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Influence of Heavy Metals on Seed Germination and Early Seedling Growth in *Eruca sativa* Mill.

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Abstract

Heavy metals present in soil and water naturally or as contaminants from human activities can cause bioaccumulation affecting the entire ecosystem and pose harmful health consequences in all life forms. Some famous non-food hyperaccumulators such as Thlaspi caerulescens, Sedum alfredii, Pteris vittata, Arabidopsis halleri and Athyrium yokoscense are of very little economic value, making it difficult for them to be used for phytoremediation. In this paper, the influence of heavy metals Cu, Ni, Zn, Hg, Cr, Pb and Cd on seed germination and early seedling growth in oil crop Eruca sativa was evaluated under laboratory conditions. Our results indicated that among the 7 heavy metals tested, only Ni at higher concentrations (1 mM and above) significantly decreased the Eruca seed germination in a dose-dependent manner. All heavy metals except Zn and Ni decreased the root length first, then the shoot length or the fresh seedling weight, and seed germination was always the last to be influenced. With Ni, the root length, shoot length and fresh seedling weight were stimulated when Ni concentrations were under 1 mM; with Zn, the root length, shoot length and fresh seedling weight were increased by all concentrations tested (0.20 - 5.0 mM). Our results indicated that Eruca is tolerant or moderately tolerant to Cu, Hg, Cr, and Cd and highly tolerant to Pb, Ni and Zn, and can be developed as an industrial oil crop for phytoremediation of soils contaminated by heavy metals.

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Keywords

Eruca sativa, Heavy Metal, Phytoremediation, Seed Germination, Early Seedling Growth

1. Introduction

Heavy metals such as Cu, Zn, Ni, Hg, Cd, Cr and Pb present in soil and water naturally or as contaminants from human activities can cause bioaccumulation affecting the entire ecosystem and pose harmful health consequences in all life forms [1]. Application of phytoextraction can reduce phyto-available metals in the soil and thereby diminish toxic metal contents in agricultural products. Some famous hyperaccumulators have been deeply researched such as Cd/Zn hyperaccumulator *Thlaspi caerulescens* [2] [3], *Sedum alfredii* [4] [5], As hyperaccumulator *Pteris vittata* [6] [7], Cd hyperaccumulator *Solanum nigrum* [8], *Arabidopsis halleri* [9], *Athyrium yokoscense* and a number of ferns belonging to the genus *Pteris* [10]. However, these hyperaccumulators are of very little economic value, making it difficult for them to be used in phytoremediation.

Eruca sativa Mill. in Brassicaceae family is an important marginal crop grown on soil with reduced fertility and is preferred over other relative species for its tolerance and adaptability to unfavorable environmental conditions [11]-[15]. Eruca lines with larger and yellow seeds, higher plant and seed yield were available [16]. Its seed oil is used for human nutrition, medicinal and cosmetic properties, and as a lubricant [14] [17]. By using its regeneration [18] and genetic transformation system [19] available, Eruca can be developed into a safe industrial oil crop, because of its low cross ability with the edible oilseed rape [20] and its resistance to powdery mildew [12], stem rot [21] and salt [22]. The present study was made to determine the influence of heavy metals Cu, Ni, Zn, Pb, Cd, Cr and Hg on Eruca seed germination, early seedling growth and the potential of using Eruca for phytoremediation of soils contaminated by heavy metals.

2. Materials and Methods

Healthy seeds of *Eruca sativa* cv. hubu-14 from Hubei University were inoculated on sand cultures with different concentrations of heavy metals. The heavy metals except Pb were dissolved in liquid MS without sugar and organic components. Pb was dissolved in ddH₂O to avoid precipitation. Different concentrations of heavy metals were prepared from CuSO₄·5H₂O, ZnSO₄·7H₂O, NiSO₄·6H₂O, K₂Cr₂O₇, Pb(NO₃)₂, HgCl₂ and CdCl₂·2½H₂O. Seed germination rates were scored 4 days after inoculation and root length, shoot length and fresh seedling weight were measured 7 - 8 days after seed inoculation. The relative seed germination, root length, shoot length and fresh seedling weight were calculated as that of treatments with heavy metals divided by that of controls. The incubation temperature was set at 25°C with a 16-hr photo period under 2000 lx. The experiment was arranged in a completely randomized design with three replicates, each replicate with about 50 *Eruca* seeds. Variance analyses and multiple comparisons were carried out on SPSS 19.0.

