Ameliorative role of *Trichoderma* fungi on cadmium and copper toxicity in wheat (*Triticum aestivum* L)

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**Key words:** Cadmium, copper, germination, Trichoderma, wheat.

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**Abstract**

Soil contamination with heavy metals may threaten the sustainability of crop production, food safety and human health. It has been well documented that the use of microorganisms such as *Trichoderma* spp. can significantly ameliorate the heavy metals toxicity in soils and plants. This study investigated the efficiency of Trichoderma application on optimizing wheat (Morvarid cultivar) germination parameters under cadmium (Cd) and copper (Cu) nitrate toxicity at four levels (0, 50, 100 and 150 mg L⁻¹). Through a completely randomized design with four replications using a factorial experiment. Some germination parameters such as germination percentage and germination rate, seed vigor index, germination index, seedling length, allometric coefficient (shoot to root ratio), fresh and dry weight of seedling were determined. The Results revealed the significant effect of Trichoderma on germination percentage (up to 10%), root length (up to 10%), seedling length (up to 15%), fresh and dry weights of seedling (nearly 12 and 11%, respectively). The application of Trichoderma in Cd contaminated treatments (up to 100 mg L⁻¹) markedly improved wheat germination index (16, 10 and 12% respectively) and seedling length (5, 26 and 0.5% respectively). On the other hand, increasing the amount of Cu up to 50 mg L⁻¹ in Trichoderma inoculation treatment resulted in higher germination index (20% compared to non-inoculated seeds) and seedling length (30% more than non-inoculated seeds). In conclusion, *Trichoderma longibrachiatum* could increase seedlings’ chance of surviving under heavy metals’ toxicity.

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Introduction

In recent years, contaminations of soils and plants with toxic heavy metals such as cadmium, copper and nickel have increasingly constrained the production of sustainable crops (Mehmet et al., 2008; Houshmandfar and Moraghebi, 2011). The application of sewage sludge and municipal waste along with Cd-containing fertilizers cause the increase of Cd content in soils. Cadmium (Cd) is a toxic heavy metal which is easily taken up by plants. It inhibits root and shoot growth, affects nutrient uptake, enters into the food chain and causes serious health problems (Dong et al., 2005; Dell’Amico et al., 2008). Cadmium stress in plants leads to a series of stress symptoms that includes chlorosis, necrotic lesions, wilting, disturbances in mineral nutrition and carbohydrate metabolism, which as a result may strongly reduce biomass production (Azevedo et al., 2005; Santos et al., 2010). The responses of plants to heavy metal stress, however, depend on the growth stages at which they were exposed to the stress factor.

It is well documented that different levels of Cd can inhibit the root and shoot growth of different plant species such as Brassica juncea (0.06 mg L⁻¹), Phaseolus vulgaris (0.6 mg L⁻¹) and Avicennia marina (Zhang et al., 2013). Cd⁺⁺ toxicity, for instance, not only does reduce root and shoot growth but also sometimes associates with chlorosis and root damage (Abu Muriefah, 2008). Photosynthesis and Fv/Fm reduction are common responses in plants exposed to Cd such as lactuca sativa (Burzynski and Klobus, 2004; Celeste Dias et al., 2013) and Cucumis sativus L. (Feng et al., 2010), chlorophyll content reduction in barley (Vassilev et al., 2002), tomato (Ammar et al., 2008; Lopez Millan et al., 2009), maize (Ekmekc et al., 2008), mustard (Mobin and Khan, 2007) and garden cress (Gill et al., 2012). On the other hand, copper (Cu) is an abundantly found element in soil and is considered as an essential element for all living organisms including plants. It is one of the common heavy metals in industrial discharge of aeronautic, metal and metallurgy and refinery industries and shows toxic effects on plants and animals (Houshmandfar and Moraghebi, 2011; Singh et al., 2007). In recent years, bioremediation composed of phytoremediation and biosorption was introduced as an innovative heavy metal removal process (Vankar and Bajpai, 2008; Hoodchi and Jalalian, 2004; Parsadoost et al., 2007; Rezvani et al., 2007; Zafar et al., 2007; Sarkar et al., 2010). This method is an effective and efficient way to restore and remediate contaminated soils (Cao et al., 2008). The biosorption process has been studied extensively using microbial biomass as a biosorbent for heavy metal removal (Zafar et al., 2007; Yien Ting and Choong, 2009; Shams Khorraramabadi et al., 2010; Vankar and Bajpai, 2008). Various types of microbial biomasses including both heterotrophs (bacteria and fungi) and photoautotrophs (algae and cyanobacteria) are reported to possess metal-binding properties and have been studied as potential biosorbents for selected metals (Akhtar et al., 2007; Yien Ting and Choong, 2009; Hoodchi and Jalalian, 2004; Parsadoost et al., 2007; Rezavani et al., 2007; Zafar et al., 2007; Sarkar et al., 2010).

