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Full Length Research Paper

Effect of mixed cadmium, copper, nickel and zinc on seed germination and seedling growth of safflower

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Elevated levels of heavy metals in contaminated soils are widely spread and concerns have been raised over the potential risks to humans, animals and agricultural crops. This study was conducted to evaluate the effect of mixed cadmium, copper, nickel and zinc on seed germination and seedling growth of safflower under controlled light and temperature conditions. Treatments were included 0 (control), 60, 120, and 180 mg kg $^{-1}$ which were made by equal amounts of cadmium (¼), copper (¼), nickel (¼), and zinc (¼). The heavy metal mixture treatment showed toxic effects on seed germination and seedling growth of safflower. Increasing the concentration of heavy metal mixture to 180 mg kg^{-1} showed a significant decrease in seed germination as compared to control treatment (p<0.01). Heavy metal mixture concentration of 60 mg kg^{-1} reduced shoot fresh and dry weight (p<0.01). Root fresh and dry weight was diversely affected by the heavy metal treatment. However, the heavy metal mixture concentration of 180 mg kg^{-1} produced the lowest amount of both root fresh and dry weight. A negative response of root and shoot length of safflower to heavy metal mixture application relative to control treatment was observed at 120 mg kg^{-1} (p<0.01). The study suggests that cultivation of safflower in metal polluted soils should be avoided or appropriate control measures be adopted to maintain the heavy metal content of the soil below the damage threshold level. The heavy metal mixture treatment of 60 mg kg^{-1} exhibited the lowest percentage of tolerance in germination and seedling growth characteristics of safflower as compared to control.

Key words: Germination, seedling growth, heavy metals, safflower.

INTRODUCTION

Modern civilization introduces a wide range of pollutants to the atmosphere through various anthropogenic activities such as industry, mining, transportation, etc. Despite the fact that, it is almost impossible to visualize a soil without trace levels of heavy metals and most of the heavy metals are essential elements for living organisms, but their excess amounts are generally harmful to plants, animals and human health (Azevedo and Lea, 2005; Jarup, 2003). Currently, contamination of soil in cultivated fields with toxic heavy metals such as cadmium, copper, nickel and zinc has emerged as a new threat to agriculture (Singh et al., 2007). Cadmium is an unnecessary element for both plants and animals and has toxic effects when its concentration has exceeded a limit. Generally, it makes negative effect on their metabolisms by influencing the activity of cellular enzymes (Yang et al., 1986). Many studies have been carried out on the effects of cadmium on plants.

They showed that, cadmium in certain amounts inhibited the germination and development of the plants (Aydinalp and Marinova, 2009). Cadmium caused chlorophyll aberrations at very high concentrations (Reddy and Vaidyanath, 1978), reduction of mitotic index in root cells (Zhang and Yang, 1994), chromosomal abnormalities and micronucleus formation (Li and Zheng, 1992), disorder in nucleus structure (Jiang et al., 1994) and abnormalities in DNA and RNA synthesis (Enger et al., 1997) . Copper is widely prevalent and was considered as an essential element for all living organisms including plants (Underwood, 1977; Goyer, 1991). Copper occurs in the environment as hydrated ionic species, forming complex compounds with inorganic

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and organic legends. Copper causes injury at cellular level by the formation of free radicals. Cellular injury by this type of mechanism is well documented for copper as well as other metals (Shi et al., 1993; Gupta and Kalra, 2006). Copper being one of the common heavy metals in industrial discharge of aeronautic, metal and metallurgy, and refinery industries shows toxic effects on plants and animals (Dharam et al., 2007). Nickel is naturally present in soil and water, usually in trace amounts. Several plants nutritionally require nickel for various metabolic activities (Welch, 1981).

