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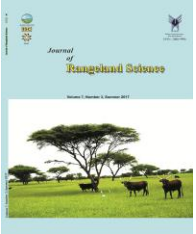

Research and Full Length Article:

Impact of Heavy Metal Stress on *In Vitro* Seed Germination and Seedling Growth Indices of Two Turfgrass species

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Received on: 07/07/2016
Accepted on: 04/11/2016

Abstract. Turfgrass is one of the important components of ornamental plant in the construction of parks and landscapes so that the purpose of this study was to investigate the influence of type and different concentrations of Copper, Zinc and Cobalt on germination and seedling growth of perennial ryegrass *(Lolium perenne* L.) and red fescue *(Festuca rubra* L.) seeds in *in vitro* conditions. This study was carried out in 2014. A factorial experiment was conducted based on a completely randomized design with three replications in laboratory. Seeds were sown in the sterilized medium containing 7 g/L of agar under the laminar air flow. Then, three solutions of ZnSO4, $CuSO_4$ and $COCl_2$ in five concentrations of 0, 20, 50, 100 and 200 mg/L were prepared. Seeds were placed in the incubator at 24°C and 50% humidity for 25 days. Seed germination traits and seedling growth were recorded. Results showed that zinc and copper had minimum and maximum inhibitory effects on seedling growth, respectively. At the presence of copper, by increasing the concentration from 0 to 200 mg/L, the germination and seeding growth were sharply reduced. Inhibitory effects on seedling growth were found in higher concentrations than 50 mg/L at the presence of zinc and cobalt metals. The minimum rate of germination and seedling growth in both red fescue and perennial ryegrass was observed in the concentrations of 20 to 200 mg/L of copper. Therefore, perennial ryegrass and red fescue as turfgrass cannot germinate and grow in soils with copper contamination even at low concentrations, but they can tolerate moderate concentration zinc and particularly cobalt contamination in the polluted environments.

Key words: Ryegrass, Fescue, Copper, Zinc, Cobalt

Introduction

Nowadays, the pollution of environment and soils are the most important issues in human life. Soil naturally contains small amounts of heavy metals and any increases in their concentration can cause risks to human, animal, and plant health .Terrestrial ecosystems due to human activities such as urbanization and industrialization are contaminated with heavy metals (Al-Anbari *et al*., 2015). Soil contamination with heavy metals is one of the most common pollution and its cleaning is the most difficult task. Some of these heavy metals are copper, zinc, cadmium, nickel, chromium, arsenic and selenium (Tucker *et al.,* 2005). Therefore, the release and conversion of these metals in the environment and the impact that they have on the growth of plants and other organisms is of utmost importance (Hall, 2002).

Although some elements are essential for plants and are used as a micronutrient, the increased levels of the heavy metals up to the toxic level affect the growth of plants (Moraghan and Grafton, 1999). This effect is depended on the species, variety and age of plant, concentration and duration of metals effect and its chemical and physical properties (Vassilev and Yordanov, 1997). This toxicity was primarily associated with a reduction in plant growth; then, crop decline in yield and more severe conditions may cause plant death. One of the negative effects of heavy metal is their accumulation in the seed of plants. Plants absorb the elements through their root system. These effects occur in the form of genetic mutations in the plants, preventing seed germination, disruption in many physiological and biochemical processes such as damages to cell membranes, decreased evapotranspiration, breakdown protein synthesis, damages to the photosynthetic system, the effect on enzyme activity, increased peroxidation lipids, impaired growth of plants and even the stopped growth (Atici *et al.,* 2005; Rout and Das, 2003; Mahmood *et al.,* 2007).

Seeds through assessing external conditions play an essential protective role for plants until environmental factors are ready for growth and development. In general, heavy metal stress on the seed is in two ways: a) preventing from water absorption by seeds and b) creating conditions that led to the entrance of metal ions and toxic effects on the embryo. Generally, inhibition of germination and stop in the growth of plants has been reported in the germination stage by heavy metals toxic conditions (Ashagre *et al*., 2013; Atici *et al*., 2005; Gangaiah *et al*., 2013; Kabir *et al*., 2010; Taghizadeh *et al*., 2002; Ebrahimi, 2012).

