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Short Communication

Phytotoxic Effects of Heavy Metals on Seed Germination and Seedling Growth of Medical Plant, Hyssop (*Hyssopus officinalis* L.) in Laboratory Conditions

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Abstract

To assess the phytotoxicity of cadmium (Cd), chromium (Cr), copper (Cu), Nickel (Ni) and zinc (Zn) on seed germination and seedling growth of *Hyssop* (*Hyssopus officinalis* L.), experiments were performed in different aqueous concentrations (50, 100 and 150µM) of aforementioned heavy metals over the period of 14 sequential days. The results showed that heavy metals adversely affect the normal growth of plants by decreasing seed germination, reducing root and shoot length, and decreasing root and shoot weight. The toxicity effects of chosen heavy metals on seed germination can be organized by the grade order of inhibition as: Cr>Cd>Cu>Ni>Zn. The minimum root and shoot length were observed in Cd (150µM) and Cr (150µM) respectively. The minimum of fresh and dry root weight were recorded at Cd (150µM) and the minimum of fresh and dry shoot weight were observed at Cr (150 µM). These results illustrate a model system for different concentrations of heavy metals for their phytotoxicity effects and also for the seeds' ability to negate the harmful effects of heavy metals in different types of irrigation waters and soils.

Keywords: Germination, Seedling growth, Heavy metal, Phytotoxicity, *Hyssopus officinalis* L.

Introduction

Hyssopus officinalis L. is an important medical plant in the Labiatae. This species is cultivated in some countries such as Russia, Spain, France, Italy and Iran which is widely used in the pharmaceutical industry [1,2]. Despite its bitter taste, it is used as a flavor enhancer in sauces and foods [3]. The essential oil of this plant has anti-fungal and anti-bacterial properties [1,3]. It is used in traditional and modern medicine as an appetizer. *H. officinalis* is also used as a cure for digestive disorders, asthma, laryngitis, herpes, bronchitis, and to help heal wounds [1,2].

The presence of heavy metals in soils could be beneficial or toxic to the environment. Excess of heavy metals causes some common effects of individual metals on various plants (i.e. both micro and macro flora) [4]. The biota may need some of these elements in trace quantities but in higher concentrations of heavy metals there can be toxicity effects [4]. Many soils particularly those in hazardous waste locations are contaminated with heavy metal e.g. cadmium (Cd), chromium (Cr), copper (Cu), Nickel (Ni) and zinc (Zn). The metal ion concentration in soil depends on the total metal content in soils and the pH of the soil [5]. Metals can also penetrate from soil into groundwater and cause soil pollution and inhibit plant growth [6]. Metal pollution of soils by disposal of sewage sludge or by atmospheric deposition disposal of sewage sludge has the risk of either excessive accumulation on top of the soil or leaching of metals into the groundwater [5]. They reach in aquatic ecosystems as solid and dissolved waste from industrial, domestic, and agricultural runoff.

