

EFFECT OF SALINITY ON UPTAKE OF MICRONUTRIENTS IN SUNFLOWER AT EARLY VEGETATIVE STAGE

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Abstract

A pot culture experiment was conducted to study the effects of 4 different levels of salinity (EC = 1.19, 9.54, 16.48 and 22.38 mS/cm) on the uptake of micronutrients (viz., Cu, Mn, Fe and Zn) by 2 varieties of sunflower (*Helianthus annuus* L.) at early vegetative stage. Salinity levels were prepared by dissolving calculated amount of NaCl₂, Na₂SO₄, CaCl and MgCl₂ (4:10:5:1) in half strength Hoagland culture solution. In response to various levels of salinity, the uptake of all mentioned micronutrients of roots and shoots of sunflower exhibited significant response ($p < 0.05$ and $p < 0.01$) while only the response of Mn uptake in shoot was found non significant. A maximum significant uptake of Cu, Mn, Fe and Zn in shoot (19.50, 120.67, 1647.67 and 59.17 $\mu\text{g/g}$) is obtained under highest dose of applied salinity (22.23 mS/cm) whereas with the exception of Zn, a maximum significant uptake of Cu (25.67 $\mu\text{g/g}$), Mn (144.87 $\mu\text{g/g}$), and Fe (5837.5 $\mu\text{g/g}$) in root as well in highest dose of salinity was observed. With reference to ratio of Fe and Zn uptake in root and shoot, variety DO 730 responded well than variety DO 728. Results on the bases of grand sum values depicted 20.38 and 69.33% decrease in uptake of Cu and Fe, but 7.65 and 18.37% increase in uptake of Mn and Zn in shoot over root in both the varieties, respectively was observed.

Introduction

Salinity is a major abiotic environmental factor by reducing plant growth and productivity throughout the world. Approximately 23% of the cultivated lands are considered as saline and another 37% are sodic. It has been also estimated that salinity and water logging seriously affect one-half of all irrigated lands i.e., 2.5×10^8 hectares. About 20 million hectares of land deteriorates to zero production each year. This problem is more serious in agriculture of south and Southeast Asia (Malcolm, 1993; Francois & Maas, 1999). The recent figure for the extent of salt affected soils in Pakistan is 61,73,000 hectares (Anon., 1999). It includes both inland and coastal areas most of which are saline and not suitable for cultivation of conventional crops, forages, fuelwood and timber species.

The criteria used to appraise the salt tolerance potential of any plant species are morphological, physiological, and biochemical in nature (Rawson *et al.*, 1988; Shannon, 1997; Flowers, 2004; Ashraf & Harris, 2004). Physiological criteria include ionic contents and photosynthetic rates (Schachtman & Munns, 1992; Murrillo-Amador *et al.*, 2002; Morant-Manceau *et al.*, 2004). Induced nutrient deficiency is one of the most important aspects of salinity, leading to serious perturbation of normal cellular activities.

Research revealed that salinity inhibits the growth of plants by affecting both water absorption and biochemical processes such as N and CO₂ assimilation and protein biosynthesis (Cusido *et al.*, 1987). Under saline conditions plants fail to maintain the required balance of organic and inorganic constituents leading to suppressed growth and yield (Gunes *et al.*, 1996). Plant performance, usually expressed as a crop yield, plant