Among heavy metals, copper and zinc are actively involved in cellular metabolism since both of them, especially zinc are present in the structure of many proteins (Ashagre *et al*., 2013). Zinc as an element of the non-oxidative micronutrient has a structural and catalytic role in many proteins and enzymes related to energy metabolism. Zinc is effective in nitrogen metabolism of plants, increasing the uptake of elements at low temperatures during the growing season and increasing the production of Indole Acetic Acid (IAA) which causes cell elongation. High levels of zinc concentration in the soil can reduce plant growth, stop the metabolic activity of soil, damage the microorganisms and earthworms and slow the decomposition of organic matters which have roles in the supply of root food. Thus, a limited number of plants has a chance to survive in rich soils of zinc. Copper causes the photosynthesis, metabolism of proteins and carbohydrate. Copper is also involved in building some of the enzymes. Another element used in this study is cobalt that like other heavy metals tend to form a chelate in combination with soil organic matters. Concentration of cobalt in soils is different so that cobalt accumulation is much more in acidity and moist soils than others (Hall, 2002).

Lolium perenne L. (Ryegrass) and *Festuca rubra* L. (Fescue) as cold-season species of turfgrasses are usually used in landscapes and sport fields (Xa *et al.*, 2006). However, there are not many reports on the evaluation of germinating and early growing characteristics of turfgrass seed affected by heavy metal pollutants. Consequently, this study was conducted to determine the effects of various concentrations of different zinc, copper and cobalt on ryegrass and fescue seed germination and early seedling growth and to manage the growth and development of turfgrass in heavy metal contaminated regions.

Materials and Methods

This study was conducted in the laboratory of the department of horticultural sciences at Arak University, Iran. For this purpose, a factorial experiment was conducted based on a completely randomized design in with four replications on perennial ryegrass *(Lolium perenne* L.) and red fescue (*Festuca rubra* L.) seeds. Before starting the experiment, the sterilization of Petri dishes and laboratory equipment was done in autoclave and seeds were disinfected with a solution of 10% commercial home bleach for 10 minutes and then, washed with distilled water.

The media used in the present work were distilled water agar media with different concentrations of Zn (ZnSO4), Cu (CuSO4) and Co (CoCl2) individually. In the second factor, five levels of heavy metals concentrations such as 0, 20, 50, 100 and 200 mg/L were used. The media employed pH was adjusted to 5.7 to 5.8 and before dispensing the equal amount of media (50 ml) into test jars, 0.7 percent agar was added to the media and melted. Petri dishes containing media were autoclaved for 15 min. After the completion of sterilization, they were removed from the autoclave and the seeds were sown in sterilized medium. Then, seeds were kept in the incubator at 24°C and 50% humidity for 25 days. On the tenth day, the seed germination started and germinated seeds were counted daily. Thirty seeds were sown on sterilized medium containing three heavy metals at different concentrations in each replication. Every three days, the germinated seeds of each Petri dish were counted and after three weeks, data were collected for shoot length and root length. Then, seedlings fresh weight was recorded using a digital scale and to measure dry weight, they were placed in an oven at 60ºC for 48 hours. According to these data, the Coefficient of Velocity of Germination (CVG), Average Velocity of Germination (AVG), Mean Daily Germination (MDG), Germination Percent (GP), Germination Rate (GR) and Seed Vigor index (VI) were calculated using the formulas in Table 1. The collected data were subjected to one-way ANOVA using SAS statistical software. The means comparisons were made using Duncan's multiple range tests at α=0.05 probability level.

Table 1. The formula used to calculate the index of germination

Germination indices	The Formula used	Formula components
Coefficient of Velocity Germination (CVG)	$CVG = \frac{\sum N_i}{\sum N_i T_i} \times 100$	N_i= the number of germinated seeds T_i= the last day of germination
Average Velocity of Germination (AVG)	$AVG = \frac{\sum Nt}{\sum t}$	N_t= total number of germinated seeds in time t= number of days to maximum germination
Mean Daily Germination (MDG)	$MDG = \frac{GP}{d} \times 100$	GP=Total germinated seed d= period of germination
Germination percent (GP)	$GP = \frac{N_g}{N_t} \times 100$	N_g= germinated seeds N_t = total seeds
Germination Rate (GRI)	$GR = \frac{G_1}{1} + \frac{G_2}{2} + ... + \frac{Gi}{i}$	G_i= the germination on each day after cultured i= alternate day after cultured
Seed Vigor index (VI)	$VI = (PL + RL) \times GP$	GP= germination PL= shoot length RL= root length

Results

In this study, for lack of antagonism, synergy effects and other elements and lack of humidity fluctuations during experiment, *in vitro* culture based on a semi-solid medium was used. Results of analysis of variance showed a significant effect of all simple interactions of the metal concentration and species for most seedling traits except for the Coefficient of Velocity Germination.

