Morphophysiological characteristics of corn (*Zea mays*) affected by copper and humic acid

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**Key words:** Copper, corn, humic acid, leaf number, root dry weight, specific leaf area.

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**Abstract**

In order to investigate the phytoremediation of copper (Cu) by corn (*Zea mays*) with and without application of humic acid fertilizer, a greenhouse experiment was carried out in Faculty of Agriculture, University of Tabriz, Iran in 2013. The experiment was arranged as factorial based on randomized complete block design with four replications. The factors were soil contamination levels with copper and humic acid. Soil contamination with copper was including 0, 80, 160 and 240 mg/kg of soil and soil humic acid additive values was including 0 and 4 kg/ha. The results showed that the morphological traits, the number of leaves in 80 mg/kg copper had the highest values. The lowest corn leaf number was observed in 240 mg/kg copper without humic and the use of fertilizer reduced the toxicity of copper. In 240 mg/kg copper the greatest growth period was occurred. The maximum specific leaf area was observed in application of 240 mg/kg copper, as well as specific leaf weight in 80 mg/kg copper was the highest. The lowest root dry weight and biological yield was observed in 240 mg/kg copper without humic acid. The interaction effect of copper × humic acid was significant on corn leaf area, root dry weight and biological yield.

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Introduction
Corn (Zea mays L.) being the third most important cereal crop after wheat and rice in Iran (Amini et al., 2014). It is cultivated across a wide range of environments, from extremely stressful to favorable (Amini and Izadkhah, 2013). Progress in our civilization is invariably associated with environmental pollution. The contamination of the natural environment is mainly caused by emission of metal-bearing dusts, which are eventually deposited on the surface of earth. Other sources of trace metals are industrial waste, munici-pal sewage and wastewater, fertilizers and plant protection chemicals (Amini et al., 2009; Bowszys et. al., 2009, Amini et al., 2014).

Copper in small concentration is an essential micronutrient for all form of life such as growth, physiological process (Sharma and Madhulika, 2005), protein trafficking machinery, oxidative phosphorylation and structure element in regulatory protein (Yruela, 2005), different metabolic pathway including ATP synthesis and acting in the prothetic group of many oxidizing enzymes (Horrison et al. 1999). However, as a result of the formation of organo-copper complexes, excess copper can be consider as a toxic elements leading to reduces shoot and root growth by inhibits cell elongation and cell cycle (Ouzounidou, 1993) decrease of chlorophyll content, leaf expansion, disturbance of DNA conformation damage chromatin and the plasma causing ion efflux (Singh et al., 2007). Hansch and Mendel (2009) indicated that the excess of Cu concentrations may induce significant toxic effect by altering the protein function and enzymes activity.

Material and methods
Site description and experimental design
In order to investigate the phytoremediation of copper by corn with and without application of humic acid fertilizer, a greenhouse experiment was carried out in Faculty of Agriculture, University of Tabriz, Iran in 2013. The experiment was arranged as factorial based on randomized complete block design with four replications. The factors were soil contamination levels with copper and humic acid. Soil contamination with copper was including 0, 80, 160 and 240 mg/kg of soil and soil humic acid additive values including 0 and 4 kg/ha.

Experiment procedure
Corn seeds (double cross) were hand sown in 5 cm depth of loam soil in the plastic pots (25 cm ×35 cm). In each pot 4-5 seeds were planted and after seedling emergence, plants per pot were reduced to 1 plant /pot. The humic acid and copper at different concentrations were added to the pots based on factorial arrangements. All pots were irrigated after sowing and subsequent irrigations were applied at 7- days intervals.

Measurement of traits and statistical analysis
Corn plant height and number of leaves per plant were measured every 10 days on a regular basis and leaf area of plants at the tasseling stage was calculated and recorded according to the following equation (Keating and Wafula, 1992). Leaf area=0.75×(Length ×Wide).

In order to evaluate specific leaf area (Amanullah et...
al., 2007) and specific leaf weight (Mondal et al., 2011) the following equations was used.

\[
SLA = \frac{\text{Leaf area}}{\text{Leaf dry weight}}
\]

\[
SLW = \frac{1}{SLA} = \frac{\text{Leaf dry weight}}{\text{Leaf area}}
\]

To obtain the dry weight of roots, after plant harvest, roots were washed and then oven dried with a temperature of 80°C for 42 h and then were weighed. Additionally, the biological yield of each plant was measured and recorded after crop harvest.

Results and discussion

Table 1. Analysis of variance for different traits of corn under copper treatment and the use of humic acid.