Fungi are known to tolerate and detoxify metals by several mechanisms; including valence transformation, extra and intracellular precipitation, and active uptake. Fungal strains grouped in the genus Trichoderma possess a wide spectrum of evolutionary responses that range from very effective soil colonization, with high biodegradation potential, to non-strict plant symbiosis (Cao et al., 2008). Trichoderma spp. are fungi that are present in nearly all soils and plant roots (Vankar and Bajpai, 2008). Trichoderma is a heavy metal-tolerant and plant growth promoting fungus for remediation and bioenergy crop production on polluted soils (Babu et al., 2014). Some mycoparasitic Trichoderma strains can tolerate more than one type of metal. The use of Trichoderma-plant association may be applicable for remediating sites with multiple contaminants (Cao et al., 2008). As they are the most common cultivable fungi, they are easily propagated and can be used as biosorbents for some metals (Wang and Zhou, 2005; Anand et al., 2006; Akhtar et al., 2007; Wang et al., 2009; Vankar and Bajpai, 2008).

Seed is a stage in the plant life cycle that is well
protected against various stresses. However, soon after imbibition and subsequent vegetative developmental processes, they become stress sensitive in general (Weiqiang et al., 2005). Seed germination and seedling growth and development are important stages of entire plant life and the most sensitive stages to environmental changes (Saberi et al., 2010). Plants susceptibility and toxicity to heavy metals are not only affected by concentration, but also by their toxicity. However, it depends on plant growth and developmental stages. Seed germination, as one of the most critical stages of plant physiology, is regulated by the interaction between several hormones and environmental factors. It is one of the most susceptible stages to the contamination of heavy metals due to insufficient defensive mechanisms (Ahsan et al., 2007). Hence, the presence of heavy metals in rhizosphere area and their entrance into plant reduce growth and violate cell metabolism (Alipour Darvari et al., 2009). Based on the above mentioned reasons, this study aims to investigate the effectiveness of Trichoderma longibrachiatum on boosting wheat seed germination and growth under Cd and Cu contamination in order to analyze the potentiality of microorganism as biofactors to alleviate alleviating heavy metals absorption and to increase plant growth.

Materials and methods
The study was conducted at Sari Agricultural Sciences and Natural Resources University in 2010. Treatments were arranged in a factorial experiment based on a completely randomized design in four replications.

Trichoderma preparation
The first under investigation factor was Trichoderma longibrachiatum (including inoculated seeds or non-inoculated seeds), the second and third factors were cadmium and copper nitrate at four levels (0, 50, 100 and 150 mg L⁻¹). Trichoderma longibrachiatum was prepared from live fungi at Mycology Lab of Sari Agricultural Sciences and Natural Resources University. PDA medium (potato extract, dextrose and agar) was kept for a week at 25°C to fungal strain propagates. The used medium was previously sterilized in autoclave for 30 minutes (Yazdani et al., 2008).

Seed preparation
Wheat seeds, Morvarid cultivar, were collected from Mazandaran Agricultural Research and Natural Resources Center. First, healthy and uniform seeds were separated from rogues and infertile ones. In each treatment, 20 seeds were disinfected in hypochlorite sodium 0.5% for 5 min and then they were washed with distilled water for three times (Singh et al., 2007). After preparing fungus spore suspension with spore concentration 10⁸ per mL water, the disinfected wheat seeds were placed in the suspension for 6 h to inoculate with fungus. 20 seeds from inoculated seeds with Trichoderma were put inside petri dish (3 cm diameter and 1.5 cm height) containing one 42 Watman paper (each petri dish was regarded as a replication). Then 10 mL of cadmium and copper nitrate solution at certain concentrations was poured to each of the petri dishes for every treatment while distilled water was used for control treatment. After that, petri dishes were put in germinator (Iran Khodsaz firm IKH.RH Model, Iran) at the fixed temperature of 20°C for 10 days. Germinated seeds were counted daily on a specific time until the tenth day. During this time, the seeds with 2 mm or higher root length were considered the germinated ones (Zeinali et al., 2002).