The species by the concentration interaction effects of germination traits of turfgrass and the metal inhibitory effect on all of traits is presented in Table 2. Results showed that by increasing the concentration from 0 to 200 mg/L, the germination percent was decreased in both species and the highest and lowest germination percent and the average values 16.67 and 4.47 were observed in ryegrass at control and in fescue at 200 mg/L, respectively (Table 2). In both species, the highest average velocity of germination values were obtained at the concentration of 0 mg/L and by increasing the concentration up to 200 mg/L, it was reduced. Through increasing the concentration from 0 to 200 mg/L, the inhibition was also observed for germination rate and this inhabitation in ref fescue was higher than ryegrass. Result of vigor index showed that increasing the concentration from 0 to 200 mg/L leads to a decline in seed vigor and as a result, the highest and lowest vigor index and the average values of 1.57 and 0.05 were obtained in the control (for ryegrass) and 200 mg/L concentration (for fescue), respectively (Table 2). So, the results of this experiment suggested a direct effect between increasing the concentration of heavy metals in the media with a significant reduction in all of germination indices. In addition, ryegrass was less affected by metal treatments than fescue (Table 2).

Results of analysis of variance showed that the interaction effect of heavy metal and species on germination characteristics was significant except for the coefficient of velocity germination and seedling fresh weight. According to the results, the species by metal interaction in both species revealed that zinc had the minimum inhibitory effect and copper had the maximum inhibitory effect on the shoot and root length in both species (Table 3).

In red fescue, the maximum root and shoot length and the average values of 1.72 and 4.55 cm were obtained in zinc treatment, respectively. The results of average velocity of germination indicated that the AVG in ryegrass had a significant difference with other treatments only in the presence of zinc. By comparing three treatments (zinc, copper and cobalt elements), the highest and lowest AVG and the average values of 5.35 and 2.2 were related to the ryegrass treated with zinc and fescue treated with copper, respectively (Table 3).

The Coefficient of Velocity Germination (CVG) and Daily Germination indices had no significant differences for any of the treatments. Based on the results from Table 3, only the rate of germination in zinc treatments was significantly different with other treatments in ryegrass. For other treatments (copper and cobalt), no significant difference was observed. The data indicated that germination percent in ryegrass treated with zinc was higher than all other treatments and ranked in class a. The highest and lowest values of this trait were obtained in ryegrass treated with zinc and copper, respectively. In comparing the interaction effects of species and heavy metal, zinc had less inhibitory effect on germination than cobalt and especially copper. Therefore, the highest germination percent (6.26%) was obtained in the ryegrass with zinc treatment and the lowest value (2.53%) was obtained in the fescue with copper treatment. The vigor index had a significant difference with the other treatments only in ryegrass with zinc treatment and the highest level of vigor index was observed in ryegrass with zinc treatment (1.3). The copper and zinc with the average values of 0.85 and 1.54 mg had the maximum and minimum reduction effects on seedlings dry weight in ryegrass. Also, the highest seedling dry weight (1.54 mg) and the lowest one (0.85 mg) were observed in Fescue and Ryegrass grown in zinc and copper, respectively (Table 3).

Analysis of variance showed that the interaction effect of heavy metal and concentration on germination characteristics was significant except for the FW. The metal by concentration interaction effect indicated that for more traits, the increased concentration of cobalt, copper and zinc in media can reduce germination and seedling growth of both species. Certainly, the degree of inhibition varied according to the type of metal in a way that increasing the cobalt concentration of media did not restrict the growth but reduced growth at the presence of copper, especially in higher concentration than 20 mg/L. The highest mean values of growth characteristics such as root and shoot length, seedling dry weight, germination rate, and germination percent were observed at the presence of 0-20 mg/L of zinc. The lowest germination indices of turfgrass were obtained at copper concentrations higher than 20 mg/L (Table 4).

