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Accumulation of Organic and Inorganic Components in *Zea mays* L. Plants Under Salinity Stress using Hydrogel Polymer

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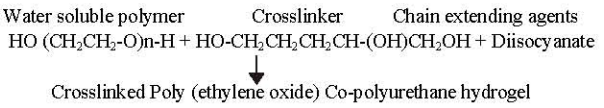
Abstract: The effects of hydrogel polymer incorporating with sand on development of selected maize grown under saline conditions has been demonstrated. Seeds of maize was germinated in sand/swollen hydrogel polymer mixture (80:20 v/v(with added Hoagland nutrient solution, then transplanted after 7 days from germination into a range of sand/swollen hydrogel polymer (90:10 v/v) in plastic grow bags. Saline solutions containing NaCl, $CaCl_2$, $MgCl_2$(0.0, 2000, 4000, 6000 and 8000 ppm) were applied to the grow bags twice per week to field capacity, alternating with a comparable watering regime. Maize adapted to salinity (<8000 ppm (was found to have undergone extensive osmotic adjustment by accumulation of organic and inorganic solutes. The monosaccharide and polysaccharide levels tended to decrease in root while as sucrose decreased in both shoot and root with salinity treatments. Organic acids, amino acids, proline and proteins were increased with salinity treatments up to 8000. The concentration of proline was positively correlated with cell osmotic potential. At 8000 ppm the mean concentration of proline in plant tissues7.3-9.3mg/100g D.W., representing about, 81%-83% of the total amino acid pool; comparable figures for unadapted plant tissues were1.2-2.0 mg/100g dry weight (D.W.) being 24%-30% of the pool respectively. Salinity concentrations tended to increase osmolality, EC_e and inorganic elements (Na^+, Ca^{+2}, Mg^{+2} and Cl) with except of K^+. The principle for osmotic adjustment, organic and inorganic solutes make significant contributions by using hydrogel polymer to improve the soil sand characters and adapted maize plant to salinization.

Key words: *Zea mays* · Hydrogel polymers · Salinity · Carbohydrate · Amino acids · Proline · Protein · Micro and Macro-elements · Ec_e · Osmolality and pH values

INTRODUCTION

The potential use of hydrogel polymers as soil conditioners or substrates for plant growth depends on a number of factors including their capacity to swell in water or water vapor, release of the contained moisture from the hydogel to the plant roots, the partitioning, binding and release of ions nutrients. Hydrogels which are commercially available and advocated for use as soil conditioners include cross linked acrylic copolymers such as polyacrylamide or polyacrylic acid and insolebilised starch. The hydrogel polymer used in this study is based on poly (ethylene oxide), a material widely used in industry and pharmacy in the forms of poly (ethylene glycol) and non ionic surface active agents. Polyethylene glycols (PEG) of high molecular weight are water soluble but can be converted into water insoluble and swell able hydrogels via the reaction of their hydroxylic end groups with diisocyanates with or without the addition of other polyols as cross linking agents. The cross linking can be by urethane, urea, allophanate or biurel group. The formation of polyurethane hydrogel is shown below:



These products absorb and hold water and are of interest as potential aids to arid land plant production [1]. The high salinity conditions were reduced the growth but appeared to be tolerant at all levels of hydrogel polymer incorporation with [2-4].

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In higher plants, salt tolerance may be achieved through the preferential accumulation of compatible osmotic solutes in the cytoplasm [5], in order to maintain equilibrium of the water potential of vacuole and cytoplasm [6, 7]. Salinity treatments are known to increase the concentration of sugars, organic acids, amino acids including proline and protein. Accumulation of chlorophyll a seems to be related to the neutral pH of the cell sap in plants of saline habitats and to the formation of sodium complexes cadaverine and putrescence. Kaymakanova and Stoeva [8], found the physiological responses of three different bean cultivars plants were treated for 7 days with NaCl and Na_2SO_4 (100 mM), starting at the appearance of the first trifoliate leaf unfolded. It was established that the applied dose of both salt types caused stress in the young bean plants, which found expression in the suppression of growth and photosynthesis activity.

In addition salts increase the activity of oxidative enzymes which also favours the biosynthesis of pigments [9]. Increase salinity appears to reduce the level of potassium uptake by plants. Ayers and Eberhard [10], found that increasing the concentration of sodium and calcium in soil resulted in a moderate decrease of potassium in the same order of magnitude for plants both broad bean and green bean plants. Uptake and accumulation of mg^{2+} and Na^{+} is increased by saline conditions [11], while accumulation of chloride may be localized in the plant [12]. Salinity is Known to retard plant growth through its influence on several facets of plant metabolism including osmotic adjustment [13], ion uptake [14], enzyme activities [15], protein and nucleic acid synthesis [16], photosynthesis [17] and hormonal balance [18]. Although much work has been done on the effect of salinity on various aspects of crop plant growth and development, little information is available regarding salt tolerance of bean (*Vicia faba*, L.). Metabolic studies in chloroplasts from salt stressed plants of peanut performed [19], revealed the sensitive nature of the plant. Several workers have noticed a decrease in net photosynthetic rate due to salt stress [20], the effect of salinity on the photophosphorylation through mediated [21]. Salinity is reported to affect the strength of the forces binding the complex of pigment-protein-lipid in the chloroplast structure [9].

Nitrogen is an element playing an initial role in plants metabolism. It is a constituent element in all cellular organelles, enzymes and energy transport and reserve compounds. Accordingly, nitrogen supply could be a limiting factor for productivity of plant species in most habitats as grasslands [22], barley fields [23]. Also, the availability of soil nitrogen governed most plant growth characteristics as dry weight of seedlings [24], plant biomass [25], or seed production. High soil nitrogen increased the plants adaptation to habitat water or salinity stresses. In this respect the nitrogen uptake important in the plant adaptation to the imposed salinity stress as nitrogen supply reduced the adverse effect and in most species nitrogen uptake did not affected by salinity [26,27]. On the other hand, high soil nitrogen (e.g. by fertilization) creates nitrogen saturation conditions differed according to plant species [24 and 28]. Also, retranslocation of nitrogen supply a great part of plant nitrogen requirements [29].

In this paper, the ability of maize (*Zea mays*, L.) to accumulate potential osmotic solute in response to osmotic stress has been examined, particularly in relation to the accumulation of organic and inorganic solutes under using hydrogel polymer as a soil conditioner.

MATERIALS AND METHODS

Hydrogel polymer: At ambient temperature the water-holding capacity of the material used in this study was 9.7 g/g of dry hydrogel polymer. The initially dry granules had a particle size of 0.5-2.0 mm and when fully swollen were rubbery grains through which liquid water could still freely drain. Typical preparations of poly (ethylene oxide)-co-polyurethane hydrogels have been reported previously [1].

