Toxicity Effect of Cr Stress on Seed Germination and Seedling Growth in Lactuca Sativa

Wan Zheng MA 1,2, Wan Min MA 3, Ying Ying DU 1, Qiong Peng DAN 1, Bing Yin 1, Shan Shan Dai 1, Xiang HAO 1

1 Resource and Environmental Engineering College, Anhui Science and Technology University, Fuyang, Anhui 233100, China
2 Key Laboratory of Bio-organic Fertilizer Creation, Ministry of Agriculture, Beng Bu, Anhui 233000, China
3 Agro-industrial-office, Qingdao West Coast New Area Working Committee, Qingdao, Shandong 266400, China

Email: Mwzujs@126.com Email: 281057370@qq.com

Abstract: The impact of Cr6+ on the growth of lactuca sativa in Greenhouse Cucumber was investigated. The seeds of lactuca sativa Italian bolting resistance lettuce were treated by different Cr6+ concentration to study the effects on its seed germination and seedling growth. The results showed that the seed germination rate, vigor index of seedlings decreased with increment of Cr6+ concentration to varying degrees, and vigor germination, vigor index, raw weight, root length significantly lower. The absorption of lettuce seedlings on different nutrient elements is impacted by the concentration of Cr6+.

1. Introduction
As one of the toxic materials that has a wide range of existence in the environment, chromium pollution degree becomes more and more grievous and now Cd is the main pollution element in the nature[1]. Cr6+ is harmful to human which can spoil human’s health. Because of its great toxicity, Cr6+ is carcinogenesis to human[2]. Plant’s roots have a great effect of absorbing and enriching Cr6+, so chromium pollution has endangered the growth of the crops and seriously affects crops production[3,4]. In order to identify the degree that different Cr6+ concentration threat lettuce, we depend on the method——ecological toxic effect method[5]. The more serious the degree of pollution, the heavier the harm to the crop and even make plants die[6]. A large number of studies show that Cd has a prevailing threaten effect to crops, plants in the nature and mainly perform in following aspects: Low concentration of Cr is helpful to their germination and growth; high concentration of Cr shows the threaten effect. Great different tolerance [7] are shown between different corps. Plants enrich the heavy metal during its growing and development. Average chromium content of normal plants is 0.2mg/kg dry weight but chromium excess will produce poisonous effect to plants[8]. Wu Yixue’s study [9] shows that with the rise of Chromium concentration, the threaten effect to lettuce is gradually strengthened and inhibits growth of lettuce, even causes plants death, especially has an obvious influence on basic seedling’s index. The effect to radicle is greater than seedling. There is a wide range of researches on chromium harm but all those researches are focus on heavy metal’s enriched effect to corps, threaten effect, physiological and biochemical effect, tolerance of corps, heavy
metal’s fouling mechanism. remedy and prevention to environmental pollution[10].

This experiment uses the method of Hoagland solution which including Cr and sandy cultivation, cultivates lettuce in illumination box for simulating growth environment of lettuce, observes status of lettuce growth, measure lettuce seeds germination, growth state of roots, seeds and leaves. In order to provide scientific basis of lettuce production, improving quality, Chromium prediction, this experiment uses statistical analysis to study lettuce status of germination and growth with different concentration of Cr.

2. Materials and Method

2.1 Material and Experimental Processing

Lettuce seeds that the experiment used is Italian very late bolt lettuce produced by Nanjing Sheng Fa Seedling limited Company.

Seed Disinfection: Soak lettuce seeds in water overnight for fully water absorption, use 30% sodium hypochlorite (30% sodium hypochlorite is prepared by volume proportion) to disinfect for 5 min, use clear water to wash cleanlily (until no smell of sodium hypochlorite, about 10 times).

Experiment Setting: Use potassium dichromate to Compound 60 mg/L mother liquor, set 6 processes Hoagland solution which including Cr of 0, 5, 10, 20, 40, 60 mg/L, each processes set 3 repetitions, CK, T1, T2, T3, T4, T5, use CK as control that is no chrome.