Germination parameters
Germination index, germination speed, germination percentage, seed vigor index, root length, allometric coefficient i.e. shoot length to root ratio (Naderi Fasarani et al., 2009) and wet and dry weights of seedlings were measured.

Germination Index (GI) = \[\frac{\sum T_i N_i}{S}\] [equation 1]

Where Ti stands for days after culture (post-culture days), Ni for the germinated seeds number at day i and S for total seeds number. The low value of this index usually indicates shorter germination period (Shariat and Asareh, 2006).

Germination speed (GS) = \[\sum \frac{n_i}{D_i}\] [equation 2]
Germination percent (GP) = \( \frac{G}{N} \times 100 \) [equation 3]

Where G is the final germinated seeds number and N is the total seeds number (Mukhtar, 2008).

Seed vigour Index = \( \frac{\text{seedling length + germination percent}}{100} \) [equation 4]

(Alizadeh and Isvand, 2004)

Seeding length (mm) = shoot + root length (Saberi et al., 2010).

Seed germination percentage and similar parameters were calculated by transforming data and normality test was done using Kolmogorov-Smirnov test. All statistical analysis were performed using SAS software (version 9.1) to arrive at the results where significant differences were observed, least significant difference (LSD) test was performed.

Results and discussion

The growth of plant in terms of germination speed, germination percentage, germination index, shoot length, root length, seedling length, allometric coefficient, and seed vigor index decreased in the presence of cadmium (Cd) in the nutrient medium (Table 1 and 2). By contrast, shoot to root ratio increased markedly by adding Cd to medium. Meanwhile, adding Cu affects all germination attributes except germination percentage as well as wet and dry weights (Table 1 and 2). The application of *T. longibrachiatum* favored the growth of plant by alleviating the toxic effects generated by cadmium that reflected as in increase of germination index and seedling length. Inoculation wheat seeds with Tricoderma alone also led to a slight increase of some characteristics as compared to those of control (germination percentage, root length, seedling length, wet and dry weights).

Application of *T. longibrachiatum* had significant effect on characteristics of germination index and seedling length not only in Cd treatments but also in Cu treatments. The results of interaction between *T. longibrachiatum* and Cd, *T. longibrachiatum* and Cu and triple interactions between *T. longibrachiatum*, Cd and Cu are shown in Table 1 and 3. As it is observed in the tables, contamination use of Cu and Cd had significant and synergistic effect on root length, shoot length, seedling length, germination index and index of seed vigour.

Shoot and root length and shoot to root ratio

The Seeds which were inoculated with *T. longibrachiatum* showed an increase in root length by 10 percent (data not shown) compared with non-inoculated seeds. By contrast, seed inoculating had no effect on shoot length (Table 1).

The results of simultaneous application of Cd and Cu (Fig 1A and B) showed that root and shoot length decreased, as expected, by increasing concentration of heavy metals in plant growth conditions, as longer roots were observed in the control treatment and the shortest ones (90 times lower than control) were

