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INTERACTIVE EFFECTS OF SALINITY AND HEAVY METAL STRESS ON ECO-PHYSIOLOGICAL RESPONSES OF TWO MAIZE (*ZEA MAYS* L.) CULTIVARS

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Abstract

Salinity and heavy metals are two important aboitic stress factors for maize crop. The present study was conducted to assess the interactive effects of salinity and two heavy metals (Cd and Cu), separately and in combination on two maize varieties: Okmass and S2000. Growth parameters, photosynthetic pigments, relative water contents and Na^+, K^+ concentrations were determined at the end of the experiment. The experiment was performed in sand culture with nutrient solution. Two weeks after the germination, the stress of 100 mM NaCl-salinity and heavy metals stress (Cu @ 0.4 mg/L and Cd @ 0.04 mg/L) were given to desired plants. The results revealed that Cu individually and in combination with salinity considerably reduced the shoot length, fresh and dry weights of shoot of both maize cultivars. However, in case of fresh and dry weights of root, both cultivars showed different response towards salinity and heavy metals. The salinity and heavy metal stresses also reduced the leaf chlorophyll contents in both cultivars. The heavy metals significantly increased the K^+ concentration in shoots of both cultivars. It may be concluded that the combined effects were more drastic as compared to single stress.

Introduction

Soil salinity and heavy metals are most threatening environmental stresses imposing hyper-osmotic and ionic stress in plants which greatly reduce the conventional agricultural production particularly in semiarid and arid regions around the globe (Chinnusamy *et al*., 2005, Li *et al*., 2010). Maize is one of the major food and fodder crop. Its grains have high food value containing edible oil, while green fodder is quite wealthy in protein. Maize is the third most important cereal after wheat and rice in Pakistan. However, this plant is intermediately sensitive to salt stress and shows deprived growth under various stress environments and ultimately reduced plant growth and yield (Ouda *et al*., 2008).

Excessive salts in rhizosphere decrease the water potential resulting in reduced water uptake efficiency leading to compromised plant growth and yield (Mansour *et al*., 2005). The accumulation of Na^+ and Cl^- in cytosol challenges the compartmentalization capacity of cells and cause osmotic stress. Salt stress also causes nutrients ion imbalance and specific ion toxicities in plants. It affects the accessibility of nutrients to plants due to change in retention, transformation and binding of nutrients like Zn^{2+},Cu^{2+}etc in the soil which affect the absorption of nutrients from the roots (Marschner and Romheld, 1994), leading to disruption in many physio-biochemical process (Ashraf *et al*., 2008; Munns and Tester, 2008).

Besides salinity stress, the accumulation of heavy metals due to industrial pollution has serious impacts on soil degradation and severely constraint the plant growth and yield that leads to agriculture losses and hazardous to human health when enter in food chain (Youssef and Azooz, 2013). Excessive malpractices of chemical fertilizers and pesticides also significantly contribute to increased heavy metal concentration in soil (Bhatia and Singh, 2012).

The injurious effects of heavy metals depends on the duration of exposure of plants to heavy metals and the chemical and physical properties of ions to penetrate the physiological barriers (Seregin and Kozhenikova, 2008). Cadmium is non-essential for plant growth, its presence in atmosphere and soil even in very low concentration cause severe problems for majority of organisms (Gupta and Abdullah, 2011). It can disrupt the various morphological and physio-biochemical attributes such as growth reduction, modification in water and ion homeostasis, reduced photosynthetic efficiency, production of reactive oxygen species and changes in enzymatic and non enzymatic antioxidant mechanisms (Pal *et al*., 2006).

Copper is essential micronutrients for plants, however, in higher concentrations it causes the toxic effect on plants such as inhibition of plant growth even causes the plants death (Chen *et al*., 2000). In recent years, copper pollution is widespread around the world. It can be accumulated in various parts of plant and disrupt photosynthetic machinery due to direct role in photosynthetic pigments and proteins. Excessive amount of Cu in soil affects the uptake of essential nutrients (Patsikka *et al*., 2002; Gupta and Abdullah, 2011). The high concentration of Cu also produces the reactive oxygen species which trigger the oxidative damages and alter the cell membrane permeability (Madejonb *et al*. 2009). The major objective of this study was to analyze the heavy morphological cum physiological response of two maize (*Zea mays* L.) cultivars (Okmass and S2000) to varied levels of Cu and Cd concentrations in the presence of NaCl-salinity. To our knowledge, it is the first study that determines the simultaneous impact of NaCl-salinity and copper and cadmium stress on growth behavior of these two maize cultivars.