biomass or crop quality (both of vegetative and reproductive organs), may be adversely affected by salinity induced nutritional disorders. These disorders may be as a result of the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant (Grattan & Grieve, 1999; Zhu, 2003; Ali *et al.*, 2006a; Nasim *et al.*, 2008). Saline conditions drastically change the environment of root aeration, osmotic potential of soil solution and normal equilibrium of the dissolved ions. The availability of most micronutrients to crop plants mainly depend upon the pH of the soil solution as well as the nature of binding sites on organic and inorganic particle surfaces. In saline and sodic soils, the solubility of micronutrients (Cu, Mn, Fe, Zn and Mo) is particularly low, and plants growing on such soils often experience deficiencies in these elements (Page *et al.*, 1990), but not in all cases. Very little attention has been diverted towards salinity's effect on Cu uptake and its accumulation in crop plants. However, in available literature salinity's influence on Cu accumulation has been reported variable. Cu concentrations in leaf and stem were found to decrease in salt-stressed maize grown in both solution cultures (Izzo *et al.*, 1991) and soil (Rahman *et al.*, 1993), but on the other hand NaCl salinity substantially increased leaf Cu in hydroponically-grown tomatoes (Izzo *et al.*, 1991). Most of the studies indicated that salinity reduces Mn level in corn shoot tissue (Izzo *et al.*, 1991; Rahman *et al.*, 1993) and tomato (Alam *et al.*, 1989). However, some studies exhibited that salinity either had no effect (Al-Harbi, 1995) or increased Mn (Niazi & Ahmad, 1984) in leaf or shoot tissue of tomato. Different plants behave differently. The majority of studies in the literature have shown salinity to increase Zn concentration in shoot tissue such as in citrus (Ruiz *et al.*, 1997), maize (Rahman *et al.*, 1993) and tomato (Knight *et al.*, 1992), but in other studies it was not affected (Izzo *et al.*, 1991) or actually decreased Zn concentration as in case of cucumber leaves (Al-Harbi, 1995). Reports on the influence of iron (Fe) concentration in plants are as inconsistent as those of Zn and Mn concentration. Reports also stated that Fe, Mn, Cu and Zn concentrations were higher in roots compared with those in leaves and stem in salt applied samples of 12 soybean cultivars (Tunçturk *et al.*, 2008).

Species and varieties of various plants differ greatly in their response to salinity of root medium (Saqib *et al.*, 2005; Ali *et al.*, 2006b; Tahir *et al.*, 2006; Nasim *et al.*, 2008). Researchers also reported that response of a plant to saline growth substrate varies with its age thereby altering the degree of salt tolerance (Ashraf, 1994; Ashraf & O'Leary, 1994; Ashraf & Khanum, 1997; Ashraf & Sharif, 1998; Ashraf & Harris, 2004; Qasim & Ashraf, 2006; Raza *et al.*, 2006), although in some other studies the reverse was true since the salt tolerance in them was not age dependant (Ashraf & Fatima, 1994, and 1995; Ashraf *et al.*, 1994; Ashraf & Tufail, 1995). However, of the various plant responses to salt stress reported in literature, pattern of ion uptake is of prime importance since it determines the means whereby plants maintain water balance and avoid Na⁺ and/or Cl⁻ toxicity under saline conditions (Munns *et al.*, 2000). Difference among species and varieties/cultivars for salinity tolerance may depend on their differences in salinity tolerance mechanism. Exploitation of these useful genetic variations in salinity tolerance particularly of crop plants is an economical approach for proper utilization of salt-affected agricultural lands. In view of the above fact, a study was conducted to appraise the effect of different salinity levels on the uptake of micronutrients in two sunflower cultivars at early vegetative stage.

Table 1. Amount of salt added in one-liter solution of various treatments.

Salinity treatments	EC mS/cm	Osmotic potential at 20°C (bars)	Amount of salts/L.				Molar concentration	pH
			NaCl	Na ₂ SO ₄	CaCl ₂	MgCl ₂		
S ₀	1.19	-	-	-	-	-	-	4.03
S ₁	9.54	-4.67	1.17	4.68	2.35	0.609	0.2	4.40
S ₂	16.48	-9.35	2.34	9.36	4.70	1.220	0.4	4.36
S ₃	22.38	-14.04	3.51	14.04	7.05	1.820	0.6	4.30

Materials and Methods

Four different levels of salinity (i.e., S₀, S₁, S₂ and S₃) having EC values of 1.19, 9.54, 16.48 and 22.38 ms/cm were used in present study to investigate their effects on the micronutrient uptake of sunflower (*Helianthus annuus* L.). The certified seeds of two varieties of sunflower viz., DO 728 and DO 730 were obtained from Agricultural Research Institute (ARI), Quetta. The above treatments/levels were prepared by dissolving calculated amount of NaCl, Na₂SO₄, CaCl and MgCl₂ (4: 10: 5: 1) in half strength Hoagland culture solution as explained by Machlis & Torrey (1956). Table 1 show the osmotic potential of each salinity treatment which was calculated by the formula as described by Ting (1981). The pH and EC of the culture solutions is given in Table 1.