3. Results and Discussion

Variance analyses indicated that among the 7 heavy metals tested only Ni (1 mM and above) decreased significantly (P < 0.05) the *Eruca* seed germination in a dose-dependent manner (**Figure 1**), suggesting that *Eruca* seed germination was pretty tolerant to heavy metals. All heavy metals except Zn and Ni decreased the root length first, then shoot length or fresh seedling weight, and finally seed germination. With Ni the root length, shoot length and fresh seedling weight were stimulated when Ni concentrations were under 1 mM; with Zn the *Eruca* root length, shoot length and fresh seedling weight were increased by 0.2 - 5.0 mM Zn (**Figures 2-4**).

3.1. The Influence of Cu

In maize, the seed germination was increased by 3.98% at 0.1 mM Cu [23]. However, most studies indicated that Cu significantly decreased the seed germination. With 10 μ M Cu treatment, wheat and rice seed germination was reduced by more than 35% and 60%, respectively [24]. In alfalfa, 40 mg·L⁻¹ Cu inhibited significantly seed germination by 39.0% [25]. In *Crambe*, higher Cu concentration (0.7 mM or 44.8 mg·L⁻¹ and above) decreased *Crambe* seed germination significantly [26]. In our experiment, all Cu concentrations tested (0.3 - 1.2)

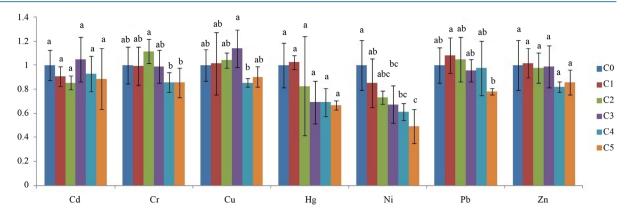


Figure 1. Influence of heavy metals on relative seed germination in Eruca. Note: Cd: C0 = 0, C1 = 0.10 mM, C2 = 0.20 mM, C3 = 0.30 mM, C4 = 0.38 mM, C5 = 0.46 mM; Cr: C1 = 0.05 mM, C2 = 0.10 mM, C3 = 0.20 mM, C4 = 0.40 mM, C5 = 0.80 mM; Cu: C1 = 0.30 mM, C2 = 0.50 mM, C3 = 0.70 mM, C4 = 0.90 mM, C5 = 1.20 mM; Hg: C1 = 0.10 mM, C2 = 0.20 mM, C3 = 0.30 mM, C4 = 0.40 mM, C5 = 0.50 mM; Ni: C1 = 0.20 mM, C2 = 0.40 mM, C3 = 1.00 mM, C4 = 3.00 mM, C5 = 5.00 mM; Pb: C1 = 0.80 mM, C2 = 3.20 mM, C3 = 4.00 mM, C4 = 5.00 mM, C5 = 5.50 mM; Zn: C1 = 0.20 mM, C2 = 0.40 mM, C3 = 1.00 mM, C4 = 3.00 mM, C5 = 5.00 mM.

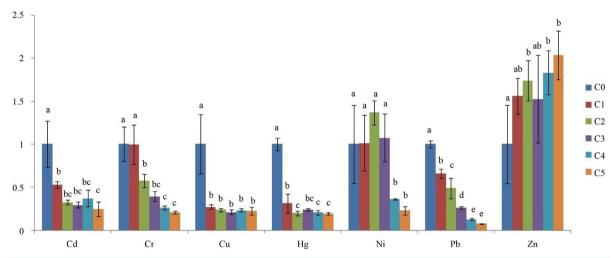


Figure 2. Influence of heavy metals on relative root length in *Eruca*. Note: the heavy metal concentrations are the same as in Figure 1.

mM) did not decrease the *Eruca* seed germination significantly (P > 0.05). Lower Cu concentration (<0.7 mM) even increased the seed germination (**Figure 1**), suggesting that *Eruca* seed germination is quite tolerant to Cu. In alfalfa Cu caused a shoot elongation reduction of 69.0% at the dose of 40 ppm [25]. Taylor and Foy [27] found 30 μ M Cu enough for reducing growth of wheat by 50%, whereas Wheeler *et al.* [28] reported that only 0.5 μ M Cu was required for a 50% growth reduction in the same species. In *Arabidopsis*, 0.2 mM Cu inhibited the seedling growth by about 60% [29]. In *Crambe*, 0.3 mM Cu decreased root length by 75.33%, shoot length by 29.44% and fresh seedling weight by 22.26% [26]. In our experiment, 0.3 mM Cu (19.2 mg·L⁻¹) decreased *Eruca* root length by 72.67%, shoot length by 24.67% and fresh seedling weight by 24% (**Figures 2-4**). This also indicated that *Eruca* is moderately tolerant to Cu regarding early seedling growth.

