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Effect of Soil Salinity on the Growth Indicators of Cassava (*Manihot esculenta* Crantz) Starch Graft Copolymers

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ABSTRACT

Graft copolymers of cassava starch and acrylonitrile were synthesized in aqueous solution using ceric ammonium ion as the initiator. Saponification of grafted copolymer was done by reaction with sodium hydroxide and precipitated with methanol. Grafting was confirmed by FTIR. The grafted copolymer and the hydrolyzed graft copolymer (hydrogel) were used as saline soil amendment to cultivate maize (Zea mays). The agricultural performance of the hydrogel was evaluated by measuring the extent of growth tolerance of maize in saline soil amended with hydrogel loadings of 0-12 g/2 kg soil and saline solution variation of 0-16,000 ppm. Sensitivity to salinity treatments was shown by maize from the earliest stage of development. Germination rate and percentage were reduced by all salinity concentrations, compared to previous findings in which there was no salinity. The findings show that a high salinity conditions reduced the growth but the plants appeared to be tolerant at all levels of hydrogel polymer incorporation with the treated soil (sandy loam soil/ hydrogel polymer).

1. INTRODUCTION

Soil salinity may be defined as any accumulation of soluble salts which may be harmful to plants (Annunziata et al., 2017). Generally, saline soils are found in arid and semi-arid regions, where the salts formed by the weathering of soil minerals are not fully leached and high evaporation rates result in accumulation of the salts (Annunziata et al., 2017). In higher plants, salt tolerance may be achieved through the preferential accumulation of compatible osmotic solutes in the cytoplasm in order to maintain equilibrium of the water potential of vacuole and cytoplasm (Britto and Kronzucker, 2015; Byrt et al., 2018).

Salinity is one of the environmental factors limiting soil fertility and plant production in arid and semiarid regions (Shabala and Cuin, 2008). This is attributed to the fact that sodium ions (Na^+) compete with potassium ions (K+) for binding sites essential for cellular function and the implication of these two macronutrients in salinity is thought to be one of the factors responsible for the reduction of the biomass and yield components of plants (Kaymakanova and Stoeva, 2008). The main cause of salinity-induced effects on growth and development of plants is accumulation of ions in soil solution and ultimately their absorption in plant cells (Kaymakanova and Stoeva, 2008). Salt-specific effects on plants are accelerated over time and by the extent of ions accumulation, which eventually rise to toxic level and impose an additional stress on physiological and biochemical processes in plant cells (Byrt et al., 2014). This implies that salt-induced osmotic effects limit the growth, predominantly through power of ion compartmentalization and energy cost in plant cells (Kaymakanova and Stoeva, 2008).

Under stress conditions, such as salinity, drought, low and high temperature, plants produce reactive oxygen species (free radical), which are harmful to plant growth and productivity. Reactive oxygen species deteriorate membrane function, limit nitrate ions (NO_3^-) uptake, damage membrane lipids, proteins and nucleic acids under saline conditions (Munnas, 2002).

As soils become more saline, plants become unable to draw as much water from the soil. This is because; the plant roots contain varying concentrations of ions (salts) that create a natural flow of water from the soil into the plant roots. As the level of salinity in the soil nears that of the roots, however, water becomes less and less likely to enter the root. In fact, when the soil salinity levels are high enough, the water in the roots is pulled back into the soil. The plants become unable to take in enough water to grow. Each plant species naturally contains varying levels of root salts. This is why some plants can continue to thrive when others die. If the salinity concentration in the soil is high enough, the plant will wilt and die, regardless of the amount of water applied, and this also decreases germination of maize (*Zea mays*).

Methods reported in literature for managing saline soils for crop growth include water leaching, chemical remediation and phytoremediation (Ahmad and Chang, 2002; Sharma and Minhas, 2005; Qadir et al., 2007). Remediation of saline soil using chemical agents such as gypsum ($CaSO_4.2H_2O$), calcite ($CaCO_3$), calcium chloride ($CaCl_2.2H_2O$), and organic matter (farmyard manure, green manure, organic amendment and municipal solid waste), has been reported in literature to be effective, low cost, and simple (Mitchell et al., 2000; Hanay et al., 2004; Sharma and Minhas, 2005; Tejada et al., 2006, Choudhary et al., 2004; Wong et al., 2009). However, contrary report by Bauder et al. (2000) established the fact that "saline soils cannot be reclaimed by chemical amendments, conditioners or fertilizers. A field can only be reclaimed by removing salts from the plant root zone. This can be done either by the leaching requirement method which involved applying more water in the root zone than the plant needs. It can also be done by combining the leaching requirement method with artificial drainage or by managed accumulation method which is the removal of salts away from the root zone to locations in the soil other than below the root zone. Although there are many treatments and management practices that can reduce salt levels in the soil, there are some situations where it is either impossible or too costly to attain desirably low soil salinity levels for effective crop yield (Hanson et al., 2006).