Salinity Treatments: Grains of maize (*Zea mays*, L.), were obtained from the Ministry of Agriculture, Egypt. In separate glasshouse experiments carried out in late spring under controlled light and temperature conditions (14 hr light, 30°C day / 20°C night), the grains of maize were germinated in sand / swollen polymer mixture (80/20 v/v) with added Hoagland's nutrient solution. After 7 days from germination, three plants were transplanting into polythene grow bag pots (25 cm x 35 cm) containing a range of sand/swollen hydrogel polymer combination (90/10, v/v) in the greenhouse. Saline solutions containing NaCl, $CaCl_2$, $MgCl_2$ as molar solutions were applied to produce final concentrations of 0.0, 2000, 4000, 6000, 8000 ppm. (Molar equivalents 0.0, 0.6×10^{-1}, 1.2×10^{-1}, 1.8×10^{-1} and 2.4×10^{-1}, respectively. Hoagland solution being used as control. The treatments were applied twice a week alternating with watering the plants with equal amounts of

water to compensate for the evapo-transpiration of water and avoid excessive salt accumulation in the plant. Harvesting was carried out at the seedling (vegetative), tussling silking (flowering) and grain filing (fruiting) stages and the effect of treatment analyzed by evaluation of growth parameters, water content, pigments, photosynthetic activity, yield production, organic and inorganic components. Data for grain filling(fruiting) was only presented in this paper.

Carbohydrate Analysis: 300 mg of oven dry plant material was extracted with 5 ml of borate buffer (28.63 g boric acid + 29.8 g KCl + 3.5 g NaOH in a liter of hot distilled water), left for 24 hr, then centrifuged and filtered. The filtrate was used for the determination of the direct reducing value (DRV) and total reducing value (TRV), while the residue was dried at 80°C for determination of polysaccharides [30, 31].

Determination of DRV, (including all free monosaccharide)was carried out by evaporation, 0.1 ml of extracted cleared borate buffer was reduced to dryness and then mixed with 1 ml of modified Nelson solution [31]. The mixture was maintaining on a boiling water-bath for 15 min, after which it was cooled rapidly using running tap water. Thereafter 1 ml of arsenomolybdate [32] was added, the mixture was diluted to a definite volume and its intensity measured at 700 nm, using colorimeter (*LKPNOVASPEC Spectrophotometrically*).

Sucrose: For determination of total reducing value (TRV), 0.2 ml of cleared extract was mixed with deionized water up to 5 ml then 0.2 ml of the diluted extract was mixed with 0.1 ml of 1% invertase enzyme solution and the mixture maintained at 37°C for 0.5 hr. Thereafter, the reducing value was determined as described before for DRV [30, 31]. The difference between the value obtained from this step and that of the DRV is an estimated of sucrose, in terms of glucose made up to 3 ml left overnight at 28°C and then centrifuged.

Polysaccharides: 10 mg of the remaining residue was mixed with 0.2 ml of 1% taka diastase enzyme and 0.1 ml acetate enzyme and ml acetate buffer (6 ml acetic acid 0.2 N + 4 ml sodium acetate buffer 0.2 N) [30]. The reducing value of 1ml of filter was estimated as above.

Nitrogenous Components: Total organic acids, total amino acids, proteins and proline were analyzed from a common extract obtained by homogenizing 50 mg dry shoots and roots materials in 2.5 ml of methanol: chloroform: water (12: 5: 3). After centrifugation, the supernatant was removed and the procedure repeated three times. Three ml of H_2O and 2 ml of chloroform were added to the pooled extracts, the aqueous phase was removed and the organic phase re-extracted with an additional 5 ml of H_2O. The aqueous extracts were pooled and evaporated to dryness at 40°C under a stream of compressed air. Amino acids and organic acids were separated by ion exchange chromatography after the residue was re-suspended in 2 ml of H_2O [33]. Half the extract was loaded of on to a 1 x 2 cm column of Dowex 50-H^+ (200-400 mesh) while the remaining 1 ml (to be used for QAC analysis) was stored at-20°C. The void and water washed (2 x 2.5 ml) were collected and stored at-20°C for analysis of organic acids. The amino acids were eluted from the column with three 2 ml compressed air at 40°C. The residue was then re-suspended in 2 ml H_2O and passed over a 1 x 2 cm column of Dowex 1-CH_3COO^-; the void and H_2O washed (3 x 2 ml) contained the neutral and basic amino acids while the acidic amino acids were eluted with 2 M CH_3COOH (3 x 2 ml). Both fractions were brought to dryness under a stream of compressed air at 40°C. The fraction from the Dowex 50-H^+ column containing the organic acids (void and water washes) was brought to dryness and re-suspended in 1 ml oh H_2O. One half ml was passed over a 1 x 2 cm column of Dowex 1-$HCOO^-$. After washing the column with 3 x 2 ml volumes H_2O the organic acids were eluted with 6N HCOOH (4 x 2 ml) and brought to dryness under a stream of compressed air at 40°C. HFBI ester of the amino acids and O-heptafluorobutyryle isobutyl and / or isobutyl ester of organic acids were prepared according to the methods of Rhodes, *et al*., (1981). α-amino-n-butyric acid (0.25 μmol) was added to the samples as an internal standard prior to derivatization. The derivatives in ethyl acetate: acetic anhydride (1: 1) were subjected to GLC (Hewlett Packard Model 5794 A) using a fused silica capillary column (DB5-30 N, 30 m x 0.2 mm ID, J and W Scientific, Rancho Cordova, CA). The split ratio at the injector port was 20: 1 column pressure 19 psi N_2 carrier gas, column flow rate 1 ml min^{-1}, 22ml N_2 sweep gas at the flame ionization detector, 222 ml min^{-1} air and 35 ml min^{-1} H_2 at the detector; 20 ml min^{-1} total N_2 flow at the split vent. The injector temperature was set at 250°C; the detector at 280°C and the column subjected to a variable temperature programme (100°C for 4 min, then increased from 100°C to

200°C at a rate of 6°C min^{-1} and held at 260°C for 4 min). Peak areas were determined by a Hewiett-Packard 3390 A reporting integrator and were related to the area of the internal standard, α-amino-n-butyric acid. The response factors for individual amino acids and organic acids were determined by GLC of HFBI esters of an amino acid standard mixture (Sigma No. AA-S-18) and HFBI esters of a 1 mM mixture of malonic, maleic, malic, citric, isocetric, tartaric and succinic acids. The only amino acids without unique retention times, as determined by GC-MS [33], were valine, which co-chromatographed with β-alanine and methionine, which co-chromatographed with tryamine. The presence of ACC in the free amino acid pool was verified by electron impact GC MS [33].

Free amino acids were determined by HPLC following extraction with sulphosalicylic acid and OPA/mercaptoethanol derivatization [34]. An aliquot of 0.1 ml plant extract was heated in a test tube with 1.9 ml of ninhydrin citrate buffer-glycerol mixture in a boiling water bath for 12 min and cooled at room temperature. Then the tube was well shaken and the optical density read at 570 nm. A blank was determined with 0.1 ml of distilled water and a standard curve obtained with 0.005 to 0.2 mM glycine Ya, *et al.*, [35].