Materials and Methods

The experiment was conducted in GC University, Faisalabad, Pakistan under the natural conditions in green house. The seeds of two maize varieties, Okmass and S2000 were obtained from the Ayub Agricultural Research Institute, Faisalabad, Pakistan. Surface sterilized seeds with 1% sodium hypochlorite were germinated in plastic pots containing pre-washed sand (1.2 Kg/pot). Hoagland solution as nutrient solution was applied regularly and two weeks after the germination, the stress of 100 mM NaCl-salinity and heavy metals stress (Cu @ 0.4 mg/L and Cd @ 0.04 mg/L) were given to desired plants. Chemically pure NaCl salts were used for NaCl salinity stress and salts of $CuCl_2$ and $CdCl_2$ were used for heavy metal stress of Cu and Cd respectively. Salinity stress and heavy metal stress were applied by dissolving appropriate quantities of above mentioned salts in nutrient solution. The experiment was carried out in a completely randomized design.

Vegetative growth parameters such as plant height, root length, fresh and dry weights of root and shoot, and other physiological parameters were at final harvest of crop after the 8 weeks of germination. For dry weights plant material was placed in oven at 70 °C for 3 days.

Photosynthetic pigments: Fresh leaf material (0.25 g) was crushed and homogenized with 5ml ice chilled 80% acetone and centrifuged twice at 2000 for 5 minutes. The absorbance of supernatant was recorded by spectrophotometer, at the wavelengths of 663nm, 645nm and 480nm, for Chl.a, Chl.b and Carotenoids, respectively (Arnon, 1949).

Relative water content: Fresh leaf was collected from the 3rd node at the apex and measured the fresh weight. The leaf was then inserted in distilled water for 6 hours and measured the turgid weight. Turgid leaf was placed in the oven for 3 days and measured the dry weight. The RWC was calculated by the following formula:

RWC (%) = [(FW – DW) / (TW – DW)] × 100

Where, FW = Fresh weight; DW = Dry weight; TW = Turgid weight.

Sodium and potassium ions: Dried powder (0.15) of each root and shoot was taken in test tube and added 3 mL of concentrated sulfuric acid. Kept the solution overnight and then heated the solution at 100˚C for 30 minutes. The solution was cooled for 15 minutes and added H_2O_2 in it. The solution was again heated until colorless solution appeared. Make the volume 50 mL by adding the distilled water. Na^+ and K^+ were determined with the help of flame photometer against the standard curve.

Results and Discussion

Morphological parameters: Data are presented in Fig 1 for the morphological parameters of two maize cultivars. Results show that salinity and heavy metal treatments affected the shoot fresh weight (Fig 1a). It was observed that the Cd^{2+} reduced the shoot fresh weight of Okmass plants while the Cu^{2+} enhances the shoot fresh weight under non saline condition. Salt stress also reduced the shoot fresh weight. The combine effect of heavy metals and salinity showed the more adverse effect as compared to controls. In S2000 and Okmass maize plants, the heavy metals reduced the shoot fresh weight under both non saline as well as saline condition as compared to their respective controls (Fig. 1a).

Cd^{2+} decreased the shoot dry weight of Okmass plants under non saline condition (Fig 1b) . The salt stress also reduced the shoot dry weight. The Combine effect of Cu^{2+} and salinity greatly reduced the shoot dry weight as compare to combine treatment of Cd^{2+} and salinity. In the S2000 cultivar, the Cu^{2+} significantly decreased the shoot dry weight. The combine treatments of Cd^{2+} and salinity, and Cu^{2+} and salinity showed the same trend to decrease the shoot fresh weight. The cadmium metal significantly reduced the shoot fresh weight in the Okmass plants as compare to S2000 cultivar plants while Cu^{2+} metal greatly reduced the shoot fresh weight in S2000 cultivar as compare to Okmass cultivar plants.

Fig. 1c shows that in the S2000 cultivars, the Cu^{2+} greatly decreased the root fresh weight of plants. The saline stress also decreased the root fresh weight. The combine stress of Cu^{2+} and salinity decreased the root fresh weight as compare to combine effect of Cd and salinity. The result showed that the treatment of Cu^{2+} and salinity significantly reduced the root fresh weight in both cultivars.

