American Journal of Plant Sciences, 2015, 6, 150-156
Published Online January 2015 in SciRes. http://www.scirp.org/journal/ajps
http://dx.doi.org/10.4236/ajps.2015.61017

Influence of Heavy Metals on Seed Germination and Early Seedling Growth in *Crambe abyssinica*, a Potential Industrial Oil Crop for Phytoremediation

Jin Hu[1], Zhaohui Deng[2], Bin Wang[1], Yuan Zhi[1], Bingxue Pei[1], Guangyu Zhang[1], Mingdan Luo[1], Banglian Huang[1], Wenhua Wu[1*], Bangquan Huang[1]

[1]Hubei Collaborative Innovation Center for Green Transformation of Bio-Resources, College of Life Science, Hubei University, Wuhan, China
[2]Vocational and Technical College of Anshun, Anshun, China
Email: [*]1305142468@qq.com

Received 12 December 2014; accepted 25 December 2014; published 15 January 2015

Abstract

The influence of essential (Cu, Ni and Zn) and non-essential heavy metals (Hg, Cr, Pb and Cd) on seed germination and early seedling growth in industrial oil crop *Crambe abyssinica* was evaluated under laboratory conditions. Our results indicated that among the 7 heavy metals tested only Cu and Hg significantly ($P < 0.01$) decreased *Crambe* seed germination in a dose-dependent manner at higher concentrations while certain Cr concentrations significantly increased the seed germination ($P < 0.05$). All the 7 heavy metals decreased significantly relative root length, shoot length and fresh seedling weight in a dose-dependent manner ($P < 0.01$). The heavy metals except Ni decreased relative root length first, then shoot length or fresh seedling weight, and finally seed germination. Ni seemed to influence the relative fresh seedling weight first, then shoot length, root length and finally seed germination at lower concentrations, but the decrease in relative root length became faster when the Ni concentrations were increased. Our results indicated that *Crambe* is tolerant or moderately tolerant to the heavy metals tested except Ni and can be improved for phytoremediation of soils contaminated by heavy metals.

Keywords

***Crambe abyssinica*, Heavy Metal, Seed Germination, Early Seedling Growth, Phytoremediation**

[*]Corresponding author.

How to cite this paper: Hu, J., *et al*. (2015) Influence of Heavy Metals on Seed Germination and Early Seedling Growth in *Crambe abyssinica*, a Potential Industrial Oil Crop for Phytoremediation. *American Journal of Plant Sciences*, **6**, 150-156.
http://dx.doi.org/10.4236/ajps.2015.61017

1. Introduction

Heavy metals such as Cu, Zn and Ni are essential micronutrients for plants, but are toxic at high concentrations. Other heavy metals like 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]. Unlike organic pollutants, heavy metals cannot be chemically degraded or biodegraded by microorganisms [2]. One alternative biological approach to deal with this problem is phytoremediation—the use of plants to remove, destroy or sequester hazardous substances from environment. It has become a topical research field in the last decades because it offers advantages of being safe, *in situ*, nondestructive and potentially cheap compared with traditional remediation techniques [3]-[8]. Application of phytoextraction can reduce phytoavailable 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* [9] [10], *Sedum alfredii* [11] [12], As hyperaccumulator *Pteris vittata* [13] [14], Cd hyperaccumulator *Solanum nigrum* [15], *Arabidopsis haller*i [16], *Athyrium yokoscense* and a number of ferns belonging to the genus *Pteris* [17]. However, these hyperaccumulators are of very little economic value, making it difficult for them to be used in phytoremediation.

Crambe abyssinica is a Cruciferae member that is a promising industrial oil crop since it shows high seed yield potential and high-erucic acid content in its seed oil [18]-[20]. The erucic acid content in *Crambe* seed oil was further increased to over 70% by genetic engineering [21]. *Crambe* can be developed into a relatively “safe” GMO industrial oil crop for phytoremediation with both environmental as well as economic values since it does not cross with the edible double-low canola [22]. Previous studies indicated that *Crambe* was not only highly tolerant to As and Cd, but also accumulated significantly higher levels of As than other Brassica species [23] [24]. The present study was made to determine the influence of essential (Cu, Ni and Zn) and non-essential metal ions (Pb, Cd, Cr and Hg) on *Crambe* seed germination, early seedling growth and the potential of using *Crambe* for phytoremediation of soils contaminated by heavy metals.