Proteins: Dry samples collected during the growth study were analyzed for protein content according to [36], after precipitating the protein with 15% Trichloroacetic acid (TCA) at 4°C. An aliquot of 0.1 ml plant extract was heated in a test tube with 1.9 ml of ninhydrin citrate buffer-glycerol mixture in a boiling water bath for 12 min and cooled at room temperature. Then the tube was well shaken and the optical density read at 570 nm. A blank was determined with 0.1 ml of distilled water and a standard curve obtained with 0.005 to 0.2 m g glycine [37].

Free Proline: This was estimated using the acid ninhydrin, 2 ml of water extract were mixed 10 ml of 3% aqueous sulfosalicylic acid. Two ml of this mixture was allowed to react with 2 ml acid ninhydrin-reagent and 2 ml of glacial acetic acid in a test tube for 1 h at 100°C; the reaction was terminated by cooling the mixture in an ice bath. The reaction mixture was extracted with 4 ml toluene and mixed vigorously for 15-20 s. The chromatophore-containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene as a blank [38]. Proline concentration was determined from a standard curve.

Osmolality ECe, pH and Ion Determination: Determination of sell-sap osmolality (mOsm/kg H_2O) was carried out on plant material using an Advanced Digimatic Osmometer Model 3D II (Advanced Instrument, INC), electric conductivity (ECe) using a Digital conductivity Meter (PTI-18) and pH using a Whatman pH U. Sensor (Electric Meter).

Inorganic Mineral Composition: Ions content measurements were carried out after extraction with 0.1 nitric acid of the ashed (powdered) milled samples at 500°C obtained after combustion in a muffle furnace, the milled samples were estimated following the "wet ashing procedure" [39]; the acid digests of the oven dried samples were analyzed. Oven dried plants were subjected to acid digestion and Sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) estimated photo-metrically using a corning-400 flame photometer [40, 41]. The levels of manganese (Mn^{2+}) and iron (Fe^{3+}) contents were determined using an atomic absorption spectrophotometer [42]. Phosphorus was estimated by Sekine *et al.*, [43] method's using the method of Molybdenum-blue [44], while the methods for chloride were determined using $AgNO_3$ titration [44, 45].

Statistical Analysis: Where relevant, the experimental data was subjected to analysis of variance. Percentage values were transformed into arcsines according to Bliss [46] and analysis of variance was carried out according to Snedecor and Cochran [47].

RESULTS AND DISCUSSION

Carbohydrate Levels: Shoot but not root monosaccharide and polysaccharide contents increased with salinity treatments, though sucrose declined in both shoot and root (Figs. 1, 2 and 3). The effects of incorporating a hydrogel polymer into sand (SS/HP) on development of maize grown under saline conditions has been observed the increasing of carbohydrate levels tended to increased of yield productions by Sand/Hydrogel polymer (SS/HP) (v/v) than pure sandy soil (SS). Carbohydrate levels in roots tended to decline more than shoots. The carbohydrate content (monosaccharide and polysaccharide) was increased with increasing salinity concentration in shoot but not in root, whereas, the sucrose content decreased. This decrease in sucrose is contrary to the findings of other workers who have suggested that the increase with increaseing salinity may

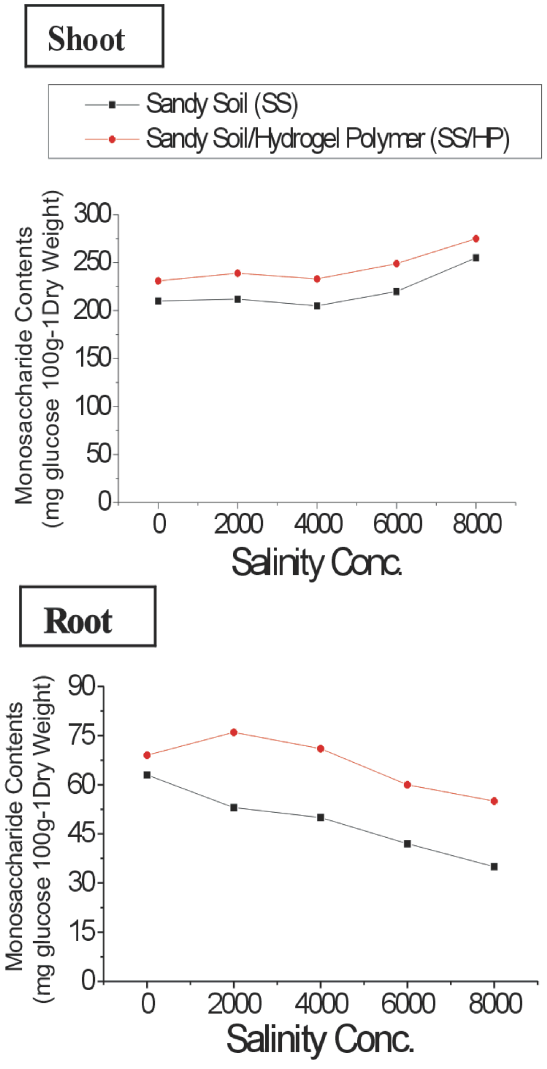

Fig. 1: Effects of Hydrogel Polymer incorporating with Sand (SS/HP -"v/v") and Pure Sandy Soil (SS) On Shoot and Root Monosaccharide Contents (mg glucose 100gG^{1}Dry Weight) Of Selected Maize Plant Under Saline Conditions.