This study therefore is employing the use of superabsorbent material, hydrolyzed graft copolymers which are slightly cross-linked, three-dimensional network polymers, cost effective water storage material which is hygroscopic and sometimes found as colloidal gels in which water is the dispersion medium. It can absorb large volumes of liquid and retain it, (Zohuriaan-Mehr and Kabiri. 2008). This is realized by increase in volume of the polymer. The effects of incorporating hydrogel polymer into soil on development of maize (*Zea mays*) grown under saline conditions have been observed in this study.

2. MATERIALS AND METHODS

2.1. Materials

Acrylonitrile (AN) used was supplied by BDH, Poole, England as reagent grade and was distilled under reduced pressure and stored in the dark at 5°C before use. Cassava starch was sourced from a local cassava starch processing factory in Benin City, Nigeria. Analar grade cerium ammonium nitrate (CAN) was supplied by BDH, Poole, England. Characterization of the cassava starch and soil, the synthesis and characterization of the graft starch acrylonitrile have been reported in previous publications (Ekebafe et al., 2011, 2012).

2.2. Experimental Details

In a glasshouse experiments carried out in November - January, under controlled light and temperate conditions (with average maximum and minimum temperatures ranging between 33 – 42 °C and 28 – 32 °C), the seeds of maize were germinated in the sandy loam soil. Measured quantities of the sand-hydrogel polymer combinations (0, 3, 6, 9, and 12 g per 2 kg soil) were thoroughly mixed and designated as Z_1, Z_2, to Z_5, placed in five-liter polyethylene containers. Saline solution containing NaCl, $CaCl_2$, $MgCl_2$, as molar solutions were applied to produce final concentrations of 0.0, 2000, 4000, 8000, and 16000 ppm (Molar equivalents 0.0, 0.6×10^{-1}, 1.2×10^{-1}, 1.8×10^{-1}, 2.4×10^{-1}, and 3.0×10^{-1} respectively, electrical conductivity 4, 8, 14, and 20 dS/m respectively, no saline solutions in the control) The treatment 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. Germinated maize grains were planted at the center of each container, arranged in a completely randomized design with three replications. Harvesting was carried out at the fruiting stage and the effect of treatment was analyzed by evaluation of growth parameters, water content, pigments, and yield production.

Grains of Maize were germinated at 28 °C in dark in 9 cm glass Petri dishes on disc of filter paper (Whatman No. 1) moistened with appropriated salinity culture solutions. Determination of plant growth (plant height, leaf area, grain yield and biomass accumulation) was carried out during harvest. Relative water content (RWC) of leaves was measured on fully expanded leaves at 8 weeks after sowing (WAS). Relative water content (RWC) of leaves was measured on fully expanded leaves at 2 weeks interval after sowing (WAS). Leaves were cut and collected at mid-day to determine fresh weight (FW). Leaf blades were then placed with their cut end pointing down into a tube containing about 15 ml of 1 mM $CaCl_2$. The $CaCl_2$ was used to increase leaf cell integrity, with the aim of reducing cell lysis due to excessive rehydration. The turgid weight (TW) was then, recorded after overnight rehydration at 4 °C. For dry weight (DW) determination, samples were oven-dried at 70 °C for 48 hr. Relative water content was calculated according to Schonfeld et al. (1988) thus:

$$\text{RWC (\%)} = [(\text{FW} - \text{DW}) / (\text{TW} - \text{DW})] \times 100 \quad (1)$$

Nitrogen content, crude protein, starch and soluble sugars were determined as follows. Dried samples were grounded and passed through a 1 mm sieve before analysis. Nitrogen content (%) was determined by the Kjeldhal method (AOAC, 1990) and crude protein (CP) content was obtained by multiplying the Kjeldahl N values by 6.25. Starch and soluble sugar contents were also determined by official AOAC method.
The fresh leaves were grounded in 80% acetone as quickly as possible at room temperature and the chlorophyll (Chl) contents (Chl.a, and Chl.b) and carotenoids were determined using a UV spectrophotometer at 420, 645 and 664 nm and by the calculations described by Wellburn (1994).

$$\text{Chlorophyll a} = 10.3\text{E}664 - 0.918\ \text{E}645 \quad (2)$$

$$\text{Chlorophyll b} = 19.7\ \text{E}645 - 3.870\ \text{E}664 \quad (3)$$

$$\text{Carotenoids} = 4.3\text{E}452 - (0.0264\ \text{Chl. a} + 0.426\ \text{Chl. b}) \quad (4)$$

Where E is absorption coefficient at the designated spectrophotometer values.
The pigment fractions (Chlorophyll a, b and carotenoids were calculated as µg Chl./mg D.W.