Table 2. Interaction effects of species by heavy metal concentration on germination indices in seedling phase

Metals concentration	Species	Germination %	Germination rate	Average velocity of germination	Mean daily germination	Shoot length (cm)	Root length (cm)	Root/Shoot length ratio	Seed vigor index	Fresh weigh mg	Dry weight mg
0	Ryegrass	16.67^{a}	5.72^{a}	4.78^{a}	13.00^{ab}	6.23^{a}	3.17^{a}	0.51^{a}	1.57^{a}	12.27^{a}	1.48^{a}
	Fescue	13.33^{ab}	4.55^{ab}	3.78^{ab}	12.80^{ab}	6.70^{a}	2.53^{a}	0.40^{bc}	1.23^{ab}	7.24^{ab}	1.50^{a}
20	Ryegrass	15.56^{a}	4.30^{ab}	3.70^{ab}	13.50^{ab}	2.90^{bc}	1.20^{bc}	0.37^{bc}	0.84^{bc}	3.00^{b}	1.00^{cd}
	Fescue	10.00^{abc}	3.8^{ab}	3.11^{abc}	13.24^{ab}	3.54^{b}	1.80^{b}	0.41^{ab}	0.70^{cd}	4.13^{b}	1.06^{cd}
50	Ryegrass	15.56^{a}	4.20^{ab}	3.70^{ab}	13.40^{ab}	3.04^{bc}	0.96^{cd}	0.30^{cd}	0.73^{cd}	3.3^{b}	0.90^{dc}
	Fescue	11.11^{ab}	2.70^{bc}	2.33^{bc}	13.56^{a}	2.77^{bc}	0.64^{cd}	0.24^{d}	0.35^{cde}	3.15^{b}	1.16^{bc}
100	Ryegrass	14.44^{ab}	4.10^{ab}	3.55^{ab}	13.55^{a}	1.90^{cd}	0.40^{d}	0.22^{d}	0.44^{cde}	2.14^{b}	0.62^{d}
	Fescue	8.33^{bc}	3.24^{bc}	2.67^{bc}	12.73^{b}	2.82^{bc}	0.64^{cd}	0.21^{d}	0.30^{de}	3.50^{b}	1.40^{ab}
200	Ryegrass	7.78^{bc}	2.30^{bc}	2.00^{c}	13.42^{ab}	1.64^{cd}	0.30^{d}	0.15^{d}	0.15^{e}	2.23^{b}	0.75^{cd}
	Fescue	4.44^{c}	1.52^{c}	1.30^{c}	13.44^{ab}	0.93^{d}	0.21^{d}	0.24^{d}	0.05^{e}	1.80^{b}	0.64^{d}

Mean values followed by different letters are significantly different

Table 3. Interaction effects of species by and heavy metals type on germination indices in seedling phase

Heavy metals	Species	Germination %	Germination rate	Average velocity of germination	Mean daily germination	Coefficient of velocity germination	Shoot length (cm)	Root length (cm)	Root/Shoot length ratio	Seed vigor index	Dry weight mg
Zn	Ryegrass	21.33^{a}	6.26^{a}	5.35^{a}	13.31^{ab}	7.52^{ab}	4.21^{a}	1.61^{ab}	0.35^{a}	1.30^{a}	1.00^{a}
	Fescue	9.67^{b}	3.80^{b}	3.13^{b}	12.85^{b}	7.81^{a}	4.55^{a}	1.72^{a}	0.35^{ab}	0.74^{b}	1.54^{b}
Cu	Ryegrass	8.33^{b}	2.70^{b}	2.31^{b}	13.25^{ab}	7.56^{ab}	2.24^{b}	0.81^{ab}	0.24^{ab}	0.36^{b}	0.85^{b}
	Fescue	10.00^{b}	2.53^{b}	2.20^{b}	13.53^{a}	7.42^{b}	2.50^{b}	0.69^{b}	0.22^{b}	0.40^{b}	0.87^{b}
Co	Ryegrass	12.33^{b}	3.37^{b}	2.98^{b}	13.50^{a}	7.42^{b}	2.97^{ab}	1.17^{ab}	0.33^{ab}	0.58^{b}	1.00^{b}
	Fescue	8.67^{b}	3.14^{b}	2.58^{b}	13.08^{ab}	7.68^{ab}	3.00^{ab}	1.07^{ab}	0.33^{ab}	0.41^{b}	1.04^{b}