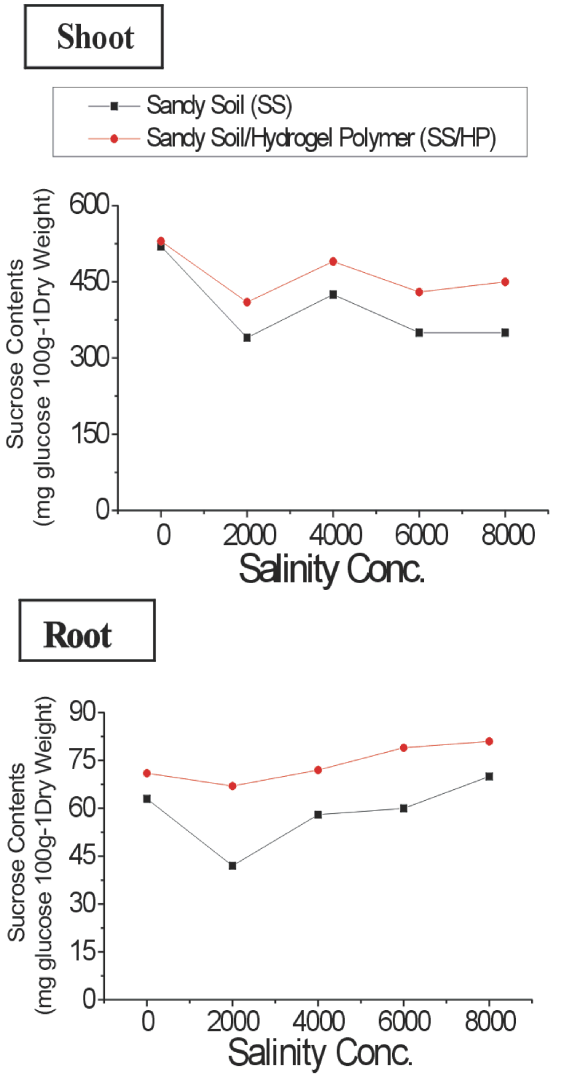

Fig. 2: Effects of Hydrogel Polymer incorporating with Sand (SS/HP -"v/v") and Pure Sandy Soil (SS) On Shoot and Root Sucrose Contents (mg glucose 100gG^{1}Dry Weight) Of Selected Maize Plant Under Saline Conditions.

protect the isolated chloroplast pigment against injury during desiccation [48]. The statistical analysis (One, two and three-way analysis of variance (ANOVA) as shown in Table 1.

Osmotic potential significantly correlated negatively with water content and polysaccharides and positively with soluble sugars, proline and quaternary ammonium compounds(QACs). Leaves water content correlated positively with amino acids and negatively with QACs. The soluble sugars correlated negatively with QACs and amino acids. The variations in polysaccharides correlated positively with that of protein and negatively with that of proline. Stepwise multiple regression equations (Table 3) pointed out the degrees of effect of each salinity, nitrogen deficiency and proline initiator on the plant metabolism of the osmotically active metabolites. However, the increase in leaves dry matter content and soluble sugars was a function of nitrogen deficiency and proline initiator with increasing salinity. Also, the metabolism of more nitrogenous compounds was a function of nitrogen content in the nutrient solution and salinity but with lower extent.

Table 1: Effects of a Hydrogel Polymer incorporating with Sand (SS/HP "v/v") and Pure Sandy Soil (SS) On Shoot and Root Inorganic Elements (Na^+, K^+, Mg^{2+}, Ca^{2+} and Cl^-) meq./100 g Dry Weight Of Selected Maize Plant Under Saline Conditions

		Pure Sandy Soil (SS)					Hydrogel Polymer incorporating with Sand (SS/HP -"v/v")				
Inorganic Elements		Salinity Concentrations (ppm)									
meq./100 g Dry Weight		0.0	2.000	4.000	6.000	8.000	0.0	2.000	4.000	6.000	8.000
Shoot System	Na^+	140	180	190	210	230	150	195	205	215	245
	k^+	125	115	100	90	87	130	125	130	125	105
	Mg^{2+}	130	135	130	125	105	140	155	140	150	155
	Ca^{2+}	530	520	550	590	600	550	555	575	605	625
	Cl^-	590	660	650	680	700	610	680	695	715	735
Root System	Na^+	270	285	300	310	330	310	325	335	345	365
	k^+	110	100	90	70	55	112	120	110	95	85
	Mg^{2+}	220	250	240	240	240	235	270	275	280	285
	Ca^{2+}	500	560	520	530	550	525	530	545	570	590
	Cl^-	495	525	535	570	590	515	240	555	579	595

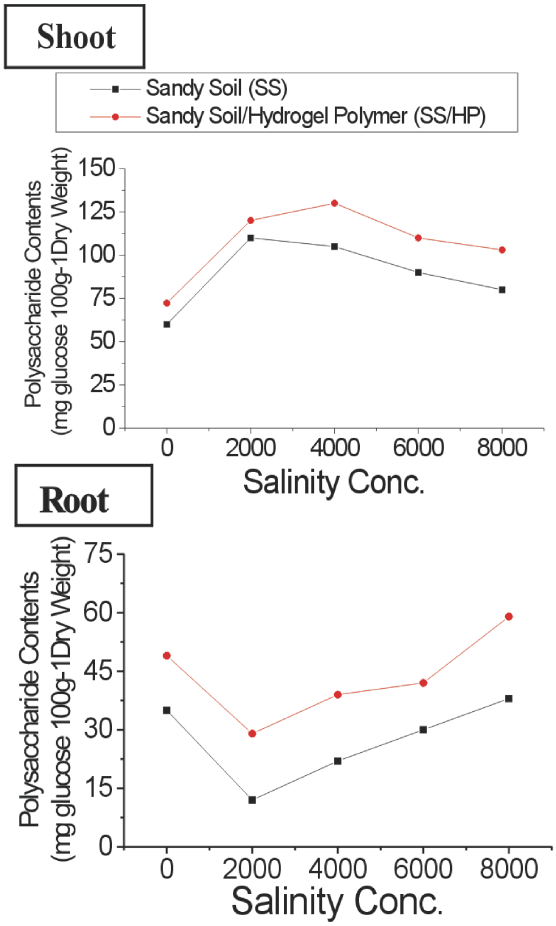

Fig. 3: Effects of Hydrogel Polymer incorporating with Sand (SS/HP -"v/v") and Pure Sandy Soil (SS) On Shoot and Root Polysaccharide Contents (mg glucose 100gG¹Dry Weight) Of Selected Maize Plant Under Saline Conditions.

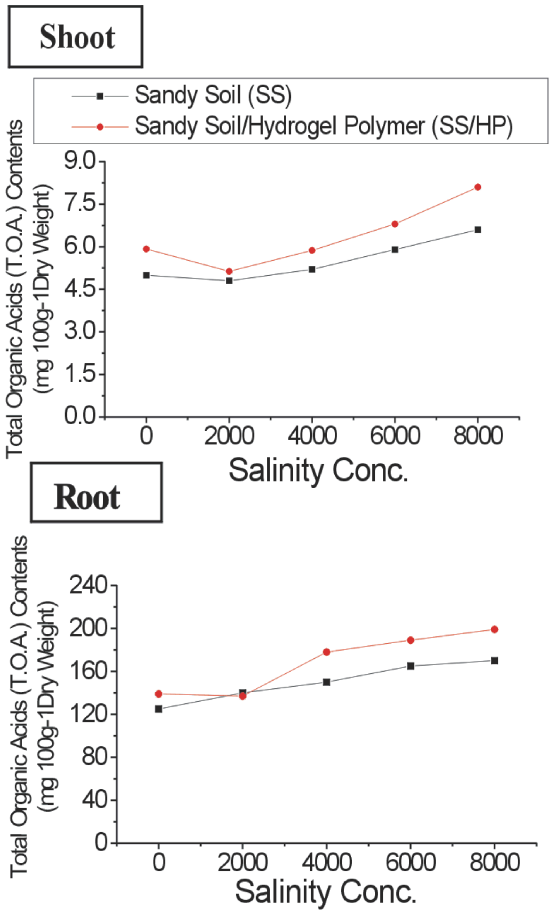

Fig. 4: Effects of Hydrogel Polymer incorporating with Sand (SS/HP -"v/v") and Pure Sandy Soil (SS) On Shoot and Root Total Organic Acids (T.O.A.) Contents (mg 100gG¹Dry Weight) Of Selected Maize Plant Under Saline Conditions.