Fig. 1d showed that the Cu^{2+} metal increased the root dry weight in Okmass plants as compare to Cd^{2+} metal. The salt stress reduced the root dry weight. The Combine treatment of Cu^{2+} and salinity greatly reduced the root dry weight. In S2000 cultivar, Cu^{2+} metal decreased the root dry weight while Cd^{2+} enhanced it. The combine stress of Cu^{2+} and salinity greatly increased the root dry weight as compare to Cd^{2+} and salinity. The combine treatment of Cu^{2+} and salinity significantly increased the root dry weight in S2000 cultivar as compare to Okmass cultivar.

Fig. 1e showed that the Cu^{2+} treatment increased the shoot length while Cd^{2+} metal decreased it in Okmass cultivar. The combine stress of Cd^{2+} and salinity significantly decreased as compare to combine treatment of Cu2+ and salinity. In S2000 cultivar, the Cu^{2+} metal greatly decreased the shoot length as compare to Cd^{2+}. The combine treatment of Cd^{2+} and salinity decreased the shoot length. The Cu^{2+} metal increased the shoot length in Okmass cultivar while in S2000 cultivar, it decreased the shoot length.

Fig. 1f showed that in Okmass cultivar the salinity greatly decreased the root length. While the Combine stressed of Cd^{2+} and salinity more decreased the root length. In S2000 cultivar, Cd^{2+} metal increased the root length while Cu^{2+} metal decreased the root length. The saline condition greatly reduced the root length. The combine treatment of Cd and salinity significantly increased the root length while the combine treatment of Cu^{2+} and salinity greatly reduced it. The Cd^{2+} and the combine treatment of Cd^{2+} and salinity increased the root length in S2000 cultivar while in Okmass cultivar it decreased the root length.

Photosynthetic pigments: Fig 2a showed that the Cu^{2+} reduced the Chlorophyll "a" (Chl.a) content in Okmass cultivar. The saline condition significantly decreased the Chl.a contents as compare to combine treatment of Cd^{2+} and salinity, and Cu^{2+} and salinity. The Cu^{2+} greatly reduced the Chl.a contents in S2000 cultivar. The combine treatment of Cd^{2+} and Salinity greatly decreased the Chl.a contents as compare to saline condition and combine treatment of Cu^{2+} and salinity. The salinity reduced the Chl.a contents in both cultivars as compare to Cd^{2+} and Cu^{2+} stress.

Fig. 2b showed that the Cu^{2+} metal greatly reduced the Chl.b contents in Okmass plant as compare to Cd stress. The salinity significantly decreased the Chl.b contents and showed the more adverse effect as compare to Cu^{2+} and Cd^{2+} metals stress. In the S2000 cultivar, the Cu^{2+} metal treatment greatly reduced the Chl.b as compare to Cd^{2+}, which little bit decreased the Chl.b contents. The saline condition significantly reduced the Chl.b contents as compare to the combine stresses of Cd^{2+} + salinity and Cu^{2+} +salinity. The result showed that the salinity significantly reduced the Chl.b contents in both cultivars.

Fig. 2c showed that the Cu^{2+} metal significantly reduced the total Chl. contents as compare to Cd stress. The salinity showed the more adverse effect and greatly reduced the total chl. contents in Okmass cultivar. In S2000 cultivar, the Cu^{2+} metal significantly reduced the total chl. contents. The salinity also greatly reduced the total Chl. contents. The combine effect of Cu^{2+} and saline greatly reduce the total Chl. contents as compare to combine effect of Cd^{2+}+salinity. The salinity was significantly reduced the total chl. contents in Okmass cultivar plants while the combine effect of Cu^{2+} and salinity greatly decreased the total chl. contents in S2000 cultivar. Fig. 2d showed that in the Okmass cultivar, ratio of a and b chl. greatly increased by the Cu^{2+} in Okmass cultivar plants. The combine effect of Cd^{2+} and salinity greatly increased the chl.a and b ratio as compare to combine effect of Cu^{2+} and salinity stress.