Plant growth studies of sunflower were carried out in plastic pots of 17.5 cm in diameter and 6.5 cm deep having drainage hole on its bottom. Twelve pots were used for each variety, and each of the salinity treatment was replicated thrice. Every pot was filled with equal volume of thoroughly washed and moist sand. Approximately uniform size and equal number of seeds were sown in each pot. They were then daily irrigated with an equal amount i.e., 50 ml of respective saline solutions. All these 24 pots were then arranged in a completely randomized design (CRD) on a Laboratory table for about 15 days. After the completion of germination, seedlings were thinned with 5 in each pot. They were then transferred to glass house. After 10 weeks of seedling growth, a set of the resultant plants was harvested from each treatment/replicate. Their roots and shoots were manually separated and washed in tap water for three times, then in Decon detergent and finally were rinsed with deionized water. Both root and shoot samples were dried in an oven at 80°C for 24 hours. They were then grounded and digested using wet acid digestion method. For this purpose HNO₃ and HClO₄ (72%) was used following the procedure as described by Tandon (1993). Standard stock solutions (100 mg L⁻¹) of Cu, Mn, Fe, and Zn were prepared from atomic absorption standards (Spectrosol, BDH, UK) in 0.01M HCl, and various working standard solutions were prepared from these stock solutions by serial dilution with 0.01M HCl. Atomic absorption spectrometer (PYE Unicon SP-9) was used for the determination of micronutrients. The absorbance for the determination of Cu, Mn, Fe, and Zn was recorded at wavelength of 324.7, 279.5, 248.3 and 213.9 nm, respectively. Similarly the digested material of roots and shoots of sunflower were then separately analyzed for their aforementioned micronutrients.

Statistical analyses of data: A randomized complete block design (RCBD) was used for setting up the experiment. The MSTAT-C computer software package was used for working out analyses of variance (ANOVA) of all variables. The least significant difference test (Snedecor & Cochran, 1980) was used to compare the mean values.

Results and Discussion

Results (Table 2) showed that in response to various levels of induced salinity (A) all mentioned micronutrients viz., Cu, Mn, Fe and Zn of sunflower roots and shoots as well as varieties (B) and their interactions too (A x B) exhibited statistically significant results at both probabilities ($p < 0.05$ & $p < 0.01$). However, in case of shoot Mn uptake, both varieties of test crop showed non-significant response. These findings are also in line with results obtained by Achakzai (2007 & 2008) in sorghum and maize seedlings subjected to various levels of water stress conditions, as well as Achakzai *et al.*, (2010) in uptake and accumulation of macronutrients by sunflower varieties of the present set of experiment.

Data presented in Table 3 showed that there was a progressive linear increase in uptake and accumulation of Cu both in root and shoot of sunflower subjected to different levels of salinity stress. Whereas, varietal response was also found to be significant. A maximum uptake of Cu by roots ($25.67 \mu\text{g/g}$) and shoots ($19.50 \mu\text{g/g}$) was recorded in highest dose of salinity (22.38 ms/cm). Based on available literature, the influence of salinity on Cu accumulation is variable. Researchers revealed that the uptake of Cu generally increased in crop plants subjected to salinity stress. Therefore, present findings in term of Cu uptake are in accordance with the results obtained by Alam (1994). However, most other researchers indicated that in saline and saline sodic soils, the solubility of Cu is particularly low, and plants grown in such soils often experience deficiency of Cu, but not in all cases. Therefore, the Cu status of present study is not in conformity with the results obtained by Page *et al.*, (1990); Izzo *et al.*, (1991) and Rahman *et al.*, (1993). They stated that leaf and stem Cu concentrations were found to decrease in salt-stressed maize grown both in solution cultures and soil. However, based on grand sum values, results also depicted that roots produced 20.38% increased Cu uptake over their respective shoots (Fig. 1). Similar results have also been reported by Tunçturk *et al.*, (2008).

