The Study Effects of Heavy Metals on Germination Characteristics and Proline Content of Triticale (*Triticoseale Wittmack*)

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ABSTRACT: In order to study, the effects of copper sulfate, cadmium nitrate and lead nitrate on germination characteristics and proline content of *Triticoseale Wittmack* an experiment in factorial-laid out complete randomized design with four replications was conducted at Seed Technology Laboratory, Faculty of Agriculture, Mashhad Branch, Islamic Azad University-Mashhad Iran, in 2014. Factors were included of heavy metals in three levels (copper sulfate, cadmium nitrate and lead nitrate) and concentrations in six levels (0, 50, 150, 300, 500, and 1000 ppm). Analysis of variance showed that the effect of heavy metal, concentration and interaction between two factors on all traits were significant at 1% probability. Means comparisons (type of heavy metal × concentration) indicated that cadmium nitrate and copper sulfate with 1000 mg/L concentration, had the highest inhibitory effect on all characteristics. So, measurement of proline content showed that the relation between proline content and heavy metal concentration (in every three metals) was linear, positive and significant. But the most accumulation of proline (3.2 µmol/gdw) observed in 1000 mg/L of cadmium nitrate.

Keywords: Cadmium nitrate, Characteristics, Copper sulfate, Germination, Lead nitrate, Proline.

INTRODUCTION

Amongst many abiotic stresses, heavy metal toxicity is very important, especially of crop species that are grown in the vicinity of sites of heavy industry, particularly in developing countries (Bi ., 2006; Ona ., 2006). Many plant species including agricultural crops can accumulate higher amounts of heavy metals than those present in the soil (Seregin and Kozhevnikova, 2008). Seedling and seed germination stage of plant life are sensitive to environmental factors such as heavy metals pollution (Abedin and Meharg, 2002). Germination inhibition is among the best-known effects of toxic impact of heavy metals (Ernst, 1998). Many plants at seed germination and seedling stages are sensitive to environmental factors. Therefore, the change of plant growth at the germination and seedling stage under heavy metal stress is often regarded as an important index to evaluate plant tolerance to heavy metals (Peralta-Videa ., 2002).

Excess copper (Cu) in soils results not only from industrial or mining activities, but also from Cu-based fungicides application (Xiong and Wang, 2005; Michaud ., 2007). Cu is an essential micronutrient for growth and development of plant and plays an important role in many biological processes such as photosynthesis, respiration and the response to oxidative stress (Burkhead ., 2009; Martínez-Peñalver ., 2012). Excessive levels of copper can cause a
range of morphological and physiological disorders. In addition, excess copper can decrease the germination rate, shoot elongation, plant biomass, and water content (Ahsan, 2007).

Cadmium (Cd) is a very toxic heavy metal which induces oxidative stress in plants (Hasan, 2009). Cd is not essential to plant growth, and it can cause various phytotoxic symptoms including leaf chlorosis, root putrescence, growth inhibition (Valentoviˇcová, 2010; Sk_rzy ska-Polit, 2010). Cd absorbed by plants accumulated in different parts of them which cause to inhibition growth and change in morphological, physiological and biochemical characteristics in plants (Benavides, 2005). Cd decreases root and shoot growth (Eshghi, 2010), by inhibition cell division and the growth of cells or both of them (Pal, 2006). It is reported that Cd inhibits plumule and radicle growth in rice (Jun-yu, 2008).

Lead (Pb), is one of the heaviest non-essential metal released into the natural environment from a range of anthropogenic activities (Ekmekçi, 2009). Sources of Pb contamination include various human activities. Despite the effort of many advanced countries to reduce emissions of Pb by using lead-free automobile fuel, the worldwide emissions of Pb continue to increase (Adriano, 2001). Pb causes retardation of plant growth and inhibition of seed germination (Iqbal and Shazia, 2004). Pb decreases germination percentage of plant, root/shoot length, tolerance index and dry mass of roots and shoots (Mishra and Choudhari, 1998). Pb has been reported to negatively affect photosynthesis, transpiration and other physiological parameters in plants (Sharma and Dubey, 2005). It has been reported that Pb induced proline accumulation and oxidative stress and reduction in chlorophyll content and plant biomass in rice plants (Zenge, 2007).