Data were analyzed by two-way Analysis of Variance (ANOVA), and the means comparisons were made using the least significant differences (LSD) at the 5% level of probability using the Genstat 12 software. The standard error of the mean (SEM), coefficient of variation (CV%), overall F- value, and its significance were also presented.

3. RESULTS AND DISCUSSION

The results of the soil physico-chemical properties, grafting and characterization of the starch and acrylonitrile have been reported in previous publications (Ekebafe et al., 2011, 2012). The growth indices as presented in Figures 1-7, indicated increasing effect of grain germination by soil/hydrogel mixture than pure sandy loam soil. The findings corroborated the results of Hui et al. (2019). The findings in Figures 1-4 showed that high salinity conditions reduced the growth of maize when compared with the control where there was no presence of salinity. However, the maize was tolerant at all levels of hydrogel polymer incorporation with the treated soil (sandy loam soil/ hydrogel polymer).

Figure 1 showed the plant heights which increased significantly even at the probability level of 0.05 in the soil with 2000 ppm of saline and the control and increases marginally with the hydrogel application as salinity increases. The application of hydrolyzed starch-g-polyacrylonitrile introduced sufficient moisture at the root zone of the plant thereby reducing the lethal effect of the salts for improved growth. The effects were significant under low and medium application of the hydrolyzed graft copolymer. The highest plant height (93 cm) was recorded at 2000 ppm salinity with 12 g hydrogel application, followed by the control plant height at 90 cm. Increased in hydrogel application from Z_1 – Z_5, increased the water level at the root zone of the plant thereby diluting the concentration and effects of the salts in the zone, causing the maize plant to grow even at high salinity level of 16000 ppm as shown in Figure 1. These results (Figure 1) confirmed earlier findings that germinating rate and capacity of maize growth in terms of the plant height were delayed with increasing salinity of substrates (Dorraji et al., 2010). Kaymakanova, and Stoeva (2008) found the reduction of the biomass in beans under saline condition was indicative of several growth limitations, so, the salinity had adverse effects not only on the biomass, but also on other morphological parameters such as plant height, number of leaves, root length and shoot/root ratio.

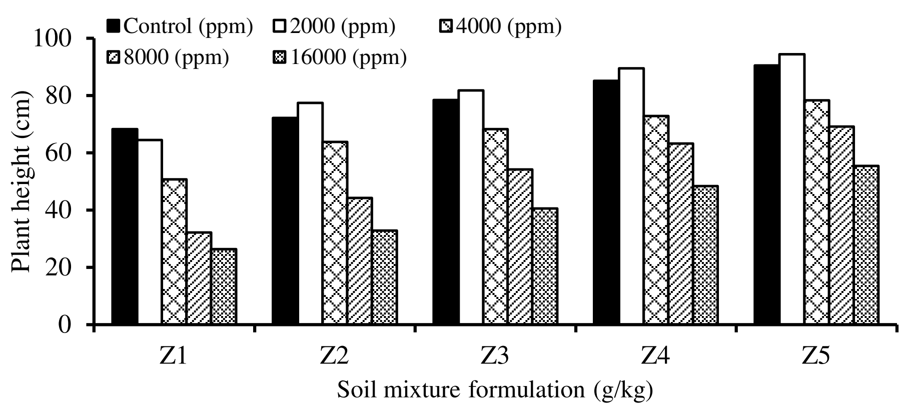

Figure 1: Effect of salinity on the plant height (cm) of maize in hydrogel amended soil

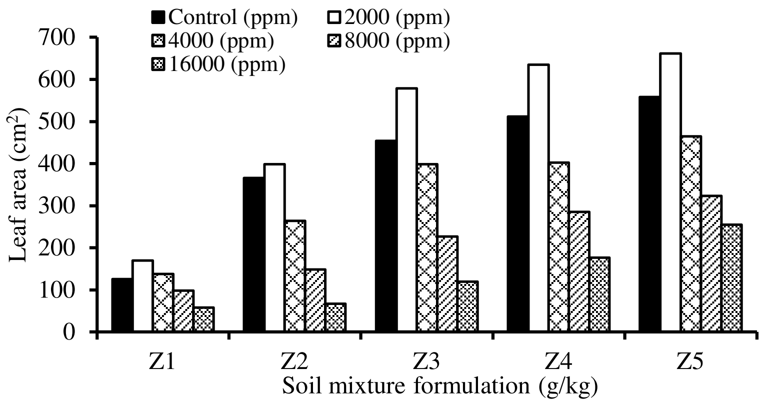

Figure 2: Effect of salinity on the leaf area (cm^2) of maize plant in hydrogel amended soil