Fig. 2d showed that there was no effect of Cu^{2+} and Cd^{2+} on the carotenoids contents in Okmass cultivar plants. The salinity showed most adverse effect and significantly the reduced the carotenoids contents. In S2000 cultivars, there was little bit effect of Cd^{2+} while Cu^{2+} reduced the carotenoids contents. The salinity greatly reduced the carotenoids contents as compare to combine effect of Cd^{2+}+ Salinity and Cu^{2+} + Salinity.

Physiological parameters: Fig 3a showed that the Cd^{2+} metal decreased the relative water content in Okmass cultivar as compare to Cu^{2+} in non-saline conditions. Salinity also reduced the relative water content. The combine effect of Cu^{2+} and salinity significantly reduced the relative water content as compare to combine effect of Cd^{2+} and salinity. In S2000 cultivar, Cd^{2+} greatly reduced the relative water content as compare to Cu^{2+}. The combine treatment of Cu^{2+} and salinity increased the relative water content while salinity and the combine treatment of Cd^{2+} and salinity had no effect. The Cd^{2+} reduced the relative water content in both cultivars. The combine treatment of Cu^{2+} and salinity decreased the relative water content in Okmass cultivar while increased in S2000 cultivar.

Fig. 3b showed that the Cu^{2+} had no effect on Na^{+} ion concentration in shoots of Okmass cultivar plants. The salt condition significantly increased the Na^{+} concentration. In the S2000 cultivar, Cd^{2+} and Cu^{2+} had no effect of Na^{+} ions concentration in shoots. The saline stress greatly increased the Na^{+} concentration in shoots. The saline stress significantly increased the Na^{+} concentration in the both cultivars.

Fig. 3c showed that the Cd^{2+} and Cu^{2+} significantly increased the K^{+} in shoots of Okmass cultivar's plants. The saline condition also increased the K^{+} ions. The combine effect of Cd^{2+} and saline increased the K^{+} as compare to the combine effect of Cu^{2+} and saline. In the S2000 cultivar, the Cu^{2+} significantly decreased the K^{+} while Cd^{2+} increased it. The saline stress greatly increased the K^{+} ions. The combine effect of Cu^{2+} and saline stress much increased as compare to Cd^{2+} and saline. The Cu^{2+} increased the K^{+} ions in Okmass cultivar as it decreased the K^{+} ions in S2000 cultivar. The saline stress increased the K^{+} ions in both cultivars.