3.2. The Influence of Zn

In alfalfa a concentration of 40 $\text{mg} \cdot \text{L}^{-1}$ Zn did not significantly reduce the seed germination [25]. In wheat and rice the seed germination was not significantly affected by Zn concentration [24], but in another study on wheat, the germination was completely inhibited at 10 $\text{mg} \cdot \text{L}^{-1}$ Zn [30]. In *Crambe* 0.85 mM (55.25 $\text{mg} \cdot \text{L}^{-1}$) Zn did not significantly decrease *Crambe* seed germination [26]. Our results indicated that all Zn concentrations tested (0.2 -

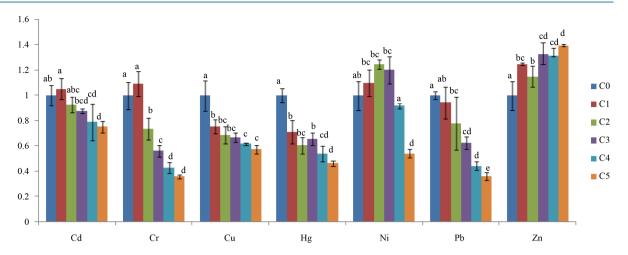


Figure 3. Influence of heavy metals on relative shoot length in *Eruca*. Note: the heavy metal concentrations are the same as in Figure 1.

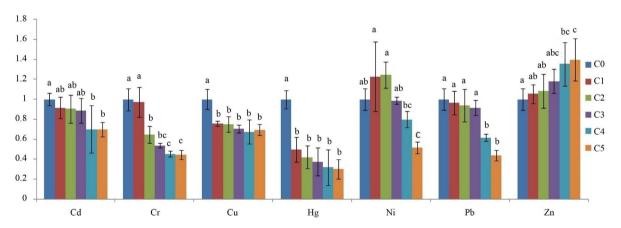


Figure 4. Influence of heavy metals on relative fresh seedling weight in *Eruca*. Note: the heavy metal concentrations are the same as in Figure 1.

5.0 mM) did not significantly decrease *Eruca* seed germination (Figure 1), suggesting that *Eruca* seed germination is very tolerant to Zn. In alfalfa all Zn concentrations increased the root length by more than 100.0%, and 40 ppm of Zn increased the shoot growth by 10% over control [25]. In Sedum alfredii the specific root length in non-hyperaccumulating ecotype was significantly reduced by 47.2%, while the SRL of hyperaccumulating ecotype was significantly increased under the treatment of 500 μM Zn [31]. In another study on S. alfredii, it was noted that root length, surface-area and volume of the hyperaccumulating ecotype increased obviously under 1224 μM Zn treatment, whereas in non-hyperaccumulating ecotype these parameter were decreased significantly [5]. In Arabidopsis thaliana the root length was decreased by about 55% while the shoot length was decreased by about 50% at 1 mM Zn [32]. In another study on Arabidopsis thaliana, the Zn concentration required for a 50% inhibition of root growth was 98 µM [33]. In Crambe, Zn inhibited root length by about 76.33%, shoot length by 43.48% and fresh seedling weight by 47.84% at 0.1 mM concentration [26]. In the hyperaccumulator species Thlaspi goesingense, the Zn concentration required for a 50% inhibition of root growth was higher than 500 μM. In a study on *Eruca* the root length was decreased by 31.54% and shoot length by 28.89% at 50 mg·L⁻¹ [34]. Ozdener and Aydin [35] found that the Eruca root length increased significantly by 54.79% and fresh root weight by 88.89% when 500 μg·g⁻¹ Zn was applied. In our experiment, the root length, shoot length and fresh seedling weight were increased by all Zn concentrations tested (0.20 - 5.0 mM). When the Zn concentration was increased to 5 mM, the root length was almost doubled, the shoot length was increased by 39.67% and fresh seedling weight was increased by 40% (Figures 2-4), suggesting that Eruca is highly tolerant to Zn regarding

early seedling growth.