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Many industries such as metal producing, textile, battery and cable manufacturing, electroplating, tannery, mining, steel, and automotive release heavy metals such as Cd, Cr, Cu, Ni and Zn in waste waters [7-8]. These heavy metals could be toxic to human health and aquatic ecosystems and they also accumulate in plants. The agglomeration of these heavy metals in plants causes biochemical and physiological changes [9,11]. Heavy metals such as Cd, Cr, Cu, Ni and Zn are highly toxic pollutants [4]. Cd interferes with homeostasis of micronutrient [12] and the ability of plant genotypes to detoxify of Cd can differ within and between the plant species [13,14]. Cr compounds have highly toxicity effects on plants and they are harmful to plant development and growth. Although some crops are not influenced by low concentration of Cr (3.8×10^{-4}μM) [15], phytotoxicity of Cr can cause inhibition of seed germination, antioxidant enzymes, nutrient balance, degrade pigment status and cause oxidative stress in plants [16-19]. Cu performs as a structural component in regulatory proteins and participates in mitochondrial respiration, photosynthetic electron transport, cell wall metabolism, hormone signaling, and oxidative stress responses [20,21]. Cu at high concentrations can become highly toxic causing symptoms such as necrosis and chlorosis, stunting, inhibition of root growth and leaf discoloration [20,22]. Ni known as a trace element, its metabolism is very decisive for maintaining proper cellular redox state, certain enzyme activities and other physiological, biochemical, and growth responses. Ni is necessary for plants [23-25] but the concentration of Ni in the majority of plants is very low (0.05–10 g/kg dry weight) [26]. In addition, increasing Ni pollution, excess Ni rather than a deficiency, is usually more observed in plant species [27-28]. Toxicity effects of Ni high concentrations in plants have been often reported, for example decreasing in plant growth [29], adverse effects on fruit quality and yield [30] and inhibition of mitotic activities [31]. Farmlands that are not suitable for growing crops, fruits and vegetables result in extremely high soil Ni concentrations [32]. Zinc acts as a plant nutrient [33,34] but at higher concentrations has toxic effects on plants [5]. Inhibition of growth is a common phenomenon associated with toxicity of zinc [36]. In plants, cellular activities that are mainly influenced by heavy metal pollution include: respiration, mineral nutrition, photosynthesis, gene expression, membrane structure, and other characteristics [11,37]. The first target of heavy metal toxicity is plant membrane structure [38]. The mutagenic ability of heavy metals leads to DNA damage and has carcinogenic effects in humans and animals [39,40]. Germination inhibition and plant growth retardation are commonly observed due to toxicity of heavy metal [41]. High concentrations of heavy metals in soils demonstrate a potential of threat for human health because it participates in the food chain principally by plant uptake [42].

The aim of this study was to investigate the phytotoxicity effects of Cd, Cr, Cu, Ni and Zn on seed germination and seedling growth of *Hyssop* (*Hyssopus officinalis* L.).

Material and Methods

Seeds of *H. officinalis* L. were obtained from the National Seed Gene Bank, Karaj, Iran. To destroy seed-borne microorganisms, all seeds were sterilized by 5% sodium hypochlorite solution for 5 minutes and washed three times with sterilized distilled water. The seeds were placed in Petri dishes on two layers of filter paper. Each dish contained 50 seeds. The experiment used a completely randomized design with four replicates per treatment.

Treatments included: control, Cd, Cr, Cu, Ni and Zn. The used concentrations were 50, 100 and 150μM prepared in distilled water. Control was without any heavy metal. The experiments were carried out in a programmed incubator at 25±2 ºC. Radicle emergence to a length of 2-5 mm was the criterion for seed germination [43]. Germination was recorded every day for 14 days, at which time the germination percentage was calculated. The seedlings were harvested after 14 days of incubation and root and shoot lengths and root and shoot fresh weights were recorded. Then the seedlings were dried in an oven at 80 ºC for 24 h and root and shoot dry weights were measured.

Statistical Analysis

Statistical analyses were carried out using SPSS 11.5. All data were analyzed by one-way analysis of variance (ANOVA) to determine the effect of treatments, and LSD multiple mean comparison test at $P\leq0.05$ was performed to determine the statistical significance of the differences between means of treatments.

Results

Effects of Heavy Metals on Seed Germination

The effects of the concentrations of Cd, Cr, Cu, Ni and Zn on seed germination of *H. officinalis* L. is presented in table 1. The results showed that seed germination decreased with increasing of heavy metals concentration (P≤0.01). The mean of seed germination values in all heavy metal treatments were significantly lower in comparison to control (P≤ 0.05). Zn had less inhibitory effect on seed germination when compared with the same concentrations of other heavy metals and maximum inhibitory were recorded in Cr (150 µM) with 43%.