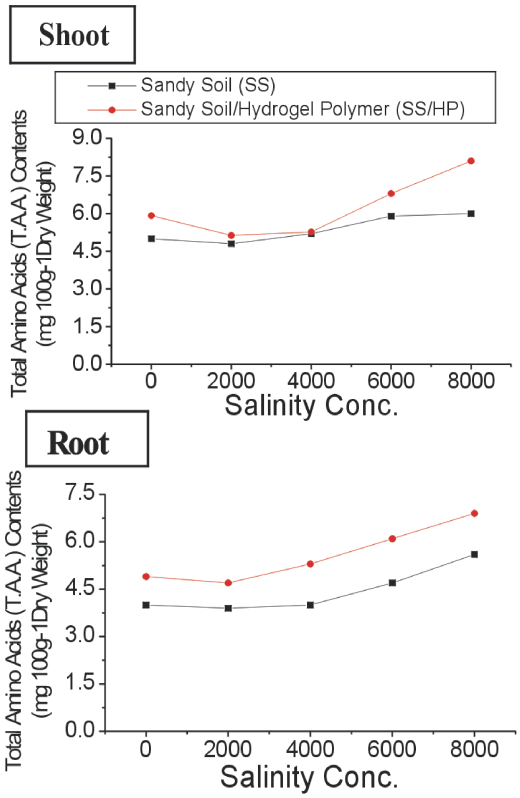

Fig. 5: Effects of Hydrogel Polymer incorporating with Sand (SS/HP -"v/v") and Pure Sandy Soil (SS) On Shoot and Root Total Amino Acids (T.A.A.) Contents (mg 100gG^{1}Dry Weight) Of Selected Maize Plant Under Saline Conditions.

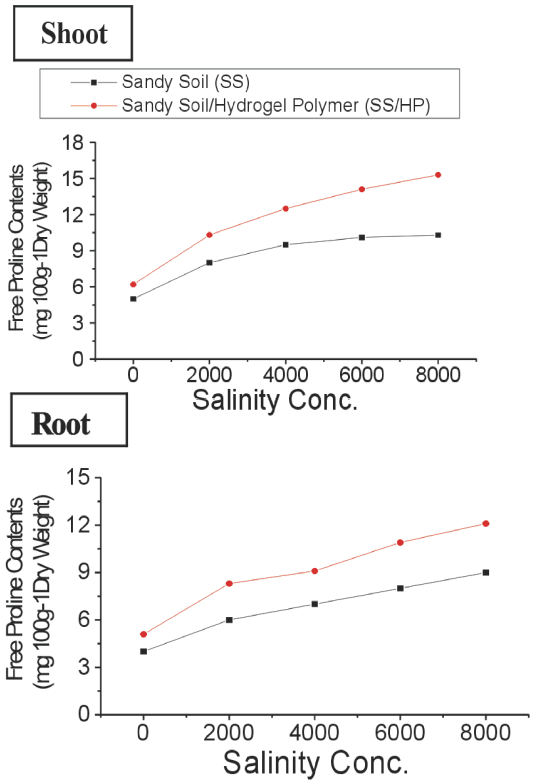

Fig. 6: Effects of Hydrogel Polymer incorporating With Sand (SS/HP -"v/v") and Pure Sandy Soil (SS) On Shoot and Root Free Proline Contents (mg 100gG^{1}Dry Weight) Of Selected Maize Plant Under Saline Conditions.

Organic and Nitrogenous Components: Total organic acids, total free amino acids, free proline and protein levels were increased with increasing salinity concentration, particularly in the shoot (Figs. 4, 5, 6 and 7). The effects of incorporating a hydrogel polymer into sand (SS/HP) on development of maize grown under saline conditions has been observed the increasing total organic acids, total free amino acids, free proline and protein levels tended to increased of yield productions by Sand/Hydrogel polymer (SS/HP) than pure sandy soil (SS). Total free amino acids and proline contents increased with salinity concentration; on average, free proline represented about 24-30 % of the total free amino acids pool in the absence of salts (control plants with polymer) and about 81-83% in the presence of salts and polymer (8000 ppm). Protein content was decreased with increasing salinity concentrations at early growth stages but later protein content increased with salinity treatments especially with added hydrogel polymer to sandy soil. In addition the contents of organic solutes, (organic acids, amino acids, proline and proteins) increased with increasing salinity concentration up to 8000 ppm, particularly in the shoot. Total free amino acid, proline, protein and organic acid contents were increased with cells osmotic adjustment, both as a function of the level of adaption and the stage of the culture growth. High concentrations of organic solutes in the cytoplasm may contribute to the osmotic balance when the concentration of electrolytes is lower in the cytoplasm than in the vacuole; they may protect the enzymes in the presence of high electrolytes in the cytoplasm [6]. The present results show that the total amino acids and proline content were increased with increasing salinity concentration up to 8000 ppm. proline which is present only in traces in the absence of salt, is increased to high levels under high saline concentrations. On average, proline represented about 24-30% of the total free amino acid pool in the absence of salt and about 81-83% in the presence of salts (8000 ppm). The rise in the content of proline was a linear function of the increase in salt concentration

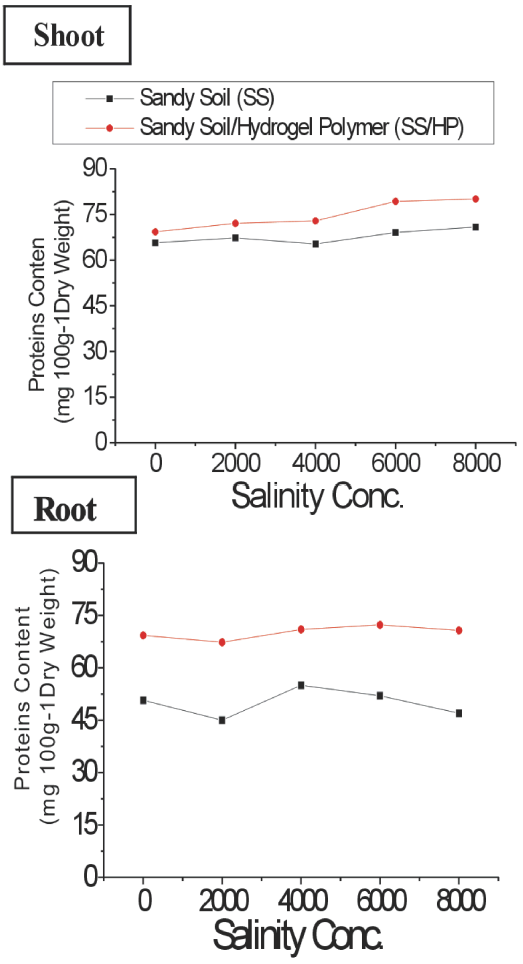

Fig. 7: Effects of Hydrogel Polymer incorporating With Sand (SS/HP -"v/v") and Pure Sandy Soil (SS) On Shoot and Root Proteins Contents (mg 100gG^{1}Dry Weight) Of Selected Maize Plant Under Saline Conditions