**Table 1.** Mean squares by ANOVA analysis for wheat germination and growth attributes.

<table>
<thead>
<tr>
<th>S.O.V</th>
<th>df</th>
<th>Germination speed</th>
<th>Germination percentage</th>
<th>Germination index</th>
<th>Shoot length</th>
<th>Root length</th>
<th>Seedling length</th>
<th>Allometric coefficient</th>
<th>Wet seedling</th>
<th>Dry seedling</th>
<th>Seed vigor index</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>0.145**</td>
<td>0.911**</td>
<td>0.27**</td>
<td>0.0003**</td>
<td>24.273**</td>
<td>0.22**</td>
<td>0.021**</td>
<td>1966.95**</td>
<td>2.27**</td>
<td>0.11**</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>21.253</td>
<td>0.204</td>
<td>2.61</td>
<td>1.732**</td>
<td>1656.69**</td>
<td>0.460**</td>
<td>2.304**</td>
<td>3348.06**</td>
<td>0.34**</td>
<td>115.00**</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>26.915**</td>
<td>0.167**</td>
<td>3.01**</td>
<td>1.886**</td>
<td>1684.75**</td>
<td>0.306**</td>
<td>2.703**</td>
<td>2361.16**</td>
<td>0.86**</td>
<td>34.57**</td>
</tr>
<tr>
<td>AxB</td>
<td>3</td>
<td>17.325</td>
<td>0.042**</td>
<td>2.04**</td>
<td>0.0182**</td>
<td>163.796**</td>
<td>0.009**</td>
<td>0.203**</td>
<td>2899.17**</td>
<td>0.27**</td>
<td>0.74**</td>
</tr>
<tr>
<td>AxC</td>
<td>3</td>
<td>2.382**</td>
<td>0.003**</td>
<td>0.88**</td>
<td>0.0479**</td>
<td>163.420**</td>
<td>0.002**</td>
<td>0.022**</td>
<td>3541.36**</td>
<td>0.33**</td>
<td>0.86**</td>
</tr>
<tr>
<td>BxC</td>
<td>9</td>
<td>4.731**</td>
<td>0.064**</td>
<td>0.70**</td>
<td>0.1435**</td>
<td>788.193**</td>
<td>0.056**</td>
<td>0.307**</td>
<td>869.65**</td>
<td>0.10**</td>
<td>15.86**</td>
</tr>
<tr>
<td>AxBxC</td>
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<td>1.772**</td>
<td>0.400**</td>
<td>3.39**</td>
<td>0.0189**</td>
<td>446.211**</td>
<td>0.009**</td>
<td>0.285**</td>
<td>783.84**</td>
<td>0.05**</td>
<td>1.77**</td>
</tr>
</tbody>
</table>

A: *Trichoderma*  B: Cd  C: Cu

ns, * and **: non-significant, significant at 5% and 1% level of probability, respectively.
observed in concentration of 150 mgL\(^{-1}\) of the heavy metals. Moreover, the highest shoot length was observed in the control treatment. However, the highest shoot length which was obtained in control treatment was not statistically different with the application of 50 mg L\(^{-1}\) Cd in the levels of 0 and 50 mg L\(^{-1}\) Cu. The shortest shoot length (100% reduction compared to control) was obtained in the treatment of 150 mg L\(^{-1}\) Cd in non-polluted Cu treatment which had no statistically significant difference with the level of 150 mg L\(^{-1}\) Cu (Fig 1A). Shoot to root ratio increased (91% and 1.2 more than control) by increasing the concentration of Cd and Cu (Table 2) due to the inhibitory effects of these heavy metals on root length. Kiran and Sahin (2006) reported that increasing the concentration of Cd would significantly decrease root length in lentil. Farooqi et al., (2009) stated that the root and shoot length and root to shoot rate in Albizia lebbeck (L.) decreased by increasing the concentration of cadmium.

**Table 2.** Simple effects of treatments on some germination attributes in wheat.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Germination speed</th>
<th>Germination percent</th>
<th>Alometric coefficient</th>
<th>Wet weight seedling (mg)</th>
<th>Dry weight seedling (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichoderma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-inoculate</td>
<td>9.40a</td>
<td>78.51b</td>
<td>4.14a</td>
<td>194.45b</td>
<td>21.55b</td>
</tr>
<tr>
<td>inoculate</td>
<td>8.08a</td>
<td>85.70a</td>
<td>3.73a</td>
<td>219.20a</td>
<td>24.04a</td>
</tr>
<tr>
<td>Cd</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>9.44ab</td>
<td>83.75ab</td>
<td>2.99b</td>
<td>207.59ab</td>
<td>21.79b</td>
</tr>
<tr>
<td>50</td>
<td>10.22a</td>
<td>87.18a</td>
<td>2.52b</td>
<td>224.31a</td>
<td>22.16ab</td>
</tr>
<tr>
<td>100</td>
<td>8.98bc</td>
<td>80.46b</td>
<td>4.53a</td>
<td>200.01b</td>
<td>23.24ab</td>
</tr>
<tr>
<td>150</td>
<td>7.88c</td>
<td>77.03b</td>
<td>5.72a</td>
<td>195.43b</td>
<td>24.00a</td>
</tr>
<tr>
<td>Cu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>9.26ab</td>
<td>81.09ab</td>
<td>2.73c</td>
<td>207.51a</td>
<td>22.67a</td>
</tr>
<tr>
<td>50</td>
<td>10.29a</td>
<td>87.18a</td>
<td>2.30c</td>
<td>216.86a</td>
<td>21.90a</td>
</tr>
<tr>
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<td>81.56ab</td>
<td>4.54b</td>
<td>207.09a</td>
<td>23.40a</td>
</tr>
<tr>
<td>150</td>
<td>7.84c</td>
<td>78.59b</td>
<td>6.18a</td>
<td>195.87a</td>
<td>22.22a</td>
</tr>
</tbody>
</table>

Average in each column and each treatment with the same letter or letters are not significantly different at 5% level according to LSD test.