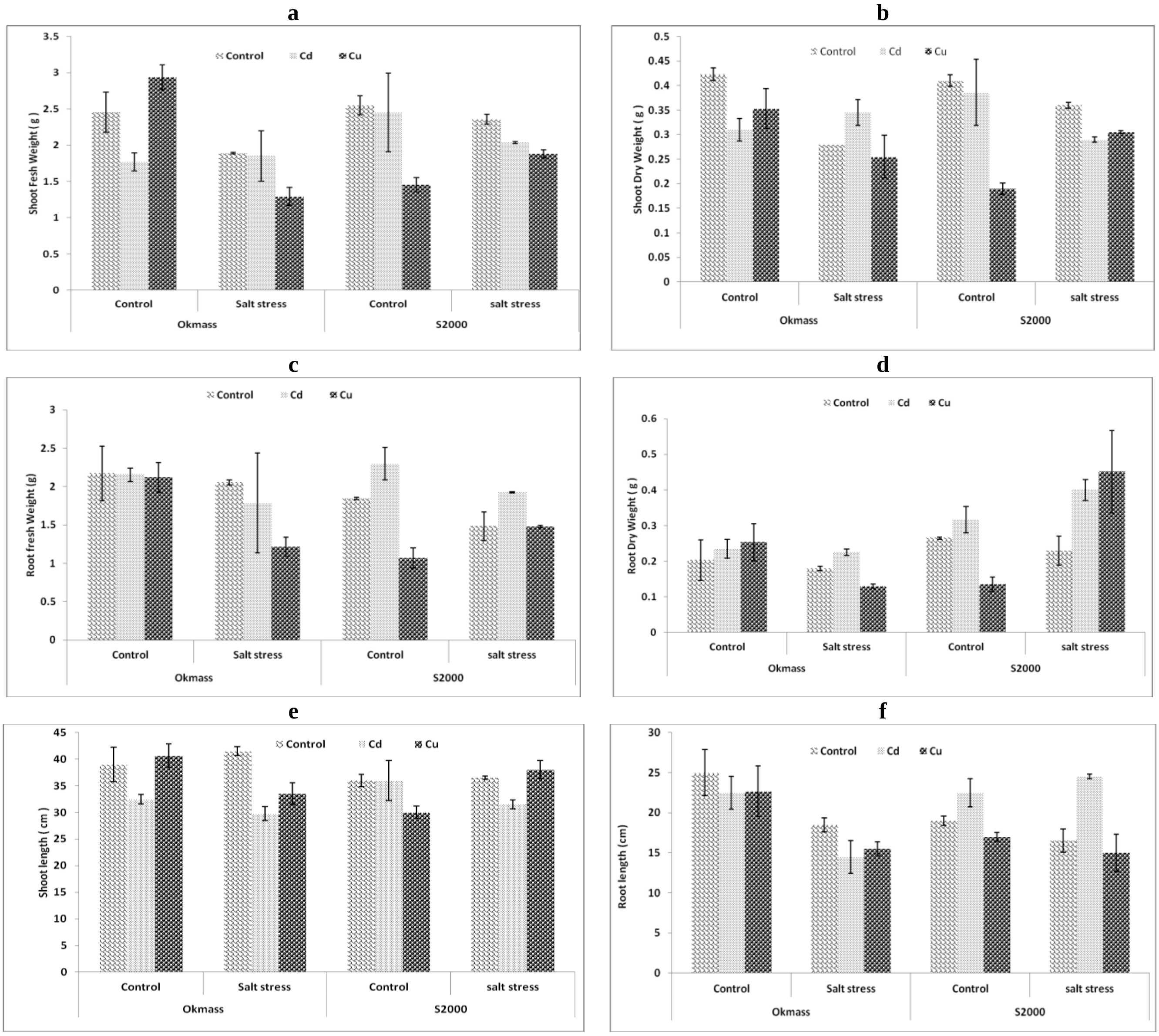
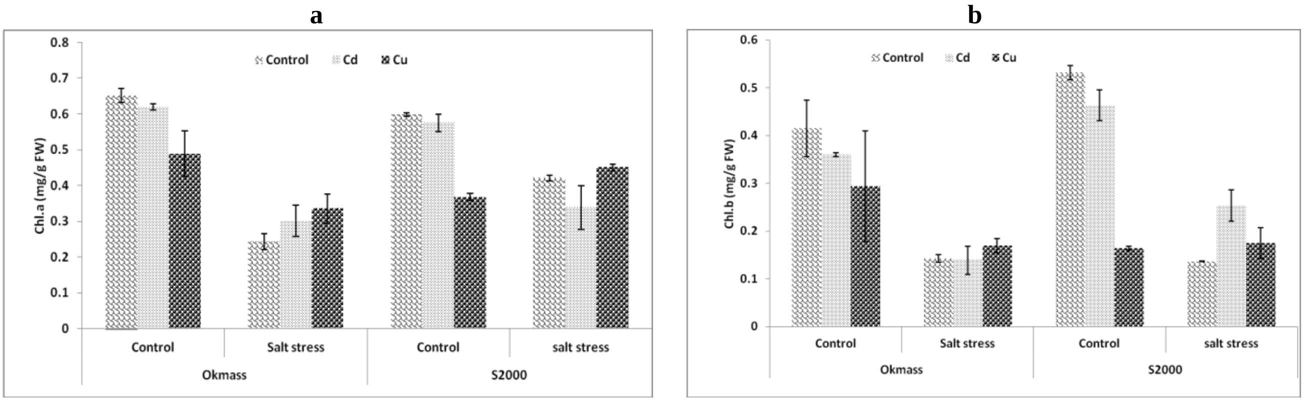


Fig.1 Effect of $CdCl_2$, $CuCl_2$, salinity, $CdCl_2$+salinity and $CuCl_2$+ salinity on growth of two Maize cultivars. a; Shoot fresh weight. b; Shoot dry weight. c; Rroot fresh weight. d; Root dry weight. e; Shoot length. f; Root length.