in the culture solutions, particularly at high salinity concentration. Accumulation of proline at high salt levels a marked in *Armeria maritime* (Mill) Wild reported [6]. The rapid increase in the level of proline in plants was associated with a threshold salt concentration in the medium [49]. Increases in the total amino acid, proline and protein levels in cytopalsmic solute under high salinity concentrations have been reported for many species [6, 50, 51]. Proteins protect chlorophyll against destruction by being strongly bound to it [9]. A large number of plant species accumulate prolinå in response to salinity stress and that accumulation may play a role in combating salinity stress. However, data do not always indicate a positive correlation between the osmolyte accumulation and the adaptation to stress [52-54]. Salt stress induces cellular accumulation of damaging active oxygen species, which can damage membrane lipids, proteins and nucleic acids [55, 56]. Lipid peroxidation, induced by free radicals, is also important in membrane deterioration [57-60]. Proline (Pro) accumulation is an important physiological index for plant response to salt stress [61], as well as to other types of stress. Salinity increased markedly the Proline content in different salt sensitive and tolerant species/cultivars: with greater pro accumulation in salt tolerant ones, which is supposed to correlate with the adaptation to salinity [52, 54]. The sulfate stress can cause higher accumulation of prîline than the chloride type [8]. The effects of incorporating a hydrogel polymer into sand (SS/HP) on development of maize (*Zea mays*, L.) grown under saline conditions has been observed the increasing total organic acids, total free amino acids, free proline and protein levels tended to increased of yield productions by Sand/Hydrogel polymer (SS/HP) than pure sandy soil (SS), this fact suggests that the induction of proline synthesis is related not only to changes in the water potential and to the salinity type-chloride and sulfate, but also resulted from metabolism interruption by high-stress intensity or from an adaptive response with special physiological function. The increase of praline content under salt stress, have been reported in two wheat cultivars [62]. It was suggested that proline accumulation may be caused by increasing proteolysis or by decreasing protein synthesis. The highest concentration of proline under salt stress is favorable to plants as proline participates in the osmotic potential of leaf and thus in the osmotic adjustment. Besides the role of osmolyte, proline can also confer enzyme protection and increase membrane stability under various condition. Proline accumulation may also help in non enzymic free radical detoxifications [63 and 64]. Nitrogen deficiency led to more linear increase in leaves OP. In opposite the increase in nitrogen by presence of nitrogen in the nutrient solution or and by application of proline initiator decrease OP. This may indicated that excess nitrogen (as in nutrient solution compared to that in the soil of the plant habitat) reduced leaves osmotic regulation promoted by salinity while nitrogen deficiency enhanced it. This also could be an adaptive response by the plant to poor nitrogen in the soil of sand dunes. The plant processes responded differently to salinity stress for survival [65]. The leaves osmotic regulation was achieved mainly by soluble sugars on the expense of polysaccharides as seen by their ratios under all salinity treatments. Nitrogen deficiency enhanced the metabolism of leaves soluble sugars in polysaccharides or nitrogenous compounds,

mainly amino acids and proteins. Different salinity (NaCl and Na_2SO_4) caused accumulation of free amino acids including free proline but reduced the protein content in the leaves at the fruiting stage [66]. On the contrary, proline initiator counteracted the metabolism into polysaccharides but not into nitrogenous compounds which increased. The tendency of amino acids accumulation in *Euphorbia paralias* leaves was inhibited or decreased by more than 100 mM salinity but nitrogen deficiency recovered or enhanced the process under the different salinity levels. The decrease in amino acids especially by higher salinity was coincided with the accumulation of proteins especially under nitrogen deficiency. Also, proline initiator doubled the leaves proteins under nitrogen deficiency. This may indicate a promoting role for nitrogen deficiency in the metabolism of amino acids and proteins in the same time in a self regulated nitrogen conserving behavior [29]. Proline was also accumulated by salinity but the ability of accumulation was hindered by more than 150 mM. Nitrogen deficiency doubled leaves proline under salinity indicating high priority of proline accumulation but proline initiator inhibited the process greatly especially when combined with nitrogen deficiency. Total of osmoregulatory metabolites increased linearly by salinity stress. Nitrogen deficiency lowered this response but its effect could be removed partly by proline initiator. The nitrogen deficiency inhibition was more pronounced on the metabolism of leaves carbohydrates as indicated by decreases in soluble sugars metabolism or enhancing their conversion into non osmotic polysaccharides. On the other hand, presence of nitrogen enhanced the accumulation of osmoregulatory metabolites by salinity and increased the role of soluble sugars over that of nitrogenous metabolites by ratios from 2-3 under low salinity to about 8 under 200 mM. This may indicate the importance of carbohydrate fraction under high salinity stress. It is notable that salinity increased these osmoregulatory metabolites on the expense of leaves dry weight under nitrogen deficiency but both dry weight and osmoregulatory metabolites increased in the same time when nitrogen is available. Nitrogen is a major limiting factor controlling productivity in the coastal area [67]. These foundations may also indicate higher adaptation of the plant to salinity than to nitrogen deficiency which could be the reason of the plant disappearance from the top of loose sand dunes and its restriction at the foot of these dune having a little high nitrogen. Correlation coefficients and regression equations pointed out salinity as the main factor for the variations in the leaves metabolism and nitrogen deficiency comes after while the role of proline initiator was significant in the metabolism of some compounds but not all. The role of proline initiator was enhancing in most cases while that of

Appendix Table 1: Effects of Hydrogel Polymer incorporating with Sand (SS/HP -"v/v") and Pure Sandy Soil (SS) On Shoot and Root Monosaccharide, Sucrose and Polysaccharide Contents (mg glucose $100g^{-1}$Dry Weight) Of Selected Maize (*Zea mays*, L.) Plant Under Saline Conditions

I tems	CO	TR	ST	CO/TR	CO/ST	TR/ST
Monosaccharide	N.S.	*	*	N.S.	N.S.	N.S.
Sucrose	N.S.	N.S.	***	N.S.	N.S.	**
Polysaccharide	N.S.	**	***	N.S.	N.S.	***
T.A.C.	N.S.	***	***	N.S.	N.S.	***

Statistical treatments, where relevant, the experimental data subjected of One, two and three -Way analysis of variance (*ANOVA*).