Germination Cultivation: Selecte the same size plumping seeds to put on the culture dishes that have 2 layers of gauze, each dish sets 15 seeds. Sock lettuce seeds into culture medium with filter paper wetted by chromium solution. Cultivate seeds in constant temperature light incubator that has 16 hour light and 8 hours dark. Each day supply distilled water to keep filter paper humid and each 2 days supply nutrient solution including chromium. Cultivate for 7 days then observe and count growth and germination status of lettuce seeds.

2.2. Testing Items and Method

According to previous research, count 3d germination energy, 7d germination rate. Germination test lasts 7 days, count sprouting number, main root length, seedling fresh weight in the end of the test.

Germination Energy = Total Peak Period Sprouting Number In Given Time/Test Seed Number (Use sprouting seed number in 3 days for calculation);
Germination Rate = Total Sprouting Number In Given Time/Test Seed Number (Use sprouting seed number in 7 days for calculation);

Germination Index = GI = \sum (Gi/Di) Gi : The sprouting number in the ith day, Di: germination days;
Vigor Index = VI = GI*s; s: Seedling fresh weight;
Inhibition Ratio On Root Length /% = (Root Length Of Ctrol Sample - Root Length Of Treated Sample)/ Root Length Of Ctrol Sample×100.

Stop cultivating seedlings until seedlings have 4-5 leaves and show obvious difference. Determine fresh weight of seedlings, put seedlings in envelope then let envelope in oven. Until temperature of oven is up to 105°C, begin timing. After deactivation of enzymes for 15 min, adjust temperature of oven to 85°C. Remove the dry matter that were dried to constant weight and weight dry matter with electronic balance (electronic balance is accurate to 1/10000 gram). Measure indexes of root length of seedlings, chromium concentration of seedlings, nutrient absorption of seedlings, etc.

3. Results and Analysis

3.1. Different concentrations Cr⁺⁺ forces to lactucasativa’s germination and vigor germination rate
Table 1: different concentrations Cr$^{6+}$ forces tolactucasativa’s germination and vigor germination rate

| Treatment | Germination rate% | Germination% |
|-----------|-------------------|--------------|
| CK        | 96±0.043a         | 82±0.02a     |
| T1        | 93±0.037a         | 80±0.10a     |
| T2        | 84±0.023a         | 80±0.00a     |
| T3        | 84±0.043a         | 78±0.02ab    |
| T4        | 71±0.058b         | 64±0.02bc    |
| T5        | 71±0.020b         | 62±0.02c     |

From Table 1, chromium concentration doesn’t have a significant influence on germination energy and germination rate of lettuce seeds but each one of them is decrease in treated sample and has a trend that decrease as the chromium concentration is increasing when its concentration is about 0-20mg/L. When chromium concentration is more than 40mg/L, germination energy and germination rate has a significant influence between chromium concentration that is about 0-20mg/L. When chromium concentration is more than 40mg/L, each treated sample doesn’t have difference.

3.2 Different concentrations Cr$^{6+}$ forces tolactucasativa’s germination index (GI) and vigor index (VI)

Table 2: different concentrations Cr$^{6+}$ forces tolactucasativa’s germination index and vigor index

| Treatment | Germination index | Vigor index |
|-----------|-------------------|-------------|
| CK        | 0.48±0.01a        | 0.16±0.004a |
| T1        | 0.42±0.04a        | 0.129±0.004a|
| T2        | 0.32±0.02a        | 0.071±0.001b|
| T3        | 0.25±0.007a       | 0.048±0.002b|
| T4        | 0.15±0.02b        | 0.019±0.001c|
| T5        | 0.11±0.02b        | 0.014±0.005c|

Tip: The different lowercase letters after numbers of the same column means that the significance of level of 5%, The same below.

From Table 2, Cr$^{6+}$ doesn’t have a significant effect of lettuce seed’s GI. The effect of VI shows a decrease trend as chromium content is decreasing. When chromium has a significant difference on vigor index when its concentration is up to 10mg/L. If chromium concentration is more than 40mg/L, it will show a significant difference on treated samples of CK, 5, 10, 20mg/L. When chromium concentration is more than 40mg/L, there is no significant difference between each two of treated samples. From Table 3, different concentration of Cr$^{6+}$ shows a significant difference on cultivation of lettuce seeds.