Proline is accumulated in cytosol and contributes to osmotic adjustment in response to dehydration stress and tends to maintain sufficient cell turgor for growth and avoid dehydration (Ku, 2012). Therefore, Proline protects folded protein structures against denaturation which may be caused by stress and stabilizes cell membranes (Claussen, 2005; Claussen, 2002). Proline is a compatible osmolyte and is known to accumulate in response to various kinds of abiotic stress, such as drought, salinity, high temperature, nutrient deficiency and exposure to heavy metals (Claussen, 2005; Nayyar and Walia, 2003; Unyayar, 2004). Therefore Proline accumulation, accepted as an indicator of environmental stresses (Alia-Saradhi, 1991). Plants develop certain physiological and morphological alteration for their survival in stress environmental situation like proline content has been reported to accumulate in such stress condition (El-Enany and Issa, 2001). The generation of proline is also one of the vital responses of plant under Cu toxicity, which is possibly associated with the protection of plant cells against oxidative damage and with signal transduction (Choudhary, 2007). Proline content in Cd-treated maize plants increased with increasing Cd concentration (Krantev, 2008).

Triticale (Triticosecale Wittmack) is an amphiploid cereal crop obtained by crossing wheat (Triticum sp.) and rye (Secale sp.). It is an important alternative crop due to its resistance to some diseases and tolerance to drought (Gustafson, 1991).

The present study was conducted to investigate the toxicological effects of Cd, Pb and Cu on biomass (seedlings fresh and dry weights), lengths (radicle and plumule lengths) and germination indices of Triticosecale Wittmack seedlings. Furthermore, accumulation of proline by Triticosecale Wittmack seedlings was also investigated.

**MATERIALS AND METHODS**

**Experimental Design**

This research was conducted in Seed Technology Laboratory, Faculty of Agriculture, Islamic Azad University, Mashhad Branch, in 2014. The experiment arranged in factorial laid out in completely randomized design with four replications. Factors including: heavy metals in three levels (copper sulfate, cadmium nitrate and lead nitrate) and concentration in six levels (0, 50, 150, 300, 500 and 1000 mg. L$^{-1}$). Triticale seeds have been provided from seed production and distribution centers. Seeds were disinfected with sodium hypochlorite 10% for 3 min and rinsed with distilled water.

25 seeds placed in each petri dish on filter paper. Then 7 ml of the prepared solution was added to each petri dish. All petri dishes were placed in germinator at 25°C.

Germination was continued for 8 days and germinated seeds were counted on a daily. Seeds were considered germinated when their radical length was 2 mm.

After 10 days, traits such as: percentage and rate of germination, seedling wet and dry weight, radical and plumule length, seed vigor and proline content were measured.
Germination Percentage (%)

Germinated seeds were counted daily according to the seedling evaluation procedure in the handbook of Association of Official Seed Analysts. The number of germinated seeds was recorded every 24 h (AOSA, 1990). Eight days after germination, the germination percentage (GP) was calculated using the formula below for each replication of the treatment:

\[
GP = \frac{\text{Germinated seed}}{\text{Total seed}} \times 100
\]

Germination Rate (seedling/day)

Germination rate (GR) was calculated according to the formula of McGuire (1962) as follows:

\[
GR = \frac{\text{Normal seedlings}}{\text{Days to first count}} + \frac{\text{Normal seedlings}}{\text{Days to final count}}
\]

Vigor Index

Vigor index (VI) was calculated by using the formula of Baki and Anderson (1973):

\[
VI = [\text{MPL} + \text{MRL}] \times GP
\]

Where, MPL is mean plumule length (cm), MRL is mean radicle length (cm) and GP is germination percentage (%).

Plumule and Radicle Length (cm)

Plumule and radicle parts of the seeds in petri dishes were separated after germination, and measured in centimeters from the point where the radicle and plumule joins together at the end of the radicle and to the top of the plumule.

Seedling Dry Weight (g)

Dry weight was also evaluated after drying the specimens (10 seedlings) for 72 hours at 76 °C.