Figure 2 shows the effect of salinity on the plant leaf area. The results showed significant increase in the leaf area of the 2000 ppm salinity level as the hydrogel increased from Z_1 to Z_5. There was 100 cm^2 increase in the leaf area of the maize with 12 g hydrogel application and 2000 ppm salinity level over the control (558 cm^2) at the same condition, representing an increase 18.18%. However, at a particular level of hydrogel application, there was an observed significant decrease in the leaf area as the salinity increased (Figure 2). Also, as the hydrogel increased ($Z_1 – Z_5$), there was an observed marginal increase in the leaf area across the salinity levels. In the maize plant, salinity treatments with hydrogel application tended to increase plant height ($p<0.05$), leaf area ($p<0.05$) and biomass accumulation ($p<0.05$) marginally (Figure 1-4). The growth indices in Figures 1 and 2 showed that the maize plant tolerated salinity increased at all levels of hydrogel polymer. Tolerance levels were maxima at < 16,000 ppm. However, plant growth (plant height, leaf area and biomass accumulation) was inhibited marginally with increase in salinity concentration (< 16,000 ppm) from Z_1 to Z_5 (Figures 1 and 2). It was also observed that the plant began to wilt above 16,000 ppm at the vegetative stage. Remediation of saline soil with the graft copolymer in this study (3 – 12 g) showed that maize plant can survive at all salinity concentrations below 16,000 ppm.

These results corroborate the earlier findings that leaf area (Figure 2) and biomass accumulation (Figure 3) generally decreased with increase in salinity concentration relating to the effect of water deficit (Moosavi et al., 2005; Reza et al., 2012; Yadav et al., 2020). Bhatia and Ashwath, (2018) found that 8,000 ppm salinity was critical, mortality being recorded at 6 weeks in oat, 7 weeks in wheat and 8 weeks in barley. At 10,000 ppm the growth of crops almost completely ceases after the addition of a second treatment of salt. Mortality began 3 weeks after treatment in wheat and oat, and after 4 weeks in barley, even before the application of the last salt treatment. Among the legumes, beans showed similar behavior towards salt concentrations both surviving <2000 ppm. At 4,000 ppm mortality occurred in pea even before the last application of salinity to the soil. In these studies, the dry weight of the specie was increased at salinity levels of 4,000 ppm. Hameda et al. (1991) observed the same results in tomato, lettuce and cucumber, barley and oat and 5-10 meq dm^{-1} of sodium increased the yield of barley (Bhatia and Ashwath, 2018; Hui et al., 2019).

Figure 4 shows the effect of salinity level on the maize yield with hydrogel application. The results showed increased yield across the salinity levels as the hydrogel application increased from Z_1 to Z_5. This observation is significant at a probability level of 5% for the control and the 2000 ppm salinity level as the hydrogel application rate increases. The maximum grain yield was recorded at 80 g for Z_4 and Z_5 for the 2000 ppm salinity level (Figure 4). This is followed by 78 g for Z_3 at 4000 ppm salinity. Maize yield decreased significantly ($p<0.05$) with increase in the salinity level for each application of the hydrogel. Bhatia and Ashwath, (2018) found that sensitivity to salinity treatments was shown by tomato, lettuce and cucumber from the earliest stages of development. Similar effects of salinity on growth have been reported in literature. A studied on the influence of four concentrations of total salts (3,500; 5,500; 8,000 and 10,000 ppm) on the development of maize was done and found that toxicity occurred at 5,500 ppm and that diluted sea water (15,000 ppm) reduced the number of leaves, tillering and plant height of barley cultivars (Abedi-Koupai et al., 2008). A 10% decrease in grain yield of different cultivars of wheat grown under saline conditions, was detected mainly reflecting a reduction in 1000 grain weight (Loescher et al., 2011). Also, of 34% recorded maximum reduction in straw yield and 49% in grain yield of barley at the highest concentration due to salinity was also reported (Loescher et al., 2011).