Mean values followed by different letters are significantly different

Table 4. Interaction effects of heavy metal type and concentration on germination indices in seedling phase

Metals	Concentration	Germination %	Germination rate	Mean daily germination	Average velocity of germination	Coefficient of velocity germination	Shoot length (cm)	Root length (cm)	Root/Shoot length ratio	Seed vigor index	Dry weight mg
Zn	0	17.50^{ab}	6.51^{a}	12.70^{c}	5.33^{ab}	7.90^{a}	6.46^{a}	2.85^{a}	0.45^{b}	1.63^{a}	1.50^{a}
	20	21.67^{a}	6.70^{a}	13.00^{bc}	5.67^{a}	7.64^{ab}	4.78^{b}	2.80^{a}	0.60^{a}	1.56^{ab}	1.15^{ab}
	50	15.83^{ab}	4.61^{abc}	13.31^{abc}	3.94^{a-d}	7.55^{abc}	4.76^{b}	1.33^{b}	0.30^{cde}	0.95^{cd}	1.40^{a}
	100	16.67^{ab}	5.19^{ab}	13.18^{bc}	4.44^{abc}	7.6^{abc}	4.07^{bc}	1.02^{b}	0.24^{def}	0.80^{cde}	1.40^{a}
	200	5.83^{cd}	2.16^{def}	13.12^{bc}	1.83^{efg}	7.67^{ab}	1.82^{ef}	0.35^{c}	0.20^{ef}	0.15^{g}	0.90^{bcd}
Cu	0	15.00^{ab}	4.98^{abc}	12.90^{bc}	4.17^{a-d}	7.76^{ab}	6.46^{a}	2.85^{a}	0.45^{b}	1.40^{ab}	1.50^{a}
	20	5.00^{d}	0.95^{f}	14.17^{a}	0.90^{g}	7.06^{c}	1.37^{ef}	0.30^{c}	0.20^{ef}	0.08^{g}	0.87^{bcd}
	50	13.33^{bc}	3.01^{b-f}	13.40^{abc}	2.67^{c-g}	7.50^{abc}	1.44^{ef}	0.22^{c}	0.16^{f}	0.23^{fg}	0.60^{cd}
	100	5.83^{cd}	2.11^{def}	13.12^{bc}	1.78^{efg}	7.65^{ab}	1.70^{ef}	0.24^{c}	0.15^{f}	0.11^{g}	0.86^{bcd}
	200	6.67^{cd}	2.01^{ef}	13.40^{abc}	1.78^{efg}	7.48^{abc}	0.90^{f}	0.16^{c}	0.20^{ef}	0.07^{g}	0.52^{d}
Co	0	12.50^{bcd}	3.91^{b-e}	13.00^{bc}	3.33^{cde}	7.70^{ab}	6.46^{a}	2.85^{a}	0.45^{b}	1.16^{bc}	1.50^{a}
	20	11.67^{bcd}	4.44^{a-d}	12.84^{bc}	3.67^{b-e}	7.82^{ab}	3.50^{cd}	1.40^{b}	0.40^{bc}	0.61^{def}	1.04^{abc}
	50	10.83^{bcd}	2.70^{c-f}	13.70^{ab}	2.44^{d-g}	7.32^{abc}	2.52^{de}	0.85^{bc}	0.34^{bcd}	0.44^{efg}	1.12^{ab}
	100	11.67^{bcd}	3.71^{b-e}	13.13^{bc}	3.11^{c-f}	7.63^{ab}	1.30^{ef}	0.30^{c}	0.25^{def}	0.18^{g}	0.76^{bcd}
	200	5.830^{cd}	1.52^{ef}	13.80^{ab}	1.33^{fg}	7.29^{bc}	1.12^{f}	0.22^{c}	0.22^{def}	0.08^{g}	0.70^{bcd}