Estimation of Proline Content

Free proline contents were measured according to the method of Bates., (1973), the required solutions such as: phosphoric acid (6M), sulphosalicylic acid (3%), ninhydrin solution and toluene were prepared. All solutions were stored on 4°C until proline measurement. Then 10 ml of sulphosalicylic acid (3%) was added to 0.05g of dry powder of each sample and dry leaves were homogenized in 10 ml of aqueous sulphosalicylic acid (3%), then homogenate was centrifuged for 20 min, at 4000 rpm. And after 24 hours it was filtered with using filter paper. 2 ml of this solution was poured into the test tube and 2 ml of ninhydrin solution and 2 ml of acetic acid (glacial) was added. The test tubes were placed in boiling water bath (100°C) for one hour, until color fixed. Then they were placed in cold water bath for 15 minute. After, the test tubes were cooled completely, 4 ml of toluene was added. The test tubes were shaken. With this, solution became two phase. The solution of color phase, containing Toluene. This solution was used to measure proline with Spectrophotometer at 520 nm wavelength.

Statistical Analysis

A completely randomized experimental design (CRD) with four replicates was involved. Data were subjected to analysis of variance using the Statistical Analysis System (SAS) software (SAS, 1997) and means comparison was performed by Duncan’s multiple range test at 5% probability.

RESULTS AND DISCUSSION

RESULTS

Analysis of variance and means comparison shown in tables 1, 2, 3 and 4. Heavy metal, concentration and interaction between two factors had significant effect on all characteristics at 1% probability (table 1).
Germination Percentage, Germination Rate, Radicle Length and Plumule Length:

The evaluation of interaction between type of heavy metal and concentration, showed that cadmium nitrate and copper sulfate with 1000 mg.L\(^{-1}\) concentration, had the highest inhibitory effect on germination percentage, germination rate, radicle length and plumule length.

In these treatment combinations, germination percentage were 46% and 48%, germination rate 4.6 and 5.1 seedling/day, radicle length 0.19 and 0.29 cm and plumule length 2.43 and 2.79 cm respectively (table 4). There were significant difference between those with the other treatment combination.

These results indicated that with increasing of heavy metal concentration, decreased mentioned characteristics. But this decreasing due to lead nitrate was very less in comparison with cadmium nitrate and copper sulfate.

Seedling Wet and Dry Weight:

The results indicated that the highest inhibitor effect on seedling wet and dry weight was due to cadmium nitrate with 1000 mg.L\(^{-1}\) concentration (table 4). In this treatment combination, seedling wet and dry weight were 0.54 and 0.049 g respectively. Based on these results decreasing in seedling wet and dry weight due to cadmium nitrate and then copper sulfate were more than lead nitrate (table 4).

Vigor Index:

Means comparisons showed that with increasing of concentration of every three heavy metals, vigor index decreased. This decreasing due to cadmium nitrate and then copper sulfate in comparison with lead nitrate was more severe (table 4).

Proline Content:

Measurement of proline content showed that, the relation between proline content and heavy metal concentration in every three metals, was linear, positive and significant. Thus the proline content increased with increasing concentrations of heavy metals. The most accumulation of proline (3.2 µmol/gdw) observed in 1000 mg.L\(^{-1}\) of cadmium nitrate. Also, proline content in copper sulfate and lead nitrate treatments was in about 2.2 and 1.9 µmol/gdw respectively (Figure 1).