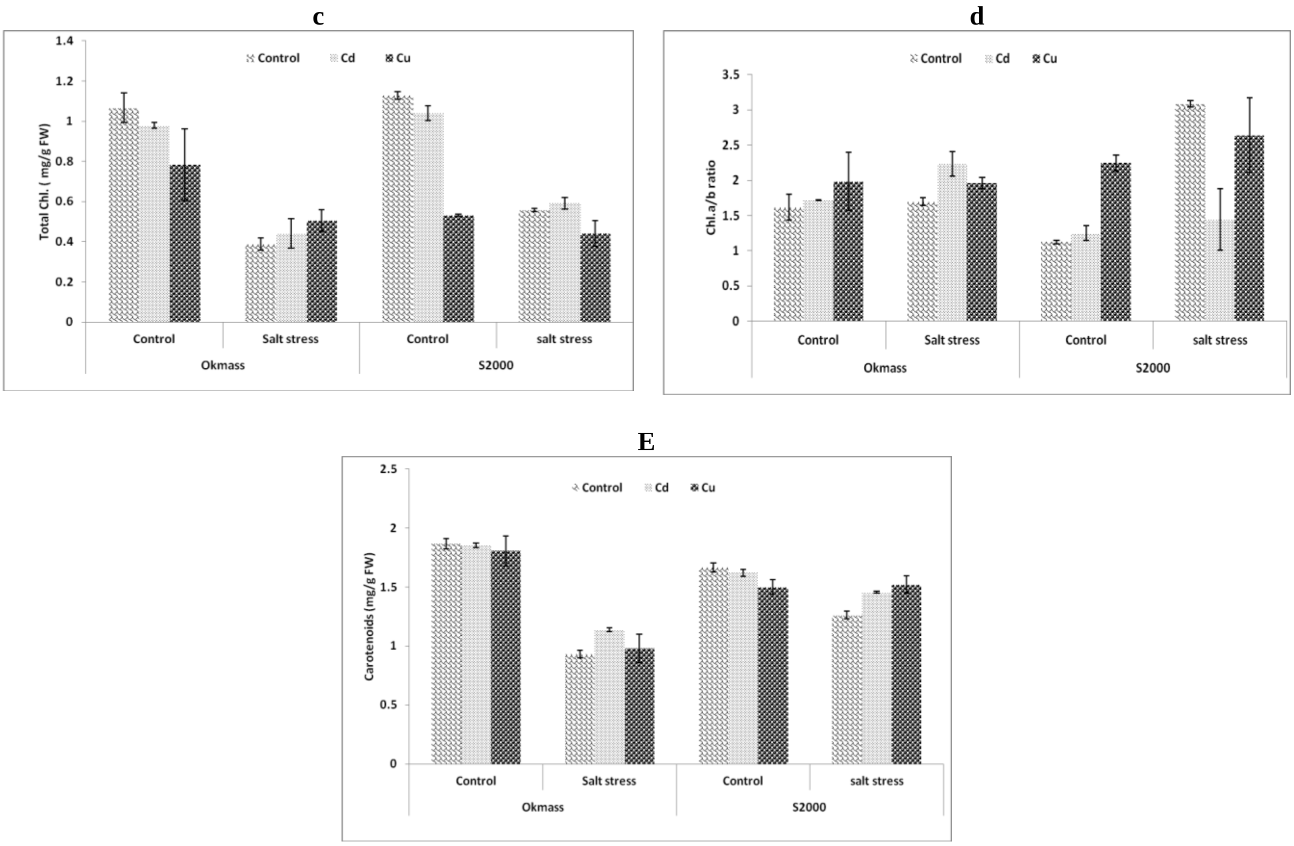


Fig.2. Effect of $CdCl_2$, $CuCl_2$, salinity, $CdCl_2$+salinity and $CuCl_2$+ salinity on photosynthetic pigments (mg/g) of two Maize cultivars. a; Chlorophyll a contents. b;Chlorophyll b contents. c; Total chlorophyll. d; Chl.a/b ratio. e; Carotenoids.