However, rapid industrialization and urbanization during the recent past have caused accumulation of nickel in varied habitats, where from the acquisition by the plants and their further transfer to human and animal population, may affect the life forms seriously. Higher concentration of nickel may cause numerous adverse effects on plants (Smith, 1996). The physiological role of nickel and its toxic effects on higher plants have been observed by many studies (Seregin and Kozhevnikova, 2006; Khan et al., 1987). Growth of most plant species is adversely affected by tissue concentration of nickel above 50 mg kg^{-1} dry weight (Rahman and Mahmud, 2010). Zinc phytotoxicity is reported relatively often, especially for acid and heavily sludged soils. The physiology and biochemistry of the toxic effects of zinc in plants are likely to be similar to those reported for other heavy metals; however, zinc is not considered to be highly phytotoxic (Kabata-Pendias and Pendias, 1973) . Although, the effects of the individual heavy metals on plants have been evaluated by many studies (Brown and Wilkins, 1986; Shafig et al., 2008; Dharam et al., 2007; Shafiq and Iqbal, 2005; Kabir et al., 2008), limited information is available on the effects of heavy metal mixture on plant species. There is the need to study the combined effects of heavy metals on plants because most of them are present in an environment at the same time or on the same environment at different times. Safflower (*Carthamus tinctorius* L.) is economically an important annual oilseed crop. It has been traditionally grown for its flowers as a source of dye for colouring food and fabrics.

Subsequently, it is grown for edible oil, animal meal, bird feed, medicinal uses, as a potential candidate crop for production of plant made pharmaceuticals, biofuel and specialty type oils (Sujatha, 2008*).* To our knowledge, limited information is available on the effects of heavy metals on seed germination and seedling growth of safflower plant. Hence, the objective of this study was to evaluate the various effects of mixed cadmium, copper, nickel, and zinc at different levels on safflower in early growth stages.

MATERIALS AND METHODS

Experimental setup

The experiment was laid out in a randomized complete block design (RCBD) with four levels of heavy metal mixture in three replications. The heavy metal mixture treatments included 0 (control), 60, 120, and 180 mg kg^{-1} made with equal amounts of cadmium (¼), copper (¼), nickel (¼), and zinc (¼). The mixtures were applied in 100 ml of a metal solution made from $Cd(NO_3)_2$ $4(H_2O)$, $Cu(NO_3)_2$ $2.5(H_2O)$, $Ni(NO_3)_2$ $6(H_2O)$, and $Zn(NO_3)_2$ $6(H_2O)$ salts diluted in deionized water. The soil was slowly mixed thoroughly with the solution using a glass rod and left to equilibrate for one day. General-purpose plastic pots were filled with 300 g of the treated soil which was classified as a clay loam with 27.9% sand, 26.2% clay and 45.9% silt, with an electrical conductivity (EC_e) of 1.4 dS m^{-1}, and a pH of 7.4 (saturated paste) . Ten seeds of safflower plant washed with deionized water and put into each pot. The plants were grown in a growth chamber at 25/18°C day/night temperature and a photoperiod of 12 h at 1500 luxes. At the planting time, 25 ml of a modified Hoagland's nutrient solution containing macro and micro elements were applied to each pot (Peralta et al., 2001). The pots were watered with deionized water as described by Houshmandfar and Tehrani (2008).

Plant sampling and analysis

Seed germination percentage was determined four times, three days interval started from fifth day after sowing (DAS). A seed was considered as germinated when root had emerged more than 2 mm. The number of germinated seeds per time was presented as seed germination rate. All the plants in every treatment were collected 25 days after planting, evaluated for root and shoot length and fresh biomass, and washed with water and deionized water, oven dried at 70°C for 48 h, weighed for determined biomass accumulation included root and shoot dry biomass. Root length was measured from the main apex to the crown, whereas shoot length was measured from the crown to the main apex. Germination percentage and tolerance indices (Iqbal and Rahmati, 1992) were determined by the following formula:

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of planted seeds}} \times 100$$

$$\text{Tolerance indices} = \frac{\text{Mean root length of polluted area seeds}}{\text{Mean root length of control area seeds}} \times 100$$

Data for various growth indices were subjected to analysis of variance using SAS/STAT software version 8 (SAS Inst., 1999). Duncan's multiple range test (DMRT) (Dancan, 1955) at the 0.01 level of probability was used to evaluate the difference among treatment means.