2. Materials and Methods

Healthy *Crambe* seeds 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 ddH_2O to avoid precipitation. Different concentrations of heavy metals were prepared from $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $NiSO_4 \cdot 6H_2O$, $K_2Cr_2O_7$, $Pb(NO_3)_2$, $HgCl_2$ and $CdCl_2 \cdot 2.5H_2O$. 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 *Crambe* seeds. Variance analyses and multiple comparisons were carried out on SPSS 19.0.

3. Results and Discussion

Figure 1 indicated that among the 7 heavy metals tested only higher concentrations of Cu (0.7 mM or 44.8 $mg \cdot L^{-1}$ and above) and Hg (0.3 mM or 60.3 $mg \cdot L^{-1}$ and above) decreased *Crambe* seed germination significantly ($P < 0.01$) in a dose-dependent manner, while certain concentrations of Cr significantly increased seed germination ($P < 0.05$). **Figures 2-4** indicated that all the 7 heavy metals tested significantly decreased *Crambe* relative root length, shoot length and fresh seedling weight in a dose-dependent manner. Heavy metals except Ni decreased relative root length first, then shoot length or fresh seedling weight, and finally seed germination. Ni seemed to influence relative fresh seedling weight first, then shoot length, root length and finally seed germination at lower concentrations, but the decrease in relative root length became faster when Ni concentrations were increased.

3.1. The Influence of Cu

Most studies indicated that Cu significantly decreased seed germination. With 10 μM Cu treatment, wheat and rice seed germination was reduced by more than 35% and 60%, respectively [25]. In alfalfa 40 $mg \cdot L^{-1}$ Cu inhi-

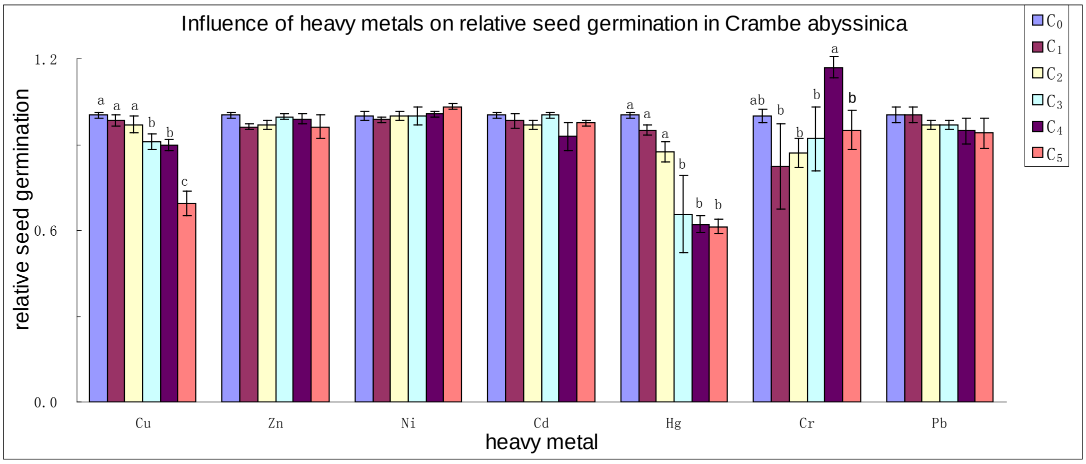

Figure 1. Influence of heavy metals on relative seed germination in *Crambe abyssinica*. Note: C_0 = without heavy metal; With Cu, C_1 = 0.3 mM, C_2 = 0.5 mM, C_3 = 0.7 M, C_4 = 0.9 M, C_5 = 1.2 mM; with Zn, C_1 = 0.10 mM, C_2 = 0.40 mM, C_3 = 0.55 mM, C_4 = 0.70 mM, C_5 = 0.85 mM; with Cr, C_1 = 0.05 mM, C_2 = 0.10 mM, C_3 = 0.20 mM, C_4 = 0.40 mM, C_5 = 0.80 mM; with Ni, C_1 = 0.4 μM, C_2 = 0.6 μM, C_3 = 0.8 μM, C_4 = 1.0 μM, C_5 = 1.2 μM; with Pb, C_1 = 0.8 mM, C_2 = 3.2 mM, C_3 = 4.0 mM, C_4 = 5.0 mM, C_5 = 5.5 mM; with Hg, C_1 = 0.1, C_2 = 0.2 mM, C_3 = 0.3 mM, C_4 = 0.4 mM, C_5 = 0.5 mM; with Cd, C_1 = 0.10 mM, C_2 = 0.20 mM, C_3 = 0.30 mM, C_4 = 0.38 mM, C_5 = 0.46 mM. Figures with same letters were not significant at 0.05 level.