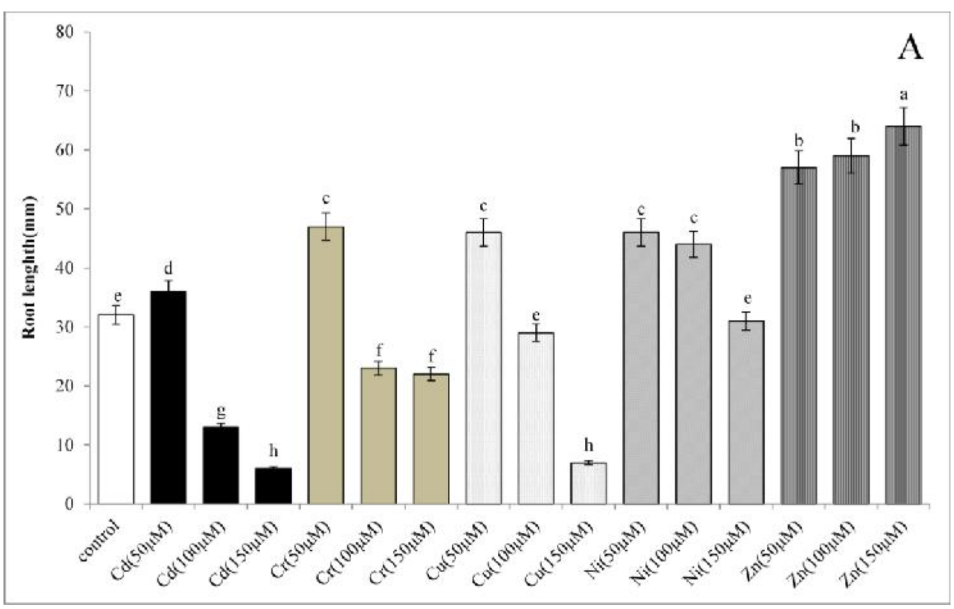
Effect of Heavy Metals on Root and Shoot Growth

The results of root and shoot growth are presented in Figure 1. A one-way ANOVA showed that the effects of heavy metal concentrations on root growth differed significantly (P≤0.01). The 50 µM of Cd, Cr, Cu, Ni and Zn significantly promoted the root growth as compared to the root growth of the control plants (P ≤ 0.05). Cd, Cr, Cu and Ni showed a concentration dependent inhibition of root growth at 100 and 150 µM. The results showed that with increasing concentration of Cd, Cr, Cu and Ni, the root length decreased but with increasing concentration of Zn the root length were increased. The maximum root length was observed at Zn (150 µM) with 64mm and minimum of root length was recorded in Cd (150 µM) with 6mm. The results showed that with increasing of heavy metal concentration, the shoot length decreased (P≤0.01). Zn had less inhibitory effects on shoot length when compared with the same concentrations of other heavy metals and maximum inhibition was recorded in Cr (150 µM). The minimum shoot length was related to Cr (150 µM) with 17mm.

Effect of Heavy Metals on Root and Shoot Fresh and Dry Weight

The effects of the concentrations of Cd, Cr, Cu, Ni and Zn on Root and Shoot fresh and dry weight of *H. officinalis* L. are presented in table 1. The results showed that with increasing of Cd, Cr, Cu and Ni concentrations, root and shoot fresh and dry weight decreased (P≤0.01). The minimum values of root fresh and dry weight were recorded in Cd (150µM) with 0.3 and 0.1 g, respectively. Also the results showed that the minimum shoot fresh and dry weights were observed at Cr (150 µM) with 0.85 and 0.28 g, respectively.