Table 1. Analysis of variance of the measured traits

<table>
<thead>
<tr>
<th>S.O.V</th>
<th>df</th>
<th>M.S Germination percentage</th>
<th>Germination Rate</th>
<th>Radicle Length</th>
<th>Plumule Length</th>
<th>Wet Weight</th>
<th>Dry Weight</th>
<th>Vigor Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy metal(A)</td>
<td>2</td>
<td>674.88**</td>
<td>122.65**</td>
<td>313.98**</td>
<td>162.05**</td>
<td>0.806**</td>
<td>0.0081**</td>
<td>945619.35**</td>
</tr>
<tr>
<td>Concentration(B)</td>
<td>5</td>
<td>2462.62**</td>
<td>264.59**</td>
<td>191.69**</td>
<td>110.18**</td>
<td>0.678**</td>
<td>0.0067**</td>
<td>6491749.41**</td>
</tr>
<tr>
<td>A x B</td>
<td>10</td>
<td>536.75**</td>
<td>50.64**</td>
<td>19.11**</td>
<td>14.57**</td>
<td>0.0571**</td>
<td>0.00057**</td>
<td>682661.28**</td>
</tr>
<tr>
<td>Error</td>
<td>54</td>
<td>10.44</td>
<td>0.618</td>
<td>0.046</td>
<td>0.088</td>
<td>0.00038</td>
<td>0.000037</td>
<td>1669.13</td>
</tr>
</tbody>
</table>

ns, * ** non significant, significant at 5% and 1% probability, respectively.

Table 2. Effect of heavy metals on germination percentage, germination rate, radicle and plumule length, wet weight, dry weight and vigor index

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Germination percentage</th>
<th>Germination Rate</th>
<th>Radicle Length(cm)</th>
<th>Plumule Length(cm)</th>
<th>Wet Weight(g)</th>
<th>Dry Weight(g)</th>
<th>Vigor Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper sulfate</td>
<td>90b</td>
<td>17.9b</td>
<td>5.8b</td>
<td>8.2b</td>
<td>0.96b</td>
<td>0.09b</td>
<td>1373 b</td>
</tr>
<tr>
<td>Cadmium nitrate</td>
<td>89 b</td>
<td>17.4c</td>
<td>5.5c</td>
<td>7.7c</td>
<td>0.91c</td>
<td>0.08c</td>
<td>1292 c</td>
</tr>
<tr>
<td>Lead nitrate</td>
<td>98a</td>
<td>21.5a</td>
<td>11.9a</td>
<td>12.4a</td>
<td>1.25a</td>
<td>0.12a</td>
<td>2417 a</td>
</tr>
</tbody>
</table>

Means with similar letters in each column are not significant at the 5% level of probability.

Table 3. Effect of concentration of heavy metal on germination percentage, germination rate, radicle and plumule length, wet weight, dry weight and vigor index
Means with similar letters in each column are not significant at the 5% level of probability.

Table 4. Effect of heavy metal and concentration of heavy metal on germination percentage, germination rate, radicle and plumule length, wet weight, dry weight and vigor index