3.3. The Influence of Ni

In alfalfa 40 ppm Ni inhibited significantly seed germination by 24.0% [25]. In maize Ni decreased the seed germination by 10.84% at $10~\mu$ M [23]. In another study on maize, at $50~mg\cdot L^{-1}$ Ni the seed germination was decreased by 11.70% [36]. In *Salicornia brachiata* the seed germination was decreased by 49.36% at $50~\mu$ M Ni [18]. In *Crambe* the root growth was completely inhibited at over $80~\mu$ M Ni [26]. In our experiment the *Eruca* seed germination was only significantly influenced by higher Ni concentrations (1 mM and above, **Figure 1**). Ni at 1 mM only decreased the seed germination by 32.67%, suggesting that *Eruca* seed germination is tolerant to Ni. In maize, $10~\mu$ M Ni decreased the root length by 19.44% and shoot length by 39.13% [36]. In hyperaccumulator species *Thlaspi goesingense*, Ni concentration required for 50% inhibition of root growth was higher than $500~\mu$ M [33]. In *Arabidopsis thaliana*, Ni concentration required for 50% inhibition of root growth was $80~\mu$ M [33]. Schaaf *et al.* [37] found that fresh weight of *Arabidopsis thaliana* was almost not affected by $50~\mu$ M Ni. In *Crambe* only $0.4~\mu$ M Ni significantly decreased root length by 5.33% and $1.2~\mu$ M Ni decreased root length by 71%, shoot length by 60% and fresh seedling weight by 73.42% [26]. In our experiment 0.4mM Ni significantly increased the root length by 24.67%, and fresh seedling weight also by 24.67%; only $3.0~\mu$ M Ni significantly decreased the root length by 63.67%, shoot length by 8.33%, and fresh seedling weight by 20.33% (**Figures 2-4**), suggesting that *Eruca* is highly tolerant to Ni regarding early seedling growth.

3.4. The Influence of Cd

In alfalfa the concentration of 40 ppm Cd inhibited significantly seed germination by 44.0% [25]. In wheat, the seed germination was decreased by 60% at 10 mg·L⁻¹ Cd [30]. In Sinapis arvensis, 1000 μM Cd significantly decreased the seed germination by 5.6% [38]. In Crambe, seed germination was not significantly affected by all Cd concentrations (0.10 - 0.46 mM) tested [26]. In our experiment the Eruca seed germination was not significantly affected by all the Cd concentrations (0.10 - 0.46 mM) tested in this study (Figure 1), suggesting that Eruca seed germination is tolerant to Cd. In castor bean 1 mg·L⁻¹ of Cd caused a reduction of 44% in the root dry mass and 53% in the shoot dry mass [39]. In Sinapis arvensis, the root length was decreased significantly by 92.62%, shoot length by 56.31% and fresh seedling weight by 49.69% at 150 µM Cd [38]. In Brassica juncea, the root length was decreased by about 37.5% at 0.20 mM Cd and fresh seedling weight by 70% at 0.05 mM Cd and more than 90% by 0.075 mM Cd [40]. In Arabidopsis halleri, 5 µM Cd decreased shoot growth by 45%, whereas 5 - 50 μM Cd had no significant effect on root biomass. At 100 μM Cd shoot and root growth were inhibited by 82% and 74%, respectively [41]. In Arabidopsis thaliana, the Cd concentration required for 50% inhibition of root growth was only 38 μM [33]. In Crambe, 0.1 mM Cd (11.2 mg·L⁻¹) decreased Crambe root length by 47.67%, shoot length by 33.67%, fresh seedling weight by 13% [26]. Plant height was significantly decreased at 50 and 100 ppm Cd in Raphanus sativus and in Eruca sativa at 50 ppm Cd only by 40.6%, 31.1%, and 14.7% respectively [42]. Fresh weight was significantly increased at 100 and 200 ppm Cd in Raphanus sativus by 17.2%, 27.6%, respectively. In Eruca sativa, fresh weight was significantly increased by addition of Cd at 200 ppm by 53.8%. Dry weight increased in Raphanus sativus at 50 - 200 ppm Cd [42]. In another study on Eruca, the root length was decreased by 27.69%, shoot length by 43.78% at 50 mg·L⁻¹ Cd [34]. In our experiment the root length was decreased by 47.33% at 0.10 mM Cd, while the shoot length and fresh seedling weight were not significantly influenced by 0.10 mM Cd (Figures 2-4), suggesting that Eruca is tolerant to Cd.

3.5. The Influence of Hg

In Festuca arundinacea, the seed germination was decreased by 4.00% at $50 \text{ mg} \cdot \text{L}^{-1}$ Hg [43]. In cucumber, the root and shoot growth was almost completely inhibited at $250 \mu\text{M}$ HgCl₂ [44]. In Brassica juncea, treatment with $2 \mu\text{M}$ Hg for 24 h inhibited the root growth by about 80% [45]. Also in Brassica juncea, treatment at $16.7 \text{ mg} \cdot \text{L}^{-1}$ Hg for two weeks decreased the dry weight of root and shoot by more than 60% [46]. In Brassica napus, the biomass was decreased by about 60% at $10 \text{ mg} \cdot \text{L}^{-1}$ Hg [47]. In Crambe, Hg significantly decreased Crambe seed germination at 0.3 mM ($60.3 \text{ mg} \cdot \text{L}^{-1}$) by 34.66%. The root length was decreased by 81.33%, shoot length by 46.34% and fresh seedling weight by 16.94% at 0.1 mM Hg [26]. In our experiment the Eruca seed germination was not significantly decreased by all the Hg concentrations tested (0.10 - 0.50 mM, Figure 1). 0.1 mM Hg