Table 1. Overall mean values for various traits of *H. officinalis* germination percentage and seedlings grown under different concentrations of heavy metals (Means followed by the same letter are not significantly different at the 0.05 level of probability by LSD test. Data are the mean ±S.E. n=4)



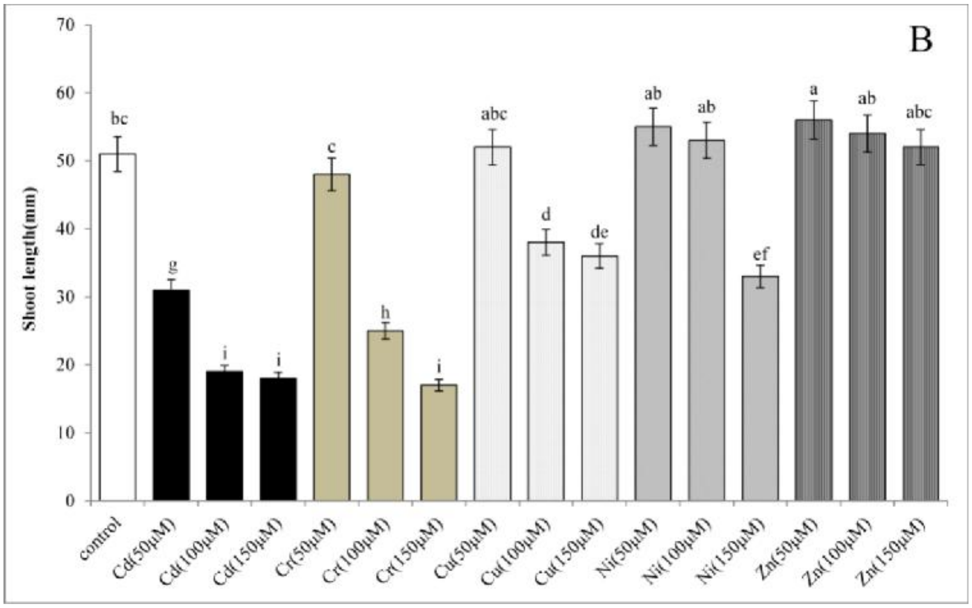


Fig. 1 Effect of different heavy metal concentrations on root length (A) and shoot length (B) of *H. officinalis*. Different letters show significant differences between means at $P \leq 0.05$ (LSD test).

Table 1 Overall mean values for various traits of *H. officinalis* germination percentage and seedlings grown under different concentrations of heavy metals (Means followed by the same letter are not significantly different at the 0.05 level of probability by LSD test. Data are the mean ±S.E. n=4)

Treatments	Concentration(μM)	Germination (%)	Root fresh weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)
Control	0	98±0.57 a	1.60±0.09 e	2.55±0.00 d	0.53±0.00 f	0.85±0.02 d
Cd	50	78±0.91 fg	1.80±0.12 d	1.55±0.01 i	0.60±0.00 e	0.51±0.00 h
	100	64±0.91 h	0.65±0.00 g	0.95±0.00 k	0.21±0.01 i	0.31±0.00 j
	150	52±1.29 j	0.30±0.00 h	0.90±0.00 kl	0.10±0.00 j	0.30±0.01 j
Cr	50	81±1.08 def	2.35±0.00 c	2.40±0.00 e	0.78±0.00 c	0.80±0.01e
	100	76±1.47 g	1.15±0.00 f	1.25±0.01 j	0.38±0.00 h	0.41±0.00 i
	150	43±1.47 k	1.10±0.00 f	0.85±0.01 l	0.36±0.00 h	0.28±0.01 j
Cu	50	86±0.91 c	2.30±0.09 c	2.60±0.11cd	0.76±0.00 cd	0.86±0.01 cd
	100	77±0.91 g	1.45±0.00 e	1.90±0.02 f	0.48±0.01 g	0.63±0.01 f
	150	58±1.47 i	0.35±0.00 h	1.80±0.01 g	0.11±0.00 j	0.60±0.01 f
Ni	50	82±0.91 de	2.30±0.12 c	2.70±0.00 ab	0.76±0.01 cd	0.92±0.01 ab
	100	78±0.91 fg	2.20±0.01 c	2.65±0.01 cd	0.73±0.01 d	0.88±0.01 bcd
	150	63±1.47 h	1.55±0.01 e	1.65±0.00 h	0.51±0.01 fg	0.55±0.01 g
Zn	50	92±0.91 b	2.85±0.00 b	2.80±0.01 a	0.95±0.00 b	0.93±0.01 a
	100	83±0.91 cd	2.95±0.00 b	2.70±0.02 bc	0.98±0.00 b	0.90±0.01 abc
	150	79±1.68 efg	3.20±0.02 a	2.60±0.02 cd	1.06±0.00 a	0.86±0.01 cd

