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Cd enrichment and nutrient variation in pine willow

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Abstract. Heavy metals can be enriched by some vegetables. However, it is not well known about their impacts on nutritional quality of vegetables. In this study, a kind of vegetable, pine willow (*Lathyrus quinquenervius*) grown hydroponically in simulated Cd contaminated culture was used to investigate both metal accumulation and its influences on nutrient content variation and growth. The results show that under Cd stress, the biomass, K, Ca, Zn and β -carotene of pine willow sprouts decreased significantly, while vitamin E and vitamin C increased slightly. Cd can be sharply enriched by pine willow shoots rather than roots. This study implies that it is absolutely necessary to remove Cd from irrigation water in order to avoid problems of both food safety and nutritional quality of pine willow sprouts.

1. Introduction

Heavy metal pollution is a global problem and could seriously affect human health. It has been reported that the accumulation of heavy metals is rapidly increasing especially in farmlands with intensive agriculture and large irrigation systems [1, 2]. Information about heavy metal accumulations in vegetables is useful for assessing their damage to human health and phytoremediation [3]. Cd is a popular heavy metal in sewage and soil, and its excessive intake is associated with cardiovascular, kidney, nervous and bone diseases. It is also one of the most common ions [4, 5] in aquatic environment.

Once toxic heavy metals enter the environment, they are enriched by plants, resulting toxic effects of plants. For example, Cd is easily taken up by plant roots and accumulates in plants with concentrations that create risks in the food chain. Food containing cadmium is generally regarded as the main environmental sources of human exposure to cadmium. Cadmium can cause renal damage, bone fracture, cancer, kidney dysfunction and hypertension. Thus, during the last decade, the toxicity of heavy metals like lead, cadmium to plants has drawn great attentions of many environmental and public health scientists notably because plants represent the main route of heavy metal entry into the food-chain, presenting a danger to human health.

Heavy metals with toxicity also have severe impacts on biological processes ranging from microbial activities to primary production of plants [6-8]. Although there were many reports on the types and contents of heavy metals in vegetables, few studies have examined whether heavy metal ions can affect the micro nutrients in vegetables. Vitamin A, C, and E are important to human health. The conventional wisdom is that vitamin contents in vegetables are often linked to photosynthesis in plants [9]. However, it is also necessary to explore whether vitamins are affected by the concentrations of heavy metals in water.

Pine willow is native to South America. Its growth cycle is usually short and it grows rapidly under proper environment. As one type of vegetables, it can provide calcium, phosphorus, potassium and



other trace elements for human. Pine willows were usually cultivated hydroponically with contaminated water containing various pollutants including heavy metals. Since the quality of vegetables is closely related to the aquatic environment, pine willow cultured hydroponically might provide a model for investigation of influences of heavy metals on their growth and nutrition.

Herein we investigated the effects of Cd^{2+} on the germination, growth of pine willow grown in hydroponic culture. Specifically, the differences of Cd uptake and accumulation as well as its impact on nutrient variation in pine willow were also explored.

2. Materials and methods

2.1. Plant material and growth conditions

Selected healthy seeds were washed with distilled water several times and soaked in distilled water for 24 hours at room temperature ($20 \pm 0.5^\circ\text{C}$). After sprouting, the seedlings were transferred to hydroponic dishes with cultivation paper soaked in the culture prepared by metal solution. Four Cd treatments (with concentration of 0, 5, 10 and 20 mg/L, as $\text{Cd}(\text{NO}_3)_2$, respectively) were applied, denoted as Control, Cd5, Cd10 and Cd20, respectively. The liquid level in the dish was slightly higher than the cultivation paper to ensure the culture paper was completely moist. All of the experiments were carried out indoor artificial breeding boxes at $25 \pm 0.5^\circ\text{C}$ with 16/8 h dark/light period. All the cultivation dishes were irrigated with corresponding metal solution once every morning. Three parallel experiments, each containing 10 plants were applied for all treatment groups.

2.2. Biomass analysis

Pine willow seeds sprouted after 5 days. At this time, 10 pine willow sprouts were randomly selected to measure their heights. It was repeated every 2 days, seven times in total. After 15 days, 10 pine willow sprouts were washed with distilled water three times and the fresh weight was measured after drying in air. Then, the roots of pine willow were cut off and weighed separately. Finally, the fresh pine willow plants were dried in an oven at 70°C for 48 hours to determine the dry weight and calculate the water content. Each group was measured three times.