3.3 Different concentrations Cr$^{6+}$ forces tolactucasativa’s root length

Table 3: different concentrations Cr$^{6+}$ forces tolactucasativa’s root length

| Treatment | Root length/cm | Root growth inhibition rate% |
|-----------|----------------|------------------------------|
| CK        | 5.47±0.088a    | 0                            |
| T1        | 1.47±0.088b    | 73.21±1.191b                 |
| T2        | 1.13±0.033c    | 79.24±0.946c                 |
| T3        | 0.73±0.088d    | 86.53±1.847d                 |
| T4        | 0.47±0.033e    | 91.44±0.713e                 |
| T5        | 0.37±0.033e    | 93.29±0.641e                 |

Growth of seedlings is reduced as concentration of Cr$^{6+}$ is increasing. High concentration of Cr$^{6+}$
will inhibit the growth of root and show withered symptom. From Table 3, control group show significant difference concentration of Cr$^{6+}$ is more than 5mg/L. When concentration of Cr$^{6+}$ is more than, there is no significant difference on each treated samples. By the analysis of the data which comes from inhibition rate of root length, inhibition rate of root length is increasing as concentration of Cr$^{6+}$ is increasing, which manifests that growth of root and stem became slow, when concentration of Cr$^{6+}$ is more than 40mg/L, inhibition rate of root length is more than 90%. Radicles can’t form and seedlings aren’t vertical.

3.4 Different concentrations Cr$^{6+}$ forces to lactucasativa’s root length and leaf length

| Treatment | Root length/cm | Leaf length/cm |
|-----------|----------------|----------------|
| CK        | 7.56±0.186a    | 8.67±0.167a    |
| T1        | 5.2±0.153b     | 5±0.115b       |
| T2        | 4.37±0.186c    | 3.7±0.058c     |
| T3        | 3.57±0.067d    | 3±0.153d       |
| T4        |                |                |
| T5        |                |                |

Tip: means that the plants is dead in the treated, the data is not shown.

From Table 4, the growth of lettuce seedlings has a significant difference with different concentration of Cr$^{6+}$ and growth tendency of root-leaf is decrease as concentration of Cr$^{6+}$ is increasing. With chromium concentration is increasing, growth of lettuce seedling’s root-leaf is continuous reduction, leaves are even withered in high chromium concentration. When chromium concentration is 40, 60mg/L, lettuce seedlings can’t sprout and growth. Table 4 shows the data analysis is more accurate, when chromium concentration is more than 5mg/L each treated sample has significant difference between blank control sample and each treated sample has significant difference from themselves.

From Table 3 and Table 4, growth of lettuce seedling roots have significant difference with threaten of Cr$^{6+}$, which shows that length of root become shorter and growth of root become worse, even seedling roots can’t form lateral root system. From those data, high concentration of Cr$^{6+}$ has a great threaten effect to the growth of lettuce seedling.

3.5 Different concentrations Cr$^{6+}$ forces to lactucasativa’s Seedling fresh weight and Seedling dry weight

| Treatment | Fresh weight/g | Leaf weight/g | Root weight/g |
|-----------|----------------|---------------|---------------|
| CK        | 4.555          | 0.142         | 0.075         |
| T1        | 2.398          | 0.0785        | 0.0324        |
| T2        | 1.306          | 0.057         | 0.0147        |
| T3        | 1.126          | 0.041         | 0.0098        |
| T4        |                |               |               |
| T5        |                |               |               |
Fig1: Rectitude of different concentrations Cr$^{6+}$ stress on lactuca sativa’s dry root leaf and CK

From Table 5, fresh weight and dry weight of lettuce seedlings dramatically reduce as chromium concentration is increasing and fresh weight and dry weight of lettuce don’t change a lot when chromium concentration is more than 10mg/L.

3.6 Effect of different concentrations Cr$^{6+}$ stress on absorbing NPK of lactuca sativa

NPK of lettuce seedling comes from nutrient solution. From Fig 2 we can see the trend of total nitrogen, potassium and phosphorus concentration, nitrogen and potassium concentration in seedlings are decreasing as chromium concentration is increasing. When chromium concentration is in 0 to 10mg/L, their concentration decreases little. When chromium concentration is more than 10mg/L, it has a large amplitude of influence which explains that chromium concentration has a great inhibitory effect to the absorption of N, P. When chromium concentration is more than 10mg/L, it will have a strong toxicity and will effect the growth of seedlings.