RESULTS

Evaluation of seed germination characteristics

Germination percentage of safflower seeds was adversely affected due to the application of heavy metal mixture at different levels. Heavy metal mixture inhibited seed germination significantly at 0.01 level of probability. The seeds germinated best in the non heavy metal mixture control. Germination percentage was markedly suppressed at a higher mixture level of 180 mg kg^{-1} (Figure 1). Variation in the set of accessions, was not possible to discern at heavy metal mixture levels of 60 and 120 mg kg^{-1} with control treatment. However, accessions differed significantly at the higher mixture

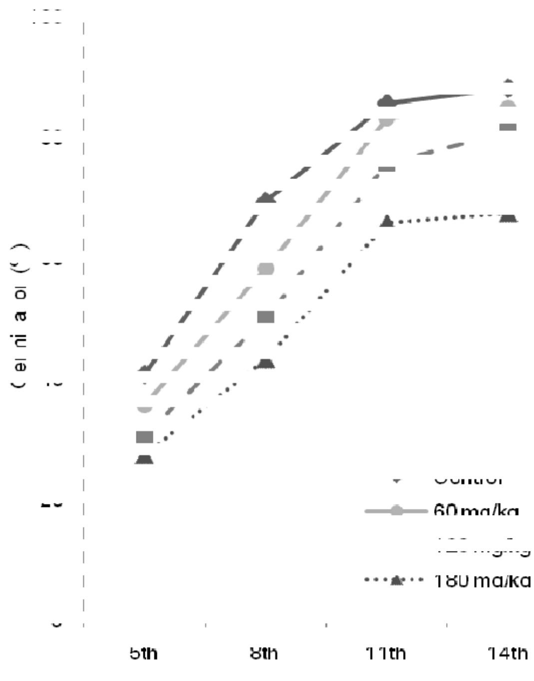

Figure 1. Germination rate of safflower plant exposed to different heavy metal mixture concentration.

Table 1. Overall mean values for various traits of safflower germination percentage and seedlings grown under different concentrations of heavy metal mixture.

Concentration (mg kg^{-1})	Germination (%)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Shoot length (cm)	Root length (cm)
0	88.33^{a} ± 5.00	4.99^{a} ± 0.35	0.45^{ab} ± 0.04	0.28^{a} ± 0.02	0.11^{b} ± 0.01	10.62^{a} ± 0.55	9.65^{a} ± 0.53
60	85.00^{a} ± 1.67	4.47^{b} ± 0.29	0.51^{a} ± 0.03	0.22^{b} ± 0.01	0.16^{a} ± 0.02	9.88^{a} ± 0.36	8.87^{a} ± 0.16
120	81.67^{a} ± 1.67	2.74^{c} ± 0.24	0.44^{ab} ± 0.02	0.20^{b} ± 0.00	0.14^{a} ± 0.01	8.42^{b} ± 0.31	7.51^{b} ± 0.38
180	67.50^{b} ± 5.83	2.19^{d} ± 0.21	0.36^{b} ± 0.05	0.21^{b} ± 0.01	0.09^{b} ± 0.00	7.48^{b} ± 0.35	6.01^{c} ± 0.18

a: Within a column, means followed by the same letter are not significantly different at the 0.01 level of probability by Duncan's multiple range test. b: SE (±), standard error.

level of 180 mg kg^{-1}. The concentration of 60, 120 and 180 mg kg^{-1} heavy metal mixture inhibited seed germination with 3.77, 7.54 and 23.59%, respectively. The onset of germination was delayed by heavy metal stress. Hence, the germination rate was better recovered final germination percentage when the seeds were treated with lower heavy metal mixture levels (Figure 1). During the first eight days, germination rate was decreased as heavy metal concentration increased. However, during the next three days, the germination rate was slightly higher at mixture concentration of 60 mg kg^{-1} as compared to control treatment.