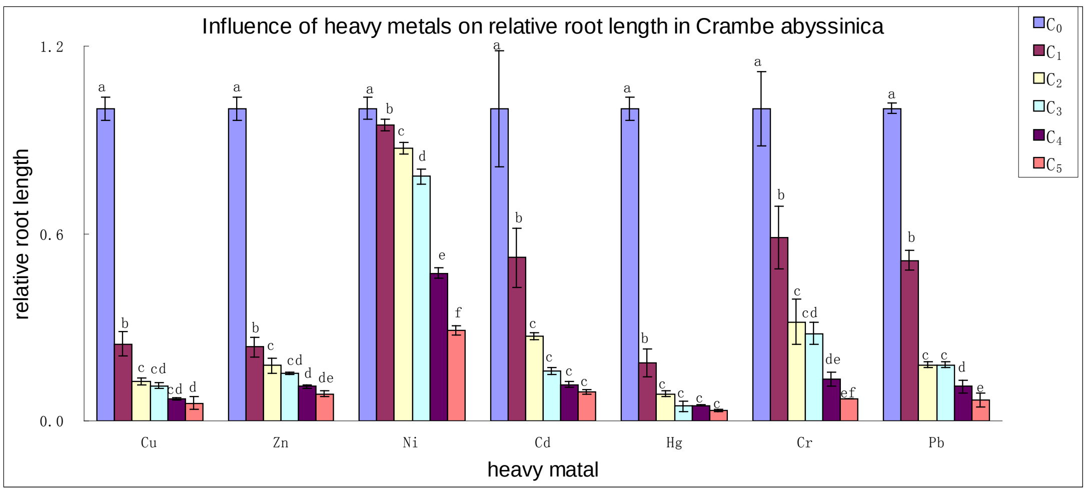

Figure 2. Influence of heavy metals on relative root length in *Crambe abyssinica*. Note: Explanations are same as in **Figure 1**.

bited significantly seed germination by 39.0% [26]. In our experiment, only higher Cu concentration (0.7 mM or 44.8 mg·L^{-1} and above) decreased *Crambe* seed germination significantly ($P < 0.01$). 0.7 mM Cu decreased *Crambe* seed germination by 9.30% and 1.2 mM Cu by 30.90% (**Figure 1**), suggesting that *Crambe* seed germination is moderately tolerant to Cu. Taylor and Foy [27] found 30 μM Cu enough for reducing growth of wheat (*Triticum aestivum* L.) by 50%, whereas Wheeler *et al*. [28] reported only 0.5 μM Cu was required for a 50% growth reduction in the same species. In *Arabidopsis* 0.2 mM Cu inhibited seedling growth by about 60% [29]. In our experiment, 0.3 mM Cu (19.2 mg·L^{-1}) decreased relative root length by 75.33% (**Figure 2**), shoot length by 29.44% (**Figure 3**) and fresh seedling weight by 22.26% (**Figure 4**), suggesting that *Crambe* is moderately tolerant to Cu regarding early seedling growth.

3.2. The Influence of Zn

In alfalf 40 mg·L^{-1} Zn did not significantly reduce seed germination [26]. In wheat seed germination was com-

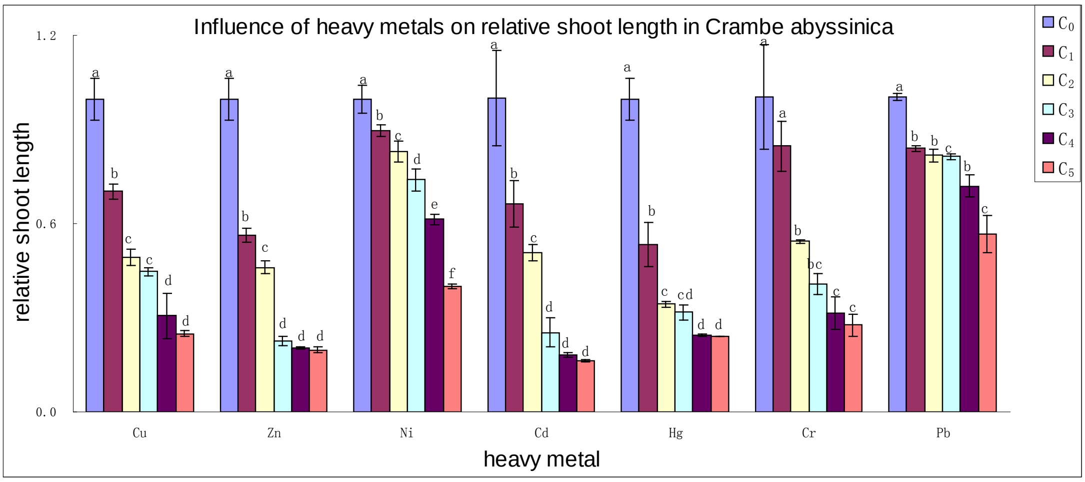

Figure 3. Influence of heavy metals on relative shoot length in *Crambe abyssinica*. Note: Explanations are same as in **Figure 1**.