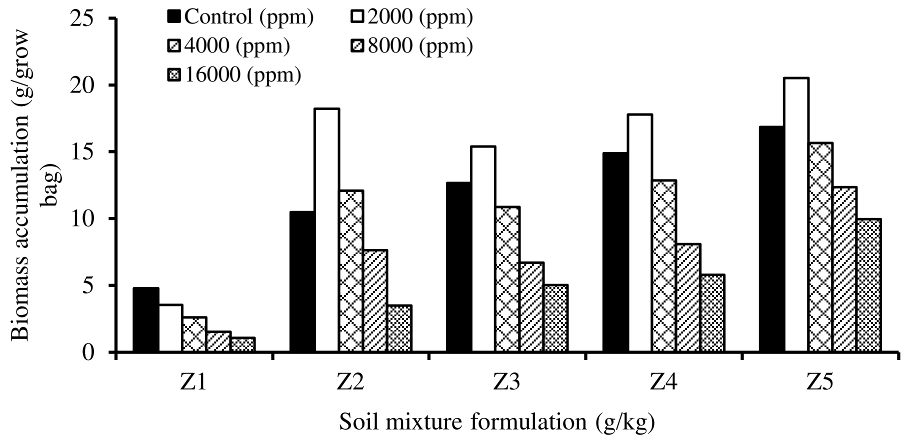

Figure 3: Effect of salinity on the biomass accumulation (g/growbag) of maize plant in hydrogel amended soil

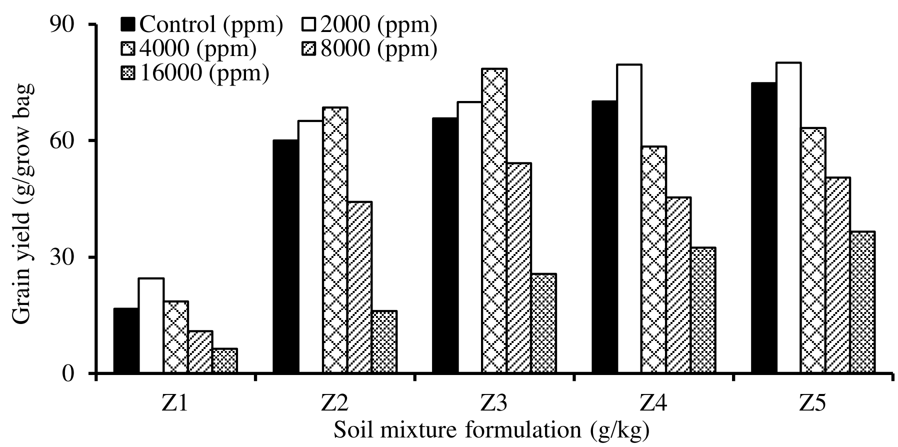

Figure 4: Effect of salinity on the grain yield (g/growbag) of maize plant in hydrogel amended soil

Figure 5 shows the effect of salinity on the relative water content of the leaves of the maize due to hydrogel application. The relative water content in plant leaves was much higher in maize plants of the control at the highest 97% for Z_5 hydrogel application. However, as the salinity level increases for each hydrogel application there was an observed decrease in the RWC. Increase in hydrogel application rate from Z1 to Z5 increased the RWC as the salinity increases as shown in Figure 5. The survival rate of plants in the graft copolymers amended soil doubles in the absence of irrigation and deficit irrigation (Abedi-Koupai et al.2008). It is also reported that graft copolymers have been used in soils in barren lands of Uganda and China for successful establishment of certain trees (Huttermann et al., 2009). Also, they have been amended to soil for grass restoration in arid regions where regular irrigation is a constraint and salinity constrain plant growth (Guzman-Lucero et al., 2010).

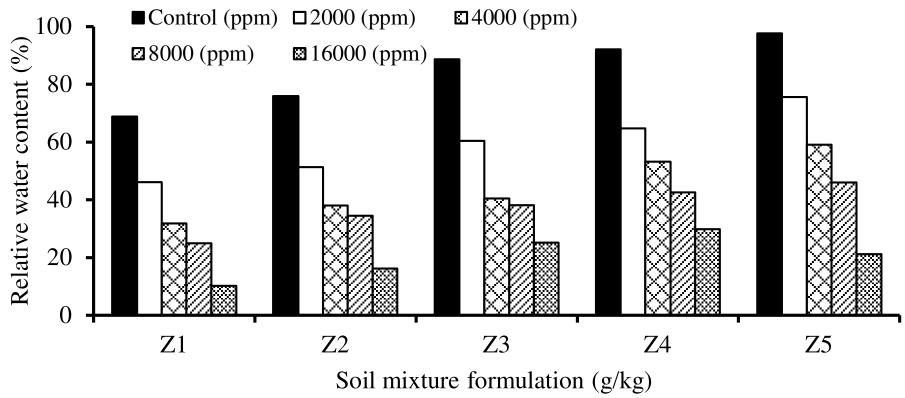

Figure 5: Effect of salinity on the relative water content (%) of the leaves of maize plant in hydrogel amended soil