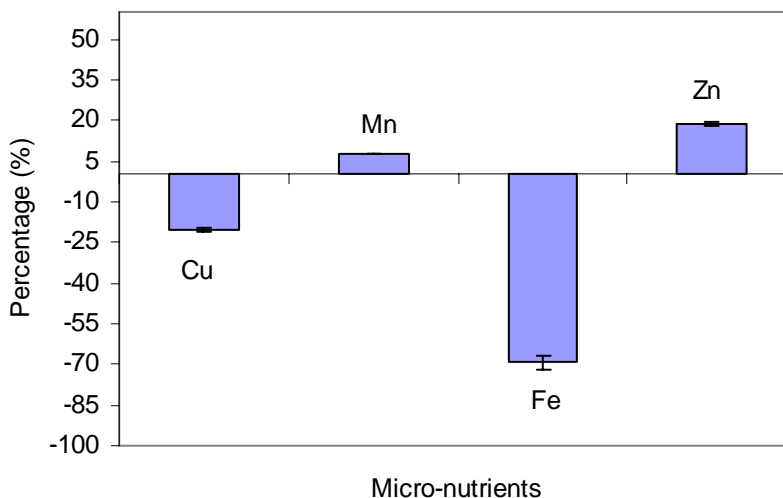


Fig. 1. Percent increase / decrease of copper, manganese, iron and zinc uptake by shoot over root of sunflower as affected by salt stress.

Table 2. Analysis of variance (ANOVA) for nutrients uptake by two varieties of sunflower (*Helianthus annuus L.*) subjected to various levels of salinity.

Variables	Sum of square			Mean square			F-value of variables at an error of 14			CV (%)
	Treatments (A)	Varieties (B)	A x B	Treatments (A)	Varieties (B)	A x B	(A)	(B)	A x B	
Root										
1. Copper	535.596	3.089	43.048	178.533	3.089	14.349	780.5354**	13.5043**	62.7345**	2.67
2. Manganese	31000.833	3360.667	3086.333	10333.611	3360.667	1028.778	1319.1844**	429.0213**	131.333**	2.96
3. Total Iron	101241916.458	1768551.042	3046600.792	33747305.486	1768551.042	1015533.597	206.5096**	10.8223**	602143.0**	17.33
4. Zinc	4917.125	630.375	1028.125	1639.042	630.375	342.708	419.1157**	161.1918**	87.6332**	6.68
Shoot										
1. Copper	481.914	1.945	41.056	160.638	1.945	13.685	702.3653**	8.5035*	59.8363**	3.35
2. Manganese	8887.5	20.167	2595.5	2962.5	20.167	865.167	349.754**	2.3809ns	102.142**	2.84
3. Total Iron	7638132.833	183050.667	181801.667	2546044.278	18050.667	60600.556	201857.215**	14512.75**	4804.575**	0.50
4. Zinc	4657.125	234.375	1668.792	1552.375	234.375	556.264	132.4525**	19.9975**	47.4618**	9.43

* and** slightly and highly significant at P<0.01 and 0.05 level of probabilities, respectively. While ns = non-significant and CV = coefficient of variation.

Table 3. Effect of salinity on the uptake of total copper ($\mu\text{g/g}$ dry weight) by root and shoot of two varieties of sunflower (*Helianthus annuus* L.).

Varieties	Salinity treatments (bars)				Mean
	0.0	-4.67	-9.35	-14.03	
Root					
1. DO 728	15.20 ef	16.00 e	18.00 c	24.00 b	18.301 a
2. DO 730	11.00 g	15.00 f	17.00 d	27.33 e	17.583 b
Mean	13.100 d	15.502 c	17.500 b	25.667 a	17.942
Shoot					
1. DO 728	7.304 e	11.007 d	19.967 a	20.000 a	14.569 a
2. DO 730	10.333 d	11.000 d	15.667 c	19.000 b	14.000 ab
Mean	8.819 d	11.003 c	17.817 b	19.500 a	14.285

LSD @ $p<0.05$ and $p<0.01$ both for varieties and treatments of the roots are 0.838 and 1.163, respectively. While LSD @ $p<0.05$ and $p<0.01$ both for varieties and treatments of the shoots are also 0.838 and 1.163, respectively.

Mean values followed by the same letter(s) within right side column (varieties) and bottom row (treatments) of the Table are not significantly different ($p<0.05$) using LSD test. Similarly, values followed by the same letter(s) within column and rows (varieties x salinity treatments) in the center of the Table are not significantly different from each other.

Table 4. Effect of salinity on the uptake of total manganese ($\mu\text{g/g}$ dry weight) by root and shoot of two varieties of sunflower (*Helianthus annuus* L.).