According to Munzuroglu and Kirbag Zengin’s (2006) findings, cadmium inhibited root and shoot growth in barley at both concentration levels (0.5 and 10 mM) while the inhibitory effect of cadmium affects root more than shoot at low concentrations. Ahsan et al., (2007) reported the reduction of shoot length, plant biomass and water content in rice as a result of Cu pollution. Moreover, root formation was not observed in concentration of 0.5 mM and shoot formation in higher concentration of 1.5 mM. Application of Trichoderma can reduce these negative effects. It may be due to enhanced nutrient uptake, enhanced root development and increased root hair formation during seedling growth and increase in hormone production (Kaveh et al., 2011).

**Seedling length, wet and dry weight of seedling**

The maximum seedling length was observed in the treatment of Trichoderma with 50 mg L\(^{-1}\) Cd (26% increase compared to the non-inoculated treatment), while in non-inoculated treatment with the level of 150 mg L\(^{-1}\) Cd, seedling length showed the lowest amount (14% compared to inoculated seeds with Trichoderma). On the other hand, in the treatment of 50 mg L\(^{-1}\) Cu with Trichoderma, seedling length had the highest amount (30% compare to non-inoculated).
While, increase in concentrations of Cd and Cu significantly affected seedling length (2 times lower than control) in simultaneous application of Cd and Cu (Fig 1E), which is consistent with the results of Saberi et al’s., (2010) studies.

Table 3. Interaction effects of Trichoderma, cadmium and copper on Germination index and Shoot, Root and Seedling length.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Germination index</th>
<th>Shoot length (mm)</th>
<th>Root length (mm)</th>
<th>Seedling length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trichoderma Cd</strong></td>
<td></td>
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</tr>
<tr>
<td>0</td>
<td>2.37b</td>
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<td>93.77abc</td>
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<tr>
<td>50</td>
<td>2.37b</td>
<td>74.88b</td>
<td>103.33ab</td>
<td>151.90c</td>
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<td></td>
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<td></td>
</tr>
<tr>
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<td>2.52ab</td>
<td>25.69g</td>
<td>78.27cd</td>
<td>97.36f</td>
</tr>
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<td>22.83h</td>
<td>67.55cd</td>
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<tr>
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<tr>
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<td></td>
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</tr>
<tr>
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<td>2.79a</td>
<td>18.55h</td>
<td>83.08a</td>
<td>93.10g</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different at (p≤0.05) according to LSD test.

In this study, wet and dry weights of seedlings were not significantly affected by Cd and Cu. This result could be due to the ability of Trichoderma in alleviating the toxic effect of heavy metals, as the highest wet and dry weights were observed in Trichoderma treatment (12 and 11 percents more than the control, respectively). Ali Khan et al., (2005) showed that wet and dry weights of seedlings increased in rice seeds which were treated with Trichoderma. In Bal and Altintas (2008) experiments, wet weight of lettuce seedling increased by the application of T. harzianum. However, reduction of wet and dry weights and length of seedling were observed in Lebbek tree (Farooqi et al., 2009), radish (Alipour Darvari et al., 2009), rice (Ahsan et al., 2007), wheat (Singh et al., 2007) and Prosopis juliflora (Victor et al., 2007) which were exposed to heavy metals such as Cu and Cd. Furthermore, Houshmandfar and Moraghebi (2011) stated that reduction occurs in wet and dry weight of safflower seedling by increasing Cd and Cu concentrations.

It is known that seedling growth is more sensitive to heavy metals compared to seed germination. Moreover, Cu caused reduction in germination but Cd
inhibited the process of germination as well as seedling growth. Using Trichoderma as a tool for an increase in potentiality of roots could help seedling to overcome these problem. More dry weight produced during seedling growth period in Trichoderma treatments may also be a beneficial point.