2.3. Vitamin analysis

Pine willow leaves (0.5 g) were crushed in a mortar. Then, 10 mL of anhydrous ethanol was added and agitated to dissolve the vitamins fully. The residue was filtered with 0.25 μm filter paper, the filtrate was transferred to a 10 mL volumetric flask and stored in a refrigerator until testing. The vitamins were extracted quickly while protected from light. The vitamin C content was determined by Waters 600 high-performance liquid chromatograph (Waters, Milford, MA, USA) equipped with a Lichrospher C18 (length 250 mm, i.d. 4.0 mm, 5 mL, Merck, Germany), which fitted with the same guard column. A mobile phase gradient composed of methanol (solvent A) and 5 mmol/L KH_2PO_4 , pH 2.65 (solvent B) was used according to the following program: linear increment starting with 5–22% A for 6 min and returning to the initial conditions within the next 9 min at a flow rate of 0.8 mL/min. The fat-soluble vitamins were extracted with anhydrous ethanol. The ratio of methanol to water in the mobile phase was 92:8. The eluate was detected by Waters 996 photodiode array detector set at 245 nm [10].

2.4. Elements analysis

Plants were harvested after treatment and the roots were washed three times with distilled water. Each seedling was divided into shoot and root, and then oven-dried at 70°C for 48 h to obtain the dry weight. Dried plant tissues from the same container were pooled into one sample and ground to fine powder. Approximately 0.5 g of the powdered material from the pooled sample per replicate was digested in a mixture of $\text{HNO}_3/\text{HClO}_4$ (4/1, v/v) at 240°C for 3 h and then dissolved in dilute HCl (50 mL, pH=3). Cd, Ca, Zn, Mg, K were analyzed by atomic absorption spectrophotometer.

2.5. Statistical analysis

The data were analysed by SPSS software (ver. 21.0, SPSS Inc., Chicago, IL, USA). Chi-square test was used to test the significance of differences. If $\chi^2 > 3.84$, the differences was significant ($p < 0.05$), meaning Cd has important effects on the index investigated.

3. Results and Discussion

3.1. Effects on growth

3.1.1. Germination As shown in Table 1, the germination rate of pine willow differed significantly ($p < 0.05$) between the Cd treatment and control. The germination rates are 92%, 87%, 85% and 81% for Cd²⁺ concentrations of 0 (control), 5 (Cd5), 10 (Cd10) and 20 mg/L (Cd20), respectively, which decline with increasing Cd concentration.

When pine willow is cultured with Cd solution, germination rate of pine willow is significantly lower than the control. Cd has high inhibition rates for percentage of germination (e.g., more than 20% for 20 mg/L Cd²⁺ after 5 days of germination). The results are in agreement with previous reports of germination investigation for other plants using either sewage or industrial effluents [11-14]. We believe that the Cd accumulation in pine willow is responsible for the toxicity observed towards reduction of germination rates, which will be discussed later on.

Table 1. Germination rate of seeds of pine willow cultivated 5 days.

Group	Sum	Budding	Germination rate	2	p
Control	300	276	92	—	—
Cd5	300	261	87	3.99	< 0.05
Cd10	300	255	85	7.222	< 0.05
Cd20	300	243	81	15.534	< 0.05

Table 2. Mean biomass of 10 pine willow sprouts cultivated hydroponically 15 days.

Group	Fresh shoot weight(g)	Fresh root weight(g)	Dry shoot weight(g)	Shoot/Root	Water content(%)
Control	4.52	1.88	0.42	2.44	90.7
Cd5	3.62	1.18	0.33	3.03	90.9
Cd10	2.96	0.65	0.26	4.55	92.6
Cd20	2.17	0.38	0.21	5.56	90.3

3.1.2. Biomass Table 2 shows the influence of cadmium on biomass of pine willow. Under Cd stress, biomass of pine willow decreases sharply in comparison with the control. With increasing Cd concentration, the fresh, dry and root weight decrease significantly. The effect is obvious on root weight, followed by dry weight and fresh weight. And hence the shoot/root ratio is increased significantly with the increasing of Cd²⁺ concentration, while water content remains the level of control group except for Cd10 group.

Typically, underground stresses decrease the shoot/root ratio of biomass, increasing the root surface area for the absorption of water and nutrients. However, under Cd stress, the shoot/root ratios behaviour adversely in hydroponic condition, and the fresh, dry, and root weights of pine willow also decrease sharply.