<table>
<thead>
<tr>
<th>S.O.V</th>
<th>df</th>
<th>Number of leaves</th>
<th>Leaf area</th>
<th>Vegetative period</th>
<th>Specific area</th>
<th>Specific leaf weight</th>
<th>leaf Root dry weight</th>
<th>Biological yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>3</td>
<td>0.208 **</td>
<td>0.005 **</td>
<td>19.28 **</td>
<td>0.0001 **</td>
<td>5×10^{-6} **</td>
<td>0.47 **</td>
<td>41.7 **</td>
</tr>
<tr>
<td>Copper</td>
<td>3</td>
<td>13.54 **</td>
<td>0.047 **</td>
<td>140.03 **</td>
<td>0.014 **</td>
<td>1×10^{-5} **</td>
<td>187.7 **</td>
<td>329.6 **</td>
</tr>
<tr>
<td>Humic acid</td>
<td>1</td>
<td>0.125 **</td>
<td>9×10^{-5}</td>
<td>34.03 **</td>
<td>0.001 **</td>
<td>7×10^{-7} **</td>
<td>8.10 **</td>
<td>6.9 **</td>
</tr>
<tr>
<td>Copper * Humic</td>
<td>3</td>
<td>2.042 **</td>
<td>0.022 **</td>
<td>21.11 **</td>
<td>0.004 **</td>
<td>3×10^{-6} **</td>
<td>165.5 **</td>
<td>151.6 **</td>
</tr>
<tr>
<td>Error</td>
<td>21</td>
<td>1.065</td>
<td>0.003</td>
<td>30.01</td>
<td>0.002</td>
<td>1×10^{-9} **</td>
<td>31.0</td>
<td>41.6</td>
</tr>
<tr>
<td>C.V (%)</td>
<td>5.71</td>
<td>1.67</td>
<td>1.65</td>
<td>2.31</td>
<td>9.97</td>
<td>22.94</td>
<td>14.5</td>
<td></td>
</tr>
</tbody>
</table>

The obtained results indicate that copper levels significantly affected the corn leaf, so that highest and lowest leaf area was observed in the control treatment and at the fourth level of copper (240 mg/kg), respectively. Humic acid fertilizer had no any meaningful effect on corn leaf by itself; however, the interaction effect of these two factors on corn leaf was significant at 1% probability level (Table 1).

Manivasagaperumal et al. (2011) concluded that increase of soil copper decreased leaf area of vetch. Barbosa et al. (2013) and Vijayarengan and Deepthi-Jose (2014) showed that by increasing amount of soil copper, corn leaf declined. As seen in Figure 4 at low concentrations of copper, humic acid had no any effect on the increase in leaf area; however, at the higher level of copper concentrations, this fertilizer caused the reduction of copper toxicity and increased corn leaf. This phenomenon was probably due to the absorption of copper cation by humic acid which consequently decreased detrimental effect of copper on the crop. At the concentrations of 80 and 160 mg Cu/kg soil, humic acid had no effect on leaf area; however at the concentration of 240 mg Cu/kg soil, application of humic acid increased this trait of the crop. Application of humic acid reduced corn’s leaf area under uncontaminated (zero level of copper) conditions.

Copper effect on the vegetative growth period was significant at the 5% level. So that, with increasing
copper concentration in the soil, duration of vegetative growth increased (Figure 3). Demaria and Rivelli (2013) showed that increasing the amount of copper in the soil, increased duration of vegetative period of sunflower. Duration of vegetative growth of corn did not have any significant difference at the copper level of 80, 160 and 240 mg Cu/kg soil; however all of these treatments had a longer vegetative growth period compared to zero level. According to the observations, with increasing concentration of copper in the soil, leaf chlorophyll index and consequently crop photosynthesis decreased and these phenomena can detrimentally affect crop growth rate. Thus, prolonged vegetative growth period can be attributed to a slowdown in the crop growth rate.

According to the analysis of variance (Table 1), it was observed that the effect of copper on the specific leaf area was significant at the 1% probability level, however acid humic did not have any significant effect on this trait. BoroumandJazi et al. (2011) demonstrated that increased levels of lead in soil, increased specific leaf area of canola. The maximum specific leaf area was observed in the treatment of 428 mg Cu/kg soil (Fig. 2) which represents an increase of leaf area compared to leaf weight. The lowest value of specific leaf area was observed in 80 mg/kg which did not have any significant difference with 160 mg/kg. Increase of specific leaf area in high level of copper treatment is the result of decrease in leaf weight. It must be mentioned that leaf area is also declined; however decrease rate of leaf area and leaf weight was not equal, thus this decline was more in leaf weight than leaf area.