Fig. 1. Interaction effects between Cu and Cd (mg L\(^{-1}\)) on (A) shoot length (B) root length (C) germination index (D) seed vigor and (E) seedling length.

Germination percentage and speed

150 mg L\(^{-1}\) Cd and Cu was the most effective level in the reduction of germination speed (15 and 16 percents lower than control, respectively) which had no significant difference with the level of 100 mg L\(^{-1}\) Cd. The highest germination speed obtained when 50 mg L\(^{-1}\) level of Cu and Cd were used. However, it was not significantly different from control. It shows that wheat seed germination speed is not sensitive to the toxicity of Cd and Cu. Nevertheless, Munzuroglu and Kirbag Zengin (2006) reported a reduction in barley germination speed as a result of increasing the concentration of Cd. Similar results have been reported by Farooqi et al., (2009) findings in Lebbek tree and Kiran and Sahin (2006) in lentil plant under Cd toxicity. In a study by Ahsan et al., (2007) Cu contamination reduced germination speed on rice. Liu et al., (2009) observed that low concentrations of Cu increased the germination speed of Chinese cabbage, while it had an inhibitory effect in high levels.

Trichoderma increased germination percentage (about 10 percent of control) (Table 2), while increasing the concentration of Cd would reduce seed germination percentage by 8 percents compared to the control. Research on Abelmochus esculentus L. (Mukhtar, 2008) and tomato seed (Mastouri et al., 2010) treated with T. harzianum demonstrated on increase in germination percentage and germination speed. Alipour Darvari et al., (2009) reported that germination percentage of radish seeds which were exposed to different levels of Cd did not show significant differences compared to the control treatment. However, Kiran and Sahin (2006) confirmed that increasing the concentration of Cd would decrease germination percentage in Lentil plant. Saberi et al., (2010) expressed that application of copper sulfate had no effect on Atriplex lentiformis germination percentage. Mahmood et al., (2005) showed that corn germination percentage was fixed in concentration of 12-13 mg Cu. While, it was reduced with an increase in concentration of Cu from 10 to 640 ppm in the mesquite plant. (Victor et al., 2007). Further studies regarding, in tomato (Jaja and Odoemena, 2004); wheat (Singh et al., 2007); alfalfa (Peralta et al., 2000) and safflower (Houshmandfar and Moraghebi, 2011) revealed that germination decreased significantly with increasing the concentration of Cu and Cd.

Germination Index and Seed Vigor

In Cd and Trichoderma treatment the lowest germination index belonged to 0 mg L\(^{-1}\) which would raise to its highest point (degree) by increasing Cd concentration up to 150 mg L\(^{-1}\) (57% growth). As it is observed in Table 3, germination index increased by Trichoderma (16% compared to non-inoculated...
However, it was not statistically different in the levels of 50 and 100 mg L\(^{-1}\). When using Cu with Trichoderma, the lowest germination index observed at the level of 50 mg L\(^{-1}\) Cu and the highest germination index was related to 150 mg L\(^{-1}\) Cu (25% growth). Unlike Cd, inoculation of wheat seeds by Trichoderma in Cu treatments reduced germination index at all levels except 100 mg L\(^{-1}\) Cu (Table 3). Mukhtar (2008) stated that okra seeds which treated with \(T.\) harzianum increased germination index. The lowest amount of germination index in simultaneous application of heavy metals belonged to the non-polluted Cd treatment with the level of 50 mg L\(^{-1}\) Cu (Fig 1C) which reveals the importance of Cu in the early stages of plant growth. Weiqiang et al., (2005) reported that the sensitivity of seedling growth to heavy metals is more than that of seed germination, as Cu caused reduction in germination but Cd inhibited the process of germination as well as seedling growth. The seed vigor index was at the highest degree in control treatment (Fig 1D).

Conclusions
The results of this study showed that Cd application had significant negative effect on all characteristics (except wet and dry weights of seedling) of wheat during germination process and early seedling stages. Meanwhile, Cu contamination significantly reduced all characteristics (except germination percentage, seedlings wet and dry weights). Application of Trichoderma, however, could decrease these negative effects. Trichoderma application significantly affected germination index and seedling length in both Cd and Cu contaminated treatments and also in the simultaneous application of Cd and Cu.

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