3.1.3. Height Figure 1 plots the height of pine willow under different treatments for 15 days. With the Cd treatment, the growth of pine willow is inhibited and this inhibitory effect increases significantly with time and Cd^{2+} concentration. With the increase of cadmium ion concentration, the inhibitory effect is also more significant. With high Cd^{2+} concentrations the growth is less than half of that of the control after 15 days.

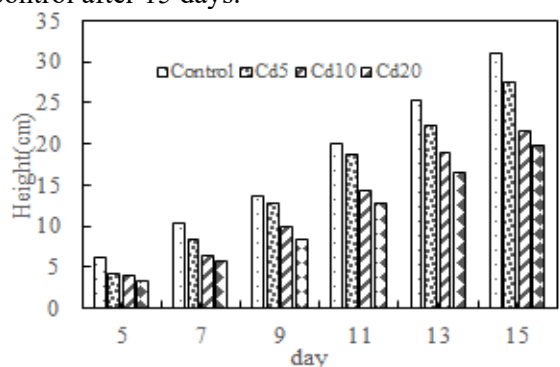


Figure 1. Height of pine willow under Cd Stress.

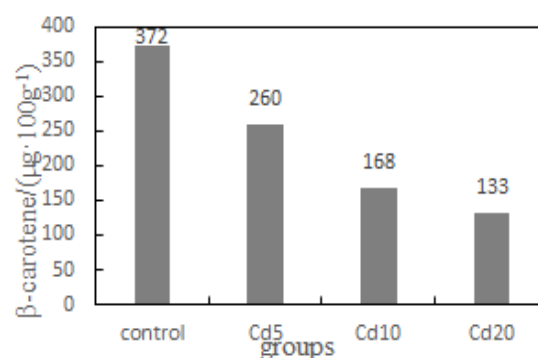


Figure 2. Variation of β-carotene in pine willow after 15 days growing under Cd stress

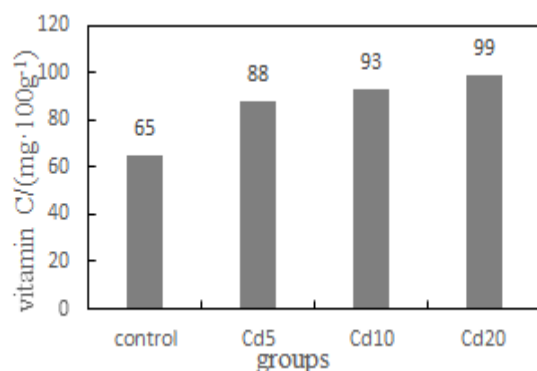


Figure 3. Variation of vitamin C in pine willow after 15 days growing under Cd stress

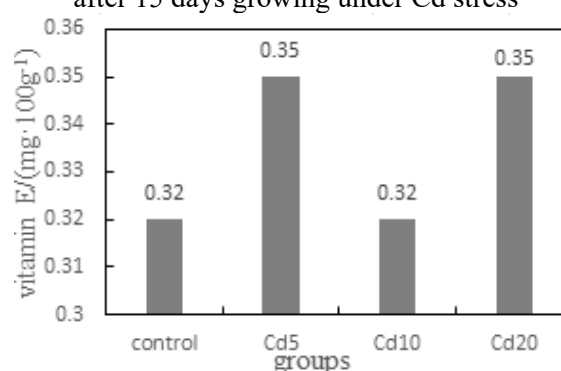


Figure 4. Variation of vitamin E in pine willow after 15 days growing under Cd stress

3.2. Effects on nutrient content

3.2.1. β-carotene As shown in Figure 2, β-carotene content of pine willow decreases significantly under heavy metals stress. When the Cd^{2+} concentration is 5, 10 and 20 mg/L, respectively, the content of β-carotene in fresh pine willow is only 260, 168 and 133 μg/100g, respectively, much lower than the content of 372 μg/100g in control sample.

3.2.2. Vitamin C The influence of cadmium on vitamin C of pine willow is shown in Figure 3. Under cadmium stress, the vitamin C contents of pine willow increase significantly with increasing of Cd^{2+} concentration. When Cd^{2+} concentration is 5, 10 and 20 mg/L, Vitamin C content increases from 65 mg/100g (control) to 88, 93 and 99 mg/100g, respectively

3.2.3. Vitamin E As shown in Figure 2, the measured vitamin E levels do not differ significantly between each experimental group and the control. After 15 days of cultivation, the vitamin content is about 0.35 mg/100g, even 9% higher than the control group.