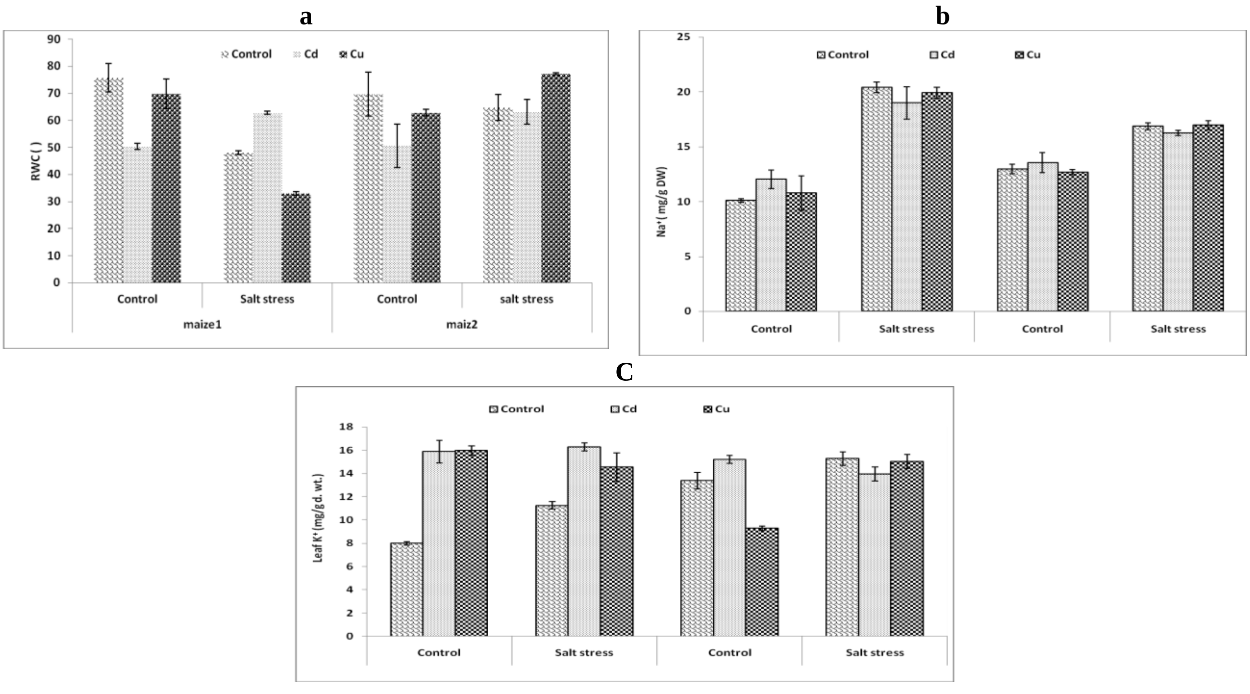


Fig.3. Effect of $CdCl_2$, $CuCl_2$, salinity, $CdCl_2$+salinity and $CuCl_2$+ salinity on physiological parameters of two Maize cultivars. a; Relative water content (RWC). b; Sodium concentration. c; Potassium concentration.

The heavy metals relatively are of high density and affect the various morphological and physiological parameters in plants. The heavy metal cadmium decrease the shoot fresh weight in maize plants (Pal *et al.*, 2006). During the present study, cadmium greatly reduced the shoot fresh weight in Okmass cultivar's while copper reduced the root fresh weight in S2000 cultivar of maize and these results are in according to Chen *et al*. (2000). The saline condition also affected the morphological parameters as well physiological parameters of various plants as reported as well by Ashraf *et al*. (2008).

Eker *et al*. (2006) has reported that salinity and heavy metals stresses cause the reduction of water uptake in the root and it increases the root length and dry weight of root in plants. Furthermore, the cadmium metal increases the dry matter production in plants as observed in our study. Copper is necessary micronutrient (Maksymiec, 2007) which enhances the growth and increased the shoot length of plants while high concentration of copper has direct effect on pigments and can reduce the chlorophyll contents (Patsikka *et al*., 2002; Gupta and Abdullah, 2011). The saline stress also greatly affects the chlorophyll pigments and reduces the chlorophyll contents in plants (Hajer *et al*. 2006).

Cicek and Cakirlar (2002) has reported the reduction of relative water content under the stress condition in maize plants. So, the cadmium stress significantly decreased the relative water content in both Okmass and S2000 cultivars of maize under the study. The salinity greatly increased the K^+ and Na^+ concentration in plants and these results are in according with Cicek and Cakirlar (2002).

Conclusion

Heavy metal pollution is a big threat to plant growth and yield but in the presence of salinity, the growth behavior of plants is greatly modified. Studying the interactive effects of heavy metals and salinity can predict the growth behavior of plants in saline industrial soils. In the current study it was found that S2000 maize cultivar was more tolerant to salinity as compared to Okmass cultivar but former was more vulnerable to high copper concentrations as compared to later. Cadmium concentrations promote the growth of S2000 cultivar. So, S2000 can be successfully grown on copper free saline soils.

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