Discussion

Heavy metals have potentially highly toxic effects on all organisms including plants and animals. Repressions of plant growth and seed germination responses have been related to the establishment of higher toxicity effect syndrome (HTES) caused by high accumulation of the metallic salts in the biomass of plant body [44-45]. In this study seed germination decreased with increasing heavy metal concentrations. The previous studies showed that heavy metals have negative effects on seed germination [46-51]. The decline in seed germination may be a result of heavy metals altering and interfering with the cell membrane penetrability properties, which is due to reduced absorption and transportation of water as well as the decrease in water stress tolerance [52-54]. In this study with increasing of Cd, Cr, Cu, Ni concentrations, root and shoot length and root and shoot weight decreased. Oncel *et al*. [55] reported similar effects of using cadmium in seedlings of wheat. Neelima and Reddy [56] investigated the effect of cadmium and mercury on seed germination, seedling growth, fresh and dry weights in *Solanum melongena*. They reported that both cadmium and mercury showed drastic effects at higher concentration on seed germination, seedling growth and fresh and dry weights. The reduction in growth performance of *H. officinalis* may be attributed to the decrease in meristamatic cells and the inhibitory effect on the hydrolytic enzyme activities included in the endosperms and cotyledon as a result of the toxicity of heavy metals. Hydrolytic enzymes are necessary for the mobilization of stored food materials to reach the plumule and radicle for the growth of seedlings [57]. The decline in growth parameters of *H. officinalis* can also be due to hastened breakdown of stored food materials in seeds by heavy metals [58,59]. The reduction in fresh and dry weight seedlings may be a result of inhibition of photosynthesis chlorophyll synthesis by increasing of heavy metal concentrations [60]. Kabir *et al*. [61] report on *Thespesia populnea* also confirms our current results. In this study, with the increase of Zn concentrations, Root length and root fresh and dry weight increased and shoot length and shoot fresh and dry weight decreased. The Zn phytotoxicity is shown by reduces in metabolism, growth, and development in different plant species such as *Brassica juncea* [62] and *Phaseolus vulgaris* [63]. High levels of Zn inhibit many functions of plant metabolism and cause retarded growth and senescence. Toxicity effects of high levels of Zn in plants limits the growth of both shoot and root [62, 64-67]. In this study Zn had less inhibitory effects on seed germination compared to other heavy metals and promoted the root and shoots growth and root and shoot fresh and dry weight as compared to the control plants. This shows that Zn does not influence seed germination but assists in radicle and plumule development in the aforementioned concentrations but it may be toxic at higher concentrations.

Conclusion

Heavy metal stress is one of the important problems affecting seed germination. Heavy metal concentrations accumulate in important agricultural crops, which are necessary components of the food chain leading to the impairment of animals and humans. The investigation of results showed that heavy metals have inhibitory effects on seed germination and early seedling growth in *H. officinalis*. The inhibition intensity was directly proportionate to the concentration of heavy metal solutions used in this study. High concentrations of heavy metals lead to decrease in percentage of tolerance indices in *H. officinalis,* which was clearly evident from the seedling growth inhibition. Uptake of heavy metals by the roots and their transmission to the shoots at higher concentrations could cause severe reduction in biomass production and seedling growth. These results illustrate a model system for different concentrations of heavy metals for their phytotoxicity effects and also for the seeds' ability to negate the harmful effects of heavy metals in different types of irrigation waters and soils.

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