Mean values followed by different letters are significantly different

Discussion

Due to the environmental conditions, the response of plants is various based on the growth stage. In relation to environmental stresses, the situation is the same. Seed germination and seedling establishment are the most critical stages to environmental stresses and in later stages, the plant resistance may be increased. In some cases, initial stress conditions may stimulate seed germination. Based on our results, the inhibitory effects of metals on seed germination characteristics of both ryegrass and fescue were proved. Previous studies have shown that heavy metals such as mercury, zinc, cadmium, cobalt, copper, lead reduced the germination of lentil, radish, mustard and rice (Ayaz and Kadioglu, 1997; Espen *et al.,* 1997; Mishra and Choudhuri, 1999). In present experiment, increasing concentration of metals delayed the germination and seedling growth. In a study of *Brassica pekinensis* seed germination treated with different concentrations of lead, it was observed that increasing the concentration of lead decreased the germination percent, and shoot and root length (Xiong, 1998), which is in agreement to our results. In another study, zinc concentrations higher than 75 m mol in medium reduced the growth traits of pea seedlings (Sharma *et al.,* 2009). Concentrations higher than 20 ppm of cobalt in medium reduced the seedling growth of *Pennisetum glacum* (Gangaiah *et al.,* 2013). Based on this study, Ryegrass and Fescue reacted differently to the presence of some metals in germination medium. Similar to our results, Wierzbicka and Obidziniska (1988) showed the lead treatment on the seed of several plant species indicating that high concentrations of lead could have inhibition effects on the germination of seeds such as pea; in contrast, lead had no significant effect on seed germination. Taghizadeh *et al.* (2002) by comparing the effects of lead on three species of ryegrass, Kentucky bluegrass and Bermuda grass in the germination stage found that ryegrass in germination and establishment stages showed more resistance to concentrations of lead. Ebrahimi and Madrid Díaz (2014) reported that copper negatively affected growth and tolerance indices of *Festuca ovina* and high concentration of copper hampered seed germination, dry weight, and root and shoot length. Mahmood *et al.* (2007) through the treatment of barley, rice and wheat with Cu, Zn, Pb, Mg and Na observed that seedlings of wheat and rice were more sensitive to toxic elements than barley. Low sensitivity of some plants such as Silene and soybean to the presence of some heavy metals such as Cd and Al in the growth medium is due to their root limitation in absorbing ions or the synthesis of a series of chelating compounds such as enzymes, and lipids which leads to the detoxification of the ions within the cell (De Knecht *et al.,* 1994). Some researchers believe that the permeability of the coat of seeds to metal ions is associated with their resistance; the more permeable is the seed coatings for metals, the presence of metals in the environment will be more which delays or prevents from the seed germination. It has been reported that seed plants inherently have the capacity for selective absorption of metals in media (Stefanov *et al.*, 1995).

In our experiment, zinc had less toxic effect than copper on the seedling growth of fescue and ryegrass and cobalt also showing the minimal inhibitory effect on seeds. Copper toxicity was higher than other metals for the seedling growth (Ouariti *et al.,* 1997). Similarly, Peralta *et al.* (2000) compared the effects of metal toxicity on the initial growth of alfalfa and found that the inhibitory effect of zinc on growth and germination of seedlings was not similar to other heavy metals such as copper. Copper concentrations higher than 20 ppm limited the growth of alfalfa seedlings (Aydinalp and Marinova, 2009). In another research, it was observed that alfalfa germinated easily at presence of 0 to 40 ppm of zinc but its germination was severely limited at the same copper concentrations. Ranking the impact of metals based on initial growth inhibitory effects on the growth of seedling included Cu, Zn and Pb in wheat, barley and rice (Wierzbicka and Obidziniska, 1988). In a research conducted by Mahmood *et al.* (2007), barley, rice and wheat were treated with Cu, Zn, Pb, Mn and Na at the concentrations of 0, 1, 5, 10μM. Among the elements, copper treatment had the most deleterious effect on seed germination and early growth of seedlings. Also, Wong and Bradshaw (1982) studied the effects of heavy metal toxicity on the growth of seedlings of ryegrass and concluded that the inhibitory effect of copper was higher than zinc on germination indices. Similarly, Aydinalp and Marinova (2009) did not report the non-toxicity of zinc on alfalfa germination at some concentrations and found that it could stimulate the growth of seedling while copper reduced the growth and germination. More inhibitory effect of copper than cobalt and zinc on growth and germination of wheat and cucumber had been proven (Munzuroglu and Geckil, 2002).