Figures 6-8 showed the effect of salinity on the maize plant chlorophylls a and b, as well as the carotenoids. In the present study, chlorophylls a and b and carotenoids increased with hydrogel concentration (Figure 6). The result indicates that at the salinity conditions, the level of chlorophyll a (Figure 6) was higher than that of either chlorophyll b (Figure 7) or Carotenoids (Figure 8), though decreasing at high saline concentrations. The highest chlorophyll a was recorded as 24 µg/mg (Figure 6) for Z_5 at 4000 ppm salinity level, whereas for chlorophyll b the highest was recorded at 12 µg/mg (Figure 7) under the same conditions of salinity and hydrogel application. The carotenoids highest value was recorded at 8.7 µg/mg under the same condition (Figure 8). Accumulation of chlorophyll a seems to be related to the neutral pH of the cell sap in plants of saline habitats which favors the growth of the maize plant (Said-Al, et al., 2011). Several researchers have noticed a decrease in net photosynthetic rate due to salt stress in plants (Sanoubar et al., 2016). According to Tavakkoli and co-workers, this is mediated through the effect of salinity on photophosphorylation (Tavakkoli et al., 2010). Salinity is reported to affect the strength of the forces binding the complex of pigment-protein-lipid in the chloroplast structure (Tavakkoli et al., 2010). Salinity treatments progressively reduced photosynthesis and this was coincident with a decline in leaf chloroplast pigments (chlorophyll a, chlorophyll b and carotenoids). Other researchers have concluded that salinity affected the rate of photosynthesis reflecting changes in pigment composition (Parvathy et al., 2014; Tong et al., 2015; Ahmadian and Bayat, 2016). Photosynthetic activity decreased with increase in salinity concentrations in pure sand soil, but increased with sand/hydrogel polymer (Figures 6-8). The most important process that is affected in plants, growing under saline conditions, is photosynthesis. Reduced photosynthesis under salinity is not only attributed to stomata closure leading to a reduction of intercellular CO_2 concentration, but also to non-stomata factors. There is strong evidence that salt affects photosynthetic enzymes, chlorophylls and carotenoids (Ahmadian and Bayat, 2016). Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate, as well as changes in plant metabolic processes (Tong et al., 2015).

Salinity treatments are known to increase the concentration of sugars, organic acids, amino acids including proline and protein. Salinity is known to retard plant growth through its influence on several facets of plant metabolism including osmotic adjustment, ion uptake, enzyme activities, protein and nucleic acid synthesis, photosynthesis and hormonal balance (Orikiriza, et al., 2009). Considering the results in Figures 6-8, it could be affirmed that salinity generally provides a slow growth and development of cells, especially in the leaves which was confirmed by Munnas (2002) who stated that salinity reduces plant growth through lessening or stopping the leaf expansion. This factor suppresses the turgor pressure and metabolic activities in the cells that are observed as low number and small size of leaves associated with short plant height. Additionally, salinity disturbs mineral nutrient absorption and ion balance in the plant organelles (Hui et al., 2018).

Therefore, a decrease in leaf and stem length could also be attributed to mineral nutrient deficiency in the root regions.

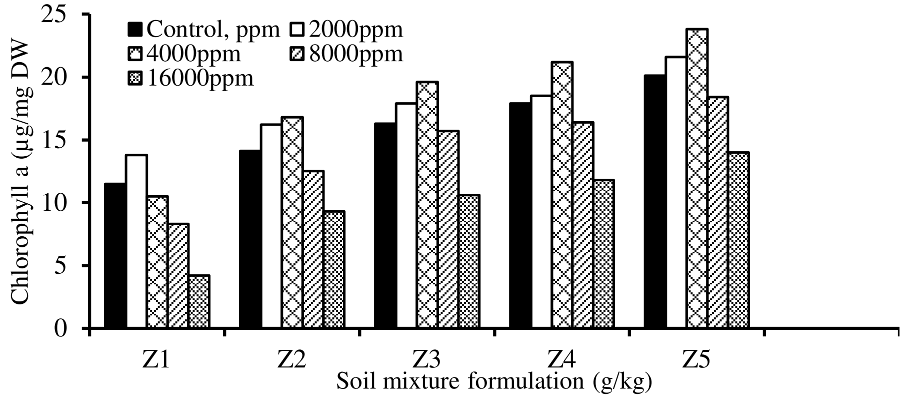

Figure 6: Effect of salinity on the chlorophyl a (µg/mg DW) of the maize plant in hydrogel amended soil