Evaluation of seedling growth

Table 1 indicates the effects of heavy metal mixture on germination percentage, shoot and root fresh weight, shoot and root dry weight and shoot and root length of safflower. The shoot fresh weight was inversely proportionally correlated to the mixture concentration added to the soil. Heavy metal mixture concentration of 60, 120 and 180 mg kg^{-1} inhibited shoot fresh weight growth with a decrease of 10.43, 45.10 and 56.12%, respectively. The highest amount of root fresh weight was obtained in mixture concentration of 60 mg kg^{-1} with no significant difference with the amount of root fresh weight of plant exposed to mixture concentration of 120 mg kg^{-1} and non heavy metal mixture control. The lowest amount of safflower root fresh weight was observed in heavy metal mixture treatment of 180 mg kg^{-1}. Shoot and root dry weight of safflower seedling was affected diversely by the different levels of heavy metal mixture. The shoot dry weight was significantly ($p<0.01$) reduced at 60, 120 and

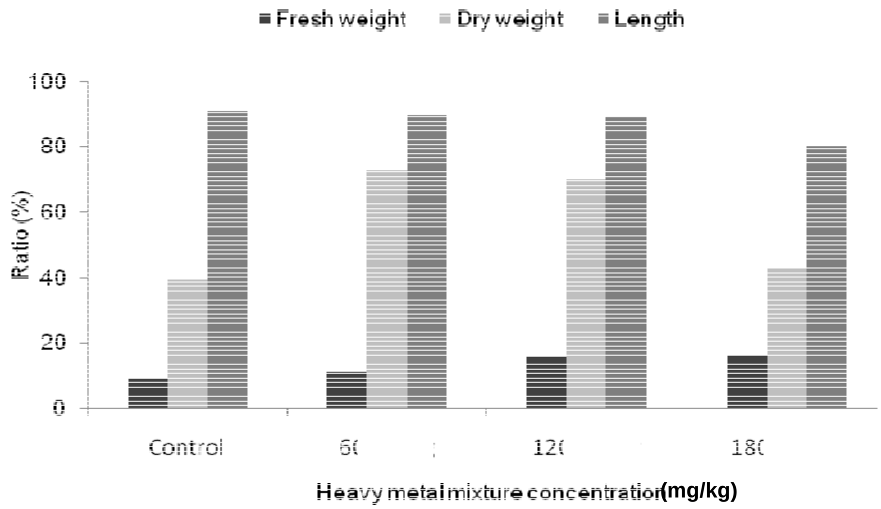

Figure 2. Root/shoot ratio of safflower exposed to different heavy metal mixture concentration.

180 mg kg^{-1} concentration of heavy metal mixture as compared to control.

However, mixture concentration of 60 and 120 mg kg^{-1} promoted root dry weight with an increase of 40.34 and 29.93%, respectively. Root dry weight of the plant exposed to the heavy metal mixture of 180 mg kg^{-1} was found to be the lowest amount. A negative response of shoot and root length of safflower to heavy metal mixture application relative to the non heavy metal mixture control was observed in all levels. The heavy metal mixture concentrations of 120 and 180 mg kg^{-1}, showed toxic effect on both shoot and root length in Safflower, as compared to control treatment. The heavy metal mixture concentrations of 120 and 180 mg kg^{-1} significantly limited shoot and root length growth with decrease of 20.72 and 29.57% for shoot length, and decrease of 22.18 and 37.73% for root length, respectively. Figure 2 indicates the effect of heavy metal mixture on root/shoot fresh weight ratio, root/shoot dry weight ratio and root/shoot length ratio of safflower plant. Heavy metal mixture was increased root/shoot fresh and dry weight ratio and slightly decreased root/shoot length ratio. Seedling of safflower also showed tolerance indices of 91.91, 77.82 and 62.27 to heavy metal mixture concentrations of 60, 120 and 180 mg kg^{-1}, respectively.

