	Humus x NaCl ns	

NaCl, mM	Manganese			
	Humus Levels, g kg^{-1}			
	0	1	2	Mean
0	1.533	1.843	1.413	1.597 c
15	2.097	2.300	1.960	2.119 a
30	1.973	1.973	1.697	1.881 b
45	1.617	1.597	1.477	1.563 c
60	1.097	1.270	1.183	1.183 d
Mean	1.663 B	1.797 A	1.546 B	
	Humus $LSD_{0.01}$ 0.119	NaCl $LSD_{0.01}$ 0.154	Humus x NaCl ns	

NaCl, mM	Sodium			
	Humus Levels, g kg^{-1}			
	0	1	2	Mean
0	3.573	3.313	3.050	3.312 c
15	3.963	3.977	3.593	3.844 c
30	4.953	3.797	4.227	4.326 bc
45	6.613	5.667	7.563	6.614 ab
60	9.340	9.367	7.770	8.826 a
Mean	5.689	5.224	5.241	
	Humus ns	NaCl $LSD_{0.01}$ 2.365	Humus x NaCl ns	

The differences between values indicated by different letters are significant. Capital letters indicate rows and small letters indicate columns. ns: not significant lowest level of NaCl, were observed as 5.27 %. The applications of higher NaCl concentrations had negative effects on the uptakes of N, P, K, Ca, Mg, Fe, Cu, Mn and Zn in maize; except for Na (Tab. 3 and 4). The decreases in the dry weight and uptake of mineral elements in the plants were evident particularly at the 45 and 60 mM of NaCl levels.

The salinity of the soil is a critical factor in plant production, as an excess of salts leads to both osmotic and ionic stresses that suppress plant growth and nutrient uptake (Grattan and Grieve, 1999; Benlloch and Gonzalez, 2005; Turan *et al.*, 2007a, b; 2010; Dhanapackiam and Muhammed Ilyas, 2010). In the present study, it has been observed a significant reduction in the growth of maize plants associated with increasing NaCl concentrations.

Salinity affects both water absorption and biochemical processes resulting in reduction of plant growth, particularly due to a decline in the rates of net photosynthesis, negative effect on the CO_2 assimilation and excessive decrease in nutrient uptake (Parida and Das, 2005; Cha-Um and Kirdmanee, 2009).

Many researchers have reported that treatment of low levels of NaCl reduce the dry weight of experimental plants (Al-Karaki, 1997; Taban *et al.*, 1999). The results obtained in our study were similar to these findings for low levels of NaCl, however, it has been found that high levels of NaCl (30, 45 and 60 mM) significantly inhibited the growth of the maize plants (Khan *et al.*, 2000; Asik *et al.,* 2009). Salinity is known to reduce the N accumulation (Alam, 1994; Turan *et al.*, 2007a, b), the P concentrations (Navarro *et al.,* 2001) and the uptake of K in plants (Lopez and Satti, 1996). High Na also has an antagonistic effect on the uptake of Ca and Mg, most likely caused by the displacement Ca and Mg by Na in the cellular membranes of the root (Yermiyahu *et al.*, 1997). Under salt stress, the solubility of micronutrients is particularly low, and plants grown in these soils often show mineral deficiencies. Furthermore, these detrimental effects are generally observed at the whole plant level (Francois and Maas, 1999).

The effects of the soil application of humus on plant growth and the uptake of plant nutrients

The soil application of humus was significantly effective on dry weight and on the uptakes of N, P, Mg, Cu and Mn (Tab. 3 and 4). The highest mean dry weight and uptakes of Mg and Mn were obtained with 1 g humus kg^{-1} treatment (82.21 and 1.797 mg pot^{-1}), and the highest amount of Cu (0.171 mg pot^{-1}) was obtained with 2 g humus kg^{-1} treatment. On the contrary, the highest means for the uptakes of N and P, 361.18 and 52.74 mg pot^{-1}, respectively, were determined at the dose of 0 g kg^{-1} of humus (Tab. 3).

Chen and Aviad (1990), Fagbenro and Agboda (1993) and David *et al.* (1994) have reported that the addition of humic substances, which act in a similar way to growth hormones, promoted the growth and nutrient uptake of plants. Plants are able to take up more mineral elements due to an increased membrane permeability, and a better-developed root system facilitates the transport of elements within the roots (Zientara , 1983).

Regardless, there is little available literature regarding the application of humic acid and its effects on salinity tolerance in plants. Liu (1998) has reported that a treatment of humic acid under salt stress did not increase the uptake of N, P, K or Ca; however, Asik *et al.* (2009), have stated that a high humic acid treatment did have a slight effect. In the present study, the uptake of minerals was negatively affected with the 2 g humus kg^{-1} treatment, in accordance with Arancon *et al.* (2006); that this was likely due to the levels of treatments, the growth medium and the origin of the humic substance.

The interaction of humic substances with salt stress

The interaction between the application of humic substances and NaCl to the soil was found significant only for the Cu uptake ($p<0.01$) in the maize plants. The effects on the dry weight and the uptake of other nutrients were not significant. The highest uptake of Cu (0.240 mg pot^{-1}) was obtained in the treatment of 2 g humus kg^{-1} + 15 mM NaCl (Tab. 4).

Although treatment of the soil with NaCl decreased the dry weight and the uptake of nutrients, the soil humus treatments ameliorated this decrease, particularly under the treatment of 60 mM of NaCl. Therefore, can be conclude that treatment of the soil with humus enhanced the uptake of nutrients in plant under conditions of 45 and 60 mM NaCl (Tab. 3 and 4).

Conclusions

Humic substances, which are generally associated with natural organic compounds comprising 50 to 90% of the organic matter of peat, lignites, sapropels and the non-living organic matter of soil and water ecosystems, can significantly reduce water evaporation and increase water availability to plants in non-clay, arid and sandy soils. Humic substances can help to improve unfavourable soil properties, plant productivity and nutrient uptake. Humic substances, holds positively charged molecules of soil surface that can be absorbed by the plant's roots and helps to improve micronutrient exchange. These benefits of humic substances may only be apperent under defined doses, thus the determination of the treatment dose is crucial under saline conditions. According to our data, the limit of application should not exceed 1 g humus kg^{-1} in the soil.

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