Phosphorus concentration is increased at first then decreased and the change range is smaller than total nitrogen, potassium’s. In the chromium concentration of 0-10mg/L, total phosphorus concentration is increased in a little range. When its concentration is up to 10mg/L, phosphorus concentration of lettuce seedlings decreases gradually which explains that 10mg/L of phosphorus concentration has a strong toxicity to lettuce seedlings.

4. Conclusion

In germination experiment, the significance of germination energy, germination rate, germination index, vigor index become large as the chromium concentration is increasing and show an obviously decrease trend. With chromium concentration is increasing, Inhibition of root is enhanced even seeds can’t form radicle and chromium concentration has a great significance on the growth of roots.

In the experiment of seedlings’ cultivation, the growth of plants is worse and worse even can’t survive. The threaten of chromium to seedling root is more serious than its to stem and leaf. It also inhibits the growth of root. With the increase of chromium concentration, the absorption and enrichment become gradually stronger; lettuce has a more concentration of chromium.

Because lowest concentration of Cr$^{6+}$ is 5mg/L and the data that the effect of low concentration of Cr$^{6+}$ to lettuce sprouting and growth of lettuce seedling, so we need further research and observation.
Acknowledgements:
This work was financially supported by the Anhui Science And Technology University Natural Science Foundation (ZRC2016483); Natural Science Key Project of An Hui Province Education Department (KJ2015A173); College Students’ Innovative and Entrepreneurial Training Program of Anhui Science And Technology University(201710879018)

References
[1] Su Qijiao, Wang Ji, Lu Xiaohui et al. Analysis and study on heavy metal content in the suburban vegetable garden soil and vegetable[J]. Journal of Guizhou Normal University (Natural Sciences), 2016, 34(6): 27-32.
[2] Yang Yali, Li Youli, Chen Qingyun et al. The research progress of lead cadmium and chromium in soil on the growth and migration of vegetables[J]. Acta Agriculturae Boreali-Sinica, 2015, 30(S1): 511-517.
[3] Li Shunjiang, Li Peng, Li Xinrong et al. The Influence of Concentration of Chromium, Cadmium in Soil-Crop System Under Different Fertilizers and Fertilization Amount[J]. Journal of Agricultural Resources and Environment, 2015, 32(3): 235-241.
[4] Li Xianzhu, Shen Yubing, Li Wanlong et al. Studies on Screening of Cr(VI) Hyper accumulator[J]. Environmental Science and Management, 2015, 40(2): 12-16.
[5] Ma Xinyan1, Li Shan, Kang Weijun. Studies on toxic effects of trivalent and hexavalent chromium onmaizes[J]. Chinese Journal of Health Laboratory Technology, 2008, 18(3): 390-392, 425.
[6] Xu Fenfen, Zhao Jing, Zhu Qizhi et al. Effects of Pb^{2+}, Cr^{6+} Single and Combined Stress on Seed Germination in Peanut[J]. Journal of Shangrao Normal University, 2016, 36(6): 83-86.
[7] Zhang Longchong, Zhang Qiuling, Zhao Yuan et al. Enrichment characteristics of four native plant species from heavy chromium pollution area and their response of seed germination to chromium (Cr6+) treatment[J]. Journal of Henan Agricultural University, 2016, 50(4): 550-557.
[8] Liang Yanpeng, Li Zonglin, Zhang Xuehong et al. Accumulation of Cr in rice under single and combined pollution[J]. Ecology and Environmental Sciences, 2016, 25(9): 1540-1545.
[9] Wu Yixue, Ai Tiancheng, Chen Chun. Effect of High Concentration Chromium Stress on Seed Germination and Seedling of Lettuce[J]. Journal of Yangtze University (Nat Sci Edit), 2013, 10(23): 70-71, 85.
[10] Wang Aiyun, Zhong Guofeng, Xu Gangbiao et al. Effects of Cr(VI) Stress on Physiological Characteristics of Brassica juncea and Its Cr Uptake[J]. Environmental Science, 2011, 32(6): 1717-1725.