DISCUSSION

Metal toxicity is an important factor governing germination and growth of plants. The effects of toxic substances on plants are dependent on the amount of toxic substance taken up from the given environment. Germination and seedling establishment are vulnerable stages in the plant life cycle (Vange et al., 2004). Seedling growth is considered as an indicator of metal stress on plant ability to survive. The toxicity of some of the metals may be large enough that, plant growth is retarded before large quantities of the element can be transferred (Haghiri, 1973). We have investigated how heavy metal mixture treatment affected germination and early growth stage of safflower plant. Mixed cadmium, copper, nickel and zinc treatment decreased seed germination and seedling growth of safflower. Reduction in seed germination and seedling growth of safflower, provided evidence that, the metal elements like cadmium, copper, nickel and zinc if present in excess amount are responsible for producing toxic effects which reduced plant development. The decrease in seed germination and seedling growth due to heavy metal treatment is in conformity with the findings of other researchers (Ayaz and Kadioglu, 1997; Morzek and Funiceli, 1982; Iqbal and Mehmood, 1991; Jamal et al., 2006). For example, Rahman et al. (2010) observed a reduction in seed germination and seedling growth in chickpea treated with 50, 100, 200 and 400 ppm of nickel and cobalt. Dharam et al. (2007) observed a reduction in germination percentage and early growth stage of wheat treated with copper at 5, 25, 50, and 100 ppm. Treatment of *Leucaena leucocephala* with 25, 50, 75 and 100 ppm of lead and cadmium showed a gradual reduction in seed germination and seedling growth (Shafiq et al., 2008).

However, germination test showed a non significant effect on germination percentage of corn treated with low levels of zinc and copper (6 to 12 ppm) (Mahmood et al., 2005). According to Shafiq et al. (2008), decrease in seed germination of plant can be attributed to the accelerated breakdown of stored food materials in seed, by the application of heavy metal mixture. Reduction in seed germination can also be attributed to alterations of selection permeability properties of cell membrane. The reason for the reduced seedling growth in metal treatments could be as a result of the reduction in meristematic cells present in this region and some enzymes contained in the cotyledons and endosperm. Cells become active and begin to digest and store food which is converted into soluble form and transported to the primary root and shoot tips for enzyme amylase which converts starch into sugar and proteases act on proteins. So, when activities of hydrolytic enzymes are affected, the food does not reach to the primary root and shoot, thereby affecting the seedling length (Kabir et al., 2008). Several authors reported that, the inhibition of root elongation caused by heavy metals may be due to metal interference with cell division, including inducement of chromosomal aberrations and abnormal mitosis (Jiang et al., 2001; Huillier et al., 1996; Radha et al., 2010; Liu et al., 2003), which can be effected on seedling growth and explain the inhabitation of seedling growth in this investigation. Tolerance test showed that, tolerance to heavy metal mixture in safflower was relatively low, especially at 60 and 120 mg kg^{-1} as compared to control. The reason for low tolerance against heavy metal mixtures might be due to changes in the physiological mechanism in seed germination and seedling growth of safflower. Shafiq and Iqbal (2005) reported similar results for low tolerance in *Cassia siamea* seedlings at 100 ppm of lead and cadmium treatments as compared to control.

General observation in this study can conclude that, heavy metal mixture treatment produced toxic impact on germination and seedling growth of safflower. Increase in the concentrations of heavy metals mixture in the soil, brought up changes in most of the growth parameters of safflower. Cultivation of safflower in metal polluted soils should be avoided or appropriate control measures should be adopted to maintain the heavy metal content of the soil below the damage threshold level. The heavy metal mixture treatment of 60 mg kg^{-1} exhibited the lowest percentage of tolerance in germination and seedling growth characteristics of safflower as compared to control. The identification of the toxic concentration of metals and tolerance indices of plant species, such as safflower, would be helpful for the establishment of an environment quality standard. The findings can also contribute to better physiological fragility, the potential of safflower in coordinating crop management programmes in metal contaminated areas. Furthermore, research studies with different metal stresses can be helpful in the solution of various problems associated with metal pollution in agricultural regions.

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