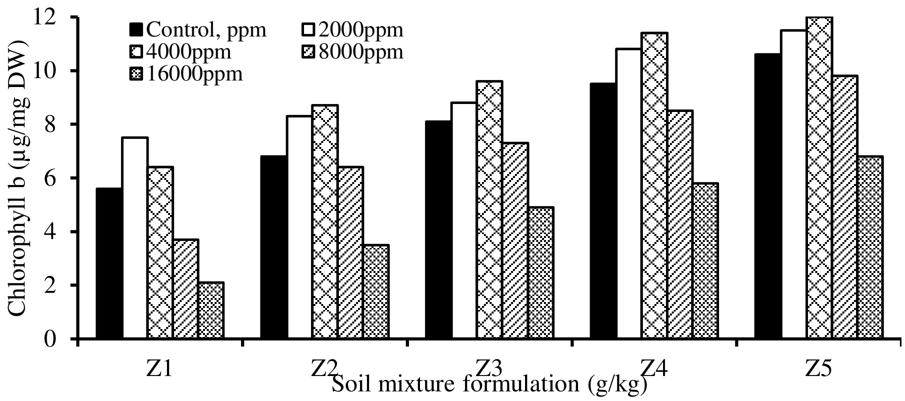

Figure 7: Effect of salinity on the chlorophyl b (µg/mg DW) of the maize plant in hydrogel amended soil

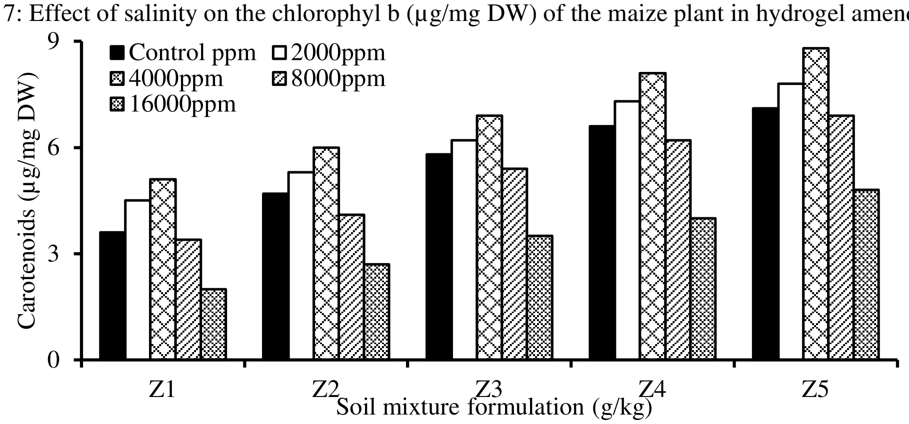

Figure 8: Effect of salinity on the carotenoids (µg/mg DW) of the maize plant in hydrogel amended soil

Figure 9 and 10 show the effect of salinity on the soluble sugars level and starch respectively in maize plant with hydrogel application. It was observed that salinity treatments increased the levels of sugars up to 85% at 4000 ppm salinity at Z_5 hydrogel application (Figure 9) and starch up to 27 g at 8000 ppm salinity level at Z_5 incorporation of hydrogel polymer. Protein levels increased with increasing salinity concentration

($p<0.05$) particularly in the presence of hydrogel (Figure 11). Protein content increased up to salinity levels of 8000 ppm ($p<0.05$).

The results in Figures 9-11 showed that under greenhouse conditions, incorporation of low concentrations of the hydrogel into sandy loam soil has enabled both species of differing salt tolerance to be grown in saline solutions of up to 16000 ppm concentration. In the absence of the hydrogel the inhibitory effects of salinity on growth, photosynthetic pigments, efficiency and protein would have be higher.

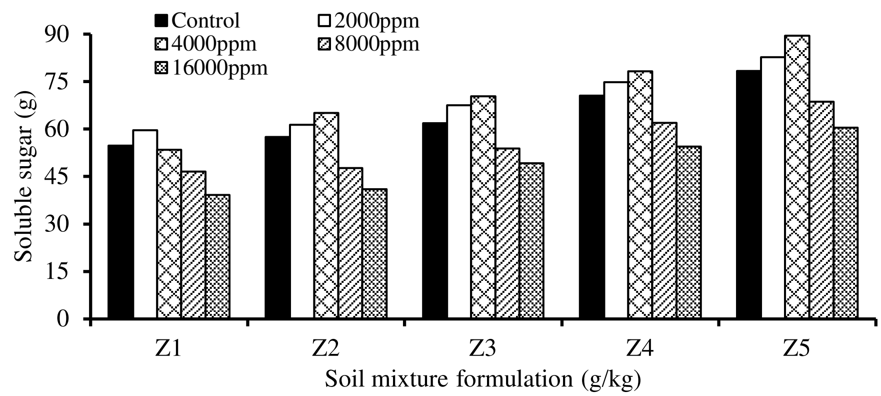

Figure 9: Soluble sugars of the maize plants under different hydrogel and salinity loading