The highest value of specific leaf weight was recorded in 80 mg Cu/kg soil and the lowest one was observed in 240 mg Cu/kg soil (Fig. 5). BoroumandJazi et al. (2011) concluded that increased levels of lead decreased specific leaf weight of canola. Posada et al. (2012) reported that zinc and manganese stress can reduce spinach specific leaf weight. The results of specific leaf area and specific leaf weight of corn suggests that with presence of moderate (160 mg/kg) and high (240 mg/kg) levels of copper in the soil, specific leaf area and specific leaf weight, were similar to treatment with no application of copper in the soil, so that no significant differences were observed between them. However, in low levels of copper (80 mg/kg), specific leaf area and specific leaf weight had a significant difference in compare with control treatment (0 mg/kg). Therefore, corn leaves had a low value of specific leaf area and a high value of specific leaf weight (Fig. 3-5).
significant, while the interaction of these two factors on root dry weight was significant (Fig. 6). Amina and Amal (2012) reported that increase of copper level decreased dry weight of root. Same results were observed by Manivvasagaperumal et al. (2011) on vetch, Zolnowski et al. (2013) and Vijayarengan and Depthy-jose (2014) on corn, Demaria and Rivelli (2013) on sunflower. According to Figure 6 application of humic acid at 0 level of copper caused a significant decrease in root dry weight in compare with treatment that this fertilizer was not used and also it had not any significant difference with 80 mg/kg and 160 mg/kg treatments, while in 160 mg/kg treatment, application of humic acid caused a significant difference compared with no-use of this fertilizer. On the basis of these results, it suggests that under high copper contamination conditions in the soil (more than 160 mg/kg), application of 2 mg/kg humic acid have a beneficent role on corn root growth and counteraction of copper toxicity. Roots are more sensitive to copper ions than shoots and perhaps it is due to the more accumulation of these ions in the roots than in the aerial parts (Pineros et al., 1998). Thus, the greater accumulation of carbohydrates in the roots consider to the accumulation of copper in this plant part, compared with the leaf, would be predictable. Other causes of more inhibition of root growth are the root tip meristem sensitivity to heavy metals (Pineros et al., 1998) and also the effect of copper on Indoleacetic acid oxidase enzyme in the root. Copper toxicity stress first occurs in the root tip, after that growth of secondary roots would be inhibited (Coombes et al., 1976).

The effect of copper treatment on corn biological yield was significant (Table 1). Humic acid fertilizer did not have any significant effect on this trait; however the interaction effect of copper and humic acid treatments was significant at 1% probability level (Table 1, Fig. 7). Zolnowski et al., (2013) showed that copper contamination in the soil reduced biological yield of corn. Vijayarengan and Depthy-jose (2014) concluded that copper stress detrimentally affected the biological yield of mustard, corn, flax and watermelon. Figure 7 demonstrates that in the zero level of copper, corn produced more biological yield in the absence of humic acid fertilizer, however, under copper contaminated conditions, this fertilizer improved biological yield of corn. There was a trifle difference between application and non-application of humic acid fertilizer, in the 80 and 160 mg Cu/kg soil treatments. However, in the 240 mg/kg treatment, biological yield had an improved increase under application of humic acid compared to non-application of this fertilizer and this increase was not significantly different.

![Fig. 5. Corn specific leaf weight in four levels of copper.](image)

The most sensitive indicators of heavy metal stress, is the changes in the activity of antioxidant enzymes which, in turn, leads to the accumulation of ROS (Hahighi et al., 2010). The growth reduction of the crop is also attributed to the effect of copper on the cell wall. As copper binding to pectin cell wall, elasticity decreases (Aidid and Okamoto, 1993). Growth slowdown under copper stress conditions can be attributed to increase in ethylene, decrease in cytokinin and polyamines (Prasad, 1995) and inhibition of cell growth caused by the direct and indirect effects of copper on auxin metabolism or carriers of this hormone (Aidid and Okamoto, 1993). Stop in some metabolic reactions can also decrease the growth, because copper inhibition of photosynthetic enzymes such as aldolase and rubisco has an inhibitory effect on the Calvin cycle and carbon dioxide reduction. Thus, the reduction in photosynthetic efficiency reduces plant growth (Mattioni et al., 1997). Growth of plant decreases through damages to membrane lipids, chlorophyll and proteins, under oxidative stress induced by high concentrations of copper (Hagedus et al., 2001).
Copper excess by means of the effect on the absorption of other essential minerals, especially iron, potassium and calcium, inhibit plant growth. In general, high concentrations of copper as a heavy metal has important effects on plant growth and inhibits the development of various aspects of physiological, biochemical and even cell functions of the crop (Hegedus et al., 2001).

Generally we can conclude that the humic acid had different effect on corn leaf area at different copper concentrations. In the absence of copper (0 mg/kg) addition of humic acid reduced the corn leaf area significantly but in 240 mg/kg copper concentration, humic acid increased the corn leaf area. About the root dry weight and biological yield in 0 mg/kg copper the humic acid reduced these traits but in 240 mg/kg copper level the humic acid had not significant effect on them.

Fig. 6. Corn Root dry weight at different levels of copper in application and without application of humic acid.

Fig. 7. Corn biological yield at different levels of copper in application and without application of humic acid.

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