During an experiment, *Thespesia populnea L.* seeds were treated with different concentrations of lead and significant effects on root growth reduction were observed and this effect was considered to be related to the decrease in mitosis and cell division in root meristematic regions (Kabir *et al.*, 2010). Other reasons for the reduction in shoot growth and seedling length in *T. populnea* at the presence of metal treatments are loss of meristematic cells in these regions, decrease in enzyme content cotyledon and endosperm cells (such as amylase enzyme that converts starch into glucose and protease involved in digestion of proteins) which digested the storage seed and converted the compounds in the form of solution and their transmission to the tip of the shoot and root (Hall, 2002; Kabir *et al.*, 2010). So, when the enzyme activity is affected, nutritional compounds do not reach to the shoot and root; therefore, shoot and root growth reduces (Kabir *et al.*, 2010).

Conclusion

Generally, in this experiment, zinc had minimal inhibitory effect, then cobalt and finally copper on turfgrass seedling growth. The effect of toxicity on germination with the increasing concentration was depended on the type of metal so that at the presence of copper, increasing the concentration from 0 to 200 mg/L greatly reduced the germination and seedling growth. Inhibitory effects on morphological traits of turfgrass were found in concentrations higher than 50 mg/L at the presence of zinc and cobalt metals. The minimum rate of germination and seedling growth in fescue and ryegrass was observed in concentrations of 20 to 200 mg/L of copper. The amount of resistance to heavy metals was not significantly different in two turfgrass species and only ryegrass was more resistant than red rescue in the presence of zinc. Therefore, ryegrass and red fescue cannot germinate and grow in soils with copper contamination even at low concentrations, but they can tolerate the average zinc followed by cobalt contamination in the polluted environments.

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تاثیر تنش فلزات سنگین بر شاخص‌های جوانه زنی و رشد گیاهچه در دو گونه چمن در شرایط آزمایشگاهی

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تاریخ دریافت: ۱۳۹۵/۰۴/۱۷
تاریخ پذیرش: ۱۳۹۵/۰۸/۱۴

چکیده. چمن‌ها از اجزای اصلی و ضروری گیاهان زینتی هستند که در ساخت پارک‌ها و مناظر از آنها استفاده می‌شود. هدف از این پژوهش بررسی تاثیر نوع و غلظت‌های مختلف مس، روی و کبالت بر جوانه زنی بذر چچم (*Lolium perenne* L.) و فستوکا (*Festuca rubra* L.) زینتی در شرایط آزمایشگاهی بود. این آزمایش در سال ۱۳۹۳ در شرایط درون آزمایشگاهی در قالب آزمایش فاکتوریل براساس طـرح کامـلا تصادفی با سه تکرار انجام شد. بذرها در محیط کشت استریل شامل ۷ g/l آگار با سه ترکیب شیمیایی؛ $ZnSO_4$ ، $CuSO_4$ و $COCl_2$ در پنج غلظت۰، ۲۰، ۵۰، ۱۰۰ و ۲۰۰ mg/L کشت شدند. کاهش بازدارندگی سمیت فلزات بر جوانه‌زنی به ترتیب Cu>Co>Zn بود. در حضور مس افزایش غلظت از ۰ تا ۲۰۰ mg/L تا حد زیادی جوانه زنی و رشد را کاهش داد. ولی اثرات بازدارندگی بر گیاهچه چمن در غلظت بیشتر از ۵۰ mg/L در حضور روی و کبالت مشاهده شد. کمترین سرعت جوانه زنی و رشد گیاهچه در فستوکا و چچم در غلظت های ۲۰ تا ۲۰۰ mg/L مس مشاهده شد. بنابراین چمن‌های چچم و فستوکا در خاک آلوده به مس حتی در غلظت‌های کم نمی‌توانند جوانه زده و رشد کنند، اما آلودگی متوسط روی و کبالت را کم و بیش تحمل می‌کنند.

کلمات کلیدی: چچم، فستوکا، مس، روی، کبالت