Note: F values * = P> 0.05, ** = P >0.01, ***= P >0.001 and N.S.= Not Significant. CO: Concentrations, TR: Treatments (SS) and (SS/HP -V"/V"), ST: Stages of growth.

Appendix Table 2: Effects of Hydrogel Polymer incorporating With Sand (SS/HP -"v/v") and Pure Sandy Soil (SS) On (a) Shoot and (b) Root Total Organic Acids (T.O.A.), Total Amino Acids (T,A.A.), Free Proline and Proteins Contents (mg $100g^{-1}$Dry Weight) Of Selected Maize Plant Under Saline Conditions

Items	CO	TR	ST	CO/TR	CO/ST	TR/ST
Citric acid	*	*	*	**	N.S.	N.S.
"-Ketoglutaric acid	***	*	*	**	*	N.S.
Succinc acid	***	***	*	**	*	N.S.
Fumaric acid	***	N.S.	N.S.	N.S.	N.S.	N.S.
Malic acid	***	**	***	N.S.	N.S.	N.S.
Oxalic acid	***	*	**	N.S.	*	N.S.
Total Organic Acids (T.O.A)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Free Proline	***	*	***	N.S.	N.S.	N.S.
Total Amino Acids (T.A.A.)	***	N.S.	***	N.S.	N.S.	N.S.
Proteins	N.S.	N.S.	***	N.S.	N.S.	N.S.

Statistical treatments, where relevant, the experimental data subjected of One, two and three -Way analysis of variance (*ANOVA*).

Note: F values * = P> 0.05, ** = P >0.01, ***= P >0.001 and N.S.= Not Significant. CO: Concentrations, TR: Treatments (SS) and (SS/HP -V"/V"), ST: Stages of growth.

Appendix Table 3: Effect of Salinity Treatments on germination, growth, yield production and endogenouse levels of some metabolites and ions in maize Plant

I tems	CO	TR	ST	CO/TR	CO/ST	TR/ST
Osmolality	***	***	***	***	N.S.	N.S.
EC_e	***	**	*	N.S.	N.S.	N.S.
pH	***	N.S.	***	N.S.	N.S.	N.S.

Statistical treatments, where relevant, the experimental data subjected of One, two and three -Way analysis of variance (*ANOVA*).

Note: F values * = P> 0.05, ** = P >0.01, ***= P >0.001 and N.S.= Not Significant. CO: Concentrations, TR: Treatments (SS) and (SS/HP -V"/V"), ST: Stages of growth.

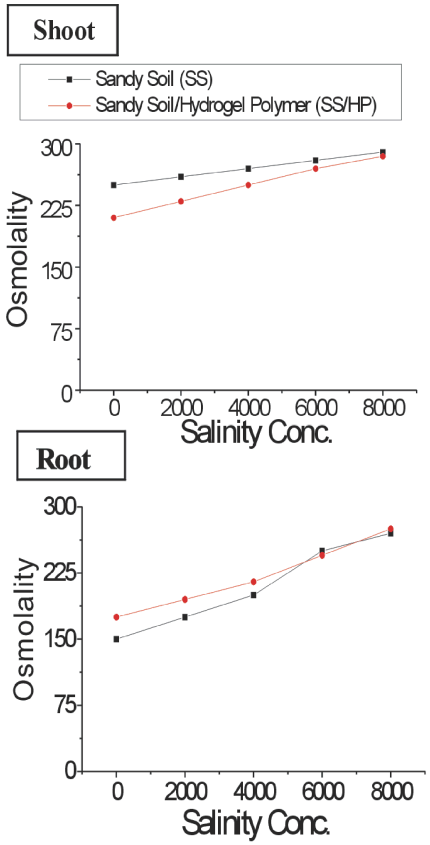

Fig. 8: Effects of a Hydrogel Polymer incorporating with Sand (SS/HP -"v/v") and Pure Sandy Soil (SS) On Shoot and Root Osmolality Of Selected Maize Plant Under Saline Conditions.

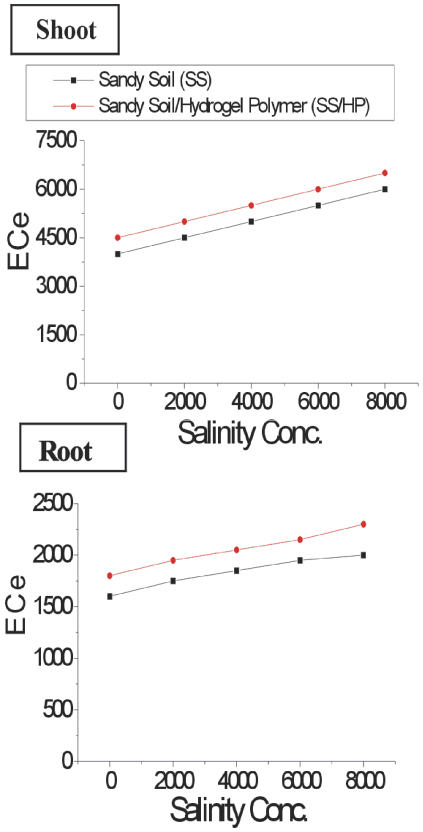

Fig. 9: Effects of a Hydrogel Polymer incorporating with Sand (SS/HP -"v/v") and Pure Sandy Soil (SS) On Shoot and Root ECe Of Selected Maize Plant Under Saline Conditions.

nitrogen deficiency was inhibitory. There was a degree of inhibition by proline initiator depending mainly on nitrogen deficiency and with less extent on salinity level. The statistical analysis (One, two and three-way analysis of variance (ANOVA) as shown in Table 2.

Osmolality, Electric Conductivity (ECe) and pH; Generally, Osmolality, EC_e but not pH of the cell-sap increased with growth stages particularly in the shoots (Figs. 8, 9 and 10). The effects of incorporating a hydrogel polymer into sand (SS/HP) on pigment contents of maize grown under saline conditions has been observed the increasing of pigment contents by Sand/Hydrogel polymer (SS/HP) than pure sandy soil (SS). Overall, osmolality and EC_e of the cell-sap of shoots and roots increased with increasing salinity concentration; pH values, however, decreased in both sandy soil (SS) and incorporated sandy soil with hydrogel polymer (SS/HP) (v/v). Shoot and root cell-sap osmolality and electric conductivity (ECe) increased with increase salinity concentrations, reflecting enhanced ion concentration in the plant tissues.The statistical analysis (One, two and three-Way analysis of variance (ANOVA) as shown in Table 3.