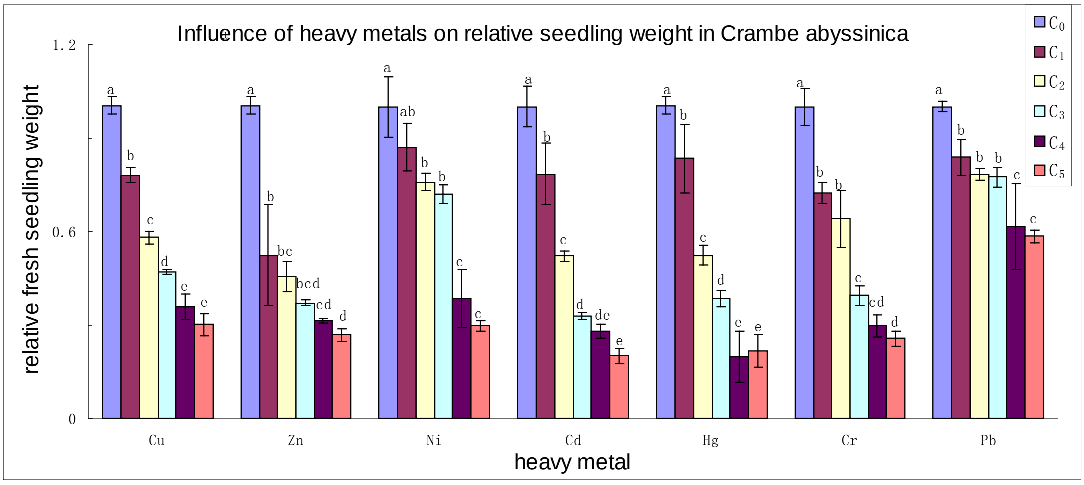

Figure 4. Influence of heavy metals on relative fresh seedling weight in *Crambe abyssinica*. Note: Explanations are same as in **Figure 1**.

pletely inhibited at 10 mg·L^{-1} Zn [30]. Our results indicated that even 0.85 mM (55.25 mg·L^{-1}) Zn did not significantly decrease *Crambe* seed germination (**Figure 1**), suggesting that *Crambe* seed germination is tolerant to Zn. In *Eruca* the root length was decreased by 31.54% and shoot length by 28.89% at 50 mg·L^{-1} Zn [31]. In hyperaccumulator species *Thlaspi goesingense*, Zn concentration required for 50% inhibition of root growth was higher than 500 μM, while in *Arabidopsis thaliana* Zn concentration required for a 50% inhibition of root growth was 98 μM [32]. In our experiment, Zn inhibited root length by about 76.33% (**Figure 2**), shoot length by 43.48% (**Figure 3**) and fresh seedling weight by 47.84% (**Figure 4**) at 0.1 mM concentration (6.5 mg·L^{-1}), suggesting that *Crambe* is only moderately tolerant to Zn regarding early seedling growth.

3.3. The Influence of Ni

In alfalf, 40 mg·L^{-1} Ni inhibited significantly seed germination by 24.0% [26]. In maize at 50 mg·L^{-1} Ni seed germination was decreased only by 11.70% [33]. In our experiment *Crambe* seed germination was not significantly influenced by Ni concentrations tested in this study (0.4 - 1.2 μM or 23.6 - 70.8 μg·L^{-1}, **Figure 1**), but our preliminary study indicated that *Crambe* root growth was completely inhibited at over 80 μM Ni (data not shown). In maize, 10 μM Ni decreased root length by 19.44% and shoot length by 39.13% [33]. In hyperaccumulator species *Thlaspi goesingense* the Ni concentration required for 50% inhibition of root growth was higher than 500 μM, while in *Arabidopsis thaliana* the Ni concentration required for 50% inhibition of root growth was 80 μM [32]. In our experiment 0.4 μM Ni significantly decreased root length by 5.33% and 1.2 μM Ni decreased root length by 71% (**Figure 2**), shoot length by 60% (**Figure 3**) and fresh seedling weight by 73.42% (**Figure 4**), indicating that early *Crambe* early seedling growth is quite sensitive to Ni.