significantly decreased the root length by 68.33%, shoot length by 28.67% and fresh seedling weight by 50.33% (**Figures 2-4**), suggesting that *Eruca* is only moderately tolerant to Hg.

3.6. The Influence of Cr

In alfalfa, the concentration of 40 ppm Cr inhibited significantly seed germination by 54.0% [25]. In wheat the germination was decreased by about 30% at 500 ppm Cr [48]. In another study on wheat, seed germination was decreased by 80% at 10 mg·L⁻¹ Cr [30]. In *Crambe*, seed germination was not significantly decreased by Cr concentrations tested (0.05 - 0.80 mM or 2.6 - 41.6 mg·L⁻¹) [26]. In our experiment the *Eruca* seed germination was not significantly decreased by the Cr concentrations tested (0.05 - 0.80 mM, **Figure 1**), suggesting that *Eruca* germination is quite tolerant to Cr. In alfalfa Cr at 10 ppm increased root growth by approximately 36.0% [25]. In soybean at 400 and 500 ppm Cr concentration there was about 83% and 85% reduction in length of seedling respectively [48]. With *Crambe* the fresh weight of plants decreased moderately at 100 μ M K₂CrO₄, whereas at 150 μ M K₂CrO₄ there was a significant reduction in biomass with no symptoms of severe cellular damage, but at higher concentration (200 and 250 μ M), plant showed chlorosis and visible necrosis on leaves [49]. Also in *Crambe*, root length was decreased by 41.33%, shoot length by 15.66% and fresh seedling weight by 27.67% at 0.05 mM Cr [26]. In our experiment 0.10 mM Cr significantly decreased the root length by 42.33%, shoot length by 26.33% and fresh seedling weight by 35.33% (**Figures 2-4**), suggesting that *Eruca* is moderately tolerant to Cr regarding early seedling growth.

3.7. The Influence of Pb

In maize, a significant increase of germination rate was observed for seeds treated with 10 µM Pb, while a significant decrease of germination by 12.48% was determined at 5 mM Pb [23]. In Sinapis arvensis, the seed germination was decreased significantly by 6.17% at 1200 µM Pb [38]. In Crambe, seed germination was not significantly decreased by 5.5 mM Pb [26]. In our experiment Eruca seed germination was not significantly influenced by the Pb concentrations tested (0.8 - 5.5 mM, Figure 1), suggesting that Eruca seed germination is very tolerant to Pb. Castor bean demonstrated to be tolerant to 0 - 96 mg·L⁻¹ Pb concentrations [39]. In Sinapis arvensis, the root length was decreased significantly by 66.46%, shoot length by 38.62% and fresh seedling weight by 33.33% at 300 µM Pb [38]. In Crambe, root growth was decreased by 48.67%, shoot length by 16.33%, and fresh seedling weight by 16.33% at 0.8 mM Pb [26]. Fresh weight was significantly increased at 50 and 100 ppm Pb in Raphanus sativus by 31.0% and 10.3% respectively. In Eruca sativa, fresh weight was significantly increased by addition of Pb at 200 ppm by 61.5% in comparison with the control. Dry weight increased in Raphanus sativus and Eruca sativa at 50 - 200 ppm Pb [42]. With Pb treatment, Eruca root length was decreased by 20.0%, the shoot length by 23.78% at 50 mg·L⁻¹ [34]. In our experiment 0.8 mM Pb only decreased root length by 33.67%, 3.2 mM only decreased shoot length by 22.67% and 5 mM Pb decreased Eruca fresh seedling weight only by 38.67%, while 4 mM Pb did not significantly decrease the fresh seedling weight (Figures 2-4), suggesting that Eruca is highly tolerant to Pb.

4. Conclusion

Our results indicated that *Eruca* is tolerant or moderately tolerant to Cu, Hg, Cr, Cd and highly tolerant to Zn, Ni and Pb, and can be developed as an industrial oil crop for phytoremediation of soils contaminated by heavy metals. The heavy metal of tolerant *Eruca* can also be used for cloning genes responsible for heavy metal tolerances.

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