Varieties	Salinity treatments (bars)				Mean
	0.0	-4.67	-9.35	-14.03	
Root					
1. DO 728	51.00 g	82.00 d	75.00 e	123.00 b	82.75 b
2. DO 730	38.00 f	123.33 b	97.67 c	166.67 a	106.417 a
Mean	44.500 d	102.667 b	86.333 c	144.83 a	94.583
Shoot					
1. DO 728	61.000 e	90.333 d	132.000 a	130.000 a	103.333
2. DO 730	87.333 d	100.000 c	107.333 b	111.333 b	101.500
Mean	74.167 c	95.167 b	119.667 a	120.667 a	102.417

LSD @ $p<0.05$ and $p<0.01$ both for varieties and treatments of the roots are 4.901 and 6.803, respectively. LSD @ $p<0.05$ and $p<0.01$ both for varieties and treatments of the shoots are 5.097 and 7.074, respectively.

Mean values followed by the same letter(s) within right side column (varieties) and bottom row (treatments) of the Table are not significantly different ($p<0.05$) using LSD test. Similarly, values followed by the same letter(s) within column and rows (varieties x salinity treatments) in the center of the Table are not significantly different from each other.

Results pertaining to Mn uptake depicted that as salinity level increases, Mn concentration also significantly increases both in roots and shoots of the test plants. This significance was much prominent in shoot over root (Table 4). A maximum uptake of Mn both in roots (144.83 $\mu\text{g/g}$) and shoots (120.67 $\mu\text{g/g}$) was also noted in highest dose of induced salinity (22.38 ms/cm). However, varietal response was found to be non-consistent. Similar findings have been obtained by very few researchers (Niazi & Ahmed, 1984; Alam, 1994). They noted that Cu generally increases in crop plants under salinity stress. Whereas most other researchers revealed that salt stress (particularly NaCl) either reduced or had non-significant effect on the Mn concentration. Therefore, present results in term of Mn uptake are not in agreement with the results obtained by most of the researchers (Alam *et al.*, 1989; Izzo *et al.*, 1991; Rahman *et al.*, 1993; Al-Harbi, 1995;

Lutts *et al.*, 1999; Mohamedin *et al.*, 2006; Huang *et al.*, 2007). Results further demonstrated that based on grand sum values, roots produced 7.65% lesser Mn uptake over the shoots of the same set of experiment (Fig. 1). These findings are contradictory with those obtained by Tunçturk *et al.*, (2008).

Results exhibited that salinity in general significantly and linearly increased the uptake of total Fe contents both by the roots and shoots of sunflower (Table 5). A significant varietal response was also recorded, and variety DO 730 produced greater Fe accumulation both in their roots and shoots when compared with other variety DO 728. Statistically a maximum concentration of total Fe contents in roots (5837.50 µg/g) and shoots (1647.67 µg/g) was recorded in highest dose of induced salinity (22.38 ms/cm). Results reported that salinity stress has stimulatory as well as inhibitory effects on the uptake of some micronutrients by plants. The uptake of Fe generally increases in crop plants under salinity stress. Therefore, present findings are in line with such reports (Alam, 1994). But most of the studies indicated that in saline and saline sodic soils, the solubility of micronutrients including Fe is particularly low and plants grown in such soils often face deficiencies of micronutrients. Therefore, our results of total Fe are not in accordance with those obtained by Page *et al.*, (1990) and Mohamedin *et al.*, (2006). Results further showed that based on grand sum values, roots produced 69.33% greater Fe content over the shoots of the same plants (Fig. 1). Similar results have also been reported by Tunçturk *et al.*, (2008). It was also noted that the uptake of Fe concentration both in roots and shoots was at par than those of Cu, Mn and Zn contents.

Table 5. Effect of salinity on the uptake of total iron (µg/g dry weight) by root and shoot of two varieties of sunflower (*Helianthus annuus* L.).

Varieties	Salinity treatments (bars)				Mean
	0.0	-4.67	-9.35	-14.03	
Root					
1. DO 728	480.333 de	235.667 e	1863.333 b	5665.333 a	2061.167 b
2. DO 730	967.667 cd	1894.667 b	1544.333 bc	6009.667 a	2604.083 a
Mean	724.000 c	1065.167 bc	1703.833 b	5837.500 a	2332.625
Shoot					
1. DO 728	94.000 h	107.333 g	718.000 c	1593.000 b	628.083 b
2. DO 730	472.333 e	394.333 f	642.000 d	1702.333 a	802.750 a
Mean	283.167 c	250.833 d	680.000 b	1647.667 a	715.417

LSD @ p<0.05 and p<0.01 both for varieties and treatments of the roots are 707.9 and 982.6, respectively.