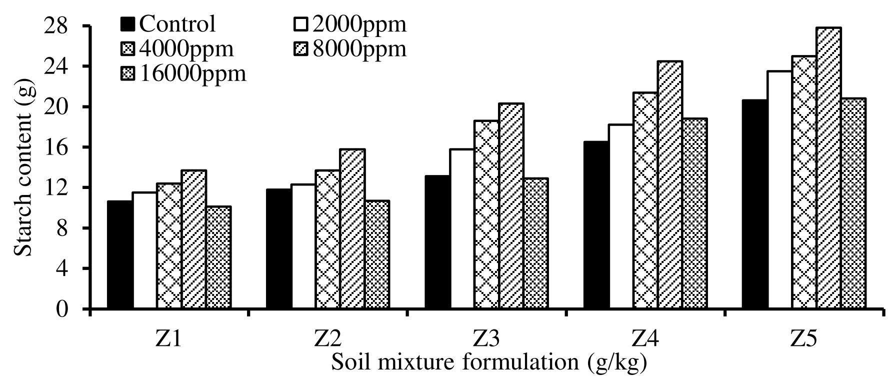

Figure 10: Starch content of the maize grain under different hydrogel and salinity loading

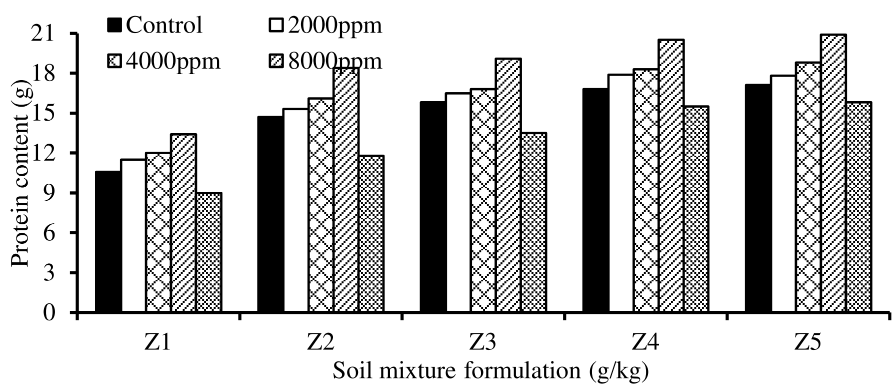

Figure 11: Protein content of the maize plants under different hydrogel and salinity loading

It is possible that metabolites which are observed to accumulate under saline conditions may provide the alternative sources of solutes in the cells to provide osmotic adjustments. The incorporation of different concentrations of hydrogel polymer appears to counteract salt inhibition. The most effective concentration being in the order (Z5 >Z4 >Z3> Z2 >Z1). Similar observation on the beneficial effect of incorporating polymeric hydrogel (aquastore) into a sandy soil on the seedling emergence of wheat cultivars in a sandy soil have also been reported (Silva et al., 2015). The study indicated that in the presence of aquastore at salinity levels of less than 6000 ppm, wheat had earlier emergence and higher germination percentage. Surface application of aquastore resulted in lower salt content of the soil than untreated. It is evident that the capacity of plants to resist water loss under saline conditions may closely associate with their ability to accumulate organic solutes under these conditions. It is possible that organic and inorganic metabolites which are observed to accumulate under saline conditions may provide an additional source of solutes in the cells in order to enable osmotic adjustment to the external environments. The reduction in growth and yield under saline stress may be due to expenditure of energy on the synthesis of organic and inorganic components for osmotic adjustment rather than for growth. Plants which have the highest ability to accumulate organic solutes under osmotic stress in the presence of hydrogel polymer show the greatest resistance to loss of water under these conditions (Seyed Doraji et al., 2010).

4. CONCLUSION

The findings show that high salinity conditions reduced the growth of maize plant but become tolerant at the application of the hydrogel polymer (sandy loam soil/ hydrogel polymer). The reduction in growth and yield under saline stress may be due to expenditure of energy on the synthesis of organic and inorganic components for osmotic adjustment rather than for growth. The application of hydrogel provides enough water at the root zone to assuage the lethal effect of salinity on the growth of the maize plant. Therefore, hydrogel is cost effective biodegradable material suitable for the remediation of saline soils for maize agriculture.

5. CONFLICT OF INTEREST

There is no conflict of interest associated in this work.

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