3.4. The Influence of Cd

In wheat 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% [34]. In our experiment *Crambe* seed germination was not significantly affected by all Cd concentrations (0.10 - 0.46 mM) tested in this study (**Figure 1**), suggesting that *Crambe* seed germination is tolerant to Cd. In *Eruca* the root length was decreased by 27.69% and shoot length by 43.78% at 50 mg·L^{-1} Cd [31]. 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 [34]. In *Arabidopsis halleri* the shoot and root growth was inhibited by 82 and 74% respectively at 100 μM Cd [35]. In *Arabidopsis thaliana* the Cd concentration required for 50% inhibition of root growth was only 38 μM [32]. In our experiment 0.1 mM Cd (11.2 mg·L^{-1}) decreased *Crambe* root length by 47.67% (**Figure 2**), shoot length by 33.67% (**Figure 3**), fresh seedling weight by 13% (**Figure 4**) without visible chlorosis, suggesting that *Crambe* is moderately tolerant to Cd regarding early seedling growth.

3.5. The Influence of Hg

In *Brassica juncea*, treatment with 2 μM Hg for 24 h inhibited root growth by about 80% [36]. In *Brassica napus* the biomass was decreased by about 60% at 10 mg·L^{-1} Hg [37]. In our experiment Hg only significantly decreased *Crambe* seed germination at 0.3 mM (60.3 mg·L^{-1}) by 34.66% (**Figure 1**). *Crambe* root length was decreased by 81.33% (**Figure 2**), shoot length by 46.34% (**Figure 3**) and fresh seedling weight by 16.94% (**Figure 4**) at 0.1 mMHg (20.1 mg·L^{-1}), suggesting that *Crambe* early seedling growth is only moderately tolerant to Hg.

3.6. The Influence of Cr

In alfalf, 40 ppm Cr inhibited significantly seed germination by 54.0% [26]. In wheat seed germination was decreased by 80% at 10 mg·L^{-1} Cr [30]. In our experiment *Crambe* seed germination was not significantly decreased by Cr concentrations tested (0.05 - 0.80 mM or 2.6 - 41.6 mg·L^{-1}), suggesting that *Crambe* seed germination is tolerant to Cr (**Figure 1**). Zulfiqar *et al*. [38] reported that *Crambe* fresh weight of plants was decreased moderately at 100 μM K_2CrO_4, whereas at 150 μM K_2CrO_4 there was a significant reduction in biomass with no symptoms of severe cellular damage, but at higher concentration (200 and 250 μM), plant showed chlorosis and visible necrosis on leaves. In our experiment *Crambe* root length was decreased by 41.33% (**Figure 2**), shoot length by 15.66% (**Figure 3**) and fresh seedling weight by 27.67% (**Figure 4**) at 0.05 mM Cr, suggesting that *Crambe* is only moderately tolerant to Cr regarding early seedling growth.

3.7. The Influence of Pb

In *Sinapis arvensis*, seed germination was decreased significantly by 6.17% at 1200 μM Pb [34]. In our experiment *Crambe* seed germination was not significantly decreased by the Pb concentrations tested (0.8 - 5.5 mM), suggesting that *Crambe* seed germination is tolerant to Pb (**Figure 1**). In *Eruca* the root length was decreased by 20.0% and shoot length by 23.78% at 50 mg·L^{-1} Pb [31]. In *Sinapis arvensis*, root length was decreased significantly by 66.46%, shoot length by 38.62% and fresh seedling weight by 33.33% at 300 μM Pb [34]. In our experiment *Crambe* root growth was decreased only by 48.67% (**Figure 2**), shoot length by 16.33% (**Figure 3**), and fresh seedling weight by 16.33% (**Figure 4**) at 0.8 mM Pb (165.6 mg·L^{-1}), suggesting that *Crambe* is quite tolerant to Pb.

4. Conclusion

Our results indicate that *Crambe* is tolerant or moderately tolerant to Cu, Zn, Hg, Cr, Pb and Cd but sensitive to Ni regarding seed germination and seedling growth and *Crambe* can be improved as a promising industrial oil crop for phytoremediation of soils contaminated by heavy metals.

Acknowledgements

This work was supported by funds from Science and Technology Department of Hubei Province; Huangshi science and technology bureau; Key Laboratory Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, China; National Natural Science Foundation of China (30771382, 30671334, 30971807, 31201238); an European Committee 7th Framework Programme (ICON, 211400) and Swedish Research Links project.

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