LSD @ p<0.05 and p<0.01 both for varieties and treatments of the shoots are 6.219 and 8.632, respectively.

Mean values followed by the same letter(s) within right side column (varieties) and bottom row (treatments) of the Table are not significantly different (p<0.05) using LSD test. Similarly, values followed by the same letter(s) within column and rows (varieties x salinity treatments) in the center of the Table are not significantly different from each other.

Data regarding Zn uptake exhibited that as salinity increases, the concentration of Zn in root decreases. While reverse was true in case of shoot of the same plants (Table 6). A significant difference in varietal response was also noted. The variety DO 730 accumulated much Zn content in roots and shoots over than those of variety DO 728. A maximum uptake of Zn by roots (54.17 µg/g) and shoots (59.17 µg/g) was recorded in salinity doses having EC 1.19 and 22.38 ms/cm, respectively. The majority of studies in the literature have shown salinity to increase Zn concentration in shoot such as in tomato, maize and citrus. Therefore, our findings are strongly in line with the results obtained by

these researchers (Knight *et al.*, 1992; Rahman *et al.*, 1993; Ruiz *et al.*, 1997), but in other studies it was not affected (Izzo *et al.*, 1991) or actually decreased Zn concentration as in case of cucumber leaves (Al-Harbi, 1995). Results further demonstrated that based on grand sum values, roots accumulated 18.37% lesser Zn contents over the shoots of the same set of experiment (Fig. 1), which are not in accordance as those explained by Tunçtürk *et al.*, (2008).

The uptake and accumulation of ions in plants is considered as an important indicator of salinity tolerance, because they are genetically regulated, though also affected by the environment (Mahmood, 1991; Chaubey & Senadhira, 1994). However, the differential pattern of ion accumulation in the two sunflower varieties clearly shows that though genes responsible for ion uptake are present in both varieties, but their expression in variety DO 730 is much greater than variety DO 728 at early vegetative stage.

Table 6. Effect of salinity on the uptake of total zinc ($\mu\text{g/g}$ dry weight) by root and shoot of two varieties of sunflower (*Helianthus annuus* L.).

Varieties	Salinity treatments (bars)				Mean
	0.0	-4.67	-9.35	-14.03	
Root					
1. DO 728	38.000 b	21.000 d	21.000 d	18.000 d	24.50 b
2. DO 730	70.333 a	28.333 c	20.000 d	20.333 d	34.75 a
Mean	54.167 a	24.667 b	20.500 c	19.167 c	29.625
Shoot					
1. DO 728	15.000 e	19.333 e	28.000 d	70.333 a	33.167 b
2. DO 730	29.333 d	39.000 c	41.333 c	48.000 b	39.417 a
Mean	22.167 c	29.167 b	34.667 b	59.167 a	36.292

LSD @ $p < 0.05$ and $p < 0.01$ both for varieties and treatments of the roots are 3.463 and 4.807, respectively.

LSD @ $p < 0.05$ and $p < 0.01$ both for varieties and treatments of the shoots are 5.995 and 8.321, respectively.

Mean values followed by the same letter(s) within right side column (varieties) and bottom row (treatments) of the Table are not significantly different ($p < 0.05$) using LSD test. Similarly, values followed by the same letter(s) within column and rows (varieties x salinity treatments) in the center of the Table are not significantly different from each other.

Conclusions

Results showed that in response to various levels of applied salinity, the uptake of all mentioned micronutrients by roots and shoots of sunflower exhibited statistically significant response both at $p < 0.05$ and $p < 0.01$. While the varietal response in term of nutrients uptake was also found significant (except of shoot Mn uptake). A maximum significant uptake of Cu, Mn, Fe, and Zn in shoot (19.50, 120.67, 1647.67 and 59.17 $\mu\text{g/g}$) is obtained in highest dose of applied salinity (22.23 mS/cm). Whereas except of Zn, a maximum significant uptake of Cu (25.67 $\mu\text{g/g}$), Mn (144.87 $\mu\text{g/g}$), and Fe (5837.5 $\mu\text{g/g}$) in root is also obtained in highest dose of salinity. Data based on root shoot Fe and Zn uptake, variety DO 730 responded well than variety DO 728. Results also based on grand sum values, depicted that there were 20.38 and 69.33% decreased uptake of Cu and Fe, but 7.65 and 18.37% increased uptake of Mn and Zn by shoots over roots of both varieties, respectively.

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