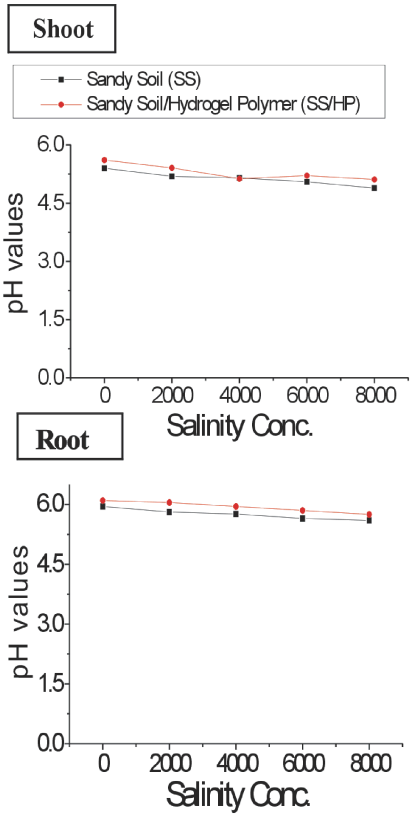

Fig. 10: Effects of a Hydrogel Polymer incorporating with Sand (SS/HP -"v/v") and Pure Sandy Soil (SS) On Shoot and Root pH values Of Selected Maize Plant Under Saline Conditions.

Inorganic Minerals Elements Content: The macronutrient and micronutrient elements in Shoot and root increased with growth stages and generally were higher in root than shoot (Table 1). The effects of incorporating a hydrogel polymer into sand (SS/HP) on pigment contents of maize grown under saline conditions has been observed the increasing of inorganic elements content by pure sandy soil (SS) than Sand/Hydrogel polymer (SS/HP) (v/v). The levels sodium (Na^{+}), calcium (Ca^{+2}) and magnesium (Mg^{+2}) increased with salinity while potassium (K^{+}) declined. Chloride (Cl^{-}) levels increased more in shoots than roots; the accumulation being more than sodium(Na^{+}), calcium (Ca^{+2}), magnesium (Mg^{+2}) or potassium (K^{+}); maize tended to accumulated chloride (Cl^{-}) (Table 2). Similarly the increased uptake inorganic elements content by used hydrogel polymer incorporated with sand soil [2-4], they observed that a high salinity conditions were reduced the inorganic elements content but appeared to be tolerant at all levels of hydrogel polymer incorporation with sand (sand/swollen hydrogel polymer; v/v). Also similar results by Ca^{2+} uptake was inhibited by $NaSO_4$ to a greater degree than NaCl [68, 69]. While Ca^{2+} is reported to play a major role in salt tolerance [70, 71], most worker have observed a depressive effect of Na^{+} on Ca^{2+} uptake [72]. According to Lessani and Marschner [72], Na^{+} and Ca^{2+} ions probably compete much more for common uptake sites. From the present results it appears that Na^{+} in combination with $SO^{4-}$is more effective than with Cl-in this respect with increasing salinity, sodium tended to accumulate, particularly in the root confirming the findings of other workers [73].

Generally, potassium content decreased, particularly in the shoots, reflecting competitive reduction in uptake due to salinity, similar observations have been [73, 74]. The calcium content increased with increasing salinity concentration, particularly in the root. This may reflect a salt induced increase in magnesium uptake [75, 76] and the indirect effect of magnesium on calcium [77]. In the present study, maize tended to retain magnesium in preference to sodium and calcium or potassium, substantiating the view that species can be classified as calcium, sodium or magnesium glycophytes. Maize affected to retain divalent more than monovalent cations perhaps reflecting the preference of secretion mechanisms ($Mg^{2+} > Ca^{2+} > N^{a+} > K^{+}$). At all levels of salinity, the chloride content increased progressively with salinity

Appendix Table 4: Effects of a Hydrogel Polymer incorporating with Sand (SS/HP "v/v") and Pure Sandy Soil (SS) On Shoot and Root Inorganic Elements (Na^{+}, K^{+}, Mg^{2+}, Ca^{2+} and Cl^{-1}) meq/100 g Dry Weight Of Selected Maize Plant Under Saline Conditions

I tems	CO	TR	ST	CO/TR	CO/ST	TR/ST
Na^{+}	***	N.S.	***	N.S.	N.S.	**
Ca^{+2}	***	***	***	*	N.S.	*
Mg^{+2}	***	*	*	N.S.	N.S.	N.S.
K^{+}	***	**	N.S.	N.S.	*	N.S.
Cl^{-}	***	N.S.	*	N.S.	N.S.	N.S.

Statistical treatments, where relevant, the experimental data subjected of One, two and three -Way analysis of variance (*ANOVA*).

Note: F values * = P> 0.05, ** = P >0.01, ***= P >0.001 and N.S.= Not Significant. CO: Concentrations, TR: Treatments (SS) and (SS/HP -V"/V"), ST: Stages of growth.

concentration, particularly in the shoot; the accumulation of chloride in both shoots and roots was higher than sodium The statistical analysis (One, two and three-Way analysis of variance (ANOVA) as shown in Appendix Table (4). The effects of incorporating a hydrogel polymer with sand on development of selected *Zea mays*, L. grown under saline conditions has been adapted to salinity (< 8,000 ppm) was found to have undergone extensive osmotic adjustment by accumulation of organic and inorganic solutes [78]. The hydrogel polymer incorporating with sand reduced the effect of salinity treatments on maize, with the exception of 2,000 ppm, salinity decreased seed germination, plant growth yield production and mitotic division and the adaptation of maize plant to salinity stress had occurred; may make significant contributions by using hydrogel polymer to improve the soil characters and adapted the maize plant [78].

CONCLUSIONS

The resulted presented here indicate that, generally, salinity treatments, pH and potassium levels; monosaccharide and polysaccharide, but not sucrose, increased under salinity stress. Most organic solutes (organic acid, amino acid, proline and protein) and inorganic elements (sodium, calcium, magnesium and chloride) also osmolality and ECe increased with increase in salinity concentrations. The accumulation of organic and inorganic solutes under saline stress may provide an alternative source and solutes in the cells in order to adjust osmotically to the conditions of the external environment. The effects of incorporating a hydrogel polymer into sand on development of selected maize grown under saline conditions has been demonstrated. The grains of maize were germinated in sand/ hydrogel polymer mixture (80: 20 v/v)with added Hoagland nutrient solution, then transplanted after 7 days from germination into a range of sand/swollen hydrogel polymer (90:10 v/v) in plastic growbags. Saline solutions containing NaCl, $CaCl_2$, $mgCl_2$ (0.0, 2,000, 4,000, 6,000, 8,000 ppm.) were applied to the growbags (to field capacity twice per week, alternating with a comparable watering regime. Hydrogel polymer incorporating with sand soil (SS/HP) (v/v) reduced the effect of salinity treatments. From those results, the reduction in growth and yield production under saline stress may be due to the expenditure of energy on the synthesis of organic or inorganic components for osmotic adjustment rather than for growth. The decrease in water content may occur because plants grown in saline soil accumulate high levels of salt and an osmotic adjustment is required to maintain root water potential lower than that of the external medium.

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