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Concentration (mg/lit)</th>
<th>Germination percentage</th>
<th>Germination Rate</th>
<th>Radicle length (cm)</th>
<th>Plumule length (cm)</th>
<th>Wet weigh (g)</th>
<th>Dry weigh (g)</th>
<th>Vigor Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper sulfate</td>
<td>0</td>
<td>100a</td>
<td>22.46a</td>
<td>14.2a</td>
<td>13.5a</td>
<td>1.39a</td>
<td>0.13a</td>
<td>2773a</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>100a</td>
<td>22.0b</td>
<td>10.5b</td>
<td>12.1b</td>
<td>1.23b</td>
<td>0.12b</td>
<td>2270b</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>99a</td>
<td>21.2c</td>
<td>7.8c</td>
<td>10.0c</td>
<td>1.06c</td>
<td>0.10c</td>
<td>1759c</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>98ab</td>
<td>20.3d</td>
<td>5.8d</td>
<td>8.7d</td>
<td>0.95d</td>
<td>0.09d</td>
<td>1425d</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>95b</td>
<td>17.3e</td>
<td>4.9e</td>
<td>7.1e</td>
<td>0.87e</td>
<td>0.08e</td>
<td>1171e</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>63b</td>
<td>10.1f</td>
<td>3.4f</td>
<td>5.4f</td>
<td>0.75f</td>
<td>0.07f</td>
<td>767f</td>
</tr>
<tr>
<td>Cadmium nitrate</td>
<td>0</td>
<td>100a</td>
<td>22.87a</td>
<td>14.27a</td>
<td>13.43a</td>
<td>1.39a</td>
<td>0.135a</td>
<td>2770a</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>100a</td>
<td>22.02a</td>
<td>8.97g</td>
<td>11.49ef</td>
<td>1.14f</td>
<td>0.109f</td>
<td>2074g</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>99a</td>
<td>20.75de</td>
<td>5.17i</td>
<td>8.47g</td>
<td>0.92h</td>
<td>0.087h</td>
<td>1351l</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>98a</td>
<td>17.3e</td>
<td>4.9e</td>
<td>7.1e</td>
<td>0.87e</td>
<td>0.08e</td>
<td>1171e</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>95a</td>
<td>16.36g</td>
<td>1.88l</td>
<td>5.62i</td>
<td>0.75k</td>
<td>0.070k</td>
<td>713l</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>48b</td>
<td>5.14i</td>
<td>0.29m</td>
<td>2.79k</td>
<td>0.58m</td>
<td>0.053m</td>
<td>147n</td>
</tr>
<tr>
<td>Lead nitrate</td>
<td>0</td>
<td>100a</td>
<td>22.74ab</td>
<td>14.26a</td>
<td>13.52a</td>
<td>1.39a</td>
<td>0.135a</td>
<td>2778a</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>100a</td>
<td>22.06a-d</td>
<td>12.93b</td>
<td>13.16ab</td>
<td>1.35b</td>
<td>0.130b</td>
<td>2609b</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>99a</td>
<td>21.52a-e</td>
<td>12.20c</td>
<td>12.75bc</td>
<td>1.25c</td>
<td>0.121c</td>
<td>2470c</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>98a</td>
<td>21.29b-e</td>
<td>11.57d</td>
<td>12.32cd</td>
<td>1.21d</td>
<td>0.117d</td>
<td>2366d</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>98a</td>
<td>21.10c-e</td>
<td>10.98e</td>
<td>11.98de</td>
<td>1.18e</td>
<td>0.113e</td>
<td>2250e</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>97a</td>
<td>20.59de</td>
<td>9.88f</td>
<td>11.09f</td>
<td>1.13f</td>
<td>0.108f</td>
<td>2033g</td>
</tr>
</tbody>
</table>

Means with similar letters in each column are not significant at the 5% level of probability.

DISCUSSION

Effect of Cu, Cd and Pb on Germination and Early Seedling Growth:

Germination and early seedling development assay has been regarded as a basic experiment for evaluating the toxicity effect of any metal or chemical type on plants (Ahsan ., 2007; Wang and Zhou, 2005). Metal toxicity is an important factor governing germination and growth of plants. Seedling growth is considered as an indicator of metal stress on plant ability to survive. Under heavy metal stress, the processes of germination, like embryo growth, will be
Effect of Cu, Cd and Pb on Proline content:

As shown in figure 1, increasing the concentrations of Cu, Cd, and Pb in the growth medium resulted in a pronounced increase in proline. In many plants, unfavorable environmental effects bring about the accumulation of proline, which is, by itself, one of the most universal poly-functional stress-protective substances (Ashraf and Foolad, 2007). Proline is known to accumulate under heavy metal exposure and is considered to be involved in the particular stress resistance (Chen., 2004; Gouia., 2003). The proline accumulation in Cd- treated seedlings can be regarded as one of the most sensitive responses to water deficiency and osmotic stress (Ashraf and Harris, 2004). The capability of plants for a heavy-metal induced proline accumulation could be brought about not only by a direct effect of Cd ions, but also by water deficiency (Shevyakova., 2003). This deficiency develops in the plant tissues under the conditions of heavy metals stress due to damage to the water-absorbing capacity of roots. It has been often suggested that proline accumulation may contribute to osmotic adjustment at the cellular level and enzyme protection stabilizing the structure of macromolecules and organelles. Besides its putative impact on osmotic adjustment processes, Proline was shown to protect enzymes and cellular structures against heavy metal damages as a consequence of the formation of Cd-Proline complexes (Sharma and Dietz, 2006) or against maintenance of the glutathione redox state, thus indirectly acting as an antioxidant (Siripornadulsil., 2002). Proline accumulation in shoots of Brassica. juncea, Triticum aestivum and Vigna radiata in response to Cd^{2+} toxicity has been demonstrated...
REFERENCES