Unlike β-carotene, vitamin E and vitamin C are antioxidative vitamins relatively more sensitive to outside stress, which may be partially responsible for the increase of the latter two types of vitamin under heavy metal stress. Under the toxic environment, the plant has stress response. Many literatures reported that plants can produce stress effects under specific conditions. Antioxidation for intoxication

is more sensitive among the stress response under the heavy metal stress. The synthesis of vitamins is mainly related to photosynthesis and enzyme, which is greatly affected by Cd toxicity. Therefore, the increase of vitamin C and E in pine willow may be mainly related to Cd Stress. Although Cd stress strongly inhibited the growth of pine willow in this study, the vitamin C and E contents were similar among the heavy metal groups.

3.2.4. Mineral elements As shown from Figure 5, Cd treatment at all concentrations reduce K, Ca and Zn contents in pine willow sprouts significantly ($p < 0.05$). Mg content in pine willow sprouts only increases significantly ($p < 0.05$) at high concentration of 20 mg/L Cd^{2+} , but they are not interfered sharply at low Cd concentrations.

Mineral elements are affected by Cd stress in the order $\text{K} > \text{Ca} > \text{Zn} > \text{Mg}$ (Figure 5). Ca is one of the most affected mineral essential elements by Cd stress. The Ca level in the high Cd group is less than half of that in the control. Even with added Ca, the influence caused by Cd ions is not reduced, implying that Cd mainly affects the uptake and transport of Ca^{2+} . The physical-chemical character of divalent Cd^{2+} , Ca^{2+} and Zn^{2+} , such as electronegativity is similar, and these three ions may compete for binding sites in the cell, leading to the large drop in the Ca^{2+} and Zn^{2+} contents.

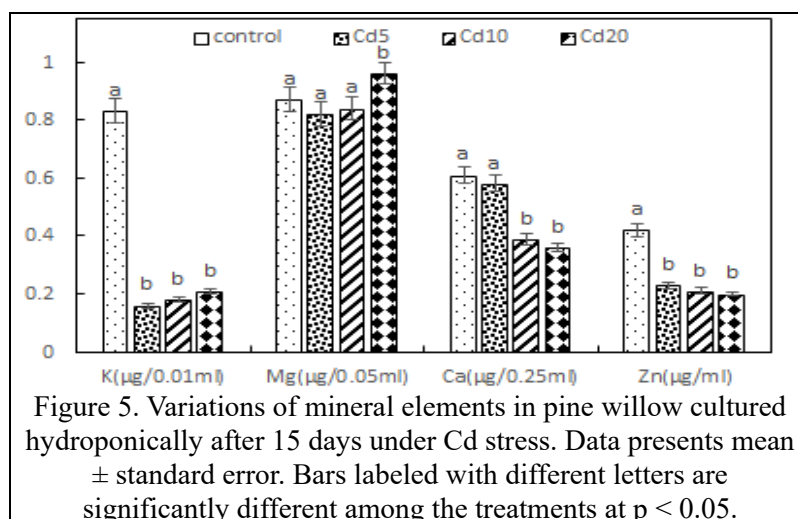


Table 3. Cd accumulation in pine willow cultivated hydroponically.

Group	Cd/(ug/ml)		Shoot/Root
	Shoot	Root	
Control	-	-	-
Cd5	0.14	0.03	4.67
Cd10	0.18	0.04	4.50
Cd20	0.23	0.05	4.60

3.3. Heavy metals accumulation

As shown in Table 3, with the increase of Cd^{2+} in hydroponic environment, the toxic cadmium content in pine willow increases at each Cd^{2+} concentration treatment significantly ($p < 0.05$, $p < 0.05$, $p < 0.05$), meaning that pine willow may enrich cadmium ion sharply. However, enrichment of cadmium in pine willow is distributed differently. Cd is detected little in root but much in shoot of pine willow, which is also observed from the high value of shoot/root ratio. Since the biomass of shoot, the edible parts of pine willow is higher than that of root (Table 2), issue of food safety concerning large exposure of Cd should be cared when the pine willow is hydroponically cultured by contaminated water.

4. Conclusion

In summary, pine willow is found very sensitive to Cd and can accumulate Cd significantly. Cd can be enriched in pine willow through shoot, the edible parts of this vegetable, causing issue of food safety of pine willow at the same time. Cd in pine willow could affect both growth and nutrients of pine willow sprout, such as mineral essential elements and vitamins, especially, K, Ca, Zn and β -carotene. This study reveals that cadmium-free water is more crucial for the safety, yield and nutritional quality of pine willow, especially in hydroponic condition.

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